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Effects of moderate-level sound exposure on behavioral thresholds in chinchillas

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EFFECTS OF MODERATE-LEVEL SOUND EXPOSURE

ON BEHAVIORAL THRESHOLDS IN CHINCHILLAS

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ABSTRACT

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Normal audiometric thresholds following noise exposure have generally been considered as an indication of a recovered cochlea and intact peripheral auditory system, yet recent animal work has challenged this classic assumption. Moderately noise-exposed animals have been shown to have permanent loss of synapses on inner hair cells (IHCs) and permanent damage to auditory nerve fibers (ANFs), specifically the low-spontaneous rate fibers (low-SR), despite normal electrophysiological thresholds. Loss of cochlear synapses, known as cochlear synaptopathy, disrupts auditory-nerve signaling, which may result in perceptual speech deficits in noise despite normal audiometric thresholds. Perceptual deficit studies in humans have shown evidence consistent with the idea of cochlear synaptopathy. To date, there has been no direct evidence linking cochlear synaptopathy and perceptual deficits. Our research aims to develop a cochlear synaptopathy model in chinchilla, similar to previously established mouse and guinea pig models, to provide a model in which the effects of cochlear synaptopathy on behavioral and physiological measures of low-frequency temporal coding can be explored.

Positive-reinforcement operant-conditioning was used to train animals to perform auditory detection behavioral tasks for four frequencies: 0.5, 1, 2, and 4 kHz. Our goal was to evaluate the detection abilities of chinchillas for tone-in-noise and sinusoidal amplitude modulated (SAM) tone behavioral tasks, which are tasks thought to rely on low-SR ANFs for encoding. Testing was performed

before and after exposure to an octave-band noise exposure centered at 1 kHz for 2 hours at 98.5 dB SPL. This noise exposure produced the synaptopathy phenotype in naïve chinchillas, based on auditory-brainstem responses (ABRs), otoacoustic emissions (OAEs) and histological analyses. Threshold shift and inferred synaptopathy was determined from ABR and OAE measures in our behavioral animals.

Overall, we have shown that chinchillas, similar to mice and guinea pigs, can display cochlear synaptopathy phenotype following moderate-level sound exposure. This finding was seen in naïve exposed chinchillas, but our results suggest the susceptibility to noise can vary between naïve and behavioral cohorts because minimal physiological evidence for synaptopathy was observed in the behavioral group. Hearing sensitivity determined by a tone-in-quiet behavioral task on normal hearing chinchillas followed trends reported previously, and supported the lack of permanent threshold shift following moderate noise exposure. As we expected, thresholds determined in a tone-in-noise behavioral task were higher than thresholds measured in quiet. Behavioral thresholds measured in noise after moderate noise exposure did not show threshold shifts relative to pre-exposure thresholds in noise. As expected, chinchillas were more sensitive at detecting fully modulated SAM-tone signals than less modulated, with individual modulation depth thresholds falling within previously reported mammalian ranges.

Although we have only been able to confirm cochlear synaptopathy in pilot assays with naïve animals so far (i.e., not in the pilot behavioral animals), this project has developed an awake protocol for moderate-level noise exposure, an extension to our lab's previous experience with high-level permanent damage noise exposure under anesthesia. Also, we successfully established chinchilla behavioral training and testing protocols on several auditory tasks, a new methodology to our laboratory, which we hope will ultimately allow us to identify changes in auditory perception resulting from moderate-level noise exposure.

CHAPTER 1 INTRODUCTION

Individuals with a history of acoustic overexposure and poor pure-tone audiometry thresholds are often clinically diagnosed as having noise-induced hearing loss and typically show poor speech intelligibility. However, even some people with normal thresholds complain of having difficulties understanding speech in noise (Hind et al., 2011). Because normal thresholds have classically been interpreted to indicate normal cochlear function, in these cases, the reduced speech intelligibility in noise has often been taken to indicate a central auditory problem.

A classic view of acquired sensorineural hearing loss suggests primary damage to sensory hair cells that leads to degeneration of the cochlear-nerve (Spoendlin, 1971). However, recent confocal imaging analyses on moderately noise-exposed animals have shown 30-50% loss of auditory-nerve synapses on inner hair cells (IHC), despite the recovery of auditory brainstem response (ABR) and distortion product otoacoustic emission thresholds to normal levels (DPOAEs; Furman et al., 2013; Kujawa and Liberman, 2009). This suggests that a "hidden" type of cochlear hearing loss may contribute to the discrepancy between having perceptual speech deficits in noise and normal audiometric thresholds.

Cochlear synaptopathy, by definition, is a biological condition that affects the auditory nerve fiber terminals (cochlear synapses). Results from a guinea-pig model indicated that cochlear synaptopathy might selectively affect auditory nerve fibers, predominantly the low-spontaneous rate (low-SR) high-threshold fibers while high-spontaneous rate (high-SR) low-threshold fibers are left intact (Furman et al., 2013). These findings are consistent with the idea that normal hearing thresholds in an audiogram depend only on having a few reliable fibers responding to low intensity, whereas speech-perception deficits may relate more to the coding of supra-threshold modulations, relying more on responses from low-SR fibers.

Human studies have shown evidence that is consistent with the idea of cochlear synaptopathy, like those observed in animal models. Supporting data have also demonstrated the difficulties of understanding speech in noisy environments with normal audiometric thresholds in humans (Zhao and Stephens, 2007; Davis, 1989). However, to date there has been no direct link between cochlear synaptopathy and perceptual deficits represented in a single model. Our goal is to create a cochlear synaptopathy animal model similar to previously established mouse and guinea pig models, but that can be easily trained and behaviorally tested. Our cochlear synaptopathy model has an advantage over previous animal models because by using chinchillas, we are able to measure perceptual deficits at lower frequencies related to human speech recognition.

The research described here seeks to better understand the effects of noise-induced cochlear synaptopathy on behavioral thresholds in a chinchilla model. We have used a combination of non-invasive physiological techniques and a behavioral approach to evaluate changes in auditory-nerve signaling and determine effects on chinchilla detection ability in acoustic behavioral tasks after moderate noise exposure. In this project, we have specifically used behavioral tasks that can be performed by both animals and humans. This will allow us to better translate our results to human psychoacoustic research and provide evidence towards better clinical diagnosis of noise-induced hidden hearing loss. These observations may lead us to reframe the concept of hearing loss to include primary cochlear synaptopathy and question its effects on the peripheral auditory pathway that can in turn result in perceptual deficits in noise and for complex acoustic stimuli.

1.1 Background

1.1.1 The Auditory System

Two major interdependent systems, the peripheral and central auditory systems, make up the mammalian hearing system. The peripheral auditory system is composed of the outer ear, middle ear, inner ear, and auditory nerve and begins the hearing process by transforming air pressure variation into mechanical energy by the middle ear. Subsequently, this energy is transformed in the cochlea into neuronal electrical signaling in the auditory nerve. Meanwhile,

the central auditory system includes brain structures to process acoustic

information carried by auditory afferent pathways.

The mammalian auditory system is tasked with processing acoustic

information over a large frequency range, e.g. the human auditory system is

sensitive to pure tones from 20 Hz to approximately 20 kHz. It also has the ability

to detect sounds that vary from very soft, 0 dB SPL, to very loud levels, 120 dB

SPL. Despite the ear's capacity to process acoustic information, it can be subject

to hearing impairment resulting from acoustic overexposure or vulnerability to

ototoxic drugs.

1.1.1.1 Spectral Decomposition

The basilar membrane, in the inner ear, displays frequency-dependent

vibration creating an auditory tonotopicity. That is, high frequency sounds cause

maximal displacement of the basilar membrane at the base of the cochlea

whereas lower frequency sounds produce maximal resonance at the apex,

establishing a particular characteristic frequency for each position along the

cochlea (Bekesy, 1960). Further, a mechanism known as the "cochlear amplifier"

provides acute sensitivity in the mammalian auditory system by amplifying the

vibrations of the basilar membrane via the fast motile response of outer hair cells.

Amplified mechanical responses over a limited range of frequencies are

transduced by individual inner hair cells, which collectively can be modeled as a

filterbank (Fletcher, 1940; Oghalai, 2004). Each filter displays a particular

characteristic frequency and increasing bandwidth toward high frequency regions of the basilar membrane (Fletcher, 1940).

1.1.1.2 Transduction Process in Hair Cells

The basilar membrane motion, as mention previously, causes IHC stereocilia to bend back and forth at various locations along the length of the cochlea transducing mechanical energy into electrical energy over a narrow range of frequencies (Hackney et al.,1993; Bekesy, 1960). Deflection of outer hair cell stereocilia results in electromotile responses, the cell shortens and then elongates, contributing to the cochlear amplifier (Brownell, 1983; Brownell et al., 1985; Zheng et al., 2000). The contribution of the outer hair cells to the mechanics of the cochlea produces high sensitivity and sharp tuning of auditory nerve responses (Brownell, 1983).

1.1.2 Responses in the Auditory Nerve

The IHC-AN signal processing complex is critical in the peripheral auditory system to transduce mechanical signal into neural signal in response to acoustic stimulation. Physiological studies have provided insight into the temporal dynamics of IHC-AN synaptic processing and the neural activity in the absence of acoustic stimulation (i.e. spikes occurring in the absence of sound-induced stimulation). A recent physiological IHC-AN model captures neural adaptation and the sensitivity to transient stimuli compared to steady-state stimuli (Meddis, 1986).

1.1.2.1 Spontaneous Firing Rates, Thresholds, and Coding of Supra-Threshold Sounds

IHC-AN complex functions as the main gate in the transmission of sound-evoked potentials (in response to release of glutamate neurotransmitters in the cochlear synapses). Spontaneous spike activity (potentials generated in the absence of sound stimulation) generated by type I spiral ganglion neurons (in the auditory nerve) are carried away by AN fibers to auditory central areas in the brain (Liberman 1978; Liberman, 1980; Kujawa and Liberman; 2009; Stöver and Diensthuber, 2011). Spontaneous potentials are classified based on firing rate; high spontaneous rates (SR > 18 spikes/second) and low-SR and medium-SR (SR < 18 spikes/second; Bharadwaj et al., 2014), and their sensitivity to sound; low-SR fibers can be as much as 80 dB less sensitive (high thresholds) than high-SR fibers (low threshold) at the same characteristic frequency (CF). Thus, physiological studies provide evidence that the major contribution of low-SR fibers is on suprathreshold sounds and in hearing in noise. (Liberman 1978; Bhardwaj et al., 2014; Taberner and Liberman (2005).

1.1.2.2 Neural Excitation Patterns

Although single-unit recordings provide valuable information about neural sound coding, they also reveal information regarding the pattern of neural responses over distinct auditory neurons. The mechanical pattern of neural activity as a function of CF is known as "excitation pattern". High level of activity

is observed in neuron with a CF close to a pure tone frequency played at low level but neural activity decreases for off-CFs. However, excitation patterns do not maintain selectivity in frequency at high sound levels, in which neural saturation is observed over a large range of frequencies (Young and Sachs, 1979; Sachs and Young, 1980). Excitation patterns become important when analyzing the internal representation of the spectrum of a stimulus.

1.1.2.3 Neural Coding, Phase Locking, and Auditory Perception

Temporal processing of acoustic information can refer to temporal fine-structure (TFS) and envelope (ENV), that relies on the ability of auditory filters to extract acoustic energy from complex sounds (Moore, 2008). The ENV corresponds to the slowly varying amplitude superimposed onto a more rapidly varying signal, TFS. The spectral information is limited by the width of the auditory filters; low frequency narrow-filters process both TFS and ENV information (here, neural spikes represent the TFS by phase locking to individual cycles of the stimulus waveform) whereas high frequency wider-filters process sound-evoked neural responses (here, responses phase lock to the ENV, but not to the TFS) (Young and Sachs, 1979; Bharadwaj, 2014). In most mammals, higher fidelity of TFS phase locking is observed below 4-5 kHz, but some evidence suggest TFS phase locking even persists up to 10 kHz (Heinz et al., 2001; Kale, 2011). Psychophysical studies have provided compelling evidence regarding the role of TFS cues on pitch perception of both pure and complex tones, speech intelligibility, and masking (Moore, 2008).

1.1.3 Psychoacoustics

Extensive research work in psychoacoustics, based on the evaluation of behavioral task responses, has been done to study and understand the correlation between the effects of acoustic signals on the auditory system and the perception of sound in both human and nonhuman listeners.

1.1.3.1 Perception of Pure Tone, Audiogram, and Absolute Thresholds

A major characteristic of the auditory system is the ability to detect low sound levels in the absence of other sounds, known as absolute thresholds. Pure tone audiometry (PTA), a clinical technique, is used to determine levels of hearing loss by presenting a repeated pure tone at specific frequencies that range from 250 to 8000 Hz in a quiet environment (Saunders et al, 1990). PTA measures the minimum audible levels in decibels (dB) at which this tone is detected 50% of the time, known as a "behavioral threshold" (Saunders et al., 1990). Behavioral threshold shifts are then quantified relative to average 'normal hearing' young individuals. In clinical settings, the use of audiograms helps to diagnose noise-induced hearing loss on individuals with acoustic overexposure history and poor speech intelligibility. However, PTA has failed to detect hearing impairment on individuals with normal thresholds, but who complain having

difficulties understanding speech in noisy environments (Hind et al., 2011). Studies have suggested that deficits of perception in the presence of normal thresholds may be an indicator of a potential type of "subclinical" hearing loss that it is undetectable by regular audiometric testing performed in clinical settings.

1.1.3.2 Perception of Complex tones and Amplitude Modulated (AM) sounds

Unlike pure tones, complex tones are the product of periodic pure tones of different frequency, amplitude, and phase, which they maintain a repetition rate similar to their fundamental frequency. Natural sounds, music, and speech are representative examples of complex tones. Research in mammals and humans subjects suggests that pitch perception of complex tones remains even when the fundamental frequency is missing (Heffner and Whitfield, 1976; Clarkson and Clifton, 1985; Shofner, 2011). Complex sound detected on a daily basis can constantly change in amplitude resulting in amplitude-modulated (AM) signals whereas in laboratory settings AM signals can be generated by changing the amplitude of the carrier signal according to the modulating signal (modulating signals have lower frequency than of the carrier signal). The carrier frequency remains constant during modulation, in this case the TFS, but its amplitude varies accordingly the amplitude of the modulator, generating then the ENV of the AM signal. Spectra of AM signals consist of three frequency components; the carrier frequency (fc), and two "sidebands" offset by the modulation frequency (fm) one above (fc+fm) and another below (fc-fm).Detection of AM signals

depends on the power contained on the sidebands and the modulation depth of the AM signal, e.g. highly modulated signals are better detected than less modulated signals. Moore and Sek (1992) used an adaptive two-alternative forced-choice task to determine the thresholds for detecting AM signals and reported that subjects were less sensitive when tested with low modulation depths (higher thresholds) but more sensitive to higher modulation depths (lower thresholds).

1.1.4 Hearing Impairment

Hearing impairment is generally defined as the inability of the ear to detect soft sounds, yet the functional state of the ear is complex and goes beyond its limitation to detect weak sounds. Two people with normal audiograms can have distinctly different degrees of hearing impairment or an individual with normal audiometric thresholds can have difficulties understanding speech in noise (Hind et al., 2011). Compelling evidence has demonstrated that hearing impairment can impact several auditory percepts such as loudness, pitch, localization, speech perception, especially in noise (Dubno et al., 1984; Hopkins et al., 2008; Moore, 2008; Moore and Glasberg, 2004).

