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The rat animal model for noise-induced hearing loss

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Rats make excellent models for the study of medical, biological, genetic, and behavioral phenomena given their adaptability, robustness, survivability, and intelligence. The rat's general anatomy and physiology of the auditory system is similar to that observed in humans, and this has led to their use for investigating the effect of noise overexposure on the mammalian auditory system. The current paper provides a review of the rat model for studying noise-induced hearing loss and highlights advancements that have been made using the rat, particularly as these pertain to noise dose and the hazardous effects of different experimental noise types. In addition to the traditional loss of auditory function following acoustic trauma, recent findings have indicated the rat as a useful model in observing alterations in neuronal processing within the central nervous system following noise injury. Furthermore, the rat provides a second animal model when investigating noise-induced cochlear synaptopathy, as studies examining this in the rat model resemble the general patterns observed in mice. Together, these findings demonstrate the relevance of this animal model for furthering the authors' understanding of the effects of noise on structural, anatomical, physiological, and perceptual aspects of hearing. https://doi.org/10.1121/1.5132553

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I. INTRODUCTION

Animal models are used in the study of human diseases because many of the lines of investigation needed to make advancements in biomedical knowledge utilize paradigms that simply cannot be employed ethically on humans. In particular, and for the purposes of this review, this is remarkably salient for the study of noise-induced hearing loss (NIHL). By this, the authors mean that in order to scientifically study the myriad effects of NIHL on the auditory system, scientists must *induce* a hearing loss in a subject by exposing them to high levels of continuous noise or some other acoustic trauma. Clearly, such investigations would be unethical if performed on human participants. Thus, animal models provide a more ethical avenue of research, and more specifically, the rat provides hearing scientists with a robust human analog for studying the detrimental effects of NIHL.

The rat (genus *Rattus*) is a general term used to refer to larger rodent species with body lengths of 5 in. or longer. As research animals, rats make excellent models for the study of medical, biological, genetic, and behavioral phenomena given their adaptability, robustness, survivability, and intelligence. From a management perspective, rats are affordable and relatively easy to maintain.

With respect to hearing, there are both similarities and significant differences between humans and rats. Developmentally, rat hearing matures only after birth whereas humans are able to hear prenatally. This very important difference makes it possible to study hearing in more ways than would be possible in humans. In other words, because the human cochlea matures before birth, scientists are unable to examine the maturation of the cochlea in regard to hearing sensitivity and other parameters in the developing human auditory system. In contrast, objective measures of hearing such as the auditory brainstem response (ABR) can be obtained from rat pups at 12 to 14 days after birth, before the rat cochlea has fully matured. This opens the opportunity for innovative developmental studies into how the mammalian cochlea matures and develops its distinct abilities and characteristics. Moreover, this unique attribute of the rat compared to humans provides researchers with a clinically relevant window into developmental, structural, and functional malformations and how these ultimately affect hearing outcomes. The frequency range of rat hearing is approximately 250 Hz to 80 kHz with the greatest sensitivity occurring between 8 and 38 kHz, a range much higher than that found in humans. In contrast, the middle ear mucosa and ossicles are remarkably similar to humans. Like humans, the rat cochlea has approximately two and a half turns with a similar arrangement of inner and outer sensory hair cells. The rat central auditory system also shares many anatomical and physiological features that are present in humans.

The use of rats for hearing research increased in popularity during the 1980s, primarily for structural and functional studies of the ear. However, and quite importantly, the rat is a popular model for studying the effects of NIHL because it embodies a reasonable compromise between the myriad genetic mutant strains available in the mouse model (there are some mutant strains that have been developed in the rat), and the behavioral training and learning attributes observed in chinchillas, gerbils, and guinea pigs. Moreover, with the increasing biomedical focus on the investigation of changes in genetic transcriptome and protein expression in sensory systems following trauma, the rat represents a much more feasible model to study changes associated with NIHL. This is because whereas many immunohistochemical antibodies and real-time quantitative polymerase chain reaction

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assays are available and well-developed for the rat, these investigative tools are comparatively scant for several of the other traditionally employed rodent models such as chinchillas and guinea pigs.

The authors' purpose in writing this review is to present a general overview of the rat model for studying NIHL and highlight several of the advancements in hearing science that have been made utilizing this animal model. First, we discuss and characterize several of the most popularly employed rat strains in hearing science. Second, we cover some important anatomical and physiological considerations when selecting the rat as an animal model with particular attention paid to hearing sensitivity, hearing range, and cochlear anatomy of the rat. Then, we review evoked potentials and behavioral hearing tests used in this animal model before examining correlations associated with noise-induced cochlear damage and hearing loss dose-effects. We then confer findings from studies on the effects of various types of noise exposure including impact and impulse noise, steady-state traumatic noise, and non-traumatic noises as well as newer data on noise-induced synaptopathy—an area of increasing attention within the field of hearing science. Finally, we discuss recently discovered changes in the central auditory system of the rat after noise overexposure. This manuscript is intended to highlight the importance of the rat animal model for furthering our understanding of the effects of noise on structural, anatomical, physiological, and perceptual aspects of hearing as well as potential genetic susceptibility to NIHL.

II. CHARACTERIZATION OF POPULAR SCIENTIFIC RAT STRAINS

Rodents, including rats and mice, are the most commonly used animal model in biomedical research. More importantly, rodents provide researchers with a variety of species and strain options. From a management perspective, many rat strains can reach maturity in 3 months and females can have up to 12 litters, each with 2 to 22 pups per year (with an average of 8 or 9 pups). Gestation periods are short and last 21 to 26 days. Because different strains present with natural hearing differences, it is important to consider and choose the most appropriate rat strain. For example, there are 51 different species of rats that are found in the wild, and they vary widely in their physical characteristics and habitat. Of these, the Norway rat is widely used within scientific settings. Over several generations the Norway rat has been inbred to fertile isolated strains, with the goal of producing multiple near identical rats that carry specific physiological traits of interest. Whereas there are numerous rat strains, a few strains have more appeal over others; these include Wistar, Long-Evans, Sprague Dawley, and Fischer 344.

The Wistar rat is an albino rat developed at the Wistar Institute in 1906 for biological and medical research and is a well-known model for both NIHL and age-related hearing loss. Notably, the Wistar rat was the first model strain developed during a time when many scientific settings were working with the common house mouse, and it remains one of the most popular rats used in research. The Wister rat was later used to develop the Long-Evans rat and the Sprague Dawley rat. The Long-Evans rat is characterized as white with a black or a brown hood and is a preferred rat model among behavioral and obesity researchers. The Sprague Dawley rat is an albino rat that was originally produced by the Sprague-Dawley farms in Madison, Wisconsin in 1925. The Sprague Dawley rat is largely used in medical and nutritional research and is known to be calm and easy to handle. Another popular model in the evaluation of changes in the auditory system is the albino Fischer 344 rat. The Fischer 344 strain was developed in 1920 by M. R. Curtis at Columbia University Institute for Cancer Research. These strains demonstrate variability within their auditory sensitivity; for example, the Fischer 344 has approximately 20 dB better hearing sensitivity at 4 kHz compared to the FBN rat (a hybrid cross between the Fischer 344 and the Brown Norway Rat) which has better hearing at 32 kHz by about 20 dB (Turner *et al.*, 2005).

Normative ABRs and audiological behavioral thresholds in rats of different ages, sex, and strains have been previously reported by Borg (1982) using 6 Sprague Dawley rats and 56 Wistar rats, 36 of those being normotensive and 20 of those being spontaneously hypertensive. Several other researchers have also obtained threshold sensitivity from white laboratory rats (Cowles and Pennington, 1943; Jamison, 1951; Gourevitch, 1965; Gourevitch and Hack, 1966; Kelly and Masterton, 1977). However, there has been poor agreement across studies with threshold variability as high as 40 dB. No significant sex differences were noted by Borg (1982) and follow up statistical analyses were performed without separating males and females. Their study found small threshold differences among the three strains only at 1500 Hz, with both good reliability and repeatability of thresholds. ABR thresholds were obtained approximately 10–20 dB higher but mirrored behavioral thresholds as expected. It is important to note that although some degree of differential hearing ability is observed between several commonly employed rat strain models, overall, the findings of the aforementioned studies suggest strain differences do not dramatically impact hearing sensitivity when thresholds are obtained behaviorally or from ABRs.

