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Effects of Age on the Adult Peripheral Auditory System

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Abstract

Age-related changes in the auditory nerve and brainstem function were studied using electrocochleography (ECochG) and auditory brainstem responses (ABR) in young and old adult age groups. Isolating the effects of aging from lifetime ototoxic exposures such as noise is challenging. For this reason, only participants with normal audiograms were studied. Extended high frequency thresholds and distortion product otoacoustic emissions (DPOAEs) were also measured. Results reveal significant age-related differences. Older adults exhibit longer ECochG AP and ABR wave I and V latencies compared to younger adults, while the amplitude of these components, and the ECochG SP latency and amplitudes were unaffected by age. Additionally, elevated extended high frequency thresholds and lower DPOAE levels were found in older adults. Age-related changes in auditory nerve and brainstem function in adults with normal clinical hearing may impact speech understanding, particularly in noisy environments.

Keywords

Age-related hearing loss, auditory function, electrocochleography, auditory brainstem response, distortion product otoacoustic emissions, pure tone audiometry

Summary for Lay Audience

Hearing loss is a common issue as people get older, but it can be hard to tell if it’s due to aging or other factors like exposure to loud noises over a lifetime. To better understand the impact of aging on hearing, this study focused on people who had normal hearing based on standard tests. The research looked at how the auditory nerve and brainstem—key components in the pathway that carries sound signals from the ear to the brain—change as people age.

To do this, the study used several specialized tests. Electrocochleography (ECochG) and auditory brainstem responses (ABR) measure how the auditory nerve and brainstem respond to sound, by recording electrical activity in these areas. Extended high-frequency hearing tests check a person's ability to hear high-pitched sounds, which are often the first to be lost with age. Distortion product otoacoustic emissions (DPOAEs) look at the health of the outer hair cells in the inner ear, which are crucial for hearing.

The findings showed that older adults, even those with normal hearing by standard tests, had differences in the way their auditory nerve and brainstem respond to sound. Specifically, certain response times were longer in older adults, indicating age-related changes in how quickly sound information travels through the auditory system. Additionally, older adults had problems hearing extended high frequency tones and worse outer hair cell activity, suggesting age-related decline in these areas. These results are important because they suggest that even if an older adult passes a regular hearing test, they might still experience difficulties with hearing, especially in noisy environments. This could affect their ability to understand speech or other important sounds in everyday life.

Overall, this study adds to our understanding of age-related changes in hearing. It underscores the importance of looking beyond conventional hearing tests to identify subtler signs of hearing decline that could impact the quality of life for older adults. By gaining a clearer picture of these changes, we can work toward better ways to diagnose and address age-related hearing issues.

Acknowledgments

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List of Abbreviations

Auditory Evoked Potentials	AEPs
Electrocochleography	ECochG
Auditory Brainstem Response	ABR
Distortion Product Otoacoustic Emission	DPOAE
Cochlear Microphonic	CM
Summating Potential	SP
Action Potential	AP
Maximum Length Sequences	MLS
Continuous Loop Averaging Deconvolution	CLAD
Extended High Frequency	EHF
Analysis of Variance	ANOVA

Chapter 1

1 INTRODUCTION

With advancing age, it is typical to develop sensorineural hearing loss beginning in the high frequencies. Approximately 55 - 65% of adults older than 75 years have this presbycusic hearing loss (Feder, 2019; Mick et al., 2020; National Institute on Deafness and Other Communication Disorders, 2024) whereas the minority of adults retain normal hearing sensitivity as they age, as defined by the clinical pure tone audiogram. The ability of speech recognition in quiet and noise can also differ with changes in age. These hearing difficulties do not always align with the degree of audiometric pure tone threshold loss, and even with matched pure tone sensitivity, older listeners often experience performance declines compared to younger counterparts (Frisina and Frisina, 1997; Pichora-Fuller and Souza, 2003).

Within the inner ear, cochlear inner and outer hair cells play a vital role in converting mechanical vibrations into electrical signals. These signals are subsequently transmitted through the inner hair cell glutamatergic synapses to the peripheral fibers of the auditory nerve. Neither sensory hair cells nor auditory neurons undergo regeneration in any mammalian ear once they are degenerated (Fujioka et al., 2015). Typically, age-related sensorineural threshold decline is associated with altered function of the afferent auditory pathway (Konrad-Martin et al., 2012; Burkard and Sims, 2002), both of which depend on sensory input from inner and outer cochlear hair cells. Extensive research efforts in both human and animal studies have sought to elucidate the pathogenesis of age-related hearing loss. The loss of cochlear hair cells, strial atrophy, and spiral ganglion neuron degeneration have all been implicated as key factors causing age-related peripheral auditory pathology and presbycusic sensorineural hearing loss (Keithley, 2020; Schuknecht and Gacek, 1993; Wu et al., 2021; Liberman and Kujawa, 2017).

However, changes in auditory nerve and brainstem responses in older people with normal pure tone thresholds, or with statistical correction for high frequency hearing loss, have also been reported by some authors (Aedo-Sanchez et al., 2023; Konrad-Martin et al.,

2012; Roque et al., 2019). Despite having normal hearing sensitivity for pure tones, some older adults report difficulty in hearing, including understanding speech in noisy environments. (Demeester et al., 2009; Lee., 2013; Jayakody et al., 2018; Pichora-Fuller and Souza, 2003). These findings suggest that age-related differences in other aspects of hearing ability or auditory physiology may be affecting auditory neural function but are not reflected by conventional pure-tone thresholds between 250-8000 Hz.

The overall purpose of the study is to better understand how age affects the peripheral auditory nervous system and brainstem in humans with normal hearing sensitivity. The research question addressed in this thesis is "What is the effect of age on the auditory nerve and brainstem function of normal hearing individuals?" In order to answer this question, the function of the auditory nerve and brainstem can be measured using auditory evoked potentials in young and old individuals with normal clinical audiograms. However, because at a young age, humans can hear up to 20 kHz, it is also important to consider hearing sensitivity above the clinical upper limit of 8 kHz. Another physiological measure, distortion product otoacoustic emissions, can be measured noninvasively in humans and provides a measure of outer hair cell status. Considering that these other aspects of hearing ability could influence the function of the auditory nerve and brainstem auditory evoked potentials, it is important to include them in investigations of age-related changes in the auditory periphery.

1.1 Organization of Thesis

The main research question of this thesis is presented here in Chapter 1. Chapter 2 provides background information about age-related studies on animal and human auditory systems, as well as an introduction to physiological measurements of the human auditory system. The methods used in this study are explained in Chapter 3, and the results of the analyzed data are given in Chapter 4. The final Chapter 5 discusses the results with respect to the current literature.

Chapter 2

2 BACKGROUND AND LITERATURE REVIEW

Older adults often experience a decline in hearing sensitivity and difficulty understanding speech, especially in background noise. Age-related hearing impairment is a prevalent condition globally, with an estimated 10-30% of adults in their 5th and 40-65% in their sixth decade living with hearing loss (Feder et al., 2015; Mick et al., 2021; National Institute on Deafness and Other Communication Disorders, 2024; World Health Organization, 2023).

This progressive sensorineural hearing loss, referred to as presbycusis, is defined clinically by abnormal hearing thresholds measured with the standard pure tone audiogram. It has been proposed that compromised hearing sensitivity and auditory processing result from peripheral damage in the cochlea and auditory nerve, central neural degeneration, or a combination of both (Billings et al., 2012; Keithly, 2020; Kohrman et al., 2020).

The structural integrity of the cochlea and auditory nerve have been studied in human temporal bones and in various animal models of presbycusis (Buckiova et al., 2007; Caspary et al., 2005; Fernandez et al., 2015; Kujawa and Liberman, 2015; Liberman et al., 2014; Möhrle et al., 2016; Nelson and Hinojosa., 2006; Parthasarathy and Kujawa, 2018; Sergeyenko et al., 2013; Wu et al., 2021). The assessment of sensory and neural function of the peripheral auditory system and brainstem in humans can be effectively captured through far-field auditory evoked potentials. Both electrocochleography and responses from the auditory nerve and brainstem have been used to study the effects of age in humans and animals. (Bester et al., 2020; Burkard and Don, 2015; Gelfand, 2016; Konrad-Martin et al., 2012; Musiek et al., 2015).

2.1 Physiological Measures of Human Auditory System Function

The timing and magnitude of physiological activity at various stages of the auditory pathway offer insights into the status of specific inner ear and brain structures involved in hearing. Key anatomical structures of the afferent auditory system (see **Figure 2.1**) include the outer and inner hair cells, Type I afferent auditory neurons, also known as spiral ganglion neurons and their cell bodies, and the synapses between inner hair cells and terminals of the auditory neuron fibers.

The outer hair cells amplify low level sound signals within the cochlea. The inner hair cells transduce the amplified sound into electrical signals, which trigger neurotransmitter release from the base of the inner hair cell. Post-synaptic fiber terminals of auditory neurons are activated via the connecting synapse, and their action potentials are conveyed to the brainstem for further processing.

These anatomical structures are crucial to the physiological integrity of the auditory system. Physiological measures in human auditory research and clinical practice are required because direct evidence of cochlear cell or auditory neuron damage in a human temporal bone and brain is only possible during surgical intervention or post-mortem. Therefore, indirect “proxy” measures such as Auditory Evoked Potentials (AEPs) and Distortion Product Otoacoustic Emissions (DPOAEs) are required to non-invasively investigate potential damage within the auditory system (Bieber et al., 2020; Burkard and Don, 2015; Gelfand, 2016; Musiek et al., 2015; Starr and Achor, 1975; Yagi and Kaga, 1979).

In this thesis, AEP recordings, specifically electrocochleography (ECochG) and auditory brainstem (ABR) component waveforms were used to assess the impact of aging on pre-synaptic and post-synaptic sites of auditory function. The ECochG SP component originates from the offset between the depolarization-hyperpolarization receptor potential response of the inner and outer hair cells, as well as the neural contribution of Type I auditory neuron fibers (Lutz et al., 2022). The ECochG AP and ABR Wave I arise from

the collective action potentials generated by individual Type I auditory neurons, particularly those with high characteristic frequency (Santarelli et al., 2021).

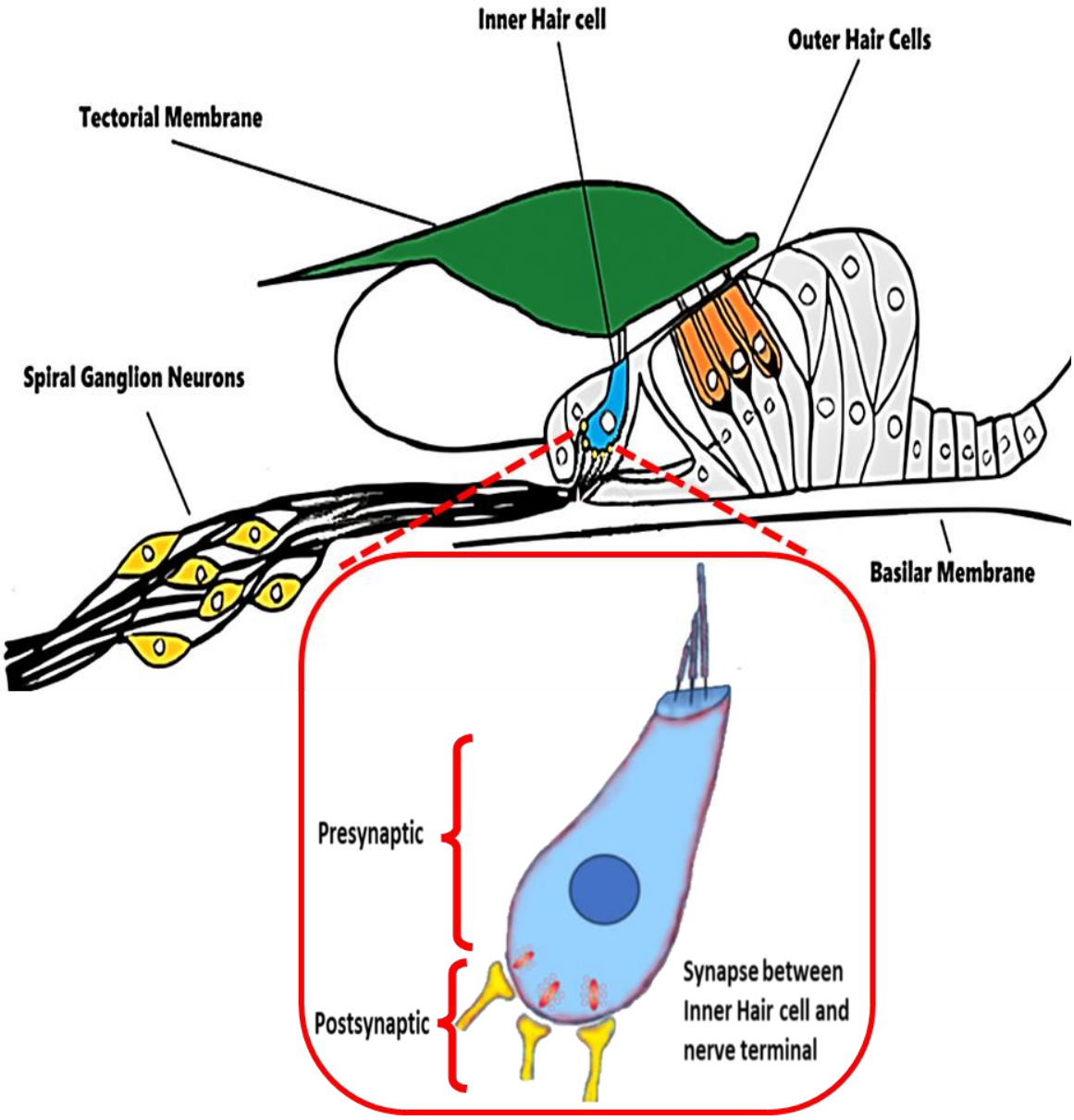

Figure 2.1: Pre and postsynaptic sites of lesion within and beyond the cochlea associated with aging.

2.1.1 Distortion Product Otoacoustic Emissions (DPOAEs)

DPOAEs were first identified by Kemp (1978). When sound permeates a healthy middle ear and cochlea, the stereocilia of outer hair cells are displaced by sound vibration. In response, the electromotile outer hair cells convert electrical receptor potentials into mechanical energy in order to amplify sound in the cochlea (Grosh et al., 2004). DPOAEs are the acoustic signals generated as a result of this outer hair cell amplification. These sound emissions propagate back along the basilar membrane to the middle and outer ear, where they can be detected in the ear canal.

DPOAEs are generated when outer hair cells are stimulated by cochlear distortion in response to two primary tones, f1/L1 (with frequency f1 and level L1) and f2/L2 (with frequency f2 and level L2), presented in the ear canal. In the cochlea, the two primary DPOAE components overlap and undergo nonlinear interaction, resulting in the generation of mechanical waves at different frequencies, such as 2f1–f2. These generated distortion products travel back toward the ear canal, where they can be recorded by a sensitive microphone (Talmadge et al., 1999; Kalluri and Shera, 2001; Knight and Kemp, 2000). DPOAEs are usually captured between 1- 6 kHz with amplitudes that vary between -10 and 30 dB SPL at approximately 5-6 dB above the noise floor (Kramer, 2014; Robinette and Glattke, 2007). DPOAEs are a valuable research and clinical tool because they reflect cochlear outer hair cell status at 2f1-f2 (Kemp, 2002).

2.1.2 Human Auditory Evoked Potentials (AEPs)

AEPs are electrical responses produced by the auditory system in response to specific acoustic stimuli. These responses can be recorded from the scalp and ear using voltage-sensitive surface electrodes. Short duration, transient AEPs are elicited by the presentation of a brief sound stimulus. The timing of different components of the AEP waveform synchronizes with the presentation of this stimulus. By averaging a suitable number of stimulus presentations together, unwanted noise is reduced while the physiological signal is enhanced. This is possible because AEP components are time-locked to the stimulus, while noise is random (Burkard and Don, 2015; Gelfand, 2016). The timing of different AEP components after stimulus presentation varies depending on

the location of their originating generators within the auditory system. Generally, electrical activity from more peripheral generators exhibits relatively shorter latencies. AEP component latencies range from one millisecond after stimulus onset for cochlear potentials up to several seconds for cortical potentials. AEPs are named based on the cochlear or neural generators responsible for their production. Two such AEPs, ECochG and ABR, were used in this thesis. The ECochG represents electrical potentials generated within the cochlea while the ABR reflects synchronous neural activity originating from the afferent auditory nerve and brainstem (Bester et al., 2020; Burkard and Don, 2015; Gelfand, 2016; Konrad-Martin et al., 2012; Musiek et al., 2015).

2.1.2.1 Electrocochleography (ECochG)

Electrocochleography (ECochG) captures electrophysiological responses from cochlear hair cells and Type I auditory neurons elicited by clicks or tone burst stimuli. The key components of ECochG are the Cochlear Microphonic (CM), Summating Potential (SP), and compound Action Potential (AP), which manifest within the first five milliseconds following the onset of the stimulus. The ECochG can be captured by electrodes inserted invasively through the tympanic membrane onto the cochlear promontory (known as the transtympanic approach) or positioned non-invasively within the ear canal or on the tympanic membrane (known as the extratympanic approach). While the promontory site offers reliable and more distinct waveforms, inserting a needle through the tympanic membrane is often impractical. The extratympanic ECochG method is generally reliable but produces smaller waveforms (Bester et al., 2020; Bieber et al., 2020; Gelfand, 2016).

In this thesis, the non-invasive method was used to record the ECochG, and the SP and AP components were used to investigate cochlear hair cell and auditory nerve activity. The SP mainly represents a direct-current component of hair cell receptor potentials that arise when sound displaces the basilar membrane and activates current flow into the hair cells (Bester et al., 2020). While the SP depends predominantly on the activation of inner and outer hair cells, recent evidence suggests that some electrical activity originating from auditory neurons also contributes to its genesis (Bester et al., 2020). On the other hand, the compound AP is a neural response evoked by the synchronous firing of action potentials by afferent fibers that make up the auditory nerve (and also by Wave I of the

ABR-see **Figure 3.2**). In individuals with normal functioning of the cochlea, the SP is relatively small compared to the AP, and the SP/AP amplitude ratio is expected to be 4.0 or less (Eggermont, 2017; Lefler et al., 2021).

An example of an ECochG waveform is shown in **Figure 2.2**, which was obtained from a tympanic membrane electrode recording. This waveform depicts the electrical activity of the cochlea and auditory nerve in microvolts across the time axis in milliseconds.

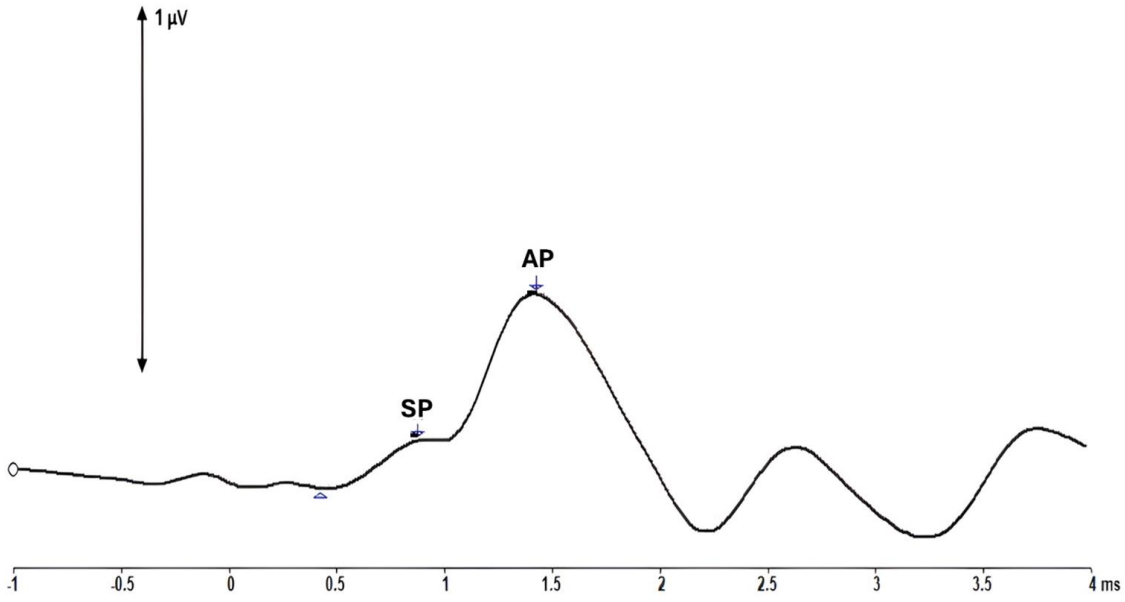

Figure 2.2: An example of ECochG recording from the study.

2.1.2.2 Auditory Brainstem Response (ABR)

The auditory brainstem response ABR refers to an AEP arising from synchronized activity in the auditory nerve and fiber tracks along the ascending auditory pathways of the auditory brainstem. The ABR recording technique involves the use of transient acoustic stimuli like clicks and tone bursts (Gelfand, 2016). Electrophysiological recordings conducted on humans with normal hearing result in a classic waveform of seven peaks that occurs within ten milliseconds after stimulus onset. Waves I, III, and V are consistently observed in humans, and primarily generated by the distal portion of the auditory nerve, cochlear nuclei, and inferior colliculus, respectively (Burkard and Don, 2015; Gelfand, 2016; Konrad-Martin et al., 2012; Musiek et al., 2015). One metric

derived from the ABR waveform is amplitude, which can be quantified by measuring the absolute amplitude of the peaks or employing amplitude ratios such as the V/I amplitude ratio. Latency is another, which involves measuring the absolute latency of Waves I, III, and V, as well the I-V interwave interval, which indicates neural transmission time along the brainstem (Burkard et al., 2012). **Figure 2.3** illustrates an example of an ABR waveform obtained from a mastoid electrode recording. This waveform depicts the electrical activity of the auditory nerve and brainstem in microvolts across the time axis in milliseconds.

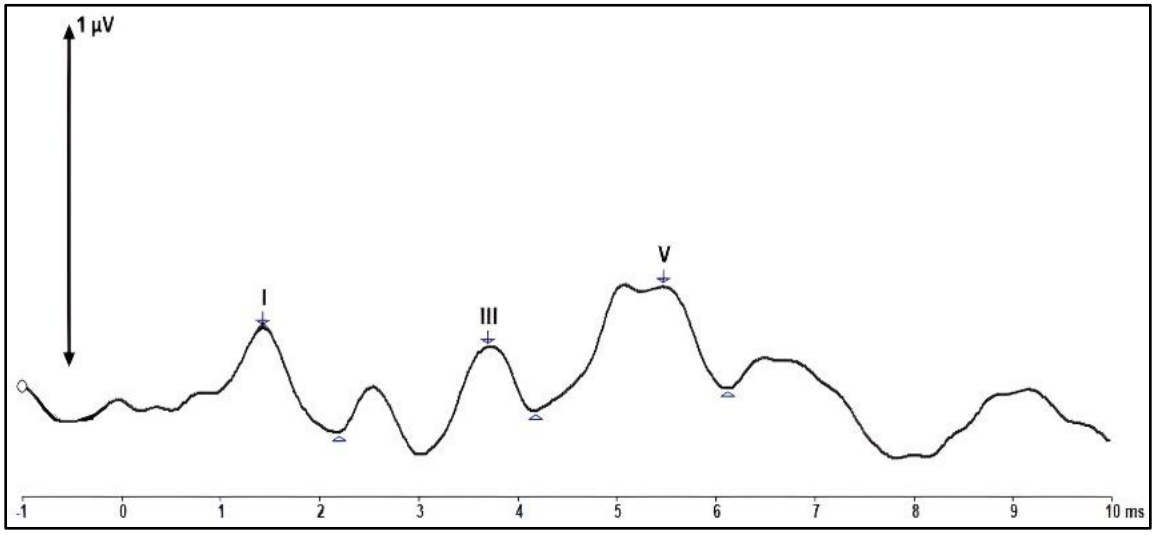

Figure 2.3: An example of ABR recording from the study.

2.2 Effects of Stimulus Rate on the ABR and ECochG

2.2.1 Conventional Stimulation Rate

Increasing stimulus rate manifests as an overall degradation of waveform morphology of the ECochG and ABR. This morphological change mainly involves the neural components, with a reduction in amplitude and prolongation of the ECochG AP and the ABR peak latencies and interwave latency intervals, and some individuals may exhibit no response at the high click presentation rates (Ankmnal-Veeranna et al., 2019; Burkard and Hecox, 1983; Don et al., 1977; Gerling and Finitzo-Hieber, 1983; Jiang et al., 1991; Lake and Stuart., 2019; Wilson and Bowker, 2002). However, high stimulation rates also have differential effects on the ECochG and ABR. While the amplitude of the AP of the

ECochG waveform is affected, the SP amplitude and latency are generally stable at higher click rates. This decrease in the AP relative to the SP leads to a rise in the SP/AP amplitude ratio (Lake and Stuart., 2019; Wilson and Bowker., 2002).