Psychophysical tuning curves (PTC) are used as clinical tools to assess frequency sensitivity and detection of dead regions in the cochlea (Sek and Moore, 2011). Shape of PTCs differs for hearing-impaired and normal hearing subjects. PTCs of normal hearing individuals are usually sharp and have narrow "V" shape. With hearing impairment and shift in thresholds, tuning curves

become broadened, decreasing the frequency resolution of the auditory system (Leshowitz, 1975, 1976; Florentine, 1992).

The spectral tuning of the auditory periphery can also be physiologically evaluated in animal models by measuring the thresholds of auditory nerve fibers in response to acoustic stimuli. As previously mentioned, the frequency tonotopicity map in the cochlea is also tonotopically represented in the auditory nerve. In single unit recordings, the frequency at which the fiber is most sensitive to is defined as the best frequency (BF). The shape of neurophysiological tuning curves are an inverse image shape of auditory nerve filters. Similar to PTCs, the bandwidth of neural tuning curves is characterized by the bandwidth located 10 dB above threshold, and the sharpness of the tuning is defined by a "quality factor", known as Q_{10dB}. Changes in thresholds and morphology of tuning curves have been observed after damage to the cochlear hair cells. Damage to OHCs is associated with broad neural tuning curves and elevated thresholds whereas damage to IHCs increases thresholds without broadening the tuning curve (Liberman and Dodds, 1984).

Discrepancy in the sharpness between a psychophysical tuning curve and neurophysiological tuning curves can be attributed to off-frequency listening during behavioral tasks. O'Loughlin and Moore (1981) used a band-rejection noise, centered on the testing frequency, to reduced off-frequency listening and improve the disagreement in sharpness between these two tuning curves. Although hearing impairment is used as a general term to describe varying

degrees of hearing loss, in this thesis, hearing loss will be used to describe elevated audiometric thresholds.

1.1.4.1 Noise-Induced Hearing Loss

Noise-induced hearing loss produced by recreational and occupational noise exposure is the second most common type of sensorineural hearing deficit after age-related hearing loss (Coad et al. 2013). At high sound levels, hearing loss spread greatly toward high frequencies regions of the cochlea creating significant damage to this region, but less toward low frequencies. The greatest damage to the cochlea is typically observed one-half to one octave above the center frequency of the noise exposure, referred to as the "one-half octave shift" (Schmiedt, 1984). Sensorineural hearing loss (SNHL) is associated with impaired hair cells (OHCs and IHCs) and supportive cochlear structures that can lead to temporary or permanent reduction in sensitivity to sounds (Liberman and Dodds, 1984; Wang et al., 2002; Kujawa and Liberman, 2009). Animal models of noise-induced hearing loss have reported poor performance in discriminating signals in noise; this is due to loss of frequency sensitivity. For detection of complex signals, degradation of the temporal coding in the cochlea leads to having difficulties in perceiving temporal information contained in complex signals after noise-induced hearing loss (Bharadwaj et al., 2014).

1.1.4.2 Permanent Hearing loss vs Temporary Hearing Loss

Permanent hearing loss, described as a permanent threshold shift (PTS), is characterized by irreversible audiometric thresholds shifts and damage to both

OHCs and IHCs (Saunders et al., 1985; Liberman and Dodds, 1984). Combined

damage to IHCs and OHCs elevates both the tip (thresholds) and tail of a tuning

curve (Leshowitz, 1975, 1976; Florentine, 1992) resulting in perceptual deficits.

Individuals with PTS usually report poor PTA thresholds, difficulties

understanding speech in both quiet and noise, and deficits in frequency

discrimination of pure and complex tones. But, peripheral temporal coding

evaluated in noise-induced hearing-impaired chinchillas is more degraded in

background noise than in quiet (Glasberg and Moore, 1989; Henry and Heinz,

2012).

Temporary threshold shift (TTS) is characterized by the temporary change

in hearing sensitivity in both humans and animal subjects (Nilson, 1991; Clark,

1991; Mills et al., 1979). Research on noise-exposed guinea-pigs indicated that

peripheral neural degeneration can occur despite full recovery of presynaptic

terminals on the IHC, recovery that explains TTS (Puel et al., 1998). However,

recent animal work on guinea-pigs and mouse challenges Puel and colleague's

work. Now, it is argued that after acoustic trauma, there is a rapid and irreversible

primary neural degeneration on IHCs and slow death of spiral ganglion cell in the

presence of recovered-thresholds (Lin et al., 2011; Kujawa and Liberman 2009).

In these animal models, an octave-band noise presented at levels that ranged

from 100-109 dB sound pressure level (SPL) for 2 hours were enough to produce

damage to the cochlea at one octave above the center frequency trauma band

(Lin et al., 2011; Kujawa and Liberman 2009). Studies in humans demonstrated

the effects of TTS on auditory percepts, including delays in recruitment of

loudness, decrease in the Békésy amplitudes, but no effects were observed on frequency discrimination (Mills, 1970).

1.1.4.3 Cochlear Synaptopathy and Implications

Cochlear synaptopathy is a type of noise-induced or age-related sensorineural hearing disorder that is characterized by the degeneration of cochlear synapses in the absence of hair cell loss or elevated thresholds. Recovered audiometric thresholds have been taken to indicate the full recovery of the cochlea to normal functioning after acoustic trauma. Recent confocal imaging analyses on moderately noise-exposed animals has challenged this view. Mice and guinea pigs have shown 30- 50% loss of auditory-nerve synapses on inner hair cells (IHC), despite the recovery of normal auditory thresholds (Furman et al., 2013; Kujawa and Liberman, 2009). Further, reduced sensitivity to speech in noise in humans with normal thresholds has long been reported and referred as "obscure auditory dysfunction" (Saunders and Haggard, 1989), a problem now known as "hidden hearing loss" (Schaette and McAlpine, 2011).

1.1.4.4 Physiological Correlates of Cochlear Synaptopathy

Animal work has provided relevant knowledge about the physiological correlates associated with cochlear synaptopathy. In a mouse model, both auditory brainstem responses (ABRs) and DPOAEs thresholds recovered to normal pre-exposure levels and remained stable between 8 and 16 weeks after acoustic trauma (Kujawa and Liberman, 2009). However, suprathreshold ABR amplitude responses of Wave 1 were reduced in the presence of recovered

thresholds, suggesting permanent loss of IHC auditory-nerve synapses in the cochlea. Permanent damage to cochlear synapses may results from glutamate excitotoxicity in response to acoustic overstimulation. Confocal imaging of the organ of Corti in mouse showed evidence of permanent damage to cochlear nerve terminals, as indicated by the absence of synaptic ribbons, but without obvious damage to either IHCs or OHCs (Kujawa and Liberman, 2009).

1.1.4.5 Neural Correlates of Cochlear Synaptopathy

A noise-induced cochlear synaptopathy guinea-pig model has suggested that cochlear synaptopathy selectively affect auditory nerve fibers (ANFs). Specifically low-spontaneous rate (low-SR) fibers that respond to high sound levels in background noise (high-threshold fibers) are potentially lost while high-spontaneous rate (high-SR) low-threshold fibers are left intact (Furman et al., 2013; Bharadwaj et al., 2014). Since, low-SR ANFs show high resistance to masking by continuous background noise, it is suggested that their acoustic driven-activity is an important cue for hearing in noisy environments (Costalupes et al., 1984)

1.1.4.6 Perceptual Correlates of Cochlear Synaptopathy

Aforementioned, evidence has demonstrated that cochlear synaptopathy selectively targets ANFs. Since normal hearing thresholds only depend on having a few reliable fibers responding to low intensity levels (recruitment of high-SR fibers), but speech-perception deficits arise when coding of supra-threshold, amplitude-modulated signals are compromised (presumably from damage to low-

SR fibers; Bharadwaj et al., 2014). Human studies have shown evidence that is consistent with the idea of cochlear synaptopathy (Schaette and McAlpine, 2011). Difficulties understanding speech in a challenging environment have been reported by humans with normal audiometric thresholds (Zhao and Stephens, 2007; Davis, 1989).

As we know, damage to low-SR ANFs is detrimental to the coding of supra-threshold amplitude-modulated signals (Bharadwaj et al., 2014), and thus individuals with a history of noise exposure, but with normal hearing thresholds, have decreased ability to discriminate complex signals (Stone et al., 2008). While the dysfunction of IHC and OHC that leads to PTS has taken a great deal of attention, there is now also enough evidence from animal studies to demonstrate that even with TTS there is a significant loss of the ANF synapses that compromises neural coding and perception. However, to date there has been no direct link shown between cochlear synaptopathy and perceptual deficits.

1.2 Motivation, Purpose, Goal, and Rationale

Our *motivation* is to create a cochlear synaptopathy chinchilla model similar to previously established mouse and guinea pig models with the *purpose* to evaluate the effects of noise-induced cochlear synaptopathy on perceptual tasks. We have as a *goal* to evaluate the changes in performance on perceptual tasks following exposure to moderate noise levels that can potentially produce cochlear synaptopathy in chinchillas without PTS.

We specifically use behavioral tasks that can be performed by both animals and humans to be able (in collaboration with UK colleagues) to translate our results to human psychoacoustic research. Results from this study will help us to better understand the prevalence and real-world consequences of cochlear synaptopathy in humans. Human studies have used similar non-invasive physiological techniques in listeners with tinnitus and normal thresholds to provide evidence suggestive of cochlear synaptopathy (Schaette and McAlpine, 2011) as well for perceptual deficits in intensity discrimination (Epp et al., 2012) and tone-detection in noise (Weisz et al., 2006). Thus, we will examine simple tone detection in noise and AM modulation-detection tasks in chinchillas, and stimulus conditions (e.g., high SPLs for signals and noise) chosen to emphasize reliance on low-SR ANFs.

1.3 Research questions and Hypothesis

In this project, we aim to answer the following research questions:

1. Can exposure to moderate sound levels produce neurophysiological changes that disrupt the fidelity of neural coding in the auditory periphery, and result in perceptual deficits?

2. Will animals with cochlear synaptopathy show deficits in detecting a tone in the presence of noise?

3. Will animals with cochlear synaptopathy have difficulty discriminating an amplitude-modulated signal of varying modulation depth post-noise exposure?

We *hypothesize* that perceptual tasks that depend on coding of suprathreshold sound levels will be most affected.

1.4 Specific Aims

Rationale

Based on the contribution of low-SR high-threshold ANFs, to hearing in noise, this study aims to evaluate the detection abilities of chinchillas in a tone-in-noise behavioral task. By evaluating pre- and post-exposure behavior along with non-invasive physiological thresholds in the same animal, it will help us to correlate supra-threshold abilities with sequelae of cochlear neuropathy. By comparing results from the tone-in-quiet task (a task relevant to a clinical audiogram) with more complex perceptual tests, such as tone-in-noise and amplitude-discrimination-depth detection, this project aims to provide evidence for which clinical tests are also relevant in the diagnosis of noise-induced hearing loss, particularly, cochlear synaptopathy. Human subjects have shown compelling evidence that supports the idea of cochlear synaptopathy and its effects on perception. Poor behavioral thresholds from a tone-detection in noise behavioral tasks were reported by individuals with high-frequency tinnitus and normal audiometric thresholds (Weisz et al., 2006).

1.4.1 Aim 1

Evaluate the effects of cochlear synaptopathy on the detection abilities of chinchillas in a tone-in-noise behavioral task.

Rationale

Based on the contribution of low-SR, high-threshold ANFs to hearing in noise and rate-coding of tone in noise and the decrease of low-SR fibers after moderated acoustic trauma, this study aims to evaluate the detection abilities of chinchillas in a tone-in-noise behavioral task. Cochlear neuropathy with resulting loss of low-SR fibers may underline auditory peripheral impairments that can compromise supra-threshold listening, without jeopardizing audiometric thresholds (Bharadwaj et al., 2014; Young and Barta, 1986).

1.4.2 Aim 2

Evaluate the effects of cochlear synaptopathy on the ability of chinchillas to detect the amplitude modulation (AM) depth for SAM tones in a behavioral task.

Rationale

Based on the expected role of low-SR ANFs to temporal modulation coding (Bharadwaj et al., 2014) and the potential participation in temporal modulation coding at high sound levels coding (Lorenzi and Moore, 2008; Hopkins et al., 2008; Zeng et al., 2005), this study aims to evaluate the effects of cochlear synaptopathy on the behavioral ability of chinchillas to detect AM depth of SAM tones.

CHAPTER 2. DESIGN AND METHODS

2.1 Subjects

Evidence has demonstrated that chinchillas are an appropriate subject to design animal models to study noise-induced hearing loss. This is because of their audibility frequency range being similar to that observed in humans (Clark, 1991; Heffner and Heffner, 1991). In this study, ten chinchillas subjects were enrolled at the ages of around 6 months to be tested in non-invasive physiological and psychophysical tasks of increasing difficulty. Animals were carefully food restricted for the length of the study to increase motivation for behavioral food rewards. Animals' body weight was monitored daily to maintain a range between 80-95%, and water was provided *ad libitum* in the home cage. The Purdue University Laboratory Animal Program (LAP) provided a fully accredited (AAALAC-I) central animal facility to house the chinchillas, implement scheduled feeding, cage cleaning, and overall health monitoring. The Purdue Animal Care and Use Committee (PACUC) reviewed protocols to assure that the animal care was performed in accordance with established standards.

2.2 Determining Noise Exposure Levels

In this study, cochlear synaptopathy in chinchillas was produced according to the noise-induced cochlear synaptopathy models previously developed in mouse and guinea pig (Lin et al. 2011; Hickox and Liberman, 2014). This experimental step was performed by a postdoctoral fellow, and the results were presented in a poster session at the 38th Annual Midwinter Meeting of the Association for Research in Otolaryngology (Hickox *et al.*, 2015). In this experiment, several naïve chinchillas were exposed in a reverberant chamber to carefully calibrated sound levels to determine the appropriate noise-exposure sound level that would produce cochlear synaptopathy without PTS.

Naïve chinchillas were randomly assigned to groups of varying exposure levels that ranged from 98 to 107 dB SPL (2-hour exposures while animals were awake). Noise exposure was designed to use an octave-band centered at 1 kHz (0.707-1.414 KHz) to cause significant synaptic degeneration one to two octaves above the trauma band (Kujawa and Liberman, 2009; Lin et al., 2011). Physiological measures (ABRs and DPOAEs) were applied to determine the effects of cochlear synaptopathy. ABRs threshold shift and high-level ABR wave-1 amplitude were measured 2 weeks after exposure.

These naïve animals were sacrificed and underwent transcardial perfusion with 4% paraformaldehyde for immunohistological evaluation. Cochlear synaptopathy was confirmed with counts of pre-synaptic ribbons, by confocal micrographs, at distinct cochlear locations and compared with physiological assays to determine the appropriate noise-exposure level to produce

synaptopathy). At each corresponding level, histological assessment was completed on 2 ears whereas physiological measurements were performed on 3-4 ears. Figure 2.1 illustrates the physiological threshold shift (dB) assessed 2 weeks after noise exposure.

As expected, high sound levels produced significant PTS within and approximately one octave above the noise band, but low levels only produced about 5-dB PTS within the noise band. Noise-exposure level of 98 or 99 dB SPL produced some effect on the wave 1 amplitude depicted in Figure 2.2. The ABR wave 1 amplitude was reduced between 15 and 30% at 2 and 4 kHz correspondingly, but not at lower frequencies. Change in amplitude was taken as evidence for some degree of cochlear synaptopathy, related to low-SR fibers damage. Histological assessment showed significantly reduced ribbon count for all high levels at frequencies below and above the noise band. The lowest sound level, 98-99 dB SPL, also produced ribbon count damage at all frequencies, except at 8-16 kHz as shown on Figure 2.3.