III. THE RAT MODEL: ANATOMICAL AND PHYSIOLOGICAL CONSIDERATIONS

The rat is a commonly used animal model of human diseases, and nearly all steps in auditory processing have been studied in depth in this laboratory animal. As is typical of many rodents, the general anatomy and physiology of the rat's auditory system is broadly similar to that observed in humans, and this has resulted in extensive use of the rat model for investigating the effect of noise overexposure on the mammalian auditory system (e.g., Abbott *et al.*, 1999; Milbrandt *et al.*, 2000; Chen and Fechter, 2003; Cheung *et al.*, 2012; Han *et al.*, 2012; Kang *et al.*, 2013; Yang *et al.*, 2016).

Hearing sensitivity, hearing range, and cochlear anatomy vary considerably among commonly used laboratory animals (Muller, 1991; Greenwood, 1996; LePage, 2003). For example, cochlear tonotopic maps have been developed for the cat (Liberman, 1982), guinea pig (Greenwood, 1990), gerbil (Muller, 1996), chinchilla (Greenwood, 1990), mouse

(LePage, 2003), rat (Muller, 1991), and several other animals. Each model possesses different characteristics regarding the aforementioned parameters, and these differences must be calculated when designing any investigation into the effects of noise overexposure on the auditory system. Behavioral audiograms of the rat (Kelly and Masterton, 1977; Borg, 1982), in conjunction with physiological characterization of cochlear neurons via injection of the neuronal tracer horseradish peroxidase (Muller, 1991), indicate the rat possesses hearing from 250 Hz to 80 kHz when stimuli are presented at sufficient suprathreshold levels. Though maximum hearing sensitivity in the rat occurs at 8 kHz, a broad range of frequencies from 8 to 38 kHz are shown to be nearly as sensitive (Kelly and Masterton, 1977).

Other factors to consider when using the rat as a model include some particular characteristics of the rat's auditory anatomy. Generally, a great many similarities can be drawn between the auditory anatomy of the rat, other rodents, and humans. For example, rats possess both inner hair cells (IHCs) in a single row and outer hair cells (OHCs) in three rows arranged along the organ of Corti (Chen and Fechter, 2003), and these sensory cells are more compressed in the basal turn than in the apical turn of the cochlea (Burda *et al.*, 1988). More specifically to the rat, this particular animal model possesses a cochlea with a maximum modiolar height of approximately 2.4 mm and is comprised of two and a half cochlear turns. The basilar membrane length varies according to strain and from individual animal to animal, from 9.4 mm in the Wistar rat up to 12.1 mm in the wild *Rattus rattus*. The total number of OHCs in the rat ranges from approximately 3600 to 4500 cells, the number of IHCs vary from approximately 980 to 1300 cells, and the total number of cochlear neurons has a range of approximately 16 500 to 18 400 (1500–1750 neurons/mm) (Burda *et al.*, 1988). For comparative purposes, a human and rat cochlea are shown side by side in Fig. 1. The general similarities can be seen both in the shape and relative location within temporal bone.

Both old world rats and mice (Muridae) are among the most commonly utilized animal models of noise overexposure. However, when compared to the much smaller dimensions of the mouse (e.g., with a typical cochlear height of approximately 1.3 mm), the rat's larger size offers several advantages. Because the cochlea and auditory centers of the central nervous system (CNS) are substantially larger in the rat, this animal model provides for comparative ease of handling and microdissection of otologic and CNS tissues. However, there are several peculiarities of rat anatomy that can also make it a difficult model for otologic investigations. In particular, rats possess a fragile junction between the auditory bulla, and their tympanic membranes do not form a complete seal between the intratympanic space and the external auditory meatus, making them particularly susceptible to otitis media (Albuquerque *et al.*, 2009; Reis *et al.*, 2017). Additionally, though most anatomic structures of the rat cochlea parallel those found in humans, including the presence of the organ of Corti, the tectorial membrane, Reissner's membrane, Deiter's phalangeal cells, etc., Hensen's cells are absent in rats (Albuquerque *et al.*, 2009). Thus, the recent surge of interest in more fully illuminating the complex roles played by cochlear supporting cells may make the rat slightly less ideal for modeling the functionality of cochlear supporting cells in humans.

FIG. 1. (Color online) Human cochlea and temporal bone from a de-identified cadaver gifted to the UT Southwestern Medical Center Willed Body Program is shown on the left. Sprague Dawley rat cochlea and temporal bone is shown on the right. White bar scale is 10 mm.

IV. NOISE VARIABLES

A. Environmental noise

A great deal of effort is taken to control environmental variables within animal laboratory facilities; this includes regulating lighting, temperature, humidity levels, airborne dust and pollution, food and water supply, along with the acoustic environment. Typically, the acoustic ambience is given less consideration, possibly suggesting that acoustic surroundings have little impact on laboratory animals. However, acoustic noise levels within laboratory facilities may result in behavioral and physiological effects within subjects, providing one potential explanation for the large variability seen in animal models within and across studies. Noise is commonly a present unintended environmental variable rather than a controlled independent variable purposefully manipulated, possibly confounding experimental data.

Currently, the *Guide for the Care and Use of Laboratory Animals* outlined by the National Research Council (Clark *et al.*, 1997) provides guidelines regarding noise in animal care facilities. Recommendations state that researchers and animal care personnel should take into account sound intensity, frequency, onset of noise exposure (quick vs slow onset), noise duration, noise oscillation, and subject specific considerations such as history of noise exposure, hearing range (as many species can hear frequencies that are inaudible to humans), and susceptibility of species and strain. These guidelines further state that noisy animals should be housed away from quieter animals, unnecessary noise should be minimized, and exposure

to sounds louder than 85 dB may have auditory as well as non-auditory effects. The impacts of noise exposure on non-auditory systems are commonly overlooked within animal-based research (Turner *et al.*, 2005). A large body of literature implicates noise exposure impacting the level of arousal, therefore impacting several organ systems and therefore biomedical and behavioral research that involves these mechanisms. Turner *et al.* (2005) have suggested that the potential impact of noise in the environment should be taken into account regardless of the specific field of research. Efforts should be made to either minimize noise as a confounding variable or include noise as an experimental variable. Researchers and animal care personnel should be aware of the range of hearing sensitivity in the specific species they are working with in order to help minimize noise as a confounding variable. Along with hearing range differences across animal species and strains, some may also differ in hearing function.

Less attention is given to sound intensities less than 85 dB as they have not been conclusively shown to cause hearing loss or trigger autonomic stress reactions. But these lower level exposures may alter auditory processing if presented chronically. More importantly, these lower levels may be masking communication signals among animals via ultrasonic vocalizations (Cohen and Weinstein, 1981).

Some environmental noise within the animal facility cannot be avoided; for example, facilities are typically constructed of concrete walls and floors, meant to minimize surfaces that can collect dust and dander, but result in increased noise reverberation times and lack of noise absorption. Other unavoidable environmental noise sources include technical devices, maintenance operations, and the animals themselves. Technical devices include air conditioners, ventilated rack systems, lab equipment, and fire alarms; maintenance noise includes opening/closing room and cage doors, push carts, and conversations between animal care workers; and animals themselves contribute to environmental noise via rattling, climbing, and chewing on cages and animal vocalizations. The effects of environmental sounds are an important consideration for studies of noise exposure in nearly any animal model (including rats), particularly when studying the effects of subclinical noise exposures. These subclinical exposures refer to intensity levels that generally induce only temporary threshold shifts and do not produce any evidence of permanent changes in hearing sensitivity (Kujawa and Liberman, 2009; Sheppard *et al.*, 2017; Frye *et al.*, 2018; Zhao *et al.*, 2018).

B. Experimental noise

Most of the studies performed to analyze noise overexposure effects in rats involve intense noise exposures that permanently damage the cochlea and can lead to altered transmission of acoustic information along the central auditory pathway. Traumatic noise exposure typically results in both acute and chronic changes in the auditory system, and noise overexposure can cause either a temporary threshold shift (TTS) or both a TTS and permanent threshold shift (PTS). Noise exposures greater than 100 dB sound pressure level (SPL) can often produce PTS. The loss of peripheral hearing from both noise and aging leads to reduced sensory input to the brain that can result in alterations along the ascending auditory pathway. These alterations are often manifested by an imbalance between excitatory and inhibitory processes in rats and other rodents (Wang *et al.*, 1996; Schatteman *et al.*, 2008; Scholl and Wehr, 2008; Bender and Trussell, 2011). This imbalance is also thought to play a role in hyperacusis and tinnitus (Milbrandt *et al.*, 2000; Browne *et al.*, 2012; Sun *et al.*, 2012; Sturm *et al.*, 2017). However, such effects have not been sufficiently substantiated.

