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Effect of Electrocochleography Stimulus Rate and Intensity on Identification of Noise Induced Hidden Hearing Loss in Humans

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EFFECT OF ELECTROCOCHLEOGRAPHY STIMULUS RATE AND INTENSITY ON IDENTIFICATION OF NOISE INDUCED HIDDEN HEARING LOSS IN HUMANS

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Doctor of Audiology, Communication Sciences and Disorders

By

Amanda McCarthy

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ABSTRACT

Noise exposure has been known to cause both temporary and permanent shifts in hearing thresholds in humans. Animal and human studies have shown noise exposure to lead to damage to the ribbon synapses of the cochlea. This damage, referred to as noise induced hidden hearing loss (NIHHL), is not detectable with standard hearing assessments, though can be the cause of difficulties understanding speech in the presence of background noise. Recent studies have begun to explore the use of electrocochleography (ECochG) to detect this neural damage in humans. Such studies strive to aid in the development of a clinical tool for the diagnosis of NIHHL in humans. To investigate the effects of stimulus intensity and presentation rate on ECochG responses, male and female participants were recruited and separated into high and low noise exposure groups based off noise exposure questionnaires. Individuals then underwent audiometric testing, speech-in-noise testing, and ECochG testing. All participants had hearing thresholds within normal limits. Speech testing was not found to be clinically significantly different between groups. While both the stimulus rate and intensity significantly affected the AP amplitude, there was only a borderline significant difference between effects of the stimulus intensity on the AP amplitude of the low-risk group as compared to the high-risk group. These results agree with previous human studies and indicate ECochG may be a potential diagnostic tool for NIHHL. No significant difference in SP amplitude was seen between groups with changes in stimulus intensity or rate was seen between groups. Stimulus intensity did, however, have an effect on SP amplitude. While ECochG shows promise as a potential diagnostic tool for NIHHL, further research is necessary both to confirm the usefulness of the measure and to develop a clinical diagnostic protocol.

KEYWORDS: noise induced hidden hearing loss, electrocochleography, noise exposure, cochlear synaptopathy, ribbon synapse, electrophysiology, hearing loss

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INTRODUCTION

Hearing loss caused by noise exposure has been well documented historically. It is accepted that noise induces hearing loss via damage to the outer hair cells (OHCs) of the cochlea leading to a peripheral threshold shift which can be seen following exposure to damaging noise (Liberman & Kiang 1978). This threshold shift can be temporary or permanent, with hearing thresholds returning to normal after a period of time in the case of temporary threshold shift (TTS), leading to the idea no permanent damage occurs to the auditory system (Nordmann et al. 2000). However, recent research suggests this may not be the case. Clinically, it is not uncommon for a patient to be seen with the complaint of hearing loss and difficulties with hearing in noise despite having hearing thresholds within normal limits. It is possible these complaints stem from limited damage to the auditory nerve which is not apparent on standard audiometric test and therefore is called hidden hearing loss (HHL), noise induced hidden hearing loss (NIHHL), or cochlear synaptopathy (CS) (Furman et al. 2013; Kujawa & Liberman 2009; Liberman et al. 2016; Lin et al. 2011; Liu et al. 2019).

The auditory nerve is comprised of two main types of afferent auditory nerve fibers (ANFs): Type I which synapse to the inner hair cells (IHCs) of the cochlea, and Type II which synapse to the OHCs (Spoendlin & Schrott 1988; Spoendlin & Schrott 1989). The IHCs and Type I ANFs are responsible for encoding most of the auditory signals which are perceived in mammals (Dallos 1992; Spoendlin & Schrott 1988). In a simplified version, this process occurs when sound waves travel through the outer and middle ear to the cochlea where they are amplified by the OHCs (Liberman et al. 2002); this then causes the tectorial membrane to shear the stereocilia of the IHCs, leading the IHCs to depolarize (Moser et al. 2006; Spoendlin &

Schrott 1988). When the IHCs depolarize, the neurotransmitter glutamate is released into the synapse between the IHC and ANFs (Moser et al. 2006; Nouvian et al. 2006; Ruel et al. 2007). The synapse between the IHCs and the ANFs is referred to as a ribbon synapse, and the auditory signal is encoded by the auditory nerve when glutamate is released into the synapse and consequently received by a postsynaptic terminal (Moser et al. 2006; Nouvian et al. 2006; Ruel et al. 2007).

Auditory nerve fibers all fire constantly, regardless of the presence of an auditory stimulus (Liberman & Kiang 1978). This spontaneous firing occurs at different rates for different nerve fibers, leading to the fibers being classified as having low, medium, or high spontaneous firing rates (Bourien et al. 2014; Liberman & Kiang 1978; Moser et al. 2006). In mammals, 60-75% of the ANFs are high spontaneous rate fibers (SRF) fibers and have a spontaneous firing rate greater than 18 spikes per second (Liberman & Kiang 1978; Taberner & Liberman 2005). Medium SRFs comprise 15-30% of ANFs and have a firing rate of greater than 0.5 spikes per second but less than 18 spikes per second (Liberman & Kiang 1978; Taberner & Liberman 2005). Lastly, 10-16% of ANFs are low SRFs and have a spontaneous firing rate of less than 0.5 spikes per second (Liberman & Kiang 1978; Taberner & Liberman 2005). Loud intensity sounds are encoded by low SRFs, while lower intensity sounds are encoded by medium and high SRFs (Heut et al 2016).

Low SRFs have been shown to play a large role in the encoding of speech in noise. Heut et al. (2016) have shown the low SRF in gerbils are more resistant to background noise than medium or high-rate fibers because the low SRF do not saturate in noise. Differences in temporal processing have been seen in humans with high noise exposure as compared to those without noise exposure (Bharadwaj et al. 2015). Bharadwaj et al. (2015) propose the importance of low

SRFs in auditory perception in the presence of noise and other competing signals. Low SRFs are likely vital to the ability of the auditory system to encode speech in noise because they continue to fire despite background noise, even high intensity background noise. Therefore, low SRFs have been implicated in NIHHL (Kujawa & Liberman 2009).

Numerous animal studies have supported the presence on NIHHL in mammals. Such studies all have similar findings of normal hearing thresholds, or a recovery of normal hearing thresholds, after noise exposure despite a decrease in the neural output in response to acoustic stimuli at a supra-threshold level (Furman et al. 2013; Kujawa & Liberman 2009; Lin et al. 2011; Liu et al. 2019). This neural output is typically measured in these studies via compound action potential (CAP) amplitudes, or the amplitude of wave I of auditory brainstem responses (ABRs). Kujawa and Liberman (2009) demonstrated damage to the auditory system beyond the OHCs in noise exposed mice; specifically these authors noted reduced IHC ribbon synapses following noise exposure in addition to delayed damage of spiral ganglion cells. Studies by Lin et al. (2011) and Liu et al. (2019) showed similar results with a return of ABR thresholds to normal within two weeks of noise exposure while ABR amplitudes remained reduced and also confirmed there was no loss of IHCs seen following noise exposure. Such results further enforce the IHC synapses as the site of lesion of NIHHL, thus causing a reduction in the wave I amplitude of the ABR. It is possible the ribbons which are not extensively damaged by noise exposure are still changed. Changes in the size and location of remaining ribbons synapses in noise exposed mice have been repeatedly reported (Kujawa & Liberman 2009; Liberman & Liberman 2015; Lin et al. 2011). Therefore, while noise exposure can lead to the loss of ribbon synapses, there is also a high likelihood of morphological changes to the remaining synapses affecting the transmission of auditory signals along the pathway. Given this, NIHHL could be

due to a combined effect of the loss of ribbon synapses as well as the change in the function of the ribbon synapses which remain, and simple counts of remaining ribbons after noise exposure may not illustrate the extent of the damage. Furthermore, Kujawa and Liberman (2009) found a slow death of the ANFs in noise exposed mice after the loss of ribbon synapses. Since the authors reported a reduction in neural firing in this study while thresholds returned to normal, it can be inferred most ANF loss was of the low SRFs while the high and medium SRFs persisted thus allowing the threshold to auditory stimuli to remain unchanged. The loss of the low SRF could therefore lead to difficulties with hearing in noise despite normal audiometric results.

In humans, evidence for NIHHL is not as conclusive. ABR wave I amplitude has been used to investigate NIHHL in humans as it has in animal studies. Many studies have shown a reduction in suprathreshold ABR wave I amplitudes in participants with known history of noise exposure as compared to those without (Bramhall et al. 2016; Schaette & McAlpine 2011). However, many human studies have failed to find evidence to support the presence of NIHHL in humans through the use of ABR testing. It has been reported a lack of significant difference between ABR response amplitudes in participants who report a history of noise exposure as compared to those who report no history of noise exposure (Prendergast et al. 2016; Guest et al. 2017). Given the variability of results seen in studies utilizing ABR responses to attempt to confirm a presence of NIHHL in human participants, it is possible ABR is not the best measurement of NIHHL in humans.

The SP/AP amplitude ratio in electrocochleography (ECochG) has been used by Liberman et al. (2016) to detect the presence of NIHHL in participants at high risk of noise exposure. ECochG was the measure of choice to probe for evidence of NIHHL as opposed to ABR wave I amplitude in an attempt to circumvent the variability seen in wave I of ABR

responses in humans. In this study it was found the SP/AP amplitude ratio of the high-risk group was about double that of the low-risk group. These results indicate ECochG may be a suitable measurement for NIHHL in humans. This is likely due to the electrode placement utilized in ECochG being closer to the response generators which produce either the AP of the ECochG response or wave I of the ABR response. The use of ECochG in the diagnosis of NIHHL in humans requires further exploration.

The effect of different stimulus parameters could be used to measure ECochG in humans with a history of noise exposure. Manipulation of different stimulus parameters such as presentation rate and stimulus intensity may help in investigating the firing patterns of the ANFs. Specifically, increasing stimulus intensity would result in recruiting more ANFs to fire, as well as increasing their firing patterns (Huet et al. 2016), leading to increase in AP amplitude and decrease in AP latency with increased stimulus intensity (Schoonhoven et al. 1995). SP amplitude would also increase with stimulus intensity, but SP latency would not change as the SP is not primarily a neural response (Ferarro & Durrant 2006; Zheng et al. 1997). Therefore, loss of ANFs responsible for encoding the increase in intensity may help detecting traces of NIHHL. Furthermore, increasing presentation rate enforces adaptation of neural responses (i.e. AP) while not impacting pre-neural responses (i.e. SP) (Kaf et al. 2017; Zheng et al. 1997). It could be inferred that in participants with a loss of neural fibers due to NIHHL, the adaptation of the AP amplitude would be reduced as compared to controls while the SP amplitude would not be significantly different. Measuring ECochG at a high rate while limiting the significant break in wave morphology can be done through the continuous loop averaging deconvolution (CLAD) algorithm (Delgato & Ozdamar 2003; Kaf et al. 2017). Furthermore, stimulus intensity will also affect the SP and AP of ECochG.

The objective of this study is to investigate ECochG as a method to detect the presence of NIHHL in young adults with normal hearing. The relationship between stimulus parameters (rate and intensity) and AP amplitude, SP amplitude, and SP/AP amplitude ratio of the ECochG responses will be investigated in controls and participants with noise exposure history. Currently, no study has been published which investigated the effect of both rate at intensity of stimulus on ECochG responses of patients with normal audiograms. This study could be beneficial in the development of a diagnostic tool for the presence of NIHHL in the future.

LITERATURE REVIEW

The Auditory System

The human auditory system is comprised of three major sections: the outer ear, middle ear, and the inner ear. The inner ear houses the snail shaped cochlea (Dallos 1992; Spoendlin & Schrott 1988). Within the fluid filled cochlea lies the organ of Corti which is the end organ for hearing (Dallos 1992; Spoendlin & Schrott 1988). The organ of Corti contains the basilar membrane on which both the outer and inner hair cells rest (Spoendlin & Schrott 1988). There are nearly four times as many (OHCs) as there are inner hair cells (IHCs) within the mammalian cochlea (Kimura et al. 1964). The top of each of the hair cells contain stereocilia, while the bottom portion is the area in which the synaptic region of the hair cells is contained (Kimura et al. 1964; Pau & Pau. 2006). While there are substantially more OHCs than IHCs, 90-95% of the auditory nerve afferent fibers synapse to IHCs, with multiple fibers per IHC; conversely approximately 10% of auditory nerve afferent fibers synapse to OHCS, leading to multiple OHCs per nerve fiber (Spoendlin & Schrott 1988; Spoendlin & Schrott 1989). The fibers which connect to the IHCs are known as Type I fibers and are on average twice the diameter of the fibers which connect to the OHC and are known as Type II fibers (Spoendlin & Schrott 1988; Spoendlin & Schrott 1989).

As sound vibrations enter the ear from the environment, they strike the tympanic membrane, thus setting it, and the ossicles of the middle ear, into motion (Mason 2016). The final ossicle of the three which comprise the ossicular chain—the stapes—then pushes into the oval window of the cochlea, compressing the fluid inside and setting into action within the cochlea a mechanical wave which travels along the basilar membrane via fluid compression

(Mason 2016). As the basilar membrane is tonotopically organized—meaning due to a reduction is stiffness progressively along the length of the basilar membrane, each area responds best to a specific or characteristic frequency—the mechanical wave will continue to travel until it reaches the corresponding frequency area along the basilar membrane (Spoendlin & Schrott 1989). Specifically, the cochlea of mammals is said to be sharply tuned with the base of the basilar membrane responding best to high frequency sounds, and the apex responding best to low frequency sounds (Dallos 1992; Spoendlin & Schrott 1989).

The OHCs play a large role in what occurs as environmental sounds reach the cochlea in the form of mechanical energy. As the basilar membrane moves, the OHC stereocilia are sheered by the tectorial membrane, thus causing the OHCs to depolarize (Pau & Pau 2006). However, as the OHC synapse with relatively few of the afferent fibers, this is not the only role they play in the sound processing of the cochlea. The OHCs are also responsible for active motion which amplifies the movement of the traveling wave (Liberman et al. 2002).