Based on the reduced wave 1 amplitude and degraded synaptic ribbon count, it was determined that the lowest sound exposure level, 98-99 dB SPL, produced the desired phenotype. Thus, it was decided that this sound exposure level was the most appropriate to replicate the cochlear synaptopathy phenotype, without producing PTS, on the behavioral animal group.
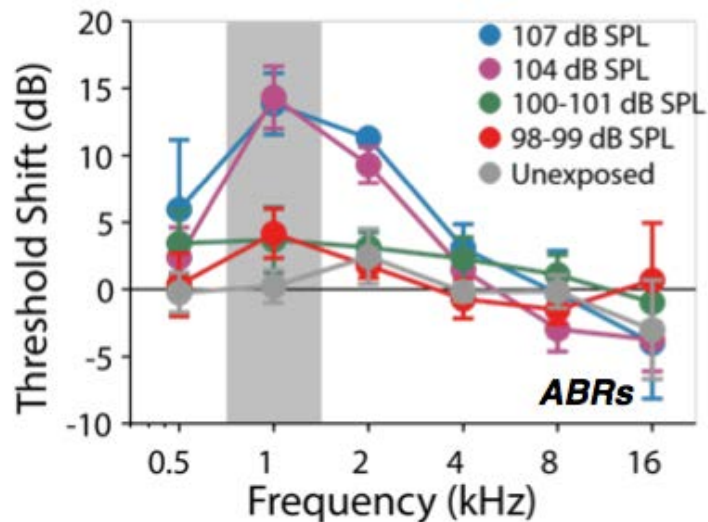
Figure 2. 1 ABR threshold shift (dB) measured on noise-exposed naive animals at various levels of noise. Figure from Hickox *et al.*, (2015).
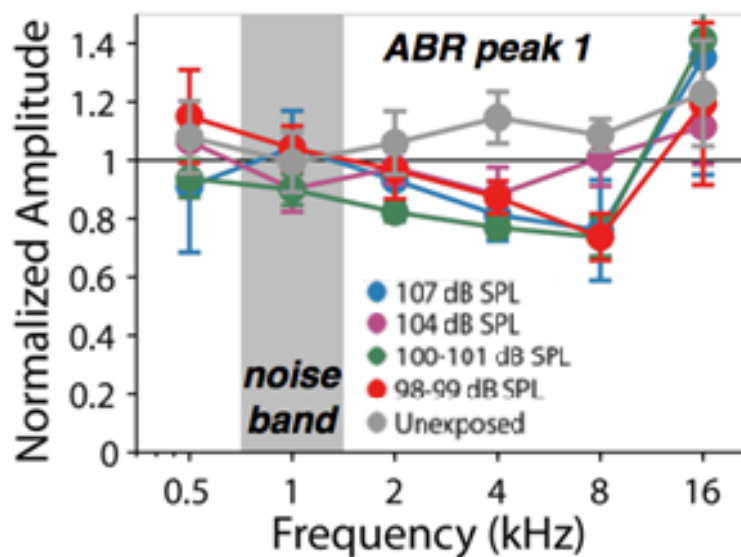
Figure 2. 2 Normalized ABR wave-1 amplitude measured on noise-exposure naïve animals at various levels of noise. Figure from Hickox *et al.*, (2015).
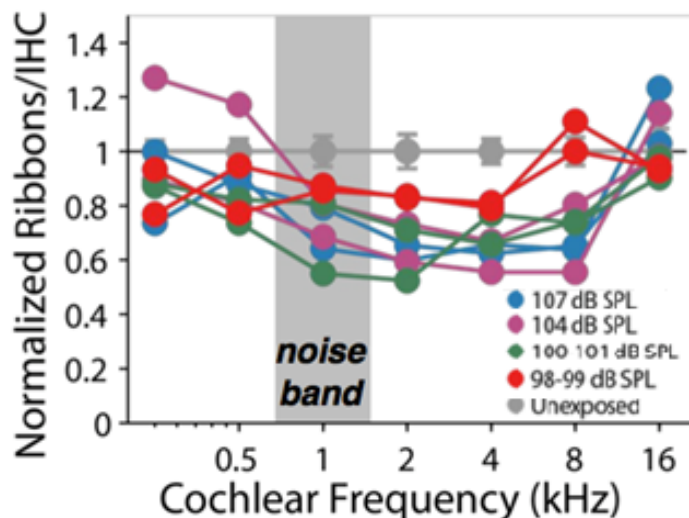
Figure 2. 3 Synaptic ribbon counts on IHC measured on naive exposed animals at varibale sound levels, normalized by unexposed data. Figure from Hickox *et al*., (2015).

2.3 Noise Exposure Rationale and Procedure to Create TTS

In this study, chinchillas were exposed in a reverberant chamber to a moderate-level octave-band noise centered at 1 kHz for 2 hr to produce a representative cochlear-synaptopathy, model similar to the one established in mouse and guinea pig. An awake exposure is scientifically advantageous for creating TTS models for three reasons: 1) Greater accuracy in the extension of the current mouse- and guinea-pig hidden hearing loss models, which exclusively employ unanesthetized noise exposures, 2) it provides a more realistic noise exposure to those typically predicted to lead to hidden hearing loss in humans, and 3) it controls for the protective effects of anesthetics (ketamine/xylazine; used during exposure) on acoustic trauma (Olney et al., 1986; Giraudet et al., 2002). The 1-kHz center frequency used for the TTS model is a tradeoff between the higher-frequency noises used in the mouse and guinea-pig TTS studies.

Also, using a low enough frequency we ensure to produce synaptopathy at frequencies in which robust temporal coding takes place.

2.4 Non-invasive physiology

Minimally invasive physiological measures (ABR thresholds and ABR wave 1 amplitude) were measured within a week before and two weeks post exposure to confirm threshold recovery with reduced suprathreshold wave 1 amplitude. Signal-induced neural responses were measured by averaging scalp potentials, which are measured by subdermal needle electrodes. These measurements were repeated on the same ear before and after acoustic trauma for each animal. ABR wave 1 amplitudes were calculated as the mean amplitude across responses to stimulus levels of 60 and 70 dB SPL. ABRs were measured with tone pips at 0.5, 1, 2, 4, and 8 kHz (by using a 5-ms tone pips with 0.5-ms rise/fall times with a repetition rate of ~19/sec).

2.5 Behavior and Controls

Animals were food-restricted to encourage behavioral work in the test chamber during both training and testing sessions. Operant conditioning paradigm and detection techniques based on positive reinforcement (food reward) were used to evaluate perceptual deficits (Shofner, 2000; 2011). Animals were tested daily in a sound-attenuating chamber, with a 60/40% or 80/20% signal/catch trial ratio. During all the behavioral training and test sessions,

flashing light located above the lever response indicated to the animals that a trial was ready to begin.

Animals were trained to detect changes in sounds in an AAAA vs BABA task and tested on three distinct behavioral tasks (TIN, TIQ, and SAM) at three signal frequencies (0.5, 2, and 4 kHz) to provide for within-animal controls in addition to pre-exposure assessments acting as controls. Frequencies were tested one octave below (0.5 kHz; no PTS or synaptopathy expected), one octave above (2 kHz; no PTS, moderate synaptopathy expected), and two octave above (4 kHz; no PTS, maximal synaptopathy expected) the noise exposure band. Animals started a trial by pressing a response lever at variable holdtimes (1-6 s) and releasing it within the response window (2 s) in response to a played sound. Sounds were presented by using the "alternating paradigm", in which alternation of the signal and the standard stimuli seem to improve behavioral performance. This paradigm has an advantage upon a non-alternating because the animal has 'multiple looks' at the signal before responding to the sound change (Shofner, 2000).
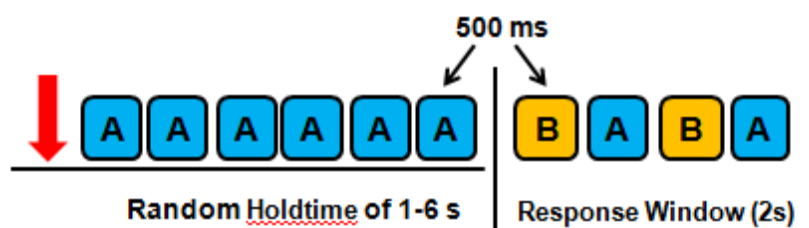
Figure 2. 4 Schematic diagram illustrating the alternating sound presentation paradigm. The red arrow indicates the animal begins a trial by pressing down on the response lever and releasing it within the response window. Figure modified from Hickox *et al.*, (2015).

The method of constant stimuli was used during testing to generate psychometric functions to evaluate the animals' sensitivity and determine behavioral thresholds pre-and-post exposure (Shofner, 2000). Psychometric functions were generated by varying the sound level or modulation depth. Sensitivity index (d-prime) was used to remove any effects of potential response bias, calculated as d' values based on [z(hit rate) - z (false alarm rate)] (Stanislaw and Todorov, 1999). We use z-scores ordinate, known as "correction for response bias", to correct for bias responses (guessing) in the yes/no response behavioral task (Klein, 2001). A total of 30 repetitions per level or per depth were collected for each animal pre-and-post exposure to determine thresholds corresponding to a d' of 1, where d-prime represents stimulus sensitivity by factoring in hit and false-alarm rates. Table 2.1 summarizes the parameters used in this study.

	Training and Testing	Ability to detect tone-detection (Aim 1)	Discrimination of AM depth (Aim 2)
Behavioral Tasks	Operant conditioning paradigm with positive reinforcement and detection techniques. Rewards for hits and correct rejections at 80% rate. Daily testing with 60/40% and 80/20% signal/catch trials. Holdtime: 1-6 sec Method of constant stimuli and BABA task (pre-and-post exposure) **Parameters:** *Noise:* *Tone:* Type: Pink Noise Pure tone Bandwidth: 24 kHz Freq: 0.5, 1, 2, 4 kHz Level: 55 dB OAL 20 - 80 dB SPL Duration: 500 ms 500 ms	**Standard:** Broad-band noise masker (24 kHz BW) Level = ~37 dB SPL spectrum level Presented alone during the random hold time and during a catch trial (AAAA) **Signal:** Noise masker with an embedded pure tone presented as BABA (signal trial) or AAAA (catch trial) Pure tone levels: 20–80 dB SPL Frequencies: 0.5, 2, and 4 kHz	**Standard:** Pure tone (no modulation) Fixed level: 70 dB SPL Repeated presentation (hold time) Alternated with signal (signal trail) **Signal:** SAM tone embedded Carrier frequency: 0.5, 2, 4 kHz Modulator frequency: 20 Hz Fixed level: 70 dB SPL Modulation depth: -30 to 0 dB varied by 3 dB steps.

Table 2. 1 Parameters for Tone-in-noise and AM SAM-tone signals.

2.5.1 General Behavior Information

Animals were trained to release a lever in response to sound in exchange for a reward (food pellet). Food rewards were delivered only when a hit (Ht) and correct response (CR) were scored, but not for a miss (Ms), false alarm (FA) or aborted trial (AB). Behavioral training involves distinct stages in which animals were presented with tasks of increasing challenge (e.g., longer hold times).

Animals were trained to hold the lever for a randomized variable hold time (1 -6 seconds) prior to trial initiation. Training lasted until consistent performance on an easy detection task was established (i.e., high hit rates during signal trials and high correct- rejection rates during catch trials). Overall, animals were trained in distinct testing conditions for least 5 consecutive days with a performance at least 81% correct before being tested on the next step (see formula). Food reward system is summarized on Table 2.2.

Ht	Ms	CR	FA	AB
If the animal held the lever until the sound was present, at the desired hold time, he received a reward, 'hit'. Ht rewards were delivered at 80% rate.	If the animal released the lever after the tone was switched off and beyond the response window (1850 ms), this response was considered a 'miss', and the animal was not rewarded.	If the animal held the lever during the length of a blank trial (no sound) and beyond the response window (1850 ms), this response was considered a 'CR', and the animal was rewarded. CR rewards were delivered at 80% rate.	If the animal released the lever during the length of a blank trial (no sound) and within the response window (1850 ms), this response was considered a 'FA, and the animal was not rewarded.	If the animal released the lever before the sound was played, it was scored as an 'aborted trial', and the trial started again. There was not time-out after incorrect responses.

Table 2. 2 Positive reinforcement-food reward system used in a method of constant stimuli.

2.5.1.1   General pre-training information

I.   Restraint and Handling

Proper animal restraint and handling were applied to reduce stress and avoid injuries to the animals. Effective handling reduced abnormal behavior, fear, and built trust and bonding between an animal, and researcher. Chinchillas were first handled in their home cage and then introduced to the test chamber.

II.   Free Feed Weight

A free feed weight (FFW), the stable weight maintained by a mature chinchilla with unlimited access to food and water, was calculated before beginning any

training. Weights of adult chinchillas were collected every other day over the course of at least a month. FFWs were then calculated by averaging the animals' weights once they had plateaued.

III.   Food restriction

Animals were food restricted by decreasing 1g of both chow (minimum 10 g) and hay (minimum 5 g) every other day until the body weight target was reached. Animals' daily diet consisted of Timothy hay and chow pellets that were adjusted daily in weight to maintain the desired daily body weight. Body weights were carefully monitored and maintained at 80-95% for the length of the study. The body weight range is based on the animals temperament, some animals work better with lower body weight.

2.5.1.2 Behavioral Training

I.   Magazine training

Once the animals reached a desirable target body weight, they were introduced to the behavioral chamber where they spent some time inside the chamber for two consecutive days. This served to let them to acclimate to a novel environment. Animals were trained to find a food dispenser and rewarded 100% of the time. Chinchillas then learned to find, approach, and touch the lever for a food reward at fixed ratio of 1:1. Chinchillas were then trained to press the lever down for at least five times for food reward before sound was presented. In order to produce a strong association between the lever and reward, food pellets

were delivered within 1-2 seconds following the lever press. This strong

association was necessary for progressive shaping of behavior.

   II.   Lever release in response to sound with increasing hold time

       Animals were trained in this task by starting a trial when pressing the lever

down, in a quiet condition, for variable hold times, 1000 ms – 6000 ms , and then

releasing it in response to two tone bursts (1 kHz, 500 ms duration, 5 ms with 10

ms rise/fall) presented at the highest sound level, 70 dB SPL. Hold times were

progressively increased by 1000 ms or 2000 ms upon a consistent performance

for at least 5 days. Animals held the lever down until the sound was played + 150

ms (i.e.[hold-time + response window] = (1000 -6000 ms)+[(1850 ms + 150 ms)]

= (1000 -6000 ms + 2000 ms).
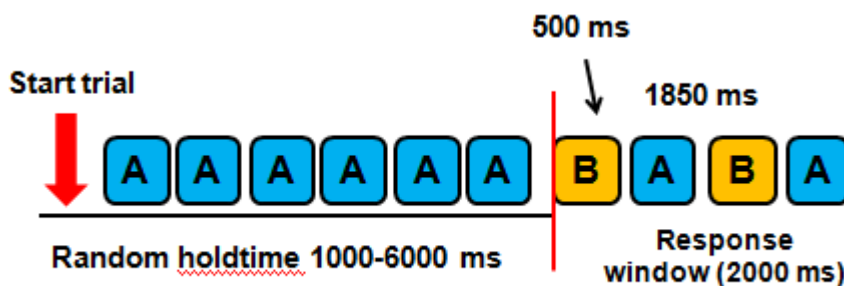
Figure 2. 5 Schematic diagram illustrating the alternating sound presentation paradigm and presentation of the standard stimulus (A) within the random holdtime and the sigmal (B) within the response window. Figure modified from Hickox *et al.*, (2015).

