ELECTROCOCHLEOGRAPHY AS A DIAGNOSTIC TOOL FOR NOISE-INDUCED COCHLEAR SYNAPTOPATHY

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Abstract

**Purpose:** Recent studies suggest synaptic connections between hair cells and the auditory nerve may be more vulnerable to noise-induced damage than the cochlear hair cells. The resulting neuropathy may be associated with an increased difficulty with speech perception in noise than expected from a normal audiogram. This type of hearing loss has been named “hidden hearing loss”. Studies with animal models indicate that suprathreshold wave I auditory brainstem responses are sensitive to the loss of synaptic ribbons in mice, but humans studies remain inconclusive. This work aims to identify the diagnostic potential of tympanic membrane electrocochleography on noise-induced cochlear synaptopathy.

**Design:** We recruited 18 music students (n = 32; mean age = 21.7) with normal hearing (≤ 25 dB, HL 250-8000 Hz) and 19 normal hearing controls (n = 35; mean age = 22.5). Lifetime noise exposure was obtained using the Noise Exposure Structured Interview. Electrocochleography was measured in response to 95 dB nHL broadband click with tympanic membrane electrodes. A factorial ANOVA was used to investigate the effect of music background and sex on the lifetime noise exposure. Mixed model ANOVA was used to analyze effect of noise exposure, sex, and ear on absolute SP and AP amplitudes, SP/AP amplitude ratio, and SP/AP area ratio.

**Results:** There was a trend for higher lifetime noise exposure scores for the music students but the difference did not reach significance. There were no significant group differences on any of the electrocochleography measures.

**Conclusion:** In the present study, there is no evidence that AP amplitude, SP/AP amplitude ratio, or SP/AP area ratio are associated with noise exposure. It is possible that the effects of noise exposure may be observed in individuals with greater lifetime noise exposure than the cohort
tested within this study. The use of tympanic membrane electrocochleography to assess cochlear synaptopathy in humans remains inconclusive. Additional research would be needed to develop a diagnostic protocol for early cochlear damage that precedes sensory hair cell loss in humans.
Lay Summary

Recent animal studies suggest that noise exposure could cause significant damage to the auditory nerve without any changes in hearing sensitivity. Despite normal hearing sensitivity, it is possible that this damage may contribute to listening difficulties in noise. This type of hearing loss has been termed “hidden hearing loss” because it is not detectable by standard threshold testing. Neural responses to loud signals, rather than soft sounds, have been proposed as a sensitive measure in animals. However, findings in human studies were inconclusive. Additional research is required to identify clinical test protocols that can differentially diagnose hidden hearing loss in humans. This study investigates the diagnostic potential of an electrophysiological assessment called electrocochleography. The development of a clinical test may help detect early noise-induced neural damage before hearing sensitivity is affected.
Preface

The work described in this thesis is part of an ongoing research project at the University of British Columbia’s Middle Ear Laboratory, conceived by Dr. Navid Shahnaz. The study design, as well as the data analysis, was a collaborative process between the principal investigator, Dr. Shahnaz, and myself, the co-investigator. Participant recruitment and study execution were carried out by Ainsley Ma, Stéphanie Monette, Natalie Tran, and myself. All related data and outcome measures were collected by me (co-investigator), along with the writing contained in this manuscript. Dr. Navid Shahnaz assisted on all aspects of the study’s development.

This study was approved by the UBC Clinical Research Ethics Board, under the project title of “Hidden Hearing Loss - HHL” and ethics certificate number H16-02052.
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List of Abbreviations

ABR Auditory brainstem response
ANOVA Analysis of variance
AP Action potential
CM Cochlear microphonic
dB Decibels
DPOAE Distortion product otoacoustic emissions
ECochG Electrocochleography
FFR Frequency-following response
HL Hearing level
Hz Hertz
nHL Normal hearing level (in auditory brainstem response studies)
peSPL Peak-equivalent sound pressure level
RETSPL Reference equivalent threshold sound pressure levels
SD Standard deviation
SP Summating potential
SPL Sound pressure level
SR Spontaneous rate
TBI Traumatic brain injury
TM Tympanic membrane
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Finally, to my family, thank you for your unwavering support and encouragement throughout my years of education. Your moral support enabled me to accomplish more than I had imagined.
Dedication

To my family.
Chapter 1: Introduction

Current hearing assessment protocols may be underestimating neural damage within the cochlea. The current conventional hearing assessment is pure-tone audiometry between 250 to 8000 Hz, which is sensitive to outer hair cell damage (Katz, 2015). However, animal studies have shown that significant neuropathy of the inner hair cells can occur without any noticeable changes in hearing thresholds at conventional audiometric frequencies (Furman, Kujawa, & Liberman, 2013; Kujawa & Liberman, 2009; Lobarinas, Salvi, & Ding, 2013). Lobarinas et al. (2013) induced inner hair cell loss in chinchillas with carboplatin and found that up to 80% of the inner hair cells could be lost without changes in the behavioural thresholds at conventional audiometric frequencies. The auditory system can also sustain neural damage through permanent loss of the cochlear synapses between the inner hair cells and the auditory nerve fibers as a result of noise exposure or aging (Kujawa & Liberman, 2009; Sergeyenko, Lall, Liberman, & Kujawa, 2013; Viana et al., 2015). Cochlear synaptopathy is the term used for pathology affecting the cochlear synapses, particularly the synapses between the inner hair cells and the afferent auditory nerve fibers, rather than damage of the sensory hair cells in the cochlea. Although cochlear synaptopathy does not affect conventional pure-tone audiometry, persisting neural damage in the cochlea may impact individuals’ ability to understand speech in background noise (Plack, Barker, & Prendergast, 2014).

The cochlea is the inner ear organ in which sound transduction happens within the auditory system. The organ of Corti is composed of sensory hair cells, the inner hair cells and outer hair cells. The inner hair cells are the main afferent receptors and are in direct synaptic contact with auditory nerve fibers (Picton, 2010). The auditory neurons are also referred to as
spiral ganglion cells, as their cell bodies form the spiral ganglion in the cochlea. These neurons are mostly bipolar and have a peripheral axon that form a synaptic connection with the inner hair cells. Each afferent nerve is in synaptic contact with one hair cell, but an inner hair cell can be innervated by up to 20 afferent nerve endings (Picton, 2010). Thus, the number of presynaptic ribbons that facilitate information transfer across the synapses can serve as a measure of inner hair cell innervation. The following sections describe the animal studies and histological analysis that confirmed cochlear synaptopathy can occur preceding sensory hair cell loss.

1.1 Mechanisms of Cochlear Synaptopathy

Research on animal models have shown that intensive noise exposure can cause cochlear synaptic damage without affecting hearing sensitivity (Furman et al., 2013; Jensen, Lysaght, Liberman, Qvortrup, & Stankovic, 2015; Kujawa & Liberman, 2009). Kujawa and Liberman (2009) exposed mice to two hours of high intensity noise (100 dB SPL, 8-16 kHz) and observed elevated thresholds in auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAEs) 24 hours post-exposure. The threshold shift was temporary and ABR and DPOAE thresholds recovered after 16 weeks. Histological analysis at one-year post-exposure revealed no loss of inner or outer hair cells due to the noise exposure. However, the neural damage from the noise exposure was immediate and permanent. The noise exposure resulted up to 50% loss of the synapses between the inner hair cells and the auditory nerve fibers in the basal half of the cochlea and the authors observed the neuropathy as early as 24 hours post-exposure. Subsequent neural degeneration was also confirmed through a significant loss of spiral ganglion cells two years post-exposure. This suggests that cochlear synapses may be more vulnerable to noise damage than sensory hair cells, and the loss of synapses could accelerate auditory neuron
loss. In addition, evidence suggests specific auditory nerve fibers are more vulnerable to noise-induced damage.

Dynamic range is encoded in the auditory system by auditory fibers with different spontaneous firing rates (Katz, 2015). A high-spontaneous rate (high-SR) fiber has low thresholds and responds to sounds at soft levels, such as those used in pure-tone audiometry. Low-spontaneous rate (low-SR) fibers have high thresholds and encode acoustic information at moderate to high levels (Young & Barta, 1986; Costalupes, Young, & Gibson, 1984). In a study replicating noise-induced cochlear synaptopathy in guinea pigs, Furman et al. (2013) suggested there was a disproportionate loss of low-SR fibers compared high-SR fibers. They found a significant reduction in low-SR fibers at the cochlear region where noise-induced synaptic loss was observed. At higher frequencies, there was a 38% loss of low- and medium-SR fibers in noise-exposed ears while there was no difference in high-SR fibers compared to unexposed ears. Selective damage of the low-SR fibers may affect encoding of high level sounds while hearing sensitivity remains unaffected due to the healthy high-SR fibers.

Cochlear synaptopathy can also occur as part of aging-related neural degeneration, in absence of significant noise exposure. Sergeyenko et al. (2013) documented the age-related loss of sensory hair cells and spiral ganglion cells in mice between 4-144 weeks. Inner hair cell loss was minimal, with around 80% surviving to the oldest age group observed. In contrast, the count of the synaptic ribbons and spiral ganglion cells steadily declined throughout the life span. The loss of spiral ganglion cells paralleled the synaptic ribbon loss, with the cochlear synapses and peripheral axons degenerating a few months before the cell bodies. This is consistent with the neural degeneration theory that cells degenerate after loss of activity. Similar findings were replicated in post-mortem human studies (Makary, Shin, Kujawa, Liberman, & Merchant, 2011;
Viana et al., 2015). Viana et al. (2015) found significant loss of synaptic ribbons as a function of age without significant loss of the sensory hair cells in a post-mortem analysis of human cochleae. Additional post-mortem analysis of human temporal bones (newborn to 100 years old) revealed that the spiral ganglion cells decline at a mean rate of 100 per year before any sensory hair cell loss is observed (Makary et al., 2011). Similarly, with noise-induced loss, research with gerbils suggest low-SR fibers are also more significantly vulnerable to loss as a function of aging (Schmiedt, Mills, & Boettcher, 1996). These studies suggest that the cochlea undergo significant neural degeneration as a natural progression of age before sensory hair cells are affected.