The IHCs, which have the most synapses with afferent fibers compared to OHC, are therefore responsible for the majority of the coding of auditory signals from the mechanical waves in the cochlea (Dallos 1992; Spoendlin & Schrott 1988). The mechanical waves, amplified by the OHCs, cause the tectorial membrane to shear the stereocilia of the IHCs to depolarize (Moser et al. 2006). The depolarization of the IHCs initiates the release of the neurotransmitter glutamate into the synaptic space between the IHCs and the auditory nerve fibers (ANFs) (Moser et al. 2006; Nouvian et al. 2006; Ruel et al. 2007). The synapse between the IHCs and the ANFs is referred to as a ribbon synapse. This synapse is characterized by a swelling at the base of the IHC—the ribbon—which contains the neurotransmitter vesicles tethered and ready to be released into the synaptic cleft (Moser et al. 2006; Nouvian et al. 2006).

When glutamate is received by a postsynaptic terminal, an auditory signal is encoded and sent to the auditory nerve (Nouvian et al. 2006; Ruel et al. 2007).

The auditory nerve is made up of different types of neural fibers. Auditory nerve fibers are constantly firing, even when an auditory stimulus is not present (Liberman & Kiang 1978). This firing occurs at different rates for different fibers (Liberman & Kiang 1978). The different spontaneous firing rates, and thus the different nerve fibers can be classified as being low, medium, or high (Bourien et al. 2014; Moser et al. 2006). High spontaneous rate fibers (SRF) fibers have a spontaneous firing rate greater than 18 spikes per second while the medium SRF have a firing rate of greater than 0.5 spikes per second but less than 18 spikes per second, and the low SRF have a spontaneous firing rate of less than 0.5 spikes per second (Liberman & Kiang 1978). Generally, in mammals approximately 60-75% of ANFs have a high SR while 15-30% have a medium SR and 10-16% have a low SR (Taberner & Liberman 2005). Low SRF are known to respond to loud sounds and relatively high sound pressure levels while high and medium SRFs respond at threshold and relatively low sound pressure levels (Heut et al 2016). The level of sound to which they fire is not the only difference between low, medium, and high SRFs. In general, the low SR ribbons are larger than high SR ribbons (Moser et al. 2006). In terms of location of the synaptic ribbons on the inner hair cells, histological analysis has shown the low and medium SRFs are located near the modiolus while the high SRFs are located near the pillar cells (Furman et al. 2013). There are more ribbon synapses found in mammals on the side of the IHCs near the modiolus than on the side near the pillar cells (Liberman & Liberman 2015).

The importance of low SRF for encoding and understanding of speech in noise has been demonstrated in both animals and humans. A study in gerbils conducted by Heut et al. (2016) has

shown the low SRF to be more resistant to noise than medium or high-rate fibers which saturate in the presence of noise. This was accomplished by recording the rate-intensity function of neural fibers (Huet et al. 2016). These results indicate low SRFs likely play a crucial role in encoding auditory stimuli in the presence of noise by continuing to fire to the stimulus regardless of background noise presence and levels. In humans, it has been shown individuals with a higher likelihood of noise exposure exhibit differences in temporal processing as compared to those with a lower likelihood when presented with suprathreshold stimuli (Bharadwaj et al. 2015). The authors suggest that low SRFs play a role in auditory perception in the presence of noise and other competing signals. Due to the importance of the low, medium, and high SRFs for encoding the various aspects of environmental sounds, different electrophysiological measures have been explored for assessing the integrity of such fibers and the auditory nerve as a whole.

Electrophysiological Measures of the Auditory System. Auditory brainstem responses (ABR) and electrocochleography (ECochG) are different electrophysiological methods which can be used to assess the integrity of the auditory nerve. ABR responses are auditory evoked responses which follow a time locked waveform where wave I can be expected to arise—on a very generalized level—from the compound action potential (CAP) of the distal auditory nerve, wave II from the proximal auditory nerve, wave III from the superior olivary complex, wave IV from the lateral lemniscus, and wave V from the inferior colliculus (Parkkonen et al. 2009). Due to the complexity of the auditory system, and the far field recording used to obtain ABR responses in humans, this explanation cannot encompass all neural elements which contribute to each waveform in the response. However, present responses with normal latencies would confirm functionality of all the generator sites in the auditory pathway thus described.

ECochG, in response to an auditory stimulus, such as a click or toneburst, is

characterized by the presence of three notable features on the response. The cochlear microphonic (CM) is a response generated mostly from the OHCs of the cochlea and is mainly representative of the base of the cochlea (Ferarro & Durrant 2006; Hall 2015). The CM response will follow the wave of the stimulus and is an alternating current response (Ferraro & Durrant 2006). The summating potential (SP) is said to be generated mainly by the IHCs of the cochlea and is a direct current response (Ferarro & Durrant 2006; Zheng et al. 1997). Lastly, the action potential (AP) is a neural response from the cochlear nerve and is therefore the same response as ABR wave I (Ferarro & Durrant 2006; Zheng et al. 1997). ECochG allows for more near field measures of the ABR wave I with the use electrodes such as the tiptrode electrode placed in the external auditory canal, the tympanic membrane electrode (TM electrode) placed on the tympanic membrane, or the trans-tympanic electrode placed on the promontory (Ferarro & Durrant 2006). ECochG responses have been shown to be less variable in humans than ABR responses due to the near field recording allowing for larger wave amplitudes (Ferraro & Ferguson 1989). With the near field electrodes which are used as recoding electrodes in ECochG, it is possible to track ABR wave I when this response is too small to be seen with the forehead and mastoid electrode placement typical of ABR testing (Ferraro & Ferguson 1989; Kaf et al. 2017). Since ECochG allows for the tracking of the neural responses which are too small to be seen on ABR recordings, this measure can be beneficial when a small CAP response would be expected.

Noise Induced Hearing Loss

Hearing loss due to noise exposure has been well documented and studied. Historically, it was believed that noise induces hearing loss is due almost solely to damage to the OHCs, which

has been seen in the form of a peripheral threshold shift after exposure to damaging noise (Le et al. 2017; Liberman et al. 1978). Damage happens to the higher frequencies first, specifically around 3-4 kHz (Liberman & Kiang 1978). This shift can be either temporary or permanent depending on the amount and duration of exposure (Nordmann et al. 2000). Generally, with a TTS, when the thresholds return to normal with time after the noise exposure, it was assumed there is no damage to the auditory system on a permanent level. This is due to the fact that damage is not detectable via audiometric testing after hearing thresholds have returned to normal (Nordmann et al. 2000). Recent research suggests this may not actually be the case. It is possible that damage to the auditory system that is not easily seen on a standard audiogram occurs with noise exposure. This form of damage is thus called hidden hearing loss (HHL), noise induced hidden hearing loss (NIHHL), or cochlear synaptopathy (CS) (Furman et al. 2013; Kujawa & Liberman 2009; Liberman et al. 2016; Lin et al. 2011; Liu et al. 2019). It is vital to further explore this idea, especially in humans, in the attempt to find ways to limit damage to the auditory system due to noise exposure and improve management plans for those with NIHHL.

Evidence for Noise Induced Hidden Hearing Loss in Animals

Noise induced hidden hearing loss has been demonstrated more frequently in animals than humans due to the ability to control potential confounding variables in animal studies. For example, with an animal study assessing damage caused by noise exposure, the exact duration and intensity of the noise can be controlled as is not possible in human studies. Such studies in animals have helped to confirm the existence of NIHHL after noise exposure. A study by Mulders et al. (2018) helps to support this existence of NIHHL in guinea pigs by exposing one ear of each animal to damaging noise and then measuring the CAP thresholds for each animal at

different intervals post exposure, as well as both CAP and SP input/output functions. The reduced CAP and SP amplitudes, as seen in the input/output functions, despite the recovered CAP threshold indicate a neural dysfunction from noise exposure that would not be apparent on a test of hearing thresholds (Mulders et al. 2018). Myriad other studies support similar findings demonstrating normal hearing thresholds, or a recovery of normal hearing thresholds, after noise exposure despite a decrease in the neural output in response to acoustic stimuli at a supra-threshold level (Furman et al. 2013; Kujawa & Liberman 2009; Lin et al. 2011; Liu et al. 2019). These studies therefore indicates that tests of hearing thresholds may not tell the full story of damage to the auditory system from hearing loss.

NIHHL Site of Lesion. The site of lesion causing NIHHL in animals has been explored extensively. One particular study by Kujawa and Liberman (2009) demonstrated exposing mice to noise lead to a reduction of ABR and CAP amplitudes which failed to return to their original values after distortion product otoacoustic emission (DPOAE) amplitudes did. These results suggest the damage to the auditory system is beyond the outer hair cells, specifically in the neurons. Further exploration into the cochleae of the mice revealed while hair cells were not reduced in number post noise exposure, the numbers of IHC ribbon synapses were reduced. Additionally, the authors noted while the damage to the IHC synapses was immediate after noise exposure, the damage to the spiral ganglion cells was delayed; however after two years, the amount of damage seen with each structure—the IHC synapses and spiral ganglion cells— was similar. This damage to the spiral ganglion cells was shown to be permanent in adult mice. Overall, it is demonstrated the ribbon synapses of the IHCs to be the site of lesion for NIHHL.

Damage to the Ribbon Synapse. Other potential sites of lesion have been examined for NIHHL. A study by Liu et al. (2019) exposed mice to broadband noise, following which they

assessed the ABR thresholds and amplitude as compared to the pre-exposure ABR responses and then assessed the status of the nuclei of the inner hair cells of the mice. Those authors showed the ABR thresholds were elevated post exposure then returned to pre-exposure thresholds within 14 days. However, for ABR wave I amplitude, exposure to noise lead to a decrease in amplitude which failed to recover to pre-exposure values. Failure of suprathreshold ABR wave I amplitude to return to normal despite DPOAE and ABR threshold recovery was also demonstrated in guinea pigs (Furman et al. 2013). Note that the generator site of wave I of the ABR is the afferent auditory nerve fibers (Parkonnen et al. 2009). Upon examination of the nuclei of the IHC in noise exposed animals, no evidence was found of IHC loss due to the noise exposure (Lin et al. 2011; Liu et al. 2019). Such results further enforce the IHC synapses as the site of lesion of NIHHL, thus causing a reduction in the wave I amplitude of the ABR.

Confounding results have been reported when investigating the potential for regeneration of the ribbon synapses after damage. Liu et al. (2019) reported mice exposed to moderate noise showed, after several days, a partial recovery of the number of ribbon synapses in the apical IHCs. A complete recovery of the number of ribbon synapses was seen after 14 days. These results were found in conjunction with a reduction of ABR wave I amplitude which failed to recover even at 14 days. Conversely, Kujawa and Liberman (2009) found after noise exposure, the ribbon synapses in mice in the 32 kHz region was decreased to around 40% of the original count and recovered to around 50% of the original count within a week. Notably, the loss of ribbons in the base of the cochlea was much greater than the loss in the apex of the cochlea, where the loss of ribbons was not found to be significant. Lin et al. (2011) demonstrated guinea pigs exposed to noise had a reduction of around 55% of the number of ribbon synapses in the base of the cochlea. Overall, the potential for loss of synaptic ribbons in different areas of the

cochlea, as well as the possibility of ribbons regenerating, has not been conclusively reported. Additionally, it could be possible the opportunities for both loss and regeneration of ribbon synapses differ among species of mammals.

It is possible noise exposure not only causes loss of ribbon synapses, but also leads to a change in existing synapses. Following noise exposure in mice, the ribbons which remained were reported to be larger than typical and moved toward the nucleus of the cell (Kujawa & Liberman 2009; Liberman & Liberman 2015; Lin et al. 2011). Ribbon size increase after noise exposure was also found in guinea pigs (Furman et al. 2013). Furthermore, the remaining ribbon synapses were enlarged for the IHCs with greater reduction in the high frequencies where the TTS after the noise exposure was found to be the greatest (Kujawa & Liberman 2009). Similar enlargement of ribbons was seen in noise exposed mice by Liberman and Liberman (2015). Such results would indicate a change in the structure of the IHC ribbon synapses due to the exposure to noise. Notably, it has been shown this change in ribbon size is not reversible in mice (Liberman & Liberman 2015). Additionally, the number of ribbon synapses not associated with nerve terminals doubles in mice exposed to noise compared to unexposed mice; going from around 20% in unexposed ears to 40% in exposed ears (Lin et al. 2011). In guinea pigs, the number of ribbon synapses without associated nerve terminals after noise exposure was also double, however only around 2% of ribbons had no associated terminal in unexposed ears and 4% in exposed ears (Furman et al. 2013). It is possible the ribbons which have moved from their typical location are no longer in an optimal position to function as a synapse (Lin et al. 2011). Therefore, while noise exposure can lead to the loss of ribbon synapses, there is also a high likelihood of morphological changes to the remaining synapses affecting the transmission of auditory signals along the pathway. Given this, NIHHL could be due to a combined effect of the loss of synapses

as well as the change in the function of the ribbon synapses which remain, and simple counts of remaining ribbons after noise exposure may not illustrate the extent of the damage.

Damage to the Nerve Fibers. The damage from NIHHL extends beyond the ribbon synapse to neurons. Kujawa and Liberman (2009) found that after noise exposure in mice, there was a quick loss to the terminals of the neurons which communicated with the lost ribbon synapses, following which a slow death of the cell body occurred. Furthermore, when examining the numbers of spiral ganglion cells persisting two weeks after noise exposure, there was negligible loss, which was followed by a sizeable loss of the cells from the base of the cochleae at a year post-exposure. At two years post-exposure, only roughly 50% of the original spiral ganglion cells persisted in the 32 kHz region of the cochlea for exposed animals as compared to a loss of less than 10% in control animals. Kujawa and Liberman (2009) found the loss of spiral ganglion cells corresponded with the amount of ribbon synapse loss in the frequency region, as well as the reduction in suprathreshold click ABR amplitude which was greater than 50%. Given these results, it becomes apparent the loss of the ribbon synapses can lead to the loss of the terminals of the cochlear nerve which leads then to degeneration of the nerve itself. The authors suggest this damage cannot be reversed. The failure of the cochlear nerve cells to regenerate is supported by Lin et al. (2011) who found a delayed loss of the spiral ganglion nerve cells in noise exposed guinea pigs. These studies demonstrate a long-term effect of noise exposure, despite recovered thresholds (Kujawa & Liberman 2009; Lin et al. 2011). As this loss of spiral ganglion cells was shown in different mammal species, it is likely such degeneration also is present in humans, though could not be observed without temporal bone analysis.