       Behavioral performance was estimated by dividing the number of hits by

the addition of hits and trials, at least 81% correct. Aborted trials were not

included in the behavioral performance evaluation and food rewards were

delivered for every 'Ht' response.

$$\% \text{ Correct} = \text{Hts}/(\text{ Hts} + \text{Misses})$$

III.   Lever release in response to sound in quiet with random hold time

Once animals were proficient at holding the lever down for 6000 ms, the hold time was now randomized for each trial. In this task, animals pressed the lever down as the hold time varied randomly from 1000 to 6000 ms in each trial. This was to ensure that the animals were attending to the tone bursts and not simply releasing the lever when the animal thought the time was up. The random hold time was determined by a rectangular probability function. Food rewards were delivered using the same criteria as indicated on Table 2.2.

IV.   Lever release in response to sound in quiet and behavioral challenge catch trials

Animals were trained to release the lever in response to tone bursts in quiet (1 kHz, 500 ms duration with 10 ms rise/fall, and 70 dB SPL) and challenged with catch trials, blank or non-signal presentation trials. Presentation of catch trials helped to correct for possibility of guesswork, especially in behavioral test based on yes-or-no response. In this step, animals were challenged with catch trials at ratio of 80/20% signal/catch trial. The animals' task was to release the pressed lever in response to sound during stimulus trials or continue holding during catch trials as depict on Figure 2.6 and 2.7 With randomized hold times, an animal was rewarded if it continued holding the lever down during a catch trial. This indicated that the animal had not detected the signal and the responses were scored as a 'CR'. If an animal released the lever

during a catch trial, the behavioral response was scored as 'FA'. See Table 2.2

for information about the food reward criterion.
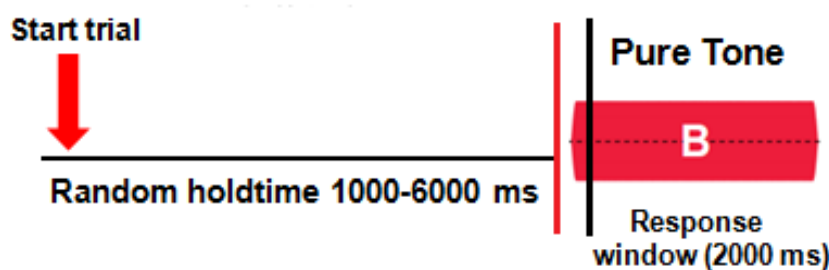
Figure 2. 6 Schematic diagram illustrating the paradigm for detection of tone signal in quiet background during a signal trial presentation. Figure modified from Hickox *et al*., (2015).
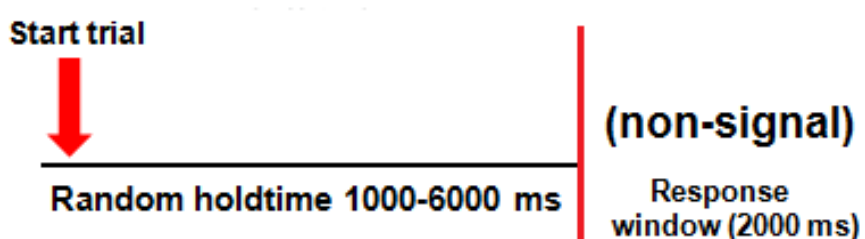
Figure 2. 7 Schematic diagram illustrating the paradigm for detection of the signal in quiet background during a catch trial. Figure modified from Hickox *et al*., 2015.

V.   Lever release in response to a tone signal in background noise

The animals' task here was to release the lever in response to a broad

noise masker of moderately high level (24 kHz BW, 37 dB SPL spectrum level)

with an embedded pure tone at the highest level (signal). Noise level was first

presented at 10 dB SPL above noise floor and increased progressively by 5 dB

SPL every other day while the tone signal was fixed at 70 dB SPL. During a

'signal trial' condition, animals were challenged with detecting burst signals

composed of the noise masker with the embedded pure tone.

The noise was presented alone in during the random hold time and during 'catch trial' condition (AAAA) and the noise masker-embedded pure tone was presented as BABA in a 'signal trial', as illustrated on Figure 2.8. Food rewards were delivered according to the criterion shown on Table 2.2 and parameters were used as shown on Table 2.1.
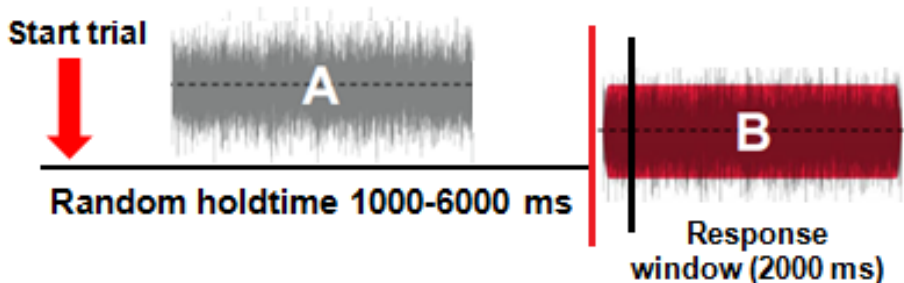
Figure 2. 8 Schematic diagram illustrating the detection of tone signal in noise background. Figure modified from Hickox *et al.*, (2015).

2.5.1.3 Behavioral Testing

Changes in the detection abilities in a tone-in-noise behavioral task (Aim 1) and discrimination of sinusoidal SAM tones of varying modulation depth (Aim 2) were assessed before and after noise exposure.

I.   Pure Tone Audiogram

Pure tone audiogram thresholds were assessed behaviorally by training the animals to indicate the presence of a pure tone in quiet. The method of constant stimuli was applied to generate psychometric functions that indicated behavioral thresholds corresponding to d-prime = 1. In a daily session, three or four distinct frequencies were tested in a session that lasted approximately 60 min. After thresholds had stabilized, thresholds were used to calculate mean

baseline pure-tone thresholds for each animal across frequency. See Table 2.1

for stimulus parameters and Table 2.2 for food rewards criterion.

II.   Behavioral Task of Tone-detection in Noise (Aim 1)

In order to determine the ability for tone-detection in noise (Aim 1),

animals had to detect a tone signal in the presence of noise. A broadband noise

masker of fixed level (37 dB SPL spectrum level), acting as a standard stimulus

(A), was presented alone during the random hold time or AAAA pattern during a

catch trial followed by the atypical signal (B) presented in a BABA pattern during

a signal trial. The atypical signal (B) consisted of a noise masker with an

embedded pure tone that varied in level from trial to trial by 5 dB steps (20-80 dB

SPL) as shown of Figure 2.9. As previously mentioned, the method of constant

stimuli was used to estimate behavioral thresholds based on a psychometric

function and a d-prime=1. Food rewards were delivered based on criterion

explained on Table 2.2, and for additional information about parameters and hold

times refer to Table. 2.1.
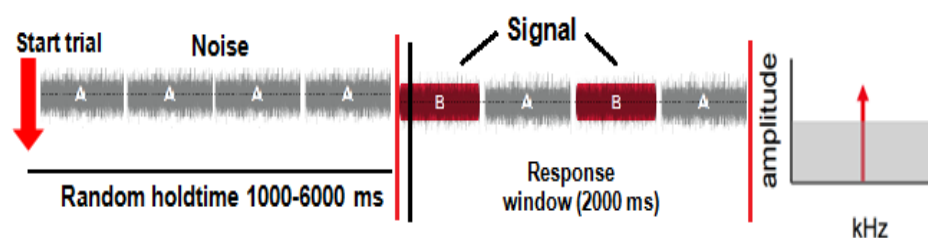
Figure 2. 9 Paradigm for detection of a pure tone signal embedded in noise and illustration of signal spectrum in noise (right). Figure modified from Hickox *et al.*, (2015).

III.   Behavioral Task of AM Depth in SAM-Tone Signals (Aim 2)

In this study, the effects of cochlear synaptopathy on the ability of animals to detect AM depths of SAM-tone signals was evaluated. This ability was assessed by repeatedly presenting an unmodulated pure tone of fixed level (70 dB SPL), acting as standard stimulus (A), during the hold time and alternated with an AM signal (B) during the signal trial as illustrated on Figure 2.10. The signal 'B' consisted of a SAM tone with variable modulation depths (-30 to 0 dB in 3-dB steps) embedded within in a notched-noise. The notched-noise masker presented with both the unmodulated pure tone (standard) and the modulated pure tone (signal) was used to avoid off-frequency listening based on high-SR ANFs (i.e., to force reliance on low-SR fibers). Psychometric functions (d-prime vs stimulus parameter) were generated to determine behavioral thresholds (d-prime=1).
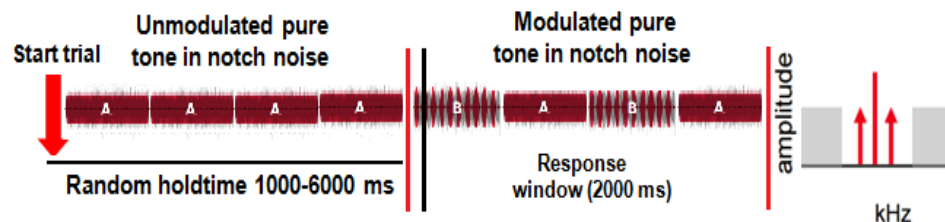
Figure 2. 10 Paradigm for detection of amplitude modulated pure tone embedded in notch noise and illustration of signal spectrum in a notch noise (right). Figure modified from Hickox et al., 2015.

CHAPTER 3. RESULTS

3.1 Physiological evaluation of the effects of moderate-level noise exposure on the peripheral auditory system

Auditory brainstem responses (ABRs) were used to evaluate the effect of moderate-level noise exposure on hearing sensitivity within animals at frequencies below, above, and within the noise band (0.5, 1, 2, and 4 kHz). For clarification purpose, animals were defined as following:

a. Unexposed: Animals tested before exposure to moderate-level sound.

b. Exposed: Same animals as the unexposed animals, but tested after exposure to moderate-level sound.

c. Sham pre-exposure: Animals that were not exposed to moderate-level sound, but experienced the same environmental conditions as the exposed animals. These animals were tested before sham noise exposure.

d. Sham post-exposure: Same animals as the sham unexposed animals, but tested after sham noise exposure.

3.1.1 Physiological assessment of hearing sensitivity

Peripheral sensitivity of normal hearing-chinchillas was evaluated by analyzing ABRs. Figure 3.1 shows individual ABR thresholds of nine chinchillas as a function frequency measured before noise exposure. ABR thresholds were more

variable across animals at higher frequencies, 2 kHz and 4 kHz, than at lower stimulus frequencies, and thresholds were consistent with previously reported on normal hearing chinchilla. Overall, physiological thresholds at 2 kHz and 4 kHz showed a wider range than 0.5 kHz or 1 kHz. On the average, thresholds fall within a range ~10 dB (0.5 kHz), ~8 dB (1 kHz), ~12 dB (2 kHz), and ~18 dB (4 kHz). Table 3.1 summarizes group mean of ABR thresholds measured at four frequencies (test group, n=7; sham group, n=2) before noise exposure.
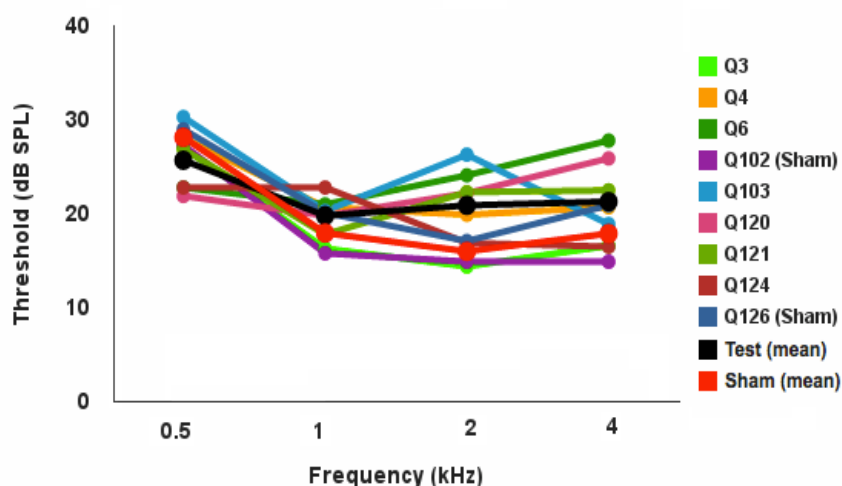
Figure 3. 1 Physiological thresholds of chinchillas measured before noise exposure (test group, n=7 and sham group, n=2).

| | Frequencies (kHz) | | | | | | | | | | | |
| | 0.5 | | | 1.0 | | | 2.0 | | | 4.0 | | |
Groups	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM
Test (n=7)	25.7	3.2	1.2	19.8	2.2	0.8	20.9	4.1	1.6	21.3	4.4	1.7
Sham (n=2)	28.2	1.2	0.9	18.0	3.0	2.2	16.0	1.6	1.1	17.9	4.2	3.0

Table 3. 1 Positive reinforcement-food reward system used in a method of constant stimuli.

3.1.2 Effects of moderate-level noise exposure on ABRs thresholds

The effects of noise exposure were evaluated on ABR thresholds two week after noise exposure. Figure 3.2 depicts the ABR thresholds of chinchillas before and after acoustic trauma (n=9 and n=7 correspondingly). Individual analysis of ABR thresholds pre-and post exposure showed significant overlap. For within-animal comparisons, on average, there were not perceivable ABR threshold changes before and after noise exposure as depicted in Figure 3.2. ABR thresholds of sham animals (unexposed animals, n=2) evaluated before noise exposure were averaged and included within the group mean of unexposed animals (n=9).

Individual threshold shifts were slightly lower in some animals after noise exposure as shown on Figure 3.2 and Table 3.2, but overall, no mean group threshold shift was observed as indicated on Figure 3.3, mean group ABR thresholds shifts as a function of frequency. Table 3.3 summarizes the group mean ABR thresholds for unexposed, exposed and sham animals (STD, and SEM +/-). Change between individual ABR thresholds was minimum on the sham animals when evaluated after noise exposure, but no pronounced group mean ABR threshold changes were observed (see Figure 3.2 and Table 3.2).
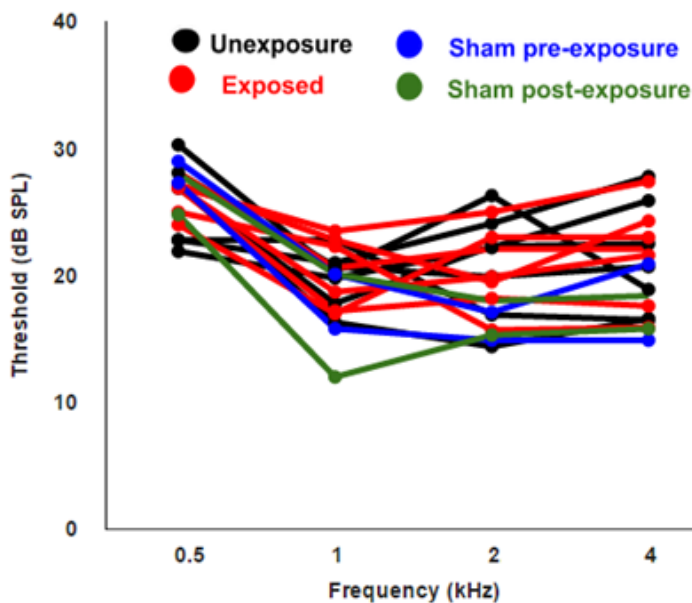
Figure 3. 2 Individual ABR thresholds pre-and-post noise exposure (unexpected, n=9; exposed, n=7; sham pre-noise exposure, n=2; sham post-noise exposure, n=2).