Intermittent, moderately intense noise exposures (85 to 96 dB SPL) can cause TTS but have also demonstrated "ear toughening" or conditioning effects, suggesting that under some noise conditions the auditory system can become less prone to damage from subsequent noise exposures (Pukkila *et al.*, 1997; Niu and Canlon, 2002; Fernandez *et al.*, 2015). The damage induced by all noise exposures are greatly dependent on the acoustic characteristics of the noise, including the duration of the exposure, the frequency content and bandwidth, the intensity, and the pattern of repetition and predictability (Mills and Going, 1982; Sullivan and Conolly, 1988). The major focus in most noise exposure studies is the intensity of the exposure, with results often suggesting that less intense sounds ($<$85 dB) are safe. However, more recent work has shown that the effects of noise are more complex than can be predicted by overall noise intensity alone (Hamernik *et al.*, 1974; Dunn *et al.*, 1991; Lei *et al.*, 1994; Hamernik and Qiu, 2001; Ising and Kruppa, 2004; Goley *et al.*, 2011).

Less is known about the effects of lower-intensity sounds relative to high intensity exposures. Chronic exposures to stimulus levels $\leq$70 dB SPL are not thought to be harmful to the cochlea and may actually enhance responsiveness to acoustic stimuli. For example, a series of experiments has demonstrated that exposure to broadband noise improved responses to suprathreshold sounds in mice (Turner and Willott, 1998; Willott and Turner, 1999, 2000; Willott *et al.*, 2000). Improved auditory performance was demonstrated by enhanced sound evoked pre-pulse inhibition (PPI) of the acoustic startle and lower ABR thresholds in mice exposed to the $<$70 dB SPL broadband sound when compared to unexposed mice (Turner and Willott, 1998; Willott and Turner, 1999, 2000; Willott *et al.*, 2000). Interestingly, these findings were shown to occur as animals in both the exposed and unexposed groups began to naturally lose their hearing as a function of genetic predisposition or age. That is, animals exposed to the lower level noise retained more robust responses to acoustic stimuli and lower thresholds than unexposed peers, suggesting increased resistance to hearing loss.

Although noise is typically associated with hearing loss it is believed that it can also act as a psychosocial stressor in humans and subhuman species (Rabat, 2007). Information garnered from the auditory system provides important knowledge regarding the surrounding environment, plays a role in the sympathetic autonomic nervous system, and is essential for survival (i.e., mating calls and signals indicating a predator or prey is nearby).

Consistent findings across noise studies suggest that exposure to noise produces increased stress hormone levels as well as a variety of secondary problems including

cardiovascular effects, elevated cholesterol (Henkin and Knigge, 1963; Rosecrans and De Feo, 1965; Smookler and Buckley, 1970; DeJoy, 1984; Cransac *et al.*, 1998; Barzegar *et al.*, 2015), and suppression of neurogenesis (Kraus *et al.*, 2010). Noise induced audiogenic seizures have also been reported in other species, as well as in immature rats (Iturrian and Fink, 1969; Pierson and Swann, 1991; Pierson and Liebmann, 1992; Ross and Coleman, 1999). Whereas the full breadth of non-auditory effects of noise are still unclear, care should be taken to use caution with unnecessary background noise to minimize these possible systemic changes potentially impacting biomedical research outcomes and introducing confounding variables.

V. CONVENTIONAL METHODS FOR DETECTING AND ASSESSING NIHL IN THE RAT

A. Auditory evoked potentials and distortion product otoacoustic emissions

The most common method for measuring hearing thresholds in humans is the behavioral audiogram. However, there is a continuous need to optimize methods for rapid assays of hearing sensitivity for both clinical and scientific purposes. For the purposes of research and specifically for animal work, behaviorally derived threshold measures require training animals to respond to low level acoustic stimuli. These measures require specific equipment, knowledge of behavioral measures, may involve long training times, and often cannot provide ear specific information. In contrast, relatively sensitive objective measures can provide good ear specific estimates of hearing sensitivity in animals.

One of the most commonly used objective electrophysiological measures of hearing is the ABR. The ABR is a far-field auditory evoked potential occurring within the first 10 ms after presentation of a stimulus and provides a non-invasive measure of the health of the auditory system from the cochlea up to the brainstem. In rats, the ABR shows the characteristic pattern of four to five waves. Wave II is noted as being the most robust wave, whereas Wave III is the smallest (Overbeck and Church, 1992; Jamesdaniel *et al.*, 2008; Alvarado *et al.*, 2012; Church *et al.*, 2012). Variations in stimulus parameters including frequency and intensity can evoke significant changes in the overall waveform morphology and measurable characteristic changes in wave amplitude and latencies.

The ABR is a rapid measurement for the assessment of hearing loss in rats and can help distinguish the site of lesions along the auditory pathway based on differential characteristics noted along the ABR waves (Melcher and Kiang, 1996). Tone burst stimuli or click stimuli are commonly used to estimate hearing thresholds in rats using the ABR. Tone bursts have the advantage of providing frequency-specific information, whereas clicks are thought to synchronously activate a broader region of the basilar membrane instantly, providing a quick overall estimate of hearing ability. However, the actual cochlear response to a click stimulus is not an ideal broadband response (Dau *et al.*, 2000). To overcome this limitation, rising frequency stimuli, commonly referred to as a "chirp," were developed as an alternative stimulus that provides a more synchronous activation of the basilar membrane by compensating for the temporal dispersion of the basilar membrane frequency response (Dau *et al.*, 2000; Fobel and Dau, 2004; Elberling *et al.*, 2007). Because responses from the apex of the basilar membrane require more time than those from the base, a temporally compensated frequency response such as the chirp is essential to eliciting a true broadband and synchronous response.

Chirps produce Wave V results that represent low, mid, and high frequency responses via temporal compensation. This temporal compensation is gained by presenting low frequencies earlier than the high frequencies in the chirp, resulting in a larger response. Spankovich *et al.* (2008) adapted and demonstrated the use of chirp ABRs in the rat model as an alternative to click stimuli. The study concluded that chirps (specifically the A-chirp) were the optimal broadband stimulus for obtaining rapid ABR thresholds and amplitude measures in the rat. Chirps were found to elicit more robust waveform amplitude responses and were suggested to be a more sensitive estimate of hearing sensitivity (Spankovich *et al.*, 2008). Thus, in the assessment of hearing sensitivity in the rat, click and chirp ABRs can be used to rapidly assay hearing status whereas tone bursts can be used to determine frequency specific sensitivity. Such measures can be used to establish baseline hearing and subsequently to assess transient and permanent changes in hearing following noise exposure.

Researchers obtaining ABR measures as NIHL outcomes have the option of using the aforementioned auditory stimuli as well as the ability to modify a number of parameters within the ABR metric in order to specifically study different phenomena. This flexibility provides advantages for individual studies to adapt their protocols accordingly.

Choosing the appropriate parameters for the ABR metric are essential because the pattern and degree of hearing loss after noise depends on the energy content of the noise across frequency, the intensity of the noise, exposure time, repetition rate, interval length between exposures (Clark, 1991), as well as subject characteristics such as age during exposure, previous exposures, and genetic susceptibility. Although these variables can produce differential changes in the ABR waveforms, noise trauma typically results in increased latencies, decreased amplitudes, and poor morphology. In fact, decreased Wave I amplitudes may be present even when PTS is not recorded. These ABR wave-I reductions in the absence of threshold changes are thought to be precursors of hearing loss in rats and mice (Kujawa and Liberman, 2006, 2009; Wang and Ren, 2012; Jensen *et al.*, 2015; Altschuler *et al.*, 2016).

In addition to deleterious effects on the periphery, noise exposure can also affect higher order central auditory function (Sheppard *et al.*, 2017, 2019). These higher order effects can be assessed with the middle latency response (MLR), a series of auditory evoked potentials that occur between 10 and 80 ms in humans and ~13 ms in rats following stimulus onset (Miyazato *et al.*, 1996). The MLR follows earlier auditory evoked potentials (such as ABR) but occur before late evoked responses (~300 ms). The MLR is made up of

multiple neural generators, including auditory [inferior colliculus (IC), medial geniculate body (MGB), and primary auditory cortex (AC)] and nonauditory generators, with greater contribution coming from the thalamocortical pathways (MacDonald and Barth, 1995; Miyazato *et al.*, 1996). The MLR is considered the most promising evoked potential test for the identification of changes in the CNS (Musiek and Nagle, 2018) whereas late evoked potentials are more useful in evaluating conscious discrimination of stimuli. For the purposes of translational research it is important to note that although the MLR has reasonable sensitivity and specificity it also has great variability in latency and amplitude values among individuals and well-established normative values have not been well developed (Musiek and Nagle, 2018).