To better understand how neural degeneration can occur in the spiral ganglion nerve cells while auditory thresholds can remain unaffected, it is necessary to delve deeper into the different

types of auditory nerve fibers. Given that high and medium SRFs fire to low level stimuli, or those near threshold, and low SRFs fire to suprathreshold sounds, it can be inferred the damage seen in NIHHL is damage to the low SRFs (Heut et al 2016). Such selective damage to low SRFs following noise exposure was demonstrated via a significant loss of low SRFs fibers in guinea pigs after noise exposure which induced a TTS and resulted in a suprathreshold decrease in ABR wave I amplitude (Furman et al. 2013). Similarly, Liberman and Liberman (2015) found that in noise exposed mice, there was synaptic loss with the majority of loss on the modiolar side, which is where the low spontaneous rate neurons are housed. Damage which is focused on low rate neural fibers helps to better explain what has been seen in electrophysiological measures of animals exposed to noise in terms of neural threshold recovery despite evidence neural damage at a suprathreshold level.

To explain damage to the low spontaneous rate synapses and fibers without damage to the OHCs, it must be considered the synapses are damaged by noise levels which are not sufficient to cause permanent damage to the OHCs. Repeated demonstration of this concept has been seen in animals with a return of DPOAE thresholds despite changes in suprathreshold ABR responses (Furman et al. 2013; Kujawa & Liberman 2009; Liberman & Liberman 2015; Lin et al. 2011; Liu et al. 2019). Additionally, the noise has been shown to not be sufficient to damage the high SRFs as shown by a study in gerbils where after the animals were exposed to noise the ABR thresholds had returned to normal (Furman et al. 2013). In these animals the auditory nerve thresholds of the high SRFs were similar to that of unexposed ears, the auditory nerve thresholds of the medium SRFs were elevated slightly, and the auditory nerve threshold for the low SRFs were reported to be challenging to assess based off of the large loss of fibers (Furman et al. 2013). It is possible the amount of noise was therefor enough to cause damage to the low SRFs,

only insignificantly affect the medium SRFs, and not affect the high SRFs. Additionally, these results can be used to enforce the fact the low spontaneous rate ribbon synapses and fibers do not contribute to auditory thresholds.

Selective Damage of Low Spontaneous Rate Fibers. While the ribbon synapses of low rate auditory nerve fibers have been shown to be the primary site of lesion for NIHHL, the mechanism behind the damage to the ribbons caused by noise exposure is less clear. Excitotoxicity has been suggested as a trigger for such damage (Huet et al. 2016; Nouvian et al. 2006). As the high SRFs contain more glutamate transporters, the low SRFs are more susceptible to an overabundance of glutamate during extended neural firing which would lead to excitotoxicity (Nouvian et al. 2006; Ruel et al. 2007). The excitotoxicity has been shown to affect the synapses while leaving the IHCs themselves to appear unaffected (Ruel et al. 2007). Such extended neural firing could occur in instances such as the presence of background noise. Additionally, it was found in gerbils that an ouabain injected into the cochlea did not affect high spontaneous rate—low threshold—fibers while destroying low spontaneous rate—high threshold—fibers (Huet et al. 2016). Susceptibility to the substance ouabain could be due to the low spontaneous rate fibers containing reduced number of mitochondria as opposed to high rate fibers, or to the axon diameter of the low SRFs being smaller (Huet et al. 2016).

To further support that low SRFs are more vulnerable to ototoxicity, Bourien et al. (2014) investigated the effects of applying ouabain to the round window of gerbils and guinea pigs. When 10-33 μM of ouabain was used, there was no change in the CAP input/output function, however when 66 μM of ouabain was used, there was a reduction seen in the amplitude of the CAP while the thresholds remained unaffected. Furthermore, as the ouabain was increased to 80-100 μM, CAP amplitudes were decreased further, and threshold shifts were seen. The results

show low to mild amounts of ototoxic drugs fail to damage the neurons responsible for thresholds while affecting the suprathreshold response. Notably, the shifts in threshold and reduction in CAP amplitude was seen more in the high frequency fibers as opposed to the low frequency fibers, however it was suggested this was due to the location to which the ouabain was applied. It remains possible the neural fibers in the basal end of the cochlea are more susceptible to damage than those in the apex. This study also included recordings from single nerve fibers. When comparing the cochleae of animals which received ouabain to control animals, it was found the area most damaged by ouabain was also the area in which the low SRFs were located. Additionally, this study then reports there is a larger number of low SRFs in the base as opposed to the apex of control gerbils. Overall, it can be suggested the low SRFs are more susceptible to ototoxicity, and as there are more low SRF found in the base of the cochlea, the effects of such neural loss could be seen more in high frequency responses.

Damage to the auditory system that is limited to the slow SRF ribbon synapses—and eventually the neural fibers to which they communicate—affects how the system is able to process incoming sound. Furman et al. (2013) suggest low SRFs must be utilized for optimal encoding of auditory signals in background noise. In relation to the selective loss of low SRF synapse loss, Kujawa and Liberman (2009) suggested the loss of the neural transmission from low SRF synapses would affect hearing when there is low signal to noise ratio because of lack of summation from the affected neurons in response to the stimulus. In other words, the remaining fibers after noise exposure may not be adequate to encode the signal with integrity in the presence of noise.

Noise Induced Hidden Hearing Loss in Humans

Unlike animal studies, it is not possible to control the noise exposure in humans to make an exact measure of the damage to the auditory system based off of exposure level. Instead, for studies assessing the presence of NIHHL in humans, or any form of noise induced hearing loss for that matter, it is necessary to go off of participant report of history of noise exposure. This can be performed through many different methods such as interviews or questionnaires. Additionally, unlike in animal studies, histological assessment of changes in the auditory system from the hair cells to the neural fibers is not always possible in humans. Hence, the assessment of the presence of NIHHL and the mechanism behind it in humans in complicated. Many audiological and electrophysiological tests have been suggested to clinically assess NIHHL in humans.

NIHHL and Speech Understanding. The loss of slow SRF synapses and neurons which characterize NIHHL have an effect on the auditory system. Specifically, with the loss of neurons responsible for coding suprathreshold sounds, a difference in coding would be expected to be seen on a subjective level with both the electrophysical measures and measures of speech and temporal processing. Liberman et al. (2016) demonstrated such an effect in participants who were at high risk for noise exposure. When the NU-6 word lists were presented to a single ear at 35 dB HL, there was no significant difference between the low and high risk group's scores. When noise was presented simultaneously with the NU-6 word lists at both a 0 and 5 dB SNR, the high risk group scored significantly lower in word recognition than the low risk group. Furthermore, those researchers found a significant difference in word recognition scores, with the high risk group performing inferiorly, when the NU-6 word lists were compressed temporally by both 45% and 65% and reverberation was created with a 0.3 second time delay. Overall, the SP/AP amplitude ratio correlated with word recognition scores in noise and reverberation

conditions. Results show noise exposure, and subsequent changes to the neural response to auditory stimuli in humans with suspected NIHHL, lead to a change in the ability to perceive speech in noise. This is significant in the indication NIHHL can affect the auditory abilities of those exposed to noise, despite normal audiograms.

ABR to Detect NIHHL in Humans. ABR wave I amplitude may indicate NIHHL in humans as it has been used to do in animal studies. A study by Bramhall et al. (2016) demonstrated a difference in suprathreshold ABR wave I amplitudes for Veterans with self-reported high noise exposure as opposed to non-Veterans and Veterans with self-reported low noise exposure. It is necessary to note all participants in the Bramhall et al. (2016) study had hearing thresholds within normal limits. Originally, the participants were grouped by low and high noise exposure via a questionnaire, however an interview about past noise exposure revealed numerous participants in the non-Veteran group had firearm exposure which was not revealed in answering the questionnaire and lead to the creation of a non-Veteran high noise exposure group. Participants answering similar questions about noise exposure differently when the questions were presented in differing formats indicate a need to delve deeper into the noise exposure history when testing human participants. This could be done by ensuring participants fully comprehend questions presented in questionnaire format, or by utilizing various formats of taking noise history from participants. Overall, Bramhall et al. (2016) found the two high noise exposure groups had the smallest ABR wave I amplitudes in response to each a 1, 3, 4, and 6 kHz tone burst stimulus presented at 110 dB p-peSPL with the veteran high noise exposure group displaying the smallest wave I amplitudes overall. Additionally, when each the 1 kHz and 4 kHz tone burst ABR were presented at increasing intensity in 10 dB steps from 80-110 dB p-pe SPL, greater differences in amplitude for the high noise exposure groups compared to the low

noise exposure groups was found as the intensity was increased. Interestingly, the amplitude difference between the group of veterans who reported high noise exposure as compared to the non-Veteran group was only seen as a 29% decrease which is not as large of a reduction as what has been reported with animals. Animal studies have reported a difference in noise exposed and non-noise exposed group ABR amplitudes to be up to 40% (Kujawa & Liberman 2009). Despite difference in the ABR wave I amplitude reduction in participants who were suspected to be at risk for NIHHL as compared to the reduction seen in animals, these results suggest impact noise could be a cause for NIHHL in humans. It is possible the differences between animals and humans could be explained by the types of noise exposure experiences. While in animal studies noise is controlled in terms of exposure amount and frequency range, this does not hold true for humans, especially when the noise exposure in question is a blast such as from a gunshot.

ABR has also been used to explore a possible link between tinnitus and NIHHL in humans. Schaette and McAlpine (2011), in their study of female participants with normal hearing thresholds from 0.25-8 kHz, found the group who reported tinnitus had ABR wave I amplitudes which were notably reduced in comparison to the group who reported not experiencing tinnitus. The reduction seen in the amplitude of wave I— in response to click ABR at both 90 and 100 dB SPL—indicates a reduction in neural firing, and thus hints at NIHHL. The authors suggest a change in the number of functioning auditory nerve fibers in the human cochlea could lead to the perception of tinnitus. Contrarily, between groups there was not a significant difference in the amplitude of ABR wave V. Therefore, while neural firing is reduced at the level of the auditory nerve, there appears to be some form of compensation for this reduced neural activity in a higher center of the auditory system. The results of this study may need to be interpreted with a degree of caution, however, as the functioning of the OHCs was not

confirmed. While it may not be likely, it remains possible the participant in the tinnitus group, despite hearing thresholds within normal limits, have damage to the OHCs leading to the perception of tinnitus and a reduction of the output of the auditory nerve.

Controversies about NIHHL and ABR in Humans. Many studies have utilized ABR responses to provide evidence NIHHL occurs in humans—and even possibly lends to the perception of tinnitus—some studies, however, fail to support the presence of NIHHL in humans. A study by Prendergast et al. (2016) looked at both ABR and frequency following responses (FFR) to probe for the loss of synapses which characterizes NIHHL in humans. This study divided 126 participants by both lifetime noise exposure and gender. All participants had hearing thresholds within normal limits from 0.25-8 kHz as well as at 16 kHz. While the authors reported technical difficulties limited the testing of transient evoked otoacoustic emissions (TEOAEs) to only 79 participants, a relationship was not seen between the participants' noise exposure history and the signal to noise ratio of the TEOAE response. The lack of relationship between TEOAE responses and reported noise exposure suggests the high noise exposure group does not have a disproportionate amount of OHC damage as compared to the low noise exposure group. As all participants were not able to be tested however, it is possible a relationship was missed. Furthermore, Prendergast et al. (2016) recorded ABR responses to click stimuli at 80 and 100 dB peSPL. There was no significant difference in wave I, III, and V amplitudes found between the male or female high noise exposure groups compared to the male and female low noise exposure groups. Additionally, the researchers found no significant difference in the FFR response signal to noise ratio between groups. Such results could demonstrate a lack of NIHHL in humans or could be the product of the assessments being examined, specifically ABR wave I amplitude and FFR response, not being sensitive to the presence of NIHHL.

Another study by Guest et al. (2017) investigated the high-pass click ABR and EFR responses of those with tinnitus matched to those without and also took into account each individual's reported lifetime noise exposure. While the group who reported tinnitus did have increased levels of reported noise exposure, neither ABR wave I amplitude nor wave I to V amplitude ratio was found to be significantly different between the groups when the stimulus was presented at 102 dB peSPL. Additionally, there was no significant difference found with EFR responses between groups. Similarly, no correlation was found between noise exposure and ABR or EFR responses. Such results indicate no relationship between ABR and EFR responses and NIHHL and no relationship between tinnitus and the same electrophysiological responses. This would show a lack of NIHHL in humans thus implying tinnitus in conjunction with normal hearing thresholds is not caused by damage to the cochlear synapses or nerve fibers. At the same time, it could be possible the measures used were simply unfit for the assessment of NIHHL in humans. Conflicting results regarding the potential for NIHHL in humans, as well as appropriate methods to measure the presence of NIHHL are evidence for a need for further research in the field.