In the healthy auditory system, rate-induced changes in the ECochG AP and ABR Wave I may be related to different effects at various sites in the auditory periphery (Chimento and Schreiner, 1991; Don et al, 1977; Gillespie and Muller, 2009; LeMasurier and Gillespie, 2005; Stauffer et al., 2005; Woo et al., 2009). These effects on auditory system structures include:

- changes in synaptic transmission at the pre-synaptic inner hair cell
- changes in synaptic transmission at the post-synaptic membrane of the Type I auditory nerve fiber terminal
- refractory period limits and recovery time in Type I auditory neuron (spiral ganglion cell) axons and cell bodies
- disruption of the synchronized firing and transmission of action potentials across the population of Type I auditory neuron (spiral ganglion cell) axons and cell bodies that form the auditory nerve

Current evidence indicates that the processes of synaptic transmission and action potential generation by the Type I spiral ganglion neurons are mainly responsible for the rate-induced adaptation of the ECochG and ABR. This rate-induced “stress” could reduce or block synaptic transmission and generation of action potentials by the Type I spiral ganglion neurons. Consequently, the number of activated neurons contributing to the overall population response of the auditory nerve is reduced. In addition, changes in the synchronized firing of auditory neurons in the neural population can be induced by increasing the stimulus rate. These factors, in turn, contribute to shifts in ECochG AP and ABR latency and reduced response amplitude with increasing stimulus rate (Chimento and Schreiner, 1991; Don et al., 1977; Gillespie and Muller, 2009; LeMasurier and Gillespie, 2005; Stauffer et al., 2005; Moser and Starr; 2016; Woo et al., 2009).

2.2.2 Ultra-High Stimulation Rates for AEPs

Conventional methods for investigating the effects of stimulus rate have used click stimuli with repetition rates up to 100/s and a 10ms recording for the ABR. This stimulus rate limit is imposed because conventional recording and averaging methods require waiting for the completion of one electrophysiological response within the recording time-window before presentation of the next stimulus, otherwise the waveforms evoked by each stimulus will overlap in the averaged response.

The auditory system is capable of precisely responding to rapidly changing sound stimuli. Individual afferent Type I spiral ganglion neurons has refractory periods of <1 ms, and only a short recovery time is required before the neuron can respond reliably to another stimulus (Bruce et al., 2018; Burkard et al., 1990; Heil and Peterson, 2015). However, because of conventional averaging limitations, standard stimulation rates do not significantly "stress" the auditory system. For a repetition rate of 100/s for 100-microsecond clicks, the interstimulus interval is 10 ms, which is considerably longer than the estimated refractoriness and recovery period of Type I spiral ganglion neurons. These afferent auditory neurons have sufficient time to recover between stimulus presentations and reliably produce action potentials to the click stimulus. For this reason, conventional stimulus rates are of limited value when the goal is to explore the timing limits of the auditory system (Burkard et al., 1990).

Therefore, when using the ECochG or ABR to study adaptive or recovery processes, click repetition rates above 100/s are needed. Using higher stimulation rates would allow for a more comprehensive assessment of neural transmission and adaptation in peripheral synapses, as well as afferent Type I auditory neurons and brainstem pathways (Burkard et al., 1990). To overcome the restrictions of conventional time-domain averaging, various methods have been employed to increase the stimulation rate above 100 clicks/s.

2.2.2.1 Ultra-High Rates: CLAD AND MLS Methods

It has been proposed that the nervous system's response to a sequence of stimuli can be modeled as a complex combination or "convolution" of individual responses to each stimulus within a sequence of overlapping responses. Based on this idea, different ultra-

fast stimulation methods have been developed for recording AEPs, such as pseudo-random pulse trains known as maximum length sequences (MLS) and continuous loop averaging deconvolution (CLAD) (Delgado and Ozdamar, 2004; Eysholdt and Schreiner, 1982; Kaf et al., 2017). With these techniques, the interval between click stimuli is very short, and ongoing stimulation with an ultra-fast stimulus sequence causes each click-evoked electrophysiological response to overlap. This produces a complex waveform generated by these overlapping electrophysiological responses. However, the ECochG and ABR can be extracted by comparing the special click stimulation sequence with the unique pattern of overlapping electrophysiological activity generated by this specific pattern of stimuli (Lina-Granade et al.,1994). These methods using ultra-high stimulation rates and deconvolution of the waveform enable a more detailed investigation of neural adaptation and recovery processes in the auditory periphery (Burkard et al., 1990).

Several studies using ultra-high rate methods report that ECochG and ABR findings are consistent with conventional recording methods. Deconvolved ABRs using either MLS or CLAD in normal adults were generally similar in waveform morphology to those acquired with conventional techniques (Burkard et al., 1990; Delgado and Ozdamar, 2004). Waves I, III, and V decreased in amplitude and increased in latency as the stimulus rate increased, and the robust wave V remained discernible even at the highest CLAD stimulation rate of 507.8/s (Burkard et al., 1990; Delgado and Ozdamar, 2004; Eysholdt and Schreiner, 1982). Kaf et al. (2017) simultaneously recorded ECochG and ABR with CLAD stimulation rates in young normal hearing adults. The ABR waveforms exhibit prolongation of all peaks with decreasing amplitudes, especially wave I, as the click rate increased, in agreement with Delgado and Ozdamar (2004). Similar to Wave I, the ECochG AP increased in latency, with widening and decreasing amplitude as the click rate increased. In contrast, the amplitude and latency of the SP wave remained stable across different click rates, which was consistent with the ECochG findings using conventional stimulus rates. A major advantage of ultra-fast stimulation with ECochG was better differentiation of the SP and AP components, which overlap at traditional stimulation rates.

2.3 Evaluating Auditory System Dysfunction using ECochG and ABR

In patients with various auditory system disorders, the ECochG and ABR are used to investigate the pathophysiology of the cochlea or afferent neural pathways. The AP of the ECochG and ABR are used to diagnose vestibular schwannoma and monitor the condition of the cochlea and auditory nerve during surgical treatment (Stanton et al., 1989; Youssef and Downes, 2009). Changes in the SP to AP amplitude ratio support the diagnosis of cochlear disorders such as endolymphatic hydrops and Meniere's disease (Santarelli and Arslan., 2015; Gelfand, 2016). With respect to neural pathology, specific changes in latency, reduced amplitude, or absence of ECochG AP or ABR peaks can differentiate between disorders affecting the cochlear synapse, auditory nerve, or brainstem pathways (Lefler et al., 2021; Santarelli and Arslan., 2015).

When subjected to an increased stimulus rate in the presence of lesions in the auditory system, AEP waveform morphology distortions become even more pronounced. Stimulus rate-induced “stress” techniques have been applied clinically for detecting dysfunction in patients with various auditory disorders. Rate induced latency shifts, as a measure of auditory system dynamics and adaptation, have been shown to improve the sensitivity of ABR to detect peripheral auditory system pathology, particularly in cases of VIII cranial nerve lesions (e.g. vestibular schwannoma) which compress the auditory nerve or brainstem (Tanaka et al., 1996). Auditory neuropathy is a pathology characterized by absent or very abnormal ABR due to inner hair cell, synaptic, or auditory nerve pathology. McKnight et al. (2018) revealed that a slower ABR stimulation rate did not yield the expected improvement in waveform morphology in patients with auditory neuropathy. With demyelination associated with multiple sclerosis, increased stimulus rate revealed prolonged or absent ABR wave I and significant increases in latencies of waves III, V, and I-III interwave interval (Jacobson et al., 1987; Santos et al., 2004). At higher click rates, Jiang (1999) reported more abnormalities in patients with purulent meningitis for the ABR at 90/s compared to 50/s and 10/s. Abnormal ABR patterns with increased stimulation rates have also been found in children with auditory processing disorders (Allen and Allan, 2014; Ankmnal-Veeranna et al., 2019).

In summary, using conventional rates (below clicks 100/s), these studies illustrate that increased physiological “stress” can be a valuable approach to studying auditory system lesions. The utilization of ultra-high stimulation techniques like CLAD has the potential to facilitate the investigation of various types of auditory system pathology, including presbycusis, that affect the peripheral system and central auditory pathway (Ozdamar et al., 2006; Ozdamar and Bohórquez, 2006).

2.4 Effects of Age on Human Auditory System Structure and Function

2.4.1 Human Temporal Bone Studies

The causes and precise pathophysiological changes underlying the aging process in the peripheral human auditory system and whether the primary degeneration occurs in sensory hair cells, the auditory nerve and synapses, or the stria vascularis continue to be the subject of controversy and ongoing research. Most studies in humans are based on temporal bone studies in adults with significant sensorineural hearing loss.

In a classical series of studies, Schuknecht and colleagues documented the effects of aging on the human auditory system by dissecting human temporal bones and correlating their histopathologic findings with audiometric profiles (reviewed by Nelson and Hinojosa., 2006). Based on their research at the Massachusetts Eye and Ear Human Temporal Bone Bank, presbycusis was divided into six categories.

1. Sensory: This type is characterized by atrophy of the organ of Corti in the basal end of the cochlea and is associated with steeply sloping high frequency hearing loss due to the loss of sensory hair cells in the cochlea.

2. Neural: Neural presbycusis is characterized by a loss of cochlear neurons and poor word discrimination relative to the degree of pure tone threshold loss, if any. In this type, the primary site of pathology is the cochlear nerve, leading to reduced neural input to the brain.

3. Metabolic: Metabolic presbycusis is associated with atrophy of the stria vascularis, which maintains the ionic balance necessary for cochlear function. This type is typically characterized by a flat audiogram and good word discrimination.

4. Mechanical: Mechanical presbycusis is characterized by a high frequency sloping audiogram despite normal morphologic findings in the cochlea. Stiffening of the basilar membrane was proposed, but this categorization has been refuted.

In subsequent work examining additional temporal bones, Schuknecht and Gacek (1993) added two additional categories that capture the true complexity of presbycusis:

5. Mixed: Mixed presbycusis manifests diverse hearing loss profiles, including flat, and sloping high frequency audiograms, and different patterns of hair cell, spiral ganglion nerve cell, and stria vascularis loss.

6. Indeterminate: Indeterminate presbycusis lacks consistent morphologic findings and may be associated with more subtle cellular dysfunction that could not be observed with histological techniques at the time.

Numerous studies on human temporal bones and animal studies have supported the original findings by Schuknecht and his team. For example, in the low-frequency apical turn, metabolic changes and strial pathology are common, with loss of lateral wall fibrocytes essential for maintaining the endocochlear potential and inner ear ion and fluid balance (Schuknecht and Gacek, 1993; Kusunoki et al., 2004; Nelson and Hinojosa, 2006; Wu et al. 2020a & 2020b). With trial damage and a reduced endocochlear potential, the inner and outer hair cells are unable to generate the sensory receptor potentials necessary for hearing (Lang et al., 2010; Wu and Marcus, 2003; Schmiedt et al., 2002).

Recently, specimens from the Massachusetts Eye and Ear Human Temporal Bone Bank have been re-evaluated using more sensitive imaging techniques (Wu et al., 2019; 2021), including high-resolution light microscopy and optical sectioning for more accurate and detailed hair cell analysis. In a group 1-49 years of age, with no history of noise exposure and relatively normal audiograms (mean thresholds < 30 at 8kHz), Wu et al. (2021)

report the loss of outer hair cells and auditory nerve fibers, worse in the basal cochlea; inner hair cell loss was less significant but mild strial degeneration was apparent throughout the cochlea. These results support Kusunoki et al. (2004), who found spiral ganglion cell loss in addition to hair cell loss in the high frequency basal turn of the cochlea with aging. However, Viana et al. (2015) report synaptic and neural pathology even in ears with no history of auditory disease and without significant cochlear hair cell loss. These studies suggest that as the human inner ear ages, there is a decline in outer hair cells, accompanied by the deafferentation of inner hair cells due to the loss of type I afferent neurons. This degeneration can occur even in individuals with relatively good pure tone sensitivity (for review, see Shehabi et al., 2022).

Some hypothesize that the decline in auditory perception with age, for example, difficulty hearing in background noise, may also be linked to changes beyond the cochlea, with degeneration occurring in the central auditory system. However, Christov et al. (2018) examined the superior olivary nucleus in the human auditory brainstem and found no correlation between neuron populations in the superior olivary nucleus, cochlear histopathology, or hearing sensitivity in individuals with presbycusis.

2.4.2 Human Electrocochleography (ECochG) and Auditory Brainstem Responses (ABR)

ECochG studies in older adults with sensorineural hearing loss have shown alterations indicative of cochlear dysfunction. Specifically, these studies have demonstrated decreased amplitude of the SP and AP components when compared to younger individuals with normal hearing and increased latency of the AP but relatively stable latency of SP. These changes suggest pathology that contributes to age-related hearing loss, affecting the function of hair cells, Type I auditory neurons, and the synapses between them (Soucek and Mason, 1992; Chatrian et al., 1985).

ABR studies in older adults with presbycusic sensorineural hearing loss have consistently shown prolongation of Waves I, III, and V and the I-V interwave interval latencies. Decreased amplitudes of these ABR waves have also been reported in older hearing impaired adults compared to younger individuals with normal hearing. These

alterations in ABR wave characteristics indicate impaired neural encoding and transmission along the auditory pathway from the distal portion of the auditory nerve to the upper brainstem, likely due to age-related degenerative changes, which include sensory cell loss in the cochlea, neuronal and synaptic loss, and alterations in myelination (Burkard and Sims, 2002; Konrad-Martin et al., 2012; Pürner et al., 2022).

Only a few studies have investigated variable degrees of hearing sensitivity and the relationship to auditory aging. ECochG findings for the SP component indicate no effect of age on latency or amplitude for older adults 46-60 years old with normal clinical audiograms when compared to younger normal hearing individuals 18-45 of age, suggesting minimal cochlear dysfunction for this peripherally generated response (Wilson and Bowker, 2002). As noted above, ABR studies have shown that for older adults with hearing loss, waves I, III, and V latencies and I-V interwave intervals, are prolonged and reduced in amplitude. Several authors report similarly delayed latencies and smaller amplitude ABR components in "normal hearing" older adults compared to younger individuals (Burkard and Sims, 2002; Konrad-Martin et al., 2012; Pürner et al., 2022). Reduced amplitude and prolonged latency of the ECochG AP component, generated by the auditory nerve, have also been found in old "normal hearing" adults (Soucek and Mason, 1992; Oku and Hasegewa, 1997). However, these studies of "normal hearing" older adults use mid-frequency or averaged pure tone threshold criteria to define hearing sensitivity, which obscured significant high frequency loss in many of the "normal hearing" subjects, presumably with presbycusis (Burkard and Sims, 2002; Konrad-Martin et al., 2012; Oku and Hasegewa, 1997; Pürner et al., 2022; Soucek and Mason, 1992). It is well established that high frequency components of broadband click stimuli contribute to the generation of the AP, Waves I, and V components; when these high frequency sound components are reduced by high frequency hearing loss, the amplitude and latency of these ECochG and ABR components are affected (Don, 2007; Don and Eggermont, 1978; Pantev et al., 1985). In conclusion, many of the so-called "normal hearing" old subjects in these studies actually have significant high-frequency sensorineural hearing loss, which can affect the ECochG and ABR, regardless of age (Don, 2007).

2.5 Effects of Age on Auditory System Structure and Function in Animal Models

Animal models, particularly rodents, have been essential in studying the mechanisms of presbycusis, as they allow for controlled experiments using both invasive anatomical and functional measures of the auditory system not possible in humans (Sun et al., 2021). Buckiova et al. (2007) explored cochlear cell death and found the density of apoptotic cells in the stria vascularis was significantly higher in aged rats compared to young rats, supporting the metabolic type of age-related cochlear pathology. Caspary et al. (2005) report that aged rats have significant outer hair cell loss of up to 90% loss, compared to inner hair cell loss of less than 5%. Studies in mouse models extensively document age-related deterioration of cochlear synapses, and loss of Type I spiral ganglion cell bodies and nerve fibers (Fernandez et al., 2015; Kujawa and Liberman, 2015; Liberman et al., 2014; Möhrle et al., 2016; Parthasarathy and Kujawa, 2018; Sergeyenko et al., 2013). Age-related synapse loss generally ranges from 2 to 53% in different studies. The decline of outer hair cells parallels synapse loss, with minimal impact on inner hair cells over time. Likewise, spiral ganglion cell deterioration follows a consistent pattern across various cochlear regions (Sergeyenko et al., 2013; Parthasarathy and Kujawa, 2018) with low to medium spontaneous rate cells predominantly affected. (Kujawa and Liberman, 2015).

Age-related changes in auditory function, as assessed through DPOAEs, ECochG, and ABR, revealed increased thresholds and abnormalities in response amplitudes and latencies, with the most significant impacts observed in the middle and high frequency ranges. The ECochG and ABR have been used to study auditory dysfunction in rodents with cochlear and Type I spiral ganglion cell damage. Aged rats with mainly outer hair cell loss exhibited significantly higher thresholds than young controls, measured electrophysiologically by ABR (Caspary et al., 2005). Möhrle et al. (2016) found that old and middle-aged rats exhibiting increased ABR thresholds also had lower DPOAE levels and input/output functions compared to their younger counterparts. Auditory dysfunction was attributed to a decline in outer hair cells in these subjects. However, a reduction in ABR wave 1 response amplitudes was observed in older rats showing a normal ABR

threshold. Anatomical studies of the cochlea showed changes in presynaptic inner hair cell structure with decreased ribbon structures critical for neurotransmitter release. Deafferentation and loss of synapses between inner hair cells and auditory neurons was also found. Sergeyenko et al. (2013) suggests that ECochG components, including the ratio of the SP and AP amplitude can been used to index these forms of cochlear synaptopathy. A compromised auditory nerve AP, without changes in the SP, resulted in an increased SP/AP ratio, indicating the possible presence of cochlear synaptopathy (Sergeyenko et al., 2013). Further investigation is required to determine the relationship between age-related outer hair cell loss, synapse loss or damage, and AP or ABR wave 1 amplitude reduction (Sergeyenko et al., 2013; Parthasarathy and Kujawa, 2018).

Animal models of presbycusis support the human temporal bone studies and demonstrate that age-related hearing loss is a complex condition, with metabolic changes in the stria vascularis and spiral ligament, sensory cell and neural damage all contributing to pathological changes in the aging inner ear. ABR threshold elevations can indicate potential dysfunction in inner hair cells and auditory neurons that are not fully captured by DPOAEs (Sergeyenko et al., 2013; Fernandez et al., 2015). On the other hand, age-related auditory changes are not necessarily apparent through electrophysiological threshold measurements alone.

Chapter 3

3 METHODS

3.1 Overview

The purpose of this study was to investigate the effects of age on auditory nerve and brainstem measures as part of a larger study of normative phenotyping methods for genetic hearing research, "Auditory Evoked Potentials Phenotyping Procedures for Genetic Hearing Research". After receiving informed consent, a brief health history was obtained from each participant. The health history survey included inquiries about neuro-psychological disorders, hearing loss, previous treatment with ototoxic drugs, exposure to prolonged loud noise, and the presence of hearing loss in family members. A basic audiological assessment was then used to determine hearing sensitivity and middle ear status, followed by advanced physiological testing procedures as described below. Measurements were conducted in one or more sessions (2.5-3 hours total duration) at the convenience of the participant. The study protocol was approved by the institutional review board at Western University (see Appendix A).

3.2 Participants

To be eligible for inclusion in this study, individuals had to meet the following criteria: (1) adult between 18-30 years of age or between 50-70 years of age, (2) normal otoscopic examination, (3) normal middle ear evaluation based on tympanometry, (4) normal hearing for conventional audiometry and (5) health history. A total of 20 participants met all inclusion criteria and were assigned to the Young Group (mean age = 26.4 years; range = 19-30 years old; 6/10 female) or the Old Group (mean age = 56.2 years range = 50-67 years old; 7/10 female).

3.3 Basic Audiological Assessment

The basic audiological evaluation included otoscopy, tympanometry, pure tone threshold audiometry, and word recognition testing. Otoscopy was used to verify normal tympanic membrane anatomy and unobstructed ear canals. Normal tympanometry was defined as middle-ear compliance (≥ 0.2 mmho and tympanometric peak pressure from -100 to 100

decapascals), determined using the clinical Interacoustics Titan version 2.0 tympanometer. Tympanograms were recorded using a measurement frequency of 226 Hz and at pressures ranging from +200 daPa to -200 daPa. Normal pure-tone air conduction thresholds were defined as $\leq$ 25 dBHL from 0.25 to 8k Hz, with no significant conductive component ($\leq$ 15 dB air-bone gap). Pure tone audiometric procedures were used to measure hearing thresholds in the conventional frequency range (0.25 to 8 kHz) and in the extended high frequencies (9, 11.2, 12.5, 14, 16, and 18 kHz). Thresholds were measured in a sound-treated booth with the Interacoustics AC40 audiometer. Below 8 kHz, ER-3A insert earphones were employed, while above 8 kHz, Sennheiser HDA200 headphones were used. Those with abnormal results for otoscopy or tympanometry, hearing loss defined by conventional audiometry (0.25-8 kHz), or a notable history of neurologic, otologic, or communication disorders were not included in the study.

3.4 Advanced Testing Procedures

3.4.1 Distortion Product Otoacoustic Emissions (DPOAEs)

DPOAE measurements were performed using the HearID+DP system manufactured (Mimosa Acoustics, USA), equipped with software version 10.0.19042. The ER-10C probe microphone was employed for the measurements. DPOAEs were elicited by two tones, referred to as f1 and f2, and the responses were assessed at a frequency of 2f1 – f2. DPOAEs were measured at specific frequencies (see **Table 3.1),** where f2 was between 0.75 kHz and 8 kHz approximately. The f2/f1 ratio used for the measurements was 1.2. Two different stimulus level conditions were used: (1) Mid-Level L1=55, L2=40 dB SPL, (2) High-Level L1 =61, L2=55 dB SPL.

	f2 frequency (kHz)
Low frequencies	0.75, 0.84, 0.93, 1.07
Mid frequencies	1.17,1.35, 1.5, 1.68, 1.87, 2.1, 2.39, 2.67, 3
High frequencies	3.37, 3.79, 4.26, 4.78, 5.34, 6, 6.75, 7.54

Table 3.1: DPOAE f2 frequencies.

A disposable foam ear tip and probe assembly were inserted into the participant's ear. Prior to each measurement, calibration was conducted in a middle ear cavity, followed by in-ear calibrations using equipment and protocols according to the manufacturer's specifications.

3.4.2 Electrophysiological Measures: ECochG and ABR

During the electrophysiological recordings, subjects were instructed to recline and remain as quiet and still as possible and encouraged to fall asleep to ensure optimal data collection. ABRs and ECochG were recorded simultaneously using the IHS System (Intelligent Hearing System, Smart EP, FL, USA) hardware and software (version 5.41.02).

Tables 3.2 and **3.3** show stimulus and recording parameters for the electrophysiological recordings employed with the Intelligent Hearing System-SmartEP system. Simultaneous recordings of the ABR and ECochG were conducted in response to click stimuli presented at different rates, between 11.3 to 507.81 clicks per second. Ultra-high rates above 100 click/s were designed by the manufacturer to minimize jitter and reduce noise amplification during the deconvolution process. To ensure the reliability of the recorded responses, a minimum of two traces were acquired at each rate.

Gold surface electrodes and a commercial tympanic membrane electrode (Lilly TM-Wick IHS) were used to record the ABR and ECochG simultaneously. Surface electrodes were applied to the high (Fz) and low forehead (Fpz) and both mastoids (ipsilateral and contralateral to the test ear), and the TM-Wick electrode was placed on the tympanic membrane. The TM-Wick electrode was soaked in a saline solution and coated with Signa-Gel Electrode Gel (Parker Laboratories, Fairfield, NJ). The cotton tip of the TM-Wick electrode was positioned on the tympanic membrane using an otoscope (Welch Allyn, 3.5V Halogen Operating Otoscope). The distal end of the TM-Wick electrode was secured in place by tape and the foam tip of the insert earphone. Impedance levels for TM-Wick electrodes were typically below 7 kΩ. Interelectrode impedances were assessed immediately after placement and at intervals during the recording session.

Stimulus	100-μs click
Polarity	Alternating
Rate	11.3/s, 19.53/s, 97.66/s, 234.38/s, 292.97/s, 507.81/s
Intensity	80-dB nHL
Presentation	electrically shielded ER-3A insert earphones

Table 3.2: ECochG and ABR Stimulus Parameters.

Gain	100,000
Electrode	**Non-inverting:** high forehead or non-test ear mastoid **Inverting:** test ear tympanic membrane or test-ear mastoid **Ground:** low forehead
Window	10 msec
Sweeps	2048
Filter	30-3000 Hz
Averaging Technique	**Conventional:** for rates 11.3/s, 19.53/s, 97.66/s **CLAD technique:** for rates 234.38/s, 292.97/s, 507.81/s

Table 3.3: ECochG and ABR Recording Parameters.