Several studies have demonstrated that following acoustic trauma, neurons in the cochlear nucleus (CN) and IC have diminished driven firing rates near thresholds but increased spontaneous firing rates and enhanced neural responses at suprathreshold acoustic stimulation (Kaltenbach and Zhang, 2007; Pilati *et al.*, 2012; Baizer *et al.*, 2015; Sheppard *et al.*, 2018). The increase in neural activity has also been recorded from the AC from MLRs. The mechanisms that lead to an increase in neural excitation and/or a lack of inhibition are still poorly understood. Popelar *et al.* (2008) looked at the effects of noise exposure on the central auditory system by comparing ABR and MLR changes following acoustic trauma on nine adult female Long Evans rats. Rats were noise-exposed with a 1-h broadband noise of 118 dB SPL for the first exposure and 122 dB SPL for the second exposure, performed 3 weeks after the first. The researchers found that ABR amplitudes were reduced as a function of threshold shift, reflecting peripheral NIHL, while MLR amplitudes increased as a function of threshold shift—suggesting the presence of loudness recruitment. Together, these data suggest that noise exposure can differentially affect the function of individual structures along the auditory pathway.

ABR and MLR represent far field responses that provide information regarding sound driven activity from the distal end of the auditory nerve up through and beyond the IC. The data garnered from these evoked potentials can be supplemented with secondary ancillary information from the cochlea. One of the most widely used indirect measures of cochlear health is the distortion product otoacoustic emission (DPOAE). The presence of distortion products to pairs of primary tones presented to the healthy ear reflect active nonlinearities associated with OHC motility. DPOAEs have been used to quickly screen animals for evidence of cochlear pathology as conductive hearing losses and loss of OHC significantly reduce or abolishes DPOAE. Because OHCs are vulnerable to otologic insults including noise exposure, DPOAEs are a popular measure in studies of NIHL. In rats, DPOAEs have been shown to be a sensitive measure of early stages of NIHL due to the vulnerability of OHCs to mechanical and metabolic stresses induced by noise. When exposed to noise, poorer DPOAEs occur before evidence of NIHL can be observed on ABR measures (Fraenkel *et al.*, 2001). ABR and DPOAEs can be used together to detect NIHL particularly in early stages as well as evaluation of long term effects of noise (Cappaert *et al.*, 2000b; Fraenkel *et al.*, 2003b; Zhao *et al.*, 2018). In addition to the frequency specific information provided by DPOAE, experimenters can also quickly screen cochlear non-linarites using transient evoked otoacoustic emissions (Khvoles *et al.*, 1996; Fraenkel *et al.*, 2001, 2003a). The effects of noise thus can be assessed rapidly over a broader range of frequencies to quickly screen animals for potential early markers of NIHL.

The methods presented in this section highlight some of the more commonly used objective assays of NIHL. A number of other methods such as measuring the compound action potential (CAP), a measure of neural output, and direct measurements along auditory structures, such as the IC are also available that require surgical implantation for either short term or long term studies (Clopton and Winfield, 1974; Moller, 1983, 1985; Pierson and Snyder-Keller, 1994; Henley and Rybak, 1995; Luo *et al.*, 2017; Sheppard *et al.*, 2017, 2019).

Taken together, studies of NIHL can be readily performed in the rat with several objective tests available to assay the effects of noise. These can be obtained in anesthetized rats before, during, and after noise exposure providing the ability to evaluate NIHL from its inception to its long term consequences.

B. Behavioral hearing tests

Behaviorally derived hearing thresholds across frequency (audiograms) as well as suprathreshold measures can be obtained from rats in a variety of ways. Rats can be trained to respond to sound using shock avoidance techniques (Campbell, 1957; Heffner *et al.*, 2008), shuttle-box paradigms (Blackwell and Schlosberg, 1943; Rohrbaugh *et al.*, 1971; Borg, 1982), operant conditioning (Gourevitch *et al.*, 1960; Gourevitch and Hack, 1966; Harrison and Turnock, 1975; Bauer *et al.*, 2000).

Alternatively, PPI of the acoustic startle reflex to a loud sound can also be used to estimate thresholds. Under this paradigm, presentation of a loud sound elicits a strong large motoric startling response in the rat. However, if an audible signal (pre-pulse) is presented prior to the loud startling stimulus, the large motoric response is attenuated. This provides the opportunity to manipulate both the frequency and intensity of the pre-pulse and estimate hearing thresholds. The use of the PPI paradigm has been used to study auditory thresholds, suprathreshold auditory perception, tinnitus, and hyperacusis and can be used to evaluate changes induced by exposure to noise (Ison, 1982; Young and Fechter, 1983; Lobarinas *et al.*, 2013; Turner and Larsen, 2016; Lobarinas *et al.*, 2017; Ouyang *et al.*, 2017).

One of the main disadvantages of behavioral assays is that it is not possible to obtain ear specific information unless one ear is deafened. Thus studies with asymmetrical hearing loss are difficult to perform because behavioral responses will reflect hearing from the better ear. Depending on the nature of the experiment, it may be advantageous to use objective measures such as the ABR for ear specific information or alter the experimental design to monaural experiments. Objective measures such as the ABR are well

established and do not require any behavioral training to obtain assays of threshold and suprathreshold hearing. However, objective measures in rats typically require anesthesia and the obtained thresholds can significantly differ from behavioral responses by either overestimating or underestimating the threshold by as much as 28 dB (Heffner *et al.*, 2008). This was particularly evident when using tone burst ABR, which is the optimal measure for frequency specific sensitivity. Consequently, experimenters will need to determine the acceptable trade-offs between behavioral and non-behavioral measures for hearing as their research studies dictate.

Behavioral measures may be preferred when using complex stimuli or for obtaining other non-threshold auditory perception measures that cannot be readily obtained under anesthesia or with objective techniques. In addition, the aforementioned studies demonstrate that the rat's ability to learn complex behavioral paradigms combined with well-designed experiments can be leveraged as an efficient animal model for auditory research (King *et al.*, 2015).

The structure and function of the rat subcortical auditory system is similar to that of other mammals and humans but with frequency sensitivity shifted toward higher frequencies and larger auditory systems relative to their brain size (Glendenning and Masterton, 1998). The extensive scientific literature on rat models in both auditory and non-auditory fields (Willott, 2007) show that these rodents are commonly used mammals in research and their auditory system is relatively well understood. The rapid and reliable training in rats enable direct observation and the development of robust experimental designs aimed at further understanding the peripheral and central auditory system have been well established. These strengths offer researchers opportunities to supplement their research studies and to study complex auditory perceptual deficits associated with NIHL and other hearing loss models.

C. Correlations between NIHL and anatomical changes

The leading cause of NIHL is damage sensory hair cells in the cochlea (IHC and OHC). PTS represents permanent damage to, or loss of, hair cells as well as damage to adjacent supporting cells and afferent synapses. However, the degree of hearing loss following noise exposure or other otologic insults and the amount of observed hair cell loss does not always show a strong correlation. For example, in rats treated with styrene, an ototoxic industrial chemical, OHC losses of up to 30% did not produce any significant change in thresholds (Chen *et al.*, 2008). In contrast, noise exposed rats with 30 dB PTS showed no evidence of OHC loss and hair cell loss was only evident as PTS began to exceed 30 dB (Borg, 1987). One explanation for these results is that NIHL reflects both surviving and functioning hair cells. That is, the number of surviving hair cells may not represent the number of *functioning* hair cells. In a series of experiments that evaluated CAP thresholds following noise exposure, the data showed that high frequency hair cell death in the basal region of the rat cochlea varied linearly with CAP threshold elevation (Chen and Fechter, 2003). In contrast threshold elevations of up to 50 dB were present before there was evidence of hair cell loss in the low frequency apical regions of the cochlea. These findings suggest that single metrics such as hair cell counts are insufficient for studying the relationships between changes in auditory function and anatomical correlates of hearing loss.

