THE EFFECTS OF MIDDLE-EAR STIFFNESS ON THE AUDITORY BRAINSTEM NEURAL ENCODING OF PHASE

By

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Dissertation

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

In

Hearing and Speech Sciences

January 31, 2022

Nashville, Tennessee

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ACKNOWLEDGMENTS

It takes a village to raise a Ph.D. student, and I would like to take this opportunity to acknowledge my village. I would like to begin by extending my deepest gratitude to the 43 individuals who participated in this study that required several hours of in-person data collection in the midst of a global pandemic. This study would not have been possible without their generosity. I also acknowledge the Vanderbilt Institute for Clinical and Translational Research (VICTR) for providing funding for this study (grant number: VR55626).

I am gratefully indebted to the members of my dissertation committee (Linda J. Hood, Ph.D., René H. Gifford, Ph.D., Ramnarayan Ramachandran, Ph.D., and Rafael E. Delgado, Ph.D.) for their guidance during each step of the scientific process related to this study. To Dr. Linda Hood, thank you for serving as the chair of my dissertation committee and for supporting me throughout my academic endeavors. I am a thoughtful and well-rounded scientist, in part, because of you. Dr. René Gifford, you are an outstandingly accomplished scientist and a wonderful mentor. It has been an honor to learn under your guidance over the past several years. Your support and excitement for this study, especially in its early stages, means more to me than I can express. Dr. Ramnarayan Ramachandran, thank you for the intellectually stimulating discussions that we have shared and for always challenging me to grow in my scientific thinking. I am honored to have had you as a member of my committee. Dr. Rafael E. Delgado, thank you for your generous support in developing the ASSR stimulus files and data acquisition programs used in this study as well as for your guidance in interpreting ASSR phase data. This dissertation study was made stronger by having you on board.

I would also like to acknowledge my colleagues in the Auditory Physiology Laboratory, especially Lauren Roberts, Au.D., Mary Ferguson, Au.D., Janice Creel, Sarah Powell, Au.D., Ph.D., and Diana Kloiber, Au.D., for their unwavering support throughout my Ph.D. program. I owe special gratitude to Dr. Lauren Roberts and Dr. Mary Ferguson with whom I gladly shared a small office space with for more than 4 years. You both have been my unending cheerleaders and sources of greatest support throughout my Ph.D. program. I am thankful to call you colleagues and honored to consider you both family. To the Au.D. students that I've worked with throughout my time at Vanderbilt, particularly Jeffrey Wise, Au.D., Sophie Werk, and Krystina Schultz, thank you for teaching me how to be an effective teacher and mentor.

I owe deep gratitude to my mentors and friends from my time at the University of Texas at Dallas, especially to Jackie Clark, Ph.D., Edward Lobariñas, Ph.D., and Ross Roeser, Ph.D. The support and mentorship that I received from each of you during my Au.D. studies was foundational in my decision to pursue a Ph.D. You all taught me to believe in my capabilities. I also want to thank Katharine Fitzharris, Au.D., Ph.D. Your friendship and support over the past several years has meant the world to me.

Most importantly, I would like to thank my loved ones. Thank you to my mother, father, and brothers for always believing in me and always supporting me. To my fellow Ph.D. candidate, Sarah Schneck, you are my soul friend. I am constantly in awe of your intelligence and your seemingly endless capacity to be supportive and kind. Your friendship is, without a doubt, the most valuable thing that I will take away from my time as a Ph.D. student. Finally, to my handsome and loving partner, Robert Troy Nall, I will never have the words to adequately express how grateful I am for you. You have been my safest space and my greatest supporter. Thank you for always reminding me that there is a beautiful life to be lived outside of work. I love you.

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CHAPTER 1

Introduction

The purpose of this study was to investigate the effects of increased middle-ear stiffness on the auditory brainstem neural encoding of phase in human adults with a typically functioning auditory system (to the extent that can be measured) using clinically translatable, non-invasive assays. The rationale supporting this study is based on the middle ear's role in transmitting mechanical sound waves from the low-impedance air-filled space of the outer and middle ear to the high-impedance fluid-filled cochlear organ of hearing (Merchant & Rosowski 2003).

Changes in middle-ear stiffness and mass can alter the middle-ear transmission of sound. The transmission of phase information is one aspect of sound that can be altered by increased middle-ear stiffness. Phase is the alternating current representation of an acoustic waveform and is reported in degrees. In normal-hearing listeners, increased middle-ear stiffness altered the cochlear encoding of phase by an average of 30 degrees as measured using distortion product otoacoustic emissions (DPOAEs; Avan et al., 2000; Büki et al., 2000; Sun 2008). The binaural neural encoding of phase (i.e., interaural phase difference, or IPD) is important for spatial hearing and sound localization (Hughes 1938; Garner & Wertheimer 1951; Klumpp & Eady 1956; Sayers & Cherry 1956; Zwislocki & Feldman 1956; Mills 1960; Yost 1974). In fact, Yost (1974) showed that human listeners with normal hearing could detect that a 0.5-kHz tone shifted from midline when presented with an IPD of as little as 5 degrees.

Two audiologic clinical populations that may demonstrate changes in middle-ear stiffness are cochlear implant (CI) patients and patients with otosclerosis. Recent work showed increased middle-ear stiffness measured using wideband acoustic immittance (WAI) following cochlear implantation (Merchant et al., 2020; Saoji et al., 2020; Scheperle & Hajicek 2020). Increased middle-ear stiffness post-cochlear implantation may alter the transmission and encoding of acoustic information by an ear with a CI, leading to impaired binaural processing of acoustic cues in CI patients who use combined electric and binaural acoustic stimulation (EAS). Middle-ear stiffness-induced changes in the encoding of phase in the CI ear may contribute to the poor spatial hearing abilities observed in some CI EAS patients (Dunn et al., 2010; Grantham et al., 2012; Gifford et al., 2014; Plant & Babic 2016).

Additionally, otosclerosis is an auditory disorder characterized by increased middle-ear stiffness due to fixation of the stapes (Chole & McKenna 2001). Redfors et al. (2015) found that patients with otosclerosis self-reported difficulties with spatial hearing and sound localization that persisted even in patients treated with hearing aids.

The investigation of middle-ear stiffness effects on the auditory brainstem neural encoding of phase may have important implications for understanding binaural cue sensitivity and spatial hearing for CI EAS patients and patients with otosclerosis. Understanding the effects of increased middle-ear stiffness on the neural encoding of phase in a normal auditory system will form a basis for future work to understand the effects of stiffness-induced changes in clinical populations.

In this study, contralateral elicitation of the middle-ear muscle reflex (MEMR) was used to increase middle-ear stiffness in the test ear and changes in acoustic absorbance were measured using WAI to quantify the effects of middle-ear stiffness. Auditory Steady-State Responses (ASSRs) quantified the neural encoding of phase through the level of the auditory brainstem. ASSRs were compared with and without contralateral elicitation of the MEMR in order to quantify the effects of increased middle-ear stiffness on the auditory brainstem neural encoding of phase.

1.1 Specific Aims and Hypotheses

Specific Aim I: To investigate the effects of increased middle-ear stiffness on ASSR phase elicited by tonebursts presented separately at 3 stimulus levels in adults aged 18 to 50 years with normal pure-tone threshold sensitivity. *Rationale:* This study aim established whether middle-ear stiffness, elicited via activation of the MEMR, altered the auditory brainstem neural encoding of phase, as measured using the ASSR. By focusing on the auditory neural encoding of phase, this aim extended work that investigated middle-ear stiffness-induced changes in cochlear phase encoding in human adults (Avan et al., 2000; Büki et al., 2000; Sun 2008). *Hypotheses:* (i) Increased middle-ear stiffness will decrease ASSR phase, consistent with studies that demonstrated changes in DPOAE phase with increased middle-ear stiffness (Avan et al., 2000; Büki et al., 2000; Sun 2008) and (ii) larger middle-ear stiffness-induced changes in ASSR phase will occur at the highest ASSR stimulus level compared to lower stimulus levels.

Specific Aim II: To investigate correlations between middle-ear stiffness-induced changes in WAI measures (i.e., acoustic absorbance and power transmittance) and ASSR phase. *Rationale:* This study aim established whether middle-ear stiffness-induced changes in middle-ear function correlated with changes in the auditory brainstem neural encoding of phase. *Hypothesis:* A positive correlation will be observed between middle-ear stiffness-induced changes in acoustic absorbance and changes in ASSR phase and between stiffness-induced changes in power transmittance and changes in ASSR phase.

Specific Aim III: To investigate, on a preliminary basis, ear-specific differences in stiffness-induced ASSR phase changes towards developing an auditory evoked potential measure of IPD in adults aged 18 to 50 years with normal pure-tone threshold sensitivity. *Rationale:* This study aim established middle-ear stiffness-induced effects on ASSR phase for both left and right ears. This investigation may have important implications in the future development of an auditory brainstem neural IPD measure. Unilateral changes in

middle-ear stiffness secondary to cochlear implantation or otosclerosis may produce ear-specific differences in the auditory brainstem neural encoding of phase (i.e., larger auditory evoked potential IPD). *Hypothesis:* For this study sample of adults with normal pure-tone threshold sensitivity, the amount of middle-ear stiffness-induced ASSR phase shift will be comparable for the right and left ears.

CHAPTER 2

Background

2.1 Overview

Changes in middle-ear forward transmission, such as increased stiffness and mass, can alter the acoustic properties of sound arriving at the cochlea. This may result in changes in the cochlear and auditory neural encoding of acoustic features of a target sound source. One acoustic property of sound that may be modified by the middle-ear forward transmission system is phase. Phase is the alternating current representation of an acoustic waveform that provides information related to the proportion of the periodic waveform that has been completed and the number of cycles of alternating current in one second is defined as the frequency of the waveform. The phase of an acoustic stimulus is encoded monaurally in contribution to pitch and speech perception in quiet and in the presence of background noise. Phase is also encoded by both ears and is transmitted via the auditory neural system in contribution to spatial hearing. The IPD is defined as the comparison of phase encoding between ears and is a metric utilized in describing human spatial hearing.

The surgical placement of a CI electrode array in the inner ear has recently been shown to decrease low-frequency acoustic absorbance, consistent with a post-operative increase in middle-ear stiffness (Merchant et al., 2020; Saoji et al., 2020; Scheperle & Hajicek 2020). While previous studies have shown that increased middle-ear stiffness changes the cochlear encoding of phase (Avan et al., 2000; Büki et al., 2000; Sun 2008), this study provides the first evaluation of the effects of middle-ear stiffness on the auditory brainstem neural encoding of phase in humans. Understanding the effects of middle-ear stiffness on the auditory brainstem neural encoding of phase in adults with normal pure-tone threshold sensitivity will provide a foundation for interpretation of findings from future studies involving CI EAS patients. Furthermore, the present study used non-invasive clinical assays, WAI-MEMR and ASSR, in order to increase its future clinical translation.

Background information in the following areas is provided to support the rationale, methodology, and interpretation of findings for this study: (i) the anatomy and physiology of the middle-ear transmission system, including physiologic assays of the MEMR; (ii) animal and human studies investigating the auditory neural encoding of phase; and (iii) studies investigating the effects of middle-ear stiffness on the cochlear encoding of phase. Limitations associated with investigating middle-ear stiffness on the cochlear encoding of phase in human adults is discussed to provide a rationale for focusing on auditory brainstem neural phase encoding in this study.

2.2 The Middle-Ear Transmission System

2.2.1 Anatomy and Physiology of the Middle-Ear Transmission System

The middle ear is a low-impedance, air-filled cavity that is responsible for transmitting mechanical acoustic energy from the external auditory canal to the high-impedance, fluid-filled cochlea (Merchant & Rosowski 2003). The middle-ear transmission system primarily consists of the tympanic membrane, three ossicles, two muscles, and joints and ligaments connecting the ossicles. Air pressure within the middle-ear cavity is maintained by the opening and closing of the Eustachian Tube. Mechanical sound waves travel along the external auditory canal to reach the tympanic membrane and cause it to vibrate. Tympanic membrane vibrations are amplified and transmitted to the cochlea via the ossicular chain. The three middle-ear ossicles, named the malleus, incus, and stapes, articulate together via joints and ligaments to form the ossicular chain. The umbo of the malleus is attached to the tympanic membrane at the distal end of the middle-ear cavity while the stapes footplate is attached to the oval window of the cochlea. Thus, vibrations of the tympanic membrane result in displacement of the umbo and vibrations of the entire ossicular chain. The vibrations of the stapes footplate introduce pressure waves into the fluid-filled cochlea (Rosowski 1996; Zhang & Gan 2011; Bohnke et al., 2013).

The high impedance of the fluid-filled cochlea results in partial reflection of mechanical acoustic energy back towards the external auditory canal. The middle ear compensates for this impedance mismatch by amplifying the mechanical acoustic pressure wave as it is transmitted through the middle ear. Decreased middle-ear volume at the stapes footplate compared to that at the tympanic membrane contributes to increased pressure and sound amplification as middle-ear transmission occurs. The middle-ear transmission system is frequency-specific providing a resonance frequency in human adults at approximately 1 kHz (Hanks & Rose 1993; Margolis & Goycoolea 1993; Shanks et al., 1993). Increased middle-ear stiffness can decrease the amount of low-frequency energy that is absorbed into the middle-ear space and can shift the middle-ear resonance frequency to a higher spectral region (Colletti 1975, 1976, 1977; van Camp et al., 1983; Funasaka et al., 1984; van Camp & Vogeleer 1986; Shanks et al., 1988).

The pressure of the mechanical acoustic wave at the stapes footplate relative to that at the tympanic membrane is known as middle-ear forward transmission. Similarly, the middle-ear transmission system transmits pressure waveforms travelling in the reverse direction from the cochlea towards the external auditory canal (i.e., otoacoustic emissions). The pressure in the external auditory canal at the level of the tympanic membrane relative to the pressure at the stapes footplate is known as middle-ear reverse transmission.

2.2.2 Anatomy and Physiology of the Middle-Ear Muscle Reflexes

The auditory system contains two acoustic reflexes that moderate the function of the middle-ear

transmission system: (i) the tensor tympani reflex and (ii) the stapedius reflex. The tensor tympani reflex involves the tensor tympani muscle which articulates with the malleus and is innervated by the mandibular branch of the trigeminal nerve (Cranial Nerve V). Contraction of the tensor tympani muscle results in a stiffening of the ossicular chain, in turn, dampening middle-ear transmission. The tensor tympani reflex has a comparatively slower reaction time relative to the stapedius reflex and thus the primary function of the tensor tympani reflex is to soften reverberant, loud sounds produced by actions of the oral cavity, such as chewing and talking.

The stapedius reflex involves the stapedius muscle which articulates with the neck of the stapes and is innervated by the stapedius branch of the facial nerve (Cranial Nerve VII). The afferent arm of the stapedius reflex arc begins with the ipsilateral auditory nerve (Cranial Nerve VIII) and includes the ipsilateral cochlear nucleus and bilateral superior olivary complexes in the caudal auditory brainstem. The efferent arm of this reflex arc includes the bilateral motor nuclei of the facial nerve and bilateral facial nerves. The stapedius reflex thus consists of an ipsilateral and contralateral reflex pathway. The reaction time of the stapedius reflex is comparatively faster than the tensor tympani reflex and primarily plays an otoprotective role by increasing the stiffness of the ossicular chain (Pilz et al., 1997; Geisler 1998). The stapedius reflex may also enhance the detection of target signals in background noise (Liberman & Guinan 1998).

2.2.3 Wideband Acoustic Immittance

WAI is a term used to describe a collection of measures related to primarily middle-ear function (though cochlear function may also contribute) across a broad spectral range. WAI measures are dependent on each other and include acoustic absorbance, acoustic reflectance, power transmittance, admittance, and impedance (e.g. Feeney et al., 2003; Allen et al., 2005). Portions of acoustic energy reaching the tympanic membrane are absorbed into the middle-ear space for transmission to the cochlea while other portions of acoustic energy are reflected back into the external auditory canal. Acoustic energy reflected back into the external auditory canal by the tympanic membrane is separate from middle-ear reverse transmission related to otoacoustic emissions described above. Acoustic absorbance values of 1.0 (or 100%) indicate complete absorbance of acoustic energy into the middle-ear cavity while values of 0.0 (or 0%) indicate total reflectance into the external auditory canal (Feeney et al., 2003; Allen et al., 2005). The resonance frequency of the middle-ear transmission system can be ascertained as the frequency at which acoustic absorbance is maximal. Furthermore, power transmittance represents acoustic power that is absorbed by the middle ear and is reported in decibels (Allen et al., 2005). The decibel reference used in reporting power transmittance has the distinct advantage of audiologic relevance and ease of clinical translation and interpretation.

The effects of both adult participant sex and age on WAI measures remain controversial. Related to participant sex, Margolis et al. (1999) found higher middle-ear impedance in males and Wiley et al. (1999)

found higher middle-ear resonance frequencies in females. In contrast, Shahnaz & Davies (2006) did not find an effect of participant sex on middle-ear resonance frequencies. Previous studies have also reported that females have lower reflectance in the higher frequencies compared to males (Shahnaz & Bork 2006; Shaw 2009). The potential effect of older adult age on WAI measures is less well understood, though Feeney & Sanford (2004) showed that older adults had reduced acoustic reflectance compared to younger adults for frequencies lower than the middle-ear resonance frequency and greater reflectance for frequencies higher than the resonance frequency. Studies utilizing middle-ear measures should account for potential effects of participant sex and age.

WAI has been applied to the assessment of the ipsilateral and contralateral MEMR in human adults (Feeney & Keefe 1999, 2001; Feeney et al., 2017). Feeney & Keefe (1999) demonstrated that contralateral elicitation of the MEMR resulted in decreased acoustic absorbance for lower frequencies consistent with a stiffening of the middle-ear transmission system. While previous researchers have applied WAI measures to improve MEMR threshold estimation, work by Feeney & Keefe (1999) also showed that contralateral elicitation of the MEMR results in demonstrable changes in acoustic absorbance that reflect changes in middle-ear stiffness.

2.3 Auditory Neural Encoding of Phase

2.3.1 Neural Encoding of Phase in Animal Models

Phase is one aspect of sound that both middle ears transmit in contribution to binaural and spatial hearing. Phase encoding occurs at the level of the cochlea and is also encoded at the level of the auditory nerve via neural firing patterns that are synchronized to the phase of an acoustic stimulus, known as phase-locking (Tasaki 1954; Kiang et al., 1965; Rose et al., 1967; Johnson 1980). The ability of the auditory nerve to encode phase has been investigated in various animal models including chinchilla (Javel 1980), gerbil (Smith & Brachman 1980; Smith et al., 1985), rat (Møller 1976), guinea pig (Palmer 1982, Yates 1987), and cat (Joris & Yin 1992). The findings from animal studies suggest that (i) the auditory nerve encodes phase via phase-locked neural firing related to both the stimulus and modulation frequencies of stimuli and (ii) the ability of the auditory nerve to encode phase is dependent on spontaneous firing rate and characteristic frequency of individual auditory nerve fibers, with maximal neural phase-locking to amplitude modulated tones occurring for low-spontaneous firing rate, high characteristic frequency auditory nerve fibers (e.g., Joris & Yin 1992).

The auditory neural pathway is capable of phase-locking to the temporal fine structure of a stimulus as well as to the temporal envelope. The ability of the auditory neural system to encode the phase of the temporal fine structure of a stimulus (i.e., inter-aural time difference and inter-aural phase difference cues) is of particular relevance for interpreting the findings of this study. It is generally well accepted that the upper frequency limit of auditory nerve phase-locking that is useful for binaural auditory processing in humans is

1.5 kHz (e.g., Brughera et al., 2013; Verschooten et al., 2019). The auditory nerve may encode monaural phase information that could contribute to pitch perception. The frequency range over which the auditory nerve is capable of phase-locking for the monaural encoding of phase remains controversial with the upper frequency limit broadly estimated to be between 1.5 and 10 kHz (Verschooten et al., 2019).

The auditory neural encoding of phase extends beyond the level of the auditory nerve. Singe-unit studies in various animal models have shown that the neural encoding of phase is maintained throughout auditory brainstem structures, including the cochlear nuclei and inferior colliculi. Previous studies have demonstrated that the upper frequency limit of neural phase-locking decreases with central progression along the auditory brainstem neural pathway (Møller 1972; Goldberg & Brownell 1973; Kuwada et al., 1984; Winter & Palmer 1990; Liu et al., 2006). Single-unit fibers in the cochlear nucleus of rats demonstrated an ability to encode the phase of amplitude modulated tones for frequencies between 50 and 200 Hz (Møller 1972). Winter & Palmer (1990) demonstrated the ability of single-unit fibers in the guinea pig cochlear nucleus to encode phase information related to the temporal fine structure of a stimulus in a manner that depended on both neuronal cell type and spontaneous firing rate with some fibers capable of encoding fine-frequency phase information for frequencies above 1 kHz. Compared to chopper neurons, primary-like cochlear nucleus neurons with lower spontaneous firing rates showed greater encoding of fine-frequency phase structure for lower characteristic frequency stimuli (Winter & Palmer 1990). Differences in the range of neural phase encoding at the level of the cochlear nucleus reported by these studies are likely due to differences in the type of phase-locking measured – neural phase-locking to an amplitude modulated tone vs. to the temporal fine structure of a stimulus.

Single-units from the inferior colliculus of various animal models have been shown to encode phase for a broad range of frequencies ranging from 80 to 1000 Hz (Kuwada et al., 1984; Liu et al., 2006). Furthermore, Liu et al. (2006) demonstrated that the upper frequency limit of phase-locking for inferior colliculus neurons differed based on anatomical location. The highest upper frequency limit for phase-locking was obtained from neurons in the central nucleus and the lowest upper frequency limit was obtained from fibers in the external nucleus.

To summarize findings from animal studies, the auditory nerve and single-units from the cochlear nuclei and inferior colliculi encode phase over a broad range of frequencies via phase-locked neural spiking. The upper frequency limit of phase-locking decreases centrally along the auditory neural pathway though specific structures along the neural pathway respond to a broad range of frequencies. These findings can help to inform understanding and interpretation of the physiologic assessment of phase in humans. Particularly, these animal studies demonstrate that phase encoding is maintained throughout the auditory nervous system, especially through rostral auditory brainstem structures that are likely generators of the human ASSR elicited by faster presentation rates.

2.3.2 Physiologic Assessment of Phase in Humans

The ASSR is a non-invasive auditory evoked potential that can be used as an assay of the auditory brainstem neural encoding of phase in humans (e.g., Picton et al., 2003). The human auditory nervous system encodes the phase of the repetition rate or modulation frequency of an acoustic stimulus, resulting in periodic neural activity at the fundamental and harmonic frequencies. One metric of the ASSR that can be analyzed is physiologic response phase. Though there are many ways to analyze ASSR phase, most analyses report the onset phase of the physiologic response relative to the phase of a cosine (Picton et al., 2003). The phase of the ASSR also can be compared to the phase of the eliciting stimulus in order to determine phase delay/lead in the physiologic encoding of phase (Picton et al., 2003). The amplitude of the ASSR also can be analyzed. ASSR amplitude provides an assessment of neural recruitment and the synchronicity with which a population of auditory neurons encode the phase of the repetition rate of an acoustic stimulus.

The ASSR can be recorded in human adults at repetition rates of up to 400 Hz (Rees et al., 1986) with the largest amplitude observed at 40 Hz in human adults (Galambos et al., 1981). Traditionally, the ASSR elicited by repetition rates near 40 Hz has been interpreted as representing primarily thalamic/cortical contributions to the physiologic response, whereas repetition rates near 80 Hz represent primarily auditory brainstem contributions (e.g., Kuwada et al., 2002). However, recent studies in humans suggest that cortical contributions to the physiologic encoding of phase exist for faster repetition rates (e.g., Coffey et al., 2019). These previous studies in combination with studies in animal models suggest that faster repetition rates should be used when targeting the sub-cortical neural encoding of phase in humans.

Specific to repetition rates that target primarily auditory brainstem generators, the ASSR is optimally recorded at repetition rates between 70 and 110 Hz in humans (Rees et al., 1986; Lins et al., 1995; Picton et al., 2003). These studies provide a rationale for using fast repetition rate ASSRs as a non-invasive assay of the auditory brainstem neural encoding of phase in humans.