Four channels were used to record the ECochG and ABR simultaneously with electrode placement. Channel A was designated the tympanic membrane Horizontal channel for recording the ECochG, where the TM electrode served as the inverting (-) electrode placed on the tympanic membrane of the test ear, and a non-inverting (+) electrode was positioned on the contralateral mastoid bone. Channel B was designated the mastoid Vertical channel for recording ABR, with the inverting (-) electrode placed on the mastoid bone of the test ear and the non-inverting electrode located on the high forehead (Fz). Channel C was designated the tympanic membrane Vertical channel for recording

ECochG/ABR, with the inverting (-) electrode placed on the tympanic membrane and the non-inverting (+) electrode placed on the high forehead. Lastly, channel D was designated the mastoid Horizontal channel ABR, connected to the inverting (-) electrode placed on the ipsilateral mastoid to the test ear and the non-inverting (+) electrode placed on the mastoid contralateral to the test ear. The common electrode (ground) was positioned on the low forehead (Fpz). **Figure 3.1** provides a schematic representation of electrode placement and channel configurations utilized in the study. This figure illustrates the arrangement of electrodes for the 4 channels: Horizontal ECochG (channel A), Vertical ABR (channel B), Vertical ECochG/ABR (channel C), and Horizontal ABR (channel D). Each channel specifies the inverting and non-inverting electrodes along with their corresponding anatomical landmarks, and the ground electrode for all channels is positioned at the low forehead (Fpz).

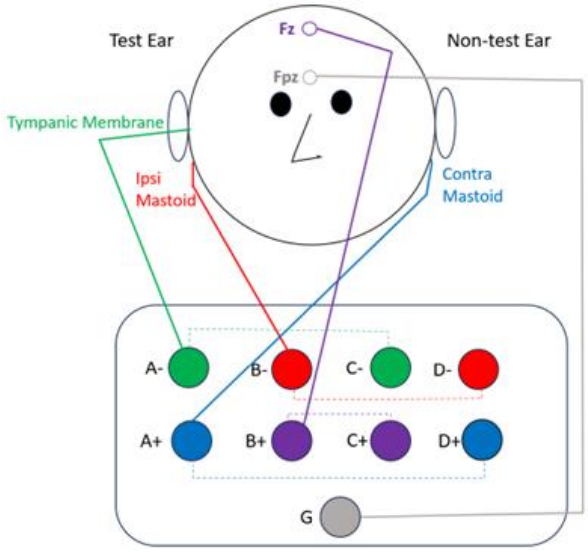

Figure 3.1. Schematic Representation of Electrode Placement and Channel Configuration: This figure depicts the arrangement of electrodes and channel configurations utilized in the study. Channel A, representing Horizontal ECochG, features the ipsilateral tympanic membrane as the inverting electrode (A-) and the contralateral mastoid as the non-inverting electrode (A+). Channel B, dedicated to Vertical ABR, comprises the ipsilateral mastoid as the inverting electrode (B-) and the

high forehead (Fz) as the non-inverting electrode (B+). Channel C, corresponding to Vertical ECochG/ABR, incorporates the tympanic membrane as the inverting electrode (C-) and the high forehead (Fz) as the non-inverting electrode (C+). Channel D, representing Horizontal ABR, utilizes the ipsilateral mastoid as the inverting electrode (D-) and the contralateral mastoid as the non-inverting electrode (D+). The ground electrode for all channels is positioned at the low forehead (Fpz). Dashed lines within the figure illustrate the connections between channels using jumpers.

3.4.2.1 ECochG and ABR Analyses

The standard ECochG trace waveform is delineated using baseline-to-peak labeling (**Figure 3.2)**, where the baseline corresponds to the trough of the SP wave directly preceding the peak of the SP wave. The absolute wave amplitudes of the SP and AP components of the ECochG recording are defined as the absolute voltage difference between the baseline point and the most positive peak. However, the absolute wave I amplitude was defined as the voltage difference between the most positive peak related to the distal portion of the auditory nerve and the following trough. The standard ABR trace was analyzed using peak-to-trough labeling (**Figure 3.3)**. The absolute wave amplitudes of the wave I, III, and V components of the ABR recording are defined as the absolute voltage difference between the peak point and the following trough.

For ECochG analysis derived from Horizontal and Vertical tympanic membrane electrode montages), SP and AP were marked. The SP was defined as the first positive peak that occurred before the AP, within one msec post-stimulus onset. SP and AP amplitudes were defined as the voltage difference between the baseline and the maximum peak deflection of the SP and AP. The SP/ AP amplitude ratio was calculated as the ratio of these two measures. For ABR analysis (derived from the Vertical mastoid (Ch B) and tympanic membrane (Ch C) electrode montage), waves I and V were marked on the average response. The ABR amplitudes were defined by the difference between the maximum peak deflections for waves I and V and their respective troughs following the peaks. The I/V amplitude ratio was calculated as the ratio of these two measures.

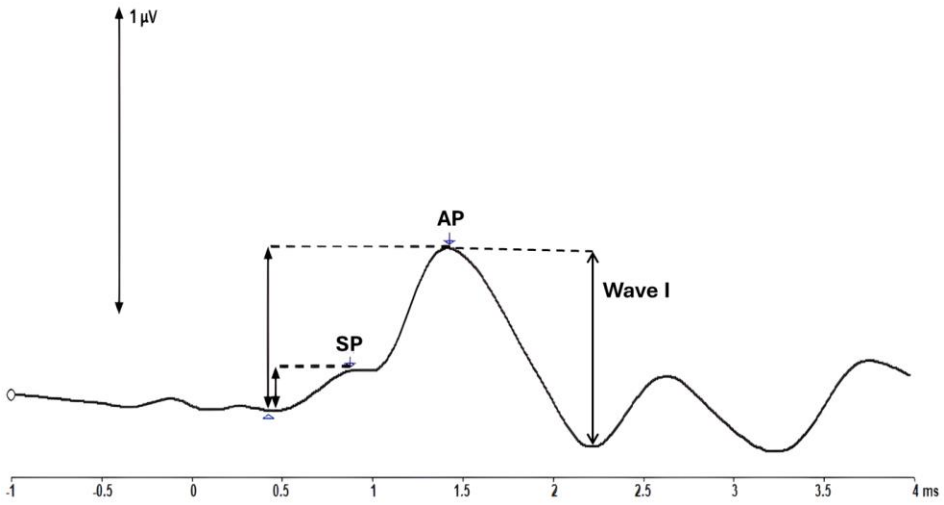

Figure 3.2: This figure presents an example of ECochG recorded with a tympanic membrane electrode, depicting SP and AP and wave I. Additionally, the figure illustrates the approach for SP, AP, and wave I amplitude measurements.

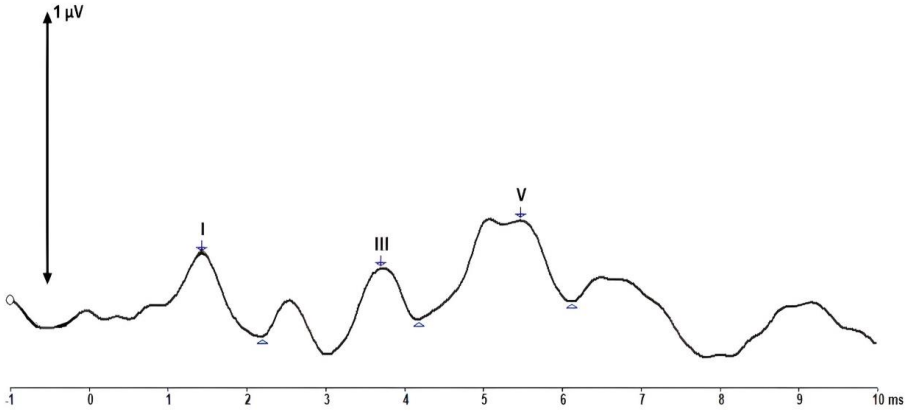

Figure 3.3: This figure presents an example of ABR recorded from a young participant, showcasing waves I, III, and V. ABR waveforms provide a graphical representation of the electrical activity generated by the auditory nerve and brainstem in response to auditory stimuli.

3.5 Data Processing and Statistical Analysis

For DPOAEs, the frequency range was divided into three categories, and DPOAE levels were averaged for these ranges: Low frequencies (.75-1.08 kHz), Mid frequencies (1.17-3 kHz), and High frequencies (3.38-7.55 kHz).

Repeated measures of analysis of variances (ANOVAs) were performed to evaluate group and frequency differences in DPOAE levels, as well as group and rate differences in ECochG and ABR components. Effect sizes were reported as partial Eta-squared (η^2_p). The Tukey's post-hoc analyses was carried out on significant main effects. The Cochran-Mantel-Haenszel test was performed to analyze the waveforms (SP, AP, Wave I, and V) with the number of present/absent responses in ABR and ECochG recordings. Post-hoc analyses using the Chi-square test were performed to indicate the rates with significantly higher absent responses. For all analyses, a significance level of $p < 0.05$ was chosen. The analysis was conducted using RStudio software (R Core Team, RStudio, Inc., Boston, USA).

Chapter 4

4 RESULTS

4.1 Pure Tone Hearing Thresholds

The mean pure-tone hearing threshold results are shown in **Figure 4.1** for the young and old groups for the standard clinical audiogram and for the extended high frequency audiogram. Mean thresholds on the clinical audiogram (0.25-8 kHz) indicate normal hearing for both groups, as expected, given this was an inclusion requirement for the study. Mean thresholds were 4–12 dB lower for the older group, not a significant difference given the ±5dB test-retest for this standardized test. However, older adults have higher mean pure-tone thresholds in the extended high-frequency region (9-18 kHz) compared to the young group, with absent responses above 14 kHz.

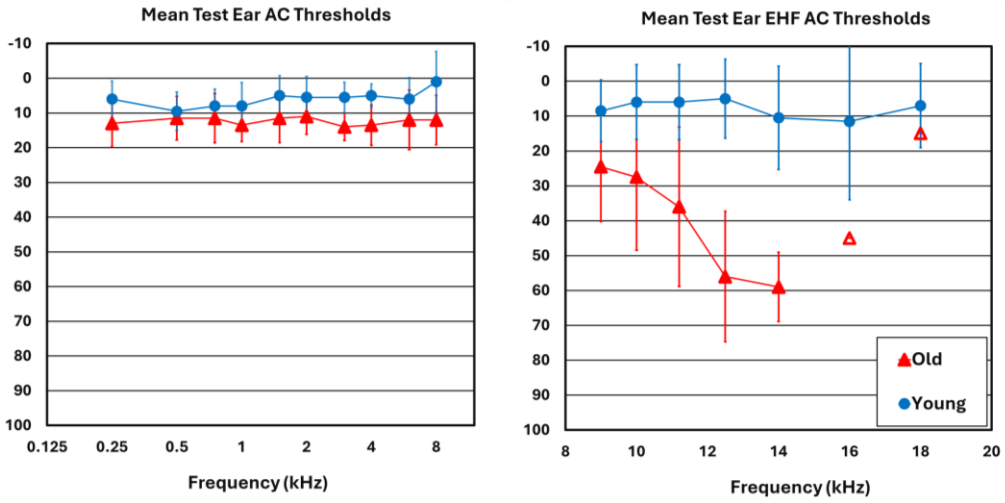

Figure 4.1: Mean audiometric thresholds for young adult and older adult age groups. Error bars represent ±1 standard deviation of the mean and are offset for visualization purposes. No connecting line between 14 to 18 kHz indicates no response at 16 and 18 kHz for any subject in the old group.

4.2 Simultaneous Electrocochleography (ECochG) and Auditory Brainstem Response (ABR)

Figure 4.2 illustrates waveform examples of auditory evoked potentials obtained from both young (blue) and old (red) participants across various click rates ranging from 11.3 to 507.8 clicks per second. The recordings were acquired using Horizontal (channel A) and Vertical (channel C) tympanic membrane electrode configurations, as well as a Vertical mastoid (channel B) electrode configuration. In both young and old examples, it is apparent that as click rates increase, the latencies of the ECochG AP, ABR waves I and V increase, while amplitudes decrease. For some subjects (not shown), one or more of these components disappeared at very high rates. Conversely, the ECochG SP latency and amplitude remain stable. Overall, for this example, the amplitudes of all components are smaller for the old participant compared to the young participant.

4.3 Electrocochleography (ECochG)

4.3.1 ECochG: Action Potential (AP)

4.3.1.1 AP Presence/Absence

The AP of the ECochG was most reliably identified for the tympanic membrane electrode recordings, so the data was evaluated for both Vertical montage (channel C) and Horizontal montage (channel A). **Figure 4.3 (A)** shows the number of subjects with an absent AP for channels A and C across all rates for the young and old age groups.

Using the Cochran-Mantel-Haenszel test, the results for the presence/absence of the AP responses in channel A are not significant ($X^2 = 0.375$, df = 1, $p = 0.5403$). However, in both the old and young groups, there is one instance of a missing AP at a rate of 234.38/s and two instances at a rate of 507.81/s, exclusively observed in the old group.

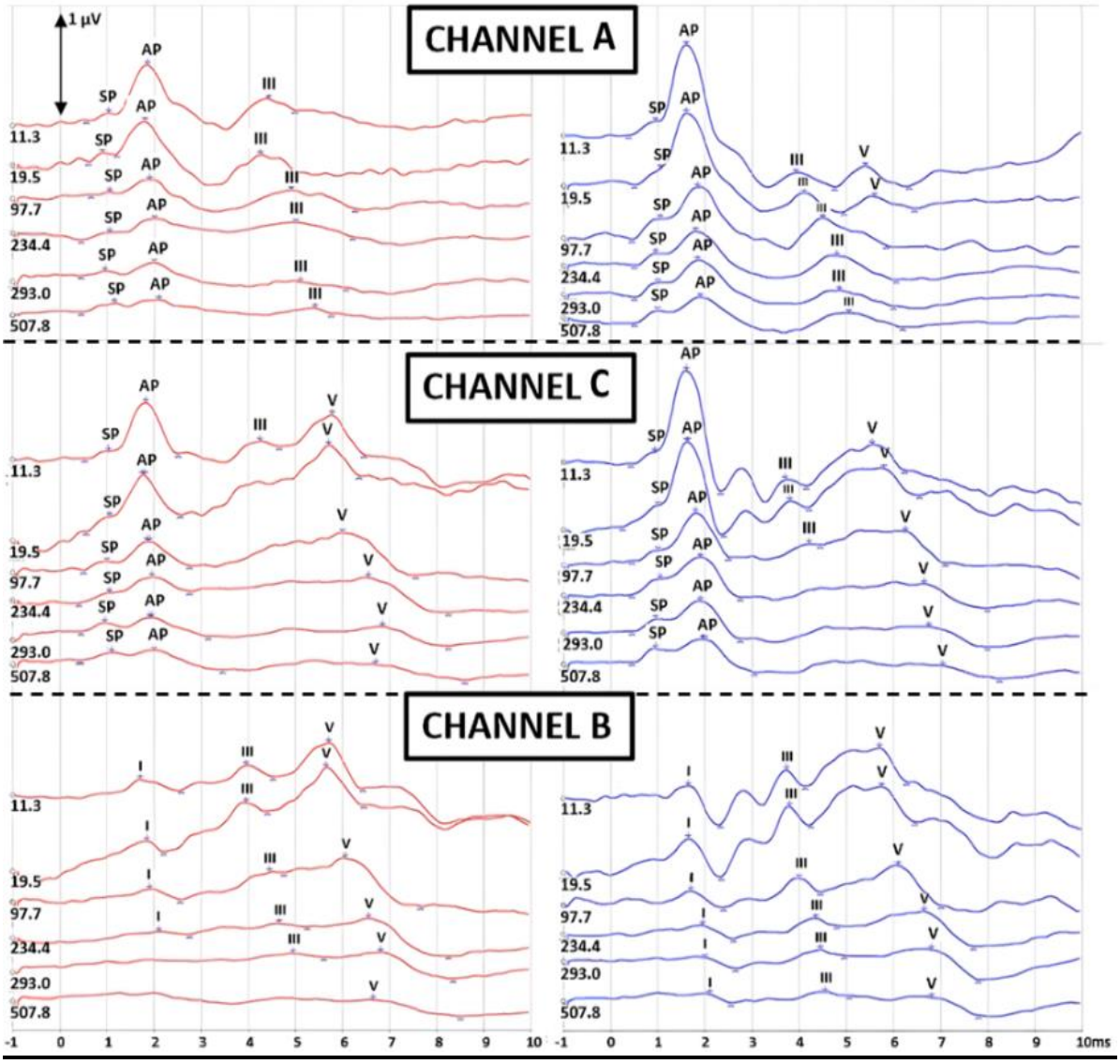

Figure 4.2: This figure presents waveform examples of auditory evoked potentials from an old participant (in red) and a young participant (in blue) across various click rates ranging from 11.3 to 507.8 clicks per second. The recordings were obtained using Horizontal (channel A) and Vertical (channel C) tympanic membrane electrode configurations, as well as a Vertical mastoid (channel B) electrode configuration.

For channel C, the results of the Cochran-Mantel-Haenszel test were also deemed not significant ($X^2 = 0.86957$, df = 1, p = 0.3511). However, in the old group, there is one instance of a missing AP at a rate of 234.38/s and two instances at rates of 292.97/s and 507.81/s. Additionally, the young group exhibits one missing AP at rates of 292.97/s and 507.81/s. Although these absent responses could be an important finding, absent APs are deemed "missing data" and could not be incorporated into the statistical analyses of AP latency and amplitude.

4.3.1.2 AP Latency

As shown in **Figure 4.3 (B),** the mean AP latencies for tympanic membrane recordings in channels A and C are prolonged in the old group compared to the young group. Also, as the stimulus rate increased, the mean latency of the AP increased for both the young and old groups. This rate-induced increase in AP latency affected the young and old groups in the same way across all stimulus rates, and this trend was apparent in both channels A and C.

In the analysis of AP latency in channel C, with age and rate as the two factors in a two-way ANOVA, a significant effect was found for both age [F (1, 94) = 37.44, p < .001, ŋ2p = .28] and stimulus rate [F (5, 94) = 13.89, p < .001, ŋ2p = .42]. These results confirm a statistically significant difference between the old and young adults, with the longer AP latencies in older adults. This outcome also indicates that as the stimulus rate increased, there was a significant increase in AP latency. However, there was no significant interaction between the age and stimulus rate factors [F (5, 94) = 0.08, p = .996, ŋ2p < .01]. This outcome indicates that as the stimulus rate increased, the increase in AP latency was similar with age. Post-hoc comparisons using Tukey's-HSD test revealed significant differences in AP latency between the following rates: 97.66/s-11.3/s (p < .001), 234.38/s-11.3/s (p < .001), 292.97/s-11.3/s (p < .001), 507.81/s -11.3/s (p < .001), 97.66/s -19.53/s (p = 0.004), 234.38/s -19.53/s (p < .001), 292.97/s-19.53/s (p < .001), and 507.81/s -19.53/s (p < .001). (Shapiro-Wilk test, p= 0.1419034; Levene's test, p= 0.1895482)

In the analysis of AP latency in channel A, the results of the ANOVA were similar, but due to the violation of normal distribution of data, as indicated by $p < .05$ in the Shapiro-Wilk test, these results should be interpreted with caution (see **Appendix C**).

4.3.1.3 AP Amplitude

Mean AP amplitudes for tympanic membrane recordings are provided in **Figure 4.3 (C)** for channels A and C at all stimulus rates for the young and old groups. The AP amplitude is similar for both young and old subjects, and there is a trend for the mean AP amplitude to decrease as the stimulus rate increases.

Results of a two-way (age × rate) ANOVA for both channels A and C suggest a significant effect of rate, but no significant effect of age, or interaction between the two factors (see Appendix **C**). However, due to the violation of normal distribution of data, as indicated by $p < .05$ in the Shapiro-Wilk test, these results should be interpreted with caution.

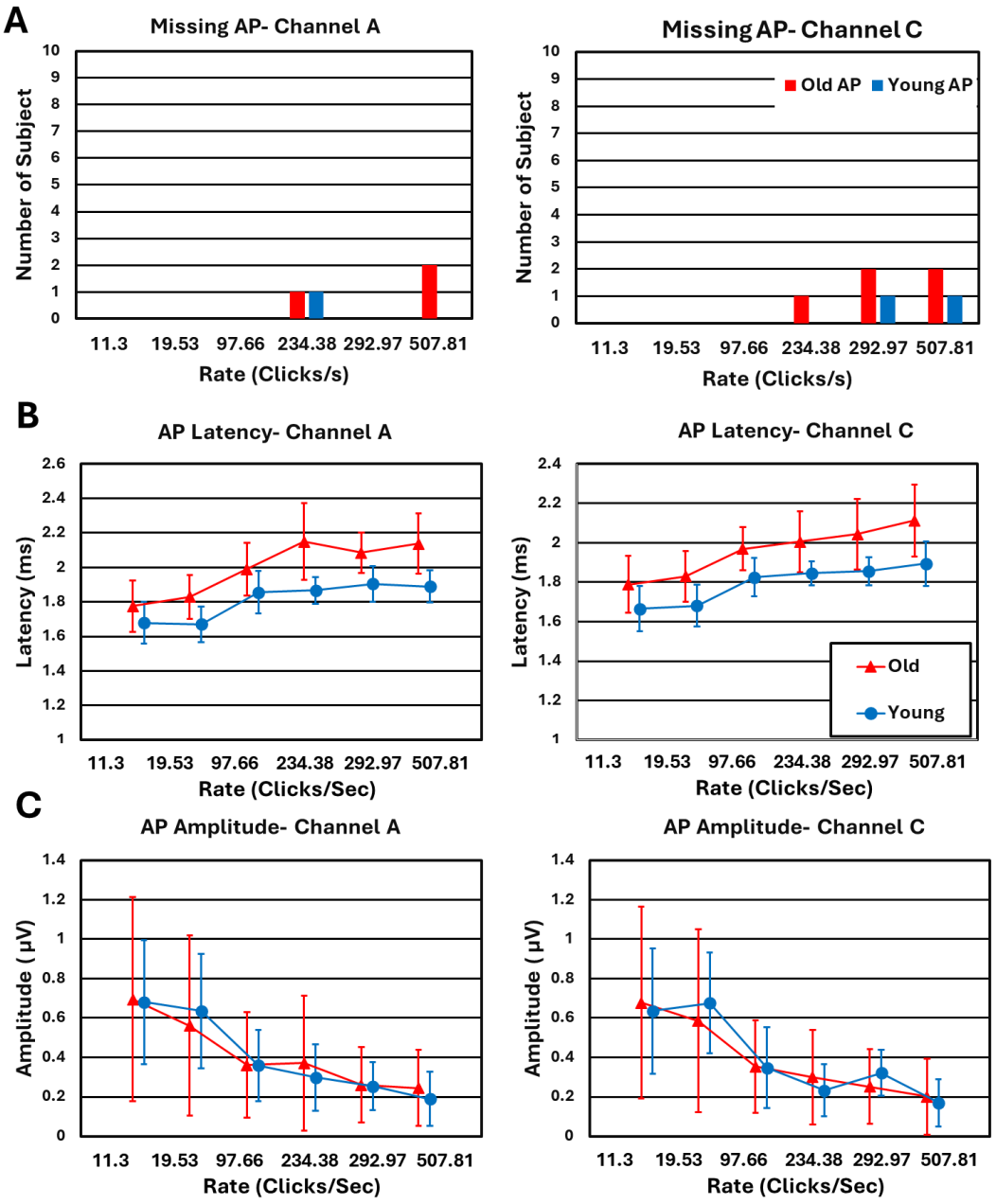

Figure 4.3: This figure illustrates various analyses comparing AP responses between the old and young groups across different click rates for Horizontal (channel A) and Vertical (channel C) tympanic membrane electrode configurations. Panel (A) presents the number of absent AP responses at varying click rates per second in both age groups. Panel (B) shows the mean AP latency across different click rates for both groups. Panel (C) demonstrates the mean AP amplitude across different click rates for both age groups. Error bars represent ±1 standard deviation.

4.3.2 ECochG: Summating Potential (SP)

Figure 4.4 shows overlapped tympanic membrane electrode recordings to demonstrate the influence of various stimulus rates on the ECochG SP and AP responses obtained from a young subject. As stimulus rate increases, the AP latency increases, contrasting with the stable SP responses. Moreover, the SP amplitude remains relatively consistent across different stimulus rates, while the AP exhibits a reduction in amplitude with flattening of the waveform as rate increases. This figure demonstrates how higher stimulus rates aid in separating the SP and AP components, facilitating the evaluation of the isolated SP latency and amplitudes in auditory evoked potential recordings.

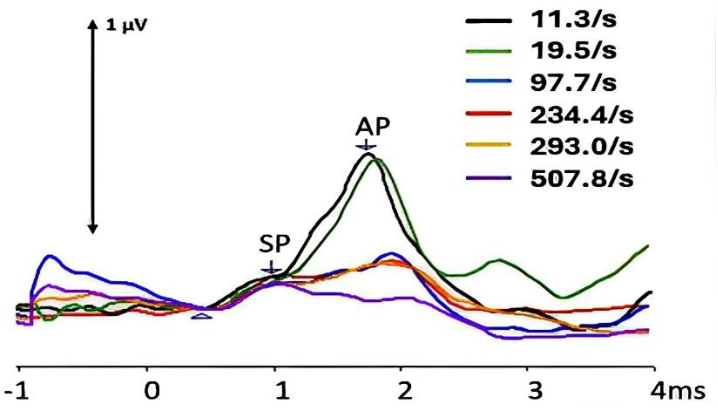

Figure 4.4: This figure displays tympanic membrane electrode recordings overlapped on each other to illustrate the effect of different stimulus rates on SP and AP responses obtained from a young subject.