Recent evidence suggests that blast exposure may also induce damage in the afferent synapses and spiral ganglion neuron loss in mice (Cho et al., 2013). Mice were exposed to compressed air to mimic blast exposure. This resulted in a rupture of the tympanic membranes which completely healed for only half of the mice. At the end of the three months period, half of the mice still had partial perforation of the tympanic membrane (at least 80% healed). ABR and DPOAE thresholds were elevated immediately post-exposure but partially recovered after 28 days. Partial recovery of the ABR and DPOAE thresholds may be explained by 1) partial recovery of the tympanic membrane in some of the mice, and 2) the outer hair cell loss that was observed in all of the blast-exposed mice. Most of the outer hair cell damage was around the cochlear base, and there was no sensory hair cell loss in the apical half of the cochlea. However, a significant reduction of afferent synapses and spiral ganglion neurons was observed in the apical half. This finding suggests that exposure to blasts and traumatic brain injury (TBI) may also cause cochlear synaptopathy in cochlear regions where sensory hair cells were preserved. The results from Cho et al. (2013) may also be related to the conductive hearing loss caused by the blast exposure. Liberman, Liberman, and Maison (2015) compared mice without tympanic
membranes to age-matched controls. The mice without tympanic membranes showed an increased threshold of around 25 dB as expected of a conductive hearing loss. At 64 weeks, they observed a reduction in cochlear nerve synapses in mice that suffered through a conductive hearing loss, either from a missing tympanic membrane or from chronic otitis media. There was no additional sensory hair cell loss compared to age-matched mice. The researchers hypothesized that auditory deprivation such as conductive hearing loss and chronic otitis media reduces the efferent innervation, which could accelerate the loss of cochlear nerve synapses.

1.2 Clinical Presentation

The previous section outlines how individuals with noise exposure, increased age, TBI, and chronic otitis media could be at risk for cochlear synaptopathy. One of the main complaints in those populations is the difficulty with understanding speech in the presence of background noise. Previous research has documented the hearing profile of patients with normal conventional audiograms but increased difficulty of understanding speech in background noise (Plack et al., 2014; Zhao & Stephens, 2007). Individuals with noise exposure history performed significantly worse in speech recognition and word identification in background noise (Liberman, Epstein, Cleveland, Wang, & Maison, 2016), detecting deviant sounds in presence of background noise, and the duration pattern test, in absence of a reduction in hearing sensitivity (for a review, see Plack et al., 2014). A possible explanation is that the low-SR fibers that are more sensitive to noise-induced damage contribute critically to speech understanding in noise due to their high thresholds and slower rate of saturation. Costalupes et al. (1984) recorded auditory fiber responses to test tones in continuous background noise. They found that high-SR fibers reach saturation levels quickly. In contrast, low- and mid-SR fibers continued to respond
to changes in the test tone at the highest noise level they tested. Thus, low-SR fibers are more resistant to mild-to-moderate masking noise (e.g. background environmental sounds) and could contribute to understanding speech in noise. Meanwhile, healthy high-SR fibers continue to respond to low levels of sound, resulting in normal levels of hearing sensitivity. The characteristic of difficulty with speech in noise in the absence of audiometric threshold elevation had been termed “hidden hearing loss”, with cochlear synaptopathy proposed as a factor.

Tinnitus is also a possible indicator for cochlear synaptopathy. Electrophysiological measures suggested that listeners with a normal audiogram and tinnitus might also be suffering from cochlear synaptopathy (Schaette & McAlpine, 2011). The researchers suggested that central neural gain may be a compensatory measure to the deafferentation of the auditory nerve fibers due to synaptic loss, resulting in the perception of tinnitus. Schaette and McAlpine (2011) proposed that a decrease in the auditory nerve fiber activity would trigger homeostatic mechanisms to restore neuronal activity to normal levels by increasing the excitatory gain and reducing the inhibitory gain at the level of the brainstem. This excitatory gain was supported by no observable changes in wave V amplitudes although wave I amplitudes were reduced. A central neural gain may also result in a faster growth of loudness perception, leading to reduced sound tolerance. Sanchez et al. (2016) found an association of tinnitus perceived during psychoacoustic assessments and a reduced sound level tolerance. The participants reported risky leisure habits such as listening to music with ear buds and attending events with loud sounds. These findings suggest that tinnitus and reduced sound tolerance could be additional effects of cochlear synaptopathy of the low-SR auditory nerve fibers.

Conductive hearing loss, such as chronic otitis media, has been associated with deficits in spatial processing which could contribute to difficulty with speech perception in noise. Spatial
processing requires the integration of binaural auditory information to localize the source of a sound. It is advantageous in understanding speech in noise because binaural cues can help parse out the speech cues from the background noise if the source of the speaker and the noise are from different directions. Children with a history of conductive hearing loss performed significantly poorer on speech perception in noise tests relying on binaural cues (Graydon, Rance, Dowell, & Van Dun, 2017; Tomlin & Rance, 2014). Specifically, they had difficulty using inter-aural cues to aid in speech perception in noise. The spatial processing deficit correlated with the age and duration of chronic otitis media, with more deficit observed in children with earlier onset of middle ear pathology and those with longer periods of conductive hearing loss (Tomlin & Rance, 2014). Spatial processing disorder is categorized as a subtype of auditory processing disorder, but it presents similarly as hidden hearing loss where the auditory perception is poorer than expected based on the hearing thresholds (Dillon, 2018).

Studies have found that veterans with a history of TBI often report as having some degree of disturbance in daily living due to hearing difficulty, such as problems understanding speech in difficult listening environments (Saunders et al., 2015; Oleksiak, Smith, Andre, Caughlan, & Steiner, 2012). Oleksiak et al. (2012) looked at a large pool of veterans with mild TBI. These authors found that 87% of the veterans reported some degree of hearing difficulty and 74% complained of tinnitus, although two-thirds of the participants who completed an audiological assessment had normal hearing. In addition, 16% were diagnosed with central auditory processing disorder. In another study with normal-hearing veterans who were exposed to blasts, Saunders et al. (2015) found that almost 60% of the participants performed one or more standard deviations poorer than normal-hearing norms in speech recognition in noise tests. Additional central auditory tests revealed that veterans with TBI often had deficits in binaural processing.
abilities and gap detection. Some researchers argued that central auditory processing disorder is the cause of “hidden hearing loss” (Musiek, Chermak, Bamiou, & Shinn, 2018). Thus, recent work has turned to electrophysiological assessments as a way to differentially diagnose noise-related cochlear synaptopathy from other potential mechanisms of dysfunction, including aging and central auditory processing disorders.

1.3 Electrophysiological Assessments for Cochlear Synaptopathy

1.3.1 Auditory Brainstem Response

The auditory brainstem response (ABR) measures the electrical changes generated along the auditory brainstem pathway in response to sounds (Picton, 2010). The first peak (wave I) is likely to be representative of the activity of the spiral ganglion cells in the cochlea (Picton, 2010). Suprathreshold wave I amplitude has been found to be sensitive to cochlear synaptopathy in rodent models. Kujawa and Liberman (2009) found that ABR thresholds and distortion product otoacoustic emissions (DPOAEs) in noise-exposed mice recovered to normal after 2 weeks post-exposure. However, suprathreshold (90 dB SPL) wave I amplitude at 32 kHz only returned to around 40% of the pre-exposure amplitude, and 12 kHz recovered around 80%. The reduction of suprathreshold wave I amplitude was correlated with the loss of presynaptic and postsynaptic degeneration of the inner hair cells. In addition, the reduction of wave I amplitude of suprathreshold responses but not thresholds is consistent with the finding that low-SR fibers are particularly vulnerable to noise-induced damage. Similar findings of presynaptic ribbon loss with a decrease in wave I amplitude was found in noise-exposed mice (Paquette, Gilels, & White, 2016), guinea pigs (Furman et al., 2013), guinea pigs with noise-induced temporal threshold shift (Song et al., 2016), and aging mice (Sergeyenko et al. (2013).
In human studies, there is conflicting evidence for the use of wave I ABR for cochlear synaptopathy. Stamper and Johnson (2015a) found that the suprathreshold (90 dB nHL) wave I ABR amplitude decreased with an increase in noise exposure background within the last 12 months. However, a follow-up analysis showed that the relationship only held true for females and not males (Stamper & Johnson, 2015b). Bramhall, Konrad-Martin, McMillan, and Griest (2017) examined 1, 3, 4, and 6 kHz wave I amplitude across four different groups: veterans with high noise exposure, veterans with low noise exposure, non-veterans with history of firearm use, and non-veterans with no history of firearm use. All participants had a normal conventional audiogram up to 8000 Hz and no evidence of a noise notch. They found smaller mean wave I ABR amplitudes in veterans with higher reported lifetime history of noise exposure and in those with history of firearm use. The difference remained significant after adjusting for sex and outer hair cell differences. Reduction of suprathreshold wave I ABR amplitudes was also found in aging and tinnitus studies. Konrad-Martin et al. (2012) observed a reduction in ABR amplitude with age even when audiometric thresholds were controlled for. In a study where cochlear synaptopathy was suspected to cause tinnitus in normal-hearing females, Schaette and McAlpine (2011) also found reduced ABR wave I while wave V was preserved.