Many stimulus-related and participant-related factors can impact ASSR phase and amplitude. The following review will focus only on the adult population as this is the relevant population in this study. Stimulus-related factors include carrier stimulus frequency and level. ASSR onset phase increases and phase delay decreases with increasing stimulus frequency. Stimulus frequency produces different effects on 40- versus 80-Hz ASSR amplitude. The amplitude of the ASSR decreases with increasing stimulus frequency for modulation rates near 40 Hz; however, ASSR amplitude is largest near 1- to 2-kHz for repetition rates near 80 Hz. Related to stimulus level, ASSR onset phase increases, phase delay decreases, and amplitude decreases with lower stimulus levels for all repetition rates (Picton et al., 2003).

Participant-related factors in adults that can impact ASSR phase and amplitude are state of arousal and attentional state. An individual's state of arousal primarily affects slow-rate ASSRs (near 40 Hz; e.g.,

Galambos et al., 1981; Galambos & Makeig 1988), but has minimal to no effect on fast-rate ASSRs (between 70 and 180 Hz; Cohen et al., 1991). Diverted attentional resources away from the ASSR stimulus and towards other relevant changes in the acoustic environment may decrease ASSR onset phase and amplitude for repetition rates near 40 Hz (Picton et al., 2003). The previously reported effects of sleep and attentional state on 40-Hz ASSR phase and amplitude are consistent with the response being primarily generated by thalamic/cortical structures. State of arousal and attention likely had minimal to no impact on the findings of this study since the ASSR was elicited using a fast modulation rate (78.125 Hz), likely to be primarily generated by auditory brainstem structures.

2.4 Middle-Ear Stiffness and Auditory Encoding of Phase

2.4.1 Cochlear Encoding of Phase

The cochlea is the sensory organ of hearing that receives mechanical sound pressure waves from the middle ear and is responsible for converting mechanical energy into electrical impulses for neural transmission. The motility of the outer hair cells (OHCs) is part of an active process within the cochlea in response to acoustic mechanical input (Brownell 1990; Dallos et al., 1997; He et al., 2003). The active cochlear process emits sound that travels through the middle-ear cavity via middle-ear reverse transmission. This energy can be measured non-invasively in humans using otoacoustic emissions (OAEs; Brownell 1990; Kemp et al., 1990; Kemp 2002). OAEs are associated with primarily OHC function with small contributions from basilar membrane micromechanical properties (Brownell 1990; Kemp et al., 1990; Kemp 2002).

As a result of their positioning on the basilar membrane, OHCs respond to and encode basilar membrane displacement in response to cochlear sound pressure waves produced by vibration of the stapes footplate or via bone-conducted stimuli. Since OAEs are associated with OHC function, measures of OAE phase provide a non-invasive assay of the cochlear (i.e., OHC) encoding of phase.

Other investigators have demonstrated that contralateral elicitation of the MEMR results in increased DPOAE phase, especially for frequencies below 2 kHz (Avan et al., 2000; Büki et al., 2000; Sun 2008). Furthermore, Sun (2008) showed that low-level contralateral broadband noise (BBN) below MEMR threshold resulted in little change in DPOAE phase; however, higher level contralateral broadband noise above MEMR threshold resulted in significant DPOAE phase shifts at 1 and 2 kHz. Avan et al. (2000) demonstrated an average 30-degree DPOAE phase shift at 0.9 kHz with elicitation of the MEMR.

2.4.2 Limitations to Assessing Middle-Ear Stiffness Effects on Cochlear Phase Encoding

Studies that have investigated the effects of increased middle-ear stiffness on the cochlear encoding of phase have several limitations. The primary limitation of these studies is the lack of defining the position of the DPOAE frequency tested in the fine structure. DPOAEs measured in the ear canal represent the sum of

two cochlear source components: the generator component and the reflection component (Shera & Guinan 1999; Talmadge et al., 1999). The DPOAE generator component arises from the area of maximal overlap of the two primary elicitor tones along the cochlear basilar membrane. The phase of the DPOAE generator component rotates slowly with changes in primary tone frequencies (e.g., Henin et al., 2011). The DPOAE reflection component, on the other hand, arises when the cochlear energy generated at the overlap region travels to its own characteristic frequency location along the basilar membrane. The phase of the DPOAE reflection component changes rapidly with manipulations to elicitor frequencies (Shera & Guinan 1999). DPOAE fine structure maxima occur when the generator and reflection components are summed while in phase, while fine structure minima occur when the two components are summed while out of phase (Shaffer et al., 2003; Dhar et al., 2005). The relationship between DPOAE component phase and fine structure highlights that any scientific investigation assessing DPOAE phase needs to account for DPOAE position in the fine structure.

A second limitation related to investigating the effects of increased middle-ear stiffness on the cochlear encoding of phase in humans is that DPOAE recordings reflect both middle-ear forward and reverse transmission (reviewed in section 2.2.1). Studies using an OAE assay have relied on modeling techniques to estimate the effects of middle-ear stiffness on middle-ear forward and reverse transmission, but could not directly measure these differential effects. Thus, it remains unclear whether the DPOAE phase shifts observed in previous studies are due to a stiffer middle-ear forward transmission system, a stiffer middle-ear reverse transmission system, or both.

A measure of cochlear OHC function such as the cochlear microphonic (CM) that relies only on middle-ear forward transmission would begin to address limitations associated with DPOAE assays of the cochlear encoding of phase discussed above. The alternating current response of the electrocochleography (ECochG) waveform represents contributions from the CM and auditory nerve neurophonic (ANN; Fontenot et al., 2017). Thus, the alternating current response of the ECochG represents a combination of cochlear and auditory nerve encoding of phase. The ECochG alternating current response is often low in amplitude when recorded using TipTrodes, which are most often used in ECochG recording in humans in the USA, as compared to the larger amplitude ECochG response obtained when recorded using transtympanic or tympanic electrodes. The complex combination of cochlear OHC and auditory nerve contributions to the alternating current response of the ECochG limits its utility as a measure of the cochlear encoding of phase in humans.

One final limitation related to previous studies that investigated increased middle-ear stiffness effects on the cochlear encoding of phase involves potential co-activation of the MEMR and medial olivocochlear reflex (MOCR). Büki et al. (2000) and Sun (2008) investigated the effects of contralateral BBN levels above and below MEMR threshold on DPOAE phase. These investigators reported small changes in DPOAE phase that did not reach statistical significance when the contralateral BBN was below MEMR threshold and large,

statistically significant DPOAE phase shifts with presentation of the BBN at levels high enough to elicit the MEMR. The BBN stimulus employed by these studies can elicit the MOCR. It is likely that the MOCR was elicited at BBN levels below MEMR threshold in previous studies as suggested by reduction in DPOAE amplitudes with low-level BBN elicitors (Büki et al., 2000; Sun 2008). However, contralateral presentation of low-level BBN did not significantly change DPOAE phase, suggesting that the observed DPOAE phase shifts were likely attributable to increased middle-ear stiffness due to MEMR elicitation. While studies by Büki et al. (2000) and Sun (2008) suggested that elicitation of the MOCR did not change DPOAE phase, Francis & Guinan (2010) showed that contralateral elicitation of the MOCR using a BBN elicitor did change click-evoked and stimulus-frequency OAE phase.

2.4.3 Rationale for Investigating the Auditory Neural Encoding of Phase

While previous studies demonstrated that increased middle-ear stiffness changed the cochlear encoding of phase, this study focused on investigating increased middle-ear stiffness effects on the auditory brainstem neural encoding of phase. The auditory neural encoding of phase does not rely on middle-ear reverse transmission – a significant confound in DPOAE assays. Furthermore, the auditory brainstem neural encoding of phase can be assessed using the ASSR in clinical populations where cochlear responses may be absent (i.e., CI EAS patients), potentially increasing the clinical applications of the findings of this study.

2.5 Significance

This work extended previous findings related to the effects of increased middle-ear stiffness on the cochlear encoding of phase. Knowledge related to this topic was meaningfully extended by investigating the effects of increased middle-ear stiffness on the auditory brainstem neural encoding of phase as measured using the ASSR. A normal-hearing human model of increased middle-ear stiffness was employed using non-invasive, clinically translatable assays in order to accomplish the aims of this work. Increased middle-ear stiffness was elicited by activation of the MEMR in order to assess for stiffness-induced effects in the ASSR test ear. This work was completed in adults with normal pure-tone threshold sensitivity as defined by current audiometric standards. This provided foundational understanding of the effects of increased middle-ear stiffness on the auditory brainstem neural encoding of phase in a presumably normal auditory system. The foundational knowledge provided by this work can be applied to understanding and interpreting the effects of increased middle-ear stiffness in the presence of non-typical auditory systems, such as CI EAS patients. This study is significant because changes in middle-ear stiffness may change the interaural neural encoding of phase and negatively impact spatial hearing abilities. This study is innovative because it employed non-invasive clinical assays in a novel paradigm to address the effects of increased middle-ear stiffness on the auditory brainstem neural encoding of phase – a topic with high clinical relevance.

CHAPTER 3

Related Work and Pilot Studies

3.1 Changes in Acoustic Absorbance in CI Patients Over Time

Recent studies demonstrated decreased low-frequency acoustic absorbance in CI patients who were tested at a single post-operative time point (Merchant et al., 2020; Saoji et al., 2020; Scheperle & Hajicek 2020). This finding is consistent with a stiffer middle-ear system following cochlear implantation. We extended these previous findings by investigating changes in acoustic absorbance at several post-operative time points through 6-months post-activation with the goal of obtaining as much within-participant, longitudinal information as possible within the constraints of a clinical CI visit schedule (Racca et al., In Review).

17 adult CI recipients within 6-months of device activation participated in this study. Wideband acoustic absorbance was measured in both ears across 6 potential time points – corresponding to the clinical CI follow-up schedule – with the goal of each participant completing testing for as many time points as possible. The time points were: (i) pre-CI baseline, (ii) CI activation, (iii) 1-week post-activation, (iv) 1-month post-activation, (v) 3-months post-activation, and (vi) 6-months post-activation. One participant completed testing at all 6 time points; 1 participant completed testing at 5 time points; 5 participants completed testing at 4 time points; 4 participants completed testing at 3 time points; 5 participants completed testing at 2 time points; and 1 participant completed testing for 1 post-operative time point. Analyses examined (i) changes in acoustic absorbance for the implanted ear compared to pre-implantation and (ii) differences in acoustic absorbance between implanted and non-implanted ears over time.

We replicated the finding of decreased low-frequency acoustic absorbance for CI patients following surgery. Figure 3.1. shows acoustic absorbance in the implanted ear across time points. Data for only 2 participants were available at the 1-week post activation time point, so this time point was excluded from implanted ear analyses. Using 95% confidence intervals based on median acoustic absorbance difference scores, we identified significantly decreased acoustic absorbance in the implanted ear at all post-surgery time points. Decreased acoustic absorbance was primarily observed in the spectral range below 1 kHz and became narrower with longer time post-activation (CI activation: 0.328 kHz, from 0.445 to 0.586 kHz, 0.656 kHz, 0.750 kHz, and from 0.797 to 0.938 kHz; 1-month post-activation: from 0.305 to 0.938 kHz and from 1.078 to 1.336 kHz; 3-months post-activation: from 0.609 to 0.867 kHz; 6-months post-activation: from 0.563 to 0.680 kHz and from 0.797 to 0.820 kHz).

Figure 3.2. compares acoustic absorbance between the implanted and non-implanted ears within participants across time points. We identified significantly decreased acoustic absorbance in the implanted compared to the non-implanted ears through at least 3-months post-activation. The spectral range of decreased

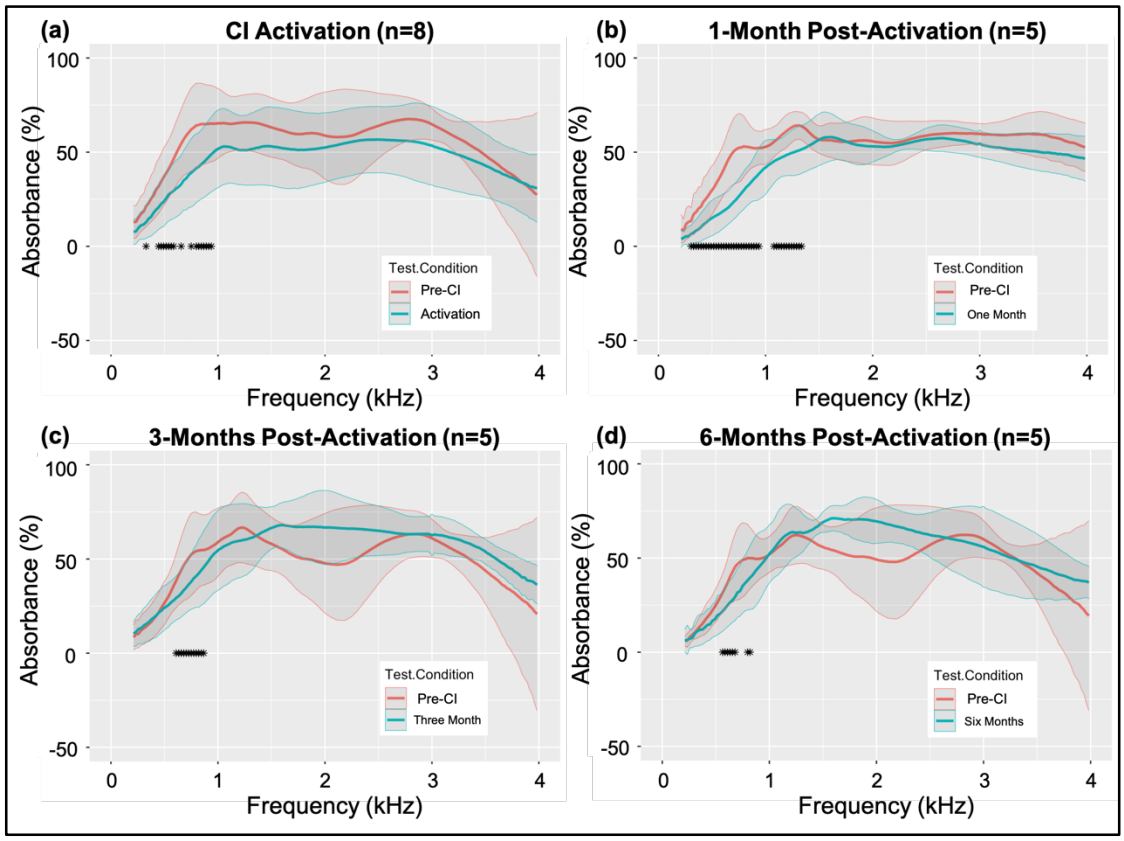

Figure 3.1: Line graphs showing decreased post-operative low-frequency acoustic absorbance in CI patients for the CI activation (a), 1-month post-activation (b), 3-months post-activation (c), and 6-months post-activation (d) time points. The salmon shaded lines in each panel represent pre-surgical acoustic absorbance and the teal shaded lines represent post-operative absorbance. The gray shaded regions represent $\pm$1 standard deviation surrounding the mean. Black asterisks below the absorbance tracings represent frequencies where a statistically significant decrease in post-operative acoustic absorbance occurred. The number of participants included at each time point is shown in the title of each panel.

acoustic absorbance in the implanted ear compared to the non-implanted ear became narrower and emphasized lower frequencies with longer time post-surgery (CI activation: from 0.469 to 0.891 kHz, from 1.148 to 1.336 kHz, from 1.547 to 2.039 kHz, from 2.133 to 2.203 kHz, from 2.250 to 2.578 kHz, from 2.977 to 3.281 kHz, and from 3.375 to 3.984 kHz; 1-week post-activation: from 0.258 to 1.641 kHz, from 2.250 to 2.297 kHz, from 2.766 to 2.859 kHz, from 3.258 to 3.305 kHz, and from 3.586 to 3.773 kHz; 1-month post-activation: from 0.609 to 1.172 kHz and from 1.711 to 2.180 kHz; and 3-months post-activation: from 0.703 to 0.984 kHz and at 1.406 kHz). No statistically significant differences in acoustic absorbance were found between ears at the 6-months post-activation time point, though we observed a trend for decreased acoustic absorbance in the implanted ear for frequencies below 1 kHz. The lack of a statistically significant difference between ears at the 6-months post-activation time point may not negate a clinically significant effect since the lack of statistical significance is likely due to high between-participant variability for this small sample size.

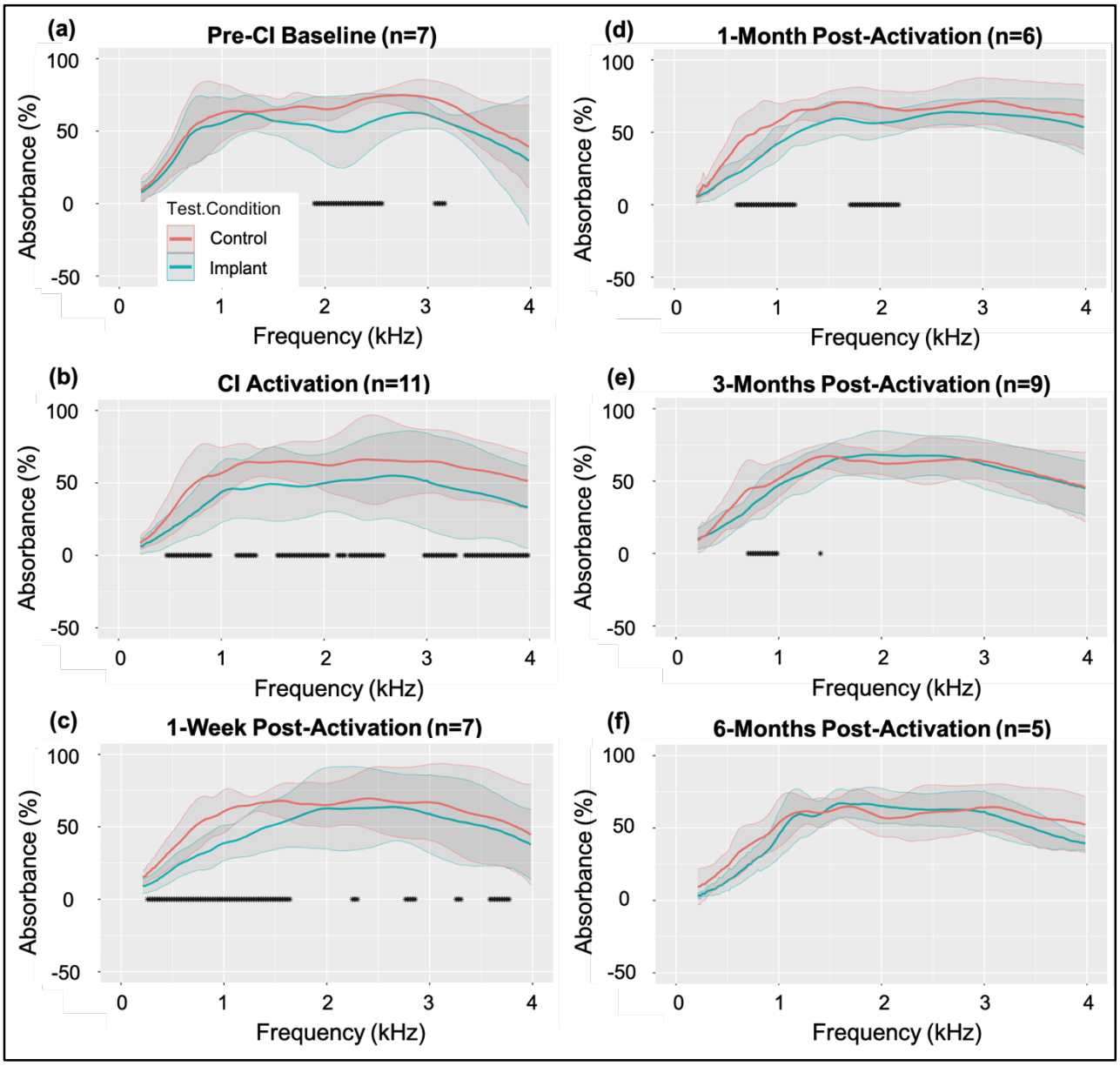

Figure 3.2: Line graphs showing decreased post-operative low-frequency acoustic absorbance in implanted compared to non-implanted ears for the pre-surgical (a), CI activation (b), 1-week post-activation (c) 1-month post-activation (d), 3-months post-activation (e), and 6-months post-activation (f) time points. The salmon shaded lines in each panel represent acoustic absorbance in the non-implanted ear and the teal shaded lines represent acoustic absorbance in the implanted ear. The gray shaded areas represent $\pm$1 standard deviation surrounding the mean. Black asterisks below the absorbance tracings represent frequencies where a statistically significant decrease in acoustic absorbance occurred for the implanted ear. The number of participants included at each time point is shown in the title of each panel.

The finding of decreased post-operative acoustic absorbance in CI patients is consistent with previous studies. Saoji et al. (2020) demonstrated decreased acoustic absorbance post-operatively for frequencies between 0.6 and 1.1 kHz for CI patients tested between 45- and 60-days post-surgery. Additionally, Scheperle & Hajicek (2020) showed decreased acoustic absorbance for frequencies between 0.25 and 0.891 kHz in the

implanted ears of CI patients compared to a different group of ears with normal hearing. Participants in their study were long-term CI patients (duration of CI use at the time of test = 3 to 25 years).

The findings of our study extend current knowledge by showing decreases in post-operative low-frequency acoustic absorbance through at least 6-months post-activation consistent with increased middle-ear stiffness post-surgery in CI patients. Several factors related to CI surgical procedures may contribute to a stiffer middle-ear system. Increased volume of the middle-ear cavity by introduction of the facial recess during CI surgery can cause the middle-ear transmission system to become dominated by ossicular chain stiffness (Mason 2016; Saoji et al., 2020; Scheperle & Hajicek 2020). Additionally, the sealing of the electrode array at the round window and the accumulation of bone dust post-surgery can lead to neo-osteogenesis, potentially increasing the stiffness of the middle-ear transmission system (Saoji et al., 2020; Scheperle & Hajicek 2020).

Increased middle-ear stiffness post-cochlear implantation may also be secondary to changes in cochlear function following surgery. For example, the development of intra-cochlear fibrotic tissue in response to surgically implanted CI biomaterials over time post-cochlear implantation may contribute to changes in cochlear physiology at the level of the oval window at the articulation of the Stapes footplate. This may contribute to increased stiffness of the ossicular chain and would be consistent with decreased acoustic absorbance at longer times post-surgery as intra-cochlear fibrotic tissue develops.

Considering that CI patients can have preserved post-operative low-frequency acoustic hearing, it is important to understand the implications of a potentially stiffer middle-ear forward transmission system on the encoding of acoustic information. Previous studies showed that increased middle-ear stiffness via elicitation of the contralateral MEMR resulted in changes in the cochlear encoding of phase (reviewed in Section 2.4.1.). This may indicate impaired interaural phase encoding in CI patients with preserved low-frequency acoustic hearing that could lead to impaired binaural cue sensitivity and spatial hearing.

3.2 Middle-Ear Stiffness and the Auditory Brainstem Neural Encoding of Phase

Many CI patients do not have measurable cochlear responses even in the presence of preserved acoustic hearing, complicating the investigation of the relationship between middle-ear stiffness and the cochlear encoding of phase in this population. However, neural measures of phase encoding, such as the ASSR, can often be elicited acoustically in CI EAS patients. This provides further rationale for the investigation of the effects of increased middle-ear stiffness on ASSR phase and amplitude investigated in this study and highlights the potential for future application of these metrics in CI EAS patients.

Four adults (2 females; 2 males; age range: 28 to 48 years) with normal pure-tone threshold sensitivity (≤15 dB HL from 0.25 to 8 kHz) participated in a pilot study investigating the effects of increased middle-ear stiffness on the auditory brainstem neural encoding of phase. ASSRs were elicited by Blackman-windowed toneburst stimuli (2-0-2 cycles in duration) centered at 0.5 and 2 kHz presented using a 95-Hz repetition rate.

The ASSR stimulus level was presented at 70 dB pSPL to the right ear of all pilot participants. The MEMR was elicited by 100-μS click stimuli presented to the left ear at 27.7/s with levels ranging from 60 to 85 dB pSPL in 5-dB steps. Elicitation of the MEMR resulted in reduced acoustic absorbance, averaged across a frequency band from 0.25 to 0.75 kHz, in the right ear for all participants. Reduced acoustic absorbance in this frequency range is consistent with elicitation of the MEMR increasing middle-ear stiffness in the test ear.