Frequency (kHz)	Q3 pre	Q3 post	Q4 pre	Q4 post	Q6 pre	Q6 post	Q103 pre	Q103 post	Q120 pre	Q120 post	Q121 pre	Q121 post	Q124 pre	Q124 post	Q102 pre	Q102 post	Q126 pre	Q126 post
0.5	27	27	28	27	23	27	30	28	22	24	27	27	23	25	27	25	29	28
1.0	16	23	21	17	21	19	20	21	20	17	18	24	23	22	16	12	20	20
2.0	14	20	20	18	24	20	26	22	22	23	22	25	17	16	15	15	17	18
4.0	17	24	21	18	28	22	19	22	26	23	23	27	17	16	15	16	21	18

Table 3. 2 Individual ABR thresholds for test and sham animals measured pre-and post noise exposure.

	0.5 Mean	0.5 STD	0.5 SEM	1.0 Mean	1.0 STD	1.0 SEM	2.0 Mean	2.0 STD	2.0 SEM	4.0 Mean	4.0 STD	4.0 SEM
Unexposed (n=9)	25.7	3.2	1.2	19.8	2.2	0.8	20.9	4.1	1.6	21.3	4.4	1.7
Exposed (n=7)	26.5	1.4	0.5	20.3	2.7	1.0	20.5	3.1	1.2	21.7	3.9	1.5
Sham post-exposure (n=2)	27.3	1.8	14.1	17.0	3.9	9.0	16.3	1.5	8.0	17.5	2.7	9.0

Table 3. 3 Comparison of group mean ABR thresholds for unexposed, exposed, and sham groups.
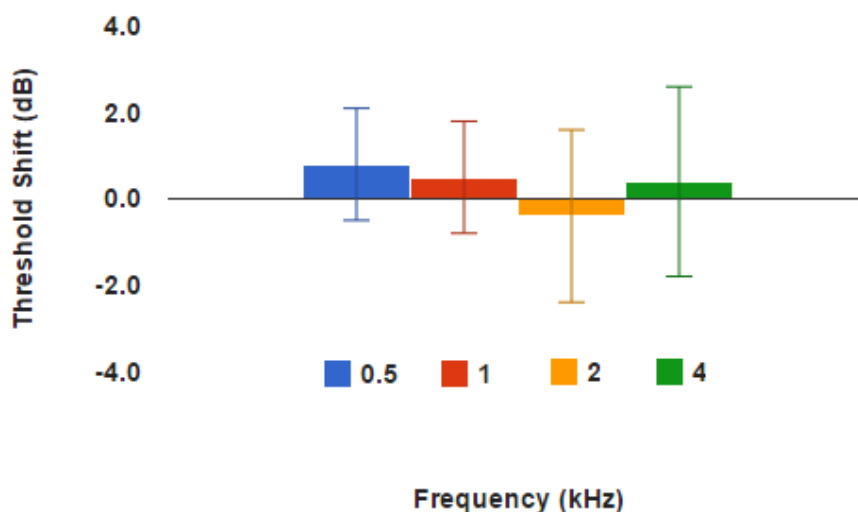
Figure 3. 3 Group mean ABR threshold shift of test animals after noise exposure (n=7).

3.1.3 Effects of moderate-level noise exposure on ABR wave 1

and later wave amplitudes

ABR wave 1 supra-threshold amplitudes were determined by measuring

the distance between the crest and bottom of the trough. The amplitude was

calculated as the mean amplitude across stimulus levels of 60 and 70 dB SPL.

Fig. 3.4 shows ABR wave 1 amplitude (μV) as a function of frequency measured

before and after noise exposure. Moderate-level noise exposure generally results

in a decrease in ABR wave 1 amplitude, yet no noise-induce change in amplitude

was observed in this study.

Table 3.4 summarizes the individual ABR wave 1 amplitude for both the

test and sham animals measured pre-and post noise exposure. Individual ABR

wave 1 amplitudes measured for both test and sham animals showed significant

overlap across frequencies and tended to be more variable at 1 kHz as depicted on Figure 3.4.

Further, we calculated the group mean normalized ABR wave 1 amplitude ratio by dividing the group mean post-exposure amplitude by group mean pre-exposure amplitude. On average, we did not observed change in the group mean ABR wave 1 amplitude on both the exposed and sham post-exposed animals across frequencies. However, as indicated on Figure 3.5, the normalized amplitude of ABR wave 1 at 4 kHz is slightly reduced after noise exposure in comparison to lower frequencies.
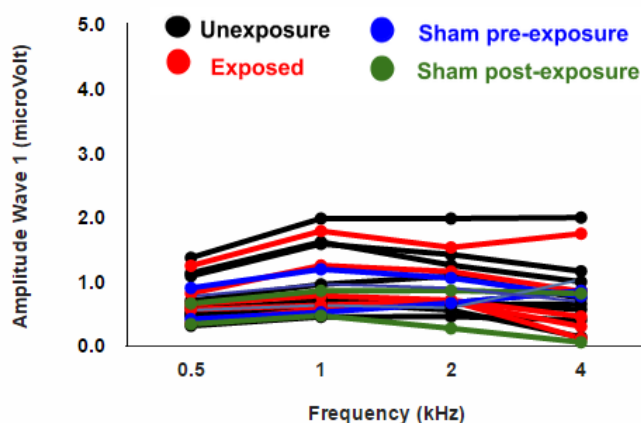
Figure 3. 4 Individual ABR wave 1 amplitude responses as a function of frequency pre-and post noise exposure.

Frequency (kHz)	Q3		Q4		Q6		Q103		Q120		Q121		Q124		Q102 (sham)		Q126 (sham)	
	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post
0.5	0.5	0.5	1.1	1.1	0.5	0.7	0.3	0.4	0.6	0.6	0.7	0.8	1.4	1.3	0.4	0.6	0.9	0.8
1.0	0.7	0.6	1.6	1.6	0.7	0.8	0.5	0.6	0.7	0.6	1.0	1.3	2.0	1.8	0.5	0.6	1.2	1.0
2.0	0.7	0.6	1.4	1.3	0.7	0.7	0.5	0.7	0.6	0.7	1.1	1.2	2.0	1.5	0.7	0.6	1.1	0.9
4.0	0.6	0.7	1.2	1.0	0.9	0.5	0.4	0.3	0.1	0.1	0.7	0.9	2.0	1.8	0.9	1.0	0.8	0.7

Table 3. 4 Individual ABR wave 1 amplitude for unexposed, exposed, and sham animals.

Groups	Frequencies (kHz)											
	0.5			1.0			2.0			4.0		
	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM
Unexposed (n=7)	0.7	0.4	0.1	1.0	0.6	0.2	1.0	0.6	0.2	0.8	0.6	0.2
Exposed (n=7)	0.8	0.3	0.1	1.0	0.5	0.2	1.0	0.3	0.1	0.7	0.5	0.2
Sham pre-exposure (n=2)	0.7	0.3	0.2	0.9	0.2	0.3	0.9	0.3	0.2	0.8	0.1	0.0
Sham post-exposure (n=2)	0.7	0.2	0.1	0.8	0.2	0.2	0.8	0.2	0.2	0.9	0.2	0.2

Table 3. 5 Group mean ABR wave 1 amplitude for unexposed, exposed, and sham groups.
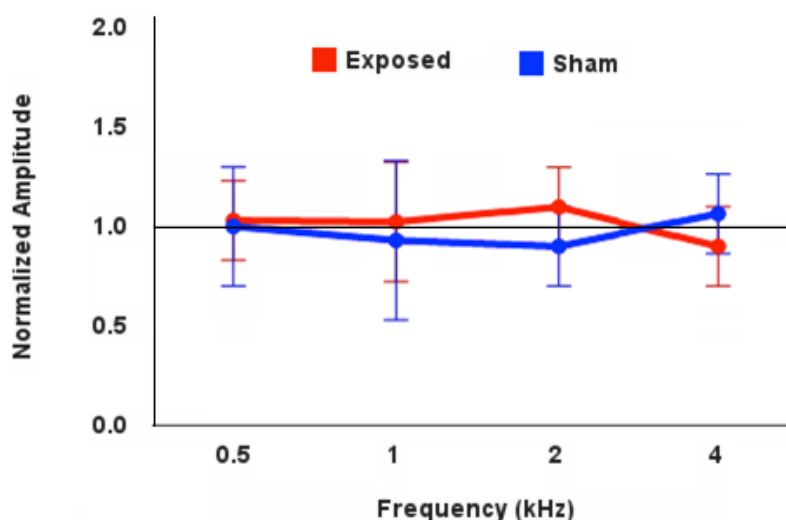
Figure 3. 5 Group mean normalized amplitude of ABR wave 1 (Normalized = the average ratio between post-and pre exposure amplitude responses).

The effects of noise exposure on later evoked potentials was further analyzed. For most mammalian species, it is commonly accepted that neural activity in the auditory brainstem and midbrain correspond to ABR wave 4/5 responses (Alvarado et al., 2012). Pre-and post ABR wave 4/5 amplitudes were analyzed on both test and sham animals, and individual ABR amplitudes show significant overlap pre-and post noise exposure as indicated in Figure 3.6.

This trend was observed on both test and sham animals. Table 3.6 summarizes the group mean ABR wave 5 amplitude for both the test and sham

animals. Figure 3.7 illustrates the normalized amplitude of ABR waves 4/5 for exposed and sham animals (Normalized=ABR wave 5 amplitude post-exposure/ABR wave 5 amplitude pre-exposure).

Analysis of normalized group mean ABR wave '4/5' amplitude depicts no group mean difference on neither the exposed group nor the sham group. Figure 3.7 suggests that normalized ABR wave 5 amplitudes of exposed animals seems to be greater at 2 and 4 kHz than sham animals, but no significant group mean difference was observed. Further, we then calculated the ratio for group mean ABR wave 1 (E=early) amplitude and group mean ABR wave '4/5' amplitude (L=late) pre-and post noise exposure correspondingly (ELpre=wave 1/ wave '4/5'; ELpost=wave 1/wave '4/5') to determine any effect of noise exposure on later evoked response. These two ratios were then compared to determine any effect of noise exposure on later ABR wave responses, expressed as "ELratio" (ELratio=ELpost/ELpre). Fig. 3.8 suggests that the ABR wave '4/5' amplitude may be reduced at 4 kHz after acoustic trauma, but no trend was observed for lower frequencies 0.5, 1, and 2 kHz. However, similar trend was observed in sham animals.
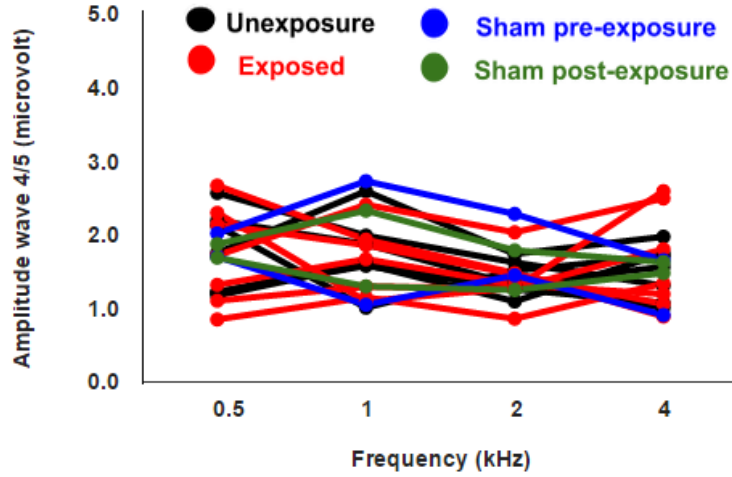
Figure 3. 6 Individual ABR wave 4/5 amplitude responses as a function of frequency for unexposed, exposed, and sham groups pre-and post noise exposure.

	Frequencies (kHz)											
Groups	0.5			1.0			2.0			4.0		
	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM
Unexposed (n=7)	1.8	0.6	0.2	1.8	0.5	0.2	1.4	0.2	0.1	1.5	0.4	0.1
Exposed (n=7)	1.8	0.7	0.3	1.6	0.5	0.2	1.4	0.4	0.1	1.6	0.7	0.3
Sham pre-exposure (n=2)	1.9	0.2	0.2	1.9	1.2	0.8	1.9	0.6	0.4	1.6	0.5	0.4
Sham post-exposure (n=2)	1.8	0.1	0.1	1.8	0.1	0.5	1.5	0.4	0.3	1.6	0.1	0.1

Table 3. 6 Group mean ABR wave '4/5' amplitude for unexposed, exposed, and sham group.
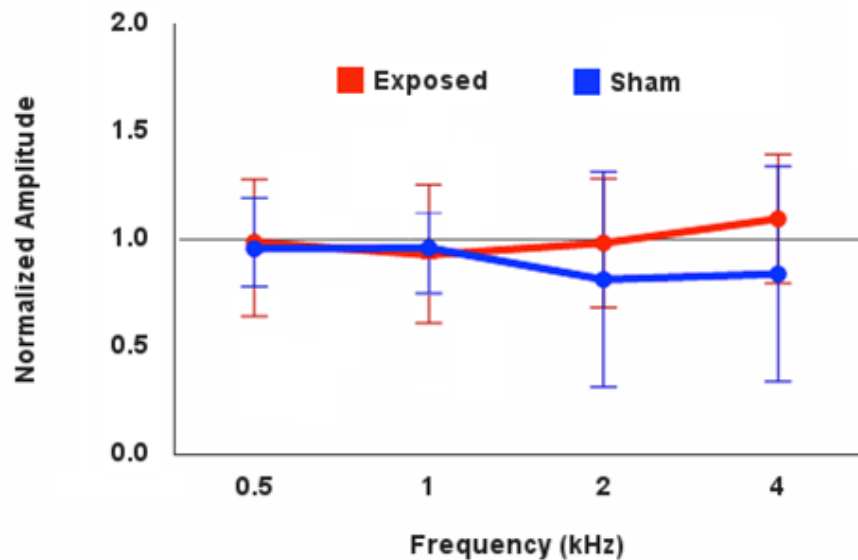
Figure 3. 7 Group mean normalized ABR wave '4/5' amplitude before and after noise exposure calculated for both test group (exposed animals) and sham group (unexposed animals). Normalized=the average ration between post-and pre exposure amplitude responses.
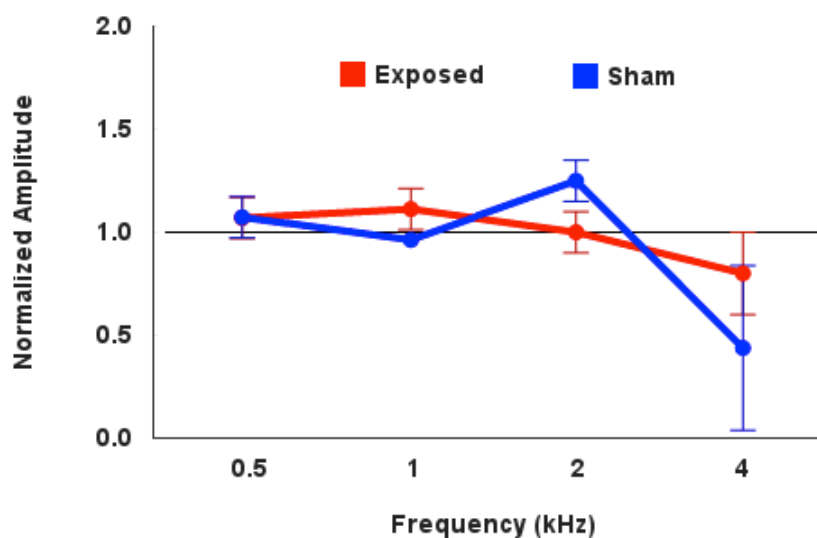
Figure 3. 8 Average ratio between post-ABR wave 1 and wave 4/5 amplitude and average ratio between pre-ABR wave 1 and ABR wave 4/5 amplitude responses.