A detailed study by Prendergast et al. (2017) found no significantly attenuated wave I amplitude due to noise exposure. They recruited 126 normal-hearing participants (thresholds of < 25 dB HL up to 8 kHz) with a wide range of lifetime noise exposure. Individuals with the highest noise exposure had about 300 times more exposure than those on the lower end of the scale. ABR was recorded with mastoid electrodes and high-pass (1.5 kHz) filtered diotic clicks at 80 and 100 dB peSPL. There was no effect of lifetime noise exposure on ABR wave I amplitude measures. Recent work also found no evidence of wave I amplitude being associated with
cochlear synaptopathy (Fulbright, Le Prell, Griffiths, & Lobairnas, 2017; Grinn, Wiseman, Baker, & Le Prell, 2017; Spankovich, Le Prell, Lobainas, & Hood, 2017). Furthermore, many factors unrelated to cochlear synaptopathy may contribute to wave I variability, such as gender-related differences in head size and temperature (Picton, 2010), individual variation in synchronization across the cochlea (Don, Ponton, Eggerment, & Masuda, 1994), and variation in physiological noise and other sources of electrical noises. Inconclusive and mixed findings in human subjects underscore the need to identify clinical tests that can diagnose cochlear synaptopathy in humans. However, Liberman et al. (2016) found intergroup differences using electrocochleography with tiptrodes, an electrode that is placed in the ear canal. It is worth exploring if this new measure is a possible clinical test because ear canal electrodes may minimize some of the individual variability from scalp-mounted mastoid electrodes such as head size.

1.3.2 Electrocochleography

Electrocochleography (ECochG) is a method of recording the electrical potentials from the cochlea as it transduces sound (Picton, 2010). An ECochG has three main components: the cochlear microphonic, summating potential (SP), and action potential (AP). The cochlear microphonic reflects the electrical depolarization and repolarization of the sensory hair cells as the basilar membrane is displaced by the stimuli (Picton, 2010). The SP is a direct current potential which reflects the nonlinearities of sound transduction in the cochlea (Katz, 2015; Ferraro, 2000). The depolarization of the inner hair cells has greater voltage than hyperpolarization, and this asymmetry results in the direct current of the SP. The AP is a summed response of the synchronous firing of thousands of auditory nerve fibers (Picton, 2010).
The AP is predominately a large negative wave (N1) and the same components as wave I of the ABR (Ferraro, 2000).

The ratio of the SP/AP amplitudes is used as a diagnostic measure for Meniere’s Disease (Picton, 2010) and also suggested for cochlear synaptopathy (Liberman et al., 2016). The loss of synaptic ribbons reduces the response of the auditory nerve fibers. Therefore, the AP is reduced, predicting that individuals with cochlea synaptopathy would have larger SP/AP ratios. The SP is not affected by cochlear synaptopathy as the response is generated by the intact hair cells (Kiang and Peake, 1960) and was found to remain robust in animal work on noise-induced and age-related synaptopathy (Sergeyenko et al., 2013). Furthermore, ABR measures are affected by a variety of subject factors independent of cochlear synaptopathy. These factors include variation in sex and head size, individual variation in tissue conductivity, and variation in physiological noise (Liberman et al., 2016; Plack et al., 2014). Normalizing the AP amplitude to the SP amplitude can help minimize between-subject variability in the outcome measure.

Liberman et al. (2016) grouped students into high-risk and low-risk groups based on their noise exposure history and recorded ear canal ECochG. The study found that the high-risk group had a significantly larger SP/AP amplitude ratio than the low-risk group. However, the difference in the SP/AP ratio difference between two groups was driven mostly from a difference in SP amplitudes rather than any significant differences between the AP amplitudes. Although contrary to the hypothesis, this trend of an increased SP was also found by Kim, Nam, and Park (2005) and Ridley, Kopun, Neely, Gorga, and Rasetshwane, (2018). A follow-up study was unable to replicate the results of any significant SP/AP ratio differences with mastoid electrodes (Prendergast et al., 2017) or ear canal electrodes (Prendergast et al., 2018). More evidence is required to determine whether suprathreshold SP/AP ratios are sensitive to noise-induced
cochlear synaptopathy in a human subject. Thus, an objective of my thesis was to investigate the SP/AP ratio with an electrode placed on the tympanic membrane which is closer to the generator sources of the SP and AP. This should increase the signal-to-noise ratio, reduce variability in the results, and improve chances of finding statistical differences between those with and without cochlear synaptopathy.

1.3.3 Frequency-Following Response

Another electrophysiological assessment for cochlear synaptopathy that has been proposed is frequency-following response (FFR). FFR is a sustained auditory-evoked potential that reflects the neural activity as the auditory system is synchronized to the stimulus envelope (Picton, 2010). This measure is sensitive to amplitude modulation of the stimulus and is a measure of auditory temporal coding. Auditory temporal coding and temporal fine structure is believed to be important in speech comprehension, especially in complex listening situations. Individuals who are at high-risk for cochlear synaptopathy are also likely to have difficulty with speech in noise. Plack et al. (2014) showed that FFR is reduced in noise-exposed ears. FFR was also predictive of behavioural performance on tasks that are affected in listeners with cochlear synaptopathy, such as frequency discrimination and modulation discrimination (as cited in Plack et al., 2016). However, recent research showed no effect of lifetime noise exposure on FFR (Prendergast et al., 2017). The source of FFR is at the level of the auditory brainstem, particularly in the region of the inferior colliculus, thalamus, and auditory cortex (Picton, 2010). Thus, changes in central auditory processing and aging may also contribute to differences in FFR. The evidence for FFR as a diagnostic tool for cochlear synaptopathy remains inconclusive.
1.3.4 Medial Olivocochlear Reflex Circuit

Hyper-responsive medial olivocochlear systems have been proposed to be evidence for noise exposure. The medial olivocochlear reflex (MOCR) circuit is one of the two auditory efferent systems that influence cochlear function. It regulates cochlear gain by modifying activity of the outer hair cells and subsequently the activity of the auditory nerve. One of the hypothesized functions of the MOCR circuit is to protect the cochlea from noise. The medial olivocochlear reflex is measured by comparing otoacoustic emissions (OAEs) between continuous broadband noise in the background and no background noise. Previous studies suggest a stronger olivocochlear efferent suppression in musicians (Bidelman, Schneider, Heitzmann, & Bhagat, 2017; Brashears, Morlet, Bertain, & Hood, 2003). Bhatt (2017) also found that higher noise exposure history within the last 12 months is positively correlated with stronger MOCR circuit. This further supported the notion that musicians, who have higher noise exposure due to the nature of their occupation, showed increased strength of contralateral OAE suppression. There is a hypothesis that increased MOCR strength is a temporary “top down” adjustment to protect the cochlea from noise damage and the MOCR could stay hyper-responsive permanently if noise exposure is not removed or reduced (Bhatt, 2017). Additionally, tinnitus was found to be associated with an increase in contralateral suppression of otoacoustic emissions (Knudson, Shera, & Melcher, 2014). However, results of MOCR and noise exposure remain inconclusive as further research is needed to investigate the functional significance of MOCR strength.
1.4 Rationale for the Current Study

ECochG has been proposed as a more direct method of measuring wave I ABR because the electrode is placed closer to its generator source – the spiral ganglia. Human wave I amplitudes recorded at the mastoid are fairly low in amplitude and have high variability among people, potentially contributing to the conflict in the current literature. Unlike rodent studies, the use of subcutaneous electrodes are not used in human studies due to the invasiveness of the procedure. On the other hand, ECochG can be recorded with an extratympanic or tympanic membrane electrode, which rests on the skin of the ear canal or the surface of the tympanic membrane. A tympanic membrane has the closest proximity to the response generator and can record significantly larger wave I amplitudes at better signal-to-noise ratio than the conventional ABR electrode configuration without being invasive (Ferraro & Ferguson, 1989). This can also help eliminate some of the variability found in human wave I ABR recordings, due to head size and individual variation in synchronization (Plack et al., 2016).

The current literature on the diagnostic ability of ECochG on cochlear synaptopathy in humans remain inconclusive. Liberman et al. (2016) found a significantly larger SP/AP amplitude ratio in music students who are at high-risk for cochlear synaptopathy versus non-music students. However, other researchers did not find a difference (Prendergast et al., 2018). The aim of this study is to further investigate the use of electrocochleography as an electrophysiological measure for cochlear synaptopathy. The results of this study may contribute to the development of a diagnostic protocol for noise-induced cochlear synaptopathy, possibly leading to treatment for individuals with normal audiograms but experiencing poor speech understanding in background noise.
1.5 Measurements for the Study

Previous research on noise-induced cochlear synaptopathy found a greater SP/AP amplitude ratio in music students compared to non-music students (Liberman et al., 2016). Participants in the current study will be music students due to their risk of cochlear synaptopathy from regular noise exposure from concert performances and rehearsals. They are also a relatively coherent group in terms of noise exposure due to regular school activities. We seek to improve the reliability of the SP and AP responses from the ECochG recording by using tympanic membrane electrodes instead of tiptrodes. In addition to the SP/AP amplitude ratio, which is the main outcome variable measured in this study, an additional measure of interpreting the electrocochleogram was incorporated.

1.5.1 Variability in Sex

ABR norms for amplitudes and latencies differ between genders (Picton, 2010). Previous literature found that females tended to have larger mean AP amplitudes than males (Chatrian et al., 1985). Some research also suggest that females may be more sensitive to noise-induced cochlear synaptopathy. When comparing high frequency audiometry up to 16 kHz, Prendergast et al. (2017) found the highest frequency thresholds were elevated with increasing noise exposure for females but not males. The relationship between higher noise exposure and decrease in wave I ABR was also true for females but not males (Stamper & Johnson, 2015b). Thus, the effects of gender will be analyzed in the current study.
1.5.2 SP/AP Area Ratio

The SP/AP area ratio was proposed by Ferraro and Tibbils (1999) as another method of interpreting the ECochG. Studies have shown that SP/AP area ratio has greater sensitivity for Meniere’s disease than SP/AP amplitude ratio alone because it accounts for the widening of the SP-AP complex (Al-momani, Ferraro, Gajewski, & Ator, 2009; Devaiah, Dawson, Ferraro, & Ator, 2003). Although this method was developed for patients with Meniere’s disease, we will include the SP/AP area ratio as an outcome variable to examine whether this methodology is also sensitive to cochlear synaptopathy.

1.6 Aims of the Current Study

The current study aims to: 1) investigate the effects of noise exposure on SP/AP amplitude ratios; and 2) determine the test performance of SP/AP area ratio as a measurement to use for cochlear synaptopathy.