Figure 3.3. shows ASSR phase as a function of the MEMR elicitor level for 0.5- (Figure 3.3.a) and 2-kHz (Figure 3.3.b) ASSR stimulus frequencies. The findings of this pilot study showed that increased middle-ear stiffness resulted in decreased ASSR phase. ASSR phase decreased when the MEMR elicitor level was presented at 85 dB pSPL (i.e., when the MEMR was activated). It is important to note that all 4 pilot participants demonstrated a decrease in ASSR phase with increased middle-ear stiffness.

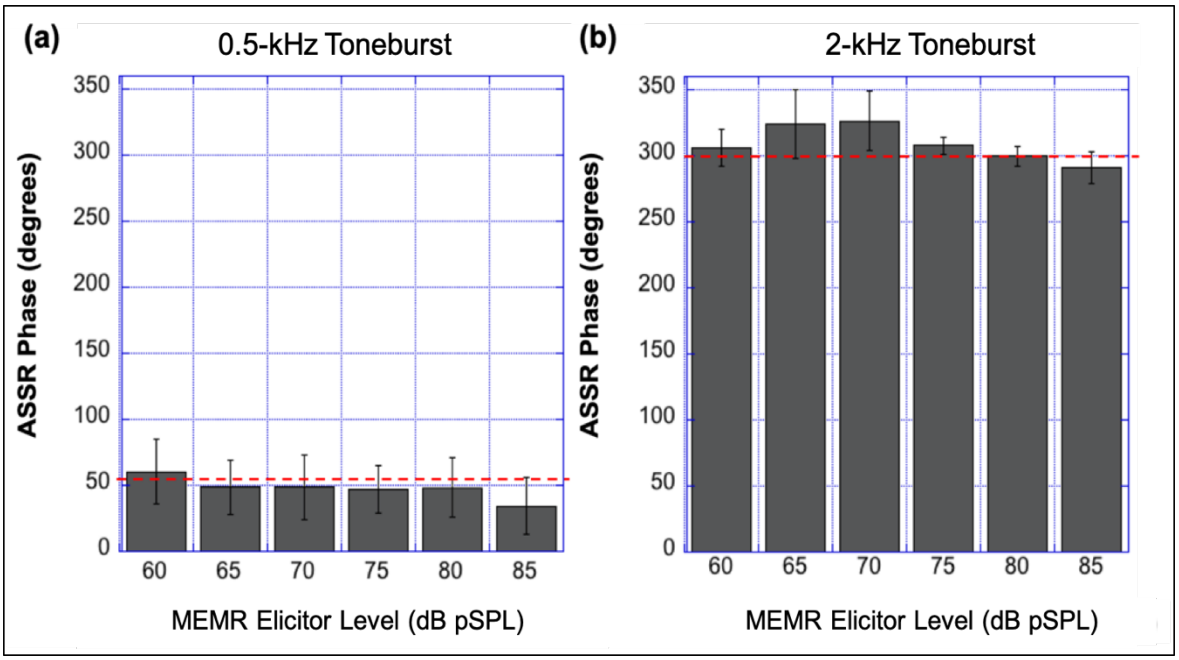

Figure 3.3: Bar graph showing group mean (±1 standard error) ASSR phase across MEMR elicitor levels for 0.5-kHz tonebursts (a) and 2-kHz tonebursts (b). The red dashed line in each panel represents the baseline group mean ASSR phase without an MEMR elicitor.

Figure 3.4. shows a larger middle-ear stiffness-induced ASSR phase shift for the 0.5-kHz toneburst frequency (average with 85 dB pSPL MEMR elicitor: 20-degree phase shift; range: 3- to 52-degree shift) compared to 2 kHz (average with 85 dB pSPL MEMR elicitor: 10-degree phase shift; range: 7- to 12-degree shift).

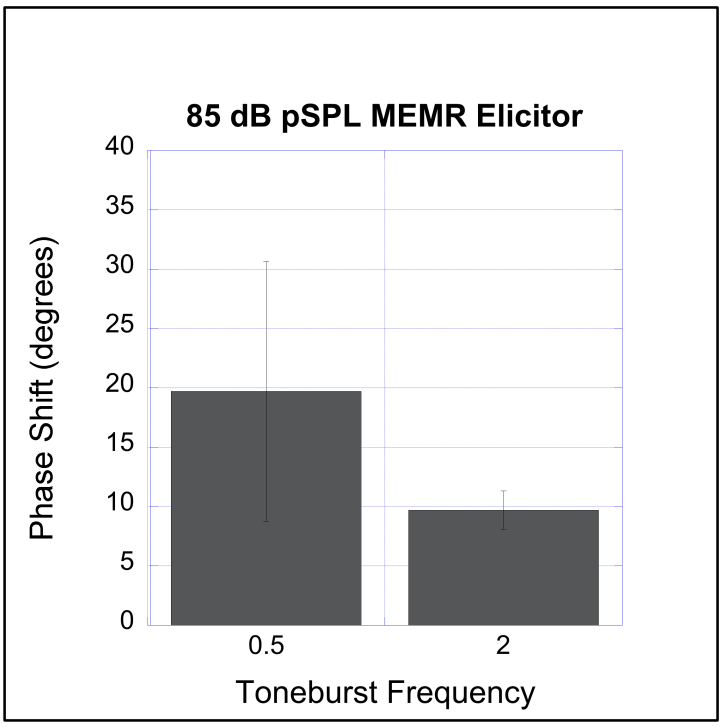

Figure 3.4: Bar graph showing group mean (±1 standard error) ASSR phase shift across toneburst frequency.

ASSR phase shift was calculated by subtracting ASSR phase with an 85 dB pSPL MEMR elicitor (right-most gray bars in Figures 3.3.a and 3.3.b) from ASSR phase without an MEMR elicitor (red dashed lines in Figure 3.3.). These findings are consistent with previous work at the cochlear level which showed DPOAE phase shifts for frequencies of 2 kHz and below with the largest shift observed at 0.9 kHz (Avan et al., 2000; Büki et al., 2000; Sun 2008).

The findings of this pilot study demonstrated middle-ear stiffness-induced changes in ASSR phase, especially for a 0.5-kHz toneburst frequency. This provided a rationale for focusing further investigation of stiffness-induced effects on ASSR phase on the 0.5-kHz spectral region.

3.3 Effects of ASSR Stimulus Level on ASSR Phase

ASSR phase was a key measure used to investigate the effects of middle-ear stiffness on the auditory brainstem neural encoding of phase. A pilot study was completed to investigate the sensitivity of ASSR phase to changes in stimulus level using the ASSR acquisition paradigm employed in the larger study. Three pilot participants (2 females; 1 male) aged 28 to 48 years with normal pure-tone threshold sensitivity (≤15 dB HL from 0.25 to 8 kHz) participated in a pilot study to examine the effects of ASSR stimulus level on ASSR phase. ASSRs were elicited by 0.75-kHz tonebursts presented using a toneburst sequence. Each toneburst in the sequence was Blackman-windowed and 4 ms in total duration. Tonebursts were presented at a rate of 78.125 Hz with a total of 15 tonebursts presented in each sequence. One sweep consisted of a 15-toneburst

sequence and a minimum of 1,160 sweeps (at least 4 minutes of recording time) were averaged together for pilot analyses. The stimulus level of the toneburst sequence was presented from 60 to 70 dB pSPL in 1-dB steps. Figure 3.5. shows group mean ASSR phase as a function of stimulus level.

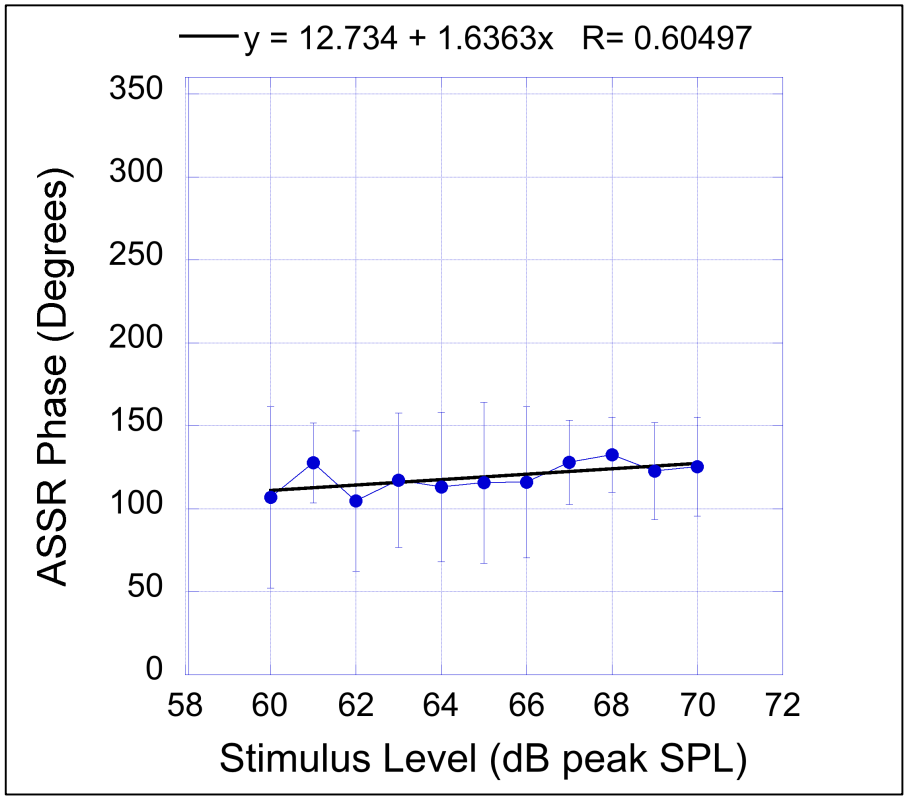

Figure 3.5: Line graph showing group mean (±1 standard error) ASSR phase as a function of ASSR stimulus level elicited by a 0.75-kHz toneburst sequence. The Ordinary Least Squares (OLS) linear regression line fit to the data is shown in black.

The slope of the linear regression line fit to the group mean data suggests a 1.64 degree increase in ASSR phase with every 1 dB pSPL increase in ASSR stimulus level from 60 to 70 dB pSPL. The key finding of this pilot study is that the ASSR acquisition paradigm demonstrated sensitivity to changes in ASSR phase equivalent to 1-dB pSPL increases in stimulus level.

Previous studies showed that group mean ASSR phase decreased with increased ASSR stimulus level (Picton et al., 2003). Possible explanations exist for the differences between this and previous studies related to ASSR phase trends as a function of stimulus level. First, previous studies have noted high between-participant variability in the ASSR phase trend as a function of stimulus level, including for the 60 to 70 dB range. Differences in the trend observed between studies may reflect the small sample size in this pilot study and the high degree of variability in ASSR phase among participants.

Differences in the ASSR acquisition paradigm among studies may also partially account for differences in the observed ASSR phase trends as a function of stimulus level. Previous studies used either amplitude

modulation or a toneburst repetition rate in order to elicit the ASSR, whereas this pilot study employed averaging of toneburst sequences to record the ASSR. Figure 3.6. compares toneburst repetition rate and toneburst sequence ASSR acquisition paradigms. A toneburst repetition rate ASSR acquisition paradigm consists of a constant series of tonebursts presented at the ASSR repetition rate (Figure 3.6.a). The physiologic response represents the average of all tonebursts presented. ASSR phase and amplitude measures are acquired for the averaged physiologic response at the frequency of the repetition rate. On the other hand, a toneburst sequence consists of groups of tonebursts presented at the ASSR repetition rate with an inter-stimulus interval between each sequence (Figure 3.6.b). The final physiologic response represents the average of each physiologic response generated by each toneburst sequence. ASSR phase and amplitude measures are acquired at the frequency of the toneburst repetition rate for each toneburst sequence. In theory, the toneburst sequence acquisition paradigm should result in lower physiologic noise levels and larger signal-to-noise ratios due to the averaging of multiple physiologic responses. A future within-participant investigation of ASSR phase and amplitude measures acquired using toneburst repetition rate and toneburst sequence ASSR paradigms will allow for direct comparisons between the two acquisition paradigms. There is no ready physiologic explanation for why different ASSR acquisition paradigms would result in different ASSR phase trends as a function of stimulus level.

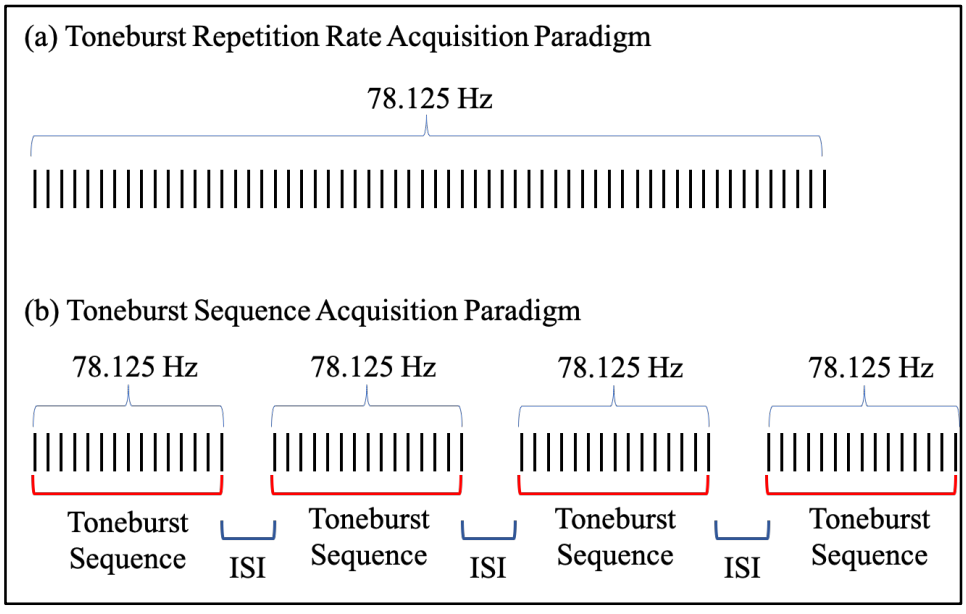

Figure 3.6: Comparison of the toneburst repetition rate ASSR acquisition paradigm (a) and the toneburst sequence ASSR acquisition paradigm (b). The toneburst repetition rate illustration represents 60 tonebursts presented at a constant rate of 78.125 Hz. ASSR phase and amplitude measures are acquired at the repetition rate of all 60 tonebursts. The toneburst sequence acquisition illustration represents 4 toneburst sequences containing 15 tonebursts in each sequence. The tonebursts in each sequence are presented at a rate of 78.125 Hz with an inter-stimulus interval (ISI) between each sequence. ASSR phase and amplitude are measured at the 78.125 Hz toneburst repetition rate for each toneburst sequence and the responses from each sequence are averaged together.

CHAPTER 4

Methods

4.1 Study Sample

4.1.1 Participant Demographics and Study Inclusion Criteria

Forty-three adults (32 females and 11 males; 38 White, 3 Asian, 2 Hispanic/Latinx) between 18 and 50 years of age participated in this study. Seven participants were excluded after completing baseline audiometric testing because they did not meet the criteria for normal pure-tone threshold sensitivity defined below. Four additional participants were excluded because their ASSR data were acquired using a shorter duration toneburst stimulus. Data from 1 final participant were excluded because they decided not to return for ASSR testing after completing baseline audiometric testing due to concerns related to the COVID-19 pandemic. Thus, the final participant cohort included data from 31 participants (24 females and 7 males; 27 White, 3 Asian, 1 Hispanic/Latinx).

The 50-year age limit employed in this study was selected in order to focus on young and middle-aged adults with normal hearing. Studies of age-related hearing loss have used the 50- to 55-year age limit as the delineation between younger and older adulthood (e.g., Blue Mountains Hearing Study, Gopinath et al., 2009; Baltimore Longitudinal Study of Aging, Lin et al., 2011). Participants were recruited from the Vanderbilt University and Vanderbilt University Medical Center community and from the greater Nashville, Tennessee area. All participants completed a Vanderbilt University Medical Center IRB-approved consent process (IRB#210138) and were compensated for their time consistent with IRB guidelines. Inclusion criteria for this study included normal pure-tone threshold sensitivity from 0.25 to 8 kHz including 3 and 6 kHz and normal middle-ear function as defined below. Exclusion criteria included any type or degree of hearing loss, a history of traumatic brain injury, or a history of a neurological disorder.

4.1.2 Baseline Testing

All participants completed baseline testing that consisted of: (i) pure-tone audiometric threshold testing in standard and extended high-frequency ranges; (ii) tympanometry and ipsilateral and contralateral MEMR testing; (iii) DPOAE testing; and (iv) MOCR testing using transient-evoked OAEs and contralaterally presented BBN and click elicitors.

Normal hearing was defined as behavioral pure-tone thresholds ≤20 dB hearing level (HL) at octave frequencies from 0.25 to 8 kHz and inter-octave frequencies of 3 and 6 kHz, bilaterally. Extended high-frequency thresholds were measured at 9, 10, 11, 12, 14, and 16 kHz, but participants were not excluded based on having elevated extended high-frequency pure-tone thresholds. All behavioral audiometric testing was

completed using a calibrated Grason-Stadler Instruments (GSI) 61 audiometer with ER-3A insert earphones (0.25 to 8 kHz) and Sennheiser supra-aural headphones (9 to 16 kHz).

Participants were required to have evidence of a mobile tympanic membrane as evidenced by middle-ear compliance ≥0.2 mmho and normal tympanometric peak pressure between -100 and 100 decapascals for both ears to be included in this study. Participants' standard ipsilateral and contralateral MEMR thresholds were measured for both ears using a 226-Hz probe tone and pure-tone elicitor stimuli from 0.5 to 4 kHz and BBN. MEMR threshold was defined as the level at which a change in middle-ear compliance of ≥0.02 mmho occurred. MEMR elicitors were presented 5-dB below and 5-dB above MEMR threshold to confirm growth of the MEMR with increased elicitor level. All tympanometry and MEMR testing was completed using the GSI Tympstar middle-ear test system.

DPOAEs were measured at the cubic distortion frequency of 2f1-f2 with f2 primary tones from 0.5 to 8 kHz at 2 points per octave. Primary tone levels of L1=65 dB SPL and L2=55 dB SPL and a frequency ratio of f2/f1=1.22 were used. All DPOAE testing was completed using the Mimosa Acoustics HearID hardware and software platforms coupled to an ER-10C probe with a foam ear-tip.

MOCR strength was assessed using a transient-evoked OAE (TEOAE) and contralateral MOCR activator paradigm. TEOAEs were elicited by 100-μS click stimuli presented at 65 dB pSPL. All click stimuli were presented at the same level and the same polarity (sometimes referred to as a "linear" click paradigm). The MOCR was activated by two different elicitors, continuous BBN and clicks at a rate of 20/s, presented contralaterally at 60 and 65 dB SPL (BBN) or pSPL (clicks). All MOCR data were acquired using the Intelligent Hearing Systems USB hardware and SmartTrOAE software coupled to an Etymotic Research ER-10D OAE probe with a foam ear-tip. The order of MOCR conditions for click and BBN elicitors was (i) without contralateral elicitor (test), (ii) with contralateral elicitor (test), (iii) without contralateral elicitor (retest), (iv) with contralateral elicitor (retest). Whether participants received the click or BBN elicitor first was counterbalanced among participants.

The Suppression Analysis Module in the SmartTrOAE software was used to analyze the MOCR data. MOCR strength was quantified for test and retest conditions as the difference in TEOAE amplitude in the 8- to 18-ms post-stimulus time window for responses with versus without the contralateral elicitor. MOCR strength for test and retest conditions were averaged together for all participants and used as the dependent variable for MOCR analyses. Quantifying TEOAE amplitude in the 8 to 18 ms range served two purposes: (i) previous studies showed that MOCR strength is maximal in this temporal region (Berlin et al., 1993, 1995) and (ii) quantifying TEOAE amplitude between 8 and 18 ms minimized the potential confound of stimulus artifact.

Obtaining measures of MOCR strength with a contralateral BBN elicitor allowed for comparison of the study sample to established metrics of MOCR strength. MOCR testing using contralateral click stimuli as the

reflex activator provided data about whether the contralateral stimuli used in the ASSR portion of this study elicited the MOCR. This aided in the interpretation of the findings of this study by allowing for differentiation between MOCR and MEMR effects on the auditory brainstem neural encoding of phase.

4.2 Experimental Data Acquisition Procedures

4.2.1 ASSR Recording Parameters

All ASSR data were acquired using the Intelligent Hearing Systems USB hardware and Advanced Auditory Research Module (AARM) software coupled to an ER-3A insert earphone with a foam ear-tip. Data were analyzed using the "Spectral Analysis" feature in the AARM software which provided polar and spectral plots of the data as well as the phase and amplitude of the physiologic response for spectral bins from 0 to 19.995 kHz with a spectral resolution of 4.89 Hz (n=4096 total spectral bins).

ASSRs were recorded using silver/silver-chloride or gold surface disc electrodes and a two-channel electrode montage (ipsilateral channel: C_Z to A_{ipsi}; contralateral channel: C_Z to A_{contra}; ground: F_{pz}, using international 10-20 system electrode position references). All electrode impedances were maintained below 5 kOhms throughout the duration of testing. Electroencephalographic (EEG) activity was amplified 100,000 times, high-pass filtered at 30 Hz, and low-pass filtered at 300 Hz. Each ASSR sweep consisted of a sequence of 15 0.5-kHz Blackman-windowed toneburst presentations. Each individual 0.5-kHz toneburst in the sequence was 8 ms in duration and tonebursts were presented at a rate of 78.125 Hz with a sampling rate of 20 kHz. The total sweep duration was 205 ms and sweeps were presented at a rate of 4.88/second. ASSR sweeps were averaged together for a total of at least 4 minutes of collection time that resulted in at least 1,160 sweeps per recording. ASSR phase and amplitude measures were derived based on the average of at least 1,160 sweeps (i.e., toneburst sequences) as opposed to being based on the number of individual toneburst presentations.

Figure 4.1. shows an example of ASSR analyses employed in this study for 1 representative participant (ME_Phase x0HOaA). ASSR data are represented in the temporal domain (Figure 4.1.a) and the spectral domain (Figure 4.1.b). Note the 15 distinct peaks representing neural responses generated by the 15 tonebursts included in the toneburst sequence when the ASSR is analyzed in the temporal domain (Figure 4.1.a). Additionally, a robust spectral peak is observed at the 78.125 Hz fundamental frequency and a secondary spectral peak is observed at the 156.25 Hz harmonic frequency demonstrating successful elicitation of the ASSR by the toneburst sequence (Figure 4.1.b). The phase and amplitude of the ASSR was obtained for the 78.125-Hz spectral region (shown by the black box at the bottom of Figure 4.1.b). These phase and amplitude measures were used to calculate slope and shift values as described below which served as the dependent variable for statistical analyses.

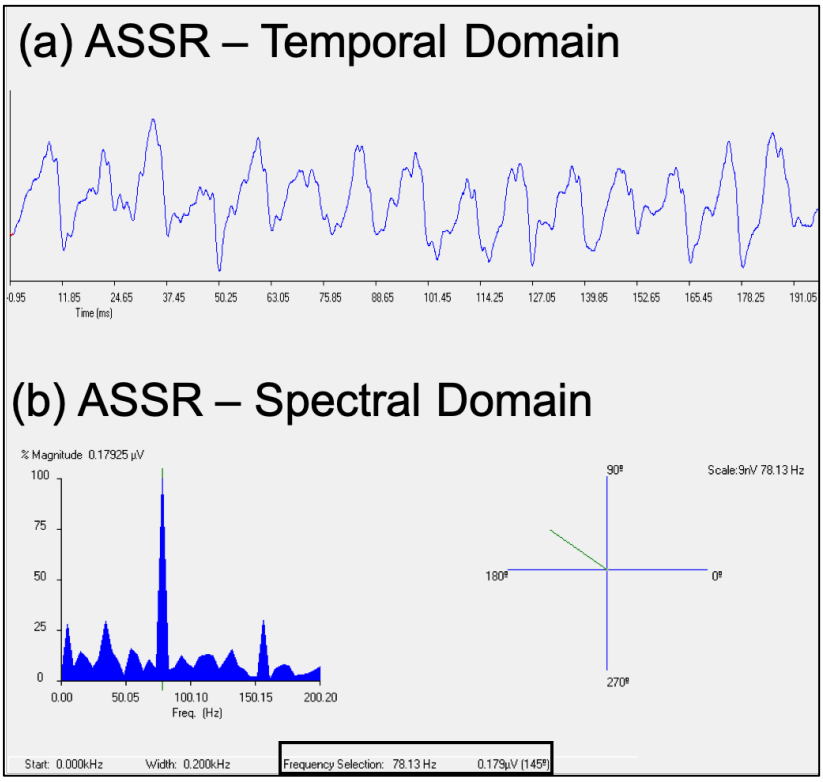

Figure 4.1: Representation of the ASSR analyzed in the temporal domain (panel a) and spectral domain (panel b) for 1 representative participant. The black box at the bottom of panel b shows ASSR phase and amplitude for the 78.125-Hz spectral region.