ECochG to Detect NIHHL in Humans. On the other hand, ABR is not the only electrophysiological measure which has been explored for usefulness in supporting the presence of NIHHL in humans. The SP/AP amplitude ratio in ECochG was used by Liberman et al. (2016) to search for differences in participants who rated themselves as low risk for damage to the auditory system as compared to those who rated themselves as high risk for such damage. All participants had normal DPOAE responses and hearing within normal limits from 0.25-8 kHz, however, the high-risk group had thresholds which were elevated as compared to those in the low risk group from 9-16 kHz. Liberman et al. (2016) hypothesized an increase of SP/AP

amplitude would be seen in the high-risk group when comparing it to the low risk group as would be caused by the same damage to the low rate ribbon synapses which characterizes NIHHL. Those researchers recorded ECochG to a 94.5 dB nHL click stimulus using a tiptrode and reported the SP/AP amplitude ratio of the high-risk group to be about double the SP/AP ratio of the low-risk group. Interestingly, the change in ratio was attributed to an increase in the SP amplitude as the decrease to the amplitude of the AP for the high-risk group was not found to be significant. A significant decrease in AP amplitude would be expected to be seen with damage to the nerve synapses, and the SP, as a pre-neural response, would be expected to remain unaffected. While it is unclear why an increase in SP amplitude was recorded, the increase in the SP/AP amplitude ratio was seen in the high-risk group and was attributed to the presence of NIHHL. Liberman et al. (2016) recorded ECochG using two presentation rates (9.1 to 40.1 Hz) but did not specify any comparison or differences between groups.

Effect of ECochG Stimulus Rate. Typically, ECochG is recorded at a slow rate, such as a rate at or below 11.3 stimuli/second, to ensure the response is complete prior to the presentation of the next stimulus (Ferraro & Durrant 2006). Presenting at a significantly higher stimulus rate could lead to overlap of responses, however can be done clinically. With an increase in stimulus presentation rate, as shown by Kaf et al. (2017), an increase of SP/AP amplitude ratio would be expected to be seen in humans. As the SP is generated in the IHCs it would be expected to maintain amplitude due to the IHCs not becoming stressed when asked to fire repetitively (Kaf et al. 2017; Zheng et al. 1997). Since the AP is a neural response, the decrease in response amplitude would be expected due to adaptation of the neurons with increased stimulus presentation rate (Kaf et al. 2017; Zheng et al. 1997). Additionally, increased rate would be expected to cause an increase in AP latency—while the SP latency is unaffected— creating

greater separation between the SP and AP and making each easier to identify (Kaf et al. 2017). It could be hypothesized that in participants with a loss of neural fibers due to NIHHL, the AP amplitude adaptation would be affected as compared to controls while the SP amplitude would not be significantly different.

As a way to record electrophysiological measures at increased rates and overcome the overlapping of responses thus resulting in the formation of a complex wave, the continuous loop averaging deconvolution (CLAD) technique has been developed (Delgato & Ozdamar 2003; Kaf et al. 2017). This technique utilizes a CLAD algorithm which allows for multiple responses to be recorded at a fast rate so that they can be averaged to improve the SNR of the response tracing, while overcoming the complications of a complex waveform of overlapped responses via deconvolution (Delgato & Ozdamar 2003). The benefit of testing electrophysiological responses at a fast rate is the ability to assess the adaptation of the auditory pathway by stressing the ANFs (Delgato & Ozdamar 2003; Kaf et al. 2017). CLAD therefore is a means to assess changes in the amplitude and latency of the AP due to adaptation (Kaf et al. 2017).

Effect of ECochG Stimulus Intensity. Changes in the intensity of the stimulus used to elicit ECochG responses will also have an effect on the AP amplitude. As the stimulus intensity is increased, an increase in the amplitude of the AP can be expected in conjunction with a decrease in AP latency (Schoonhoven et al. 1995). Conversely, as the SP is not a neural response, it would be expected there would be no shift in latency seen with an increase in stimulus intensity, but an increase in response amplitude would be seen (Ferarro & Durrant 2006; Zheng et al. 1997). The increase in response amplitude seen with an increase in stimulus intensity is due to both an increase in the firing rate of the neurons, and recruitment of a larger number of nerves to fire in response to the stimulus (Huet et al. 2016). Measuring ECochG

responses as various intensity levels can give insight into the functioning of the cochlear nerve by looking at the AP amplitude changes. Specifically, it could be hypothesized damage to low SRFs may be seen as a growth of AP amplitude smaller than typical growth with an increase in intensity. This would be due to a loss of low SRFs which are expected to fire in response to high intensity and increase in firing rate as the intensity of the stimulus is increased.

OBJECTIVES

Numerous animal studies have demonstrated evidence of decreased neural output to supra-threshold stimuli after noise exposure, despite CAP thresholds being normal (Furman et al. 2013; Kujawa & Liberman 2009; Lin et al. 2011; Liu et al. 2019; Mulders et al. 2018). This damage to the auditory system, NIHHL, has not been conclusively supported in human studies. However, many studies in humans support the presence of such neural damage to the auditory system, despite normal audiograms, in participants with noise exposure (Bramhall et al. 2020; Liberman et al. 2016; Schaette & McAlpine 2011).

Furthermore, the NIHHL supported in these studies has been shown to lead to tinnitus and difficulties with speech in noise (Bharadwaj et al. 2015; Schaette & McAlpine 2011). As young adults are likely to have a history of noise exposure through common activities such as listening to music through headphones, leisure activities like concerts, and certain work environments for young adults, NIHHL could be prevalent in this population. NIHHL is therefore a probable contributing factor to the complaints of young adults who report difficulty hearing in noise, as well as complaints of tinnitus. For these reasons, a diagnostic tool for NIHHL in humans would be of great value.

The results reported by Liberman et al. (2016) are promising in the use of the SP/AP amplitude ratio of ECochG for NIHHL in humans. Specifically, ECochG may be more successful for assessing the presence of NIHHL in humans; however more research is required. Currently, there are no available studies in the literature that investigate the effect of both rate and intensity of stimulus on ECochG responses of patients at risk for NIHHL. This could be a valid way to assess the presence of NIHHL in these patients. To do this, CLAD could be an

invaluable tool, as it would allow for the assessment of neural adaptation to high right stimuli to be assessed.

The aim of this study is to investigate ECochG as a method to diagnose the presence of NIHHL in young adults who have hearing within normal limits. To accomplish this, a relationship between stimulus rate and intensity and the AP amplitude of the ECochG will be assessed, as well as the relationship between the changes in ECochG SP/AP amplitude ratio and reported noise exposure history of the patients. The null hypothesis is: 1) no significant difference exists between the SP/AP amplitude ratio of the low and high risk groups, 2) no significant difference exists between the AP amplitude of the low and high risk groups with an increase in stimulus rate 3) no significant difference exists between the AP amplitude of the low and high risk groups with a decrease in stimulus intensity. The alternative hypothesis is: 1) a significant difference exists between the SP/AP amplitude ratio of the low and high risk groups, 2) a significant difference exists between the AP amplitude of the low and high risk groups with an increase in stimulus rate, 3) a significant difference exists between the AP amplitude of the low and high risk groups with a decrease in stimulus intensity.

METHODS

Participants

Young adults, ages 18-30 were recruited from the Missouri State University campus to participate in this study. Flyers were hung around campus and emails were used to recruit participants. Participants were healthy and reported no significant otologic history or hearing loss. The inclusion criteria included: 1) normal audiometric thresholds from 0.25-8 kHz 2) normal 226 Hz tympanometry 3) present and normal ipsilateral and contralateral acoustic reflexes 4) normal otoscopic exam 5) present and normal DPOAE responses from 1500-8000 Hz 6) present and recordable ECochG responses in the right ear. Participants were separated into groups of high and low risk of noise exposure based off of self-reported history of noise exposure via the Noise Exposure Questionnaire (NEQ) and informal interview. All participants gave voluntary consent prior to participating in this study. Testing was completed in the audiology research laboratory on the Missouri State University Campus. IRB approval for this study was received on 11/10/2020 (see appendix for IRB approval: IRB-FY2021-267).

Participant Grouping. Participants were separated by their reported noise exposure history into a low-risk and high-risk group. Participants were assigned to the low-risk group if they did not report history of TTS and had <100% dose on the NEQ which was completed by all participants. This questionnaire is focused only on noise exposure from the past 12 months (Johnson et al. 2017). Participants who had >100% dose on the NEQ were assigned to the high-risk group regardless of history of TTS. Additionally, participants who reported experiencing TTS on two or more occasions were assigned to the high-risk group regardless of noise dose as calculated from NEQ responses.

Equipment

Otoscopy was performed using a Welch Allyn otoscope to visually assess the status of the external ear canal and the tympanic membrane (TM). Tympanometry was performed with an InteracoutsicsTitan to assess the middle ear status. Acoustic reflexes were performed with an Interacoustics Titan to assess the acoustic reflex pathway. Hearing thresholds were assessed in a sound treated booth using pure tone stimuli from 250-8000 Hz presented via a GSI AudioStar Pro audiometer with ER-3A insert earphones, and at ultra-high frequencies using circumaural headphones. DPOAEs were measured using the Intelligent Hearing Systems Corporation SmartDPOAE system. ECochG measurements were made using an Intelligent Hearing Systems Smart–Evoked Potential, version 5.10, with ER-3A insert earphones. EcochG was recorded using a TM electrode, which was handmade in the lab following the method by Ferraro and Durrant (2006). Taflon-insulated silver wire (0.008” bare diameter and 0.011” insulated diameter) with cotton tied to one end was inserted into medical grade silicon tubing (0.058” inner diameter and 0.077” outer diameter) to create the TM electrode. The cotton wad was socked in conductive gel, and a copper microalligator clip was attached to the other end of the TM electrode.

Audiometric Testing Procedures

Prior to audiometric testing, otoscopy was performed to ensure a clear view of an intact tympanic membrane. Tympanometry was performed using a Titan tympanometer with a probe tone of 226 Hz to ensure normal middle ear function, bilaterally. Ipsilateral and contralateral acoustic reflex thresholds were measured with a Titan tympanomter and 0.5, 1, 2, and 4 kHz, bilaterally. Audiometric thresholds were obtained with a Grason-Stadler Inc. AudioStar Pro

audiometer in a sound treated booth. Pure tone air conduction thresholds were obtained using ER-3A insert earphones at 0.25, 0.5 1, 2, 3, 4, 6, and 8 kHz, bilaterally. Ultra-high frequency pure tone air conduction thresholds were found bilaterally with circumaural headphones at 10 and 12.5 kHz. Word recognition scores were assessed bilaterally using insert earphones and Northwestern University Auditory Test Number 6 (NU-6) word lists of 50 words under two conditions: speech in quiet, speech in noise with 0 dB SNR. Speech testing was performed in each condition at both 40 dB SL and 60 dB SL as calculated based off of the participant's pure tone average. Pure tone average was calculated as the average of the puretone air conduction thresholds at 0.5, 1, and 2 kHz. For the speech in noise testing, a speech noise was utilized.

Electrophysiological Testing Parameters and Procedures

DPOAEs were assessed, bilaterally, in a sound treated booth. DPOAEs were elicited with an L_1 at 65 dB SPL and L_2 at 55 dB SLP and a frequency ratio (F_2/F_1) of 1.22. The primary frequencies (F_2) ranged from 1.5-8 kHz and were swept from high frequencies to low frequencies with two repetitions. ECochG was recorded bilaterally using SmartEP, version 5.10, from Intelligent Hearing Systems Corporation. ECochG was recorded via a single channel using a horizontal electrode montage. The non-inverting electrode was placed on the mastoid of the non-test ear, the ground electrode on low forehead (Fpz), and the inverting electrode was placed on the tympanic membrane of the test ear. For the ground and non-inverting electrodes, disposable surface electrodes were used. The inverting electrode was a homemade TM electrode. Electrode impedance was <7 KΩ for all electrodes. The stimulus used was a 100 μsec click presented at a rate of 11.1 clicks/sec, 37.1 clicks/sec, 58.59 clicks/sec, 78.13 clicks/sec, and 97.66 clicks/sec. Each click rate was presented at four intensity levels: 50, 60, 70, and 80 dB

nHL. The 11.1 clicks/sec and 37.1 clicks/sec recordings were run using a standard ECochG recording while the higher rates were recorded using the CLAD sequence from the SmartEP system (Delgato & Ozdamar 2004). A bandpass filter of 10-3000 Hz and a gain of 100,000x was used. Two traces of 1024 sweeps were obtained for each recording.

To prepare for ECochG, conducting gel was placed onto the TM electrodes. Participants were then seated in a reclining chair. Once the participant was comfortable in the chair, the participants' forehead and mastoid regions were scrubbed with an alcohol wipe followed by Nu-prep. Disposable electrodes were then placed on low forehead and both mastoids, and the TM electrodes were slowly placed into each ear until touching the tympanic membrane which was confirmed by the participants' report of hearing the electrode contact the tympanic membrane. Insert earphones were then placed in each ear, and the ends of the TM electrodes were secured by taping them down. An alligator clip was then attached to the end of the TM electrode, and impedances were measured for all electrodes. A continuous click stimulus was then presented bilaterally at 80 dB nHL, and participants were asked to verbally report if the sound is equally loud between ears to confirm the TM electrode was not affecting the ability of the stimulus to travel through the outer ear. Participants were instructed to relax and nap if possible. Recordings consisting of two repeatable traces were then obtained, beginning with the right ear, for each rate (11.1, 37.1, 58.59, 78.13, and 97.66 clicks/sec) at each intensity (50, 60, 70, 80 dB nHL) in a randomized order. The CLAD sequence was used for the stimulus rates of 58.59, 78.13, and 97.66 clicks/second. CLAD recordings were not deconvolved in real time but instead were deconvolved upon conclusion of testing, after the participant has left.

Data Analysis

All data analysis was performed offline by the researchers. For each recording, the two traces obtained were averaged to create an averaged response, the waveform of which was then labeled with the baseline, SP, and AP. The baseline to peak approach was utilized to label the waveforms to limit variability (Ferraro & Durant 2006; Kaf et al. 2017). This approach was completed by marking the baseline of the response at 0 msec, marking the SP at the highest deflection from baseline that falls with the first msec after the stimulus presentation, and marking the AP as the largest deflection from baseline between 1-2 msec from stimulus presentation. The amplitude of the SP was recorded as the distance between baseline and the SP peak, and the amplitude of the AP was recorded as the distance between baseline and the AP peak

Descriptive statistics were calculated and provided for each test under each condition. A two-way (5 x 4) repeated measures analysis of variance (ANOVA) with mixed effects was used to evaluate the effects of the stimulus rate and intensity on the AP amplitude for both groups. A one-way repeated measures ANOVA with mixed effects was used to evaluate the speech in noise performance between groups.