D. Direct and secondary correlates of NIHL

Noise exposure produces immediate direct mechanical stress causing permanent cochlear damage. The mechanical stress is followed by secondary damage that progressively induces OHC death via both necrosis (unplanned cell death) and apoptosis (programmed cell death) for several weeks following noise exposure. The exact cause of secondary cellular damage resulting in NIHL is not completely understood, but there are several proposed mechanisms, including overproduction of free radicals. Several studies have demonstrated that reactive oxygen species (ROS) are generated in the cochlea following injury caused by noise exposure (Ohlemiller *et al.*, 1999; Yamashita *et al.*, 2005; Le Prell *et al.*, 2007). Rats offer a possible model to examine the apoptosis regulatory genes expressed following noise exposure, due to the moderate correlations between NIHL and OHC in this animal model. Melgar-Rojas *et al.* (2015) used a continuous high-level broadband white noise (118 dB SPL for 4 h for 4 consecutive days) to produce NIHL in Wistar rats in order to assess cochlear injury at different time points post-exposure and correlate molecular findings with hearing loss assessed by ABR. Following noise exposure, ABR thresholds could no longer be obtained in the noise-exposed rats regardless of presentation level, whereas the control group was in good agreement with previous reports. Cochlear histopathology revealed that as post-exposure time increased, the amount of OHC loss also increased for rats in the experimental groups relative to the control group. The data showed that the permanent change in threshold correlated with the loss of OHCs and spiral ganglion neurons as well as fibrocyte damage and a reduced blood supply with structural damage progressing as post-exposure time lengthened.

Oxidative stress is an important mechanism involved in NIHL and is evident in pharmacological studies that demonstrated the ability of antioxidant drugs to block or reduce the degree of NIHL (Seidman *et al.*, 1993; Yamasoba *et al.*, 1999; Henderson *et al.*, 2006). In addition to pharmaceutical implications, genetic studies have shown that laboratory animal models with reduced ability to buffer antioxidants are more susceptible to NIHL than their wild-type controls (Ohlemiller *et al.*, 1999, 2000). Whereas these data are promising in further understanding the mechanisms involved in NIHL and possibly identifying successful pharmaceutical preventions and interventions for NIHL, important questions concerning the extent of ROS production as a function of noise severity remain unanswered.

In addition to cochlear damage following acoustic trauma, recent findings have indicated that noise injury likely also contributes to alterations in neuronal processing within the CNS. Several studies have demonstrated that both

the absence of input as well as over-stimulation of the peripheral auditory system can result in reorganization of structures within the CNS (Kaas and Garraghty, 1991; Popelar *et al.*, 1994). Several studies have indicated that the IC may be the primary anatomical location in the ascending auditory pathway where noise-induced neuronal plasticity occurs, resulting in changes in higher up processing of auditory information (Gerken *et al.*, 1991; Lonsbury-Martin and Martin, 1981; Salvi *et al.*, 1978; Salvi *et al.*, 1982, Salvi *et al.*, 1990; Willott and Lu, 1982).

VI. EFFECTS OF NOISE EXPOSURE IN THE RAT

A. NIHL dose-effects

The sensitivity to noise-induced injury varies with animal species. Sullivan and Conolly (1988) reported functional and morphological changes following broadband white noise exposures at 85, 95, 100, and 110 dB SPL for 6 h per day, 5 days a week for 4 weeks in 8 male Sprague Dawley rats. Based on ABR threshold shifts and sensory hair cell counts, no observable changes were noted following the 85 dB SPL exposure. Increased ABR thresholds were noted at 4, 8, 16, and 32 kHz following the 100 and 110 dB SPL noise exposures. However, increased thresholds for the 95 dB SPL noise were not statistically significant, even though significant OHC loss was observed following the 95, 100, and 110 dB SPL exposures. In order to examine the relationship between hair cell loss and threshold elevations, the experimenters converted threshold shifts to percentage change in order to allow comparisons of the linearity of changes following noise exposures for both percent threshold change and percent hair cell loss. Based on the study's extrapolation of the data, noise exposure of 117 dB SPL produced approximately 50% OHC loss, but exposures as low as 104 dB SPL produced a 50% loss as measured by ABR. These results again suggest that measurable changes in hearing occur prior to measurable OHC loss.

A dose-effect curve for NIHL in 24 adult rats was also investigated by Cappaert *et al.* (2000a) using similar broadband noise parameters at 90, 100, and 110 dB SPL for 8 h a day for 5 days. The results showed detrimental effects of noise measured as a function of electrocochleography (ECOG) thresholds, DPOAEs, and morphological changes in the organ of Corti. No changes were noted in the 90 dB SPL group. The 100 dB SPL noise exposure showed ECOG threshold changes only at 12 kHz. The 110 dB SPL noise-exposed group showed changes in the DPOAE growth curve at 4, 8, and 16 kHz that mirrored ECOG growth curve changes at 4, 8, 12, 16, and 24 kHz. Although these findings suggested loss of OHC, all OHCs were present and damage to these cells was not evident under light-microscopy. Thus, these results suggested that the function of OHCs was likely substantially impaired. It is important to note, however, that damage to IHCs, IHC synapses, or afferent fibers were not investigated. Other studies have also indicated that, depending on the metric used, the relationship between functional and morphological loss following NIHL is not linear (Borg *et al.*, 1995). Overall, noise exposures of 100–110 dB SPL appear to be needed to cause consistent measurable hearing loss in the rat model. Thus, noise exposure level and dose play a critical role in the damage profile to cochlear structures as well as the sensitivity of individual metrics and correlates of hearing loss.

B. Impulse and impact noise

Noise can be classified as steady, non-steady, impact, or impulse depending on its temporal characteristics. Impulse noise is characterized by one or more high intensity, short duration bursts with broad spectral widths that are often associated with sudden pressure changes in gases. Impact noise is caused by the collision of solids. The temporal features of impulse noise are best described as an N shaped wave whereas impact noise consists of a high initial peak followed by positive and negative oscillations generated by the residual motion of the colliding objects. In a reverberant environment, impulse noise may also show some of the residual oscillatory activity typically shown by impact noise (Atherley *et al.*, 1970).

Because impulse noise occurs almost instantaneously with a very rapid rise time, the resulting sharp sounds are often characterized as cracks, clicks, or pops. High levels of impulse noise have been shown to cause permanent hearing loss and in some cases more damage than continuous noise exposures of similar intensities (Mantysalo and Vuori, 1984). In guinea pigs, even when the degree of hearing loss and energy content is similar between continuous and impulse noise, the anatomical damage differs with more damage to IHC produced by continuous noise and more damage produced to OHC by impulse noise (Nilsson *et al.*, 1987). Similar results have been reported in rats; animals exposed to impulse noise, that had lower energy than continuous noise, showed more cochlear damage, particularly to OHC (Carreres Pons *et al.*, 2017). Given the prevalence of clinical populations effected by impulse noise, such as military personnel discharging weapons, it is imperative that noise exposure models continue to be replicated within animal models to deepen our understanding of these exposures (Dancer *et al.*, 1999). Indeed, impulse, impact, continuous, and blast noises (high level impulse noise) have been extensively studied in chinchillas, guinea pigs, and other rodents with less work performed on the rat. In the chinchilla, for example, an empirical impulse noise model has been developed that provides TTS and PTS dose response curves as well as TTS recovery. This model has shown good agreement with previously published results (Chan *et al.*, 2016). Data from the chinchilla have been summarized in at least one major critical review (Henderson and Hamernik, 1986) as well as the correlation between impulse noise and audiometry (Slepecky *et al.*, 1982), the correlation between temporal patterns of impulse noise and hearing loss (Danielson *et al.*, 1991), and the relationship between apoptotic cell death and impulse noise (Hu *et al.*, 2006). In guinea pigs, several studies have reported on the damaging effects of impulse noise on hair cells (Poche *et al.*, 1969; Nilsson *et al.*, 1980), degeneration of the CN (Theopold, 1975), and on the different patterns of damage induced by continuous

noise versus impulse noise (Hamernik and Henderson, 1974; Erlandsson *et al.*, 1980; Emmerich *et al.*, 2005).

In rats, several studies have evaluated the effects of blast exposure on both the peripheral and central auditory system, Race *et al.* (2017) investigated differences between a mild blast exposure (a maximum direct overpressure equivalent to 198 dB SPL) and a single impulse exposure (animals placed outside the direct path of the blast to experience the noise alone but not the blast wave front) among 18 Sprague-Dawley male rats (aged 3 to 4 months old) with DPOAEs, ABRs, envelope following responses, and MLRs. The study concluded that the acute trauma of the direct blast exposure caused a greater degree of hearing impairment and central auditory processing deficits than in the rats exposed to the impulse noise component of the blast. These data were consistent with previous studies (Ewert *et al.*, 2012; Cho *et al.*, 2013; Luo *et al.*, 2014a,b) and extended findings by providing comparisons between the two acoustic trauma effects for higher order auditory processing with the assessment of the MLR reflecting alterations in the thalamic, thalamocortical, and cortical processing (Kraus *et al.*, 1992; Kraus and McGee, 1994; Phillips *et al.*, 2011; Suta *et al.*, 2011). Minimal MLR changes were observed for noise-exposure alone, but the blast exposure resulted in amplitude and latency changes in the waveforms. Acute blast exposures appear to result in different pathologies than impulse-alone and steady-state continuous noise exposures of similar or even higher intensities. These data highlight important variables to consider when designing experiments that mirror blast trauma conditions in humans whereby the direct and indirect paths of the blast wave front can cause differential effects on the auditory system. Blast exposures in the rat model may provide important translational insights for those with blast induced traumatic brain injury, particularly among the veteran population.