The 78.125-Hz toneburst presentation rate was selected to maximize contributions from rostral auditory brainstem structures in the generation of the physiologic response (e.g., Kuwada et al., 2002, but see Coffey et al., 2019). This study employed a 0.5-kHz toneburst frequency because increased middle-ear stiffness primarily affects lower frequencies. Additionally, unpublished data from the Auditory Physiology Laboratory (P.I. Linda J. Hood, Ph.D.) at Vanderbilt University Medical Center showed that WAI-MEMR magnitude is largest for 0.5- and 0.75-kHz half-octave bands. The decision to use a 0.5-kHz ASSR stimulus rather than 0.75 kHz was based on increasing the clinical translation and future scientific application of the findings of this study. Specifically, CI EAS patients are more likely to have acoustic hearing preservation in the lower audiometric frequencies (i.e., 0.5 kHz and below). Thus, the methodology developed by this study can be applied by future studies including CI EAS patients.

The decision to utilize a toneburst presentation rate rather than amplitude modulated tones to elicit the ASSR is supported by findings from Dobie & Wilson (1998) who demonstrated no difference in ASSRs generated by toneburst presentation rates and amplitude modulated tones in adults with normal hearing. Additionally, all pilot participants for the current study had recordable ASSRs to 0.5-kHz tonebursts presented using a 95-Hz presentation rate; however, 1 pilot participant did not have a recordable ASSR to a 0.5-kHz

amplitude modulated tone despite having a robust ASSR in the toneburst presentation rate paradigm.

Noise in the physiologic recordings was estimated using the average amplitude of electrical activity for the 6 spectral bins immediately below and above the target spectral bin (78.125 Hz). Thus, noise was estimated based on the 12 spectral bins from 48.83 to 107.42 Hz, excluding the 78.125 Hz spectral bin. The signal-to-noise ratio (SNR) of each ASSR recording was calculated by comparing the response amplitude at the target frequency (78.125 Hz) to the amplitude of the averaged noise estimate ($20*\log[A_{target}/A_{noise}]$). ASSR recordings were required to have a dB SNR value ≥6 dB in order to be included in further analyses.

4.2.2 Specific Aim I: Effects of Middle-Ear Stiffness on ASSR Phase and Amplitude

ASSRs were elicited at stimulus levels of 75, 80, and 85 dB pSPL in the right ear of all participants. Increased middle-ear stiffness in the ASSR test ear (right) was elicited by presenting an MEMR elicitor to the contralateral ear (left) for every participant. To elicit the MEMR, 100-μS click stimuli with a presentation rate of 20/s were presented to the left ear at levels from 60 to 90 dB pSPL in 5-dB steps (Johnsen & Terkildsen 1980). ASSRs were acquired with and without the MEMR elicitor at each level for a total of 8 ASSR recordings for each of the 3 ASSR stimulus levels. The order in which ASSR stimulus levels were acquired and MEMR elicitor levels were presented was randomized among participants.

The click MEMR elicitor used in this study was selected in order to minimize elicitation of the MOCR as much as possible. Johnsen & Terkildsen (1980) showed that click stimuli presented contralaterally at rates from 8 to 128/s elicited the MEMR in human adults with normal hearing. Additionally, Veuillet et al. (1991) demonstrated that contralateral click rates ≤20/s did not elicit a measurable MOCR. Thus, the findings of Johnsen & Terkildsen (1980) and Veuillet et al. (1991) support the use of contralateral click stimuli at 20/s to elicit the MEMR (Johnsen & Terkildsen 1980) while minimizing elicitation of the MOCR (Veuillet et al., 1991) in adults with normal hearing.

ASSR phase and amplitude were quantified for each recording and were plotted as a function of MEMR elicitor level separately for each of the 3 ASSR stimulus levels. A linear regression model was generated for each condition for each participant using the "lm" function from the R base package. For each ASSR stimulus level, participants were required to have ASSR data with an amplitude of ≥0.4 μV and an SNR of ≥6 dB for at least 3 out of the 7 conditions with presentation of the MEMR elicitor in order to be included in analyses.

Potential outlier data points in the linear regression models for each participant were evaluated by plotting (i) residual vs. fitted plots, (ii) normal Q-Q plots, (iii) scale location plots, and (iv) residual vs. leverage plots for each condition. There are many methods for determining statistical outliers in a dataset. These 4 outlier analyses were chosen because they are part of a standard outlier analysis provided by the "lm" function in R that was used to generate the linear regression models. Using 4 different outlier analyses, as opposed to relying

on a single outlier analysis, provided a more thorough identification of potential outliers.

All potential outlier data points were reviewed to ensure accurate data input and to ensure that they met the ASSR amplitude and SNR criteria. There was no justification for removing potential outlier data points. Thus, a robust linear regression model was generated for each participant using the "rlm" function from the "MASS" package in R. Robust linear regression adjusts the fit of the linear regression line for potential outliers by weighting the residuals. Using robust linear regression, data points with a larger residual are assigned a lower weight in the calculation of the linear regression line. The slopes of the robust linear regression lines for each participant were obtained from the models and used as the dependent variable for statistical analyses. A negative phase slope value indicated decreased ASSR phase with increased MEMR elicitor level (consistent with the hypothesis of increased middle-ear stiffness).

We observed a high degree of between-participant variability in baseline ASSR phase values recorded without presentation of the MEMR elicitor. To be able to reference differences among test conditions to individual participants, ASSR phase shifts and amplitude shifts were calculated by subtracting ASSR phase or amplitude with contralateral presentation of the MEMR elicitor from ASSR phase or amplitude without contralateral presentation of the MEMR elicitor. The ASSR phase shift measures quantified middle-ear stiffness-induced changes in ASSR phase normalized for each individual participant to the condition without the MEMR elicitor.

The primary goal of this aim was to determine whether middle-ear stiffness affected the auditory brainstem neural encoding of phase. Four dependent variables were available for statistical analyses for this Specific Aim: (i) ASSR phase slope; (ii) ASSR amplitude slope; (iii) ASSR phase shift; and (iv) ASSR amplitude shift. One-sample t-tests were calculated using the "t.test" function from the base R package to determine whether the group mean of each dependent variable differed significantly from zero. A slope or shift value that differed significantly from zero indicated statistically significant effects of middle-ear stiffness on the auditory brainstem neural encoding of phase.

Additionally, Pearson correlation coefficients were calculated to examine the effects of participant age on stiffness-induced changes on ASSR phase slope for each ASSR stimulus level. The effects of ASSR stimulus level on stiffness-induced changes in ASSR phase slope also were investigated for the cohort of participants with ASSR data for all 3 stimulus levels (n=19). A one-way repeated measures ANOVA with post-hoc pairwise t-tests with pooled standard deviation corrected for multiple comparisons using the Bonferroni correction were calculated using the "aov" and "pairwise.t.test" functions in R to assess for differences in ASSR phase slopes among stimulus levels.

4.2.3 Specific Aim II: Relationships Between WAI Measures and ASSR Phase

All WAI data acquisition was completed using the Mimosa Acoustics HearID hardware and software

platforms coupled to an Etymotic Research ER-10C probe with a foam ear-tip. WAI recordings were acquired in the right ear using 70 dB SPL chirp stimuli with and without 100-μS click stimuli presented at a rate of 20/s in the left ear from 60 to 90 dB pSPL in 5-dB steps. Acoustic absorbance and power transmittance measures were acquired from 0.21 to 6 kHz. Stiffness-induced changes in acoustic absorbance and power transmittance were calculated for a half-octave band with a 0.5-kHz center frequency (range of 421.88 to 585.94 Hz). Furthermore, a 0.5-kHz half-octave band was selected for WAI analyses in order to provide overlap with the spectral composition of the 0.5-kHz ASSR toneburst used in Specific Aim I.

Acoustic absorbance and power transmittance were plotted as a function of MEMR elicitor level and a linear regression model was generated using the "lm" function from the base R package. Potential outliers were identified and reviewed using the same procedures described for Specific Aim I in section 4.2.2 above. A robust linear regression model was generated using the "rlm" function from the "MASS" R package in order to adjust the fit of the linear regression line for potential outlier data points. The slopes of the robust linear regression lines were obtained for each participant and used as the dependent variable for correlational analyses described below. A negative acoustic absorbance and power transmittance slope indicated decreased absorbance and transmittance with increased MEMR elicitor level consistent with activation of the contralateral MEMR and the hypothesis of increased middle-ear stiffness.

Additionally, acoustic absorbance and power transmittance shifts were calculated by subtracting acoustic absorbance and power transmittance values with contralateral presentation of the MEMR elicitor from absorbance and transmittance values without contralateral presentation of the MEMR elicitor. This provided a quantification of stiffness-induced changes in acoustic absorbance and power transmittance that were normalized to each individual participant's baseline absorbance and transmittance without presentation of the MEMR elicitor.

The primary goal of this aim was to investigate correlations between WAI measures and ASSR phase. Correlations between the following variable pairs were investigated: (i) ASSR phase slope and acoustic absorbance slope and (ii) ASSR phase slope and power transmittance slope. Pearson correlation coefficients for the variable pairs listed above were calculated using the "cor.test" function from the base R package.

4.2.4 Specific Aim III: Ear-Specific Analyses of Stiffness-Induced Effects on ASSR Phase

The primary goal of this aim was to investigate whether middle-ear stiffness-induced effects on ASSR phase are comparable for both left and right ears. ASSRs, as described in relation to Aim I, were elicited from the left ear at an ASSR stimulus level of 85 dB pSPL with the MEMR elicitor presented to the right ear at 90 dB pSPL. ASSR phase shift was quantified for the left ear using procedures described for Specific Aim I which focused on the right ear.

The ASSR stimulus level (85 dB pSPL) and MEMR elicitor level (90 dB pSPL) conditions were chosen

to maximally elicit middle-ear stiffness-induced effects on the auditory brainstem neural encoding of phase for preliminary investigations into ear-specific differences. The 85-dB pSPL ASSR stimulus level was chosen because this was the highest stimulus level used in this study and resulted in the largest amplitude ASSRs. The MEMR elicitor presented at 90 dB pSPL was at the highest elicitor level used in this study and thus had the greatest likelihood of eliciting middle-ear stiffness.

The dependent variables available for statistical analyses related to this aim were ASSR phase shifts for the left and right ears (85 dB pSPL ASSR stimulus level and 90 dB pSPL MEMR elicitor level). Paired t-tests were calculated using the "t.test" function from the R base package to compare the group mean ASSR phase shift between the left and right ears. Paired t-tests that did not reach statistical significance would be consistent with comparable middle-ear stiffness-induced effects on ASSR phase for both ears.

4.3 Scientific Rigor and Reproducibility

All stimuli used for testing in this study were calibrated using a Bruel & Kjaer Pulse calibration system Type 3560C with Type 4157 coupler to increase study accuracy. Biologic listening checks and calibrations of all equipment were completed before each test session. All physiologic data were analyzed and reviewed by two individuals experienced in the analysis of auditory physiologic responses to increase the validity of acquired data. Based on the objective nature of ASSRs, the expert review focused on the quality of the ASSR data rather than on determining the presence of the ASSR.

4.4 Participant Cohorts and Power Analyses

Of the 31 participants meeting the overall inclusion criteria for this study, 3 cohorts were derived based on ASSR data meeting amplitude and SNR criteria. For each of the 3 ASSR stimulus levels (75-, 80-, and 85-dB pSPL), each participant completed 7 ASSR recordings with presentation of the MEMR elicitor at levels ranging from 60 to 90 dB pSPL in 5-dB steps. Participants were required to have ASSR data with an amplitude ≥0.4 μV and an SNR value ≥6 dB for 3 or more ASSR recordings with presentation of the MEMR elicitor. These requirements were implemented in order for a linear regression line to be fit based on at least 3 ASSR data points. The composition of each cohort included 28 participants meeting inclusion criteria for the 85-dB ASSR stimulus level, 24 participants meeting inclusion criteria for the 80-dB ASSR stimulus level, and 20 participants meeting inclusion criteria for the 75-dB ASSR stimulus level. 19 participants had ASSR data that met inclusion criteria for all 3 ASSR stimulus levels.

Post-hoc power analyses were calculated for the ASSR phase slope analyses outlined under Specific Aim I related to the investigation of middle-ear stiffness effects on the auditory brainstem neural encoding of phase. Power analyses were calculated for one-sample t-tests using the "pwr.t.test" function from the "pwr" package in R. 95% power was achieved for the 75-dB ASSR stimulus level cohort; 54% power was achieved for the

80-dB ASSR stimulus level cohort; and 64% power was achieved for the 85-dB stimulus level cohort. Recruiting additional participants to increase statistical power for the 80- and 85-dB ASSR stimulus level cohorts was not feasible given the limitations associated with the COVID-19 global pandemic during data collection for this study.

CHAPTER 5

Results

5.1 Baseline Measures

5.1.1 Behavioral Pure-Tone Threshold Sensitivity

Data will be presented for each ASSR stimulus level cohort (75, 80, 85 dB) individually in this section and the sections to follow. The behavioral pure-tone audiograms for both the left and right ears of all 3 cohorts are shown in Figure 5.1.

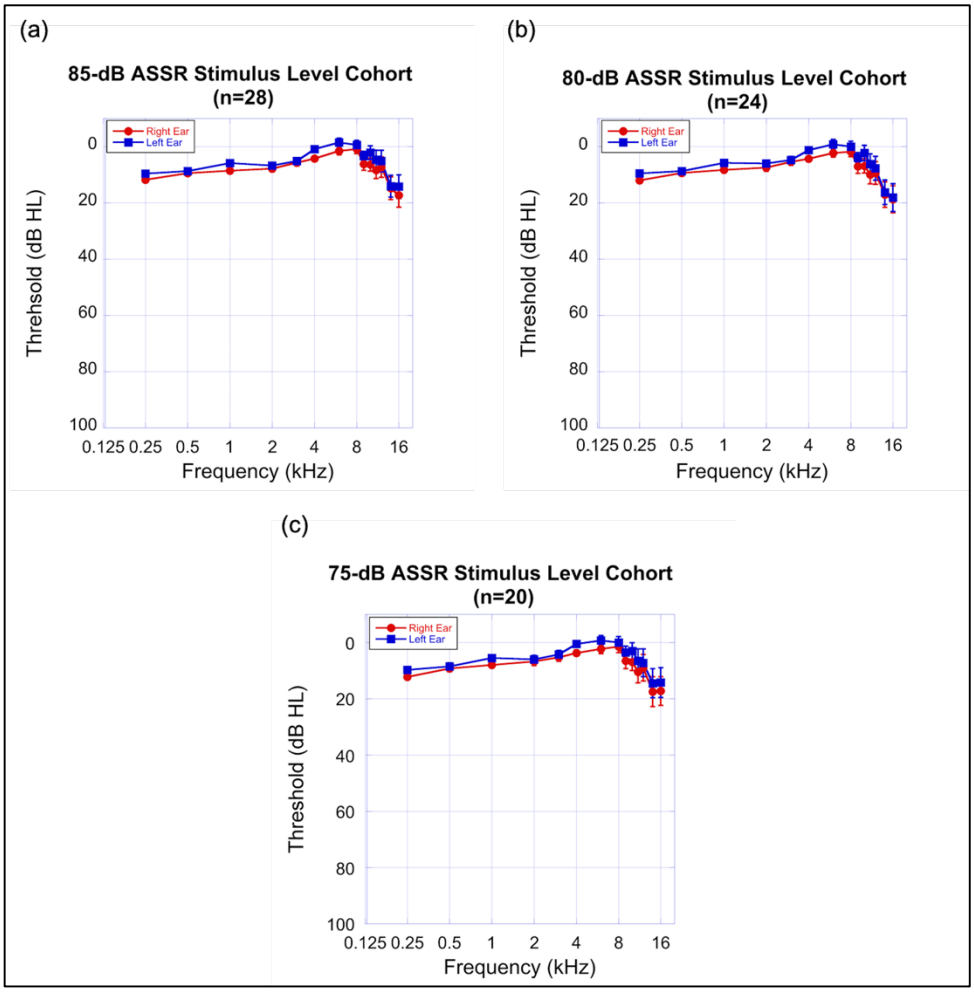

Figure 5.1: Line plots showing group mean (±1 standard error) behavioral pure-tone audiograms for the right (red) and left (blue) ears across ASSR stimulus level cohorts.

Average pure-tone thresholds were within the normal range (≤15 dB HL) from 0.25 to 8 kHz and were within the near-normal range (≤ 25 dB HL) from 9 to 16 kHz for both ears of all cohorts. All participants had individual pure-tone thresholds ≤20 dB HL for both ears in the standard audiometric frequency range (0.25

through 8 kHz). Differences in group mean pure-tone thresholds between ears and among cohorts did not exceed 5 dB, which is widely accepted as within test-retest repeatability. These behavioral pure-tone thresholds indicate typical pure-tone threshold sensitivity for both ears among all participants for the investigation of middle-ear stiffness and the auditory brainstem neural encoding of phase.

5.1.2 Standard MEMR

Standard ipsilateral and contralateral MEMR thresholds were elicited by 0.5, 1, 2, and 4 kHz pure-tones and BBN in order to assess baseline MEMR function. Figure 5.2. shows MEMR thresholds for both ears across all elicitor conditions for the 3 ASSR stimulus level cohorts.

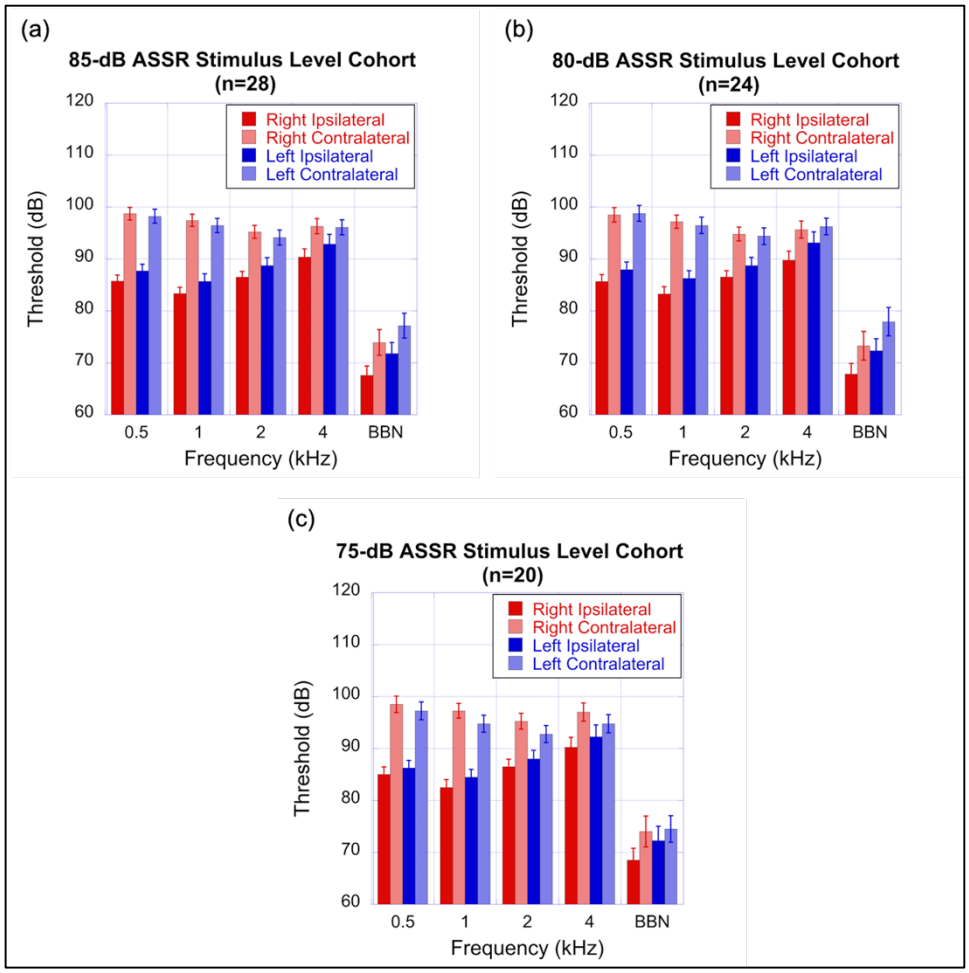

Figure 5.2: Bar plots showing group mean (±1 standard error) MEMR thresholds for right (red bars) and left (blue bars) ears and for ipsilateral (darker bars) and contralateral (lighter bars) conditions.

Group mean ipsilateral MEMR thresholds were within the expected normal range (≤95 dB HL) across all elicitor conditions for both ears. Contralateral MEMR thresholds were elevated (i.e., poorer) compared to ipsilateral thresholds for all conditions. Compared to pure-tone elicitors, BBN elicited lower (i.e., better)

ipsilateral and contralateral MEMR thresholds for both ears.

5.1.3 WAI-MEMR – Quantifying Middle-Ear Stiffness

Acoustic absorbance and power transmittance were measured in the right ear of all participants with and without an MEMR elicitor (20/s click stimuli) presented contralaterally in the left ear. This was then used as an indication that the contralateral stimulus used during ASSR testing increased middle-ear stiffness in the test ear. A decrease in low-frequency acoustic absorbance and power transmittance with presentation of the MEMR elicitor indicated middle-ear stiffness. Figure 5.3. shows acoustic absorbance and Figure 5.4. shows power transmittance for a half-octave band centered at 0.5 kHz as a function of MEMR elicitor level for the 3 ASSR stimulus level cohorts.

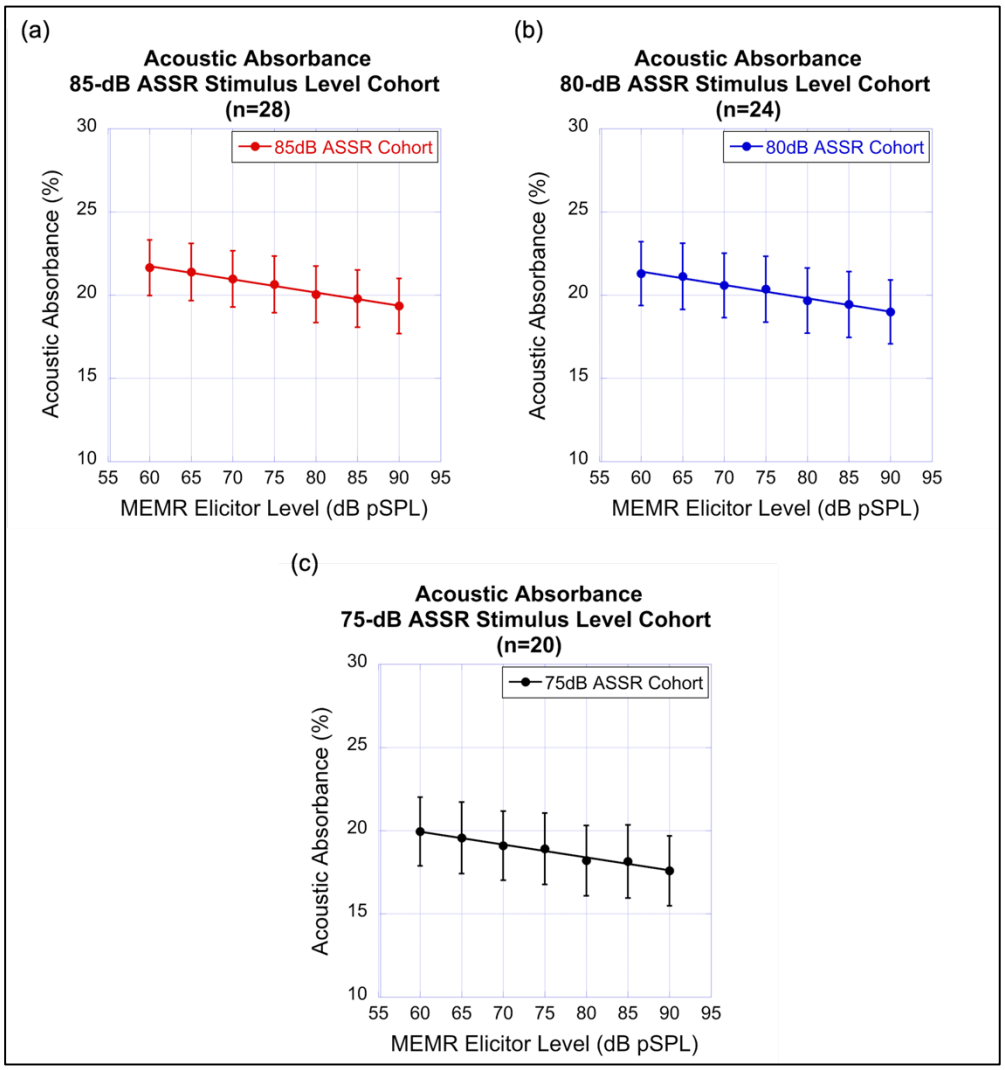

Figure 5.3: Line plots with OLS linear regression lines showing group mean (±1 standard error) acoustic absorbance across MEMR elicitor levels. The number of participants are shown in the titles of each panel.