RESULTS

Behavioral audiometry thresholds, acoustic reflex thresholds, DPOAEs, word recognition in quiet, word recognition in noise, and ECochG recordings were obtained from the right ear of 25 participants (11 males, 14 females). The participants were divided into low-risk and high-risk for noise exposure groups based off of NEQ scores and self-reported case history questionnaire answers. The low-risk group was comprised of three males and eight females (n=11). The high-risk group was comprised of eight males and six females (n=14). Pure tone thresholds from 250-8000 Hz for all participants were found to be 25 dB HL or better at all frequencies tested indicating all participants have hearing thresholds within normal limits. Additionally, pure tone thresholds to ultra-high frequencies (10000 and 12500 Hz) were measured at 25 dB HL or better in all participants. An independent samples *t*-test revealed no significant difference in pure tone thresholds between groups for all frequencies tested (see Figure 1).

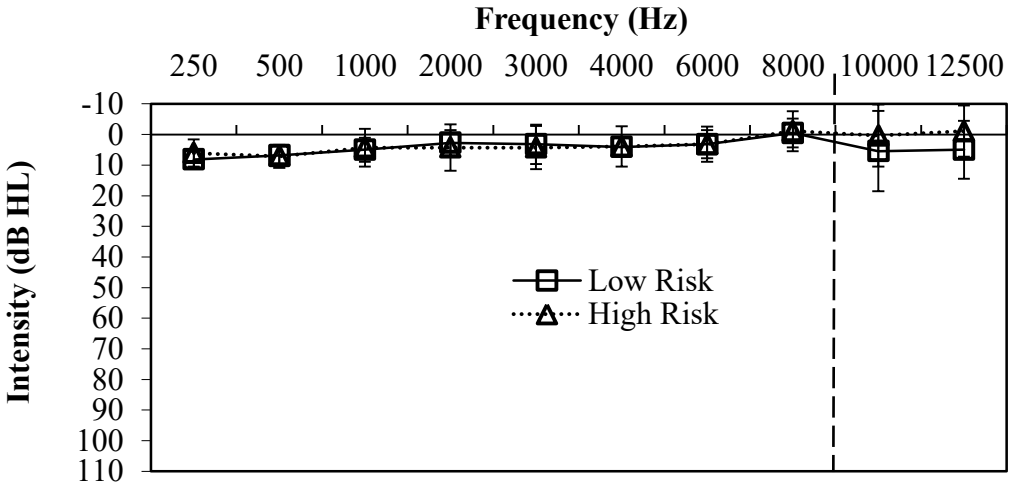


Figure 1. Mean pure tone air hearing thresholds for both groups (low-risk group is solid line and high-risk group is dotted line) from 250-12500Hz. No statistically significant difference was found between the hearing thresholds of the two groups. [±SD].

DPOAEs were tested from 1500-8000 Hz and were present within normal limits for all participants at all frequencies tested. An independent samples *t*-test revealed no significant difference in DPOAE responses between groups for all frequencies tested (see Figure 2). Acoustic reflex thresholds were obtained for all participants ipsilaterally at 500, 1000, 2000, and 4000 Hz and contralaterally at 500, 1000, 2000, and 4000 Hz. An independent samples *t*-test revealed no significant difference between groups for all frequencies tested with the exception of 500 Hz presented contralaterally which showed a statistical difference between the low-risk group (M= 83.18, SD= 4.04) and the high-risk group (M= 88.93, SD= 6.84) [$t(23) = -2.46$, $p < 0.05$]. However, the acoustic reflex thresholds for all participants at all frequencies and conditions tested were within normal limits (see Figure 3).

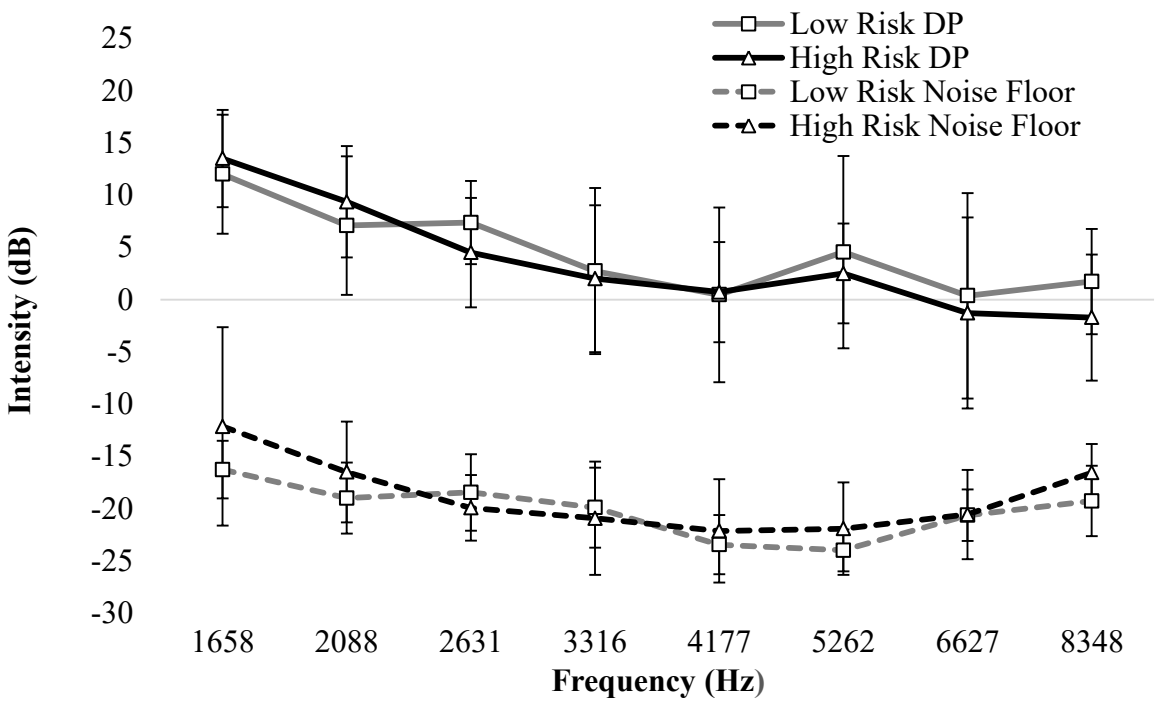


Figure 2. Mean DPOAE amplitudes (solid lines) and noise floor (dashed lines) for all frequencies tested for both the low-and high-risk groups. No significant difference in DPOAEs was found between the low and high-risk groups. [±SD]

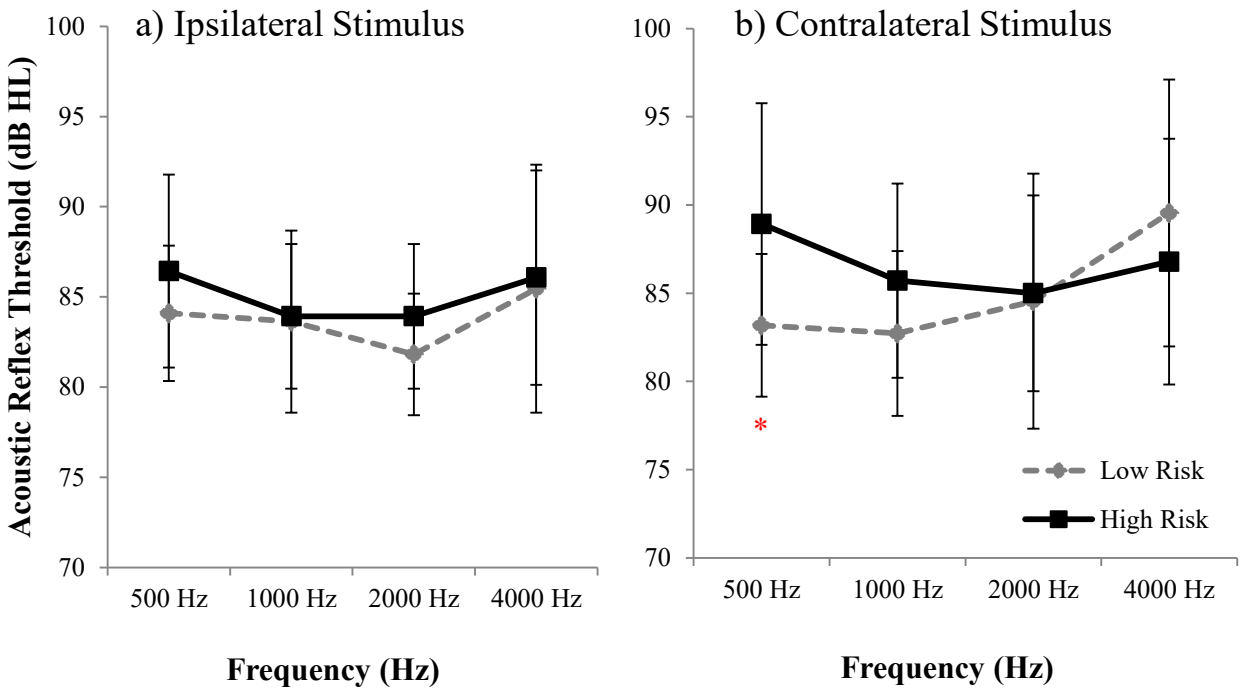


Figure 3. Mean acoustic reflex thresholds to ipsilateral and contralateral stimuli for both low-risk and high-risk groups. a) Mean ipsilateral acoustic reflex thresholds at 500, 1000, 2000, and 4000 Hz. The acoustic reflex thresholds of the low- and high-risks groups were similar at all stimulus frequencies. b) Mean contralateral acoustic reflex thresholds at 500, 1000, 2000, and 4000 kHz. The high-risk group had higher acoustic reflex threshold at 500 Hz compared to the low-risk group, while acoustic reflex thresholds are similar between both groups at 1000, 2000 and 4000 Hz. [*p< 0.05; ±SD]

Word Recognition in Quiet and Noise

Word recognition scores were obtained at two levels—at 40 and 60 dB SL—both in quiet and with 0 dB SNR for all participants. An independent samples *t*-test revealed a significant difference between the word recognition scores for the 40 dB SL speech in quiet condition for the low-risk (M= 99.81, SD= 0.60) and high-risk (M= 98.57, SD= 1.65) groups [t(23)= 2.37, p= 0.026], however the difference between scores between groups is not clinically significant. No significant difference was found between the word recognition scores for the 60 dB SL speech in quiet condition for the low-risk (M=99.09, SD= 1.38) and high-risk (M= 98.43, SD= 1.79)

groups [$t(23)= 1.015$, $p= 0.32$]. Additionally, no significant difference was found between the word recognition scores for the 40 dB SL speech in 0 dB SNR noise for the low-risk (M= 39.45, SD= 8.68) and high-risk (M= 41.14, SD= 6.74) groups [$t(23)= -0.55$, $p= 0.59$], and no significant difference was found between the word recognition scores for the 60 dB SL speech in 0 dB SNR noise condition for the low-risk (M=29.27, SD= 5.88) and high-risk (M= 28.57, SD= 5.29) groups [$t(23)= 0.31$, $p= 0.76$] (see Figure 4).

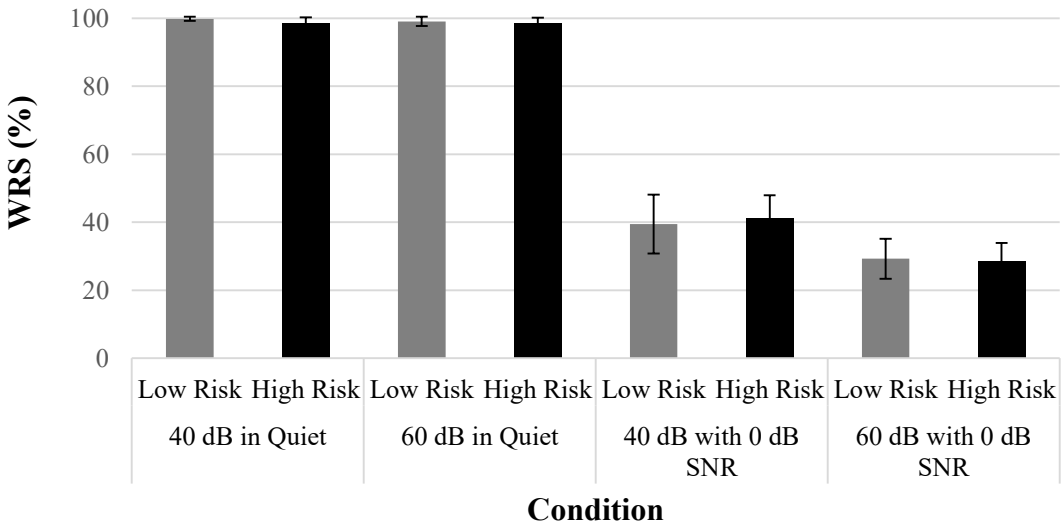
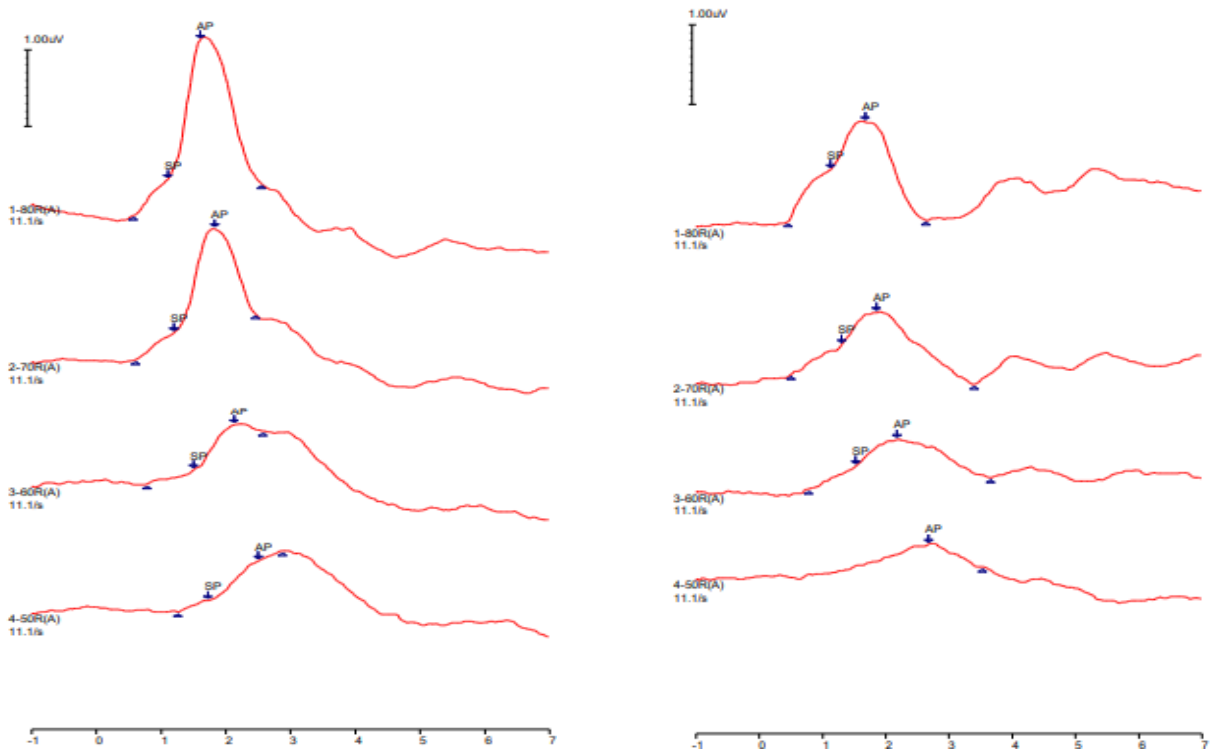