4.3.2.1 ECochG: SP/AP Amplitude Ratio

Individual differences in factors such as cranial anatomy and sex are associated with high intersubject variability in the AP and SP amplitudes. The SP/AP ratio is utilized to manage these potential sources of measurement variability (Liberman et al., 2016). **Figure 4.5** shows the SP/AP ratio for channels A and C at all rates for the young and old age groups.

For the dependent variable SP/AP amplitude ratio for channel A, a two-way ANOVA considering the factors of age and stimulus rate revealed non-significant effects of age [F (1, 94) = 2.76, p = .100, ŋ2p = .03], stimulus rate [F (5, 94) = 1.60, p = .168, ŋ2p = .08], and no significant interaction between these factors [F (5, 94) = 0.68, p = .638, ŋ2p = .04]. (Shapiro-Wilk test, p= 0.3568438; Levene's test, p= 0.007248607)

For the SP/AP amplitude ratio in channel C, a two-way ANOVA indicated no significant effect of age [F (1, 94) = 1.93, p = .168, ŋ2p = .02], no significant effect of stimulus rate [F (5, 94) = 1.22, p = .304, ŋ2p = .06], and no significant between-factors interaction [F (5, 94) = 0.23, p = .948, ŋ2p = .01]. (Shapiro-Wilk test, p= 0.3304237; Levene's test, p= 0.005106322)

The results of these analyses indicate that there is no significant effect of increasing the stimulus rate or effect of age on the SP/AP amplitude ratio.

4.3.2.2 SP Presence/Absence

The SP of the ECochG was most reliably identified for the tympanic membrane electrode recordings, so the data were evaluated for both the Vertical montage (channel C) and the Horizontal montage (channel A). The presence/absence of the SP, as well as the latency and amplitude of this component, were analyzed. SP responses were present at all rates in channel C for all young and old participants, but this was not true for channel A.

Figure 4.6 (A) shows the number of subjects with an absent SP for channel A at all rates for the young and old age groups. The Cochran-Mantel-Haenszel test revealed non-significant results ($X^2 = 0.3$, df = 1, p = 0.5839). We fail to reject the null hypothesis, indicating that there is no significant difference in the proportion of subjects with absent SP between the young and old groups in channel A. Although not statistically significant, the absence of SP is observed at the click rate of 11.3/s for both a young and an elderly subject, with additional instances of one case with absent SP at rates of 23.438/s and 507.81/s, specifically within the old group. Consequently, these absent SP results are considered "missing data" and could not be included in the statistical analyses of SP latency and amplitude.

4.3.2.3 SP Latency and Amplitude

Figure 4.6 (B) shows the mean SP latencies and **(C)** SP Amplitudes for the young and old groups across all stimulus rates in channels A and C. In both channels, the mean SP latencies are unaffected by both age and stimulus rate, while there is a trend for SP amplitudes to increase slightly.

However, separate two-way (age × rate) ANOVA for both channels A and C and for SP Amplitude and SP Latency indicate no significant main effects of rate or age or significant interactions (see **Appendix C**). These results suggest there was no significant change in SP latency or amplitude with respect to age or stimulus rate in either channel A or C. However, due to the violation of normal distribution of data, as indicated by $p < .05$ in the Shapiro-Wilk test, these results should be interpreted with caution.

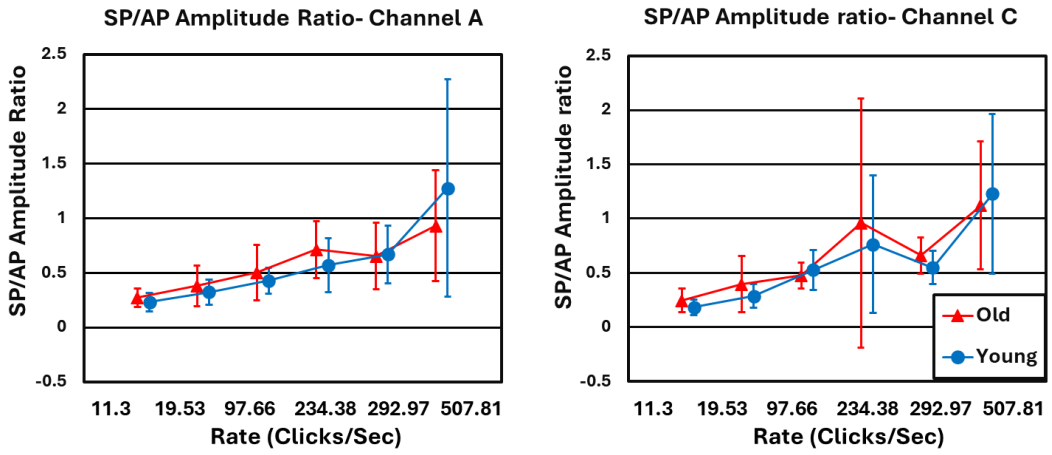

Figure 4.5: This figure illustrates the mean SP/AP amplitude ratio across various click rates for both the old and young groups in Horizontal (channel A) and Vertical (channel C) tympanic membrane electrode configurations. Error bars represent ±1 standard deviation.

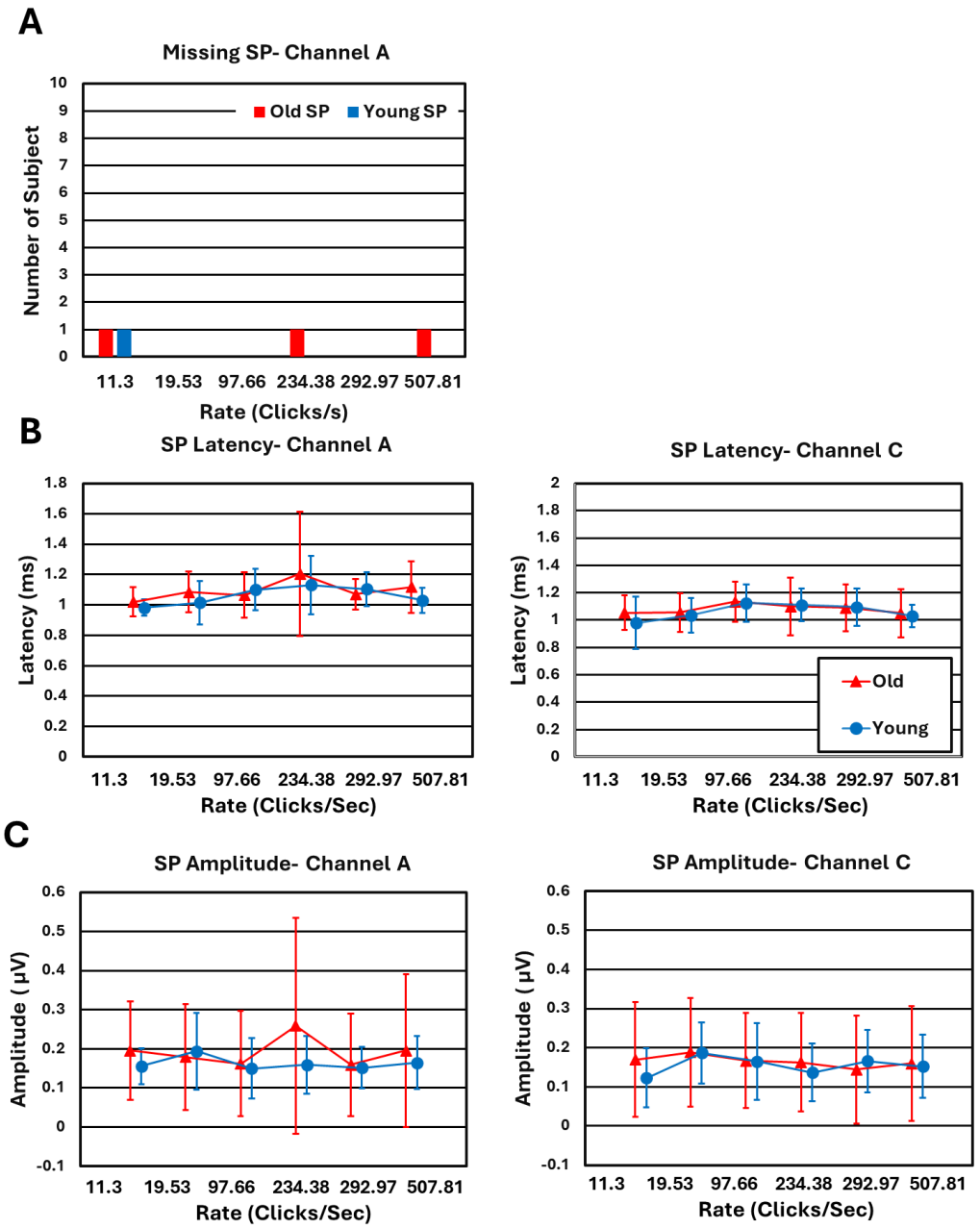

Figure 4.6: This figure presents various analyses comparing the SP responses between the old and young groups across different click rates for Horizontal (channel A) and Vertical (channel C) ECochG recording configurations. Panel (A) illustrates the number of absent SP responses at varying click rates per second in both groups. All SP responses were present in Chennel C. Panel (B) displays the mean SP latency across different click rates for both groups. Panel (C) showcases the mean SP amplitude across different click rates for both age groups. Error bars represent ±1 standard deviation.

4.4 Auditory Brainstem Response (ABR)

4.4.1 ABR: Wave I

4.4.1.1 Wave I Presence/Absence

Figure 4.7 (A) shows the number of subjects with missing wave I across different rates in channel B (mastoid electrode- Vertical configuration) and channel C (tympanic membrane electrode- Vertical configuration) for the young and old group.

For wave I recorded in channel B, the Cochran-Mantel-Haenszel test results were found to be statistically significant ($X^2 = 18.156$, df = 1, p = 2.036e-05). The analysis of Wave I indicates a higher incidence of absent responses in channel B (mastoid electrode recording) for old subjects compared to young subjects. In the old group, there are subjects with the number of missing waves I observed at the following rates: one at 19.53/s, three at 97.66/s, four at 234.38/s, five at 292.97/s, and seven at 507.81/s. Meanwhile, the young group exhibited 4 cases of a missing wave I, all at a rate of 507.81/s (see **Figure 4.7 (A)**). Post-hoc analyses using the Chi-square test revealed that a greater proportion of old subjects exhibited absent responses at rates 234.38/s (X2 = 8, p = 0.02799) and 292.97/s (X2 = 10, p = 0.01049) compared to young subjects at these corresponding rates. As a result, these observations have been designated as "missing data." Despite this significant finding, due to the absence of Wave I in channel B, data for these subjects could not be included in the statistical analyses of latency and amplitude.

For Wave I in channel C, the Cochran-Mantel-Haenszel test result is not significant. (X^2= 1.8243, df = 1, p = 0.1768). We fail to reject the null hypothesis that the proportion of subjects with absent Wave I is the same for the young and old groups. However, in the old group, there are two instances of missing wave I observed at rates of 234.38/s, 292.97/s, and 507.81/s, while in the young group, there is only one missing wave I at rates of 292.97/s and 507.81/s. Consequently, these findings have been classified as "missing data," and these data were omitted from the statistical analyses of Wave I latency and amplitude.

4.4.1.2 Wave I Latency

Figure 4.7 (B) shows the mean absolute latency of wave I across different stimulus rates in channels B (mastoid electrode) and C (tympanic membrane electrode) for the young and old group. This finding indicates a significant difference between the young and old groups for Wave I latency in both channels A and B in stimulus rate and age. Furthermore, the rate had a significant effect on Wave I latency. However, there was no significant interaction, which meant the rate effect was similar for both age groups.

A repeated measures ANOVA was employed to examine wave I latency in channel B, utilizing stimulus rate as a within-subjects factor and age as a between-subject factor. The analysis revealed a significant effect of age on wave I latency [$F(1, 64) = 18.10$, $p < .001$, $\eta 2p = .22$]. Furthermore, the effect of stimulus rate was also found to be significant [$F(5, 64) = 11.89$, $p < .001$, $\eta 2p = .48$]. However, there was no significant interaction effect between age and stimulus rate [$F(5, 64) = 1.18$, $p = .331$, $\eta 2p = .08$]. Post-hoc comparisons utilizing Tukey's SHSD test disclosed significant differences in wave I latency among various rates. Specifically, the following pairwise comparisons demonstrated statistically significant differences: 97.66/s-11.3/s ($p = .005$), 234.38/s-11.3/s ($p < .001$), 292.97/s-11.3/s ($p < .001$), 507.81/s -11.3/s ($p = .004$), 97.66/s -19.53/s ($p = .031$), 234.38/s -19.53/s ($p < .001$), 292.97/s-19.53/s ($p < .001$), 507.81/s -19.53/s ($p = .009$). (Shapiro-Wilk test, $p= 0.1456548$; Levene's test, $p= 0.1680826$)

A repeated measures ANOVA was conducted on wave I latency in channel C, with stimulus rate as a within-subjects factor and age as a between-subject factor. The analysis revealed a significant effect of age on wave I latency [$F(1, 92) = 37.42$, $p < .001$, $\eta 2p = .29$]. Additionally, the effect of stimulus rate was found to be significant [$F(5, 92) = 13.88$, $p < .001$, $\eta 2p = .43$]. However, there was no significant interaction effect between age and stimulus rate [$F(5, 92) = 0.09$, $p = .994$, $\eta 2p < .01$]. Post-hoc comparisons utilizing Tukey's SHSD test identified notable differences in wave I latency among various rates. Specifically, the following pairwise comparisons demonstrated statistically significant differences: 97.66/s-11.3/s ($p < .001$), 234.38/s-11.3/s ($p < .001$), 292.97/s-11.3/s ($p < .001$), 507.81/s -11.3/s ($p < .001$), 97.66/s -19.53/s ($p = .009$), 234.38/s -

19.53/s ($p < .001$), 292.97/s-19.53/s ($p < .001$), 507.81/s -19.53/s ($p < .001$). (Shapiro-Wilk test, $p= 0.1061552$; Levene's test, $p= 0.3572434$)

4.4.1.3 Wave I Amplitude

Figure 4.7 (C) shows the mean wave I amplitude across different rates in channels B (mastoid electrode) and C (tympanic membrane electrode) for the young and old group. There is a trend for the mean wave I amplitude to decrease with increasing stimulus rate in both channels. However, the results appear similar for both the old and young group.

Results of a two-way (age × rate) ANOVA for both channels B and C suggest a significant effect of rate but no significant effect of age or interaction between the two factors. However, due to the violation of the normal distribution of data, as indicated by $p < .05$ in the Shapiro-Wilk test, these results should be interpreted with caution (see Appendix **C**).

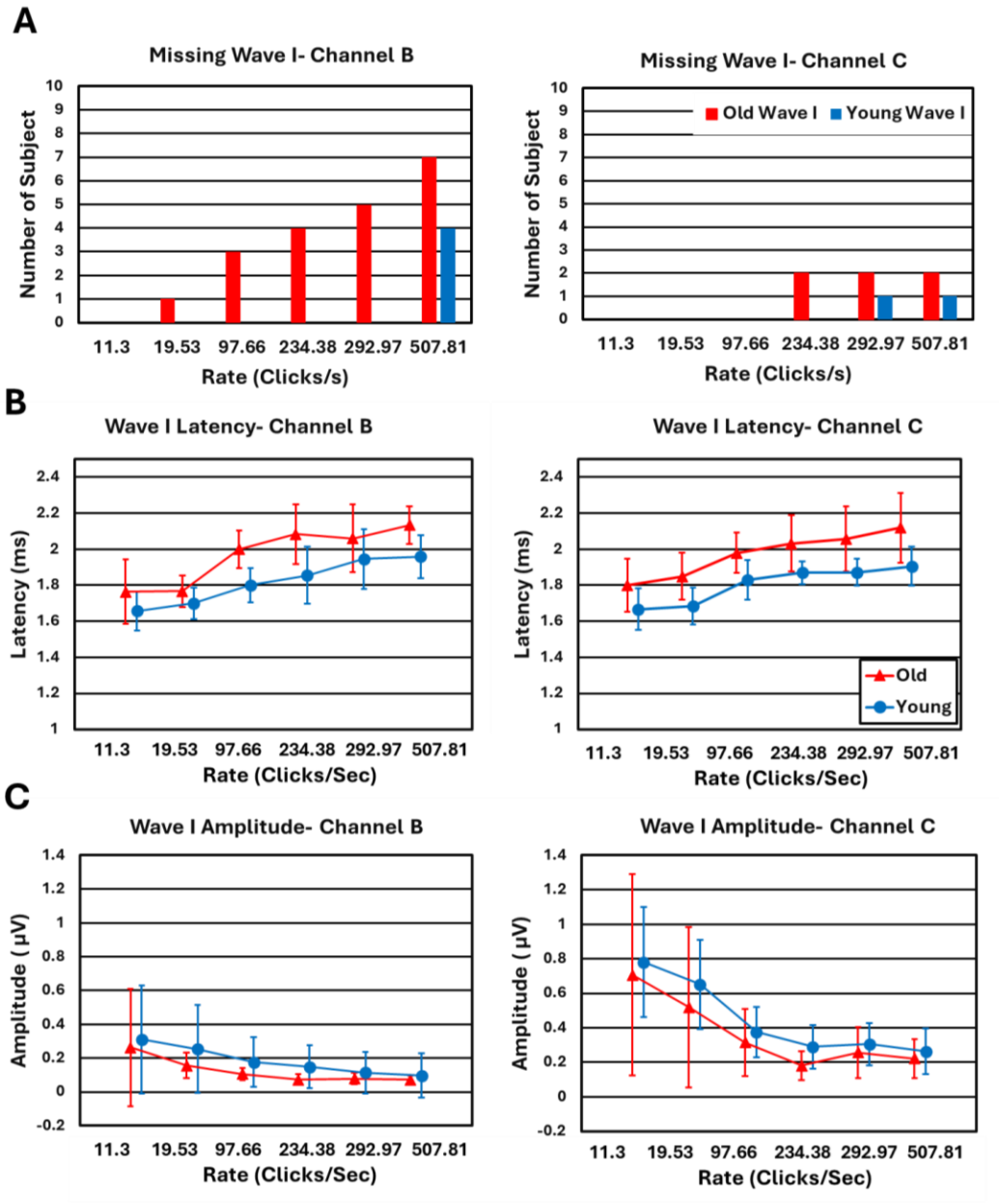

Figure 4.7: This figure presents Wave I responses for the old and young groups across different click rates using Vertical configurations for the mastoid electrode (channel B) and the tympanic membrane (channel C) electrode. Panel (A) displays the number of absent Wave I responses at various click rates per second in both age groups. Panel (B) illustrates the mean Wave I latency across different click rates for both groups. Panel (C) depicts the mean Wave I amplitude across different click rates for both age groups. Error bars represent ±1 standard deviation.

4.4.2 ABR: Wave V

4.4.2.1 Wave V Presence/Absence

The number of subjects with an absent Wave V was analyzed for the mastoid electrode channel B and the tympanic membrane channel C recordings. Histograms showing the number of subjects with an absent Wave V are presented in **Figure 4.8 (A)**.

The Cochran-Mantel-Haenszel test results indicate that there is no significant difference in channel B between the old and young groups ($X^2 = 1.2174$, df = 1, $p = 0.2699$). Overall, the old group of subjects does not have a significantly higher proportion of absent wave V than the young group. Although not statistically significant, it should be noted that in the old group, Wave V was absent in 1 subject at a rate of 292.97/s and in 2 subjects at a rate of 507.81/s. Additionally, there is one instance of missing Wave V in the young group at a rate of 507.81/s.

The Cochran-Mantel-Haenszel test for channel C results indicates that there is no significant difference in the number of subjects with absent Wave V between the old and young groups ($X^2 = 1.9539$, df = 1, $p = 0.1622$). However, in the old group, Wave V is absent in 2 subjects at a rate of 292.97/s and in 4 subjects at a rate of 507.81/s. Additionally, there are two instances of missing Wave V in the young group at a rate of 507.81/s.

For both channels, absent Wave V responses were classified as "missing data" and, therefore, excluded from the statistical analyses of Wave V latency and amplitude.

4.4.2.2 Wave V Latency

For young and old subjects with a Wave V component, the absolute latency was evaluated, as shown in **Figure 4.8 (B)**. The mean absolute latency of wave V in channels B and C appears to be slightly longer for the old group in the channel C recording using the tympanic membrane electrode. In both groups, the Wave V latency also increased as the stimulus rate increased; this is apparent in both channels B and C.

For both channels B and C, separate two-way ANOVAs of Wave V latency as the dependent variable (age × rate) were performed, with age as a between-participant factor and stimulus rate as a within-participant factor. A significant effect of age was found for channel C but not channel B. A significant effect of stimulus rate was found for each channel. However, due to the violation of the normal distribution of data, as indicated by $p < .05$ in the Shapiro-Wilk test, these results should be interpreted with caution (see Appendix **C**).

4.4.2.3 Wave V Amplitude

The amplitude values for wave V in channels B and C (shown in **Figure 4.8 (C)**) indicate a decrease in the mean Wave V amplitude with an increase in stimulus rate in both channels. However, the mean Wave V amplitude appears similar for both the young and old groups, across the range of stimulus rates.

In the analysis with the dependent variable as Wave V amplitude in channel C, a two-way ANOVA indicated no significant effect of age (between-participant factor) [$F (1, 92) = 0.63$, $p = .431$, $\eta 2p < .01$]. However, a significant effect of stimulus rate (within-participant factor) was observed [$F (5, 92) = 12.82$, $p < .001$, $\eta 2p = .41$], with no significant interaction between the two factors [$F (5, 92) = 1.28$, $p = .279$, $\eta 2p = .07$]. Post-hoc comparisons using Tukey's-HSD test identified significant differences in Wave V amplitude among various rates. The following pairwise comparisons showed statistically significant differences: 234.38/s-11.3/s ($p = .01$), 292.97/s-11.3/s ($p = .001$), 507.81/s -11.3/s ($p < .001$), 234.38-19.53 ($p = .018$), 292.97/s-19.53/s ($p = .002$), 507.81/s -19.53/s ($p < .001$), 234.38-97.66 ($p = .002$), 292.97-97.66 ($p < .001$), 507.81-97.66 ($p < .001$), 507.81-234.38 ($p = .036$). These findings suggest that an increase in stimulus rate is associated with a statistically significant decrease in Wave V amplitude. (Shapiro-Wilk test, $p= 0.3822233$; Levene's test, $p= 0.08982703$)

In the analysis of Wave V amplitude in channel B, the results of the ANOVA were similar, but due to the violation of normal distribution of data, as indicated by $p < .05$ in the Shapiro-Wilk test, these results should be interpreted with caution (see **Appendix C**).

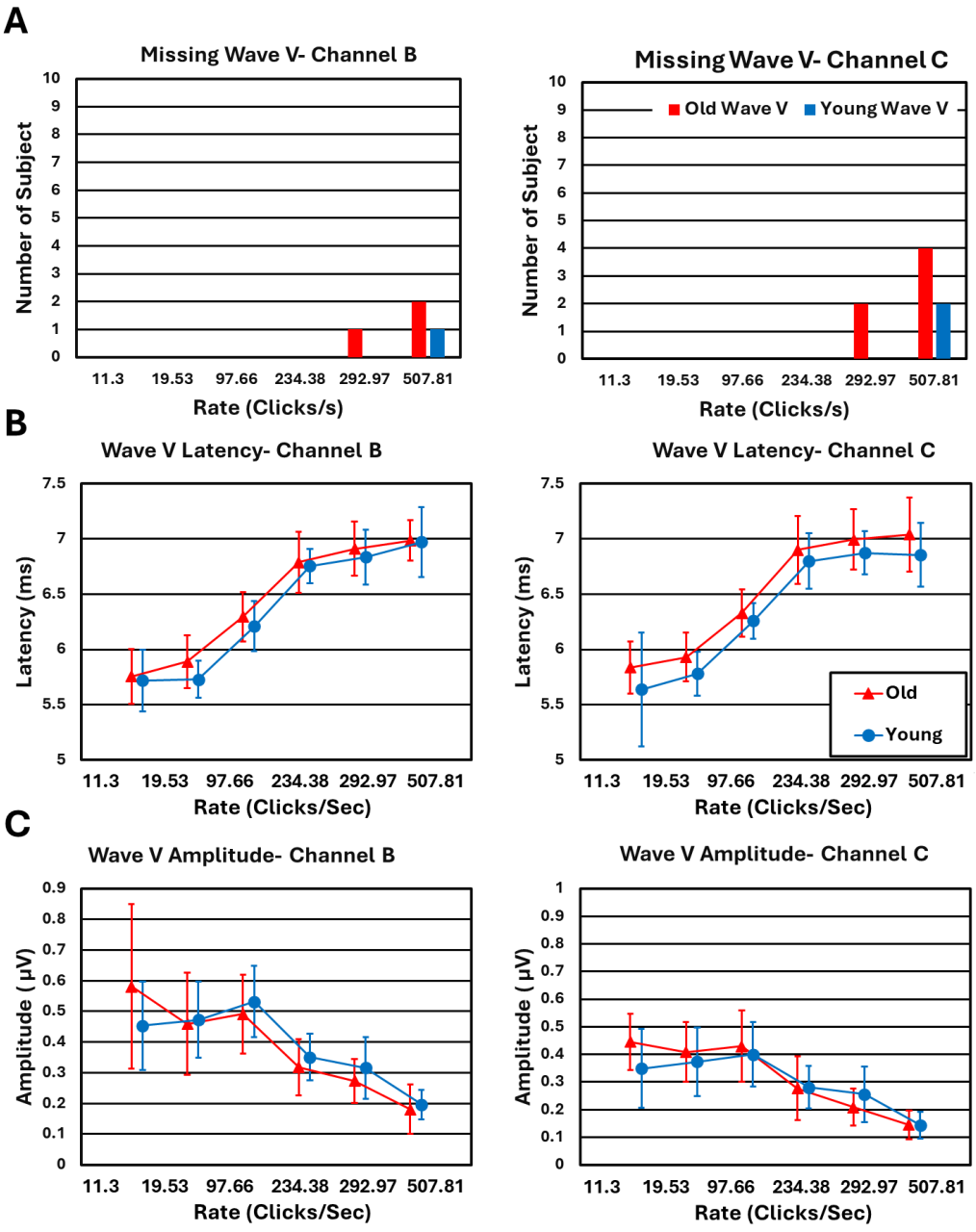

Figure 4.8: This figure illustrates analyses comparing Wave V responses between the old and young groups across various click rates and for Vertical ABR (channel B) and Vertical ECochG (channel C) recording configurations. Panel (A) depicts the number of absent Wave V responses at different click rates per second in both age groups. Panel (B) presents the mean Wave V latency across different click rates for both the old and young

groups. Panel (C) demonstrates the mean Wave V amplitude across different click rates for both age groups. Error bars represent ±1 standard deviation.