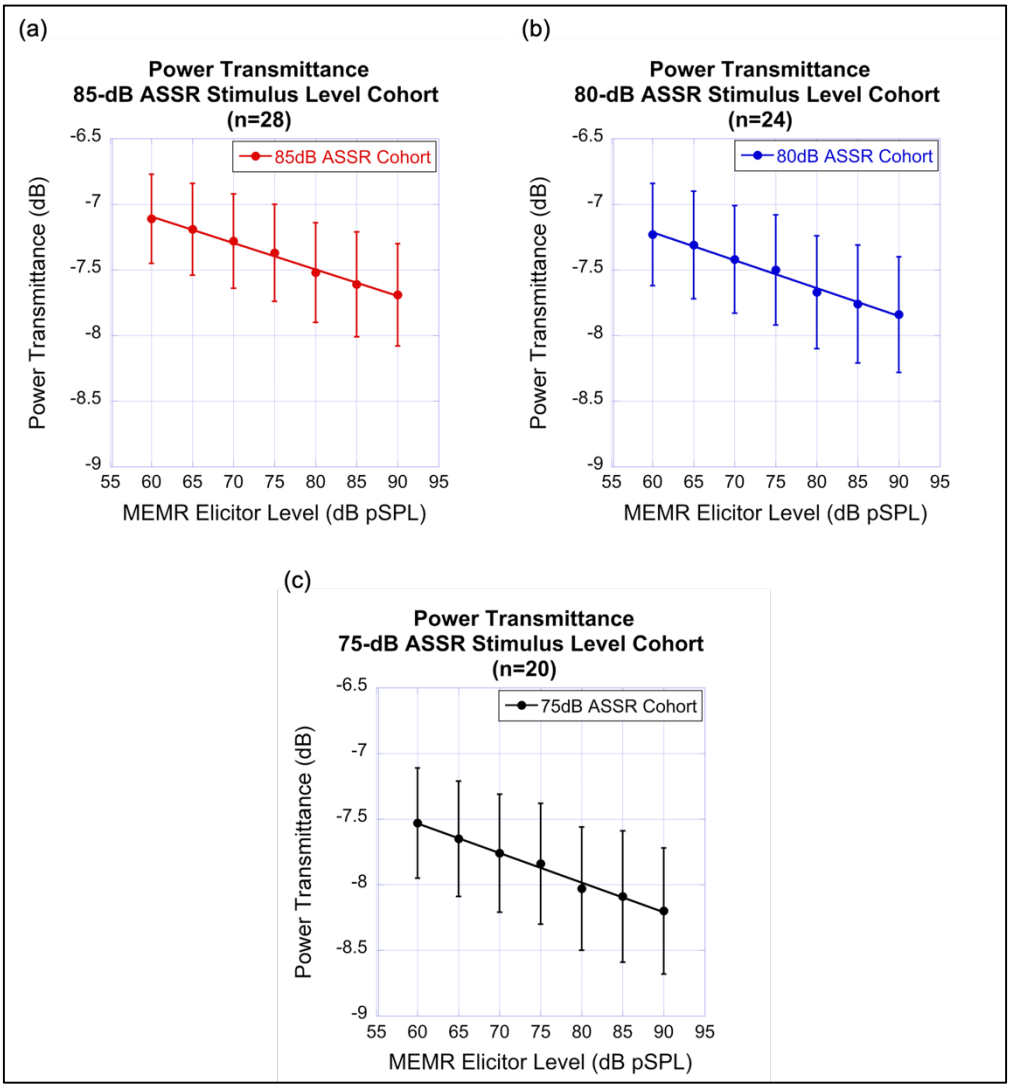

Figure 5.4: Line plots with OLS linear regression lines showing group mean (±1 standard error) power transmittance across MEMR elicitor levels. The number of participants are shown in the titles of each panel.

Acoustic absorbance and power transmittance for the half-octave band centered at 0.5 kHz decreased with increasing MEMR elicitor level for all 3 cohorts. The slopes of the robust linear regression lines demonstrated a statistically significant 0.8% decrease in acoustic absorbance for every 10 dB pSPL increase in MEMR elicitor level for the 85-dB ASSR stimulus level cohort (slope=-0.08, R^2=0.60, t=-8.88, df=27, p<0.0001) and statistically significant 0.9% decreases in acoustic absorbance for every 10 dB pSPL increase in MEMR elicitor level for the 80-dB and 75-dB ASSR stimulus level cohorts (80-dB cohort: slope=-0.09, R^2=0.62, t=-8.58, df=23, p<0.0001; 75-dB cohort: slope=-0.09, R^2=0.61, t=-7.43, df=19, p<0.0001). Additionally, the slope analyses showed a statistically significant 0.2 dB decrease in power transmittance for every 10 dB pSPL increase in MEMR elicitor level for all 3 ASSR stimulus level cohorts (85-dB cohort: slope=-0.02, R^2=0.60, t=-6.49, df=27, p<0.0001; 80-dB cohort: slope=-0.02, R^2=0.62, t=-6.08, df=23, P<0.0001; 75-dB cohort:

slope=-0.02, R^2=0.61, t=-5.43, df=19, p<0.0001).

5.1.4 MOCR

The contralateral MOCR pathway was activated separately by 2 elicitors, continuous BBN and 20/s click stimuli, presented at 2 levels (60 and 65 dB SPL for BBN and pSPL for clicks) to assess whether the contralateral stimulus used to elicit middle-ear stiffness in this study also elicited the MOCR. MOCR strength across elicitor conditions for the 3 ASSR stimulus level cohorts is shown in Figure 5.5.

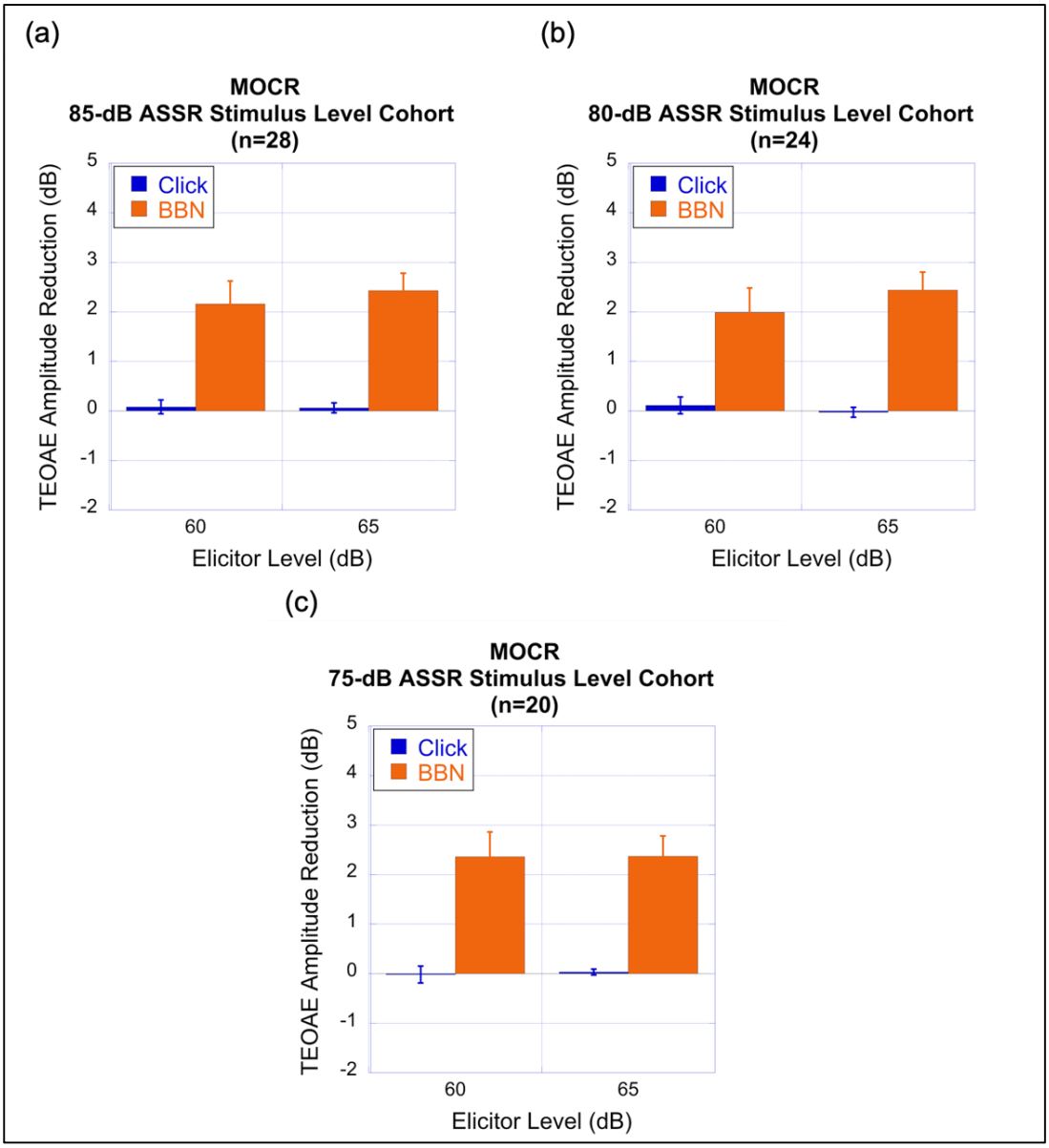

Figure 5.5: Bar plot showing group mean (±1 standard error) contralateral MOCR function elicited by BBN (orange bars) and 20/s click stimuli (blue bars). The number of participants are shown in the titles of each panel.

The contralateral presentation of the BBN elicitor at 60 and 65 dB SPL resulted in statistically significant reductions in TEOAE amplitude for all cohorts, consistent with the presence of MOCR activation in these participant samples (orange bars in Figure 5.5.; 85-dB cohort with 60 dB SPL BBN: $t=4.87$, $df=27$, $p<0.0001$; 80-dB cohort with 60 dB SPL BBN: $t=4.09$, $df=23$, $p<0.001$; 75-dB cohort with 60 dB SPL BBN: $t=4.69$, $df=19$, $p<0.001$; 85-dB cohort with 65 dB SPL BBN: $t=7.04$, $df=27$, $p<0.0001$; 80-dB cohort with 65 dB SPL BBN: $t=6.69$, $df=23$, $p<0.0001$; 75-dB cohort with 65 dB SPL BBN: $t=5.78$, $df=19$, $p<0.0001$). In contrast, no significant reductions in TEOAE amplitude were observed with contralateral presentation of the click elicitor for any condition (blue bars in Figure 5.5.; 85-dB cohort with 60 dB pSPL clicks: $t=0.57$, $df=27$, $p=0.57$; 80-dB cohort with 60 dB pSPL clicks: $t=0.67$, $df=23$, $p=0.51$; 75-dB cohort with 60 dB pSPL clicks: $t=-0.12$, $df=19$, $p=0.90$; 85-dB cohort with 65 dB pSPL clicks: $t=0.60$, $df=27$, $p=0.55$; 80-dB cohort with 65 dB pSPL clicks: $t=-0.32$, $df=23$, $p=0.75$; 75-dB cohort with 65 dB pSPL clicks: $t=0.60$, $df=19$, $p=0.56$). The minimal impact of contralateral click stimuli on TEOAE amplitude is consistent with minimal to no co-activation of the MOCR by the stimulus used to elicit middle-ear stiffness in these participant samples.

5.2 Middle-Ear Stiffness and Auditory Brainstem Neural Encoding of Phase

5.2.1 ASSR Signal-to-Noise Ratio (SNR)

Figure 5.6. shows ASSR SNR as a function of MEMR elicitor level for all 3 ASSR stimulus level cohorts.

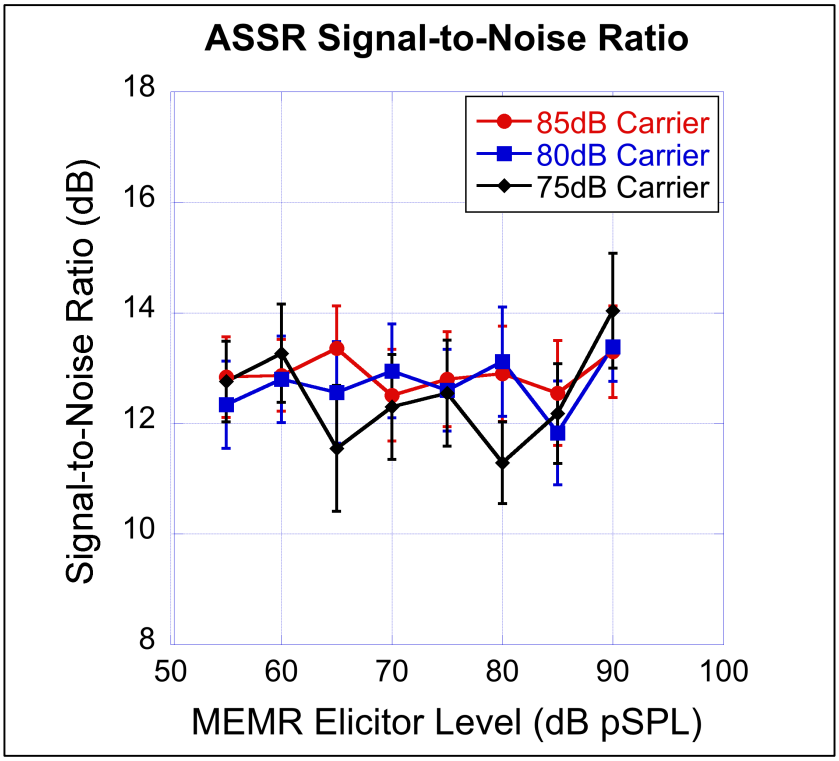

Figure 5.6: Line plot showing group mean (±1 standard error) ASSR SNR across ASSR stimulus levels and MEMR elicitor levels.

Group mean SNRs remained constant across MEMR elicitor levels and no differences were observed among participant cohorts. Additionally, the group mean SNR values across conditions and participant cohorts were well above the 6 dB SNR inclusion criterion used in this study, providing evidence of robust physiologic responses for the analysis of the effects of middle-ear stiffness on the auditory brainstem neural encoding of phase.

Figure 5.7. shows group mean ASSR amplitude compared between included versus excluded participants for each ASSR stimulus level cohort and Figure 5.8. shows group mean estimated noise compared between included versus excluded participants for each cohort.

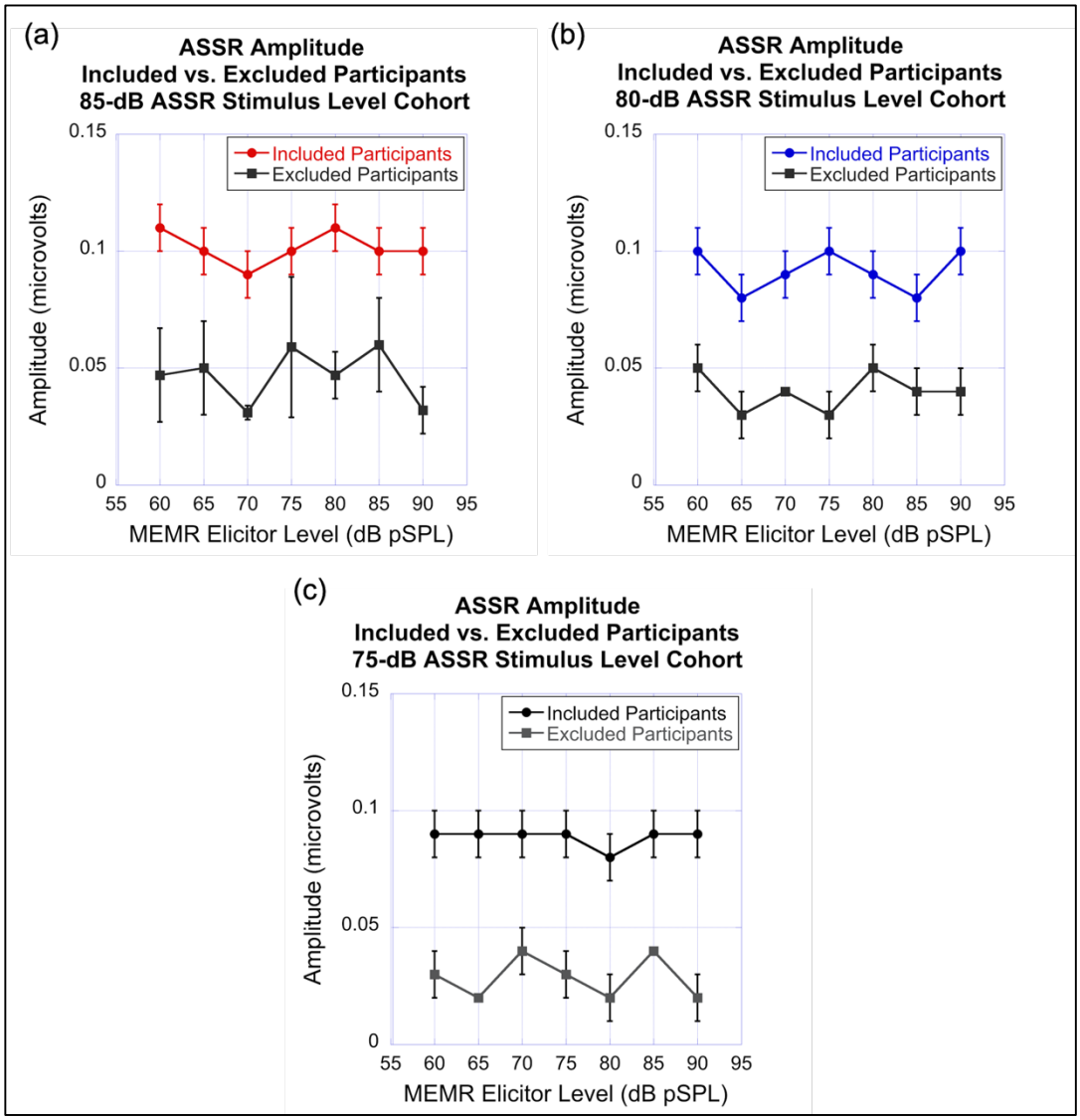

Figure 5.7: Line plots showing group mean (±1 standard error) ASSR amplitude across MEMR elicitor levels for included and excluded participants for the 3 ASSR stimulus level cohorts.

Three participants were excluded from the 85-dB ASSR stimulus level cohort, 7 participants were excluded from the 80-dB ASSR stimulus level cohort, and 3 participants were excluded from the 75-dB ASSR stimulus

level cohort due to not meeting ASSR signal-to-noise ratio and amplitude criteria for at least 3 test conditions with contralateral presentation of the MEMR elicitor. Figure 5.7. shows that the excluded participants in each ASSR stimulus level cohort had group mean ASSR amplitudes less than half the amplitudes of the included participants across MEMR elicitor levels. Figure 5.8. shows that group mean estimated noise amplitudes across MEMR elicitor levels were comparable for included compared to excluded participants. Reduced ASSR amplitudes were the primary contributing factor in the exclusion of participants from each of the ASSR stimulus level cohorts.

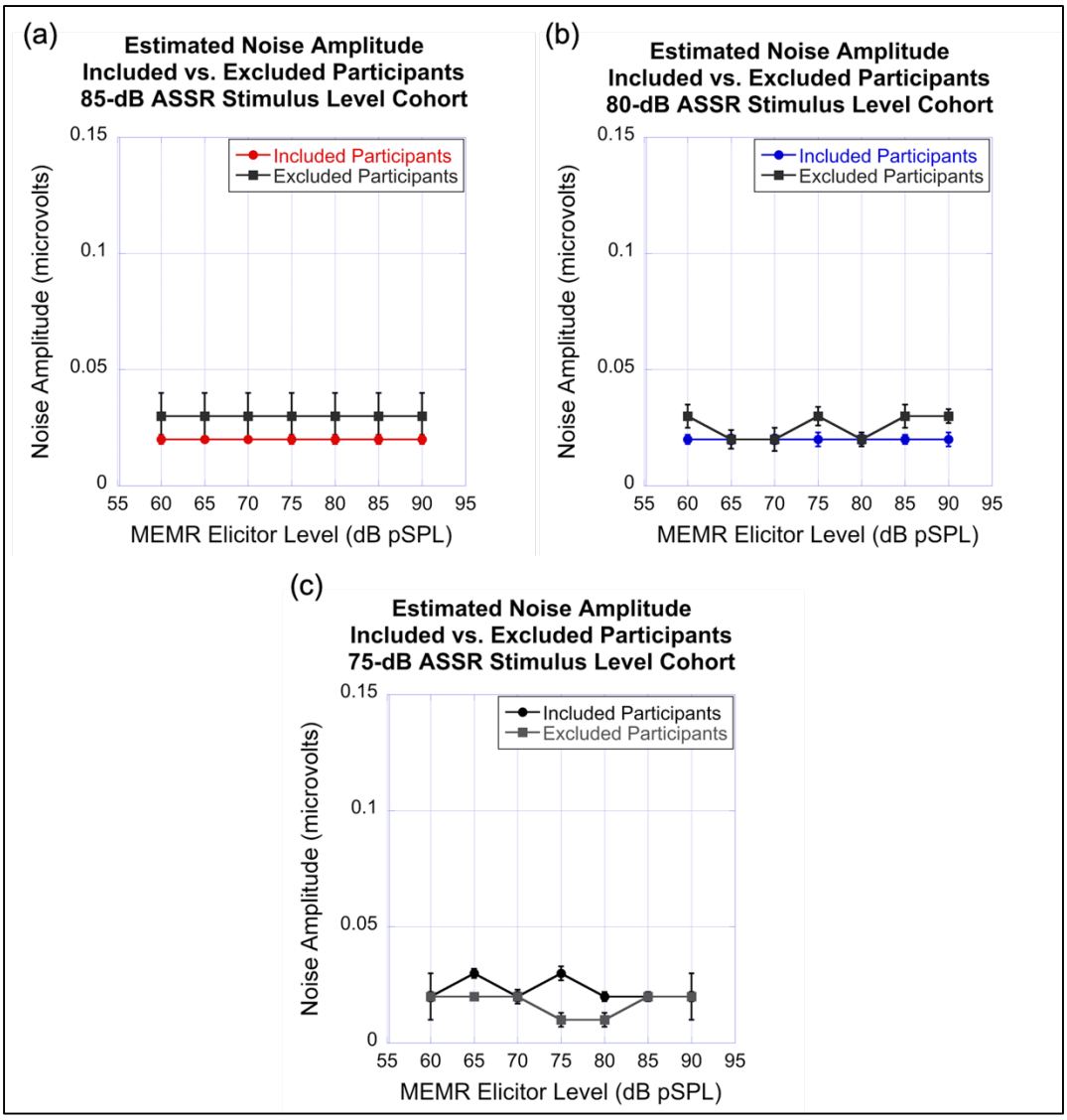

Figure 5.8: Line plots showing group mean (±1 standard error) estimated noise across MEMR elicitor levels for included and excluded participants for the 3 ASSR stimulus level cohorts.