3.2. Evaluation of animal's sensitivity in the detection of a tone in quiet measure pre-noise exposure

Behavioral thresholds of normal hearing chinchillas were evaluated with a tone-in-quiet behavioral task before noise exposure to determine its sensitivity to a pure tone and generate a chinchilla's audiogram. Behavioral sensitivity was also examined in the same animals after moderate-level noise exposure.

3.2.1 Behavioral thresholds of tone-in-quiet (TIQ) pre-noise exposure

Data analysis was based on a minimum of 10 blocks (about 30 repetitions of each stimulus) at each frequency. Psychometric functions were generated to determine behavioral thresholds by converting 'hit' rate, at each stimulus level, into d-prime values. Behavioral thresholds were determined by selecting sound level (dB SPL) corresponding to d-prime=1. Fig. 3.9 shows both individual (color) and group mean (black) psychometric functions for a tone-in-quiet behavioral task pre-noise exposure. Overall, individual behavioral thresholds fall within a range of ~30-40 dB across frequencies.

Group mean audiometric thresholds of test animals measured in a tone-in-quiet behavioral task before noise exposure (n=12) are depicted on Figure 3.10. Group mean ABR thresholds were similar across all experimental frequencies, but slightly improved at 4 kHz, see Figure 3.10. Behavioral thresholds seem to be consistent with thresholds previously reported chinchilla audiograms. However, absolutes thresholds were about 10-20 dB greater than previously reported (Heffner and Heffner, 2007; Lobarinas et al, 2013). Figure 3.10 depicts similar

trend on group mean thresholds of sham pre-exposure animals (n=2), but

behavioral thresholds were ~10-15 dB higher than unexposed animal.
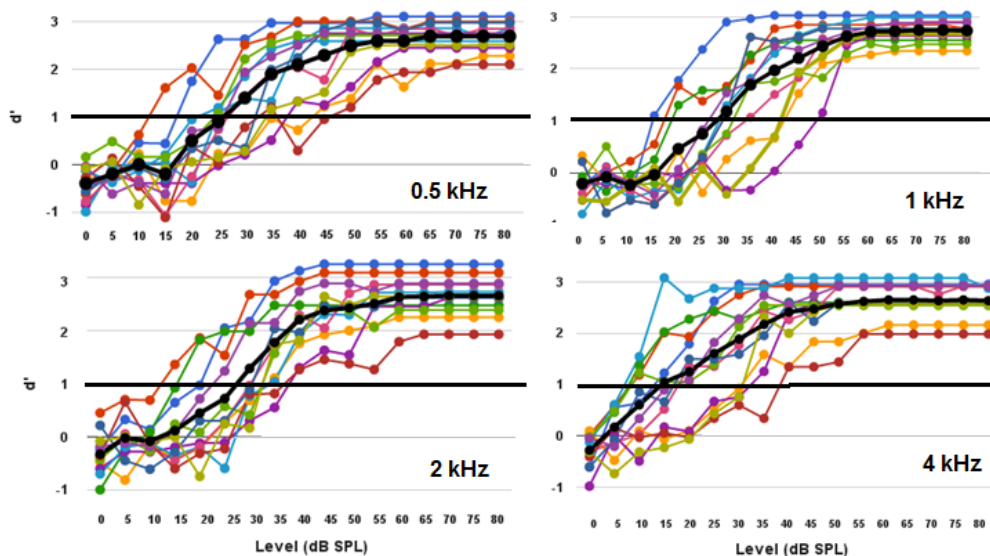
Figure 3. 9 Individual behavioral thresholds (color) and group mean (black) measured in a tone-in-quiet behavioral task for four frequencies pre-noise exposure (n=12).
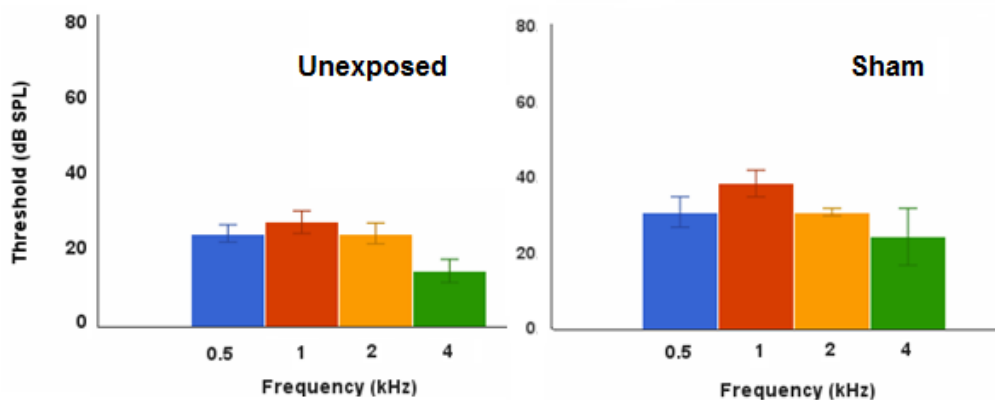
Figure 3. 10 Group mean audiometric thresholds of unexposed (n=12; left) and sham pre-exposure (n=2; right) animals measured in a tone-in-quiet behavioral task pre-noise exposure.

3.2.2 Effects of noise exposure on behavioral thresholds for a tone-in-quiet behavioral task

Behavioral thresholds were also evaluated, within animal, with a tone-in-quiet behavioral task post-noise exposure. Figures 3.11 depicts individual (color) and group (black) mean psychometric functions for exposed animals (n=7). Behavioral thresholds post-noise exposure showed similar trend that the behavioral thresholds pre-noise exposure.

Overall, individual behavioral thresholds fall within a range of ~25-30 dB across frequencies as illustrated on Figure 3.12. We only observed a small threshold shift (~5 dB) after noise exposure at 4 kHz in the exposed animals, while thresholds improved between 5-10 dB in the sham post-exposure animals after noise exposure as illustrated on Figure 3.13. Table 3.7 summarizes the group mean behavioral threshold measures in tone-in-quiet task pre-and-post noise exposure for unexposed, exposed, sham animals.
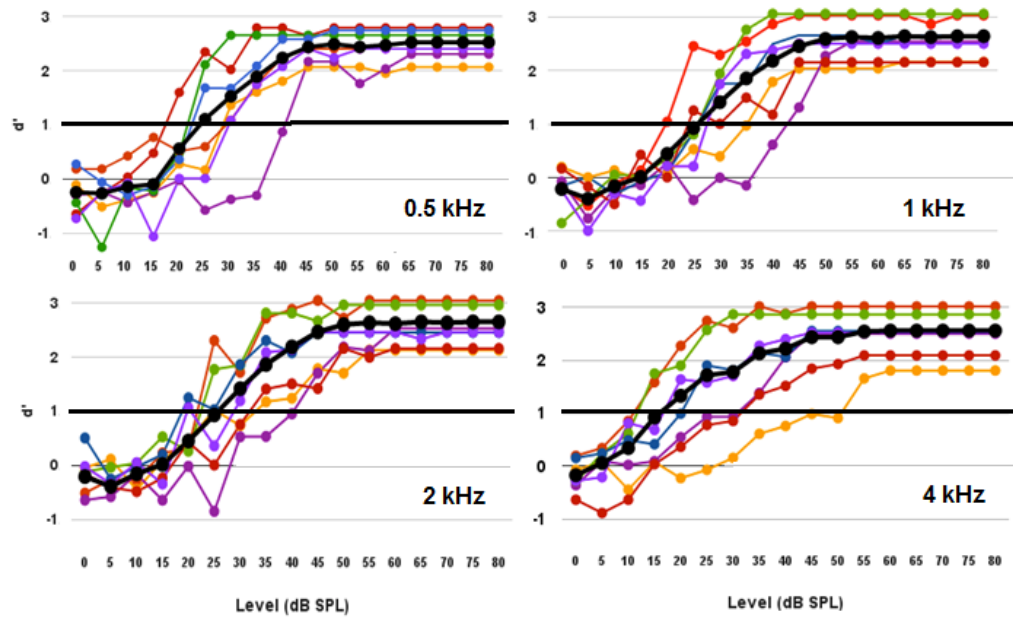
Figure 3. 11 Individual behavioral thresholds measured in a tone-in-quiet behavioral task post-noise exposure (n=7).

Figure 3. 12 Group mean audiometric thresholds (dB SPL) of exposed animals (n=7) and sham post-exposure (n=2) animals measured in a tone-in-quiet behavioral task.

Figure 3. 13 Threshold shift between group mean pre vs post exposure for exposed and sham post exposed animals.

| Groups | Frequencies (kHz) | | | | | | | | | | | |
| | 0.5 | | | 1.0 | | | 2.0 | | | 4.0 | | |
	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM
Unexposed (n=12)	27.9	7.5	2.2	30.0	10.0	3.0	29.1	9.2	2.8	20.9	10.2	3.1
Exposed (n=7)	25.7	8.4	3.2	28.6	9.0	3.4	27.1	7.0	2.6	24.4	11.7	4.4
Sham pre-exposure (n=2)	31.0	5.7	4.0	38.5	4.9	3.5	31.0	1.4	1.0	24.5	10.6	7.5
Sham post-exposure (n=2)	22.5	3.5	2.5	26.0	8.4	6.0	27.5	3.5	2.5	19.5	10.6	7.5

Table 3. 7 Group mean behavioral thresholds (dB SPL) for unexposed, exposed, and sham groups measured in a tone-in-quiet behavioral task after noise exposure.

3.3 EXPERIMENT 1: Behavioral Task of Tone-detection in Noise (Aim 1)

Behavioral thresholds were measured in a tone-in-noise behavioral task pre-noise exposure to determine the effects of noise on tone detection. Animals tested in the quiet condition (see section 3.2.1) were also evaluated in a tone-in-noise pre-and-post noise exposure to determine the animals' sensitivity in the detection of tone in noise pre-and post acoustic trauma.

3.3.1 Behavioral thresholds of tone-in-noise pre-noise exposure

Behavioral thresholds of tone-in-noise pre-noise exposure were determined at the same frequencies as for the tone-in-quiet task (0.5, 2, 4 kHz). However, behavioral thresholds were not evaluated at 1 kHz (band noise exposure) as we suspected some permanent threshold shift. Figures 3.14 shows the individual (color) and group mean (black) psychometric functions of test animals generated in a tone-in-noise behavioral task pre-noise exposure. As expected, behavioral thresholds increased in the presence of noise and were less variable across animals. As illustrated in Figure 3.14, both the individual thresholds increased in the presence of noise in comparison to individual thresholds measured in tone-in-quiet. Group mean thresholds were increased ~20-40 dB relative to tone-in-quiet pre-exposure as depicted on Figure 3.15 (see 3.12 for threshold in tone-in-quiet).
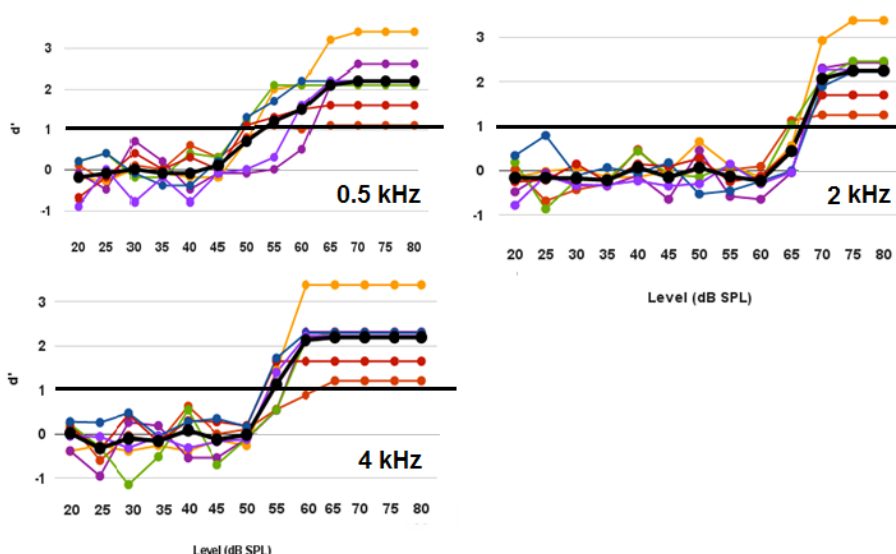
Figure 3. 14 Individual (color) and group mean (black) behavioral thresholds for a tone-in-noise behavioral task measure pre-noise exposure at 0.5 kHz (n=7) and 2 kHz (n=7), and 4 kHz (n=7).
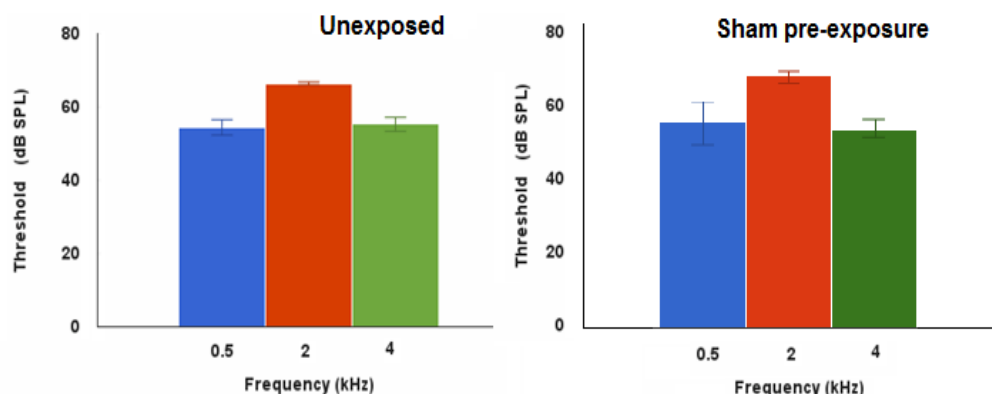
Figure 3. 15 Group mean thresholds (dB SPL) for unexposed (n=7) and sham pre-exposure (n=2) animals measured in tone-in-noise pre-noise exposure.