4.4.3 ABR: I-V Interwave Interval

The mean I-V interwave interval in channels B and C for the young and old groups are shown in **Figure 4.9.** This graph suggests that in both channels, the I-V interwave interval increased as stimulus rate increased for both the young and old groups. However, there was no effect of age observed.

Due to significant number of cases in the old group with missing Wave I and/or V, especially for the higher rates >97.66/s (see **Figures 4.7 (A) and 4.8 (A)**) statistical analyses of I-V interwave interval was not completed.

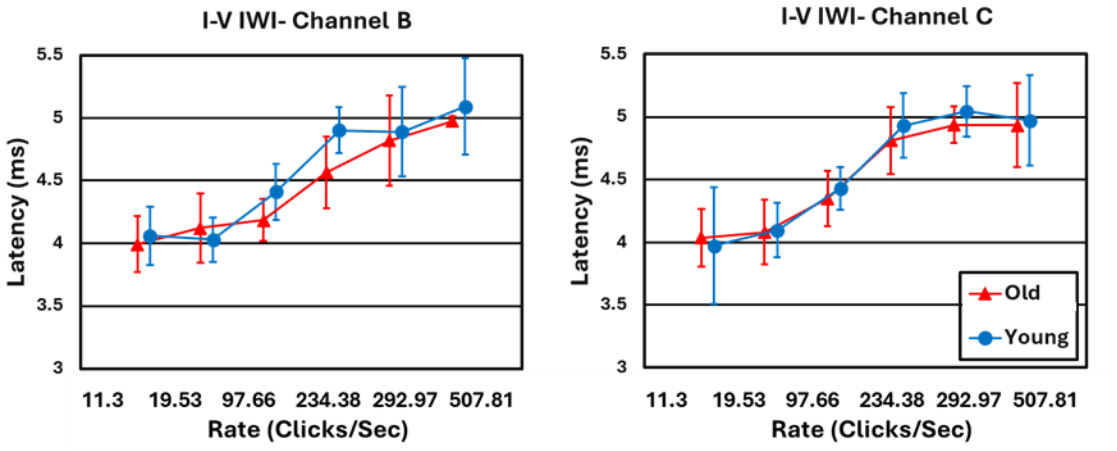

Figure 4.9: This figure illustrates the mean I-V interwave interval across various click rates for both the old and young groups in Vertical mastoid electrode (channel B) and Vertical tympanic membrane (channel C) recording configurations. Error bars represent ±1 standard deviation.

4.4.4 ABR: I/V Amplitude Ratio

The mean I/V amplitude ratio across different stimulus rates for the young and old groups are shown in **Figure 4.10**. This graph shows no apparent change in I/V with stimulus rate or different between age groups for this metric.

Due to significant number of cases in the old group with missing Wave I and/or V, especially for the higher rates >97.66/s (see **Figures 4.7 (A)** and **4.8 (A)**) statistical analyses of I/V amplitude ratio was not completed.

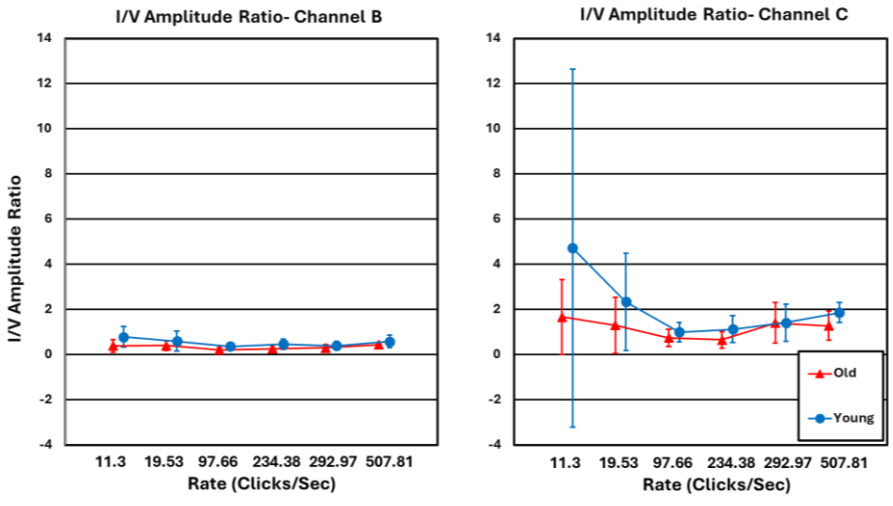

Figure 4.10: This figure illustrates the mean I/V amplitude ratio across various click rates for both the old and young groups in Vertical ABR (channel B) and Vertical ECochG (channel C) recording configurations. Error bars represent ±1 standard deviation.

4.5 Distortion Product Otoacoustic Emission (DPOAE) Levels

The mean DPOAE levels and noise floor for the young and old groups at each test frequency are shown in **Figure 4.10 (A)**. In **Figure 4.10 (B)**, the mean DPOAE levels across three frequency regions (low, mid, and high frequency) are provided for the young and old groups. These results are shown separately for the two different stimulus level conditions (Mid-level primary tones: L1= 55, L2= 40 dB SPL and High-level primary tones: L1= 61, L2= 55 dB SPL). There appears to be an age effect on the DPOAE levels, which were lower overall for the old compared to the young subjects.

4.5.1 DPOAE: Mid-Level Primary Tones

With the dependent variable being DPOAE levels evoked by L1= 55, L2= 40 dB SPL tones, a two-way ANOVA revealed significant effects of age (between-participant factor) [$F (1, 54) = 31.79$, $p < .001$, $\eta 2p = .37$] and frequency (within-participant factor) [$F (2, 54) = 3.21$, $p = .048$, $\eta 2p = .11$], with no significant interaction between the two factors [$F (2, 54) = 0.49$, $p = .617$, $\eta 2p = .02$]. The frequency range was divided into three categories, and DPOAE levels were averaged for these ranges: Low frequencies (.75-1.08 kHz), Mid frequencies (1.17-3 kHz), and High frequencies (3.38-7.55 kHz). Post-hoc comparisons using Tukey's-HSD test revealed a significant difference in DPOAE levels between low and high frequencies ($p = .047$). Considering both age and frequency factors, there were significant differences between the young and old groups in DPOAE levels at high frequencies ($p = .002$), as well as a significant difference at low frequencies ($p = .041$) (Shapiro-Wilk test, p= 0.04950162; Levene's test, p= 0.006521789).

4.5.2 DPOAE: High-Level Primary Tones

In the analysis of DPOAE levels in the High-Level condition (L1= 61, L2= 55), the results of the ANOVA were similar, but due to the violation of normal distribution of data, as indicated by $p < .05$ in the Shapiro-Wilk test, these results for should be interpreted with caution (see **Appendix C**).

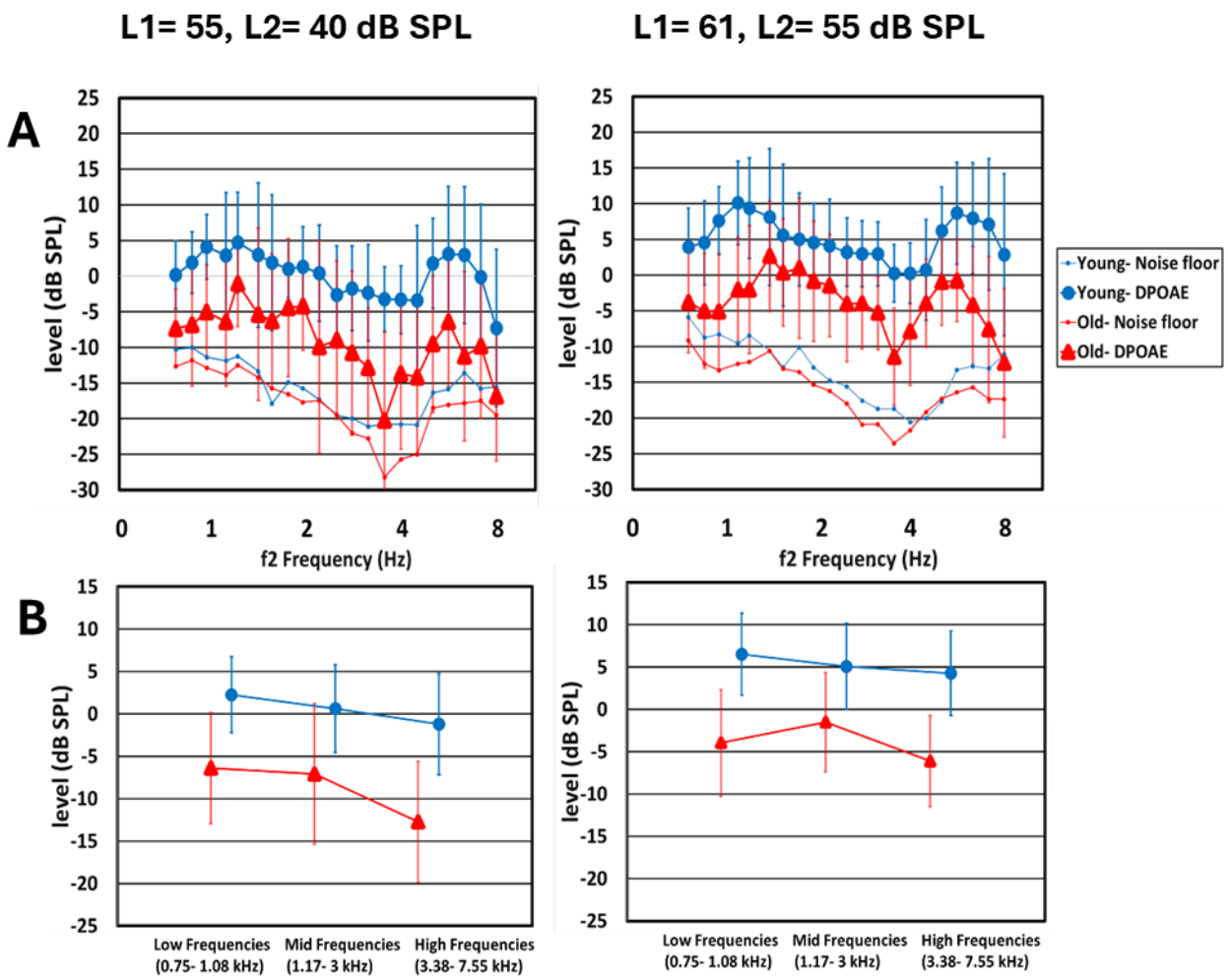

Figure 4.11: Panel (A) displays the mean DPOAE levels for both the young and old groups, recorded with two different sets of primary tone levels: L1=55 and L2=40 dB SPL, and L1=61 and L2=55 dB SPL, across all f2 frequencies. Panel (B) shows the mean DPOAE levels for both age groups, but analyzed across low (0.75-1.08 kHz), mid (1.17-3 kHz), and high (3.38-7.55 kHz) frequency regions. Error bars represent ±1 standard deviation.

Chapter 5

5 DISCUSSION

Physiological measures of human cochlear hair cells and auditory neural function are currently of interest because they can serve as noninvasive markers of sensory hair cell, synaptic and neural pathology in the auditory periphery and brainstem **(**Carcagnoa and Plack, 2020; Grant et al., 2020**).** When ABR, ECochG, and DPOAEs are obtained from the same individual, prolonged latencies or reduced amplitudes of different components relative to normative data can together indicate pathology in the cochlea, auditory nerve, and/or brainstem.

5.1 Age Effects on Auditory Nerve Function

The AP of the ECochG and ABR Wave I arise from the collective extracellular components of action potentials generated by individual auditory nerve fibers, particularly those with high characteristic frequency (Santarelli et al., 2021). The AP of the ECochG and ABR Wave I were recorded simultaneously in this study; because these components are generated by auditory nerve activity, they are discussed together in this section.

5.1.1 AP and Wave I

This thesis revealed a significant effect of age on the ECochG AP latency when measured with the tympanic membrane electrode (channels C). Likewise, there was also a statistically significant age-related increase in the Wave I latency for the simultaneously recorded ABR in channel B using a mastoid electrode. This is expected because the AP of the ECochG and wave I of the ABR are both measures of synchronized action potentials from the auditory nerve. Two previous studies using a tympanic membrane electrode to record the ECochG reported a similar association between age and AP latency as in this study, with prolongation of AP latency with increasing age (McClaskey et al., 2018; Soucek and Mason, 1992). These results also agree with reports using the transtympanic recording method (Oku and Hasegawa, 1997) or ear canal electrodes (Chatrian et al., 1985). Similarly, the longer Wave I latencies in older adults found in this

study are consistent with prior investigations of wave I using a transtympanic (Oku and Hasegawa (1997), tympanic membrane (Burkard and Sim, 2002) or mastoid surface electrodes (Konrad-Martin et al., 2012) to record the ABR. However, not all previous investigations were in agreement with these results. Wilson and Bowker (2002) found no age-related effect on AP latency when recorded with tympanic membrane electrodes, similar to Carcagnoa and Plack (2020), who reported no impact of age on ABR Wave I latency.

Although the AP and Wave I are often recorded with different electrode types and montages, they both represent synchronous action potentials generated by the auditory nerve and appear as a single peak with the same latency when a combined ECochG/ABR waveform is acquired. Different labels are used because for amplitude measurements, the ECochG AP and ABR Wave I are calculated using different criteria (see **Figure 3.2** and **3.3**). The AP amplitude is usually identified as the difference between a preceding baseline and the positive peak, whereas the ABR Wave I amplitude is measured from the peak to the following negative trough.

In this thesis, the ECochG and ABR were acquired simultaneously across different channels. No age-related group difference in AP amplitude was found for the tympanic membrane electrode (channel A or C) or for wave I recorded from either the mastoid (channel B) or tympanic membrane (channel C). Although this finding aligns with previous results for the AP (Wilson and Bowker (2002), numerous other investigations have found a reduced amplitude with increasing age for the AP (Chatrian et al., 1985; Soucek and Mason, 1992; Oku and Hasegawa, 1997) and Wave I (Konrad-Martin et al., 2012; Burkard and Sim, 2002; Aedo-Sanchez et al., 2023; Grose et al., 2019; Johannesen et al., 2019; Carcagnoa and Plack, 2020; Roque et al, 2019; Purner et al., 2022).

5.2 Age Effects on Auditory Brainstem Function

5.2.1 Wave V latency

The ABR Wave V absolute latency and amplitude, I/V amplitude ratio and I-V interwave interval were also evaluated in order to assess the effects of age on central auditory function, specifically at the level of the auditory brainstem.

For Wave V latency, a significant age effect was identified, with longer latencies in older adults. However, this age effect was only significant when using the tympanic membrane electrode in channel C; no age effect was observed with the mastoid electrode recording in channel B. This age effect on ABR Wave V latency is supported by previous research identifying longer Wave V latencies in older adults when using a variety of different electrode types and montages, including transtympanic (Oku and Hasegawa, 1997) tympanic membrane (Burkard and Sim, 2002; Soucek and Mason,1992), ear canal (Carcagnoa and Plack, 2020), or mastoid surface electrodes (Konrad-Martin et al., 2012; Aedo-Sanchez et al., 2023; Jerger and Hall, 1980).

With respect to the amplitude of the ABR Wave V component, this study did not find evidence of an age effect when recorded with either a mastoid or tympanic membrane electrode (channels B and C), which corresponds with the results reported by Carcagnoa and Plack (2020) who acquired the ABR from the ear canal. Using a mastoid electrode, Jerger and Hall (1980) also found that Wave V showed no consistent change in Wave V amplitude per decade as age increased from 20-59 years. However, many others have found that aging was associated with a reduction in Wave V amplitude (Konrad-Martin et al., 2012; Burkard and Sims, 2002; Aedo-Sanchez et al., 2023; Jerger and Hall, 1980; Soucek and Mason, 1992), using a variety of electrode sites for recording the ABR.

5.2.2 I-V Interwave Interval

The ABR I-V interwave interval is interpreted as a metric of central auditory afferent integrity because brainstem transmission from the auditory nerve to the lateral lemniscus is isolated from peripheral auditory function. From **Figure 4.9**, there is no apparent age effect on the I-V interwave interval for either the mastoid or tympanic membrane

(channel B or C) recordings, suggesting the auditory brainstem is not altered by aging. However, due to the number of missing Wave I and/or V components, particularly in the older subject group, statistical analyses of I-V interval data were not completed. Previous investigations of the I-V interwave interval are conflicting, with some finding no effect (Burkard and Sims, 2002; Konrad-Martin et al., 2012; Soucek and Mason, 1992) and others reporting an increased I-V interwave interval with aging (Pürner et al., 2022; Carcagnoa and Plack, 2020; Oku and Hasegewa, 1997) which has been interpreted as an increase in brainstem transmission time.

5.2.3 I/V amplitude ratio

Few studies have examined the amplitude ratio between the ABR Waves I and V, which provides within-subject standardization as a way to control for between-subject amplitude variability, especially for Wave I. Grose et al. (2019) using an ear canal recording technique reported a reduction in the I/V amplitude ratio with aging. From **Figure 4.10**, there is no apparent difference between the young and old subjects for the mastoid (channel B) or the tympanic membrane electrode recordings (channel C) in this study. However, the I/V amplitude data is based on a small number of participants due to the number of missing Wave I and/or V components, as noted above.

5.3 Presbycusic Hearing Loss - A Confounding Factor

For broadband click stimuli, the ECochG AP and ABR components are differentially affected by reduced hearing sensitivity, with the high frequency cochlear base having a greater influence on the AP and Wave I compared to Wave V, which derives from a more widespread cochlear region (Don and Eggermont, 1978).

Most evidence supporting an age-related prolongation of absolute latency or reduced amplitude of the AP or Waves I and V does not adequately account for the effects of presbycusic hearing loss. Furthermore, even though the ABR I-V interwave interval is recognized as a measure of neural transmission time in the auditory brainstem, it can also be confounded by peripheral damage in those with sensorineural hearing loss (Jerger and Hall, 1980). With the exception of Aedo-Sanchez et al. (2023), Roque et al. (2019), and Wilson and Bowker (2002), studies cited in the previous sections include older subjects

with significant high-frequency sensorineural hearing loss. Some, like Konrad-Martin et al. (2012), made efforts to control for hearing loss by averaging pure-tone thresholds at 2, 3, and 4 kHz to define a “better hearing” group. However, averaged 2-4 kHz thresholds obscured sharply sloping high frequency impairment, and thresholds beyond 4 kHz were also excluded so that even the better hearing group included subjects with significant hearing loss above 3kHz. Statistical models have also been used to control for confounding factors but often use pure tone averages to account for sensorineural hearing loss, with differing outcomes (Bramhall et al., 2021; Konrad-Martin et al., 2012; Carcagno and Plack, 2020; Grant et al., 2020; Johannesen et al., 2019). Consequently, discrepancies between prior age-related studies likely reflect, in part, threshold differences above 2 kHz between young and old participants rather than age-related effects specifically.

In the current study, a notable advantage lies in the absence of hearing loss in the clinical pure tone range of 0.25-8 kHz for all participants, both young and old. This allowed for a more controlled examination of the specific effects of age on the ECochG and ABR parameters without the confounding influence of significant hearing loss.

5.4 Rate Effects on Auditory Nerve and Brainstem

Ultrahigh rates are important because the time interval between stimuli is close to the recovery time required following synaptic transmission at the cochlear synapse and refractory period of auditory neurons. Only a few studies have compared the effects of adaptation using ultrahigh click rate above 100/sec in aging humans with their younger counterparts (Burkhard and Sims, 2012; Soucek and Mason, 1992; Wilson and Bowker, 2002) and report reduced AP and ABR amplitudes and prolonged latencies in older subjects, using increasing rates up to 500/s.

5.4.1 AP latency and amplitude

The results of this study indicated a significant rate effect on AP latency (channels A and C) using the tympanic membrane electrode**.** This finding corresponds with results from Kaf et al. (2017) and Soucek and Mason (1992). Wilson and Bowker (2002) also reported longer AP latencies with increased rates. AP amplitudes were also reduced with

an increased rate in both Horizontal and Vertical montages (channels A and C) using the tympanic membrane electrode. Smaller AP amplitudes are consistent with the results reported by Kaf et al. (2017), Soucek and Mason (1992), and Wilson and Bowker (2002).

5.4.2 Wave I latency and amplitude

Wave I, like the AP, was also significantly longer and decreased in amplitude with increasing rate for both mastoid and tympanic membrane electrode (channels B and C) configurations. This aligns with the findings of longer latency with increasing rate for young normal hearing adults (Kaf et al., 2017; Maele et al., 2021; Kaf et al., 2022; Soucek and Mason, 1992; Burkard and Sim, 2002; Konrad-Martin et al., 2012), older adults with good hearing (Burkard and Sim, 2002) and adults with high-frequency hearing loss (Soucek and Mason, 1992; Burkard and Sim, 2002; Konrad-Martin et al., 2012).

5.4.3 Wave V latency and amplitude

In the assessment of auditory brainstem function, significantly longer wave V latencies and smaller amplitudes for both the tympanic membrane and mastoid electrode configurations (channels C and B) occurred with an increasing rate, as reported by previous investigations (Burkard and Sim, 2002; Jiang et al., 2009; Kaf et al., 2017; Kaf et al., 2022; Konrad-Martin et al., 2012; Maele et al., 2021; Picton et al., 1992; Soucek and Mason, 1992; Yasmin et al., 2020).

5.5 Absence/Presence of ECochG and ABR

Analyses of age and click rate effects on ABR and ECochG latency and amplitude measures depend on the ability to detect these physiological responses. Cochlear and neural deterioration, together with test conditions like high click rate that “stress” auditory processing, have the potential to reduce physiological signals to the point where responses are absent. Analyses of latency and amplitude data alone excludes the results for some participants – this occurs because this data is missing when ABR or ECochG

components are absent. Yet, for participants with the most severe damage, absent AP or ABR waves represent the most abnormal outcome.

The complete absence of one or more components of the ECochG and ABR is an unusual finding when hearing is normal and cochlear outer hair cells are healthy. When hearing sensitivity is normal, as in this thesis, absent ECochG or ABR components are significant, suggesting pathology at a corresponding level of the auditory pathway. For example, in clinical practice, an absent ABR combined with present DPOAEs for any range of hearing sensitivity, from normal to profound, indicates functioning outer hair cells and auditory neuropathy.

Simultaneous recordings across channels in the same individual enable the comparison of ECochG and ABR waveforms acquired with different electrodes and montages under otherwise similar recording conditions. In this thesis, channels using the tympanic membrane electrode provided larger response amplitudes and fewer absent responses, as noted in several previous studies (Soucek and Mason, 1992; Burkard and Sims, 2001; Wilson and Bowker, 2002; Kaf et al., 2017; Kaf et al., 2022). Few studies consider or report absent responses. Not only is potentially meaningful data excluded, but the sample size is also reduced, providing a less representative estimate of age and click rate effects on the evoked ECochG and ABR. For this reason, evaluating the presence vs. absence of ABR and ECochG components alongside ANOVA provided a more comprehensive understanding of auditory response results in this study. Significantly more absent Wave I components occurred with older subjects compared to younger subjects, and also as stimulus rate increased in both groups, but only for mastoid electrode recordings in channel B. This was not the case for the ECochG AP component for recordings acquired simultaneously with the tympanic electrode in channels A and C. No significant differences in presence/absence of the ECochG SP component or the ABR Wave V were found in any recording channel.