5.2.2 ASSR Phase Analyses

ASSR phase was measured at the toneburst presentation rate of 78.125 Hz with and without contralateral

presentation of the MEMR elicitor to assess the impact of middle-ear stiffness on the auditory brainstem neural encoding of phase. ASSR phase as a function of MEMR elicitor level is shown for the group mean in Figure 5.9 and for each individual participant in Figure 5.10.

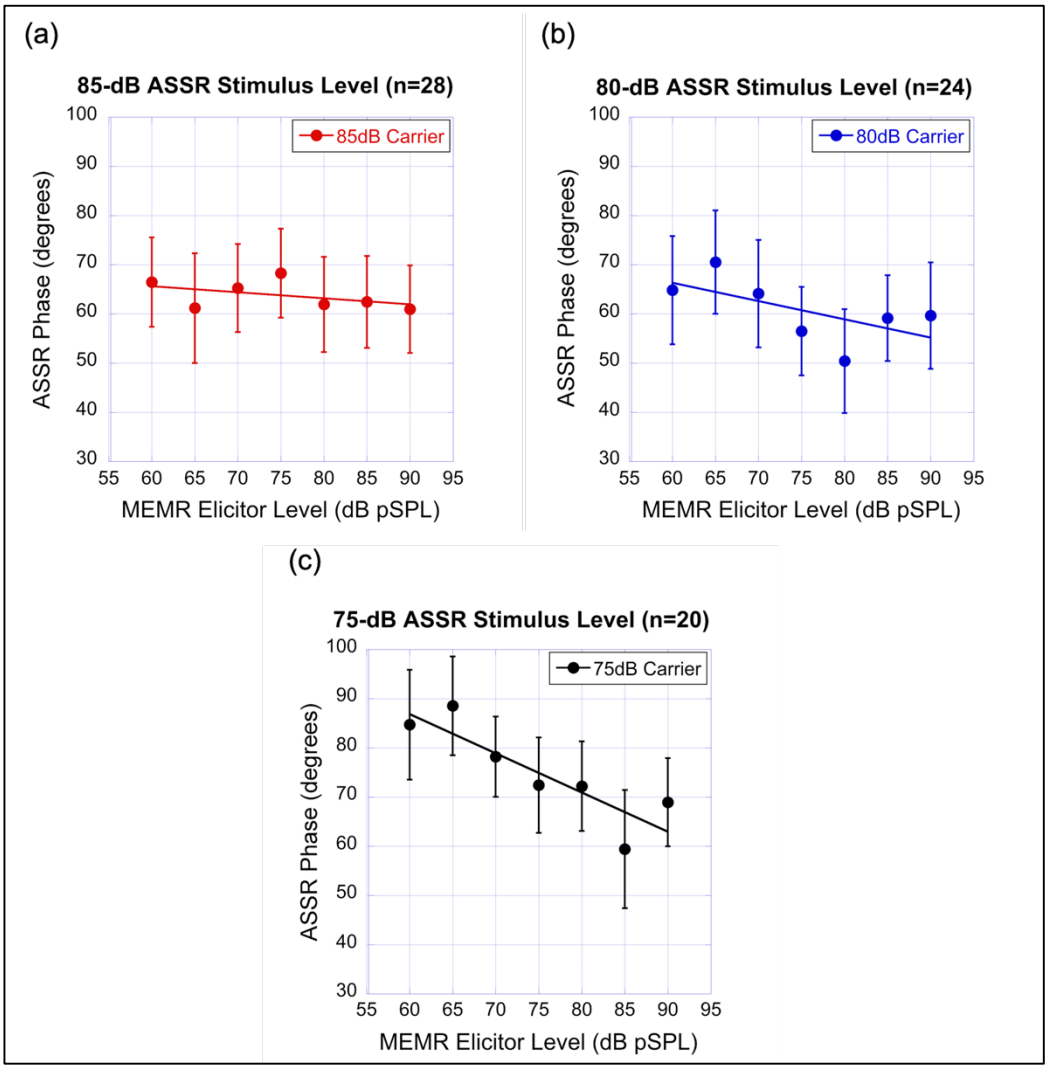

Figure 5.9: Scatter plots with OLS linear regression lines showing group mean (±1 standard error) ASSR phase across MEMR elicitor levels. The number of participants are shown in the title of each panel.

Average ASSR phase decreased with increasing MEMR elicitor level for all ASSR stimulus level cohorts. Robust linear regression slope analyses demonstrated that for every 10 dB pSPL increase in MEMR elicitor level, ASSR phase decreased by 2.8 degrees for the 85-dB ASSR stimulus level cohort (slope=-0.28, R^2=0.24, t=-2.36, df=27, $p<0.05$), ASSR phase decreased by 3.7 degrees for the 80-dB ASSR stimulus level cohort (slope=-0.37, R^2=0.34, t=-2.16, df=23, $p<0.05$), and ASSR phase decreased by 6.5 degrees for the 75-dB ASSR stimulus level cohort (slope=-0.65, R^2=0.27, t=-3.75, df=19, $p<0.01$).

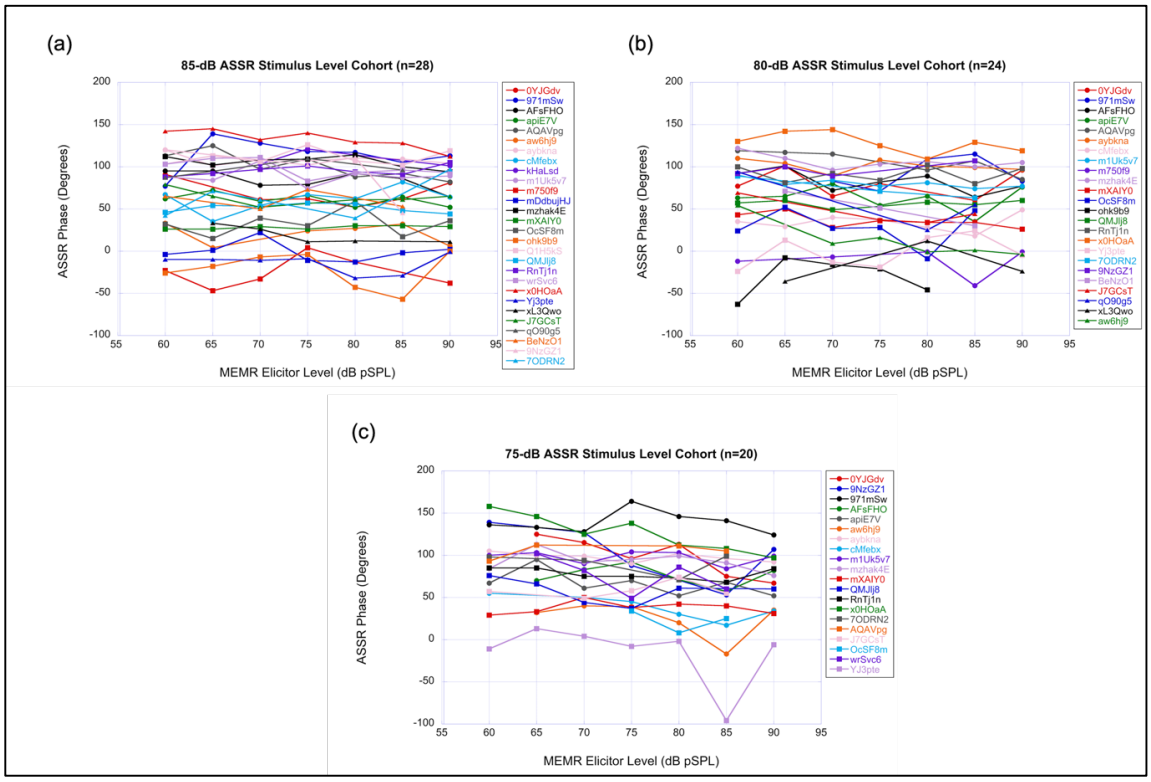

Figure 5.10: Line plots showing ASSR phase across MEMR elicitor levels for individual participants included in the 3 ASSR stimulus level cohorts. The de-identified alpha-numeric participant IDs are shown to the right of each panel and the number of participants are shown in the title of each panel.

Due to observed between-participant variability in baseline ASSR phase values, phase data were normalized to each participant's ASSR phase value obtained without the MEMR elicitor. Thus, ASSR phase shifts were calculated by subtracting phase with presentation of the MEMR elicitor from phase without presentation of the MEMR elicitor (see Section 4.2.2. for details related to the calculation of ASSR phase shift). Figure 5.11. shows ASSR phase shift as a function of MEMR elicitor level for the 3 ASSR stimulus level cohorts.

Statistically significant ASSR phase shifts occurred at the 2 highest MEMR elicitor levels for the 75-dB ASSR stimulus level cohort (85 dB pSPL MEMR elicitor level: $t=3.52$, $df=16$, $p<0.05$; 90 dB pSPL MEMR elicitor level: $t=4.08$, $df=13$, $p<0.01$). ASSR phase shifts were consistently larger with higher MEMR elicitor levels for the 75-dB and 80-dB ASSR stimulus level cohorts, whereas phase shifts remained constant across MEMR elicitor levels for the 85-dB ASSR stimulus level cohort. For the 2 highest MEMR elicitor levels, less ASSR phase shift occurred for the 80-dB ASSR stimulus level compared to the other stimulus levels.

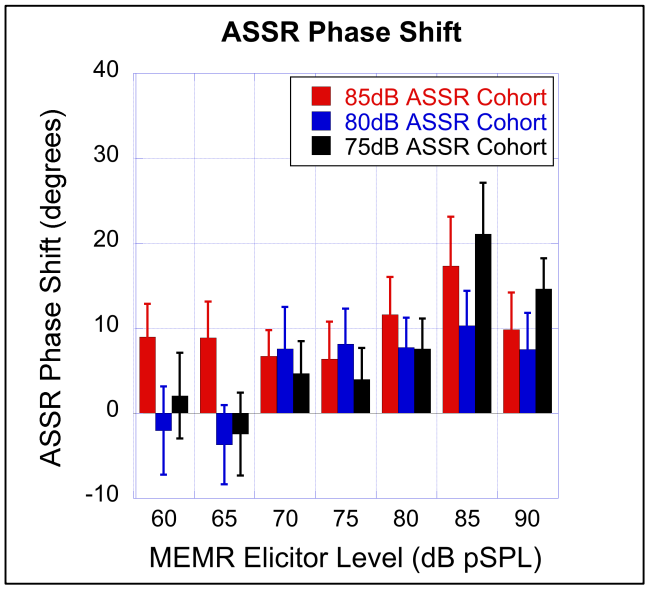

Figure 5.11: Bar plot showing group mean (±1 standard error) ASSR phase shift across MEMR elicitor levels for the 3 ASSR stimulus level cohorts.

5.2.3 Effects of Participant Age on ASSR Phase Slope Analyses

Scatter plots representing ASSR phase slope as a function of participant age are shown for the 3 ASSR stimulus level cohorts in Figure 5.12.

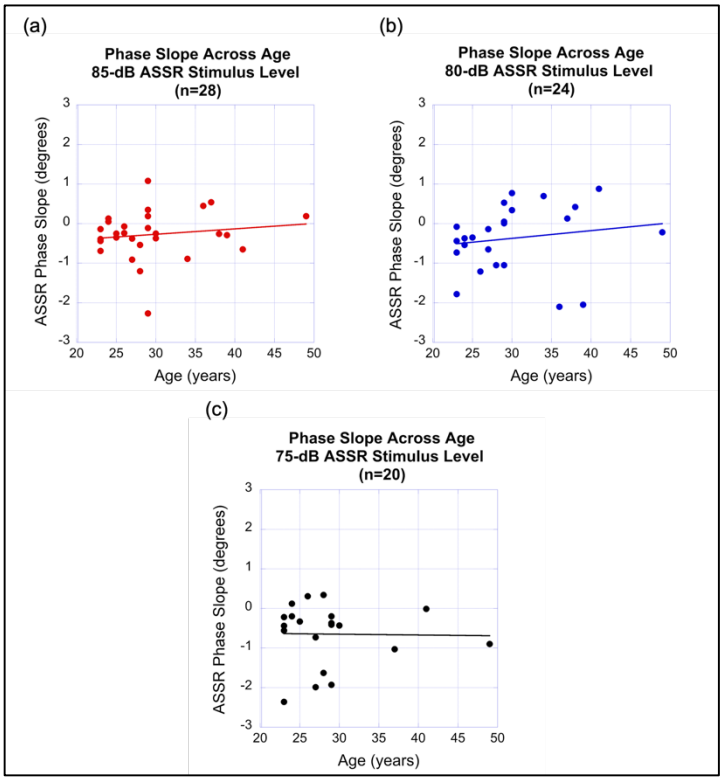

Figure 5.12: Scatter plots with OLS linear regression lines showing the effects of age on ASSR phase slope across ASSR stimulus level cohorts. The number of participants are shown in the title of each panel.

Pearson correlation coefficients were calculated to examine the effects of participant age on ASSR phase slope for the 3 ASSR stimulus level cohorts. No statistically significant correlations were observed between participant age and ASSR phase slope (85-dB ASSR stimulus level cohort: r=0.14, t=0.73, df=26, p=0.47; 80-dB ASSR stimulus level cohort: r=0.16, t=0.75, df=22, p=0.47; 75-dB ASSR stimulus level cohort: r=-0.02, t=-0.07, df=18, p=0.94). Note the fewer number of participants in the older age range, especially for the 75-dB ASSR stimulus level cohort. Additionally, between-participant variability in ASSR phase slope occurred across all ages.

5.2.4 Within-Participant Effects of ASSR Stimulus Level on ASSR Phase Slope

Nineteen participants had ASSR data available for all 3 ASSR stimulus levels allowing for within-participant investigation of the effects of ASSR stimulus level on ASSR phase slope. ASSR phase as a function of MEMR elicitor level for the 19 participants with ASSR data at all 3 ASSR stimulus levels is shown in Figure 5.13.

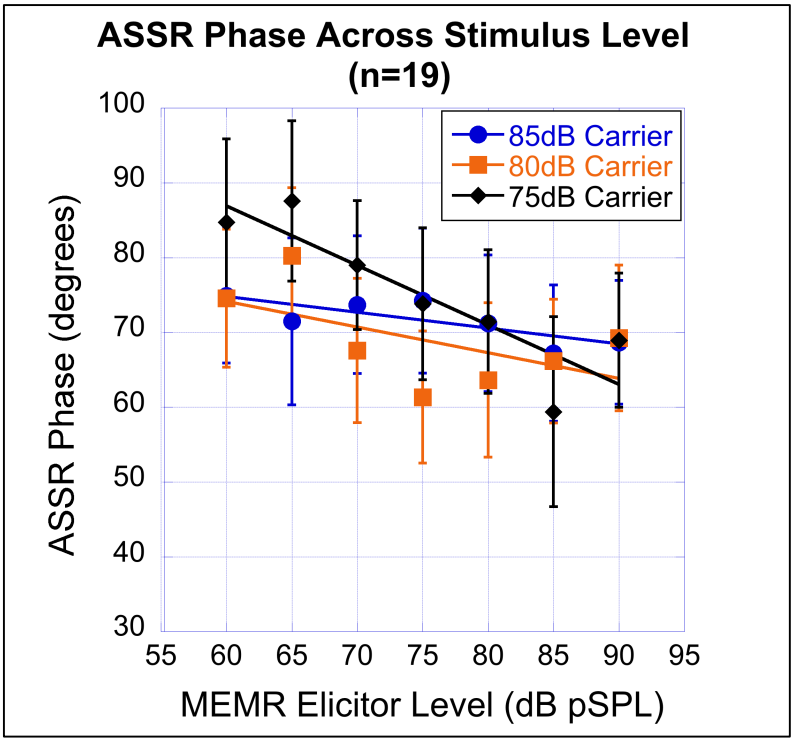

Figure 5.13: Scatter plots with OLS linear regression lines showing group mean (±1 standard error) ASSR phase across MEMR elicitor levels. The number of participants is shown in the title.

A one-way repeated measures ANOVA (dependent variable: ASSR phase slope; independent variable: ASSR stimulus level) demonstrated no statistically significant differences among ASSR phase slopes for the 3 ASSR stimulus levels (f=2.34, df=1, p=0.14; all pairwise post-hoc t-tests were not significant). Although no

statistically significant differences were noted, lower ASSR stimulus levels tended to have steeper ASSR phase slopes consistent with a greater effect of middle-ear stiffness on the auditory brainstem neural encoding of phase for lower ASSR stimulus levels.

5.2.5 Relationship Between Acoustic Absorbance and ASSR Phase Without an MEMR Elicitor

Figure 5.14. shows the relationship between acoustic absorbance and ASSR phase acquired without an MEMR elicitor in order to investigate whether baseline middle-ear status may partially contribute to between-participant variability in ASSR phase. A moderately strong, positive correlation occurred between acoustic absorbance and ASSR phase acquired without an MEMR elicitor for the 85-dB ASSR stimulus level cohort ($r=0.47$, $df=26$, $t=2.73$, $p<0.05$). For the 80-dB ASSR stimulus level cohort, the opposite trend occurred wherein a negative correlation was observed between acoustic absorbance and ASSR phase, though this trend did not reach statistical significance ($r=-0.38$, $df=22$, $t=-1.94$, $p=0.07$). No relationship was observed between acoustic absorbance and ASSR phase acquired without an MEMR elicitor for the 75-dB ASSR stimulus level cohort ($r=-0.07$, $df=18$, $t=-0.29$, $p>0.7$).

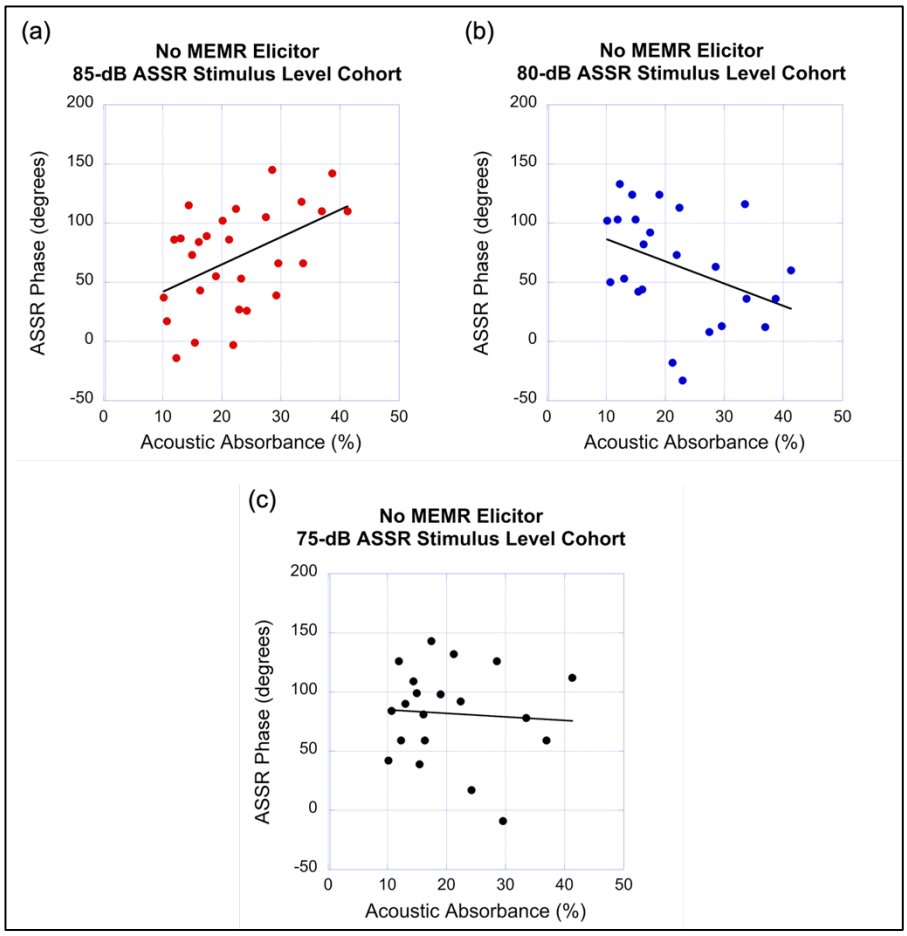

Figure 5.14: Scatter plots with OLS linear regression lines showing the relationships between acoustic absorbance and ASSR phase acquired without an MEMR elicitor for all cohorts.

5.2.6 ASSR Amplitude Analyses

ASSR amplitude as a function of MEMR elicitor level is shown for the 3 ASSR stimulus level cohorts in Figure 5.15.

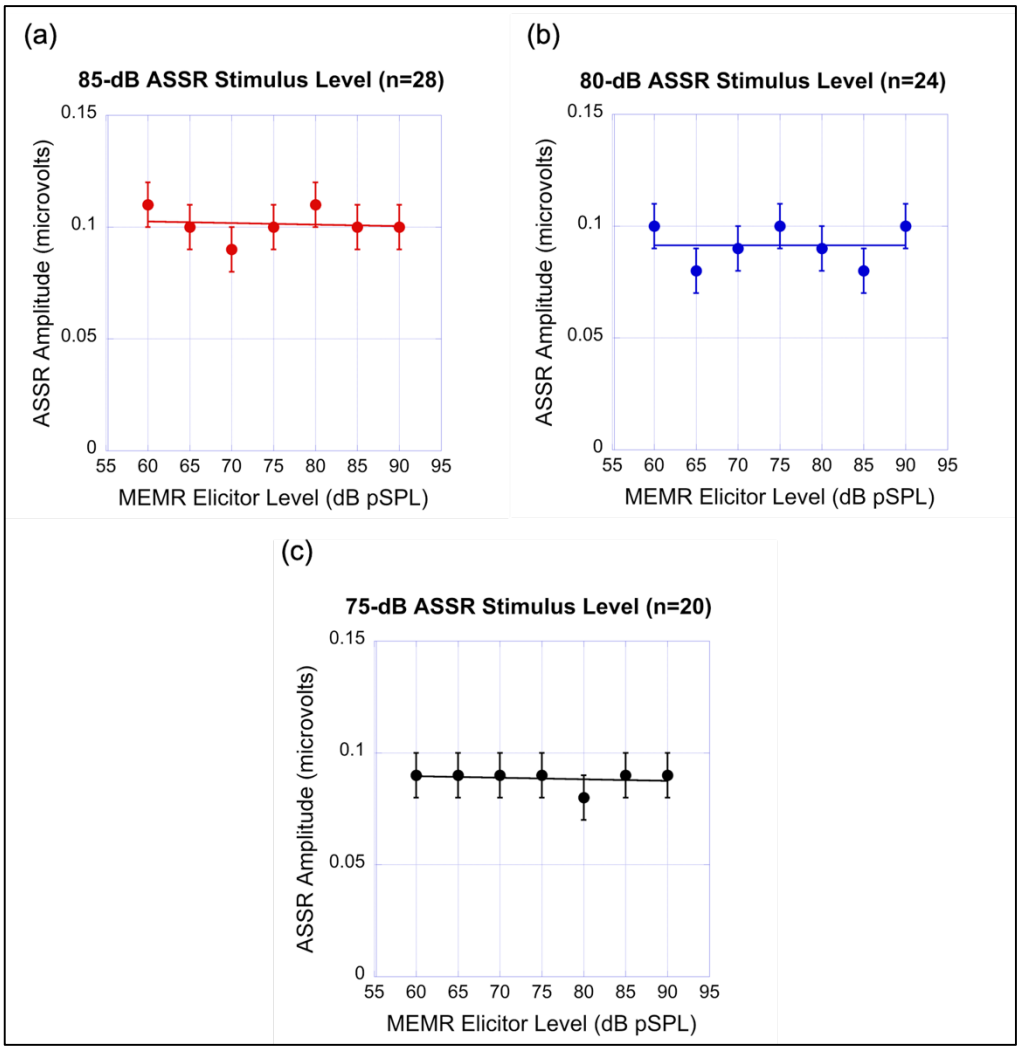

Figure 5.15: Scatter plots with OLS linear regression lines showing group mean (±1 standard error) ASSR amplitude across MEMR elicitor levels. The number of participants are shown in the title of each panel.