Figure 4. Average WRS for each condition tested for both the low and high-risk groups. Despite a statistically significant difference being found between the WRSs for the low and high-risk groups at 40 dB for speech in quiet, no clinically significant difference between groups was present at any condition. [±SD]

11.1/s Presentation Rate at 80 dB nHL

ECochG recordings were obtained at 80 dB nHL at a rate of 11.1/s. AP amplitude, SP amplitude, and SP/AP amplitude ratios were measured for all participants in both the low and high-risk groups at this rate, which was the slowest rate assessed, and intensity, which was the highest intensity assessed. Independent samples *t*-tests were performed to assess each of the AP

amplitude differences between groups, the SP amplitude differences between each group, and the SP/AP amplitude ratio differences between each group. No significant difference was found in AP amplitude between the low-risk group (M= 1.02 µV, SD 0.44) and the high-risk group (M= 0.78 µV, SD= 0.34) [$t(23)= 1.54$, $p= 0.14$]. No significant difference was found in SP amplitude between the low-risk group (M= 0.26 µV, SD= 0.04) and the high-risk group (M= 0.20 µV, SD= 0.05) [$t(23)= 0.85$, $p= 0.41$]. And lastly, no significant difference was found in SP/AP amplitude ratio between the low-risk group (M= 0.26, SD= 0.04) and the high-risk group (M= 0.25, SD= 0.04) [$t(23)= 0.34$, $p= 0.74$] (see Figure 5 for example of responses for each group).



Low Risk High Risk

Figure 5. ECochG recordings from a low-risk and high-risk participant at a rate of 11.1/s showing decreasing intensities from 80-50 dB nHL demonstrating the difference in AP amplitudes between low and high-risk participants.

Stimulus Presentation Rate and Intensity Level Effects on AP Amplitude

ECochG recordings were made at five different rates (11.1, 37.1, 58.59, 78.13, and 97.66 clicks/sec) and at four different intensity levels (50, 60, 70, and 80 dB nHL) at each of the five rates. Mean AP amplitude values for each rate and intensity for the low-risk group and the high-risk group can be found in Table 1. A two-way (5 x 4) repeated measures analysis of variance (ANOVA) with mixed effects was performed to evaluate the effects of the stimulus rate and intensity on the AP amplitude for both the low and high-risk groups. The between-subject analysis showed a significant main effect of noise risk group [$F(1, 23) = 5.88$, $p < 0.05$, $\eta_p^2 = 0.20$] (see Figure 6). The two-way (5 x 4) ANOVA analysis revealed a significant main effect of stimulus rate on the AP amplitude [$F(4, 92) = 28.19$, $p < 0.001$, $\eta_p^2 = 0.55$]. The results showed the AP amplitude decreased with increasing the presentation rate (see Figure 7). Post-hoc analysis using the Least Significant Difference (LSD) revealed a significant difference in AP amplitude between the different rates, as shown in Table 2, with the exceptions of the mean AP amplitude obtained at a rate of 58.59 compared to 78.13 [$p = 0.15$], and those obtained at a rate of 78.13 compared to 97.66 [$p = 0.23$]

The two-way (5 x 4) repeated measures ANOVA with mixed effects also showed a significant main effect of stimulus intensity on the AP amplitude [$F(3, 69) = 46.25$, $p < 0.001$, $\eta_p^2 = 0.67$]. The results showed the AP amplitude decreased with decreasing the intensity (see Figure 6). Post-hoc analysis using the LSD revealed significant difference in AP amplitude between the different intensities, as shown in Table 3, with the exception of the difference in AP amplitude at 60 compared to 50 dB nHL.

Furthermore, multiple interactions were evaluated. The two-way repeated measures ANOVA with mixed effects revealed a significant two-way interaction between presentation rate

and stimulus intensity [$F(12, 276) = 9.04$, $p< 0.001$, $\eta_p^2= 0.28$]. Additionally, the analysis revealed a marginal, yet not significant two-way interaction between presentation rate and noise exposure risk group [$F(4, 92) = 2.22$, $p=0.07$], and revealed a not significant two-way interaction between stimulus intensity and noise exposure risk group [F(3, 69) = 0.05, p=0.98]. Finally, the analysis revealed a not significant three-way interaction between presentation rate, stimulus intensity, and noise exposure risk group [$F(12, 276) = 0.84$, $p= 0.61$].

Table 1. Mean (±SD) of the AP amplitude for each rate at each intensity (80, 70, 60, and 50 dB for the low-risk (LR) and high-risk groups (HR).

	80		70		60		50	
Rate (clicks/s)	LR	HR	LR	HR	LR	HR	LR	HR
11.1	1.02 (±0.44)	0.78 (±0.34)	0.63 (±0.33)	0.41 (±0.27)	0.34 (0.±24)	0.20 (±0.18)	0.25 (±0.23)	0.15 (±0.15)
37.1	0.85 (±0.40)	0.64 (±0.28)	0.48 (±0.24)	0.30 (±0.23)	0.37 (±0.23)	0.15 (±0.15)	0.27 (±0.21)	0.14 (±0.16)
58.59	0.66 (±0.34)	0.51 (±0.24)	0.34 (±0.17)	0.18 (±0.17)	0.24 (±0.21)	0.11 (±0.12)	0.22 (±0.20)	0.05 (±0.08)
78.13	0.52 (±0.23)	0.51 (±0.27)	0.28 (±0.14)	0.18 (±0.18)	0.17 (±0.18)	0.12 (±0.14)	0.21 (±0.25)	0.12 (±0.11)
97.66	0.48 (±0.26)	0.42 (±0.26)	0.26 (±0.20)	0.18 (±0.17)	0.18 (±0.18)	0.13 (±0.10)	0.20 (±0.27)	0.08 (±0.08)

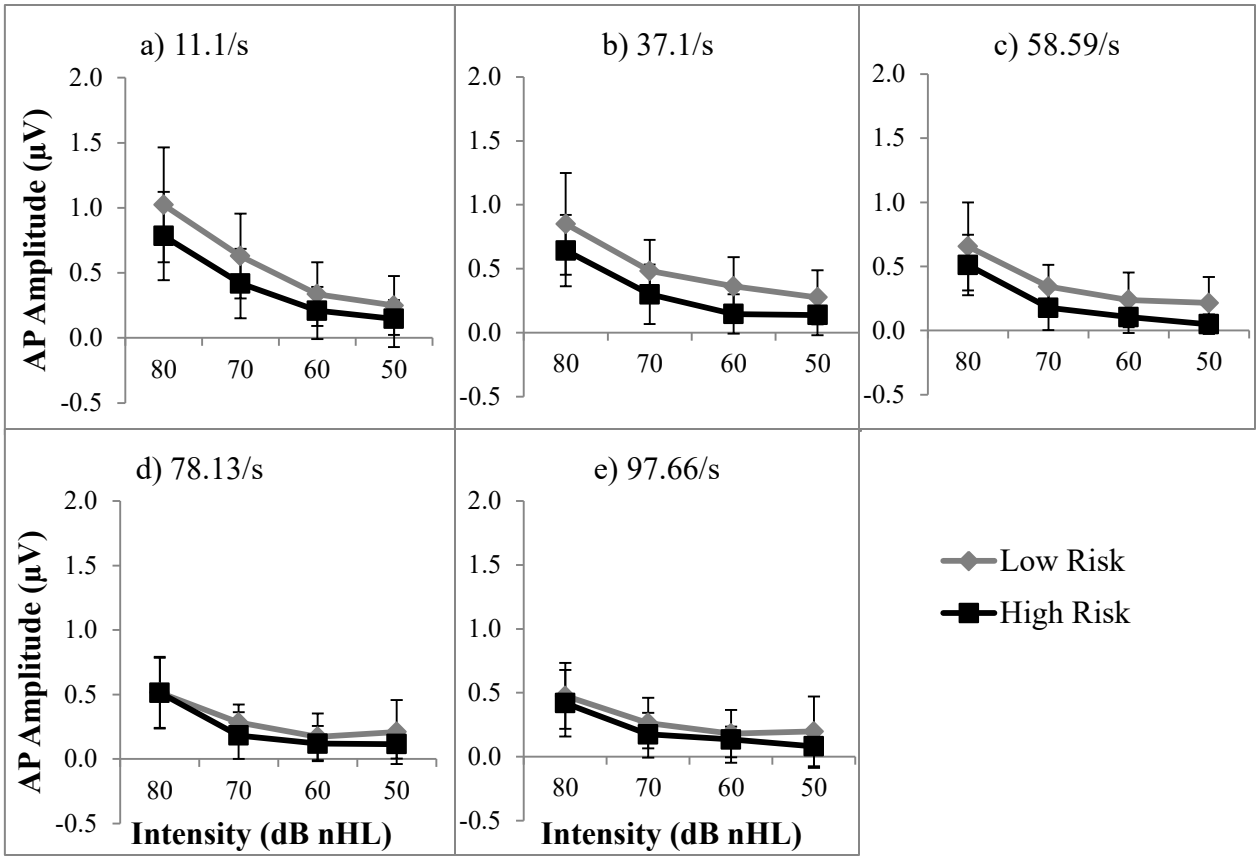


Figure 6. Mean AP amplitude for the low-risk (gray lines) and high-risk (black lines) groups at each intensity for each rate: A) 11.1/s, B) 37.1/s, C) 58.59/s, D) 78.13/s, and E) 97.66/s. The figure shows the low-risk group to have larger amplitude than the high-risk, which is more pronounced with the slower presentation rates [±SD].

Table 2. Pairwise comparison of AP amplitude based off rate. *P*-values highlighting the significant relationships between the rates.

Rate (clicks/s)	11.1	37.1	58.8	8.9	97.7
11.1	-	0.011*	0.000*	0.000*	0.000*
37.1	0.011*	-	0.000*	0.000*	0.000*
58.8	0.000*	0.000*	-	0.146	0.013*
78.9	0.000*	0.000*	0.146	-	0.229
97.7	0.000*	0.000*	0.013*	0.229	-