5.6 Interaction Between Age and Stimulus Rate

Pathologies affecting the transduction or pre-synaptic functions of inner hair cell (Salvi et al, 2017) or neural encoding and transmission by spiral ganglion auditory neurons

(Jacobson et al., 1987; Santarelli et al., 2021 and 2019; Tanaka et al., 1996; Santos et al., 2004) can reduce their physiological signals to the point where the auditory evoked responses are abnormal or absent. Similarly, increasing stimulus repetition rate, especially into the ultrahigh range above 100 clicks/s, will reduce and delay auditory evoked potentials by decreasing synaptic and neural recovery time between stimuli, causing adaptation (Burkhard and Sim, 2002; Grant et al., 2020; Kaf et al., 2017). High click rates below 100 clicks/s have been utilized clinically to improve the diagnostic sensitivity of the ABR to the auditory nerve and brainstem lesions (Jacobson et al., 1987; Santos et al., 2004; Stanton et al., 1989; Youssef and Downes, 2009).

One of the aims of this study was to evaluate ultra-high rate stimuli, with the expectation that increasing physiological stress on synaptic and neural structures compromised by the aging process would enhance adaptation effects and reveal dysfunction. As a result, AP or ABR component latencies and amplitudes would be more seriously affected in older subjects versus younger adults. However, this study revealed no significant interaction between subject age and stimulus rate for any of the ECochG and ABR components. Even though absolute latencies were significantly delayed and amplitudes decreased in older subjects, the size of these rate-induced AP and ABR changes was the same for young and old groups. This was also true for the number of absent ABR Wave I components which grew with increasing rate in both older subjects and younger subjects for mastoid electrode recordings in channel B.

This outcome is consistent with previous research, which has found no significant interaction between age and stimulus rate on ECochG and ABR components for either conventional (Konrad-Martin et al., 2012) or ultrahigh stimulus rates (Burkard and Sims, 2002; Soucek and Mason, 1992). However, each of these investigations included subjects with significant sensorineural hearing loss in older subjects, which complicated interpretation - cochlear damage associated with sensorineural hearing loss is potentially more severe and different from purely age-related pathology. Only one study controlled for hearing loss by measuring the ECochG in normal hearing adults 18-60 years of age. Wilson and Bowker (2002) evaluated the AP component in 3 different age groups using click rates between 7.1 – 151.1 clicks/s and found no interaction between age and

stimulus rate. The results of this thesis are important because they support the AP results of Wilson and Bowker (2002) and extend the results by simultaneously measuring ABR Waves I and V and using ultrafast stimulus rates up to 507.81 clicks/s. The absence of the rate by age interaction reported in previous work (Burkard and Sims, 2002; Konrad-Martin et al., 2012; Soucek and Mason, 1992) is confirmed here, but in a group of older adults with normal hearing.

5.7 Cochlear Function: SP & DPOAE

In this thesis, the physiological integrity of the cochlea was assessed by the ECochG SP and by DPOAEs. The main generators of these potentials are the cochlear hair cells, which are important for sensory transduction in the cochlea.

5.7.1 SP

The click-evoked SP appears to be a sustained voltage difference due to asymmetric hair cell receptor potentials. Far-field recordings appear to originate in the basal cochlea, but the generation of the SP is complex with input from multiple cell populations. The current consensus is that inner hair cells are the major source, with contributions from outer hair cells and neural activity arising from the peripheral spiral ganglion terminals (Eggermont, 2019; Lutz et al., 2022; Russell, 2008). Together, the SP and AP components of the ECochG are used as clinical and research tools to diagnose and monitor pathology affecting the cochlear hair cells, synapses, and distal auditory nerve fibers of spiral ganglion neurons. In this study, the SP was used in combination with the AP and ABR waves to assess the impact of age-related pre-synaptic and post-synaptic auditory dysfunction (Eggermont, 2019; Lutz et al., 2022; Russell, 2008).

Results show that the SP and SP/AP ratio were not affected by age or stimulus rate. No effect of age on SP latency or amplitude was observed for tympanic membrane electrode configurations. Chatrian et al. (1985) noted a decrease in the SP amplitude with age when using ear canal electrodes. However, there was a 4 - 8 kHz hearing loss in subjects older than 50 years, with the potential to confound the observed age effect on the SP. Wilson and Bowker (2002) controlled for hearing sensitivity by only including subjects with normal hearing in the conventional range from 0.25 to 8 kHz, as was done in this thesis.

Using tympanic membrane electrodes, subjects 18-60 years old had no age-related differences in the absolute SP latency or amplitude. Together, these results indicate that the SP is not significantly influenced by age when hearing thresholds are clinically normal.

The effects of stimulus rate on the SP were also examined, with no significant impact found for the latency or amplitude when the SP was recorded with the tympanic membrane electrode (channels A and C). This outcome aligns with the findings of Aleksandra et al. (2015) and Kaf et al. (2017), both of whom reported no rate effects on SP latency or amplitude. However, a discrepancy emerged with Wilson and Bowker (2002), who observed an increase in both SP amplitude and latency with increasing stimulus rate using a tympanic membrane electrode.

One of the limitations of using the SP is the high between-subject variability, which also exists for the AP component of the ECochG (Coats, 1986; Eggermont, 2017; Chatrian et al., 1985). However, amplitudes of the SP and AP tend to covary, so that people with low AP amplitudes usually have low SP amplitudes and vice versa. Methods to limit this variability include calculating the SP/AP ratio to normalize the SP relative to the AP amplitude. Chatrian et al. (1985) found a significant increase in the SP/AP amplitude ratio with age, but as noted above, the interpretation of this age effect was complicated by significant high frequency sensorineural hearing loss in older subjects. Several investigators also report a significant increase in the SP/AP amplitude ratio with increasing rate (Kaf et al., 2017; Kaf et al., 2022; Wilson and Bowker, 2002), which was not observed in this thesis.

5.7.2 DPOAE

It is well established that hearing thresholds exhibit a systematic increase with advancing age, with the most notable increase observed at higher frequencies. DPOAEs are generated by the outer hair cells, and human temporal bone studies show that presbycusic pathology includes significant cochlear outer hair cell damage (Viana et al., 2015; Wu et al., 2020a & b; Wu et al., 2021). Similarly, DPOAE investigations have considered the effects of pure tone hearing thresholds in addition to subject age and found evidence to

support age-related outer hair cell dysfunction. Several studies show lower DPOAE levels across the frequency range (between 1-8 kHz) in older adults with mid-high frequency sensorineural hearing loss when compared to younger age groups (Abdala and Dhar, 2012; Jedrzejczak et al., 2023; Uchida et al., 2008).

An important research question has focused on the cochlear status of older adults with normal hearing sensitivity between 0.25-8 kHz and whether their corresponding DPOAEs below 8 kHz are also normal. Several studies report that age-related differences in standard frequency DPOAES (1-8kHz) can occur even when conventional hearing thresholds are normal (Abdala and Dhar, 2012; Hunter et al., 2020) or near-normal (Bramhall et al., 2021; Märcher-Rørsted et al., 2022). For normal hearing middle age (Jedrzejczak et al., 2023) and older adults (Abdala and Dhar, 2012; Hunter et al., 2020; Märcher-Rørsted et al., 2022), the DPOAEs are reduced, specifically in the high frequency range at 2 kHz or above.

In this study for the Mid-Level test condition, the DPOAE results also show an age-related difference, with significantly lower DPOAE levels overall for the old group compared to the young group. In addition, there was a frequency effect with lower DPOAEs in the f2 “High Frequency” range compared to the “Low Frequency” range. Similar trends were found for the High-Level test condition. These collective findings support the notion that DPOAE levels are influenced by age, with younger individuals typically displaying higher level DPOAEs compared to their older counterparts. In the standard DPOAE frequency range below 8 kHz, DPOAE levels decrease with increasing age, even though the standard clinical audiogram thresholds in this frequency range are normal.

5.8 Extended High Frequency Hearing Thresholds (EHF)

Another important age-related consideration is hearing sensitivity beyond the standard clinical audiogram. Hearing loss in the EHF region can signal damage or dysfunction within extreme basal regions of the human cochleotopic map (Viana et al., 2015; Wu et al., 2020a & b; Wu et al., 2021). The results of this thesis show that pure tone thresholds in the extended high frequency range were higher overall in the older group compared to the younger group and progressively declined between 9-14 kHz, with absent responses

at 16 and 18 kHz. EHF pure tone thresholds beyond the 8 kHz upper limit of the clinical audiogram are worse in the old group, which also has reduced DPOAE levels < 8 kHz compared to their younger counterparts.

5.9 EHF Thresholds and DPOAE Levels in Normal Hearing Old Adults

This study found that an age-related decline occurred in both the EHF between 9-16 kHz and in the DPOAE levels measured below 8 kHz, even though clinically normal thresholds are retained between 0.25- 8 kHz. In support of our findings, there is growing evidence showing that adults with normal conventional audiograms (0.25-8kHz) but poor EHF hearing sensitivity also have reduced DPOAE levels in the standard frequency range between 1-8 kHz. Similar to this study, Hunter et al. (2020) found that standard DPOAEs and EHF thresholds are both affected by age and that as age increased, DPOAE levels below 8kHz declined along with the EHF thresholds above 8 kHz, despite normal standard audiometric hearing (.25-8 kHz). Mishra et al. (2021) focused specifically on younger adults (19–38 years) and whether EHF audiometric thresholds above 8 kHz could affect standard 2-5 kHz DPOAEs. Study participants all had normal conventional hearing thresholds but were subdivided based on the degree of EHF hearing impairment (> 20 dB HL from 10 - 16 kHz). The EHF impaired group (median 28 years) exhibited an overall reduction in 2-5 kHz DPOAE levels compared to the group with normal EHF hearing (median 22 years). There was a correlation between DPOAEs and EHF thresholds, with worse overall DPOAE levels as EHF thresholds decreased. Glavin et al. (2021) studied five different age groups, all with normal conventional hearing up to 8 kHz, and found reduced frequency-specific DPOAE (2, 4, 8 kHz) levels with increasing age. These reduced DPOAE levels were related to the mean EHF thresholds, which decreased in every age decade from 10 to 50+ years. The authors conclude that adults with normal conventional audiograms up to 8 kHz can manifest reduced DPOAEs below 8 kHz, and this DPOAE decline correlates with impaired threshold sensitivity in the EHF range above 8 kHz.

Both EHF and DPOAE measures indicate dysfunction with increasing age, but it is difficult to disentangle the effects of age alone from pathology associated with EHF

hearing loss. Jedrzejczak et al. (2023) recently explored this research problem by studying standard DPOAE levels (1, 1.5, 2, 4, 6, 8 kHz) and DPOAEs in the extended high frequencies (10, 12, and 16 kHz) in three different age groups that differed in extended high frequency hearing sensitivity. A young group (aged 29 years + SD 7) had better hearing (normal up to 16 kHz) than an old group with a normal clinical audiogram but impaired EHF (aged 47 years +10 SD). A second older group (51 years + 11 SD) had much worse hearing across the entire frequency range, including significant clinical impairment on the standard audiogram (> 25 dB HL below 8 kHz). The authors found that DPOAE levels were negatively correlated with age, with a stronger effect observed for DPOAEs in the 10–16 kHz range compared to the 1–8 kHz range. The study concluded that DPOAEs, both within the standard frequency range (DPOAEs 0.125–8 kHz) and the extended high-frequency range (DPOAEs 10–16 kHz), decrease as behavioral hearing thresholds deteriorate and as age increases.

5.10 EHF Thresholds, DPOAEs and Human Temporal Bone Research

There is a growing body of evidence from human temporal bone research that is relevant to the interpretation of age-related effects on EHF and DPOAEs in this and other investigations of cochlear aging. With aging, frequency-specific sensitivity declines progressively from low to high along the length of the cochlea, with the basal cochlea responsible for detecting and transmitting high frequency sound. Consistent with prior research, this study demonstrated that older adults exhibited greater hearing thresholds compared to younger adults in the EHF region >8 kHz, which becomes progressively worse from 9 -18 kHz (Aziz et al., 2020; Glavin et al., 2021; Märcher-Rørsted et al., 2022; Jedrzejczak et al., 2023; Roque et al., 2019; Aedo-Sanchez et al., 2023; Bramhall et al., 2021; Hunter et al., 2020). Given the cochleotopic organization of sound frequency, hearing loss in the EHF range in older adults indicates that extreme basal cochlear structures are the most susceptible to the initial stages of aging. Collectively, human temporal bone studies show progressively more age-related degeneration towards the basal cochlea, with the greatest loss of all cell types (strial cells, inner and outer hair cells, and Type I spiral ganglion neurons) in the extreme (>10 kHz) high frequency

region of the cochleogram (Nelson and Hinojosa, 2006; Ramadan and Schuknecht, 1989; Viana et al., 2015; Wu et al., 2020a & b; Wu et al., 2021).

The lower DPOAE levels observed for normal hearing older adults in this thesis were most pronounced in the high frequencies (3-8 kHz) and also signify age-related functional deterioration of basal outer hair cells, which aligns with evidence from human temporal bone studies (Kusunoki et al., 2004; Nelson and Hinojosa, 2006a; Ramadan and Schuknecht, 1989; Viana et al., 2015; Wu et al., 2019; Wu et al., 2020a & b; Wu et al., 2021). Wu et al. (2021) conducted a human temporal bone study with modern histopathological techniques to measure cell loss across the cochleotopic frequency map in a young-middle age (1-49 years old) and an old group (50-74 years old). Mean pure tone thresholds for the young group were normal from 2-8 kHz, while the old group had a mild loss at 2-4 kHz, sloping to moderate at 8 kHz, with approximately 20 dB lower thresholds overall. Outer hair cell loss was greater for the old group at 45% versus 20% for the young group for the 2-6 kHz cochleotopic region. Between the 10-16 kHz region, the outer hair cell loss increases from 50%-80% for the older group and from 30% - 65% for the younger group. Together, these findings indicate a significant loss of outer hair cells, which increases rapidly towards the cochlear base, even in the young group with normal hearing.

In this thesis, both the young and the old groups had normal hearing sensitivity in the 0.25-8 kHz range. However, there was a slight difference in their standard clinical audiograms, with mean thresholds of 5 dB (at 2 kHz) to 12 dB (at 8 kHz), worse in the old group. Hearing thresholds declined rapidly in the EHF range and were again worse in the old group, which corresponds to the % cell loss in the extreme basal region of the cochleotopic map, as reported by Wu et al. (2021). A similar trend in mean DPOAE levels was present in this study, with the old group having 10 dB (at 2 kHz) to 20 dB (at 8 kHz) lower DPOAE levels compared to the young group. These group differences in hearing sensitivity and DPOAEs, which rely on outer hair cell function, are interesting and parallel the group threshold differences and patterns of outer hair cell loss reported by Wu et al. (2021).

5.11 Hearing Thresholds, Auditory Nerve Function (AP and Wave I) and Human Temporal Bone Research

Wu et al. (2021) also compared the loss of inner hair cells and auditory nerve (spiral ganglion) fibers in the normal hearing young-middle age (1-49 years) and the mildly hearing impaired old group (50-74 years) with 20 dB worse pure tone hearing thresholds. Inner hair cell loss was 15-20% more severe for the old group across the cochleotopic frequency map, ranging from 30% to 60% (2-6 kHz vs. 16 kHz cochlear regions) compared to 15% to 40% (2-6 kHz vs. 16 kHz cochlear regions) for the young group. There was a similar trend but more significant loss of auditory nerve fibers across the 2-8 kHz cochlear region, with 45-60% for the old group compared to 30-35% for the normal hearing young group. Viana et al. (2015) analyzed five human temporal bones (54-89 years of age) and found a decrease in the number of auditory nerve fibers per inner hair cell with increasing age, with a similar age effect noted by Wu et al. (2019) for 29 ears. The number of intact synapses also decreased, as indicated by the reduction in synaptic ribbons per inner hair (Viana et al., 2015). These histopathology results indicate loss of functional synaptic connections between inner hair cells and auditory neurons with increasing age.

Whole nerve population responses of the AP or ABR wave I arise from the summation of the synchronized activity of many auditory (spiral ganglion) neurons, which in turn depend on the normal function of inner hair cells and their synaptic connections. The loss of inner hair cells, auditory (spiral ganglion) nerve fibers, and their synaptic connections, as noted above (Viana et al., 2015; Wu et al., 2019; Wu et al., 2021), would likely reduce electrophysiological activity in the whole nerve compound action response, and result in a reduction of the AP and Wave I amplitudes. The loss of synapses and synaptic ribbons could lead to variable delays in synaptic transmission time and impact the generation of action potentials remaining post-synaptic auditory (spiral ganglion) terminal nerve fibers. Reduced number of actional potentials and desynchronization of activity in the whole nerve population response would prolong the latency and reduce the amplitude of the AP and ABR wave I (Moser and Starr, 2016).

In this study, older age was associated with prolonged latency of AP and ABR Waves I and V, indicating potential abnormalities in the auditory nerve and the brainstem pathway. The observed prolongation of these waves suggests alterations in auditory neural processing with age and aligns with these histopathological studies, which show loss of auditory nerve fibers and synapses even in young and middle age normal hearing subjects (Wu et al., 2019; Wu et al., 2021). However, dysfunction of outer hair cells as indicated by the DPOAE levels <8kHz, and likely present in the EHF 9-16 kHz may also contribute to the delayed latencies of the AP, and ABR Waves I and V, as discussed below.

5.12 Summary and Conclusions

The purpose of this study was to measure AP/ABR to evaluate pre-post synaptic neural function in "aging ears". The role of cochlear outer hair cells is to provide mechanical amplification to downstream inner hair cells and Type I spiral ganglion neurons, which perform mechanoelectrical transduction and neural encoding of sound. Aging of the peripheral auditory system can involve dysfunction or loss of any of these cell types and their connecting synapses. ECochG AP and ABR Wave I reflect the collective electrical activity generated by the population spiral ganglion neurons that form the auditory nerve, while ABR Wave V reflects neural encoding and transmission in the auditory periphery and brainstem. Previous studies have shown age-related reduction and delay of the AP and ABR Waves, but the underlying neuropathological mechanisms are difficult to interpret because of confounding high frequency hearing loss. One strength of this study was that conventional pure tone audiograms <8 kHz were normal for all subjects. Although conventional audiometric thresholds depend on outer hair cell status, they are not very sensitive to inner hair cell-synaptic and neural pathology (Salvi et al., 2017; Moser and Starr, 2016).

1. Absolute latency of the ECochG AP neural component and ABR Waves I and V were significantly prolonged in the old group compared to the young group, despite normal hearing sensitivity (<8k). One explanation for the prolongation of the AP

and ABR Wave latencies is underlying inner hair cell, synaptic, and/or peripheral auditory nerve pathology.

Adults in the old group had longer latencies for the ECochG AP and ABR Waves I and V overall compared to young adults. The amplitude of these components was not significantly different, possibly due to large intersubject variability and small sample size. One interpretation is that ECochG AP and ABR Wave I latencies are sensitive metrics of age-related neural loss or dysfunction involving (1) pre-synaptic inner hair cells critical for mechanoelectrical transduction or pre-synaptic neurotransmitter release. (2) spiral ganglion neuron cell bodies or their terminal nerve fibers that synapse with inner hair cells. Prolongation and reduced amplitude of the ABR Wave V could reflect this peripheral damage or involve changes in auditory brainstem processing.

2. Unlike the other physiological measures, the SP did not show any significant age-related changes in latency or amplitude.

The SP component is sensitive to hair cell function, particularly inner hair cell mechanoelectrical transduction when recorded in close proximity to the cochlear base. The stability in SP with age in this study may reflect the preservation of inner hair cell function. Although the inner hair cells are considered the main generators, outer hair cells and neural activity also contribute to SP, making interpretation less straightforward (Lutz et al., 2022; Salvi et al., 2017). However, the stability of the SP aligns with recent human temporal bone research showing less inner hair cell loss in the basal cochlea relative to outer hair cells and auditory neurons (Wu et al., 2021).

3. EHF thresholds (9-18 kHz) were impaired, and conventional DPOAEs (<8k) were reduced in the old group relative to the young group, despite normal hearing sensitivity (<8k) in both age groups. In this thesis, EHF impairment coupled with DPOAE reduction <8kHz together serve as markers of subclinical cochlear pathology worse in the old group and involving the outer hair cells. Including DPOAE levels and EHF thresholds (9 – 18 kHz) contributed to the interpretation of physiological data.

One strength of this study was the strict criteria that all participants have normal clinical audiograms (0.25-8 kHz). Despite having normal pure tone thresholds <8 kHz, reduced hearing sensitivity in the extended high frequency range (EHF) >8 kHz was greater in the older group. Older adults also had lower DPOAE levels across the conventional frequency range, with a trend toward greater reductions for higher f2 frequencies (3.38 – 7.55 kHz). The elevated thresholds from 9-18 kHz support age-related cochlear pathology in the extreme basal cochlea, while conventional DPOAE results specify effects on outer hair cell function. For normal hearing older adults in this study, reduced DPOAEs, together with elevated EHF thresholds, suggest preclinical cochlear degeneration that includes the outer hair cells. This is important for the interpretation of electrophysiological measures because outer hair cell dysfunction can impair "downstream" mechanical amplification for low-moderate level stimuli. Others have reported that for young adults with normal clinical audiograms, reduced DPOAEs < 8 kHz in conjunction with elevated EHF thresholds > 8 kHz serve as an early indication of preclinical hearing decline (Dreisbach et al., 2008; Jedrzejczak et al., 2023; Mishra et al., 2021).

Temporal bone studies show that as the subject's age increased, the cochlear cell loss was progressively more severe towards the extreme basal cochleogram region. The percentage and cochleotopic extent of missing hair cells and auditory neural fibers across the cochlear frequency map was greater in older subjects and spread into the 2- 8 kHz region despite clinical audiograms < 8 kHz that were normal or only mildly impaired (Viana, 2015; Wu et al., 2019; 2020 a & b; 2021). They also found that the degree of outer hair cell loss correlated with greater damage to synapses and neurons in this cocheotopic frequency region of the underlying cochlear map, while inner hair cells were relatively less impaired. Observations of poor EHF hearing in this study, combined with this recent histological evidence (Viana, 2015; Wu et al., 2019; 2020 a & b; 2021) support the conclusion that structure and function in the most basal, high frequency cochlear regions > 8 kHz is disrupted in the older group with normal hearing clinical hearing sensitivity < 8 kHz.

4. The reduced DPOAE levels in this study indicate possible deterioration of outer hair cells below 8kHz, while EHF decline suggests an extension of cochlear damage towards extreme basal cochlea. Outer hair cells are critical for cochlear amplification required for sound detection and activation of inner hair cells and spiral ganglion neurons near the threshold. The interpretation that AP and ABR Waves are markers of underlying neural types of pathology is complicated by this evidence of outer hair cell damage.

The outer hair cells provide "cochlear" gain by amplifying and sharpening biomechanical activation in response to low level sound stimulation. The loss of outer hair cells can impair this mechanical amplification and tuning, which in turn affects "downstream" processing by inner hair cells and auditory spiral ganglion neurons. Using the derived frequency-band masking method, Don and Eggermont (1978) show that the high level click-evoked ABR arises mainly from the 2-8 kHz cochlear region. Some suggest that because outer hair cell cochlear gain is specific to lower level stimulation, the impact is diminished when high intensity click stimuli are used, as in this study, or in people with high frequency sensorineural hearing loss (Hoben et al., 2017; Gorga et al., 1985) However, Bharadwaj et al. (2019) claim that degeneration of outer hair cells in basal frequency regions > 8 kHz can still influence the amplitude and latency of the AP and Wave I components. In the healthy cochlea, outer hair cells in the 9-16 kHz cochlear region may contribute through the upward spread of high level click excitation above 8 kHz. Stimulated outer hair cells in this "off-frequency" 9-16 kHz region mechanically amplify nearby inner hair cells, which in turn excite their post-synaptic spiral ganglion neurons. With outer hair cell damage in the 9-16kHz region, this cochlear gain is lost, and spiral ganglion neurons are no longer stimulated by the click stimulus. When these 9-16 kHz neurons fail to reach their action potential threshold, they "drop out," and their contribution to whole auditory nerve electrical activity is lost. Loss of this rapid onset, highly synchronized action potentials could potentially reduce the amplitude and delay the latency of the whole nerve AP/Wave I response, even though they are outside the 2-8kHz region predominately activated by high intensity clicks (Bharadwaj et al., 2019).

5. For older adults, neural components of the ECochG and ABR (AP, Waves I, and V) did not show heightened susceptibility to high stimulus repetition rates compared to younger adults.

It was hypothesized that increasing the click stimulation rate would cause greater physiological stress on the aging cochlea and would manifest as a greater rate-induced latency delay or reduction of the ECochG or ABR in the old subject group. However, increasing stimulation rate affected the latencies and amplitudes of the AP, Waves I, and V to the same extent in old and young adults. In contrast, the ECochG SP component, generated mainly by inner hair cell receptor potentials, remained stable across different stimulation rates and, as noted above, was not affected by age.

5.13 Impacts and Limitations

Aging adults with normal hearing sensitivity may have more peripheral auditory degeneration than suggested by their clinical audiogram as indicated by DPOAE, EHF thresholds and AEP results of this study. These finding should be considered in age-related hearing research studies, and by clinicians managing older patients with hearing complaints and communication challenges.