The robust slope analyses confirmed that ASSR amplitude did not change as a function of MEMR elicitor level for any of the 3 ASSR stimulus level cohorts (85-dB ASSR stimulus level cohort: slope=-0.0001, R^2=0.35, t=-0.52, df=27, p=0.61; 80-dB ASSR stimulus level cohort: slope=-0.0001, R^2=0.23, t=-0.43, df=23, p=0.67; 75-dB ASSR stimulus level cohort: slope=-0.0001, R^2=0.34, t=-0.61, df=19, p=0.55).

ASSR amplitude shifts were calculated for each MEMR elicitor level, normalizing each participant's ASSR amplitude to their baseline amplitude without the MEMR elicitor (see Section 4.2.2. for details related

to the calculation of amplitude shifts). Figure 5.16. shows ASSR amplitude shift across MEMR elicitor levels for each of the 3 ASSR stimulus levels.

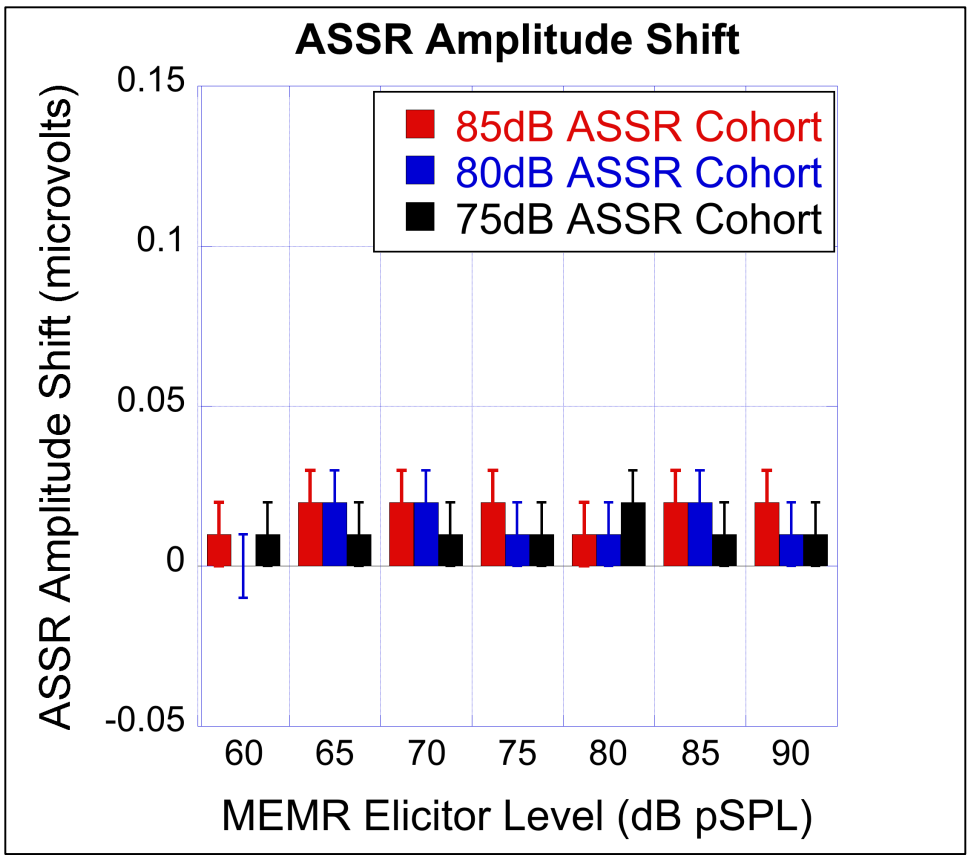

Figure 5.16: Bar plot showing group mean (±1 standard error) ASSR amplitude shift across MEMR elicitor levels for the 3 ASSR stimulus levels.

Average amplitude shifts did not exceed 0.02 μV and remained constant across MEMR elicitor levels. Slightly greater shifts occurred for the higher ASSR stimulus levels. One-sample t-tests confirmed statistically significant amplitude shifts with MEMR elicitor presentation for the 2 highest ASSR stimulus level cohorts only (85-dB ASSR stimulus level with 90-dB pSPL MEMR elicitor: $t=-2.77$, $df=24$, $p<0.05$; 85-dB ASSR stimulus level with 85-dB pSPL MEMR elicitor: $t=-3.45$, $df=22$, $p<0.01$; 85-dB ASSR stimulus level with 75-dB pSPL MEMR elicitor: $t=-2.91$, $df=24$, $p<0.05$; 85-dB ASSR stimulus level with 70-dB pSPL MEMR elicitor: $t=-3.36$, $df=23$, $p<0.01$; 85-dB ASSR stimulus level with 65-dB pSPL MEMR elicitor: $t=-2.48$, $df=22$, $p<0.05$; 80-dB ASSR stimulus level with 85-dB pSPL MEMR elicitor: $t=-3.12$, $df=21$, $p<0.05$; 80-dB ASSR stimulus level with 70-dB pSPL MEMR elicitor: $t=-3.85$, $df=18$, $p<0.01$; 80-dB ASSR stimulus level with 65-dB pSPL MEMR elicitor: $t=-3.66$, $df=16$, $p<0.01$). No statistically significant amplitude shifts occurred for the 75-dB ASSR stimulus level cohort.

5.3 Correlations Between WAI and ASSR Measures

The relationship between ASSR phase slope and 0.5-kHz-centered acoustic absorbance slope is shown in Figure 5.17. and the relationship between ASSR phase slope and 0.5-kHz-centered power transmittance slope is shown in Figure 5.18 for the 3 ASSR stimulus level cohorts.

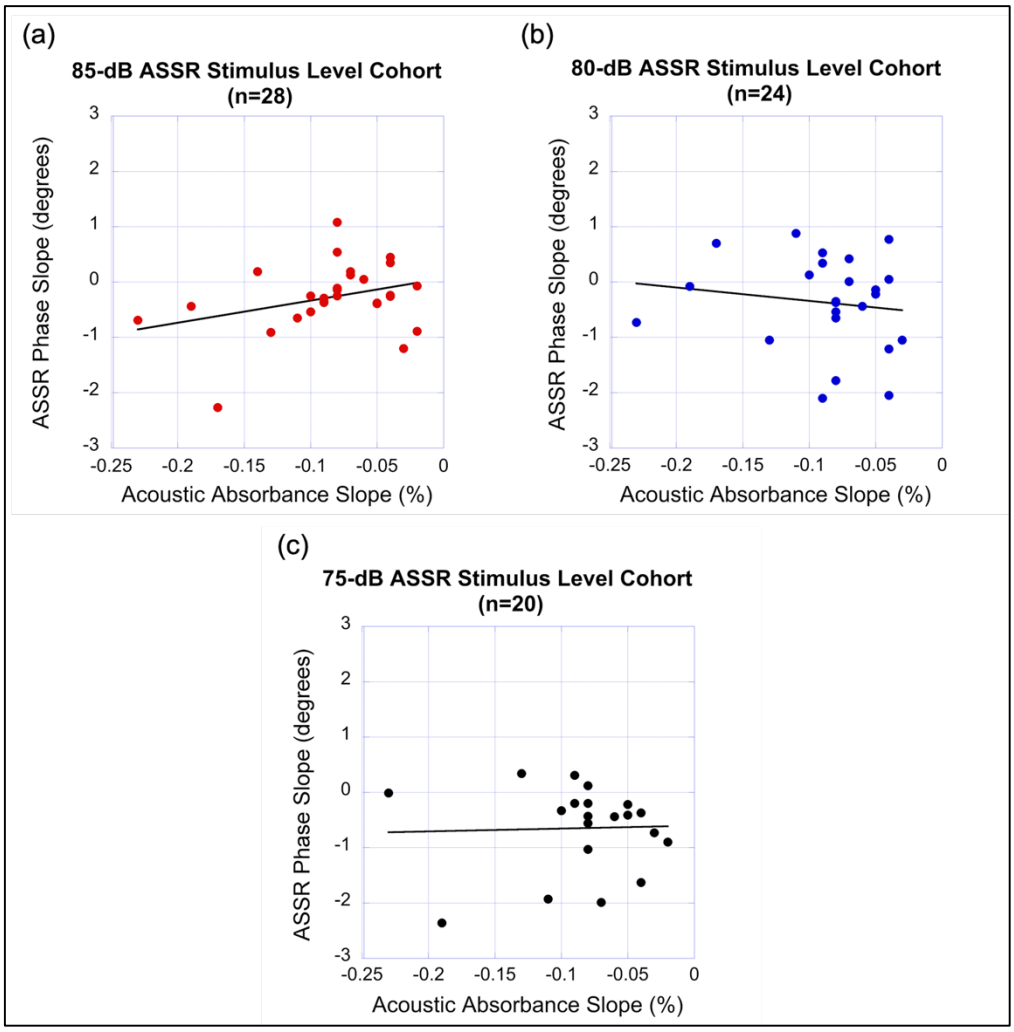

Figure 5.17: Scatter plots with OLS linear regression lines showing the relationship between ASSR phase slope and acoustic absorbance slope for the 3 ASSR stimulus level cohorts.

Pearson correlation coefficients demonstrated no statistically significant correlations between acoustic absorbance slope and ASSR phase slope for any of the ASSR stimulus level cohorts (85-dB ASSR stimulus level cohort: r=0.33, t=1.77, df=26, p=0.09; 80-dB ASSR stimulus level cohort: r=-0.14, t=0.68, df=22, p=0.51; 75-dB ASSR stimulus level cohort: r=0.03, t=0.14, df=18, p=0.89). Note the trend towards a positive correlation between acoustic absorbance slope and ASSR phase slope for the 85-dB ASSR stimulus level cohort. Additionally, no statistically significant correlations were observed between power transmittance slope

and ASSR phase slope for any of the 3 ASSR stimulus level cohorts (85-dB ASSR stimulus level cohort: r=0.15, t=0.78, df=26, p=0.45; 80-dB ASSR stimulus level cohort: r=-0.06, t=-0.29, df=22, p=0.77; 75-dB ASSR stimulus level cohort: r=-0.15, t=-0.65, df=18, p=0.53).

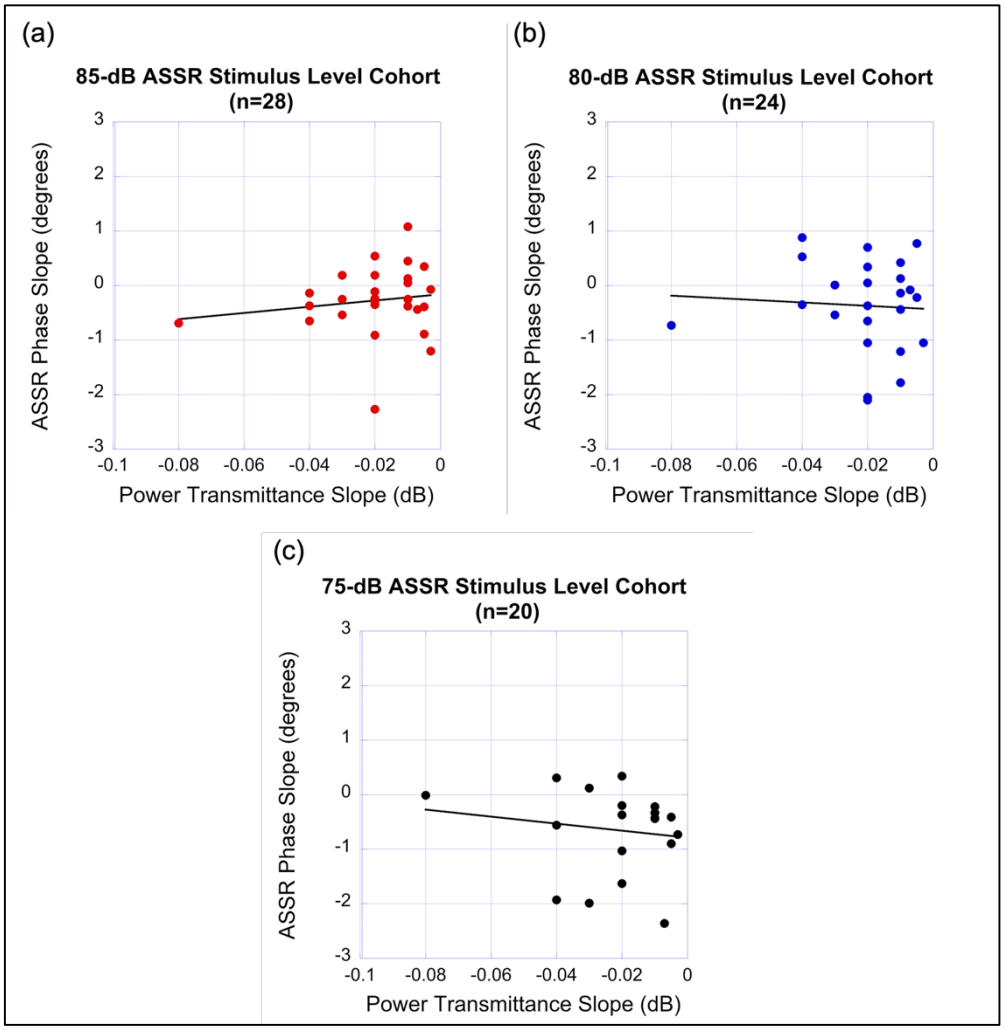

Figure 5.18: Scatter plots with OLS linear regression lines showing the relationship between ASSR phase slope and power transmittance slope for the 3 ASSR stimulus level cohorts.

5.4 Preliminary Ear-Specific Analyses

Preliminary ear-specific analysis of middle-ear stiffness-induced ASSR phase shift was investigated for an 85-dB pSPL ASSR stimulus level with 90-dB pSPL MEMR elicitor. Twenty participants had ASSR data that met all inclusion criteria described above for both the left and right ears. ASSR phase shifts for the right and left ears are shown in Figure 5.19.

A two-sample paired t-test confirmed that there was no statistically significant difference in ASSR phase shift between ears (t=-1.3, df=19, p=0.21). While no statistically significant differences in group mean ASSR

phase shifts occurred between the left and right ears, a smaller group mean ASSR phase shift was observed in the left ear compared to the right ear. Note the variability in ASSR phase shifts for both the left and right ears in Figure 5.19.

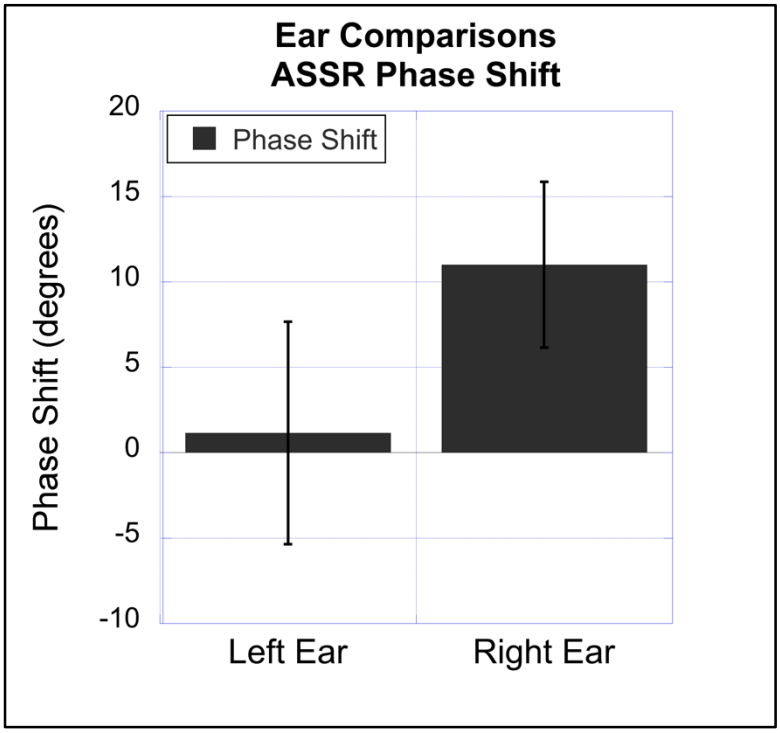

Figure 5.19: Bar plot comparing group mean (±1 standard error) ASSR phase shift between the left and right ears for an 85-dB pSPL ASSR stimulus level with 90 dB pSPL MEMR elicitor.

CHAPTER 6

Discussion

6.1 Summary of Findings

6.1.1 Middle-Ear Stiffness and Auditory Brainstem Neural Encoding of Phase

This study primarily investigated whether middle-ear stiffness changed the auditory brainstem neural encoding of phase. Middle-ear stiffness was elicited by activation of the MEMR using contralateral click stimuli and was measured using an acoustic absorbance assay. The auditory brainstem neural encoding of phase was measured by recording the ASSR elicited by 0.5-kHz toneburst stimuli presented at 78.125 Hz with and without contralateral presentation of the MEMR elicitor. As the MEMR elicitor level increased, acoustic absorbance in the 0.5-kHz half-octave frequency band and ASSR phase decreased. The findings of this study are consistent with the elicitation of middle-ear stiffness via activation of the MEMR which, in turn, changed the encoding of phase at the auditory brainstem. Middle-ear stiffness changed the auditory brainstem neural encoding of phase up to a maximum 6.5-degree decrease for every 10 dB pSPL increase in MEMR elicitor level. Furthermore, middle-ear stiffness introduced changes in ASSR phase in this study with very little to no change in ASSR amplitude as a function of MEMR elicitor level. Thus, middle-ear stiffness appears to change the auditory brainstem neural encoding of phase with minimal reduction of auditory brainstem neural recruitment and synchrony.

The findings of this study are consistent with the findings of studies that investigated the effects of middle-ear stiffness on the cochlear encoding of phase (Avan et al., 2000; Büki et al., 2000; Sun 2008). Avan et al. (2000) identified an approximately 20-degree middle-ear stiffness-induced DPOAE phase shift at 0.6 kHz. Similarly, the present study identified a 21-degree ASSR phase shift using 0.5-kHz ASSR eliciting tonebursts presented at 75 dB pSPL. Considered together, the findings of this and other studies highlight shifts in both the cochlear and neural encoding of phase that occur with comparable magnitude for a similar spectral region. Further within-participant investigations employing additional spectral and level ranges will be required in order to make scientifically valid comparisons between stiffness-induced effects on auditory phase encoding at the cochlear and neural levels.

The physiologic mechanisms that underlie and contribute to middle-ear stiffness-induced changes in the auditory system's ability to encode phase are not yet known; however, the following hypothesis is proposed. The phase of an acoustic waveform is an alternating current response. Mechanistically, alternating current (i.e., phase) is transmitted by the middle-ear system via movements of the ossicular chain. Activation of the MEMR results in contraction of the stapedius muscle and a subsequent stiffening of the ossicular chain, in

turn impeding the mobility of the ossicular chain. A stiffer and less mobile ossicular chain is consistent with reduced transmission of alternating current and decreased auditory brainstem neural encoding of phase as observed in this study. Future studies should investigate the physiologic mechanisms that underlie middle-ear stiffness-induced changes in the auditory encoding of phase.

The current study was designed to describe phase changes associated with middle-ear stiffness rather than to determine physiologic mechanisms; however, this study provides several insights into this area. First, baseline MOCR and WAI-MEMR testing completed during this study confirmed that the 20/s contralateral click stimuli successfully activated the MEMR pathway with little to no evidence supporting co-activation of the MOCR pathway. This supports middle-ear stiffness-induced changes in ASSR phase with minimal to no contribution from the MOCR pathway. Furthermore, the use of contralateral acoustic stimulation to activate the MEMR eliminated the confound of stimulus interactions in the ear canal. Contralateral acoustic stimulation also did not affect ASSR amplitude, which suggests little to no acoustic crossover of the contralateral click stimuli to the cochlea of the test ear. Thus, stimulus interactions in the ear canal and acoustic crossover do not appear to have contributed to the observed effects on ASSR phase demonstrated by this study.

6.1.2 Effects of ASSR Stimulus Level and Participant Age

ASSRs were elicited by tonebursts presented at 75, 80, and 85 dB pSPL in this study, allowing for within-participant investigation of ASSR stimulus level effects related to middle-ear stiffness-induced changes in ASSR phase. The effect of middle-ear stiffness on the auditory brainstem neural encoding of phase was quantified using ASSR phase slope. Steeper ASSR phase slopes occurred at lower ASSR stimulus levels, though this trend did not reach statistical significance. This trend opposes the hypothesized ASSR stimulus level effect and suggests more pronounced middle-ear stiffness-induced changes in auditory brainstem phase encoding at lower ASSR stimulus levels.

One potential explanation for this observed trend is that the ASSR stimulus itself may have elicited changes in middle-ear stiffness in the test ear when presented at higher levels. If the ASSR stimulus elicited middle-ear stiffness, then the addition of a contralateral MEMR elicitor may not have been as effective in eliciting additional stiffness. Support for this explanation can be found in Figure 5.13, which shows ASSR phase across MEMR elicitor levels for all 3 ASSR stimulus level cohorts. At lower MEMR elicitor levels, group mean ASSR phases for the 80 and 85 dB ASSR stimulus level cohorts were decreased compared to ASSR phase for the 75 dB ASSR stimulus level cohort. Because the MEMR elicitor was not likely to elicit middle-ear stiffness at lower levels where phase differences were observed, the higher ASSR stimulus levels in the test ear may have elicited middle-ear stiffness that contributed to decreased ASSR phase across all MEMR elicitor levels and shallower ASSR phase slopes.

ASSR stimulus level as it relates to the investigation of middle-ear stiffness-induced effects on auditory

brainstem phase encoding is a complex issue. The ASSR stimulus level needs to be high enough to elicit an ASSR with robust amplitude and SNR; however, higher stimulus levels may elicit middle-ear stiffness in the test ear, potentially confounding the investigation of stiffness-induced effects on ASSR phase when using an MEMR paradigm to experimentally manipulate middle-ear stiffness. The findings of this study related to ASSR stimulus level suggest that 0.5-kHz tonebursts presented at 75 dB pSPL may be optimal for eliciting the ASSR while minimizing changes in middle-ear stiffness by the ASSR stimulus itself. Future studies exploring additional spectral and level ranges will help to define optimal stimulus parameters for measuring the effects of middle-ear stiffness on the cochlear and auditory neural encoding of phase.

This study did not identify any statistically significant effects of participant age on middle-ear stiffness-induced changes in ASSR phase. The lack of age effects may indicate consistent effects of middle-ear stiffness on the auditory brainstem neural encoding of phase across the lifespan from 18 to 50 years. This particular finding should be considered with regard for the limited age range investigated in this study spanning only younger and middle adulthood. Additionally, fewer older participants had available ASSR data meeting inclusion criteria, especially for the 75-dB ASSR stimulus level cohort, and between-participant variability in ASSR phase slope occurred across the lifespan. The fewer number of older participants and variability among participants may have prevented the identification of statistically significant age effects for this study sample.

Feeney & Sanford (2004) showed that older adults had greater low-frequency acoustic absorbance compared to younger adults. Age-related changes in low-frequency acoustic absorbance highlight the need for continued investigation of middle-ear stiffness-induced changes in auditory phase encoding, especially for older participant populations. Although the current study focused on adults, it is worth noting that middle-ear and auditory neural development occur from birth throughout childhood. Thus, future studies investigating the interaction between middle-ear and auditory neural development throughout childhood in the context of stiffness-induced changes in auditory brainstem phase encoding are a high priority.

6.1.3 Correlations Between WAI Measures and ASSR Phase

A secondary aim of this study was to investigate relationships between middle-ear stiffness-induced changes in WAI measures and ASSR phase slope. Correlation analyses did not demonstrate statistically significant relationships between acoustic absorbance slope and ASSR phase slope or between power transmittance slope and ASSR phase slope, though a trend towards a positive correlation between acoustic absorbance and ASSR phase slope was observed for the 85-dB ASSR stimulus level cohort. The lack of statistically significant correlation between these measures may indicate differential effects of middle-ear stiffness on peripheral (i.e., middle ear) versus neural (i.e., auditory brainstem) structures. The lack of statistically significant correlation between WAI and ASSR measures may also reflect the complexity of both measures wherein contralateral presentation of an MEMR elicitor may activate cochlear and cortical efferent

neural pathways that could impact the neural ASSR measure with minimal or no impact on the middle-ear measures.