1.7 Hypothesis of the Current Investigation

There is one central null hypothesis to be investigated in the current study: The electrocochleography measures are expected not to be significantly different between the low noise exposure (low-risk control group) and high noise exposure (high-risk music students) groups.
Chapter 2: Methods

All procedures were approved by the University of British Columbia Clinical Research Ethics Board as part of a larger study, certificate number H16-02052. All participants provided informed consent.

2.1 Participants

2.1.1 Subject Recruitment

Music students were recruited as the experimental (High-risk) group. Students were recruited from the University of British Columbia School of Music through advertisement circulated by the music faculty. Participants for the control group were recruited publicly via the University of British Columbia website listing of active research projects. A total of 20 music students and 21 non-music students volunteered to participate in the study.

2.1.2 Inclusion Criteria

All participants (high-risk and control group) met the following criteria: (i) between 18 to 28 years of age; (ii) normal hearing as defined by pure-tone thresholds at 25 dB HL or better at inter-octaves between 250 to 8000 Hz; (iii) no active middle ear involvement; (iv) no asymmetry in pure-tone thresholds between 250 to 8000 Hz, defined as > 15 dB HL difference in hearing threshold in the same frequency between the left and right ear; and (v) fluency in English, as other test protocols involved required a proficiency in English, such as Multiple Auditory Processing Assessment (MAPA).
In addition to the criteria above, students recruited for the control group met the additional inclusion criteria: (i) no asymmetry (> 15 dB HL difference between ears) in pure-tone thresholds between 9 to 16 kHz; (ii) no history of loud noise exposure; (iii) no ear surgery, ear injury, or recent ear infections, (iv) no head trauma or concussions, (v) no high fevers, (vi) no history of tobacco use; and (vii) no personal listening device use above 60% of the maximum volume as recommended by the World Health Organization (2015).

2.1.3 Exclusion Criteria

Participants were excluded if they had excessive wax in their ear canals and refused cerumen management. We were unable to obtain recordings from two ears from the high-risk group and one ear from the control group due to excessive cerumen in the ear canal. The participants with only one ear excluded was still eligible to continue testing with unilateral data being collected. Ear-specific recordings were rejected from data analysis if significant electrical noise was present and prevented accurate waveform labeling for analysis. Data from two ears in the high-risk group and two ears from the control group was excluded due to electrical interference in the recording.

2.1.4 Participant Overview

A total of 18 music students (mean age: 21.7 years; range: 18-26 years) with normal hearing participated as the experimental (high-risk) group. Data from 32 ears were analyzed as the high-risk group.

The control group consisted of 19 participants (mean age: 22.5 years; range: 18-28 years) with normal hearing, no significant history of noise exposure as assessed by a standard
questionnaire, no history of tinnitus, hyperacusis, no speech intelligibility problem in quiet and noise, no head trauma or concussions, or of recurrent middle ear infection. Data from 35 test ears were included in the analysis for the control group.

We were unable to recruit the originally planned target population of 20 participants for data analysis in each group due to logistics.

<table>
<thead>
<tr>
<th></th>
<th>High-risk</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Ears</td>
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</tr>
<tr>
<td>Total</td>
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<td>35</td>
</tr>
<tr>
<td>Female</td>
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<td>23</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 2.1 Number of participants and test ears included for data analysis within each group and sex.

2.2 Procedure

2.2.1 Study Overview

This thesis was part of a larger project investigating the effect of cochlear synaptopathy on several behavioural and physiological tests. The project included several measures to assess auditory processing capabilities because central auditory processing disorder was proposed to be a factor in difficulty with speech in noise in normal-hearing listeners (Musiek et al., 2018).

Participants were required to read and sign the study’s consent form before starting any screening or testing. Each participant was assigned a unique code known only to the researchers for confidentiality. Participants filled out a case history form and completed a screening session to determine eligibility.
The screening session included otoscopy and air-conduction pure-tone audiometry (0.25-16 kHz). Otoscopic examinations were performed using a Welch Allyn clinical otoscope with Welch Allyn Universal disposal otoscope specula, adult size 4.25mm. If partial or fully occluding cerumen was observed and the participant passed the screening, the participant was asked to get the excessive cerumen flushed out by their family physician prior to the electrophysiological portion of the study. Audiometry was conducted using an Otometrics Madsen Astera² audiometer with Sennheiser HDA200 circumaural headphones in a sound-treated booth. Audiometry was conducted using the automatic Bekesy method (method of adjustment), with a fixed frequency approach and pulsed tones in 1 dB steps. The system automatically extrapolated the thresholds from the 3 samples at each frequency tested (250 Hz, 500 Hz, 750 Hz, 1000 Hz, 1500 Hz, 2000 Hz, 3000 Hz, 4000 Hz, 6000 Hz, 8000 Hz, 9000 Hz, 1000 Hz, 11200 Hz, 12500 Hz, 14000 Hz, 16000 Hz). Reference Equivalent Sound Pressure Levels (RETSPLs) was determined using the calibration set-up of a Larson Davis PRM902 preamplifier, a Larson Davis AEC101 artificial ear, a Larson David one-half inch microphone Model 2559, a flat plate attachment, and a 3.2 lb (1.5 kg) weight. The hearing thresholds in HL were converted to corresponding RETSPLs at each frequencies. Calibration was done according to ANSI standards (S3.6.1989).

Once eligible, the whole diagnostic test protocol was conducted. All participants completed a comprehensive test battery of behavioural, physiological, and speech comprehension assessments including, but not limited to: Tinnitus Handicap Inventory (THI), wideband tympanometry, acoustic reflexes, transient evoked and distortion product otoacoustic emissions, contralateral suppression of otoacoustic emissions, Noise Exposure Structured Interview (NESI), electrocochleography (ECochG), loudness scaling, Threshold in Noise (TEN)
test, Multiple Auditory Processing Assessment (MAPA), Temporal Modulation Transfer Function (TMTF), triple digits in noise, Hearing In Noise Test (HINT), and random gap detection test. The entire study comprised of three sessions at one and half hours each. Participants were presented with a monetary honorarium of $50 for their completion of the entire study.

Although several behavioural and physiological measures were collected from all the participants in this study, this paper will focus on lifetime noise exposure history and electrocochleography measurements.

2.2.2 Lifetime Noise Exposure History

Noise exposure history was estimated for each participant using the Noise Exposure Structured Interview (NESI) developed by Guest et al. (2018). This technique measures the level of exposure (SPL) based on participant’s self-reported vocal effort required during the activity. The interview was structured to elicit relevant noise exposure history without being restricted to pre-determined activities and accounted for lifestyle changes throughout one’s lifetime. Participants listed all their noisy (above ~80 dBA) recreational and occupational activities. “Noisy activities” were defined as activities that “the respondent would need to raise his or her voice to communicate (at a distance of 4 feet, communicating with a listening partner with normal hearing, with gestures and facial cues available to aid communication)” (Guest et al., 2018). Appendix A.1 is the conversion chart used to estimate environmental sound pressure level from vocal effort required. The duration, frequency, and sound pressure level of the noise exposure, as well as attenuation from hearing protection (if applicable) were obtained through self-report. Any firearm exposure without hearing protection was included in the calculation but
not used as an exclusion criterion. The number of noise exposure units for each participant is calculated by the following formulas:

For exposure activities where no hearing protection was worn:

\[ U = \frac{Y \times W \times D \times H}{2080} \times 10^{\frac{L - 90}{10}} \]

For exposure activities where hearing protection was worn and reduced sound to \( \leq 80 \) dBA:

\[ U = \frac{Y \times W \times D \times H}{2080} \times (1 - P) \times 10^{-10} \]

For exposure activities where hearing protection was worn and did not reduce sound to \( \leq 80 \) dBA:

\[ U = \frac{Y \times W \times D \times H}{2080} \times \left[ P \times 10^{\frac{L - A - 90}{10}} + (1 - P) \times 10^{\frac{L - 90}{10}} \right] \]

where \( U \) is units of noise exposure, \( Y \) is the years of exposure, \( W \) is weeks per year of exposure, \( D \) is days per week of exposure, \( H \) is hours per day of exposure, \( P \) is proportion of time that hearing protection was worn (between 0 to 1), \( L \) is the sound level in dBA as estimated from vocal effort, \( A \) is the attenuation of hearing protection in dB, and 2080 (40 hours per week x 52 weeks in a year) corresponds to the number of hours in a working year. Thus, one unit of noise exposure is equivalent to one working year of exposure to 90 dBA. For this study, the raw noise exposure units were used as a measurement of the participant’s lifetime noise exposure history.

### 2.2.3 Electrocochleography

ECochG recordings were performed in one of the two locations: (1) a quiet room at the Middle Ear Lab of Dr. Navid Shahnaz, or (2) in a sound-attenuated, electrically-shielded sound booth at the BRANE Lab of Dr. Anthony Herdman. Both labs are located within University of
British Columbia Woodward Instructional Resources Centre. Stimulus generation, data acquisition, and waveform analysis were handled by Interacoustics Eclipse (Assens, Denmark). An EPA4 pre-amplifier was used. Adult- or pediatric-sized Etymotic Research (ER) 3A insert earphones were used to deliver the stimuli. Lilly TM-Wick electrodes (Intelligent Hearing System) were used for the recording.

2.2.3.1 Protocol Settings and Test Parameters

The stimulus was a 95 dB nHL broadband click presented at a rate of 11.3/second. The filter was set between 3.3 and 5000 Hz. The online rejection criteria was ±40 µV. No contralateral masking was used. We collected 1000 stimuli in each run, recording from 2 ms pre-stimulus to 12 ms post-stimulus, and each run was repeated at least twice. We recorded rarefaction clicks and condensation clicks separately to average offline to obtain alternating polarity. A total of two rarefaction runs and two condensation runs were recorded for each ear. If the recording did not show the expected response and morphology, additional runs were recorded or the TM electrode was repositioned until a highly replicable test response with good morphology with easily identifiable peaks is achieved.