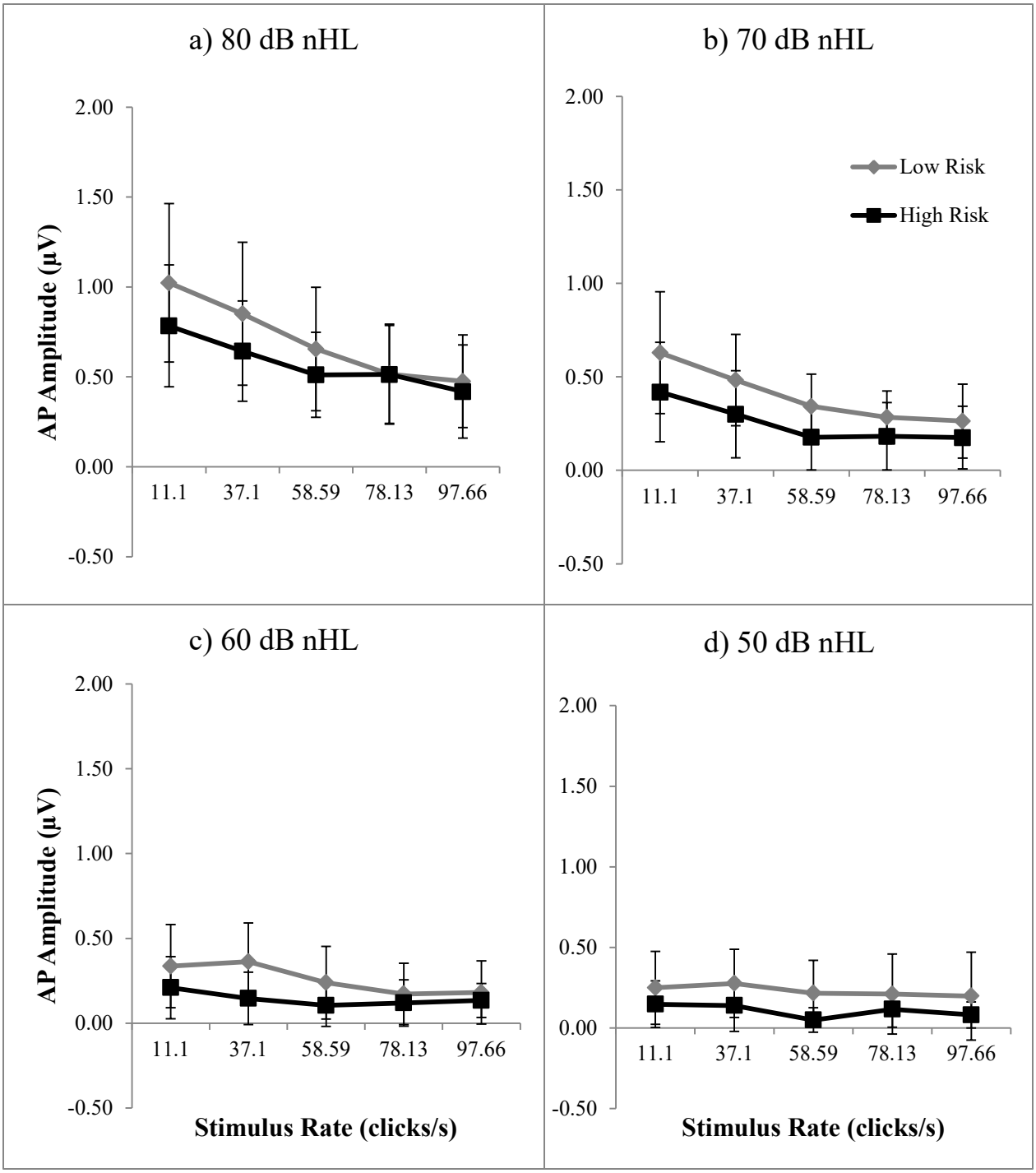


Figure 7. Mean AP amplitude for the low-risk (gray lines) and high-risk (black lines) groups at each rate for each intensity: A) 80 dB nHL, B) 70 dB nHL, C) 60 dB nHL, and D) 50 dB nHL. The figure shows the low-risk group to have larger amplitude than the high-risk, which is more pronounced with the higher intensities [±SD].

Table 3. Pairwise comparison of AP amplitude based off intensity. *P*-values highlighting the significant relationships between the intensities.

Intensity (dB nHL)	50	60	70	80
50	-	0.056	0.001*	0.000*
60	0.056	-	0.002*	0.000*
70	0.001*	0.002*	-	0.000*
80	0.000*	0.000*	0.000*	-

Stimulus Presentation Rate and Intensity Level Effects on SP Amplitude

ECochG recordings were made at five different rates (11.1 clicks/s, 37.1 clicks/s, 58.59 clicks/s, 78.13 clicks/s, and 97.66 clicks/s) and at four different intensity levels (80, 70, 60, and 50 dB nHL) at each of the five rates. SP amplitude was measured for each participant at the highest two intensities (80 and 70 dB nHL) at each rate. SP amplitude was not measured at the lower intensities due to the loss of the response for most participants at these intensities. A two-way (5 x 2) repeated measures analysis of variance (ANOVA) with mixed effects was performed to evaluate the effects of the presentation rate and stimulus intensity on the SP amplitude for both the low and high-risk groups. The between-subject analysis revealed effects was performed to evaluate the effects of the presentation rate and stimulus intensity on the SP amplitude for both the low and high-risk groups. The between-subject analysis revealed a not significant main effect of noise risk group [$F(1, 23) = 0.00$, $p=0.99$]. The two-way repeated measures ANOVA with mixed effects revealed not significant main effect of presentation rate on the SP amplitude [$F(4, 92) = 0.55$, $p=0.70$] (see Figure 8). Additionally, the analysis revealed significant main effect

stimulus intensity on the SP amplitude [$F(1, 23) = 33.47$, $p < 0.001$, $\eta_p^2 = 0.59$]. Finally, the analysis revealed no significant two-way interaction between presentation rate and stimulus intensity [F(4, 92) = 2.16, p= 0.09], presentation rate and noise exposure risk group [F(4, 92) = 0.48, p=0.75], stimulus intensity and noise exposure risk group [F(1, 23) = 0.0001, p= 0.99], and no significant three-way interaction between presentation rate, stimulus intensity, and noise exposure risk group [F(4, 92) = 0.67, p= 0.62] (see Figure 9).

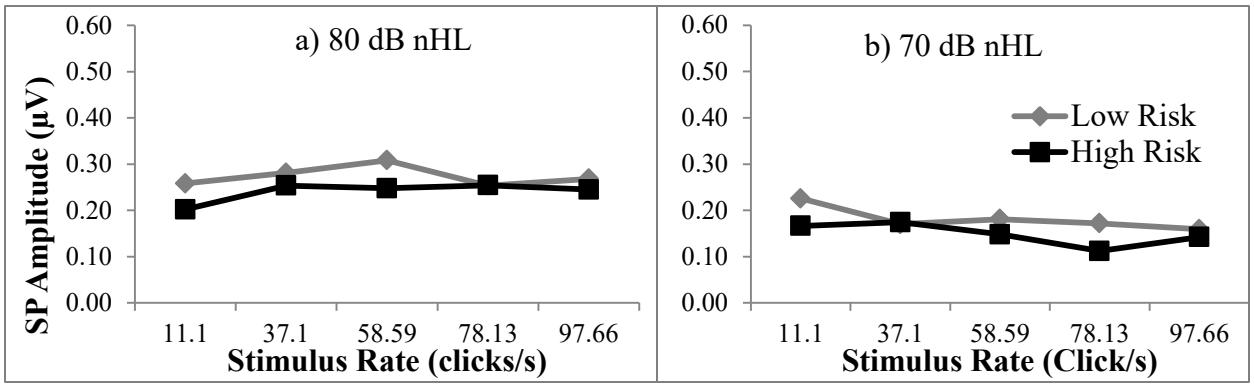


Figure 8. A) Mean SP amplitude shown at each rate tested for both the low and high-risk groups at a stimulus intensity of 80 dB nHL. B) Mean SP amplitude shown at each rate tested for both the low and high-risk groups at a stimulus intensity of 70 dB nHL.

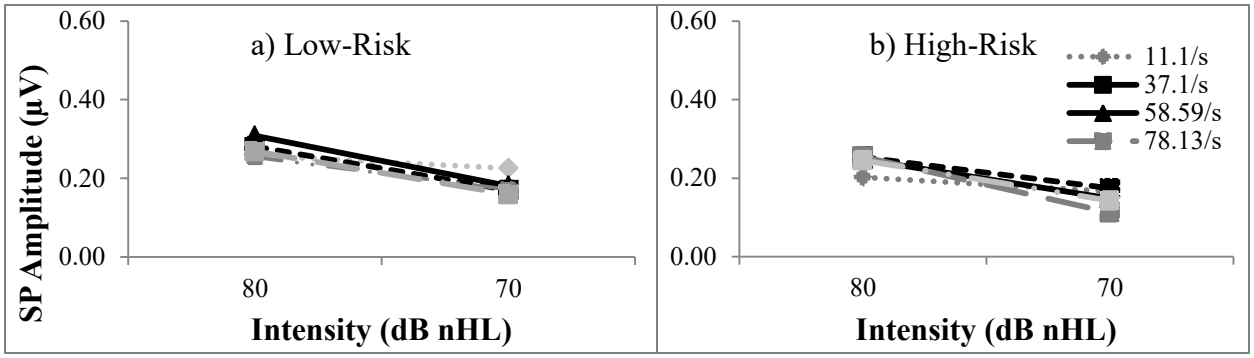


Figure 9. A) Mean SP amplitude for each rate shown at both intensities tested for the low-risk group. B) Mean SP amplitude for each rate shown at both intensities tested for the high-risk group.

DISCUSSION

The mechanism behind NIHHL is thought to be a loss of the ribbon synapses to the neural fibers of the auditory nerve despite present and functioning IHCs (Bramhall et al. 2016; Furman et al. 2013; Liberman et al. 2016; Schaette & McAlpine 2011). This damage leads to hearing difficulties in humans despite hearing thresholds within normal limits (Bharadwaj et al. 2015; Liberman et al. 2013). Currently, there is not a diagnostic protocol for the clinical diagnosis of NIHHL. The goal of this study was to assess the relationship between the SP and AP amplitudes of ECochG responses of participants at low-risk for noise exposure compared to those at high-risk as a potential diagnostic tool for NIHHL.

Pure Tone Audiometry

Testing of pure tone thresholds is designed to assess hearing sensitivity to a variety of frequencies. It has been accepted that pure tone threshold testing allows for the detection of damage to the OHCs of the cochlea such as loss of, or damage to, OHCs (Davis et al. 1989). While it has been accepted that permanent threshold shifts are correlated to damage to the OHCs, it was not previously thought that temporary threshold shifts lead to damage to the auditory system (Nordmann et al. 2000). However, it has been found that pure tone audiometry is not always sensitive to damage to the IHCs and/or the synaptic region between IHCs auditory nerve fibers (Bharadwaj et al. 2015; Lobarinas et al. 2017). It is possible to have damage to the IHCs and nerve fibers while hearing thresholds remain within normal limits or return to normal limits after noise exposure (Furman et al. 2013; Kujawa & Liberman 2009; Lin et al. 2011; Liu et al. 2019). The current study was comprised of participants with hearing thresholds 25 dB HL or less

from 250-12500 Hz. There was no significant difference between the thresholds of the high and low-risk for noise exposure groups at any frequency tested. Such findings are supported by evidence that damage to IHCs and IHC synapses in animals needs to be extensive to cause a shift in pure tone hearing thresholds outside of normal limits (Lobarinas et al. 2017). The presence of normal hearing thresholds in all participants, despite noise exposure group placement, is in support of earlier findings that NIHHL is not able to be diagnosed by pure tone air conduction testing.

Distortion Product Otoacoustic Emission Testing

DPOAE testing was performed on all participants to confirm findings of audiometric testing by showing present and functioning OHCs in all participants. OAEs are generated from the OHCs and are sensitive to OHC damage; however, they are unaffected by damage to IHCs (Trautwein et al. 1996). Past studies of NIHHL in humans have shown IHC and neural damage despite present and normal DPOAEs (Bramhall et al. 2016, Kujawa & Liberman 2009). In the present study, DPOAEs were tested from 1500-8000 Hz and were found to be present and within normal limits for all participants at all frequencies tested with no significant difference in DPOAE responses between the low and high-risk groups. This finding supports the presence of functioning OHCs in all participants as well as supports past literature finding DPOAEs to be unaffected by the IHC damage which characterizes NIHHL.

Acoustic Reflex Threshold Testing

Acoustic reflex thresholds were obtained for all participants ipsilaterally and contralaterally at 500, 1000, 2000, and 4000 Hz in the present study. All participants, regardless

of group placement, were found to have acoustic reflex thresholds within normal limits for all frequencies and conditions tested. However, a significant difference was found between the groups for 500 Hz presented contralaterally. With this stimulus, the acoustic reflex thresholds for the high-risk group were elevated compared to the thresholds of the low-risk group. It has been hypothesized that the low-SR fibers, the same fibers at risk for damage by NIHHL, are the same fibers that influence the acoustic reflex response, and this hypothesis has been supported by continued elevated acoustic reflex thresholds in noise exposed mice after hearing thresholds returned to normal (Valero et al. 2016).

Similar changes in acoustic reflexes have been found in humans. Bramhall et al. (2021b) performed wideband acoustic reflexes on veterans with high noise exposure, veterans with medium noise exposure, and non-veterans with reported minimal noise exposure. The results showed a reduction of the mean acoustic reflex magnitude of the high noise exposure group as compared to the mean acoustic reflex magnitude of the non-noise exposure group at stimuli over 70 dB SPL. This difference in means grew larger as the stimulus intensity level increased, and at the highest intensity tested, the difference in mean magnitude between groups was as large as 25% (Bramhall et al. 2021b). Similar to the current study, the results of the aforementioned study, while not statistically significant, indicate a reduction in acoustic reflex thresholds in humans with noise exposure. The elevation of 500 Hz acoustic reflexes when presented contralaterally in the high-risk group of the present study supports the findings in past literature, and the presence of acoustic reflex thresholds within normal limits for both groups is indicative of typically functioning auditory reflex pathways for all participants.

Word Recognition Testing

Clinically, many patients present with complaints of difficulties understanding speech in noise despite having normal audiometric thresholds. It has been suggested these difficulties may stem from NIHHL due to damage to the neurons which encode suprathreshold sounds (Heut et al. 2016; Liberman et al. 2016). Previous studies have shown those with a high-risk of noise exposure to have difficulties with speech comprehension in noise, conditions with reverberation, and difficulties with speech comprehension of time compressed speech signals (Liberman et al. 2016). The results of this study were not in agreement with such findings, as the WRS between the low and high-risk groups were found to not be significantly different under any condition tested.

The discrepancy in results between the present study and past studies could be due to the noise exposure levels of participants in the two studies. The study by Liberman et al. (2016) recruited most of their high-risk participants from music performance programs at local colleges and low-risk participants from a communication sciences and disorders program. Similarly, in the present study, many low-risk participants came from a CSD program, however, the high-risk group in the present study mainly reported their noise exposure to be from heavy equipment, such as lawn mowers, as well as occasional concerts. This could indicate the type of noise exposure to be different in nature. Due to the subjective nature of grouping human participants by self-reported noise exposure levels and history of TTS, it is possible that some participants did not accurately represent their noise exposure.