Unlike many studies that use either ECochG or ABR, this study simultaneously recorded both measures across different electrode configurations. This provides a more comprehensive picture of auditory function. This comprehensive approach enables the identification of dysfunction of auditory system at different cochlear and auditory nerve and brainstem components under the same recording conditions (e.g. myogenic noise artifacts). For example, by comparing recordings from mastoid and tympanic electrodes, the most sensitive recording site for detecting these components can be used. It also made it possible to compare the results of this study to others which use only one recording site, mastoid or tympanic membrane. The use of ultrahigh stimulus rates (up to 507.81 clicks/s) is relatively rare in auditory research. This approach offers insights into how aging affects neural adaptation and recovery. It also enables the separation of the SP from the AP at these high rates, which has not been widely studied except in normal subjects and not in the context of aging. The effects of aging on SP-AP separation can reveal age-

related changes in cochlear and auditory nerve function that are not evident at lower rates. The study's inclusion of participants with normal hearing in both young and old groups eliminates the confounding effects of hearing loss at 8 kHz and below. Many previous studies have not controlled for hearing status, making it difficult to determine whether observed differences were due to aging or hearing loss. The study included tests for DPOAEs and EHF hearing measures. DPOAEs provide information about outer hair cell function, and EHF hearing measures capture high-frequency cochlear function from extreme basal regions of the cochlea. The most novel aspect of this study was that all these measures and analyses were obtained from the same subjects, leading to a more complete interpretation of the study outcomes. Current studies in literature have looked at some of these factors, but not all in the same study group.

The findings of this study have limited generalizability due to the controlled selection criteria of no history of health and neurological issues, which resulted in a sample biased towards healthy older individuals, potentially including those with less affected auditory system function. Additionally, the sample size and age range for older adults were limited due to the challenges associated with recruiting older adults with normal standard audiograms. The study also observed high variability in the amplitudes of ABR waves I and V, and ECochG AP, with no significant differences between age groups. Future research could benefit from a larger sample size and the incorporation of correlation analysis with other factors such as EHF thresholds and DPOAE levels. Furthermore, longitudinal data would be valuable in identifying measures that are more sensitive to age-related changes in the auditory system of the same individuals over time. Lastly, the omission of cognitive and perceptual measures, as well as more advanced neural imaging techniques (e.g., fMRI or MEG) or neurophysiological measures, limits the ability to establish a link between peripheral auditory dysfunction and higher-order auditory processing or cognitive decline.

References

Abdala, C., & Dhar, S. (2012). Maturation and aging of the human cochlea: a view through the DPOAE looking glass. Journal of the Association for Research in Otolaryngology : JARO, 13(3), 403–421. https://doi-org.proxy1.lib.uwo.ca/10.1007/s10162-012-0319-2

Aedo-Sanchez, C., Oliveros, J., Aranguiz, C., Muñoz, C., Lazo-Maturana, C., & Aguilar-Vidal, E. (2023). Subclinical hearing loss associated with aging. Journal of otology, 18(3), 111–117. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.joto.2023.05.002

Allen, P., & Allan, C. (2014). Auditory processing disorders: relationship to cognitive processes and underlying auditory neural integrity. International journal of pediatric otorhinolaryngology, 78(2), 198–208. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.ijporl.2013.10.048

Ankmnal-Veeranna, S., Allan, C., & Allen, P. (2019). Auditory Brainstem Responses in Children with Auditory Processing Disorder. Journal of the American Academy of Audiology, 30(10), 904–917. https://doi-org.proxy1.lib.uwo.ca/10.3766/jaaa.18046

Aziz, A., Md Daud, M. K., Nik Othman, N. A., & Abd Rahman, N. (2020). Early Detection of High-frequency Presbycusis Among Normal Hearing Individuals. Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology, 41(8), e989–e992. https://doi-org.proxy1.lib.uwo.ca/10.1097/MAO.0000000000002725

Bester, C., Weder, S., Collins, A., Dragovic, A., Brody, K., Hampson, A., & O'Leary, S. (2020). Cochlear microphonic latency predicts outer hair cell function in animal models and clinical populations. Hearing research, 398, 108094. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.heares.2020.108094

Bharadwaj, H. M., Mai, A. R., Simpson, J. M., Choi, I., Heinz, M. G., & Shinn-Cunningham, B. G. (2019). Non-Invasive Assays of Cochlear Synaptopathy - Candidates and Considerations. Neuroscience, 407, 53–66. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.neuroscience.2019.02.031

Bieber, R. E., Fernandez, K., Zalewski, C., Cheng, H., & Brewer, C. C. (2020). Stability of early auditory evoked potential components over extended test-retest intervals in young adults. Ear and hearing, 41(6), 1461-1469.

Billings, C., Tremblay, K., & Willot, J. (2012). The aging auditory system. Translational perspectives in auditory neuroscience, 63-82.

Bramhall N. F. (2021). Use of the auditory brainstem response for assessment of cochlear synaptopathy in humans. The Journal of the Acoustical Society of America, 150(6), 4440. https://doi.org/10.1121/10.0007484

Bramhall, N. F., McMillan, G. P., & Mashburn, A. N. (2021). Subclinical Auditory Dysfunction: Relationship Between Distortion Product Otoacoustic Emissions and the Audiogram. American journal of audiology, 30(3S), 854–869. https://doi-org.proxy1.lib.uwo.ca/10.1044/2020_AJA-20-00056

Bruce, I. C., Erfani, Y., & Zilany, M. S. (2018). A phenomenological model of the synapse between the inner hair cell and auditory nerve: Implications of limited neurotransmitter release sites. Hearing research, 360, 40-54.

Burkard, R. F., & Sims, D. (2002). The Human Auditory Brain-stem Response to High Click Rates: Aging Effects. American journal of audiology, 11(1), 12. https://doi-org.proxy1.lib.uwo.ca/10.1044/1059-0889(2002/er01)

Burkard, R. F., Eggermont, J. J., & Don, M. (2012). Chapter 11/ The Auditory Brainstem Response. In Auditory evoked potentials: Basic principles and clinical application (pp. 229–253). Lippincott Williams & Wilkins.

Burkard, R., & Don, M., (2015). Introduction to auditory evoked potentials. In Hood L. J. Katz J. Chasin M. English K. M. Tillery K. L. & Lippincott Williams & Wilkins (Eds.). Handbook of clinical audiology (7th ed. international., pp. 187-205). Wolters Kluwer Health.

Burkard, R., & Hecox, K. (1983). The effect of broadband noise on the human brainstem auditory evoked response. I. Rate and intensity effects. The Journal of the Acoustical Society of America, 74(4), 1204–1213. https://doi.org/10.1121/1.390024

Burkard, R., Shi, Y., & Hecox, K. E. (1990). A comparison of maximum length and Legendre sequences for the derivation of brain-stem auditory-evoked responses at rapid rates of stimulation. The Journal of the Acoustical Society of America, 87(4), 1656–1664. https://doi-org.proxy1.lib.uwo.ca/10.1121/1.399413

Carcagno, S., & Plack, C. J. (2020). Effects of age on electrophysiological measures of cochlear synaptopathy in humans. Hearing research, 396, 108068. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.heares.2020.108068

Caspary, D. M., Schatteman, T. A., & Hughes, L. F. (2005). Age-related changes in the inhibitory response properties of dorsal cochlear nucleus output neurons: role of inhibitory inputs. The Journal of neuroscience : the official journal of the Society for Neuroscience, 25(47), 10952–10959. https://doi.org/10.1523/JNEUROSCI.2451-05.2005

Chatrian, G. E., Wirch, A. L., Edwards, K. H., Turella, G. S., Kaufman, M. A., & Snyder, J. M. (1985). Cochlear summating potential to broadband clicks detected from the human external auditory meatus. A study of subjects with normal hearing for age. Ear and hearing, 6(3), 130–138. https://doi-org.proxy1.lib.uwo.ca/10.1097/00003446-198505000-00002

Chimento, T. C., & Schreiner, C. E. (1991). Adaptation and recovery from adaptation in single fiber responses of the cat auditory nerve. The Journal of the Acoustical Society of America, 90(1), 263–273. https://doi.org/10.1121/1.401296

Christov, F., Nelson, E. G., & Gluth, M. B. (2018). Human Superior Olivary Nucleus Neuron Populations in Subjects With Normal Hearing and Presbycusis. The Annals of otology, rhinology, and laryngology, 127(8), 527–535. https://doi.org/10.1177/0003489418779405

Coats, A. C. (1986). The normal summating potential recorded from external ear canal. Archives of Otolaryngology–Head & Neck Surgery, 112(7), 759-768.

Delgado, R. E., & Ozdamar, O. (2004). Deconvolution of evoked responses obtained at high stimulus rates. The Journal of the Acoustical Society of America, 115(3), 1242–1251. https://doi.org/10.1121/1.1639327

Demeester, K., van Wieringen, A., Hendrickx, J. J., Topsakal, V., Fransen, E., van Laer, L., Van Camp, G., & Van de Heyning, P. (2009). Audiometric shape and presbycusis. International journal of audiology, 48(4), 222–232. https://doi.org/10.1080/14992020802441799

Don, M. (2007, December). Hearing Loss can muddy the waters of otologic disease detection. In Proceedings of the International Symposium on Auditory and Audiological Research (Vol. 1, pp. 173-186).

Don, M., & Eggermont, J. J. (1978). Analysis of the click-evoked brainstem potentials in man using high-pass noise masking. The Journal of the Acoustical Society of America, 63(4), 1084–1092. https://doi.org/10.1121/1.381816

Don, M., Allen, A., & Starr, A. (1977). Effect of click rate on the latency of auditory brainstem responses in humans. Anals of Otology, Rhinology and Laryngology, 86, 186195.

Eggermont J. J. (2017). Ups and Downs in 75 Years of Electrocochleography. Frontiers in systems neuroscience, 11, 2. https://doi-org.proxy1.lib.uwo.ca/10.3389/fnsys.2017.00002

Eggermont J. J. (2019). Cochlea and auditory nerve. Handbook of clinical neurology, 160, 437–449. https://doi.org/10.1016/B978-0-444-64032-1.00029-1

Eysholdt, U., & Schreiner, C. (1982). Maximum length sequences -- a fast method for measuring brain-stem-evoked responses. Audiology : official organ of the International Society of Audiology, 21(3), 242–250. https://doi.org/10.3109/00206098209072742

Feder, K. P., Michaud, D., Ramage-Morin, P., McNamee, J., & Beauregard, Y. (2015). Prevalence of hearing loss among Canadians aged 20 to 79: Audiometric results from the 2012/2013 Canadian Health Measures Survey.

Fernandez, K. A., Jeffers, P. W., Lall, K., Liberman, M. C., & Kujawa, S. G. (2015). Aging after noise exposure: acceleration of cochlear synaptopathy in "recovered" ears. The Journal of neuroscience : the official journal of the Society for Neuroscience, 35(19), 7509–7520. https://doi.org/10.1523/JNEUROSCI.5138-14.2015

Ferraro, J. A., & Ferguson, R. (1989). Tympanic ECochG and conventional ABR: a combined approach for the identification of wave I and the I-V interwave interval. Ear and hearing, 10(3), 161–166.

Frisina, D. R., & Frisina, R. D. (1997). Speech recognition in noise and presbycusis: relations to possible neural mechanisms. Hearing research, 106(1-2), 95–104. https://doi-org.proxy1.lib.uwo.ca/10.1016/s0378-5955(97)00006-3

Fujioka, M., Okano, H., & Edge, A. S. (2015). Manipulating cell fate in the cochlea: a feasible therapy for hearing loss. Trends in neurosciences, 38(3), 139–144. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.tins.2014.12.004

Gelfand, S. A. (2016). Physiological Methods in Audiology. Essentials of audiology (Fourth edition., pp. 302-328). Thieme.

Gerling, I. J., & Finitzo-Hieber, T. (1983). Auditory brainstem response with high stimulus rates in normal and patient populations. The Annals of otology, rhinology, and laryngology, 92(2 Pt 1), 119–123. https://doi-org.proxy1.lib.uwo.ca/10.1177/000348948309200204

Gillespie, P. G., & Müller, U. (2009). Mechanotransduction by hair cells: models, molecules, and mechanisms. Cell, 139(1), 33–44. https://doi.org/10.1016/j.cell.2009.09.010

Glavin, C. C., Siegel, J., & Dhar, S. (2021). Distortion Product Otoacoustic Emission (DPOAE) Growth in Aging Ears with Clinically Normal Behavioral Thresholds. Journal of the Association for Research in Otolaryngology : JARO, 22(6), 659–680. https://doi-org.proxy1.lib.uwo.ca/10.1007/s10162-021-00805-3

Gorga, M. P., Worthington, D. W., Reiland, J. K., Beauchaine, K. A., & Goldgar, D. E. (1985). Some comparisons between auditory brain stem response thresholds, latencies, and the pure-tone audiogram. Ear and hearing, 6(2), 105-112.

Grant, K. J., Mepani, A. M., Wu, P., Hancock, K. E., de Gruttola, V., Liberman, M. C., & Maison, S. F. (2020). Electrophysiological markers of cochlear function correlate with hearing-in-noise performance among audiometrically normal subjects. Journal of neurophysiology, 124(2), 418–431. https://doi.org/10.1152/jn.00016.2020

Grose, J. H., Buss, E., & Elmore, H. (2019). Age-Related Changes in the Auditory Brainstem Response and Suprathreshold Processing of Temporal and Spectral Modulation. Trends in hearing, 23, 2331216519839615. https://doi-org.proxy1.lib.uwo.ca/10.1177/2331216519839615

Grosh, K., Zheng, J., Zou, Y., de Boer, E., & Nuttall, A. L. (2004). High-frequency electromotile responses in the cochlea. The Journal of the Acoustical Society of America, 115(5 Pt 1), 2178–2184. https://doi-org.proxy1.lib.uwo.ca/10.1121/1.1695431

Heil, P., & Peterson, A. J. (2015). Basic response properties of auditory nerve fibers: a review. Cell and tissue research, 361, 129-158.

Hoben, R., Easow, G., Pevzner, S., & Parker, M. A. (2017). Outer hair cell and auditory nerve function in speech recognition in quiet and in background noise. Frontiers in neuroscience, 11, 157.

Hunter, L. L., Monson, B. B., Moore, D. R., Dhar, S., Wright, B. A., Munro, K. J., Zadeh, L. M., Blankenship, C. M., Stiepan, S. M., & Siegel, J. H. (2020). Extended high frequency hearing and speech perception implications in adults and children. Hearing research, 397, 107922. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.heares.2020.107922

Jacobson, J. T., Murray, T. J., & Deppe, U. (1987). The effects of ABR stimulus repetition rate in multiple sclerosis. Ear and hearing, 8(2), 115–120. https://doi.org/10.1097/00003446-198704000-00009

Jayakody, D. M. P., Friedland, P. L., Martins, R. N., & Sohrabi, H. R. (2018). Impact of Aging on the Auditory System and Related Cognitive Functions: A Narrative Review. Frontiers in neuroscience, 12, 125. https://doi-org.proxy1.lib.uwo.ca/10.3389/fnins.2018.00125

Jedrzejczak, W. W., Pilka, E., Pastucha, M., Kochanek, K., & Skarzynski, H. (2023). Extended High Frequency Thresholds and Their Relationship to Distortion Product Otoacoustic Emissions, Hearing Acuity, Age, Gender, Presence of Spontaneous Otoacoustic

Emissions, and Side of Measurement. Applied Sciences, 13(18), 10311-. https://doi.org/10.3390/app131810311

Jerger, J., & Hall, J. (1980). Effects of age and sex on auditory brainstem response. Archives of otolaryngology (Chicago, Ill. : 1960), 106(7), 387–391. https://doi-org.proxy1.lib.uwo.ca/10.1001/archotol.1980.00790310011003

Jiang Z. D. (1999). Outcome of brain stem auditory electrophysiology in children who survive purulent meningitis. The Annals of otology, rhinology, and laryngology, 108(5), 429–434. https://doi-org.proxy1.lib.uwo.ca/10.1177/000348949910800502

Jiang, Z. D., Wu, Y. Y., & Wilkinson, A. R. (2009). Age-related changes in BAER at different click rates from neonates to adults. Acta paediatrica (Oslo, Norway : 1992), 98(8), 1284–1287. https://doi-org.proxy1.lib.uwo.ca/10.1111/j.1651-2227.2009.01312.x

Jiang, Z. D., Wu, Y. Y., & Zhang, L. (1991). Amplitude change with click rate in human brainstem auditory-evoked responses. Audiology : official organ of the International Society of Audiology, 30(3), 173–182. https://doi.org/10.3109/00206099109072882

Johannesen, P. T., Buzo, B. C., & Lopez-Poveda, E. A. (2019). Evidence for age-related cochlear synaptopathy in humans unconnected to speech-in-noise intelligibility deficits. Hearing research, 374, 35–48. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.heares.2019.01.017

Kaf, W. A., Lewis, K. M., Yavuz, E., Dixon, S. M., Van Ess, M., Jamos, A. M., & Delgado, R. E. (2017). Fast Click Rate Electrocochleography and Auditory Brainstem Response in Normal-Hearing Adults Using Continuous Loop Averaging Deconvolution. Ear and

hearing, 38(2), 244–254. https://doi-org.proxy1.lib.uwo.ca/10.1097/AUD.0000000000000381

Kaf, W. A., Turntine, M., Jamos, A., & Smurzynski, J. (2022). Examining the Profile of Noise-Induced Cochlear Synaptopathy Using iPhone Health App Data and Cochlear and Brainstem Electrophysiological Responses to Fast Clicks Rates. Seminars in hearing, 43(3), 197–222. https://doi-org.proxy1.lib.uwo.ca/10.1055/s-0042-1756164

Kalluri, R., & Shera, C. A. (2001). Distortion-product source unmixing: a test of the two-mechanism model for DPOAE generation. The Journal of the Acoustical Society of America, 109(2), 622–637. https://doi-org.proxy1.lib.uwo.ca/10.1121/1.1334597

Keithley E. M. (2020). Pathology and mechanisms of cochlear aging. Journal of neuroscience research, 98(9), 1674–1684. https://doi-org.proxy1.lib.uwo.ca/10.1002/jnr.24439

Kemp D. T. (1978). Stimulated acoustic emissions from within the human auditory system. The Journal of the Acoustical Society of America, 64(5), 1386–1391. https://doi-org.proxy1.lib.uwo.ca/10.1121/1.382104

Kemp D. T. (2002). Otoacoustic emissions, their origin in cochlear function, and use. British medical bulletin, 63, 223–241. https://doi-org.proxy1.lib.uwo.ca/10.1093/bmb/63.1.223

Knight, R. D., & Kemp, D. T. (2000). Indications of different distortion product otoacoustic emission mechanisms from a detailed f1,f2 area study. The Journal of the Acoustical Society of America, 107(1), 457–473. https://doi-org.proxy1.lib.uwo.ca/10.1121/1.428351

Kohrman, D. C., Wan, G., Cassinotti, L., & Corfas, G. (2020). Hidden hearing loss: a disorder with multiple etiologies and mechanisms. Cold Spring Harbor perspectives in medicine, 10(1), a035493.

Konrad-Martin, D., Dille, M. F., McMillan, G., Griest, S., McDermott, D., Fausti, S. A., & Austin, D. F. (2012). Age-related changes in the auditory brainstem response. Journal of the American Academy of Audiology, 23(1), 18–75. https://doi-org.proxy1.lib.uwo.ca/10.3766/jaaa.23.1.3

Kramer, S. J. (2014). Audiology : science to practice (Second edition.). Plural Publishing.

Kujawa, S. G., & Liberman, M. C. (2015). Synaptopathy in the noise-exposed and aging cochlea: Primary neural degeneration in acquired sensorineural hearing loss. Hearing research, 330(Pt B), 191–199. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.heares.2015.02.009

Kurata, N., Schachern, P. A., Paparella, M. M., & Cureoglu, S. (2016). Histopathologic Evaluation of Vascular Findings in the Cochlea in Patients With Presbycusis. JAMA otolaryngology-- head & neck surgery, 142(2), 173–178. https://doi-org.proxy1.lib.uwo.ca/10.1001/jamaoto.2015.3163

Kusunoki, T., Cureoglu, S., Schachern, P. A., Baba, K., Kariya, S., & Paparella, M. M. (2004). Age-related histopathologic changes in the human cochlea: a temporal bone study. Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery, 131(6), 897–903. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.otohns.2004.05.022

Lake, A. B., & Stuart, A. (2019). The Effect of Test, Electrode, and Rate on Electrocochleography Measures. Journal of the American Academy of Audiology, 30(1), 41–53. https://doi-org.proxy1.lib.uwo.ca/10.3766/jaaa.17081

Lang, H., Jyothi, V., Smythe, N. M., Dubno, J. R., Schulte, B. A., & Schmiedt, R. A. (2010). Chronic reduction of endocochlear potential reduces auditory nerve activity: further confirmation of an animal model of metabolic presbyacusis. Journal of the Association for Research in Otolaryngology : JARO, 11(3), 419–434. https://doi-org.proxy1.lib.uwo.ca/10.1007/s10162-010-0214-7

Lee K. Y. (2013). Pathophysiology of age-related hearing loss (peripheral and central). Korean journal of audiology, 17(2), 45–49. https://doi.org/10.7874/kja.2013.17.2.45

Lefler, S. M., Kaf, W. A., & Ferraro, J. A. (2021). Comparing simultaneous electrocochleography and auditory brainstem response measurements using three different extratympanic electrodes. Journal of the American Academy of Audiology, 32(06), 339-346.

LeMasurier, M., & Gillespie, P. G. (2005). Hair-cell mechanotransduction and cochlear amplification. Neuron, 48(3), 403–415. https://doi.org/10.1016/j.neuron.2005.10.017

Liberman, M. C., & Kujawa, S. G. (2017). Cochlear synaptopathy in acquired sensorineural hearing loss: Manifestations and mechanisms. Hearing research, 349, 138–147. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.heares.2017.01.003

Liberman, M. C., Epstein, M. J., Cleveland, S. S., Wang, H., and Maison, S. F. (2016). Toward a differential diagnosis of hidden hearing loss in humans. PLoS ONE 11, 1–15. doi: 10.1371/journal.pone.0162726

Liberman, M. C., Liberman, L. D., & Maison, S. F. (2014). Efferent feedback slows cochlear aging. The Journal of neuroscience : the official journal of the Society for Neuroscience, 34(13), 4599–4607. https://doi-org.proxy1.lib.uwo.ca/10.1523/JNEUROSCI.4923-13.2014

Lina-Granade, G., Collet, L., & Morgon, A. (1994). Auditory-evoked brainstem responses elicited by maximum-length sequences in normal and sensorineural ears. Audiology : official organ of the International Society of Audiology, 33(4), 218–236. https://doi.org/10.3109/00206099409071882

Lutz, B. T., Hutson, K. A., Trecca, E. M. C., Hamby, M., & Fitzpatrick, D. C. (2022). Neural Contributions to the Cochlear Summating Potential: Spiking and Dendritic Components. Journal of the Association for Research in Otolaryngology : JARO, 23(3), 351–363. https://doi-org.proxy1.lib.uwo.ca/10.1007/s10162-022-00842-6

Maele, T. V., Keshishzadeh, S., Poortere, N., Dhooge, I., Keppler, H., & Verhulst, S. (2021). The Variability in Potential Biomarkers for Cochlear Synaptopathy After Recreational Noise Exposure. Journal of speech, language, and hearing research : JSLHR, 64(12), 4964–4981. https://doi-org.proxy1.lib.uwo.ca/10.1044/2021_JSLHR-21-00064

Märcher-Rørsted, J., Encina-Llamas, G., Dau, T., Liberman, M. C., Wu, P. Z., & Hjortkjær, J. (2022). Age-related reduction in frequency-following responses as a potential marker of

cochlear neural degeneration. Hearing research, 414, 108411. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.heares.2021.108411

McKnight, R. J., Glick, H., Cardon, G., & Sharma, A. (2018). The Effects of Stimulus Rate on ABR Morphology and its Relationship to P1 CAEP Responses and Auditory Speech Perception Outcomes in Children with Auditory Neuropathy Spectrum Disorder: Evidence from Case Reports. Hearing, balance and communication, 16(1), 1–12. https://doi-org.proxy1.lib.uwo.ca/10.1080/21695717.2017.1418803

Mick, P. T., Hämäläinen, A., Kolisang, L., Pichora-Fuller, M. K., Phillips, N., Guthrie, D., & Wittich, W. (2021). The prevalence of hearing, vision, and dual sensory loss in older Canadians: An analysis of data from the Canadian Longitudinal Study on Aging. Canadian Journal on Aging/La revue canadienne du vieillissement, 40(1), 1-22.