2.2.3.2 Software Versions for Data Collection

Two Otoaccess software versions were used for recording in the current study. Software version 4.4 was used in the first half of data collection (High-risk: 2; Control: 10). It did not have a specific research license so we were unable to run additional analysis with the waveform data outside the software. The software was updated with a research license that allows us to save the
data for additional analysis. The remaining participants (High-risk: 16; Control: 9) were recorded with software version 4.5.

### 2.2.3.3 Test Procedure

Participants were asked to relax and recline on a sofa or bed for the duration of the recording. A horizontal electrode montage was used with the surface electrodes on the contralateral mastoid and middle forehead as the vertex and ground, respectively (Figure 2.1). Patches of skin on the mastoids and forehead were scrubbed prior to attaching the surface electrodes. An impedance of 25 kΩ or less was achieved for all surface electrodes. Impedance of 25 kΩ was used as a guideline for tympanic membrane electrode placement. The forehead and mastoid electrodes most likely had impedances lower than 25 kΩ; however, individual electrode impedance measures were not available due to the set-up and equipment used in this study.

![Figure 2.1 Illustration of the horizontal electrode montage used in the current study to record ECochG.](image)

The ear canal was prepared by soaking in room-temperature saline for one minute. The tympanic membrane (TM) electrode (Lilly TM-Wick electrode, Intelligent Hearing System) was also soaked in a saline solution for at least 10 minutes before the start of the recording and coated in a thin layer of conductive gel (SignaGel Electrode Gel, Parker) prior to insertion. The TM electrode was carefully guided towards the tympanic membrane. Adequate placement of the TM electrode was determined by low impedance (at 25 kΩ or less) or a low electrophysiological noise floor. Patient comfort was prioritized over exact placement of the TM electrode. After insertion, the TM electrode was stabilized with surgical tape and the pediatric ER-3A insert earphone. The pediatric ER-3A insert earphone was trimmed by shaving off the foam section evenly for participants with a smaller ear canal for ease of insertion. If sound leakage was significant from the pediatric insert, it was replaced with an adult-sized ER-3A insert earphone.

The same TM electrode was used for both ears of the same participant. The TM electrode was re-soaked in saline for a short period of time and re-coated in conductive gel before insertion in the other ear. The order of the test ear was randomized. Post-ECochG otoscopy was conducted to ensure the tympanic membranes were healthy and that no adverse complication was observed.

2.3 Data Analysis

Two runs of rarefaction clicks and two runs of condensation clicks were collected for each ear. Each rarefaction run was averaged offline with a condensation run to obtain alternating polarity (2000 sweeps). The two alternating waveforms were then averaged for one grand average resulting in 4000 sweeps per waveform per ear. Recordings were visually examined and excluded if significant electrical interference was observed or if unable to interpret SP/AP peaks (Figure 2.2).
2.3.1 Waveform Interpretation

All of the data were labelled by the main researcher. A second research assistant labelled the waveforms to confirm the consistency of waveform interpretation. Both researchers were blinded to each other’s waveform labeling. Correlation matrixes revealed that the SP and AP labeling between the two researchers were 90.17% and 99.19% respectively, suggesting a strong positive linear relationship. Absolute SP and absolute AP amplitudes were defined as the difference between the peak and the baseline to be consistent with the previous literature in this field (Prendergast et al., 2017; Liberman et al., 2016). The waveforms were labelled accordingly to calculate the SP amplitude, AP amplitude, SP/AP amplitude ratio, and SP/AP Area Ratio.

The following markers were determined for each waveform through visual inspection: (i) baseline start (BL_st), (ii) baseline end (BL_e), (iii) SP, (iv) AP1, (v) AP peak, and (vi) AP2. Figure
2.3 is an example of the waveform labelled. BL<sub>st</sub> was marked at the onset of the response, where the SP first began to displace the waveform. This is the baseline voltage and occurs around 0.5 ms (Interacoustics, 2017b). The Eclipse software automatically marks BL<sub>e</sub> at the next point in the waveform where the amplitude crosses the baseline voltage again. If the waveform morphology does not allow this, BL<sub>e</sub> was placed immediately after AP2. The SP was labelled at the small deflection occurring immediately after BL<sub>st</sub>, around 0.7 ms (Interacoustics, 2017b). In situations where the SP was not easily observable, other waveforms of alternating polarity from the same participant were referenced for the latency and placement of the SP. AP was characterized by three different labels, AP1, AP peak, and AP2. AP1 marked the onset of the AP deflection. In cases where there is no distinct onset of the AP, AP1 was marked immediately after the SP. AP peak was labelled on the largest negative deflection around 1.2-1.5 ms (Interacoustics, 2017b). AP2 was labelled at the first positive peak following AP peak or when the waveform approaches a slope of 0, whichever comes first. AP2 is where the action potential ends and changes direction.

SP/AP area measurements were calculated by Interacoustics Eclipse and in accordance to the method described by Ferraro and Tibbils (1999). The SP area is the area under the curve from BL<sub>st</sub> to BL<sub>e</sub>. It often encompasses the AP as well. The AP area is the N1 component, calculated from the area under the curve between AP1 and AP2. Figure 2.4 shows how the Interacoustic Eclipse calculate the SP and AP area automatically once all the labels are properly added. The SP/AP area ratio is derived from subtracting AP area from the overall SP area and then dividing by the AP area, or (SP area – AP area)/AP area.
Figure 2.3 Depiction of the ECochG waveform labelled for SP/AP area ratio calculation. Screenshot from Interacoustics (2017b). Retrieved March 25, 2020.

Figure 2.4 An ECochG recording obtained from a participant in the high-risk group in the current study. The ascending diagonal lines represent the SP area and the descending diagonal lines represent the AP area used in SP/AP area ratio calculation.
2.4 **Statistical Analysis**

Statistica Software version 13 (TIBCO Inc., 2017) was used to conduct all the statistical analysis. For the current study, a $p$-value $< 0.05$ was considered to be significantly significant.

A factorial analysis of variance (ANOVA) design was used to analyze the effects of music background and sex on the lifetime noise exposure measure. In this model, group (music students, control), and gender (female, male) served as a categorical factors and noise exposure units estimated from NESI served as a dependent variable. In addition, a mixed model ANOVA approach was used to analyze the effects of group, gender, and ear on the electrocochleography measures and to assess the potential differences between Otoaccess software version 4.4 and version 4.5. In this model, group (high-risk, control), gender (female, male), system (version 4.4, version 4.5), and ear (right, left) served as between-subject factors and SP absolute amplitude, AP absolute amplitude, SP/AP amplitude ratio, and SP/AP area ratio served as within-subject factors.
Chapter 3: Results

3.1 Lifetime Noise Exposure History

Lifetime units of noise exposure were recorded for all participants using the NESI. One unit of noise exposure is equivalent to one working year of exposure to 90 dBA. For this study, the raw noise exposure units (also referred to as NESI scores) were used as a measurement of the participant’s lifetime noise exposure history. Table 3.1 shows the descriptive statistics, including mean, standard deviation, minimum and maximum of NESI scores across the High-risk and Control group. A detailed version of the descriptive statistics including the median, 5th and 95th percentile, and range can be found in Table B.1 in the Appendix B.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
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<tr>
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<td>78.96</td>
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<tr>
<td>Control</td>
<td>1.34</td>
<td>2.23</td>
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</tr>
</tbody>
</table>

Table 3.1 Mean, standard deviation, minimum, and maximum of noise exposure units recorded in the high-risk and control group in the current study.

Data for lifetime noise exposure was analyzed using a factorial ANOVA. In this model, group (2 levels) and gender (2 levels) served as categorical factors while noise exposure units served as the dependent variable. The results of this ANOVA is included in Appendix C.1. The main effect of the group, was not significant \[F (1,33) = 0.922, p = 0.344\] indicating that the lifetime noise exposure (as collected by NESI) did not differ significantly between the high-risk group and control group (Figure 3.1). There was no main effect of gender \[F (1,33) = 0.591, p = 0.448\]. The interaction of group and gender was not significant \[F (1,33) = 0.632, p = 0.432\].
However, there was a trend for higher units of lifetime noise exposure in the high-risk group although it did not reach significance. There was also a greater range of lifetime noise exposure recorded by participants in the high-risk group. The results suggest that music students are exposed to more noise throughout their lifetime although it did not reach significance, and the variability of lifetime noise exposure in the high-risk (music students) group can be quite high.

Figure 3.1 Mean and standard error of noise exposure units in the control and high-risk group. There was a trend for higher mean units of lifetime noise exposure reported in the high-risk group but it did not reach statistical significance.
3.2 Electrophysiological Measurements

Table 3.2 summarizes the grand mean absolute SP amplitude, absolute AP amplitude, SP/AP amplitude ratio, and SP/AP area ratio across High-risk and Control group. A more detailed descriptive statistics table is included in Appendix B.2.

<table>
<thead>
<tr>
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<tr>
<td></td>
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<td>M</td>
<td>SD</td>
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<td>Area Ratio</td>
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</table>

Table 3.2 Mean and standard deviation of the SP absolute amplitude, AP absolute amplitude, SP/AP amplitude ratio, and SP/AP area ratio measured in the high-risk and control group in the current study.

3.2.1 SP and AP Absolute Amplitudes

Data for electrocochleography measures were analyzed using a mixed-model ANOVA. In the first model, group (2 levels), gender (2 levels), and ear (2 levels) served as between-subject factors while SP absolute amplitude and AP absolute amplitude served as within-subject factors. The resulting design was a 2 x 2 x 2 x 2 mixed-model ANOVA design, where the first three factors are between-subject factors and the last is a within-subject factor. The results of this ANOVA is included in Appendix C.2.

The main effect of the between-subject factor, group, [F (1,62) = 2.911, p = 0.930] and interaction of group and amplitude measures [F (1,62) = 3.054, 0.086] was not significant, indicating that the mean SP and AP absolute amplitudes did not differ significantly across the high-risk group and control group. However, there was a tendency for higher AP absolute amplitude with the high-risk group (Figure 3.2). This trend did not reach significance. The main effect of gender [F (1,62) = 0.313, p = 0.578] and ear [F (1,62) = 0.130, p = 0.720] was not
significant. The interaction of amplitude measures and gender \[F (1,62) = 0.514, 0.472\] and ear \[F (1,62) = 0.457, 0.501\] also did not reach significance, indicating that there was no association of mean SP and AP absolute amplitudes with gender or ear.