C. Steady-state, traumatic noise

Continuous, or steady-state noise, is noise with negligible fluctuations of SPL that remains stable over a period of time. Exposure to high levels of steady state noise (>100 dB SPL) has been shown to damage or destroy hair cells in the rat cochlea (Engstrom and Borg, 1983; Borg, 1987; Chen *et al.*, 2000; Chen and Fechter, 2003). The consequences of cochlear damage following exposures to high levels of noise can extend beyond the peripheral auditory system. For instance, Szczepaniak and Moller (1996) demonstrated that a 30-min sound exposure of a 4 kHz tone at 104 dB SPL caused specific changes in the excitability of IC neuronal responses in 29 adult, female Wistar rats (as recorded from the external nucleus of the IC in response to clicks and tones). These changes were reported to last several hours following the termination of the tone. Data from the study supported the "two-neuron" model proposed by Gerken (1993) that suggests a simple inhibitory action to higher-order neurons occurring at longer tone durations relative to shorter durations. Whether the increased excitability noted in the IC after noise exposure was due to an increase in the excitatory communication or to a decrease in inhibitory mechanisms is still unclear.

In subsequent experiments Syka and Rybalko (2000) assessed the effects broadband, low and high frequency noise exposures (105–120 dB SPL for 1 h) on thresholds and MLR amplitudes in adult pigmented rats. Noise exposures produced TTS of 20–43 dB as a function of exposure level as expected. However, when the MLR was assessed, the amplitude of the MLR was enhanced post-exposure, suggesting central compensation for the reduced peripheral auditory input. Similar results have been reported in rats exposed to noise producing 5–70 dB TTS. Reductions in peripheral input were correlated with enhanced MLR amplitudes (Popelar *et al.*, 2008). The same group of researchers previously showed that MLR amplitudes increased with low frequency tone stimuli in the guinea pig following noise exposure, suggesting a generalized phenomenon that occurs across species (Popelar *et al.*, 1987; Syka *et al.*, 1994). Overall, findings of enhanced MLR amplitudes in rats following traumatic noise exposure indicate that peripheral injury can trigger compensatory changes in the central auditory system. To the extent and how these changes affect other aspects of auditory perception is not completely understood.

The rat cochlea does not reach anatomical maturation until postnatal day 16. The ABR is known to match fully developed, adult responses by 24 to 36 days after birth (Iwasa and Potsic, 1982). This pattern of development provides the opportunity to study the effects of noise exposure in the pre- and post-developed ear within the same animal model. Rybalko and Syka (2001) examined susceptibility to NIHL in the immature rat ear by examining MLR changes during postnatal development in 20 female Long Evans rats. Rats were exposed to a broadband noise at 120 dB SPL for 1 h at 3, 4, 5, and 6 to 7 weeks old. The MLR thresholds and amplitudes were recorded from the AC pre- and post-noise exposure. These findings were then compared to data previously gathered on effects of broadband noise exposure on MLRs in adult rats (Syka and Rybalko, 2000). Whereas adult rats showed full recovery from TTS resulting from the same noise exposure parameters previously used, the rat pups developed PTS that varied as a function of age (younger rat pups showed a larger PTS in a broader frequency range compared to older pups and the permanent changes showed greater individual variability in those 6 to 7 weeks old). Similar to the adult rats, the rat pups also demonstrated enhanced MLR amplitudes. However, younger rat pups (3 to 5 weeks postnatal) showed a delayed recovery in the enhanced cortical responses relative to older animals. Adult rats and older rat pups (age 6 to 7 weeks postnatal) recovered by 2 weeks post-noise exposure, whereas the younger pups still displayed enhanced MLR responses 4 to 8 weeks post-noise exposure. The researchers concluded that young animals were more susceptible to noise injury relative to fully matured adult animals, findings that are supported by previous studies showing auditory susceptibility in kittens, guinea pigs, hamster, mice, and rats (Coleman, 1976; Price, 1976; Bock and Saunders, 1977; Lenoir *et al.*, 1979; Rybalko and Syka, 2001). Given that this critical period of increased

susceptibility to NIHL is present following the complete cochlear development suggests immature development somewhere else along the auditory system. The site of where this immaturity difference associated with this vulnerability is present is still unknown, but the bulk of the literature suggests noise exposure influences the function of higher order central auditory processing, particularly in the IC and/or AC (Saunders *et al.*, 1972; Popelar *et al.*, 1987; Salvi *et al.*, 1990; Syka and Popelar, 1994; Salvi *et al.*, 1999; Syka and Rybalko, 2000).

D. Continuous and intermittent, non-traumatic noise

Exposure to high-level continuous, impact, or impulse noise is often associated with PTS, whereas lower intensity continuous noise exposures typically result in only a TTS, or in many cases no notable threshold sensitivity changes at all. Recent work has indicated that low-level, continuous noise may permanently destroy IHC synaptic terminals and result in degeneration of auditory nerve fibers, even in the absence of PTS (Kujawa and Liberman, 2009; Wang and Ren, 2012). Based on these findings, it has been proposed that chronic exposures at moderate intensity levels may be related to central auditory processing disorders. These discoveries have tremendous translational significance as the lower sound exposure levels that produce only TTS are not deemed to be hazardous (OSHA, 1983; NIOSH, 1998).

Many studies observing the effects of noise exposure on animals have used high-levels of acute, continuous, impact, or impulse noise. Alternatively, humans can also develop NIHL over a time span of several years via more moderate noise levels. Animal research observing the progress of inner ear damage and functional pathologies caused by moderate, long-term noise exposure has been more limited. Damage caused by long term, moderate noise exposure can produce variable functional findings, even when the damage results in hearing loss of the same degree and configuration (Bohne *et al.*, 1982; Lim *et al.*, 1982). One possible explanation comes from studies investigating auditory behavioral and neuronal changes following long-term, moderate intensity exposures that show significant functional changes at higher levels of the auditory system (Pienkowski and Eggermont, 2010; Zhou and Merzenich, 2012).

Chronic noise exposure may also lead to several systemic problems due to increased levels of stress hormones. For example, Davis (1974) showed fear induced behavior in rats following a short-duration 80 dB exposure. Long term activation of the hypothalamic-pituitary adrenal axis and increased stress hormones has been linked to immunosuppression, diabetes, cardiovascular disease, osteoporosis, and gastrointestinal problems (Spreng, 2000a,b). In addition to varying noise exposure history, noise parameters, and stress measures, another advantage of the rat model is the ability to perform reasonable long-term noise studies given the rat life expectancy of 2–3 years. The life expectancy of the rat allows for studying the whole lifespan of hearing within a reasonable time frame as opposed to other species like the chinchilla whose lifespan can be greater than a decade.

The majority of the work using objective measures to assess NIHL has focused on electrophysiological testing. More recently, however, there is a growing interest in using neuroimaging techniques, such as MRI (magnetic resonance imaging) and more specifically functional magnetic resonance imaging (fMRI). Whereas the ABR and MLR can provide a measure of changes in sound evoked activity along the auditory pathway as a function of NIHL, the fMRI allows researchers to look at not only cortical changes but the interactions of neural networks affected by NIHL. Studies using fMRI achieve this by indirectly measuring neuronal activity. This activity is based on magnetically driven changes to hydrogen atoms in living tissues that are used to create high resolution images. The images are taken sequentially to create non-invasive assays of neural activity. A full description of MRI is beyond the scope of this review but the reader unfamiliar with this technology is encouraged to read an excellent overview of diffusion-weighted MRI by Zanin *et al.* (2019).

In rats, Lau *et al.* (2015) used blood oxygenation level dependent (BOLD) fMRI (blood oxygen consumption is correlated with neuronal activity) to study the long-term effects of non-traumatic noise exposure in adult rats. Rats were passively exposed to a chronic 65 dB SPL pulsed noise for 2 months. Following the noise-exposure, control and experimental groups underwent fMRI assay with a 10 and 5 Hz pulsed acoustic stimuli. Noise-exposed rats had decreased sound-evoked BOLD fMRI signaling at the MGB and the AC for the 10 Hz stimulus but not the 5 Hz stimulus. The experimenters concluded that there were differential effects of adaptation to long term noise exposure but that this adaptation was frequency dependent. By using fMRI, the researchers were able to show long term cortical changes associated with chronic noise exposures that were non-traumatic.