Mishra, S. K., Saxena, U., & Rodrigo, H. (2022). Extended High-frequency Hearing Impairment Despite a Normal Audiogram: Relation to Early Aging, Speech-in-noise Perception, Cochlear Function, and Routine Earphone Use. Ear and hearing, 43(3), 822–835. https://doi-org.proxy1.lib.uwo.ca/10.1097/AUD.0000000000001140

Möhrle, D., Ni, K., Varakina, K., Bing, D., Lee, S. C., Zimmermann, U., et al. (2016). Loss of auditory sensitivity from inner hair cell synaptopathy can be centrally compensated in the young but not old brain. Neurobiol. Aging 44, 173–184. doi: 10.1016/j.neurobiolaging.2016.05.001

Moser, T., & Starr, A. (2016). Auditory neuropathy—neural and synaptic mechanisms. Nature Reviews Neurology, 12(3), 135-149. https://doi.org/10.1038/nrneurol.2016.10

Musiek, F. E, Gonzalez, J. E. and Baran, J. A. (2015). Auditory Brainstem Response: Differential Diagnosis. In Hood L. J. Katz J. Chasin M. English K. M. Tillery K. L. & Lippincott Williams & Wilkins (Eds.). Handbook of clinical audiology (7th ed. international., pp. 231-248). Wolters Kluwer Health.

Musiek, F. E., & Baran, J. A. (1990). Canal electrode electrocochleography in patients with absent wave I ABRs. Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery, 103(1), 25–31. https://doi-org.proxy1.lib.uwo.ca/10.1177/019459989010300104

National Institute on Deafness and Other Communication Disorders. (2024). Quick Statistics About Hearing, Balance, & Dizziness. Retrieved March 25, 2024, from https://www.nidcd.nih.gov/health/statistics/quick-statistics-hearing#6.

Nelson EG, Hinojosa R (2006) Presbycusis: A human temporal bone study of individuals with downward sloping audiometric patterns of hearing loss and review of the literature. Laryngoscope 116:1–12. https://doi.org/10.1097/01.mlg.0000236089.44566.62.associated

Oku, T., & Hasegewa, M. (1997). The influence of aging on auditory brainstem response and electrocochleography in the elderly. ORL; journal for oto-rhino-laryngology and its related specialties, 59(3), 141–146. https://doi-org.proxy1.lib.uwo.ca/10.1159/000276927

Ozdamar, O., & Bohórquez, J. (2006). Signal-to-noise ratio and frequency analysis of continuous loop averaging deconvolution (CLAD) of overlapping evoked potentials. The Journal of

the Acoustical Society of America, 119(1), 429–438. https://doi-org.proxy1.lib.uwo.ca/10.1121/1.2133682

Ozdamar, O., Delgado, R.E., Yavuz, E. and Acikgoz, N. (2006). Deconvolution of Overlapping Auditory Brainstem Responses Obtained at High Stimulus Rates. In Handbook of Neural Engineering, M. Akay (Ed.). https://doi.org/10.1002/9780470068298.ch6

Pantev, C., Lagidze, S., Pantev, M., & Kevanishvili, Z. (1985). Frequency-specific contributions to the auditory brain stem response derived by means of pure-tone masking. Audiology, 24(4), 275-287.

Parthasarathy, A., and Kujawa, S. G. (2018). Synaptopathy in the aging cochlea: Characterizing early-neural deficits in auditory temporal envelope processing. J. Neurosci. 38, 7108–7119. doi: 10.1523/JNEUROSCI.3240- 17.2018

Pichora-Fuller, M. K., & Souza, P. E. (2003). Effects of aging on auditory processing of speech. International journal of audiology, 42 Suppl 2, 2S11–2S16.

Picton, T. W., Champagne, S. C., & Kellett, A. J. (1992). Human auditory evoked potentials recorded using maximum length sequences. Electroencephalography and clinical neurophysiology, 84(1), 90–100. https://doi-org.proxy1.lib.uwo.ca/10.1016/0168-5597(92)90071-i

Pürner, D., Schirkonyer, V., & Janssen, T. (2022). Changes in the peripheral and central auditory performance in the elderly-A cross-sectional study. Journal of neuroscience research, 100(9), 1791–1811. https://doi-org.proxy1.lib.uwo.ca/10.1002/jnr.25068

Ramadan, H. H., & Schuknecht, H. F. (1989). Is there a conductive type of presbycusis?. Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery, 100(1), 30–34. https://doi-org.proxy1.lib.uwo.ca/10.1177/019459988910000105

Robinette, M. S., & Glattke, T. J. (2007). Otoacoustic emissions : clinical applications (3rd ed.). Thieme.

Roque, L., Karawani, H., Gordon-Salant, S., & Anderson, S. (2019). Effects of Age, Cognition, and Neural Encoding on the Perception of Temporal Speech Cues. Frontiers in neuroscience, 13, 749. https://doi-org.proxy1.lib.uwo.ca/10.3389/fnins.2019.00749

Russell, I. (2008). Cochlear Receptor Potentials. In The Senses: A Comprehensive Reference (Vol. 3, pp. 319–358). https://doi.org/10.1016/B978-012370880-9.00030-X

Salvi, R., Sun, W., Ding, D., Chen, G. D., Lobarinas, E., Wang, J., Radziwon, K. &Auerbach, B. D. (2017). Inner hair cell loss disrupts hearing and cochlear function leading to sensory deprivation and enhanced central auditory gain. Frontiers in neuroscience, 10, 621.

Santarelli, R and Arslan, E. (2015). Electrocochleography. In Hood L. J. Katz J. Chasin M. English K. M. Tillery K. L. & Lippincott Williams & Wilkins (Eds.). Handbook of clinical audiology (7th ed. international., pp. 207–230). Wolters Kluwer.

Santarelli, R., La Morgia, C., Valentino, M. L., Barboni, P., Monteleone, A., Scimemi, P., & Carelli, V. (2019). Hearing dysfunction in a large family affected by dominant optic atrophy (OPA8-Related DOA): A human model of hidden auditory neuropathy. Frontiers in Neuroscience, 13, 447692.

Santarelli, R., Scimemi, P., Costantini, M., Domínguez-Ruiz, M., Rodríguez-Ballesteros, M., & Del Castillo, I. (2021). Cochlear synaptopathy due to mutations in OTOF gene may result in stable mild hearing loss and severe impairment of speech perception. Ear and Hearing, 42(6), 1627-1639.

Santarelli, R., Scimemi, P., La Morgia, C., Cama, E., Del Castillo, I., & Carelli, V. (2021). Electrocochleography in Auditory Neuropathy Related to Mutations in the OTOF or OPA1 Gene. Audiology research, 11(4), 639–652. https://doi-org.proxy1.lib.uwo.ca/10.3390/audiolres11040059

Santos, M. A., Munhoz, M. S., Peixoto, M. A., & Silva, C. S. (2004). High click stimulus repetition rate in the auditory evoked potentials in multiple sclerosis patients with normal MRI. Does it improve diagnosis?. Revue de laryngologie - otologie - rhinologie, 125(3), 151–155.

Schmiedt, R. A., Lang, H., Okamura, H. O., & Schulte, B. A. (2002). Effects of furosemide applied chronically to the round window: a model of metabolic presbyacusis. The Journal of neuroscience : the official journal of the Society for Neuroscience, 22(21), 9643–9650. https://doi.org/10.1523/JNEUROSCI.22-21-09643.2002

Schuknecht, H. F., & Gacek, M. R. (1993). Cochlear pathology in presbycusis. The Annals of otology, rhinology, and laryngology, 102(1 Pt 2), 1–16. https://doi-org.proxy1.lib.uwo.ca/10.1177/00034894931020S101

Sergeyenko, Y., Lall, K., Liberman, M. C., & Kujawa, S. G. (2013). Age-related cochlear synaptopathy: an early-onset contributor to auditory functional decline. The Journal of

neuroscience : the official journal of the Society for Neuroscience, 33(34), 13686–13694. https://doi.org/10.1523/JNEUROSCI.1783-13.2013

Shehabi, A. M., Prendergast, G., & Plack, C. J. (2022). The Relative and Combined Effects of Noise Exposure and Aging on Auditory Peripheral Neural Deafferentation: A Narrative Review. Frontiers in aging neuroscience, 14, 877588. https://doi-org.proxy1.lib.uwo.ca/10.3389/fnagi.2022.877588

Soucek, S., & Mason, S. M. (1992). Effects of adaptation on electrocochleography and auditory brain-stem responses in the elderly. Scandinavian audiology, 21(3), 149–152. https://doi-org.proxy1.lib.uwo.ca/10.3109/01050399209045995

Stanton, S. G., Cashman, M. Z., Harrison, R. V., Nedzelski, J. M., & Rowed, D. W. (1989). Cochlear nerve action potentials during cerebellopontine angle surgery: relationship of latency, amplitude, and threshold measurements to hearing. Ear and hearing, 10(1), 22-28.

Starr, A., & Achor, L. J. (1975). Auditory brain stem responses in neurological disease. Archives of Neurology, 32(11), 761-768.

Stauffer, E. A., Scarborough, J. D., Hirono, M., Miller, E. D., Shah, K., Mercer, J. A., Holt, J. R., & Gillespie, P. G. (2005). Fast adaptation in vestibular hair cells requires myosin-1c activity. Neuron, 47(4), 541–553. https://doi.org/10.1016/j.neuron.2005.07.024

Sun, Z., Cheng, Z., Gong, N., Xu, Z., Jin, C., Wu, H., & Tao, Y. (2021). Neural presbycusis at ultra-high frequency in aged common marmosets and rhesus monkeys. Aging, 13(9), 12587–12606. https://doi.org/10.18632/aging.202936

Talmadge, C. L., Long, G. R., Tubis, A., & Dhar, S. (1999). Experimental confirmation of the two-source interference model for the fine structure of distortion product otoacoustic emissions. The Journal of the Acoustical Society of America, 105(1), 275–292. https://doi-org.proxy1.lib.uwo.ca/10.1121/1.424584

Tanaka, H., Komatsuzaki, A., & Hentona, H. (1996). Usefulness of auditory brainstem responses at high stimulus rates in the diagnosis of acoustic neuroma. ORL, 58(4), 224-228.

Uchida, Y., Ando, F., Shimokata, H., Sugiura, S., Ueda, H., & Nakashima, T. (2008). The effects of aging on distortion-product otoacoustic emissions in adults with normal hearing. Ear and hearing, 29(2), 176–184. https://doi-org.proxy1.lib.uwo.ca/10.1097/aud.0b013e3181634eb8

Ueberfuhr, M. A., Fehlberg, H., Goodman, S. S., & Withnell, R. H. (2016). A DPOAE assessment of outer hair cell integrity in ears with age-related hearing loss. Hearing research, 332, 137–150. https://doi-org.proxy1.lib.uwo.ca/10.1016/j.heares.2015.11.006

Viana, L. M., O'Malley, J. T., Burgess, B. J., Jones, D. D., Oliveira, C. A., Santos, F., Merchant, S. N., Liberman, L. D., & Liberman, M. C. (2015). Cochlear neuropathy in human presbycusis: Confocal analysis of hidden hearing loss in post-mortem tissue. Hearing research, 327, 78–88. https://doi.org/10.1016/j.heares.2015.04.014

Wężyk, A., Morawski, K., Delgado, R., Pierchała, K., Niemczyk, K. (2015). Evoked Auditory Potentials Recorded at Fast Stimulation Rates. Polski Przegląd Otorynolaryngologiczny. 4. 40-45. 10.5604/20845308.1150810.

Wilson, W. J., & Bowker, C. A. (2002). The effects of high stimulus rate on the electrocochleogram in normal-hearing subjects. International journal of audiology, 41(8), 509–517. https://doi-org.proxy1.lib.uwo.ca/10.3109/14992020209056071

Woo, J., Miller, C. A., & Abbas, P. J. (2009). Simulation of the electrically stimulated cochlear neuron: modeling adaptation to trains of electric pulses. IEEE transactions on bio-medical engineering, 56(5), 1348–1359. https://doi.org/10.1109/TBME.2008.2005782

World Health Organization. (2023). Deafness and hearing loss. Retrieved March 25, 2024, from https://www.who.int/health-topics/hearing-loss#tab=tab_2.

Wu PZ, O'Malley JT, de Gruttola V, Liberman MC (2020a) Age-related hearing loss is dominated by damage to inner ear sensory cells, not the cellular battery that powers them. J Neurosci 40:6357–6366. https://doi.org/10.1523/JNEUROSCI.0937-20. 2020

Wu, P. Z., Liberman, L. D., Bennett, K., de Gruttola, V., O'Malley, J. T., and Liberman, M. C. (2019). Primary neural degeneration in the human cochlea: evidence for hidden hearing loss in the aging ear. Neuroscience 407, 8–20. doi: 10.1016/j.neuroscience.2018. 07.053

Wu, P. Z., O'Malley, J. T., de Gruttola, V., & Liberman, M. C. (2021). Primary Neural Degeneration in Noise-Exposed Human Cochleas: Correlations with Outer Hair Cell Loss and Word-Discrimination Scores. The Journal of neuroscience : the official journal of the Society for Neuroscience, 41(20), 4439–4447. https://doi-org.proxy1.lib.uwo.ca/10.1523/JNEUROSCI.3238-20.2021

Wu, P. Z., Wen, W. P., O'Malley, J. T., & Liberman, M. C. (2020b). Assessing fractional hair cell survival in archival human temporal bones. The Laryngoscope, 130(2), 487–495. https://doi.org/10.1002/lary.27991

Wu, T., & Marcus, D. C. (2003). Age-related changes in cochlear endolymphatic potassium and potential in CD-1 and CBA/CaJ mice. Journal of the Association for Research in Otolaryngology : JARO, 4(3), 353–362. https://doi-org.proxy1.lib.uwo.ca/10.1007/s10162-002-3026-6

Yagi, T., & Kaga, K. (1979). The effect of the click repetition rate on the latency of the auditory evoked brain stem response and its clinical use for a neurological diagnosis. Archives of oto-rhino-laryngology, 222(2), 91–97. https://doi-org.proxy1.lib.uwo.ca/10.1007/BF00469746

Yasmin, S., Purcell, D. W., Veeranna, S. A., Johnsrude, I. S., & Herrmann, B. (2020). A novel approach to investigate subcortical and cortical sensitivity to temporal structure simultaneously. Hearing research, 398, 108080. https://doi.org/10.1016/j.heares.2020.108080

Youssef, A. S., & Downes, A. E. (2009). Intraoperative neurophysiological monitoring in vestibular schwannoma surgery: advances and clinical implications. Neurosurgical focus, 27(4), E9.

Appendices

Appendix A

Western Research

Date: 6 December 2023

To: Dr. Susan Stanton

Project ID: 112044

Review Reference: 2023-112044-86915

Study Title: Auditory Evoked Potential Phenotyping Procedures for Genetic Hearing Research

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

Date Approval Issued: 06/Dec/2023 13:35

REB Approval Expiry Date: 05/Dec/2024

Ethics Lapse: 05/Dec/2023 - 06/Dec/2023

Dear Dr. Susan Stanton,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Electronically signed by:

Mr. Joshua Hatherley, Ethics Coordinator on behalf of Dr. N. Poonai, HSREB Chair 06/Dec/2023 13:35

Reason: I am approving this document

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix B

Interview Guide

Medical History: Question Guide **Participant ID#: _____**

Interview conducted by _____ Date: _____

Interview conducted (check all that apply): _____ in-person_____by phone

A) Health Information / Past Medical history

(***Please CIRCLE the*** *responses*)

1. Have you ever had, or do you now have a neuro-psychological condition (e.g. Alzheimer's, Parkinson's, cognitive disorder, dementia etc.)? YES/NO

    a. If YES, did you ever seek professional consultation from a neurologist/ Psychologist? YES/NO Specify type of specialist:_____

    b. If YES, please specify the management plan (e.g. Drug therapy, cognitive/ behaviour therapy etc.)

    .....

2. Have you ever had, or do you now have a hearing loss? YES/NO

3. Have you ever visited an audiologist/ Otolaryngologist/Hearing instrument specialist for professional advice? YES/NO

    a. If YES, specify type of specialist:_____

    b. If YES, what was the reason (e.g. Ear surgery, hearing assessment, hearing aid etc.)?

    .....

4. Have you ever been treated with gentamycin or cancer drugs? YES/NO

    a. If YES, please explain the reason and duration of drug treatment

    .....

5. Have you ever been exposed to loud noise repeatedly, or for a long time. For example, do you work in a noisy workplace or in the military, or have a hobby that exposes you to noise (hunting, carpentry etc)?
   YES/NO

   a. If yes, do you feel that long-term exposure to the loud noise has adversely affected your hearing (do you have noise in the ear, poor hearing ability or increased sensitivity to loud sound?) YES/NO

   b. If YES, did you see your family physician/audiologist? YES/NO

   c. If YES, please explain how you were managed (e.g. hearing assessment and counselling, hearing aid, retraining etc.)

.....

B) Family history

6. Has anyone in your family (siblings, parents, grandparents etc.) have a hearing problem?

   YES/NO

7. If YES, who has affected? (***Please check all that apply***)

Child	☐
Brother/ sister	☐
Parents	☐
Uncle/ Aunt	☐
Cousins	☐
Nephew/ Niece	☐
Grandparents	☐

8. If YES, how it was managed (surgery, medication, hearing assessment or devices etc.), please explain?

   .....

9. What was the age of onset of hearing loss in your family member/ members?

   .....

Appendix C

AP Latency: Channel A (ipsiTymptrode- contraMastoid)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.82	1	0.82	45.01	.000	.31	
Rate	1.63	5	0.33	17.95	.000	.47	[.33, .54]
Age*Rate	0.07	5	0.01	0.82	.539	.04	[.00, .07]
Error	1.82	100	0.02				
Shapiro-Wilk test, p= 0.02495653 Levene's test, p= 0.03173617							

AP Latency: Channel C (ipsiTymptrode- Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.56	1	0.56	37.44	.000	.29	
Rate	1.03	5	0.21	13.89	.000	.42	[.27, .50]
Age*Rate	0.01	5	0.00	0.08	.996	.01	[.00, 1.00]
Error	1.40	94	0.01				
Shapiro-Wilk test, p= 0.1419034							

Levene’s test, p= 0.1895482

AP Amplitude: Channel A (ipsiTymptrode- contraMastoid)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.00	1	0.00	0.01	.905	.00	
Rate	3.42	5	0.68	7.96	.000	.28	[.14, .37]
Age*Rate	0.03	5	0.01	0.07	.996	.00	[.00, 1.00]
Error	8.60	100	0.09				
Shapiro-Wilk test, p= 1.549592e-08 Levene’s test, p= 0.2729728							

AP Amplitude: Channel C (ipsiTymptrode- Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.01	1	0.01	0.11	.742	.00	
Rate	3.74	5	0.75	9.35	.000	.33	[.18, .41]
Age*Rate	0.14	5	0.03	0.35	.882	.02	[.00, .02]
Error	7.51	94	0.08				

Shapiro-Wilk test, p= 1.116996e-08

Levene's test, p= 0.5653204

SP/AP Amplitude ratio: Channel A (ipsiTymptrode- contraMastoid)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.02	1	0.02	2.76	.100	.03	
Rate	0.05	5	0.01	1.60	.168	.08	[.00, .13]
Age*Rate	0.02	5	0.00	0.68	.638	.03	[.00, .06]
Error	0.57	94	0.01				

Shapiro-Wilk test, p= 0.3568438

Levene's test, p= 0.007248607

SP/AP Amplitude ratio: Channel C (ipsiTymptrode- Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.01	1	0.01	1.93	.168	.01	
Rate	0.05	5	0.01	1.22	.304	.06	[.00, .11]
Age*Rate	0.01	5	0.00	0.23	.948	.01	[.00, .00]

Error	0.73	94	0.01				
Shapiro-Wilk test, p= 0.3304237 Levene's test, p= 0.005106322							

SP Latency: Channel A (ipsiTymptrode- contraMastoid)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.02	1	0.02	0.81	.370	.01	
Rate	0.30	5	0.06	2.00	.085	.09	[.00, .15]
Age*Rate	0.07	5	0.01	0.47	.800	.02	[.00, .04]
Error	2.96	100	0.03				
Shapiro-Wilk test, p= 3.406909e-10 Levene's test, p= 0.3219015							

SP Latency: Channel C (ipsiTymptrode- Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.01	1	0.01	0.44	.510	.00	
Rate	0.20	5	0.04	1.72	.135	.08	[.00, .12]

Age*Rate	0.02	5	0.00	0.20	.962	.01	[.00, 1.00]
Error	2.45	108	0.02				
Shapiro-Wilk test, p= 0.002116472 Levene's test, p= 0.3702353							

SP Amplitude: Channel A (ipsiTymptrode- contraMastoid)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.02	1	0.02	1.14	.289	.01	
Rate	0.04	5	0.01	0.45	.809	.02	[.00, .03]
Age*Rate	0.04	5	0.01	0.39	.854	.02	[.00, .03]
Error	1.79	100	0.02				
Shapiro-Wilk test, p= 2.173396e-10 Levene's test, p= 0.00100723							

SP Amplitude: Channel C (ipsiTymptrode- Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.00	1	0.00	0.26	.610	.00	

Rate	0.02	5	0.00	0.36	.874	.01	[.00, .02]
Age*Rate	0.01	5	0.00	0.22	.953	.01	[.00, 1.00]
Error	1.37	108	0.01				
Shapiro-Wilk test, p= 3.73039e-09 Levene's test, p= 0.06978363							

Wave I Latency: Channel B (ipsi Mastoid– Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.34	1	0.34	18.10	.000	.22	
Rate	1.13	5	0.23	11.89	.000	.48	[.29, .56]
Age*Rate	0.11	5	0.02	1.18	.331	.08	[.00, .14]
Error	1.21	64	0.02				
Shapiro-Wilk test, p= 0.1456548 Levene's test, p= 0.1680826							

Wave I Latency: Channel C (ipsiTymptrode- Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**

Age	0.58	1	0.58	37.42	.000	.29	
Rate	1.08	5	0.22	13.88	.000	.43	[.27, .51]
Age*Rate	0.01	5	0.00	0.09	.994	.01	[.00, 1.00]
Error	1.43	92	0.02				
Shapiro-Wilk test, p= 0.1061552 Levene's test, p= 0.3572434							

Wave I Amplitude: Channel B (ipsi Mastoid– Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.06	1	0.06	2.62	.111	.04	
Rate	0.41	5	0.08	3.67	.006	.22	[.04, .31]
Age*Rate	0.00	5	0.00	0.04	.999	.00	[.00, 1.00]
Error	1.42	64	0.02				
Shapiro-Wilk test, p= 6.725452e-13 Levene's test, p= 0.8717909							

Wave I Amplitude: Channel C (ipsiTymptrode- Forehead)

Predictor	Sum of Squares	df	Mean Square	F	p	Partial η^2	Partial η^2 90% CI [LL, UL]
Age	0.19	1	0.19	2.22	.139	.02	
Rate	4.12	5	0.82	9.85	.000	.35	[.19, .43]
Age*Rate	0.02	5	0.00	0.04	.999	.00	[.00, 1.00]
Error	7.70	92	0.08				
Shapiro-Wilk test, p= 1.496411e-10 Levene's test, p= 0.8482949							

Wave V Latency: Channel B (ipsi Mastoid– Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.18	1	0.18	3.3	.072	.03	
Rate	25.97	5	5.19	93.54	.000	.83	[.77, .85]
Age*Rate	0.05	5	0.01	0.19	.967	.01	[.00, 1.00]
Error	5.44	98	0.06				
Shapiro-Wilk test, p= 0.0001334539 Levene's test, p= 0.7683606							

Wave V Latency: Channel C (ipsiTymptrode- Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.41	1	0.41	5.28	.024	.05	
Rate	25.23	5	5.05	64.78	.000	.78	[.70, .81]
Age*Rate	0.08	5	0.02	0.20	.960	.01	[.00, 1.00]
Error	7.17	92	0.08				
Shapiro-Wilk test, p= 0.01434612 Levene's test, p= 0.753378							

Wave V Amplitude: Channel B (ipsi Mastoid– Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.00	1	0.00	0.02	.875	.00	
Rate	1.36	5	0.27	12.86	.000	.40	[.24, .47]
Age*Rate	0.12	5	0.02	1.09	.369	.05	[.00, .09]
Error	2.07	98	0.02				
Shapiro-Wilk test, p= 1.735161e-05 Levene's test, p= 0.265164							

Wave V Amplitude: Channel C (ipsiTymptrode- Forehead)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	0.01	1	0.01	0.63	.431	.01	
Rate	0.74	5	0.15	12.82	.000	.41	[.25, .49]
Age*Rate	0.07	5	0.01	1.28	.279	.06	[.00, .11]
Error	1.07	92	0.01				
Shapiro-Wilk test, p= 0.3822233 Levene's test, p= 0.08982703							

DP levels dB: Low level tones (40, 55 dB)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	1297.20	1	1297.20	31.79	.000	.37	
Frequency	262.27	2	131.13	3.21	.048	.11	[.00, .22]
Age* Frequency	39.74	2	19.87	0.49	.617	.02	[.00, .08]
Error	2203.73	54	40.81				

Shapiro-Wilk test, p= 0.04950162

Levene's test, p= 0.006521789

DP levels dB: High level tones (61, 55 dB)							
Predictor	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial η^2**	**Partial η^2 90% CI [LL, UL]**
Age	1257.75	1	1257.75	42.47	.000	.44	
Frequency	84.21	2	42.10	1.42	.250	.05	[.00, .15]
Age* Frequency	49.18	2	24.59	0.83	.441	.03	[.00, .11]
Error	1599.28	54	29.62				

Shapiro-Wilk test, p= 0.01263933

Levene's test, p= 0.1562332

Curriculum Vitae

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