3.3.2 Effects of noise exposure on behavioral thresholds of a tone-in-noise behavioral task post-noise exposure

Behavioral thresholds were measured in a tone-in-noise behavioral task post-noise exposure to determine the effects of moderate-level noise exposure on tone detection in noise. Figures 3.16 illustrates individual and group mean psychometric functions for tone-in-noise after noise exposure. Figure 3.17 shows elevated group mean thresholds of a tone-in-noise task measured after noise exposure, but similar to unexposed animals tested in a tone-in-noise task, there was no group mean shift in noise masked thresholds post-noise exposure relative to tone-in-noise pre-exposure as depict on Figure 3.18. This trend was observed on both the test and sham animals. However, analysis of within-animal thresholds post- vs pre-exposure showed changes in individual threshold that were covered up by group mean metrics. Table 3.8 summarizes the behavioral thresholds for tone-in-noise.
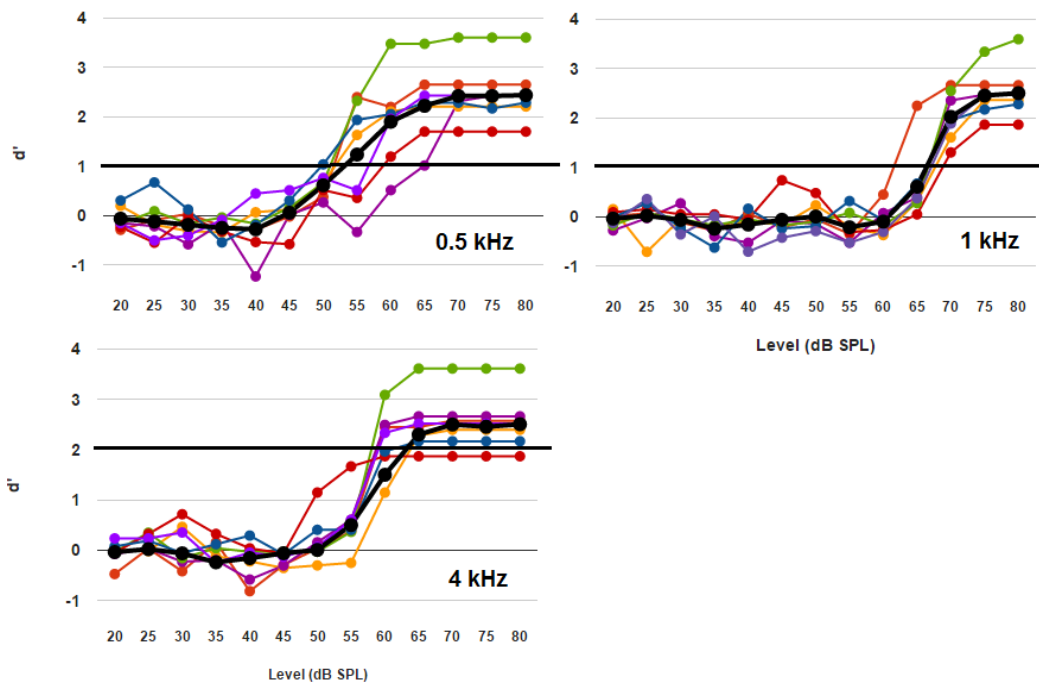
Figure 3. 16 Individual (color) and group mean (black) behavioral thresholds measured in a tone-in-noise post-noise exposure at 0.5 kHz (n=7), 2 kHz (n=7), and 4 kHz (n=7).
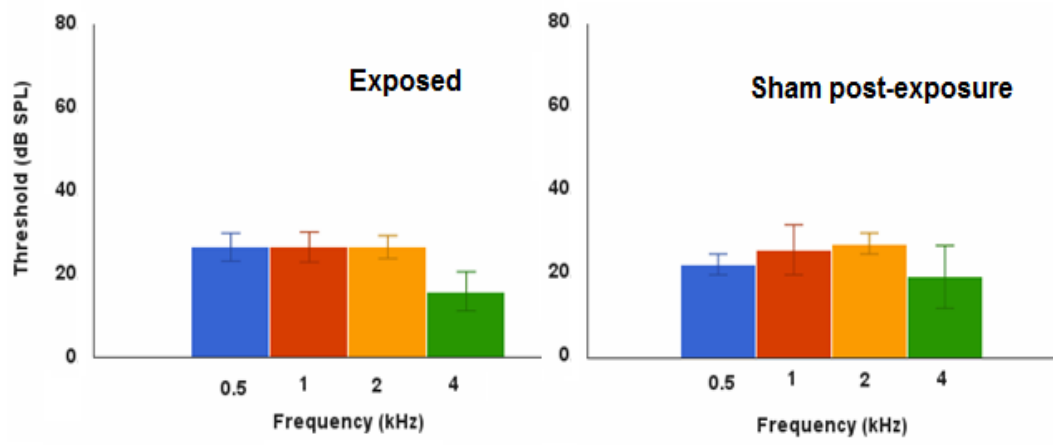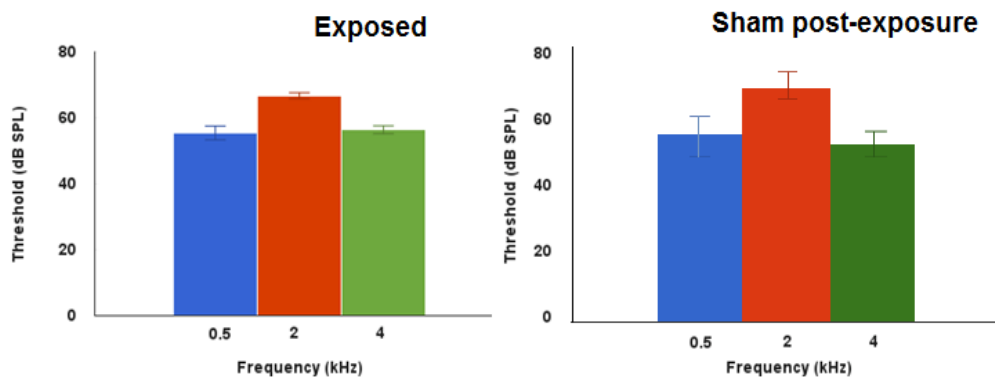

Figure 3. 17 Group mean thresholds (dB SPL) of exposed (n=7) and sham post-exposure (n=2) animals measured in a tone-in-noise behavioral task post-noise exposure.
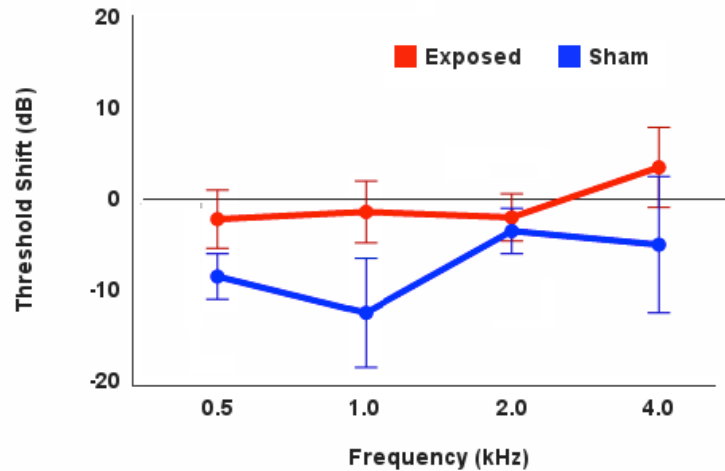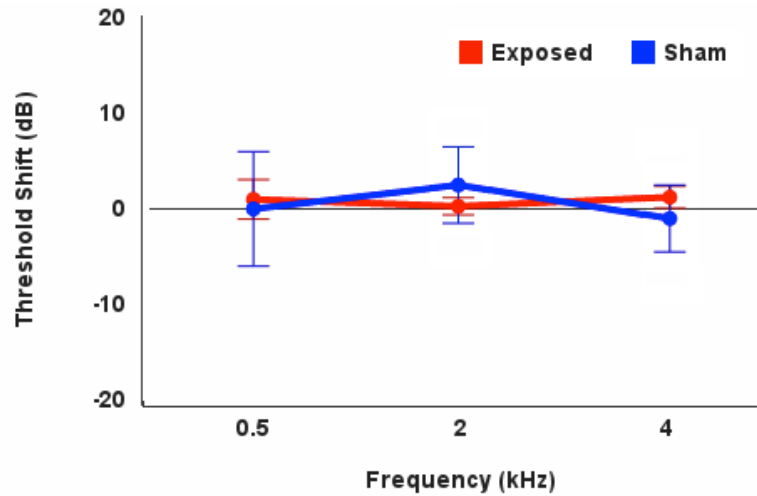
Figure 3. 18 Group mean threshold shift for exposed and sham post-exposure animals measured in a tone-in-noise behavioral task after noise exposure.

| Groups | Frequencies (kHz) | | | | | | | | |
| | 0.5 | | | 2.0 | | | 4.0 | | |
	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM
Unexposed (n=7)	54.4	5.5	2.1	66.4	1.0	0.4	55.3	4.9	1.9
Exposed (n=7)	55.4	5.5	2.1	66.7	2.4	0.9	56.4	3.1	1.2
Sham pre-exposure (n=2)	56.0	8.5	6.0	68.5	2.1	1.5	54.5	3.5	2.5
Sham post-exposure (n=2)	56.0	8.5	6.0	71.0	5.7	4.0	53.5	4.9	3.5

Table 3. 8 Group mean behavioral thresholds for unexposed, exposed, and sham groups measured in a tone-in-noise behavioral task.

3.4. EXPERIMENT 2: Behavioral Task of AM Depth in SAM-Tone Signals
(Aim 2)

We studied the effects of noise exposure on the ability of chinchillas to detect sinusoidal amplitude modulation (SAM) on tone carriers in a behavioral task.

3.4.1 Behavioral thresholds for AM depth detection in SAM-tone signals pre-exposure.

Behavioral thresholds were measured in an AM depth in SAM-tone signals before moderate-level sound exposure. For this behavioral task, from three animals were trained to discriminate a SAM tone from a pure tone embedded in a notch noise. Successfully trained animals were tested with various AM depths to determine the animals' thresholds in the detection of amplitude modulated signals (SAM 4000 kHz carrier, 20 Hz modulator).

Psychometric functions were generated to identify the modulation-depth threshold for detection. Figures from 3.19 illustrates individual (color) and group mean psychometric functions (black) generated in an AM detection task using SAM-tone signals pre-exposure. As expected, d-primes were highest for more modulated signals (less negative dB values, i.e., to the right, closer to 0 dB) as shown on Figure 3.19.

The individual modulation depth thresholds (depicted in color) varied by animal across frequencies as illustrated on Figure 3.19. Group mean AM detection thresholds fall within -5 and -12 dB across frequency and are considered to be within the range previously reported in mammals (Carney et al.,

2013). However, thresholds are slightly higher than those previously reported in chinchillas when noise carriers were used (Henderson et al., 1984). Fig 3.20 depicts group mean thresholds measured in a AM depth in SAM-tone signals (4 kHz) pre-noise exposure.
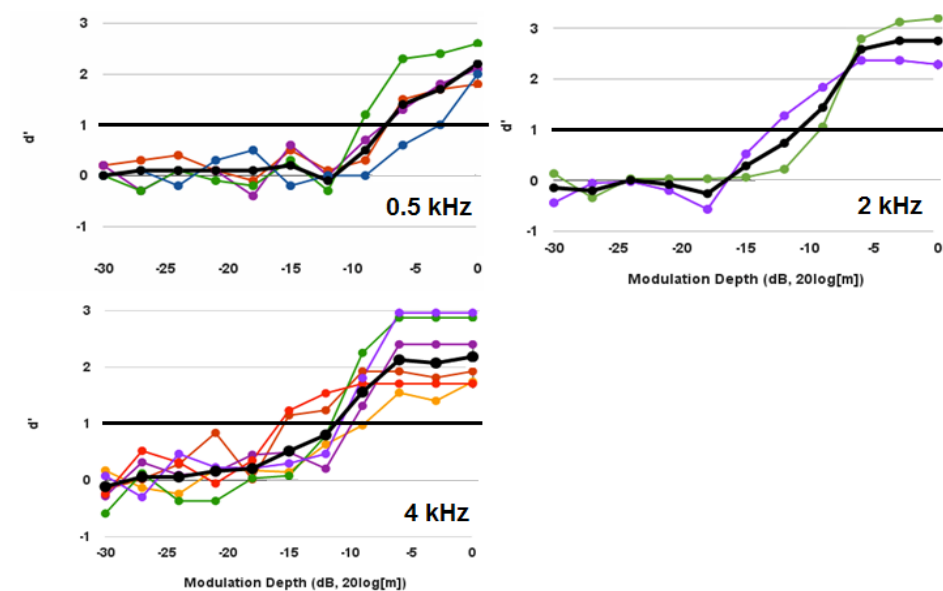
Figure 3. 19 Individual (color) and group mean (color) behavioral thresholds of AM depth measured with SAM-tone signals at 0.5 kHz (n=4), 2 kHz (n=3), and 4 kHz (n=6) pre-noise exposure.
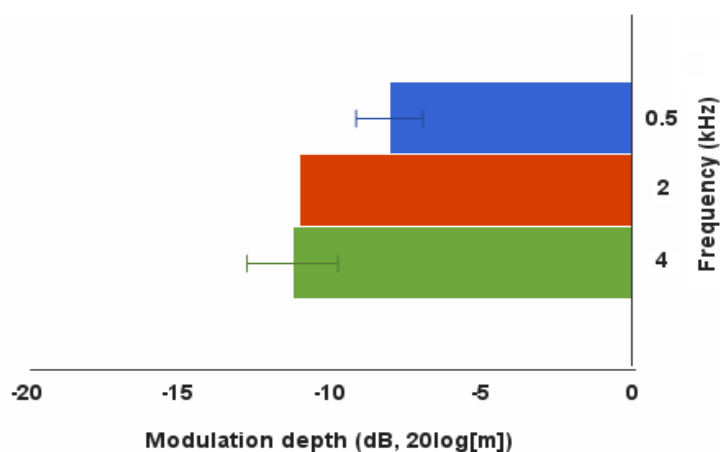
Figure 3. 20 Group mean thresholds for AM depth detection in SAM-tone signals at 0.5 kHz, 2 kHz, and 4 kHz pre-noise exposure.

3.4.2 Effects of noise exposure on behavioral thresholds for AM depth detection measured in SAM-tone signals post-exposure.

Behavioral thresholds for AM depth detection were determined by using SAM-tone signals after moderate-level sound exposure. Figure 3.21 illustrates individual (color) and group mean (black) psychometric functions generated for AM depth detection by using SAM-tone signals measured pre-noise exposure. Group mean behavioral thresholds for 0.5 and 2 kHz were lower after noise exposure relative to pre-exposure thresholds. Figure 3.22 illustrates thresholds after noise exposure for 0.5, 2, and 4 kHz.

Behavioral group mean threshold shifts were slightly higher at 0.5 kHz, but there was not significant different at 2 and 4 kHz as illustrated on Figure 3.23. Table 3.9 summarizes group mean changes in behavioral thresholds in the detection of AM depth in SAM-tone signals behavioral task pre-and post exposure to moderate sound level. A t-test analysis for repeated measurements was performed on each frequency, 0.5, 2, and 4 kHz.