The observed location of neural reorganization suggested that moderate-level noise may degrade auditory temporal rate and frequency discrimination abilities. The fMRI findings and the study conclusions support concerns of potential negative impacts of long-term, non-traumatic noise exposures.

The same group of investigators used BOLD fMRI with tonal acoustic stimulation to evaluate the effects of chronic, moderate intensity noise exposure in rats (Lau *et al.*, 2015). Rats were exposed to 30 kHz low-pass acoustic stimuli at 65 dB SPL for 60 days. Following the exposure, the response of the IC shifted dorsolaterally following the 7 kHz acoustic stimuli. When compared to the control group, the shifted region typically responded to lower frequency sound. A dorsolateral shift was also observed in response to acoustic stimuli at 40 kHz; a frequency above the noise exposure. These results suggest spatial expansion of high frequency IC regions taking place above the exposure bandwidth and shifting to lower frequency regions dorsolaterally. Along with other studies, these findings suggest that long-term, moderate level intensity exposures can affect auditory processing at various levels up to the midbrain (Zhou *et al.*, 2011; Zhou and Merzenich, 2012).

The MRI studies demonstrate that the effects of noise exposure on the central auditory system can be quite complex and can be both stimulus and location dependent. Importantly, these studies may help expand our understanding of these complex relationships as other studies using evoked potentials have shown trends that suggest that peripheral injury can drive central gain (Schormans *et al.*, 2019). This has been found in enhanced AC responses following NIHL in a model of hyperacusis in rats (Sun *et al.*, 2012). However, the enhanced central gain associated with NIHL may be a generalized response that can occur independent of tinnitus or hyperacusis (Mohrle *et al.*, 2019).

Collectively, the findings from MRI studies, using the rat noise model, have begun to reveal complex effects of noise exposure on the brain that can be used in conjunction with evoked potential experiments to answer important questions regarding central auditory processing changes driven by NIHL.

E. Imaging techniques and other noise-induced central changes

In Sec. VI D the use of fMRI was discussed to show that imaging techniques can be leveraged to understand long term cortical changes produced by noise exposures.

Other research groups have looked at the consequences of noise exposure as these relate to the development of tinnitus by employing MRI techniques. In two studies, manganese enhanced magnetic resonance imaging (MEMRI) was used to evaluate neuroplastic changes associated with noise induced tinnitus in rats. Manganese was used because it accumulates in active neurons, particularly at synapses, and is a reliable correlate of functional and structural changes (Brozoski *et al.*, 2007; Holt *et al.*, 2010). MEMRI has also been used to elucidate the role of the cerebellum in noise-induced tinnitus (Bauer *et al.*, 2016). In Sec. VI B, we discussed blast exposures and its damaging effects on the inner ear. However, blasts can also be neurotraumatic and have been associated with tinnitus. The rat model in combination with functional imaging techniques such as the MRI make it possible to evaluate auditory and neural correlates of blast induced trauma in ways not possible using auditory evoked potential techniques alone (Mao *et al.*, 2012).

F. Interaction of noise and ototoxic agents

In humans, high-level noise exposures can occur alone or in the presence of other environmental hazards. For instance, occupational noise exposures in the manufacturing sector can and often occur in conjunction with chemical exposures that can potentiate NIHL. In one study, exposures to low concentrations of hydrogen cyanide (HCN), a commercial compound used for fumigation, mining, chemical synthesis. and the production of plastic and synthetic fibers was found to potentiate NIHL in rats. In contrast HCN alone had no effect on hearing or loss of hair cells (Fechter *et al.*, 2002). The results are also indicative that compounds that do not appear to be ototoxic can interact with noise to produce increased risk of NIHL. Compounds that may not be as obvious include exposure to cigarette smoke in conjunction with noise exposure. In rats exposed to white noise (102 dB SPL, 8–10 h/day) and smoke from 20 cigarettes (daily), DPOAE amplitudes began to decline just 1 day post combined exposure. Although cigarette smoke alone also caused DPOAE reduction, it was the combination with noise that yielded long term decrements (Habybabady *et al.*, 2019). Other common environmental agents include carbon monoxide that, in addition to being poisonous, also potentiates NIHL (Chen and Fechter, 1999; Fechter *et al.*, 2000).

It is not surprising, then, that many industrial chemicals and solvents can potentiate hearing loss in a noisy environment. The mechanism of action of some of these chemicals such as acrylonitrile, are believed to potentiate NIHL by disrupting endogenous antioxidant activity (Fechter *et al.*, 2003; Pouyatos *et al.*, 2005). Industrial solvents (Hodgkinson and Prasher, 2006), such as carbon disulfide (Carreres Pons *et al.*, 2017), styrene (Chen *et al.*, 2008; Chen and Henderson, 2009), toluene (Lund and Kristiansen, 2008; Rumeau *et al.*, 2011), and ethyl benzene (Cappaert *et al.*, 2000a) have all been shown to potentiate NIHL. In some cases the solvents were ototoxic on their own whereas others were not. However, when exposed to both the solvent and noise, NIHL was greater than chemical exposure or noise alone.

Other studies show that relationships between solvents and NIHL can be more complex. For example, co-exposure to styrene and continuous noise yielded less hearing loss than noise alone. However, when exposed to styrene and impulse noise, rats showed a potentiation of NIHL. The effects of combined chemical and noise exposure may lead to neurotoxic effects even in the absence of hearing loss (Guthrie *et al.*, 2016) and although synergistic effects may be observed in one species these may not occur in another (Lataye *et al.*, 2003).

Collectively, rats have been used effectively to demonstrate the synergistic effects of noise and chemical exposures that are common in industrial settings. The use of the rat animal model to study complex interactions of noise with other environmental variables cannot be overstated.

G. Treating NIHL

The ultimate goal of animal experiments on NIHL is to further understand underlying mechanisms in order to prevent and/or develop effective treatments and interventions. To this end many studies have focused on evaluating therapeutic interventions to attenuate or prevent NIHL.

One of the metabolic consequences of noise exposure, in the inner ear, is the production of ROS which contribute to hair cell damage. When treated with either superoxide dismutase-polyethylene glycol or allopurinol, rats have been shown to exhibit less noise-induced threshold shift than control animals (Seidman *et al.*, 1993). Similarly, by blocking apoptotic pathways associated with noise-induced stress and injury, CEP-1347/KT7515, an inhibitor of c-Jun N-terminal kinase activation has been shown to have otoprotective effects in rats (Pirvola *et al.*, 2000) Using the rat model has also shown the potential protective effects of hyperbaric oxygen treatment on PTS as a result of firearm noise exposure (Kuokkanen *et al.*, 1997).

Other studies have shown that antioxidants (Lorito *et al.*, 2006; Ewert *et al.*, 2012; Loukzadeh *et al.*, 2015; Hanci *et al.*, 2016; Ogurlu *et al.*, 2017; Fetoni *et al.*, 2018; Altschuler *et al.*, 2019), steroids (Arslan *et al.*, 2012; Gumrukcu *et al.*, 2018; Mutlu *et al.*, 2018), drugs to treat diabetes (Kesici *et al.*, 2018; Paciello *et al.*, 2018) compounds with anti-inflammatory properties (Aksoy *et al.*, 2015; Soyalic *et al.*, 2017), and neuroprotectants (Kil *et al.*, 2007; Altschuler *et al.*, 2016) can all attenuate to some degree the effects of NIHL.

Collectively, these studies demonstrate the flexibility of the rat model for its use in understanding both the underlying mechanism of hearing loss as well as for evaluating preclinical treatments. There are many more studies that are beyond the scope of this review that highlight numerous strategies to prevent or attenuate NIHL that have been studied using the rat model.

H. Treating noise-induced synaptopathy

Historically, the boundary at which NIHL has been defined is the point where hair cells are damaged and there is evidence of PTS. In contrast the presence of TTS alone has been thought to represent a transient but recoverable injury. More recent data suggests that the boundary of NIHL may occur before evidence of threshold elevation or overt hair cell damage. The correlate of this subclinical hearing loss is the IHC synapse. Exposure to noise may lead to early synaptopathic degradation that precedes hair cell loss.