![Figure 3.2 Mean SP and AP absolute amplitudes recorded in the control and high-risk group. There was no significant interaction of group on amplitude measures but a trend for a higher mean AP absolute amplitude was observed in the High-risk group. Vertical bars denote 95% confidence intervals.](image)

The main effect of system \[F (1,62) = 16.565, 0.000\] was significant (Figure 3.3), indicating that the newer software version consistently recorded lower absolute amplitudes values. The interaction of system on amplitude measures \[F (1,62) = 8.967, 0.004\] was also significant. This interaction (Figure 3.4) showed that the newer software system recorded lower
SP and AP absolute amplitude values, and the difference between systems was more significant for AP absolute amplitudes. However, any statistical interaction of the system should be interpreted with caution because the sample size for the two software systems was unequal. In addition, the difference in system did not have an impact on group differences in the current analysis.

Figure 3.3 Main effect of system on amplitude measures was significant. System denotes the two software versions of Otoaccess used to record ECoG in the present study. Vertical bars denote 95% confidence intervals.
Figure 3.4 Mean SP and AP absolute amplitudes recorded with different Otoaccess software systems. The interaction of system and amplitude measures was significant. Vertical bars denote 95% confidence intervals.

3.3 SP/AP Amplitude Ratio and SP/AP Area Ratio

A second mixed-model ANOVA was used to explore SP/AP amplitude ratio and SP/AP area ratio. The group (2 levels), gender (2 levels), and ear (2 levels) served as between-subject factors while SP/AP amplitude ratio and SP/AP area ratio served as within-subject factors. The resulting design was a 2 x 2 x 2 x 2 mixed-model ANOVA design, where the first three factors are between-subject factors and the last is a within-subject factor. The results of this ANOVA is included in Appendix C.3.
Figure 3.5 Mean SP/AP area ratio and SP/AP amplitude ratio recorded in the control and high-risk group. There was no significant interaction of group on ratio measures. SP/AP area ratios had greater variance in both groups. Vertical bars denote 95% confidence intervals.

The main effect of group [$F(1,62) = 0.148, p = 0.702$], gender [$F(1,62) = 0.007, p = 0.932$], and system [$F(1,62) = 0.184, p = 0.180$] was not significant. There was also no significant interaction between ratio measures and group [$F(1,62) = 0.032, 0.859$] (Figure 3.5), gender [$F(1,62) = 0.000, 0.992$], or system [$F(1,62) = 1.536, 0.220$]. This indicates that the mean SP/AP amplitude ratio and SP/AP area ratio did not differ significantly by group, gender, or system. The effect of system found previously on absolute amplitudes measures was gone when comparing SP/AP amplitude ratio.
Figure 3.6 Mean SP/AP area ratio and SP/AP amplitude ratio for the right and left ear. The interaction of ear and ratio measures was significant, noticeably for SP/AP area ratio. Vertical bars denote 95% confidence intervals.

The main effect of ear \([F (1,62) = 9.031, 0.004]\) and the interaction between ear and ratio measures \([F (1,62) = 7.577, 0.008]\) were significant. This interaction (Figure 3.6) showed that the left ear has lower SP/AP area ratios only, and the SP/AP amplitude ratio does not differ depending on the ear.
Chapter 4: Discussion

This chapter is divided into 4 sections: (4.1) Summary of electrocochleography measures, (4.2) Possible Caveats with NESI; (4.3) Possible Explanations for Current Findings, (4.4) Study Limitations, and (4.5) Directions for Future Research.

4.1 Summary of Electrocochleography Measures

4.1.1 Comparison to Published Norms

The mean, standard deviation, and upper limits of the AP amplitude and SP/AP amplitude ratio from the present study are listed in Table 4.1 with norms from Campbell, Harker, and Abbas (1992), Margolis, Ricks, Fournier, and Levine, (1995), Wilson, Bowker and Wilson (2002), and Grasel et al., (2017). Only norms with tympanic membrane electrodes and a baseline-to-peak measurement for the amplitudes were included. The recording system for each study was noted if available.

The mean AP amplitude, SP/AP ratio, and standard deviation of the control group from the present study align with norms from the previous literature. The AP amplitudes are variable across the studies but the SP/AP amplitude ratios are comparable to previous studies done on normal subjects (for review, see Picton, 2010). Absolute AP amplitudes could be directly affected by experimenter variability in the placement of the tympanic membrane electrodes (Ferraro, 2000).

Although the effect of age on ABR latencies are well-documented (Picton, 2010), the effect of age on absolute amplitude is variable. Wilson et al. (2002) did not find any effect of age between three age groups: 18-30 years, 31-45 years, and 46-60 years. However, in an
extratympanic study with the elderly, Soucek and Mason (1992) found smaller absolute amplitudes in waveforms recorded from the elderly participants (65-88 years) than controls (15-27 years). A decrease in amplitude with increasing age is in line with the theory of age-related cochlear synaptopathy; thus, any interpretation of the norms with older participants should be interpreted carefully. The norms obtained by Wilson et al. (2002) for the age group most closely matched with the current study are described in Table 4.2. Figure 4.1 shows that the mean±SD for the amplitude ratios obtained in this study are comparable to the published norms, including the age-matched subset of data from Wilson et al. (2002).

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>Control 35</td>
<td>High-risk 32</td>
<td>200</td>
<td>102</td>
<td>53</td>
</tr>
<tr>
<td><strong>Age range</strong></td>
<td>18-28</td>
<td>18-26</td>
<td>19-71</td>
<td>18-60</td>
<td>19-49</td>
</tr>
<tr>
<td><strong>Intensity (dB nHL)</strong></td>
<td>95</td>
<td>90</td>
<td>90</td>
<td>88</td>
<td>90</td>
</tr>
<tr>
<td><strong>Rate (/s)</strong></td>
<td>11.3</td>
<td>11.3</td>
<td>7.1</td>
<td>13</td>
<td>11.7</td>
</tr>
<tr>
<td><strong>Recording system</strong></td>
<td>Interacoustics Eclipse</td>
<td>Interacoustics Eclipse</td>
<td>Biologic EP</td>
<td>-^a</td>
<td>-^a</td>
</tr>
<tr>
<td><strong>AP (µV)</strong></td>
<td>1.25±0.52</td>
<td>1.28±1.09</td>
<td>0.51±0.24</td>
<td>3.2</td>
<td>1.78±1.34</td>
</tr>
<tr>
<td><strong>AP UL</strong></td>
<td>2.34</td>
<td>4.74</td>
<td>0.99</td>
<td></td>
<td>4.46</td>
</tr>
<tr>
<td><strong>SP/AP</strong></td>
<td>0.25±0.15</td>
<td>0.19±0.11</td>
<td>0.21±0.07</td>
<td>0.32±0.14</td>
<td>0.26±0.09</td>
</tr>
<tr>
<td><strong>SP/AP UL</strong></td>
<td>0.59</td>
<td>0.35</td>
<td>0.37</td>
<td>0.60</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Table 4.1 Comparison of the AP absolute amplitude, SP/AP amplitude ratio, and test parameters from the current study to published norms. UL = upper limit, which is the 95th percentile (if listed) or mean +2 SD.

^a Did not mention. ^b Data for the left ear. ^c Data for the right ear.
### Table 4.2 Comparison of the AP absolute amplitude, SP/AP amplitude ratio, and test parameters from the current study to published age-matched norms from Wilson et al. (2002). Upper limit denotes the 95th percentile (if listed) or mean +2 SD.

<table>
<thead>
<tr>
<th></th>
<th>Current Study</th>
<th>Current Study</th>
<th>Wilson et al. (2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>High-risk</td>
<td>Left ear</td>
</tr>
<tr>
<td>n</td>
<td>35</td>
<td>32</td>
<td>20</td>
</tr>
<tr>
<td>Age range</td>
<td>18-28</td>
<td>18-26</td>
<td>18-30</td>
</tr>
<tr>
<td>Intensity (dB nHL)</td>
<td>95</td>
<td>90</td>
<td>0.59±0.18</td>
</tr>
<tr>
<td>Rate (/s)</td>
<td>11.3</td>
<td>7.1</td>
<td>0.95</td>
</tr>
<tr>
<td>AP (µV)</td>
<td>1.25±0.52</td>
<td>1.28±1.09</td>
<td>0.59±0.18</td>
</tr>
<tr>
<td>AP Upper Limit</td>
<td>2.34</td>
<td>4.74</td>
<td>0.95</td>
</tr>
<tr>
<td>SP/AP</td>
<td>0.25±0.15</td>
<td>0.19±0.11</td>
<td>0.31±0.12</td>
</tr>
<tr>
<td>SP/AP Upper Limit</td>
<td>0.59</td>
<td>0.35</td>
<td>0.55</td>
</tr>
</tbody>
</table>

**Figure 4.1** Graph comparing the mean±SD of the SP/AP amplitude ratio obtained in the control and high-risk group in the current study to published norms.
The mean, standard deviation, and upper limits results of the SP/AP area ratios of the present study are listed in Table 4.3 with norms from Ferraro and Tibbils, (1999), a retrospective chart review by Devaiah et al. (2003), and Al-momani et al. (2009). All of the studies, including the present study, calculated SP/AP area ratio with the same method as described by Ferraro and Tibbils (1999). The cut-off for SP/AP area ratio suggested by Devaiah et al., (2003) is 1.94. Several of the participants in the present study from both the high-risk and control group had an abnormal SP/AP area ratio above this cut-off value. In the current study, the variation in area ratio was also higher than published norms (Figure 4.2).