Group mean threshold for AM depth detection at 0.5 kHz pre vs post exposure were not statistical significant. Behavioral thresholds of AM detection unexposed animals were not significantly different ($M = $ -6.67. $SE = 2.02$) than group thresholds of exposed animals ($M = $ -8.67, $SE = $ 1.2), $t(2) = 0.622$, $p = $ 0.59. Similar trend was observed at 2 kHz in which pre-exposure thresholds ($M = $ -11.50, $SE = 2.5$) were not significantly different from un exposed thresholds ($M = $ -12.50, $SE = 1.50$), $t(1) = 1.0$, $p = 0.50$. On average, behavioral thresholds at 4

kHz were not significantly different for unexposed thresholds (M = -10.5, SE =

2.12) than exposed thresholds (M = -12.5, SE = 0.92), $t(5)$ = 0.9, p = 0.409.
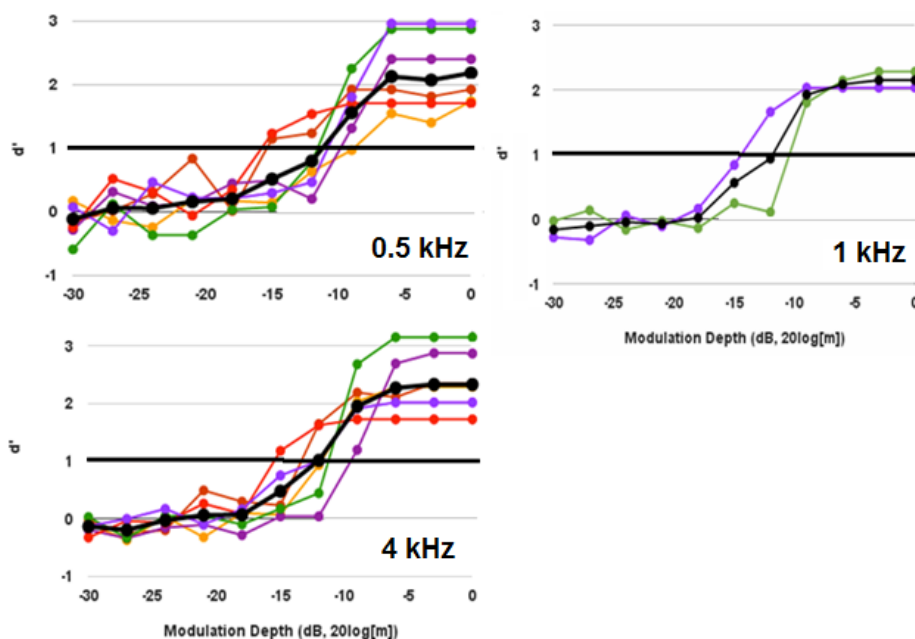
Figure 3. 21 Individual behavioral thresholds measured in an AM depth detection in SAM-tone signals measured at 0.5 kHz (n=6) , 2 kHz (n=2), and 4 kHz (n=6) post-noise exposure. Due to time constraints, two animals were only tested at 2 kHz.
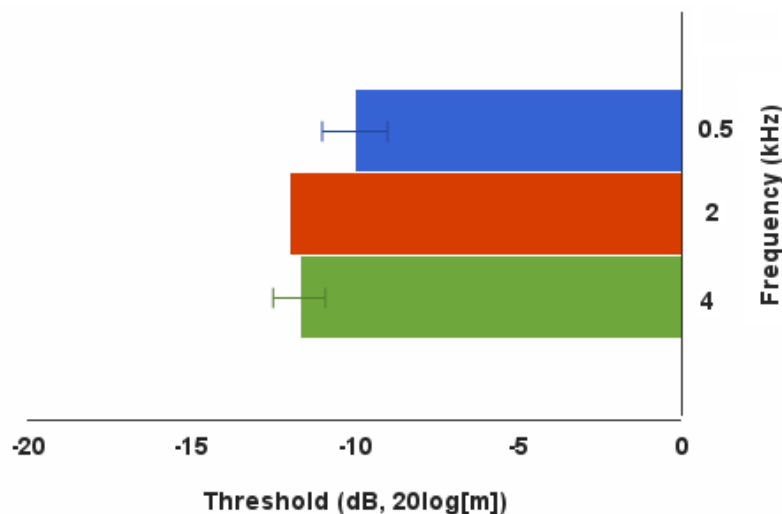
Figure 3. 22 Group mean behavioral thresholds for AM depth detection measured in SAM-tone signals at 0.5 kHz (n=6), 2 kHz (n=2), and 4 kHz (n=6) post-noise exposure. Due to time constraints, two animals were only tested at 2 kHz.
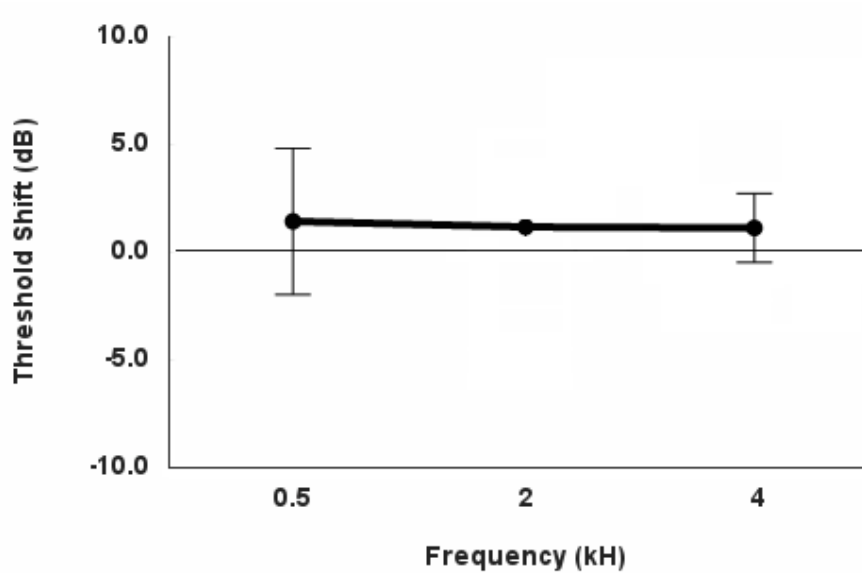
Figure 3. 23 Behavioral thresholds shift for AM depth detection in the SAM-tone signal behavioral task evaluated between pre vs post thresholds for moderate sound level exposed animals.

Groups		Frequency (kHz)										
		0.5				2				4		
		Mean	STD	SEM		Mean	STD	SEM		Mean	STD	SEM
Pre-Exposure	n=4	-8.0	2.2	1.1	n=2	-11.0	1.4		n=6	-11.2	2.8	1.1
Post-Exposure	n=6	-10.8	2.3	1.0	n=2	-12.0	2.8		n=6	-11.7	2.1	0.8

Table 3. 9 Group mean behavioral thresholds for AM depth detection in SAM-tone signals behavioral task (pre-and post-noise exposure).

CHAPTER 4. DISCUSSION

4.1 Effects of moderate noise exposure on ABR characteristics and perception

In the past, it was believed that noise-induced hearing loss simply resulted in direct damage to cochlear hair cells, and that cochlear-nerve fibers were lost after the degeneration of cochlear synapses. Recent animal work in mice and guinea pigs has challenged this view. Noise-induced hearing loss in animals produced loss of ~50% of the cochlear nerve/hair cell synapses and reversible ABR threshold (Kujawa and Liberman, 2009). This evidence raises new concerns about occult damage to the cochlea, known as cochlear synaptopathy, which is not detected by standard clinical methods.

In this study, we aimed to develop a cochlear synaptopathy chinchilla model by exposing animals to moderate-level noise, and we corroborated the effect of noise exposure on the auditory neural coding and perception by applying physiological and psychophysical measurements. Results demonstrated that ABR thresholds of normal hearing animals were consistent across frequencies with previously reported data from normal hearing chinchillas (Henry et al., 2011). Moderate level noise exposure in awake chinchillas, as a group average, did not result in permanent ABR thresholds shift. However, analysis of

individuals showed threshold shifts after noise exposure. This suggests that damage to the cochlea and the effect of noise exposure on auditory evoked potentials may differ from animal to animal. Although, individual ABR wave 1 amplitude pre-and post noise exposure overlapped greatly, on average, group mean ABR wave 1 amplitude at 4 kHz was slightly reduced in response to noise exposure in comparison to lower frequencies. Decrease of ABR wave 1 amplitude may possibly be an indication of cochlear synaptopathy in response to moderate-level noise exposure. Further analysis of ABR wave 4/5 amplitude was completed to analyze the effect of noise exposure on later evoked responses. Group mean normalized ratio of ABR wave 5 amplitudes for exposed animals was greater at 2 and 4 kHz than sham post-exposure animals. Also, we furthered our analyses to evaluate the effect of noise exposure by comparing the ratio of wave 1 and wave 5 pre-and post-noise exposure separately, then these ratios were compared to determine an overall ratio,(E = early "wave 1", L = late "wave 4/5). The 'ELratio' (ELratio = ELpost/ELpre) indicated that ABR wave '4/5' amplitude may be reduced at 4 kHz after acoustic trauma, but no trend was observed for lower frequencies 0.5, 1, and 2 kHz. However, a similar trend was observed in sham animals, which suggests that less damage to cochlea synapses occurred than expected based on our pilot data from naïve exposed chinchillas.

Although, group mean ABR thresholds measured with a tone-in-quiet behavioral task before noise exposure were similar across frequencies, yet improved at 4 kHz, results indicated that individual thresholds showed no

consistent trend, but varied from animal to animal across frequency. Hearing

sensitivity of chinchillas was determined before and after noise exposure with a

TIQ behavioral task. Results indicated that absolute TIQ thresholds were ~10-20

dB higher than previously reported (Heffner and Heffner, 2007; Lobarinas et al.,

2013; Henderson et al., 1969).

ABRs thresholds measured in the inferior colliculus of the normal hearing

chinchillas were ~ 20 dB less sensitive than behavioral thresholds (Henderson et

al., 1969). In our study, although we evaluated peripheral evoked potentials,

rather than more central responses as measured in the inferior colliculus, we

found that behavioral thresholds were less sensitive than physiological

thresholds as illustrated on Figure 3.24. Our behavioral thresholds were similar at

0.5 and 4 kHz, but ~10 dB higher at 2 kHz whereas ABRs were in about the

same range as those previously reported (Henderson et al., 1969). It is possible

that the discrepancy between our ABR and behavioral thresholds may be due to

different noise levels being present during recordings and during behavior (which

was measured in a pilot quiet environment, but which now has a sound-

attenuating booth for future work in our lab).
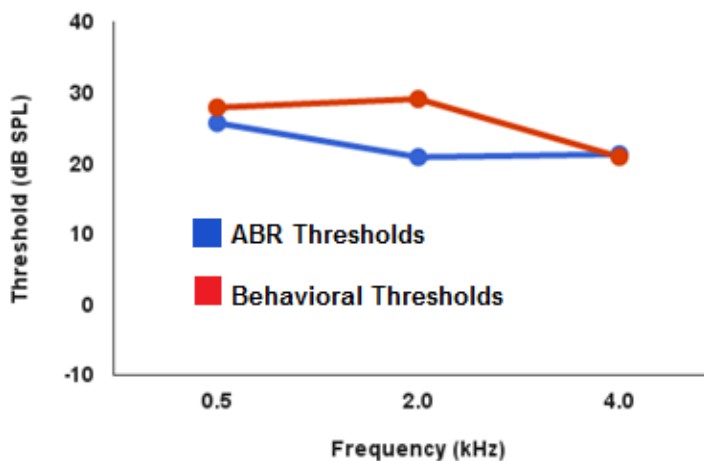
Figure 3. 24 Group means of behavioral threshold pre-exposure (positive reinforcement-food reward technique) vs ABR thresholds of pre-exposed animals.

Another possibility may be due to difference in calibration technique and physical characteristics of the operant conditioning. Our increased absolute behavioral thresholds follow a similar trend observed on absolute thresholds previously reported, but at frequencies above 4 kHz, in comparison to behavioral thresholds determined by a shock avoidance.

In this study, one may imply that the high behavioral thresholds determined by positive-reinforcement relative to those determined by conditioned-avoidance (i.e., electric-shock) are a result of differences in reinforcement conditioned behavior. Although the conditioning paradigms are different, a study that compared the auditory thresholds determined by conditioned and unconditioned responses in mammals showed that guinea pigs had worse thresholds at low frequencies (from 0.125-4 kHz) in a suppression/avoidance (training an animal to stop drinking water when it hears a

sound to avoid a shock) than thresholds determined by positive-reinforcement. Thresholds were similar at frequencies above 8 kHz (Lee, 2012).

Hearing sensitivity was also evaluated after noise exposure, and individual behavioral thresholds in TIQ after noise exposure were variable as in TIQ before noise exposure. As expected, on average, group mean TIQ threshold did not differ relative to TIQ thresholds pre-exposure. As expected, behavioral thresholds in a noisy condition measured before acoustic trauma were higher, ~20-40 dB, relative to thresholds in quiet. Increased thresholds in tone-detection in noise indicates a clear masking effect. TIN thresholds pre-and post-noise exposure differed from animal to animal, however, this difference in thresholds was obscured by group mean metrics. Analysis of TIN of pre-and post-noise data indicated that neither the exposed animals nor sham animals showed group mean thresholds shift.

Behavioral thresholds were measured in an AM depth detection task with notched-noise masked SAM-tone signals before and after moderate-level sound exposure. As expected, greater modulation depths were easier to detect. Consequently, d-prime metrics were highest for more modulated signals (less negative dB values), but d-prime was lower for less modulated signals (more negative dB values). Individual modulation depth thresholds varied from animal to animal across frequencies. Group mean thresholds for modulation depth fall within -5 and -12 dB across tested frequencies , which are considered to be within mammalian ranges previously reported (Carney et al., 2013). In this study,

however, thresholds are slightly higher than those previously reported in chinchillas when noise carriers were used (Henderson et al., 1984).

4.2 Limitations

Although the naive animal group used for determining noise exposure levels exhibited a cochlear synaptopathy phenotype following moderate-level sound exposure (98-99 dB SPL), the behavior animal group did not show changes in either ABR characteristics or acoustic sensitivity in the behavioral tasks. A major limitation in this study was to find the appropriate sound level that would successfully produce the desired cochlear synaptopathy phenotype, without permanent threshold shift. Another limitation in this study was the diverse cognitive and learning processing abilities among animals Some animals were fast learners while other were not able to work in trials with longer hold times while other animals required longer time to perform well when challenged with complex signals. One latent problem during the length of the study was the animal's health and temperament. Unexpected health issues and change in temperament resulted in some delayed or incomplete training or testing sessions. Also, differences in the ambient noise level in the ABR test chamber and the operant conditioning chamber may have prevented us from generating data entirely similar to those previously reported in the literature.

4.3 Future Research Work

Despite limitations in reproducing the noise-induced cochlear synaptopathy phenotype in the behavioral animals, this work represents several

significant steps towards our goal to better understand the effects of cochlear

synaptopathy on perception, in the presence of normal audiometric thresholds.

The next step was to re-expose the animals to higher sound levels (100-101 and

104 dB SPL) and re-evaluate changes in perception by reexamining their

auditory sensitivity with the same behavioral tasks. Group mean ABR thresholds

for exposed animals were also similar across all experimental frequencies, but

slightly improved at 4 kHz. A similar trend was observed on group mean ABR

thresholds for sham animals (n=2), but ABR thresholds were ~10-15 dB higher.

CHAPTER 5: SUMMARY

Chinchillas, similar to mice and guinea pigs, can display the cochlear synaptopathy phenotype following moderate-level sound exposure. In this study, naive chinchillas (used to determine an adequate sound level to produce cochlear synaptopathy characteristics) showed recovered evoked potentials, reduced ABR wave 1 amplitude, and significantly reduced synaptic ribbon counts after exposure to moderate-level sound. Hearing sensitivity determined by a TIQ behavioral task on normal hearing chinchillas follow the same trend across frequency, but thresholds were higher than previously reported. As we expected, threshold determined in a TIN behavioral task were higher than threshold measured in quiet.

Behavioral threshold measured in noise after noise exposure did not show threshold shift relative to threshold in noise pre-exposure. As expected, chinchillas were more sensitive at detecting fully modulated SAM-tone signals than less modulated. Individual modulation depth thresholds varied from animal to animal across frequencies, yet group mean modulation depth thresholds fell within mammalian ranges previously reported (Carney et al., 2013). Although we were able to only reproduce cochlear synaptopathy in pilot assays (naïve animals), but not in the behavioral animals, this project aimed to develop an

awake protocol for moderate-level noise exposure, an extension to our lab's previous experience with high-level permanent damage noise exposure.

Also, chinchilla behavioral training and testing protocols on several auditory tasks to identify changes in auditory perception resulting from moderate-level noise exposure was successfully established, a new methodology to our laboratory. As well, future work in the behavioral laboratory will extend the present work to evaluate direct links between cochlear synaptopathy and perceptual deficits (in collaboration with Prof. Chris Plack, who is exploring similar studies in humans as part of the MRC Programme Project grant).

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