Synaptopathy is a term describing the dysfunction and loss of synapses between the IHCs and afferent auditory nerve fibers. Recent research in animal models has provided robust evidence that the synaptic connections to low spontaneous rate auditory nerve fibers are particularly susceptible to noise, ototoxic drugs, and aging (Schmiedt *et al.*, 1996; Kujawa and Liberman, 2009; Furman *et al.*, 2013; Sergeyenko *et al.*, 2013; Bourien *et al.*, 2014; Ruan *et al.*, 2014). Noise-induced synaptopathy results in substantial loss of synapses, permanently reduced ABR Wave-I amplitudes, and long-term delayed loss of cochlear spiral ganglia. However, there is minimal to no loss of hair cells and only a TTS occurs, with hearing sensitivity generally recovering to baseline levels (Kujawa and Liberman, 2009; Lin *et al.*, 2011; Wang and Ren, 2012; Jensen *et al.*, 2015) Because synaptopathy is not readily detected with clinical pure tone audiometry (250 to 8000 Hz), it has sometimes been described as hidden hearing loss (Schaette and McAlpine, 2011) and has been purported to underlie (or at the very least be a contributing factor to) a range of symptoms such as difficulty with speech perception in noise, tinnitus, hyperacusis (intolerance to moderately loud sounds) (Schaette and McAlpine, 2011; Hickox and Liberman, 2014), and auditory processing disorders (Bharadwaj *et al.*, 2015).

Cochlear synaptopathy has been investigated in numerous animal models. Whereas the majority of studies on this topic have investigated loss of IHC synapses in the mouse model as a result of various insults (e.g., Kujawa and Liberman, 2009; Maison *et al.*, 2013; Sergeyenko *et al.*, 2013; Jensen *et al.*, 2015; Shaheen *et al.*, 2015), synaptopathy has also been reported in other mammals including the guinea pig (Lin *et al.*, 2011; Shi *et al.*, 2013), chinchilla (Hickox *et al.*, 2017), non-human primates (Valero *et al.*, 2017), and rats (Ruttiger *et al.*, 2013; Singer *et al.*, 2013; Uran *et al.*, 2014; Bing *et al.*, 2015; Rybalko *et al.*, 2015; Altschuler *et al.*, 2016; Möhrle *et al.*, 2016; Yu *et al.*, 2016; Hickox *et al.*, 2017). A brief overview of studies employing the rat model for analysis of noise-induced synaptic damage can be seen in Table I.

A number of noise exposure paradigms have been used with varying frequency bands, intensity levels, and duration. The range of noise frequencies used for the induction of synaptopathy varies substantially among studies and includes narrowband stimuli at 10 kHz (Ruttiger *et al.*, 2013; Singer *et al.*, 2013; Bing *et al.*, 2015), octave-band noise centered at 4 kHz (Altschuler *et al.*, 2016), octave-band noise from 8 to 16 kHz (Möhrle *et al.*, 2016), or broadband noise (Uran *et al.*, 2014; Yu *et al.*, 2016). Among studies, noise intensity has varied from 95 dB SPL (Uran *et al.*, 2014) to 120 dB SPL (Ruttiger *et al.*, 2013; Singer *et al.*, 2013; Bing *et al.*, 2015) and noise duration has ranged from a single exposure for 1 h (Singer *et al.*, 2013) up to a 2 h daily exposure repeated for 15 days (Uran *et al.*, 2014). Not surprisingly, the myriad of noise exposure paradigms has produced varying degrees of synaptopathy with synaptic ribbon loss ranging from as little as 28% (Möhrle *et al.*, 2016) to as much of 85% (Bing *et al.*, 2015).

Due to the differences in findings among studies that have employed the rat-based synaptopathy model, it is cumbersome to glean any trends among the frequency, intensity, and duration of noise exposure and the degree of

TABLE I. Studies reporting noise-induced cochlear synaptopathy in rats. Abbreviations: NR = not reported, OBN = octave-band noise, BBN = broadband noise.

Source	Strain	Sex	Age at noise exposure	Noise paradigm	Ribbon loss (max)	Elevated threshold	Amplitude loss in frequency region of synaptopathy
Ruttiger *et al.*, 2013	Wistar	F	NR	10 kHz, 120 dB SPL, 1 or 15 h	77%	NR	NR
Singer *et al.*, 2013	Wistar	F	NR	10 kHz, 120 dB SPL, 1 or 2 h	80%	NR	NR
Uran *et al.*, 2014	Wistar	M	P15	BBN, 95–97 dB SPL, 2 h, single exposure or daily for 15 days	61%	NR	NR
Bing *et al.*, 2015	Wistar	F	NR	10 kHz, 120 dB SPL, 2 h	85%	NR	NR
Altschuler *et al.*, 2016	Sprague-Dawley	M	NR	OBN at 4 kHz, 117 dB SPL, 3 h	38%	Yes	50% amplitude loss
Möhrle *et al.*, 2016	Wistar	F	2–3 m	OBN, 8–16 kHz, 100 dB SPL, 2 h	28%	NR	NR
Yu *et al.*, 2016	Sprague-Dawley	NR	Perinatal at 3 wks	BBN, 100 dB SPL, 2 h (subsequent to iron deficiency)	55%	NR	NR

synaptopathy produced. However, some generalizable patterns do emerge. Quite expectedly, the greater the intensity of noise exposure utilized for the study, the greater the maximum reported loss of ribbon synapses. Whereas an octave band noise at 8 to 16 kHz presented at 100 dB for 2 h produces a maximum of 28% ribbon synapse loss (Möhrle *et al.*, 2016), a 10 kHz narrowband noise presented at 120 dB SPL for 2 h can result in ribbon synapse losses up to 85% (Bing *et al.*, 2015). What emerges from a review of studies examining noise-induced synaptopathy in the rat model is that the same general patterns of cochlear synaptopathy observed in mice (e.g., Kujawa and Liberman, 2009; Maison *et al.*, 2013; Jensen *et al.*, 2015) and other animal models (e.g., Lin *et al.*, 2011; Shi *et al.*, 2013; Bourien *et al.*, 2014) by and large resemble those seen in the rat. Thus, the rat is a strong model for studying noise-induced cochlear synaptopathy, though precisely how findings of synaptopathy in animal models, including the rat, translate to human hearing dysfunction is yet to be fully elucidated. In our own lab, the functional consequences of synaptopathic noise exposure yielded changes in hearing-in-noise performance in rats that were limited to only one frequency and were only evident in the poorest signal-to-noise ratio (Lobarinas *et al.*, 2017). Thus, more work is needed to determine the functional correlates of cochlear synaptopathy.

VII. CHANGES IN THE CENTRAL AUDITORY SYSTEM OF THE RAT AFTER NOISE OVEREXPSOURE

Numerous studies have examined noise-induced central auditory system changes in rats. The auditory centers of the CNS in the rat model mirror those seen in humans and can be considered broadly similar (Cheung *et al.*, 2012). In both species, acoustic signals ascend the auditory pathway and are relayed through several auditory nuclei prior to arriving at the AC (Malmierca and Merchán, 2004). After cochlear sensory cells convert mechanical energy into electrical signals, those signals begin an ascending path through the CN, the superior olivary complex (SOC), the lateral lemniscus, and the IC which consolidate and transmit auditory signals from the CN and SOC to the MGB and AC (Malmierca and Merchán, 2004; Winer and Schreiner, 2005).

Investigations into rat auditory pathways and central tonotopic organization have revealed that the rat is a robust model for investigating anatomical, physiological, and behavioral changes in the central auditory system following noise overexposure. fMRI investigations in rats have proven useful in studies of central auditory function and plasticity (Cheung *et al.*, 2012). Subsequent to noise trauma and loss of cochlear sensory cells in the rat, long-term alterations in gain and function have been reported in the dorsal CN (Li *et al.*, 2015) and the IC (Abbott *et al.*, 1999; Milbrandt *et al.*, 2000), among other central auditory nuclei (Auerbach *et al.*, 2014). Moreover, even in the event of only intermittent lower-level noise stress, alterations in central gain have been reported in the rat model (Sheppard *et al.*, 2017; Sheppard *et al.*, 2018). Though the peculiarities of the anatomy and physiology of the rat's auditory system must be considered when employing the rat as a model of human noise overexposure, the rat has nevertheless proven a strong and reliable model to investigate NIHL.

VIII. SUMMARY

This review describe and highlight the use of the rat as an effective animal model to study NIHL. These advantages include a robust, economical, and intelligent animal suitable for a variety of anatomical and physiological studies, large scale studies, and auditory perception studies.

In general, the anatomical arrangement of the outer, middle, and inner ear are similar to humans. The pattern of damage following continuous, impact, blast, and impulse noise bears a number of similarities to humans. In addition, the post-natal development of the rat ear and its 2–3 year life span make this model ideal for studies across the life span. Finally, advanced imaging, biochemical, genetic, and neuroscience techniques are available and well suited for the rat, ensuring its utility for current, emerging, and future studies of NIHL.

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