A possible explanation is that the waveforms recorded in this study prevented accurate area calculations. Previous studies used clicks with alternating polarity in the recording, in which the cochlear microphonic from the rarefaction and condensation polarities cancelled out. We recorded rarefaction and condensation polarity separately and then averaged them offline. We were unable to see the morphology of the alternating wave during the recording session. Post-hoc visual analysis of some of the averaged waveform revealed that SP and AP components were separate in time, as seen in Figure 4.2. Interacoustic Eclipse system’s automatic area calculation was unable accurately determine the SP area with this type of waveform morphology, which may contribute to greater variability in the area ratio measurements. Amplitude ratios were not affected because we used baseline-to-peak method to measure the absolute SP and absolute AP amplitudes.
Table 4.3 Comparison of the SP/AP area ratio from current study to published norms. UL = Upper limit, which is the 95th percentile (if listed) or mean +2 SD.

*a Did not mention. b Data for the left ear. c Data for the right ear.

Figure 4.2 Graph comparing the mean±SD of the area ratio obtained in the control and high-risk group in the current study to published norms. Note the high variability in area ratio in the current study.
4.1.2 Effects of Noise Exposure

The current study found no significant AP amplitude differences between the control group and the high-risk group. The results are consistent with previous studies that did not find a significant association of AP amplitudes and noise exposure (Prendergast et al., 2017; Liberman et al., 2016). The current study also found no association between lifetime of noise exposure and the SP/AP amplitude ratio, consistent with Prendergast et al. (2018). The results do not provide evidence that electrocochleography is sensitive to noise-induced cochlear synaptopathy. There are several possible explanations for the results: (1) the high-risk group in the current study is not representative of those with noise-induced cochlear synaptopathy, (2) young adults are not...
affected by noise-induced cochlear synaptopathy, (3) lifetime noise exposure measures are an inadequate measure of noise-induced cochlear synaptopathy in humans, and (4) our measures are insensitive to cochlear synaptopathy in humans. Section 4.3 will discuss the explanations in more detail.

4.1.3 Effects of Ear

There were roughly the same numbers of right and left ears in the control group (left ear: 19; right ear: 16) and high-risk group (left ear: 15; right ear: 17). There was a significant association with higher SP/AP area ratio in the right ear than the left. A higher SP/AP area ratio is consistent with pathology. There was no effect of ear on SP/AP amplitude ratio. It is possible that the right ear is more exposed to noise such as through playing instruments such as flute or piccolo. Although instrument was recorded in the case history used in the current study, it was not sufficient for statistical analysis because 1) there were not enough subjects for each type of instrument reported, and 2) some subjects reported playing more than one type of instrument. In addition, the rationale of using SP/AP area ratio may not be appropriate for the current analysis (will be discussed in Section 4.3.3). Thus, the interaction of ear in the current study should be interpreted with caution.

4.2 Possible Caveats with NESI

Music students are exposed to more noise per day than non-music students (Washnik, Phillips, & Teglas, 2016). Although music students are exposed to noise more often, the way they judged their noise exposure on the NESI did not reveal a significant difference. The NESI estimates environmental sound pressure level using self-reported vocal effort. Speech
communication ability are a reliable method to estimate noise levels (Ferguson, Tomlinson, David, & Lutman, 2019). However, vocal effort could be harder to assess for musicians because: (1) it is unrealistic in certain cases, such as musicians who use wind or brass instruments, and (2) sound level pressure could fluctuate and vary within the same performance. In a comparative observation study by Ferguson et al. (2019), 91% of the respondents were able to estimate noise levels within 6 dB of dosimeter readings using speech communication ability. However, a few still underestimated or overestimated the noise level by more than 6 dB. Calculation for the NESI scores would amplify the extreme responses because the duration of the activity is taken into account as well. Those who reported extremely low or high noise levels, even when they are few in number, may contribute significantly to the statistical analysis in a small sample-sized study such as the present one. For example, respondents from the current study reported sound exposure levels between 87 to 110 dBA for activities such as band and orchestra rehearsal. The upper limit of the reported values is high compared to previous literature that recorded the actual sound pressure levels with dosimeters. The maximum sound pressure levels recorded with dosimeters during rehearsals and concerts were around 93-98 dBA across studies (Berger, Neitzel, & Kladden., 2006; Qian, Behar, & Wong, 2011). High variability in self-reported measures even for a homogenous group of university-level music students could be problematic in interpreting NESI scores.

Another possibility is that music students naturally have a greater variance of noise exposure. The exact noise level a musician is exposed to can vary depending on the type of music and the type of instrument played (Berger et al., 2006; Schmidt et al., 2011; Qian et al., 2011). The Noise Navigator™ Database of over 1700 measurements recorded sound levels for rehearsals ranging from 77 dBA to 98 dBA depending on the type of music: symphony (90
dBA), jazz (96-98 dBA), and opera (77-92 dBA) (Berger et al., 2006). Even within the same orchestra performance, the sound pressure level experienced by the musician can vary depending on the positioning within an orchestra. Qian et al. (2011) recorded dosimeter readings from different sections of a large orchestra during the same performance. Sound pressure level ranges from a mean Leq of 87 dB in the woodwind section to 93 dB for the flute and trombone players. Thus, the greater variability of noise exposure in the high-risk group is consistent with the literature because the type of instrument played was not controlled for in this study.

4.3 Possible Explanations for Current Findings

4.3.1 Lack of Significant Noise Exposure

Although there was a trend for greater lifetime noise exposure in the high-risk group, it did not reach significance. Thus, it is possible that we did not observe any group differences in ECochG measures because the noise exposure in the high-risk group was not significant. Preliminary work with NESI showed that the noise exposure from a cohort of 62 music industry workers ranges from 1 to over 100 (as cited by Guest et al., 2018). In our study, only two subjects in the high-risk group reported lifetime noise exposure greater than 100. Figure 4.4 shows the distribution NESI scores for all participants in the current study. Contrary to what the mean values might suggest, most of the respondents in the high-risk group had similar NESI scores as the control group. Figure 4.5 shows the overlap in NESI score distribution between the control group (n = 19, NESI range: 0.02-7.15) and the majority of the participants in the high-risk group (n = 16, NESI range: 0.34 – 20.17), who did not score above 100 on the NESI. Given that lifetime noise exposure accumulates over time, it is unsurprising that our population of young university students reported relatively low NESI scores. Thus, our high-risk group may
not be representative of those who have had accumulated significant neural damage from noise exposure. If the experimental group has greater lifetime noise exposure, perhaps the ECochG would be sensitive to the group differences.

Figure 4.4 Histogram of the distribution of NESI scores reported by all participants in the high-risk group (including outliers) and control group. X-axis: 0 – 340.

Figure 4.5 Histogram of the distribution of NESI scores reported by participants in the high-risk group (excluding outliers) and control group. X-axis: 0 – 20.
Electrophysiological measures also may only be sensitive to significant noise exposure. Kim et al. (2005) studied individuals who were exposed to music trauma and suffered from temporary threshold shifts. Extratympanic ECochG recordings indicated an increase in SP amplitude and SP/AP ratio in noise-exposed individuals, similar to the results from Liberman et al. (2016). Kujawa and Liberman (2009) also observed a similar threshold shift that preceded electrophysiological changes they observed in rodent. Fernandez, Jeffers, Lall, Liberman, and Kujawa (2015) showed that synaptopathy only occurred with extreme exposure. They observed an immediate 35–55% reduction of synaptic counts in adult mice after 2 hours of exposure to 100 dB SPL but no synaptic losses after 2 hours of 91 dB SPL. None of our participants, including the high-risk exposure group, experienced temporary threshold shifts as determined through a self-reported case history. It is possible that dramatic noise trauma, such as one accompanied by a temporary threshold shift, is required for cochlear synaptopathy to be detectable by electrophysiological measures.

4.3.2 Age Effects of Cochlear Synaptopathy

Another explanation for the lack of group differences may be because both group are young university students. Kujawa and Liberman (2009) exposed mice to intensive noise at 16 weeks of age and observed significant reduction of spiral ganglion cells 2 years post-exposure. Conversation to human age would be around 60-75 years old (Andreollo, Santos, Araújo, & Lopes, 2012). Thus, it could take up to 60 years to see such a change in humans. In the current study, both groups of participants have mean ages of 21-22 years old (control group, age range: 18-28; high-risk group, age range: 18-26). It is possible that the loss of the spiral ganglion cells and the consequent reduction of wave I ABR and the AP would not be observed until much later
in life. Currently, we don’t know how early the noise-induced cochlear synaptopathy can be detected in humans but an age difference between the rodent and human studies should be noted.

4.3.3 Perceptual Difficulties with Cochlear Synaptopathy

In addition to significant noise exposure, perceptual listening differences may also be an indication for cochlear synaptopathy. Liberman et al. (2016) reported that high-risk participants score significantly lower on word recognition in noise in addition to a higher SP/AP ratio. Similarly, Plack et al. (2014) described listeners who were exposed to more noise had more difficulty listening to speech in noise as a characteristic of hidden hearing loss. The high-risk group in the current study may not be representative of those individuals who suffer from cochlear synaptopathy since none of the participants self-reported difficulty with speech understanding in any situation. Perceptual listening difficulty may serve as a better criterion for cochlear synaptopathy in humans considering the clinical feasibility of developing a diagnostic toolset for noise-induced cochlear synaptopathy. However, introducing perceptual difficulties as a criteria may include populations with central auditory processing disorders. There is an ongoing debate of whether “hidden hearing loss” is a form of central auditory processing disorder or cochlear synaptopathy (Musiek et al., 2018). Further research would be needed to differentiate between central auditory processing and peripheral auditory neuropathy.

4.3.4 SP/AP Area Ratio

SP/AP area ratio was proposed as a method to account for both the amplitude and duration of electrocochleography components that may be affected in Meniere’s Disease (Ferraro & Tibbils, 1999). Morrison, Moffat, and O’Conner (1980) hypothesized that the widening of the
SP-AP complex found in Meniere’s Disease was due to the “after-ringing” of cochlear microphonic from the endolymphatic hydrops. In addition, a latency difference of greater than 0.38ms in the AP component between rarefaction and condensation clicks was considered to be a positive diagnosis for endolymphatic hydrops (Margolis et al., 1995). The latency difference is due to the alteration in opposing polarity clicks and the velocity of the traveling wave in the cochlea, which is filled with endolymph. The latency difference would be obscured by recording alternating clicks but may present as a prolonged SP-AP complex. Hence, the SP/AP area ratio is more efficient at capturing the amplitude and durational differences of an ECochG recording than SP/AP amplitude ratios. We were unable to verify the diagnostic value of SP/AP area ratios for cochlear synaptopathy, but we suspect this measure will likely be insensitive due to the different mechanisms between Meniere’s Disease and damage of cochlear synapses.