Additionally, 0.5-kHz-centered acoustic absorbance and power transmittance slopes may indicate the amount of middle-ear stiffness elicited by the contralaterally presented MEMR elicitor. For example, a steeper acoustic absorbance and power transmittance slope is consistent with a greater amount of middle-ear stiffness with presentation of the MEMR elicitor. A positive correlation between WAI slopes and ASSR phase slope would indicate that greater amounts of increased middle-ear stiffness contribute to greater stiffness-induced ASSR phase changes. Thus, the lack of statistically significant correlations between WAI measures and ASSR phase slope may suggest that middle-ear stiffness does not result in additional changes to phase encoding once a threshold amount of middle-ear stiffness is reached.

The use of Pearson correlation coefficients is one limitation of these analyses. Acoustic absorbance slope, power transmittance slope, and ASSR phase slope are all dependent variables. Correlational analyses comparing 2 dependent variables would be more accurately analyzed using multivariate multiple regression modeling the effects of 1 predictor variable (i.e., MEMR elicitor level) on multiple dependent variables (i.e., acoustic absorbance, power transmittance, and ASSR phase). This study did not have a sufficient number of participants to complete multivariate multiple regression analyses. Thus, the correlational analyses should be considered in the context of the limitations associated with the type of correlation employed and future studies should include a larger sample size.

6.1.4 Comparison of Stiffness-Induced ASSR Phase Shifts Between Ears

Preliminary within-participant comparisons of middle-ear stiffness-induced ASSR phase shifts between ears demonstrated no statistically significant differences in the group mean ASSR phase shift for the left and right ears. However, Figure 5.19. highlights a trend wherein a larger average ASSR phase shift occurred for the right ears (mean: 11-degree ASSR phase shift) compared to the left ears (mean: 1-degree ASSR phase shift). The high degree of between-participant variability in ASSR phase shift values, especially for the left ears, likely contributed to the lack of statistical significance for the ear comparison analysis.

Several factors may have contributed to the between-participant variability in ASSR phase shifts observed in this study. The ASSR stimulus level (85 dB pSPL) and contralateral click level (90 dB pSPL) used for preliminary ear comparisons were selected to maximize the amplitude of the ASSR and to maximize activation of the MEMR. However, as discussed above, the ASSR stimulus level may have elicited middle-ear stiffness in the test ears potentially preventing additional middle-ear stiffness from being elicited by activation of the MEMR. Between-participant variability in MEMR threshold levels in the test ears (i.e., variability in the level at which the ASSR stimulus elicited stiffness in the test ear) may be contributing to the variability in ASSR phase shifts observed.

Additionally, the greater amount of between-participant variability noted for the left ears compared to the right ears may be related to increased physiological noise at the end of the test session. The left ears of all participants were tested at the end of data collection as data acquisition in the right ear for Specific Aims I and II were prioritized. Some participants chose to complete the entire data acquisition protocol in a single test session (between 4 and 5 hours in total duration) while other participants chose to complete data collection over 2 different test sessions (between 2 and 3 hours per session). Participant fatigue, especially for those completing testing in a single session, may have contributed to greater participant-generated physiologic noise at the end of the test session when the left ear was being tested.

Finally, the trend of greater middle-ear stiffness-induced ASSR phase shifts in the right compared to left ears may be due to within-participant differences in MEMR strength between ears. WAI-MEMR was measured in the right ears only, limiting the interpretation of differences in MEMR strength between ears; however, standard MEMR thresholds elicited by BBN were lower (i.e., better) in the right ears compared to the left ears for all participant cohorts (compare red and blue bars in Figure 5.2.). While MEMR threshold is not a direct measure of MEMR strength, lower thresholds may indicate stronger MEMR activation in the right ears. While stronger right-ear MEMR activation may elicit greater middle-ear stiffness-induced effects on ASSR phase, this interpretation should be considered in the context of the lack of statistically significant correlations between WAI slopes and ASSR phase slopes, which may suggest that the amount of middle-ear stiffness is not related to stiffness-induced changes in ASSR phase. Future studies prioritizing ear comparisons of middle-ear stiffness-induced changes in ASSR phase should utilize lower ASSR stimulus levels, a larger number of MEMR elicitor levels, and quantify WAI-MEMR strength for both left and right ears.

6.2 Impact

The findings of this study have clear and meaningful impacts on audiologic practice. Yost (1974) demonstrated in adults with normal hearing that an IPD of as little as 5 degrees impacted behavioral horizontal plane sound localization of a 0.5-kHz pure-tone. The current study showed middle-ear stiffness-induced changes in the auditory brainstem encoding of phase consistent with up to a 6.5-degree decrease in ASSR phase for every 10 dB pSPL increase in MEMR elicitor level and a maximum 21-degree stiffness-induced ASSR phase shift. The findings of this study, in conjunction with those of Yost (1974), emphasize that middle-ear stiffness can result in changes in the auditory brainstem encoding of phase to a degree that may alter behavioral performance on horizontal plane localization tasks. Knowledge gained from the present study may contribute to understanding auditory function, especially spatial hearing performance, in several clinical populations, including CI EAS patients, individuals with normal pure-tone sensitivity and a history of noise exposure, and patients with otosclerosis.

6.2.1 Binaural Cue Sensitivity in CI EAS Patients

As noted in section 3.1. above, recent work showed decreased post-operative low-frequency acoustic absorbance in CI patients (Merchant et al., 2020; Saoji et al., 2020; Scheperle & Hajicek 2020; Racca et al., In Review), consistent with increased middle-ear stiffness following CI surgery that can persist for at least 6-months post-activation (Figure 3.1.; Racca et al., In Review). Additionally, decreased acoustic absorbance in the implanted ear of unilateral CI patients compared to their non-implanted ear persisted through at least 3-months post-activation (Figure 3.2.; Racca et al., In Review). While these findings suggest that increased middle-ear stiffness may occur in the implanted ears of unilateral CI patients, it is also important to note that differences between ears in the degree of post-operative middle-ear stiffness may occur in bilateral CI patients.

As suggested by the findings of the current study, middle-ear stiffness that differs between ears, whether in unilateral or bilateral CI patients, may result in changes in the auditory brainstem neural encoding of phase that contribute to larger post-operative IPDs. Stiffness-induced changes in the auditory encoding of phase and consequently larger IPDs may partially contribute to variability in binaural cue sensitivity in CI EAS patients who utilize low-frequency acoustic hearing in conjunction with electric hearing. Cochlear implantation resulted in an approximately 10-15% reduction in acoustic absorbance for the 0.5-kHz spectral region (Figure 3.1.), whereas MEMR activation in the current study resulted in a maximum 3.1% acoustic absorbance shift (see Figure 6.1. below).

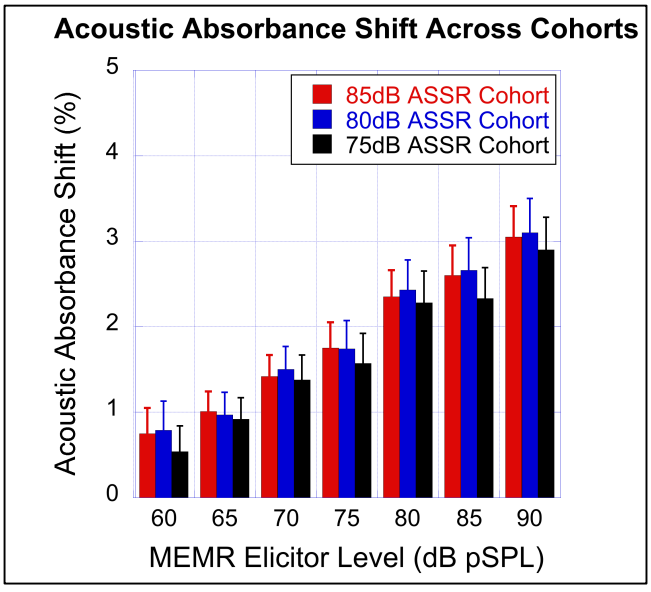

Figure 6.1: Bar plot showing group mean (±1 standard error) acoustic absorbance shift across MEMR elicitor levels for all participant cohorts.

Differences in the amount of acoustic absorbance reduction observed for CI patients (Racca et al., In Review) compared to participants with normal hearing in this study are likely due to differences in mechanisms contributing to middle-ear stiffness. Post-operative middle-ear stiffness in CI patients is likely due to a

combination of factors including changes in middle-ear volume via introduction of the facial recess during surgery, sealing of the electrode array at the round window, and the physical presence of the electrode array in the cochlea. Middle-ear stiffness in the current study was elicited via activation of the MEMR. The MEMR is not likely to be present in CI patients due to their degree of sensorineural hearing loss, though it should be noted that some CI patients with low-frequency, post-operative hearing preservation may have an MEMR. Thus, application of the findings of this study may have limited generalizability to CI EAS patients due to likely differences in mechanisms contributing to middle-ear stiffness. However, the identification of middle-ear stiffness-induced changes in the auditory brainstem neural encoding of phase identified by this study provides rationale for the investigation of middle-ear stiffness-induced effects on the auditory brainstem neural encoding of phase in CI EAS patients. This area of future discovery is a priority.

6.2.2 MEMR Contributions to Spatial Hearing in Noise for Normal-Hearing Populations

Activation of the MEMR, a normal physiologic reflex, was used to elicit middle-ear stiffness in this study. Changes in the auditory brainstem encoding of phase secondary to MEMR-induced middle-ear stiffness may indicate a role of the MEMR in spatial hearing in noise. The middle-ear transmission systems of the left and right ears may be differentially affected by activation of the MEMR when listening in background noise. For example, a background noise source may activate the MEMR. Even though the MEMR is a bilateral reflex, MEMR threshold and presumably MEMR strength differ for the ipsilateral and contralateral pathways (see Figure 5.2. showing within-participant differences in MEMR thresholds for ipsilateral versus contralateral MEMRs). Differential activation of the ipsilateral and contralateral MEMR pathways may result in differing amounts of middle-ear stiffness and differences in the middle-ear transmission of the phase of a target sound source when compared between the two ears. Thus, future studies that investigate potential MEMR contributions to binaural cue sensitivity, and specifically IPD sensitivity, in the context of spatial hearing may provide a more thorough understanding of the physiologic network employed during sound localization in noise performance.

One clinical population that may demonstrate changes in their MEMR function are individuals with normal to near-normal pure-tone threshold sensitivity and a history of noise exposure (i.e., hidden hearing loss). Kujawa & Liberman (2009) first identified a loss of inner hair cell synaptic ribbons following noise exposure that caused a temporary shift in outer hair cell function (i.e., cochlear synaptopathy). Subsequent work demonstrated primary effects of synaptopathic noise exposure on the subset of auditory nerve fibers with low-spontaneous firing rate and high thresholds (e.g., Furman et al., 2013). These low-spontaneous firing rate auditory neurons may contribute to the MEMR (e.g., Liberman & Kiang 1984; Kobler et al., 1992). Valero et al. (2016, 2018) showed weaker MEMR activation when elicited by stimuli associated with synaptopathic cochlear regions in anesthetized and un-anesthetized mice.

Weaker MEMR activation secondary to synaptopathy-inducing noise exposure may preclude middle-ear stiffness from occurring when elicited by stimuli targeting spectral regions associated with cochlear synaptopathic damage. The extent of synaptopathic-induced effects on the MEMR may differ between ears depending on the extent of cumulative noise exposure for both ears. Furthermore, synaptopathic noise exposure may affect different spectral regions along the cochlear length for the left and right ears, thus binaural cue sensitivity may be affected in a frequency-specific manner in the presence of bilateral cochlear synaptopathy. The relationships among cochlear synaptopathy, MEMR-induced middle-ear stiffness, and the auditory encoding of phase highlight a potential area for future scientific inquiry related to investigating bilateral effects of cochlear synaptopathy specifically related to understanding binaural cue sensitivity post-hazardous noise exposure.

6.2.3 Binaural Cue Sensitivity in Patients with Otosclerosis

The findings of the current study may also have relevant clinical applications for patients with otosclerosis. Otosclerosis is an audiologic disorder that can result in a stiffening of the ossicular chain due to sclerotic bone growth and fixation of the stapedial footplate about its anterior annulus (Chole & McKenna 2001). Unilateral otosclerosis can result in differing degrees of middle-ear stiffness between left and right ears wherein middle-ear stiffness only occurs for the affected ear. Bilateral otosclerosis may also lead to different degrees of middle-ear stiffness between ears depending on the time course of bone growth and disease progression for each ear.

Redfors et al. (2015) showed that individuals with unilateral and bilateral otosclerosis self-reported significant difficulties localizing sounds based on the speech, spatial and qualities of hearing scale (SSQ). Self-reported difficulty localizing sounds persisted even in individuals with otosclerosis who were treated with a hearing aid (Redfors et al., 2015). Middle-ear stiffness-induced changes in the auditory encoding of phase, as identified in the current study, may contribute to difficulties in sound localization reported by individuals with otosclerosis. Future studies investigating relationships between middle-ear stiffness, phase encoding, and behavioral spatial hearing performance in patients with otosclerosis may extend current knowledge related to spatial hearing difficulties reported by patients with otosclerosis.

6.2.4 Phase Encoding and Pitch Perception

While this study emphasizes the effects of middle-ear stiffness on the auditory neural encoding of phase in contribution to sound localization, it should also be considered that phase encoding contributes to pitch perception in quiet and in the presence of background noise. Thus, differences in middle-ear stiffness between the two ears may contribute to differences in pitch perception between ears that can negatively impact functional hearing, particularly in the presence of background noise. Future studies investigating the potential relationship among middle-ear stiffness, phase encoding, and pitch perception should employ a within-

participant design that measures ASSR phase and behavioral performance on a pitch perception task with and without contralateral elicitation of the MEMR.

6.3 Study Limitations and Future Directions

6.3.1 Between-Participant Variability in ASSR Phase

As noted throughout the preceding discussion sections, between-participant variability in ASSR phase and phase shifts were a potential limitation in several analyses, including those related to the effects of participant age and to ear comparisons. The between-participant variability may have weakened effect sizes and may have precluded identification of statistically significant effects in some analyses. Additionally, the between-participant variability in ASSR phase for the 80- and 85-dB ASSR stimulus levels contributed to functions at the group level that may not be most accurately fit using a linear regression model (Figure 5.9). Linear regression modeling was used in this study in order to calculate the slope measure used as the dependent variable for statistical analyses. Future studies and analyses should investigate other modeling techniques, such as sigmoid curve fitting, that may be appropriate for investigating the effects of middle-ear stiffness on the ASSR phase elicited by higher stimulus levels.

The between-participant variability in ASSR phase observed in this study sample is consistent with the work of Herdman & Stapells (2001) who identified a high degree of between-participant variability in ASSR phase, especially for lower frequency and lower level amplitude modulated tones. Toneburst stimuli used to elicit the ASSR in this study were centered at 0.5 kHz. The average behavioral threshold to the physiologic stimulus was 35 dB pSPL for all 3 ASSR stimulus level cohorts. Thus, ASSRs were elicited, on average, between 40 and 50 dB above behavioral threshold, which may have been near physiologic threshold for some participants.

The use of higher frequency and higher level stimuli, where ASSR phase may be less variable among participants (Herdman & Stapells 2001), is not conducive to investigating the effects of middle-ear stiffness on auditory phase encoding. Middle-ear stiffness primarily affects the transmission of low-frequency sound, so low-frequency ASSR stimuli should be used. Additionally, higher level ASSR stimuli may themselves elicit middle-ear stiffness in the test ear, precluding accurate investigations of changes in phase encoding with the elicitation of middle-ear stiffness.

Variability among individuals related to the effects of middle-ear stiffness-induced changes in auditory phase encoding may be a characteristic feature at both the cochlear and auditory brainstem levels. In fact, Avan et al. (2000) observed high between-participant variability in their DPOAE phase study. It is worth noting that both Avan et al. (2000) and the current study utilized activation of the MEMR to elicit middle-ear stiffness and examine its effects on the encoding of phase. Thus, between-participant variability in MEMR strength may be a contributing factor to the observed variability in stiffness-induced changes in auditory phase

encoding.

Factors that contribute to the between-participant variability observed in ASSR phase without an MEMR elicitor are not well understood and are minimally discussed in the current literature base. This study identified that changes in middle-ear stiffness result in changes in ASSR phase, highlighting an important role of the middle-ear transmission system in the encoding of phase. As such, variability among participants in their baseline middle-ear status in the absence of an MEMR elicitor may be one factor that contributes to between-participant variability in ASSR phase.

Figure 5.14. shows the relationship between acoustic absorbance and ASSR phase acquired without an MEMR elicitor for the 3 ASSR stimulus level cohorts. The statistically significant positive correlation between acoustic absorbance and ASSR phase acquired without an MEMR elicitor for the 85-dB ASSR stimulus level cohort is consistent with a stiffer baseline middle-ear transmission system partially contributing to decreased baseline ASSR phase. Between-participant variability in baseline middle-ear stiffness in the absence of other elicitors may partially contribute to variability in ASSR phase. However, the negative correlational trend observed for the 80-dB ASSR stimulus level cohort and lack of correlation for the 75-dB ASSR stimulus level cohort may indicate that the relationship between baseline acoustic absorbance and ASSR phase in the absence of an MEMR elicitor is partially dependent on the ASSR stimulus level.

Additionally, variability in participant state of arousal and variability in physiologic noise may also contribute to variability in ASSR phase. As of the writing of this dissertation, studies that address the effects of participant state on ASSR phase, especially for 80-Hz modulation rates, could not be identified. Future studies that seek to elucidate contributing factors to between-participant variability in ASSR phase should focus on two areas. First, investigating the relationship between acoustic absorbance and ASSR phase without an MEMR elicitor for additional toneburst frequencies and levels will help to understand whether baseline middle-ear transmission, particularly stiffness, contributes to between-participant variability in ASSR phase. Second, future studies that investigate the effects of participant state of arousal on 80-Hz ASSR phase would provide further insight into whether state of arousal contributes to between-participant variability in ASSR phase.

6.3.2 Statistical Power

This study reached 95% statistical power for one-sample t-test analyses related to middle-ear stiffness-induced changes in ASSR phase for the 75-dB ASSR stimulus level cohort; however, statistical power levels of 54% and 64% were reached for the 80-dB and 85-dB ASSR stimulus level cohorts respectively. The findings reported for the 80-dB and 85-dB ASSR stimulus level cohorts should be considered in the context of the reduced statistical power levels observed for these cohorts. Based on the magnitude of the effect size and between-participant variability observed in this study, future sample sizes of 41 and 43 participants would be

required for the 85-dB and 80-dB ASSR stimulus level cohorts in order to achieve 80% statistical power. Achieving these sample sizes in the current study was not feasible due to limitations associated with the current COVID-19 global pandemic.

6.3.3 Limitations Associated with ASSR Stimulus Parameters

The ASSR stimulus levels used in this study were a potential limitation. The findings of this study suggest that higher ASSR stimulus levels may have elicited middle-ear stiffness in the test ear potentially confounding interpretation of findings for the higher stimulus levels. On the other hand, lower ASSR stimulus levels resulted in fewer participants with ASSR data meeting amplitude and SNR criteria for inclusion in study analyses. The trade-off between ASSR amplitude and unintended elicitation of middle-ear stiffness by the ASSR stimulus is a limiting factor in this experimental paradigm and an issue that should be addressed in future studies.

6.3.4 Consideration for the Entire Auditory System

This study only investigated middle-ear stiffness-induced changes on the auditory brainstem neural encoding of phase. The auditory brainstem neural pathway is only a small portion of the auditory pathway. Studies completed by other investigators (Avan et al., 2000; Büki et al., 2000; Sun 2008) provide some insights into stiffness-induced changes in the cochlear encoding of phase, though these studies are limited by their lack of defining DPOAE position in the fine structure as discussed in section 2.4.2. above. The current study and previous studies investigating middle-ear stiffness effects at the cochlear level are limited by the lack of within-participant comparisons of effects at both the cochlear and auditory brainstem neural levels. Furthermore, this study only investigated middle-ear stiffness effects for a sub-cortical auditory neural pathway, whereas auditory cortical neural networks are required for the conscious perception of sound and provide a more comprehensive assessment of the auditory pathway.

Future studies using animal models may begin to adequately assay increased middle-ear stiffness effects on the cochlear and auditory nerve encoding of phase by employing the auditory nerve overlapped waveform (ANOW; e.g., Lichtenhan et al., 2014). The ANOW measures the cochlear response to elicitors of opposite phase. The cochlear microphonic can be derived via the subtraction of the cochlear response waveforms elicited by opposite phase elicitors, while the ANOW is derived from the average of the two waveforms. The cochlear microphonic provides a measure of the OHC encoding of phase, while the ANOW provides a measure of the auditory nerve encoding of phase (Lichtenhan et al., 2014). Thus, the ANOW potentially provides an animal model assay of the simultaneous, within-subject assessment of the cochlear and auditory nerve encoding of phase that could be compared with and without increased middle-ear stiffness. Additionally, the auditory cortical encoding of phase could be assessed with and without middle-ear stiffness using a comparable

paradigm to the one employed in this study but with a lower ASSR toneburst presentation rate. These investigations would provide valuable assessment of within-subject effects of middle-ear stiffness for a more comprehensive portion of the auditory pathway.

6.3.5 Future Directions

The over-arching long-term goal of this work is to understand whether middle-ear stiffness post-cochlear implantation contributes to variability in binaural cue sensitivity and poor spatial hearing abilities in CI EAS patients. Several areas of future investigation related to this long-term goal are warranted. First, a study utilizing a model of the middle-ear and auditory neural pathway (i.e., Verhulst et al., 2018) would allow for investigation of the mechanisms contributing to middle-ear stiffness post-cochlear implantation. The model could be used to specifically manipulate the volume of the middle-ear cavity to determine whether the creation of a facial recess during CI surgery may contribute to post-operative middle-ear stiffness. A larger post-operative middle-ear cavity volume could change the compliance of the middle-ear transmission system causing it to become dominated by middle-ear stiffness (Mason 2016).

The findings of the current study lend themselves well to future longitudinal investigations in CI EAS patients. Longitudinal investigations related to changes in acoustic absorbance and ASSR phase pre- and post-cochlear implantation would begin to provide insight into whether post-operative middle-ear stiffness changes the post-operative auditory brainstem neural encoding of phase. These longitudinal investigations would require investigations related to the long-term, within-participant stability of ASSR phase measures over time. Additionally, future studies are warranted investigating potential relationships between post-operative changes in the auditory brainstem encoding of phase and horizontal plane sound localization.

Finally, the findings of this study and future studies may be used to inform the programming of hearing aids for clinical populations where middle-ear stiffness may negatively impact binaural cue sensitivity (i.e., CI EAS and otosclerosis patients). If the degree of middle-ear stiffness-induced phase shift can be identified and if the difference in the auditory neural encoding of phase between the left and right ears can be quantified, then perhaps hearing aid technology can be programmed with individualized phase delays in order to improve patient access to binaural cues that are important for sound localization and spatial hearing.

6.4 Conclusions

This study identified a change in the auditory brainstem neural encoding of phase, as measured via the ASSR, secondary to middle-ear stiffness elicited by activation of the MEMR in adults with normal pure-tone threshold sensitivity. ASSR phase decreased by up to 6.5 degrees and acoustic absorbance decreased by approximately 1% for every 10 dB pSPL increase in MEMR elicitor level. A maximum 21-degree middle-ear stiffness-induced ASSR phase shift was observed at the group level. This magnitude of stiffness-induced

ASSR phase shift may be sufficient to alter behavioral performance on a sound localization task (e.g., Yost 1974). The findings of this study are particularly relevant for understanding the poor spatial hearing abilities of CI EAS patients who may have persistently increased post-operative middle-ear stiffness (Merchant et al., 2020; Saoji et al., 2020; Scheperle & Hajicek 2020; Racca et al., In Review) and for patients with otosclerosis who may have increased middle-ear stiffness. Additionally, the findings of this study highlight a potential role of the MEMR in contributing to spatial hearing in noise for individuals with normal hearing.

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