Furthermore, it is possible the difference in results between the study by Liberman et al. (2016) and the present study is due to the differing presentation levels. The present study presented NU-6 word lists at both 40 and 60 dB SL in both quiet and with an SNR of 0 dB while Liberman et al. (2016) used 35 dB HL and at 5 and 0 dB SNR. While it was expected the higher

presentation levels would show the greatest difference between groups in noise due to the damage to the ribbon synapses may affect the encoding of supra-threshold stimuli, it is possible that the lower levels of presentation used in previous research increased listening fatigue and this effect was seen specifically in the high-risk group. Overall, more research into the area of noise exposure and WRS scores in those at risk for NIHHL is indicated.

AP Amplitude

Stimulus Rate Effect. Because of the neural nature of the AP response, its amplitude is affected by the stimulus used to evoke the response (Ferraro & Durrant 2006; Kaf et al. 2017; Schoonhoven et al. 1995; Zheng et al. 1997). Specifically, Kaf et al. (2017) demonstrated a significant decrease in AP amplitude as the rate of the stimulus increases; notably, the decrease in AP amplitude was most prevalent between the lower rates tested and became less prevalent with the faster rates. A study by Liberman et al. (2016) also demonstrated a decrease in AP amplitude with an increase in stimulus rate from 9.1/s to 40.1/s. The results of the present study agree with the literature because a significant main effect of stimulus rate was found on the AP amplitudes with amplitudes decreasing as stimulus rate increased. Additionally, while the AP amplitudes between the lower rates were significantly different, the AP amplitudes between the rate of 58.59 compared to 78.13 as well as the rate of 78.13 compared to 97.66 were not significantly different. These findings demonstrate neural adaptation and fatigue caused by increasingly fast stimulus rates.

Stimulus Intensity Effect. Similar to stimulus rate, stimulus intensity has an impact on the AP amplitude of ECochG responses. Past research on the effects of stimulus intensity on the amplitude of ABR wave I demonstrated a roughly proportional increase in wave I amplitude with

increases in stimulus intensity (Pratt & Sohmer 1976). In agreement with past literature, the present study found a significant main effect of stimulus intensity on the AP amplitude. In the present study, the AP amplitude was found to decrease as the stimulus intensity decreased. The results indicate that the AP response is dependent on the parameters of the stimulus.

Noise Group Effects on AP Amplitude. The AP of ECochG responses is generated from the auditory nerve and the synapses of the IHCs with the auditory nerve, therefore, the AP response measured during ECochG testing is generated from the same areas as wave I of the ABR (Ferraro & Durrant 2006; Zheng et al. 1997). As ECochG testing is performed with electrodes placed closer to the response generators than with ABR testing, the AP response of ECochG testing is less variable than wave I of the ABR (Ferraro & Ferguson 1989). With damage to the auditory nerve and ribbon synapses, as would be seen in cases of NIHHL, it is expected to see a reduction in the amplitude of AP responses during ECochG testing as well as in the wave I response of ABR testing. The damage to the auditory nerve and ribbon synapses can be present without a change in hearing thresholds. This has been evidenced in animal studies in which the IHCs have been found to be intact despite noise exposure leading to a 20-60% reduction in AP amplitude after hearing thresholds have returned to baseline (Kujawa & Liberman 2009).

Many studies in both animals and humans have demonstrated a reduction in ABR responses in noise exposed populations (Bramhall et al. 2020; Furman et al. 2013; Kujawa & Liberman 2009; Lin et al. 2011; Liu et al. 2019). ECochG responses have been studied far less frequently than ABR responses in noise exposed populations. One study of ECochG responses and NIHHL in humans by Liberman et al. (2016) found a statistically insignificant reduction in AP amplitude in participants with noise exposure as compared to those without noise exposure

when using a 9.1/s rate at 94.5 dB nHL, which is consistent with previous studies using ABR responses in both animals and humans (Bramhall et al. 2016; Furman et al. 2013; Kujawa & Liberman 2009; Lin et al. 2011; Liu et al. 2019; Schaette & McAlpine 2011). The present study found a significant main effect of noise risk group on the AP amplitude with the AP amplitude of the low-risk group being larger than that of the high-risk group. These results are expected as the predicted damage to the nerve synapses with NIHHL would be expected to cause a significant reduction in the AP amplitude in the high-risk group.

11.1/s Presentation Rate at 80 dB nHL. It is noted that despite this significant main effect, a present though not significant effect was found at 11.1/s presentation rate at 80 dB nHL. These findings are in agreement with prior literature in humans where a present, but not significant decrease in AP amplitude was found between high and low noise exposure risk groups (Liberman et al. 2016). It is possible the lack of significant effects at slow presentation rates and high intensity levels in the present study and other studies of ECochG in humans, despite significant differences being found in animal studies, are due to inconsistent grouping stemming from the subjective nature of self-reports. In animal studies, noise exposure can be controlled and quantified, however, in studies of human participants subjective measures must be used to group participants which by nature introduces variability into the grouping. This variability could be the cause of the lack of significant difference between the AP amplitudes of the high and low-risk group in the current study as well as in previous studies using human participants when using high intensity, slow rate stimuli.

Noise Group and Rate. As stated previously, with an increase in stimulus rate, a decrease in the AP amplitude would be expected to be seen due to neural fatigue (Kaf et al. 2017). Such results were seen in the present study. Additionally, the results show a greater separation in

response amplitude between the high and low-risk groups at the slow rates—where there is less neural fatigue being seen—and a smaller separation between groups at the higher rates. ANOVA analysis revealed this two-way interaction between stimulus rate and noise exposure risk group to be marginal, yet not significant. Despite this, the interaction between stimulus rate and noise exposure risk group demonstrates the combined effects of neural fatigue and decreased neural output due to synaptic damage in the high-risk group.

Noise Group and Intensity. Studies in animals have shown that damage to ribbon synapses, as would be expected with NIHHL, would be expected to create a significant difference in the change of AP amplitude with decreasing stimulus intensity between the low and high-risk groups (Huet et al. 2016). Despite this, the expected difference in change in AP amplitude with decreasing stimulus intensity was not seen between the groups. These results suggest that there was no additional fatigue of the ribbon synapses of the high-risk group as the stimulus intensity was manipulated as this fatigue would result in an increase in the separation of the AP amplitude between the groups. It is possible that the lack of significant findings in this area of the present study are due to grouping of participants due to the subjective nature of the questionnaire filled out by participants to assign them to groups. Conversely, the previous literature was conducted with animals, and therefore it was possible to objectively measure the noise exposure of each group.

SP Amplitude

Stimulus Rate Effect. Due to the pre-neural nature of the SP response, the rate of the external stimulus is not expected to impact the response amplitude or latency (Dallos 1992; Ferraro & Durrant 2006; Kaf et al. 2017). Kaf et al. (2017) demonstrated only a slight decrease

in SP amplitude with increasing ECochG stimulus rate. The current study found no significant relationship between stimulus rate and SP amplitude which is in support of past literature. These findings support the idea of the pre-neural nature of the SP and reinforce that lack of stimulus rate effect on this response.

Stimulus Intensity Effect. Prior literature has shown a decrease in SP amplitude with decreasing stimulus intensity (Ferraro 2010). In agreement with the literature, the present study found a statistically significant decrease in SP amplitude as the click stimulus intensity was decreased. Due to the decrease in SP amplitude with decreasing stimulus intensity, in the majority of participants the SP amplitude was only measurable at the two highest intensities tested (80 and 70 dB nHL) and therefore was only assessed at these two intensities.

Noise Group Effect on SP Amplitude with 11.1/sec and 80 dB nHL. Unlike the AP, the SP response is believed to stem from the IHCs of the cochlea and thus is a pre-neural response (Ferraro & Durrant 2006). Because of the pre-neural nature of this response, damage to the auditory nerve and ribbon synapse would not be expected to impact the SP amplitude (Kujawa & Liberman 2009). A study with human participants by Liberman et al. (2016) showed a significantly larger SP amplitude in the high-risk group compared to the low-risk groups. Despite normative data for ECochG focusing on those with typical auditory systems and not those at risk for NIHHL, these results are not what would be expected due to the pre-neural nature of the SP response and the presence of DPOAE responses within normal limits for all participants in the study (Ferraro & Durrant 2006; Liberman et al. 2016). It is not clear why this increase in SP occurred. Studies in animals exposed to noise has shown an increase in SP amplitude with damage to the apical OHCs (Wang et al. 2016). However, there is no evidence this is the cause of the increase in SP as the participants had hearing thresholds within normal

limits as well as present DPOAEs which indicates no damage to the OHCs of the cochlea (Liberman et al. 2016). Furthermore, damage from noise exposure in humans would be expected to first impact the basal hair cells as opposed to the apical OHCs (Liberman et al. 2016; Liberman & Kiang 1978). The results of the present study indicated no significant difference in SP amplitude between the low-risk and high risk-groups. These results are not in agreement with the previous study with human participants as no significant difference was found in the SP amplitude between the low and high-risk groups, however the results agree with previous animal studies which indicate intact IHCs despite a reduction in neural responses after noise exposure.

SP/AP Amplitude Ratio with 11.1/sec and 80 dB nHL

For the slow rate stimuli, SP/AP amplitude ratio was calculated to control for overall variability in response amplitude between individuals. The SP/AP amplitude would be expected to be higher in high-risk participants compared to low-risk participants due to the anticipated decrease in AP amplitude with increased noise exposure while the SP amplitude remains similar between groups (Wilson et al. 2002). Prior research by Liberman et al. (2016) showed the SP/AP amplitude ratio of participants at high-risk for noise exposure to be about double the SP/AP ratio those at low-risk for noise exposure when responses with recorded to a 94.5 dB nHL click stimulus utilizing a tiptrode. The findings of the present study are not in agreement with past literature as the present study found with a slow rate click of 11.1/sec and suprathreshold intensity of 80 dB nHL, there was no significant difference in SP/AP amplitude ratio between the high and low-risk groups.

In the study by Liberman et al. (2016), it is important to note it was an increase in SP amplitude for the high-risk group that caused the doubling in SP/AP ratio due to the fact the

decrease in the AP amplitude seen in the high-risk group was not significant. These results were not expected as the predicted damage to the nerve synapses would be expected to be seen in the AP response with no change in the SP response as it is pre-neural (Ferraro & Durrant 2006; Kaf et al. 2017). Despite this, the increase in SP/AP ratio was suggested to indicate the presence of NIHHL.

The differing findings between the current study and past literature could be due to the fact the present study utilized a different slightly lower intensity for the click stimulus. However, in both studies the stimuli were suprathreshold and would be expected to be loud enough to activate the low SRFs, thus showing possible damage to the SRFs in the high-risk group.

Limitations

The present study is limited due to the strategy utilized to group participants into low and high-risk groups. As there is currently no way to quantify noise exposure in humans objectively, the subjective measure of a noise exposure questionnaire was used in addition to informal interview questions about the participant's history of TTS. The specific questionnaire inquired only into the noise exposure history of participants over the last year. It is possible participants were reluctant to share their true noise exposure history, inaccurately estimated their time spent around noise, or were exposed to noise from sources not mentioned in the questionnaire. Due to the events the year prior to this study, it is also possible that participants were not exposed to the degree of noise they would typically be exposed to in a year.

Another limitation of the present study is the sample size and the homogeneity of the sample. The sample size of 26 with only 14 females and 11 males is quite small and introduces uncertainty in the possibility of generalizing the results to the general population. Additionally,

as all participants were recruited from a college campus, they are not as diverse in the type and amount of noise exposure they have as the general population which again generates the need for caution in generalizing the results.

Future Studies

This study assessed the effects of stimulus rate and intensity on the SP and AP of ECochG responses in individuals at low and high-risk for noise exposure. This study utilized a relatively homogeneous sample, and therefore, it is not appropriate to generalize the results to a broader population. The current study had a small sample size of 26 participants within a limited age range of 18-30; all of whom were recruited from a college campus. The research conducted by Liberman et al. (2016) used only a slightly broader age range of 18-41, and most of the 22 high-risk participants were recruited from music programs at area colleges while most of the 12 low-risk participants were recruited from communication sciences and disorders programs at area colleges. In the current study, many participants indicated their noise exposure was from the same kinds of sources regardless of noise exposure group. Future studies should aim to not only increase the sample size, but also to select participants from broader age groups and noise backgrounds.

Due to the difficulty of quantifying noise exposure in participants, many other studies of the effects of noise exposure have focused on veteran populations (Bramhall et al. 2021a). Future studies could utilize veteran populations of those working regularly with artillery or machinery as high-risk and those with office jobs as low-risk. Another possible method would be to use veterans as a high-risk for noise exposure groups and non-veterans as low-risk. This would allow for more confidence in the separation of participants into groups over the participant's subjective

report of noise exposure.

CONCLUSION

This study was intended to aid in the search to find a clinical tool which could be utilized to diagnose NIHHL in humans. Specifically, the aim of this study was to assess the relationship between the SP and AP amplitudes of ECochG responses for participants at low-risk for noise exposure compared to those at high-risk. In the present study, it was found that while the stimulus rate and intensity significantly affected the AP amplitude, there was only a borderline significant difference between effects of the stimulus intensity on the AP amplitude of the low-risk group as compared to the high-risk group. No significant difference in the change of SP amplitude with decreasing stimulus intensity was seen between groups. Additionally, the stimulus rate had no significant effect on the SP amplitude, nor did the groups. Stimulus intensity did, however, have an effect on SP amplitude. While ECochG shows promise as a potential diagnostic tool for NIHHL, further research is necessary both to confirm the usefulness of the measure and to develop a clinical diagnostic protocol.

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APPENDIX: RESEARCH COMPLIANCE

Missouri State
UNIVERSITY

To:
Abdullah Jamos
Communication Sciences & Disor

RE: Notice of IRB Approval
Submission Type: Initial
Study #: IRB-FY2021-267
Study Title: Effect of Electrocochleography Stimulus Rate and Intensity on Identification of Noise Induced Hidden Hearing Loss in Humans
Decision: Approved

Approval Date: November 10, 2020

This submission has been approved by the Missouri State University Institutional Review Board (IRB). You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented. Should any adverse event or unanticipated problem involving risks to subjects or others occur it must be reported immediately to the IRB.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40 CFR 26 (EPA), where applicable.

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