4.4 Study Limitations

The current study suffers from a small sample size for the high-risk group (n = 32) and control group (n = 35). It could explain for the lack of differences in lifetime noise exposure, especially because of the greater variance of noise exposure that exist in music students.

There may have been variability introduced in the electrocochleography recording by using a tympanic membrane electrode. The technique applied in this study to insert the tympanic membrane electrode, as Ferraro (2010) described, was partially “blind” as the electrode tip obscured the tympanic membrane. Proper placement was verified by having the participant acknowledge feeling the electrode but it was not always achieved as some participants became highly uncomfortable. Therefore, the variability in the exact placement of the tympanic membrane electrodes could introduce inter-subject variability in the AP amplitudes recorded.
Similarly, Stamper and Johnson (2015a) found greater variability when recording with tympanic membrane electrodes compared to using mastoid electrodes to record ABR. Another source of variability in ECochG recording is the SP amplitude. The SP was difficult to observe in some of the recordings in the current study (Figure 4.6), leading to higher variability in SP measurements. Prendergast et al. (2018) also reported low test-retest reliability in SP amplitudes across sessions compared to main ABR waves. This could affect the feasibility of using an outcome measure that incorporates the SP amplitudes, such as SP/AP amplitude ratio, in a clinical diagnostic setting.

Figure 4.6 Example of an ECochG recording where the SP was difficult to observe visually.

4.5 Directions for Future Research

Further investigation is required into electrophysiological differences in individuals at high-risk for cochlear synaptopathy. It may be useful to recruit individuals with significant noise exposure as the experimental group and differentiate between industrial noise exposure versus
leisure noise exposure. This may include recruiting older musicians, in their 60s and 70s, and compare their electrophysiological results to age-matched controls. In addition, it may be of interest to see if reported temporary threshold shifts and reported difficulty listening to speech in noise has any effects. Determining whether listening difficulties in speech or a history of noise exposure is a better predictor of cochlear synaptopathy would have important future clinical implications.

For future use of the NESI, it may be useful to have an estimation of noise levels for different activities to help guide respondents to a more realistic estimate. For musicians, it may be beneficial to take into account of the type of instrument played because the exposure level could also differ. Future studies may benefit from comparing reported noise levels to dosimeter readings for a better documentation of the noise exposure level.

Following up to the current study, it would be beneficial to compare high frequency audiometric differences between groups. Wave I response to a broadband click is strongly influenced by activity in the basal region of the cochlea (Don & Eggermont, 1978). Thus, hair cell loss in high frequency regions (> 8000 Hz) could affect the amplitude of the response. In the study where an intergroup difference was found for SP/AP ratio, only conventional audiometry was considered (Liberman et al., 2016). Prendergast et al. (2017) controlled for extended high frequency differences and found no difference. Thus, high frequency audiometric difference may have contributed to the significant finding in SP/AP ratios by Liberman et al. (2016). Analysis of the high frequency thresholds from participants in the current study would allow a better comparison of the overall health of the cochlea and any possible interaction with the electrophysiological measures.
We are unable to comment on the feasibility of electrocochleography with tympanic membrane electrodes as better than ABR wave 1 measurements. Prendergast et al. (2018) reported similar test-retest reliability with either mastoid electrodes or ear canal electrodes. Future experiments may find it useful to compare simultaneous recordings of mastoid and tympanic membrane electrodes to compare the sensitivity of each recording method. More evidence is needed for the diagnostic ability of wave 1 amplitude or SP/AP amplitude ratio on cochlear synaptopathy in human studies.
Chapter 5: Conclusion

In the present study, tympanic membrane electrocochleography was measured across participants at high-risk for noise exposure and controls. Our study suggested no relation between AP amplitude, SP/AP amplitude ratio, and SP/AP area ratio with noise-induced cochlear synaptopathy. In particular, AP amplitudes did not decrease in the high-risk group as predicted. It remains possible that the effects of noise exposure may be more easily observed in participants with a greater lifetime noise exposure than the cohort tested within this study. Another interpretation from this study is that young adults may be less susceptible to noise-induced neural damage than rodents. Further research on electrophysiological assessments is required to develop a clinical test protocol that can detect noise-induced cochlear synaptopathy preceding sensory hair cell loss in humans.
Bibliography


https://www.youtube.com/watch?v=ILG6ErIjXZE


Lobarinas, E., Salvi, R., & Ding, D. (2013). Insensitivity of the audiogram to carboplatin induced inner hair cell loss in chinchillas. Hearing research, 302, 113-120.


Appendices

Appendix A  Supporting Documents for Methods

A.1  NESI Vocal Effort to SPL Conversion Chart

The following conversion chart from Guest et al. (2018) was used to estimate environmental sound pressure level based on the vocal effort required for the respondent to communicate with someone 4 feet away.

<table>
<thead>
<tr>
<th>Vocal Effort Required</th>
<th>Estimated Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talk normally from 4 feet (1.2 m)</td>
<td>≤ 80 dBA</td>
</tr>
<tr>
<td>Raise voice from 4 feet (1.2 m)</td>
<td>87 dBA</td>
</tr>
<tr>
<td>Talk loudly from 4 feet (1.2 m)</td>
<td>90 dBA</td>
</tr>
<tr>
<td>Talk very loudly from 4 feet (1.2 m)</td>
<td>93 dBA</td>
</tr>
<tr>
<td>Shout from 4 feet (1.2 m)</td>
<td>99 dBA</td>
</tr>
<tr>
<td>Shout from 2 feet (0.6 m)</td>
<td>105 dBA</td>
</tr>
<tr>
<td>Shout in listener’s ear</td>
<td>110 dBA</td>
</tr>
</tbody>
</table>
Appendix B  Descriptive Statistics

B.1  Lifetime Noise Exposure

Mean, standard deviation, median, minimum, maximum, range, upper and lower quartile, quartile range, and 5th and 95th percentile values for noise exposure units obtained with Noise Exposure Structured Interview (Guest et al., 2018) for the control and high-risk group in the current study.

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>High-risk Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Mean</td>
<td>1.34</td>
<td>30.69</td>
</tr>
<tr>
<td>SD</td>
<td>2.23</td>
<td>78.96</td>
</tr>
<tr>
<td>Median</td>
<td>0.22</td>
<td>4.21</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.02</td>
<td>0.34</td>
</tr>
<tr>
<td>Maximum</td>
<td>7.15</td>
<td>320.61</td>
</tr>
<tr>
<td>Range</td>
<td>7.13</td>
<td>320.26</td>
</tr>
<tr>
<td>Lower Quartile</td>
<td>0.06</td>
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<tr>
<td>Upper Quartile</td>
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<td>Quartile Range</td>
<td>1.46</td>
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</tr>
<tr>
<td>5th Percentile</td>
<td>0.02</td>
<td>0.34</td>
</tr>
<tr>
<td>95% Percentile</td>
<td>7.15</td>
<td>320.61</td>
</tr>
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</table>
B.2 Electrocochleography Measurements

Mean, standard deviation, median, minimum, maximum, range, upper and lower quartile, quartile range, and 5th and 95th percentile values for the SP amplitude, AP amplitude, SP/AP ratio, and SP/AP Area Ratio obtained in the current study for the control and high-risk group.

<table>
<thead>
<tr>
<th></th>
<th>SP</th>
<th>AP</th>
<th>SP/AP</th>
<th>Area Ratio</th>
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<tr>
<td></td>
<td>C</td>
<td>HR</td>
<td>C</td>
<td>HR</td>
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<tr>
<td>n</td>
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<td>32</td>
<td>35</td>
<td>32</td>
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<tr>
<td>Mean</td>
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<td>0.26</td>
<td>1.25</td>
<td>1.28</td>
</tr>
<tr>
<td>SD</td>
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<td>0.31</td>
<td>0.52</td>
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<tr>
<td>Median</td>
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<td>0.19</td>
<td>1.18</td>
<td>0.87</td>
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<td>Min</td>
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<td>0.03</td>
<td>0.49</td>
<td>0.25</td>
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<tr>
<td>Max</td>
<td>1.10</td>
<td>1.63</td>
<td>2.68</td>
<td>5.02</td>
</tr>
<tr>
<td>Range</td>
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<td>1.60</td>
<td>2.19</td>
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<td>LQ</td>
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<td>0.09</td>
<td>0.82</td>
<td>0.73</td>
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<tr>
<td>UQ</td>
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<tr>
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<tr>
<td>5th</td>
<td>0.07</td>
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<td>0.58</td>
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<tr>
<td>95th</td>
<td>0.69</td>
<td>0.92</td>
<td>2.34</td>
<td>4.74</td>
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</table>

C = control. HR = high-risk. LQ = lower quartile. UQ = upper quartile. 5th = 5th percentile. 95th = 95th percentile. QR = quartile range. SD = standard deviation.
Appendix C  Statistical Analysis

C.1  Factorial ANOVA for Noise Exposure Units

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>Degr. of (Freedom)</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
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<td>3755.717</td>
<td>1.206482</td>
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<tr>
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<tr>
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<td>1839.013</td>
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</tr>
<tr>
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<td>1967.529</td>
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<tr>
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C.2  Mixed Model ANOVA for Amplitude Measures

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<tr>
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<tr>
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<tr>
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<tr>
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</table>

*Notes: ABSOL = SP and AP Amplitudes*
### C.3 Mixed Model ANOVA for Ratio Measures

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<thead>
<tr>
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<th>p</th>
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<tbody>
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*Notes: ABVSAREA = SP/AP Amplitude Ratio and SP/AP Area Ratio*