6-21-2023

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Recommended Citation

Jha, Raghav H., "Effects of age on amplitude-modulated cVEMP temporal modulation transfer function" (2023). *Dissertations, 2020-current*. 107.
https://commons.lib.jmu.edu/diss202029/107

Effects of age on Amplitude-modulated cVEMP Temporal Modulation Transfer Function

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A dissertation submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

for the degree of

Doctor of Philosophy

Department of Communication Sciences and Disorders

August 2023

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DEDICATION

I dedicate this work to myself. It has been a journey that I will always remember and cherish.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude and appreciation to all the individuals who have played a role in the completion of this doctoral dissertation. Their support, encouragement, and guidance have been crucial in shaping my academic journey, and I would not have been able to accomplish this research endeavor without their unwavering assistance.

First and foremost, I extend my heartfelt gratitude to my PhD advisor, Dr. Erin Piker. Her expertise, insightful feedback, and continuous encouragement have been invaluable throughout the entire process. I am immensely grateful for her patience, guidance, and mentorship, as they have significantly influenced both my research and personal growth. I would also like to thank Dr. Chris Clinard for his valuable input and advice, which greatly enhanced my thinking processes. His time, expertise, and unwavering commitment to ensuring the rigor and quality of this work are deeply appreciated. Furthermore, I am grateful to another member of my dissertation committee, Dr. Kristal Riska, for her contribution to this research.

In addition to my academic mentors, I am immensely grateful to my family for their unwavering love, support, and encouragement. To my parents, Mummy and Papa, and my siblings, Keshav and Pushpam, thank you. I would also like to express my gratitude to Namitha for her unconditional love and understanding, which provided me with the strength and motivation to persevere through the challenges of this demanding endeavor. Additionally, I am indebted to my past advisors who introduced me to the field of audiology, sparking my interest and paving the way for this research.

I am also indebted to my friends and colleagues who have been constant sources of motivation and support throughout my doctoral studies. Our insightful discussions and camaraderie have made this academic journey more enjoyable and fulfilling. I would also like to acknowledge the financial support provided by CAPCSD and the American Academy of Audiology. Their assistance has facilitated my research by providing access to resources, travel opportunities, and data collection, enabling me to conduct a comprehensive study.

Lastly, I want to express my heartfelt gratitude to all the participants who willingly and generously shared their time, knowledge, and experiences. Without their active involvement, this research would not have been possible. Their contribution has been invaluable to the development of this work. In conclusion, I am humbled by the support and guidance I have received from all those who have contributed to the completion of this doctoral dissertation. Thank you all,

Raghav

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ABSTRACT

With the aging population on the rise, the need for effective assessment tools to identify risk factors for falls among the elderly is paramount. One independent risk factor for falls is vestibular impairment, but the available vestibular diagnostic tests have limitations. A promising new methodology: amplitude-modulated tones to elicit a vestibular-evoked myogenic potential (AMcVEMP), offers a more detailed examination of vestibular (sacculo-collic reflex) functions. This method assesses non-linearities, synchrony, and magnitude, providing a more thorough evaluation compared to the conventional transient cVEMP. So far, AMcVEMP has only been utilized in young adults.

The current study aimed to investigate the impact of age on the AMcVEMP temporal modulation transfer function (TMTF), determine the shape and limit of the AMcVEMP TMTF, and assess non-linearity across a range of modulation frequencies. The study included 49 healthy participants, categorized into three age groups. AMcVEMP responses were elicited using a carrier-frequency of 500 Hz and 10 modulation frequencies. An FFT-based approach was employed to analyze the responses, focusing on amplitude, signal-to-noise ratio (SNR), phase coherence (PC), and non-linearity. To measure non-linearity, harmonics of the modulation frequencies were analyzed.

The AMcVEMP responses exhibited characteristics consistent with saccular rectification. The AMcVEMP amplitude, SNR, and PC reduced with increasing age. The effects of age were less pronounced for PC, showing 100% response rates among older adults. Further, AMcVEMP TMTF range reduced with age for all measures. The shape of the AMcVEMP TMTF resembled bandpass filter among young adults and got narrower with age. Lastly, for the non-linearity measures, harmonics were robust in most young, some middle-aged and fewer older adults, indicating loss of non-linearity with aging.

AMcVEMP offers several advantages in assessing older adults compared to conventional cVEMP: higher response rates and the ability to examine and quantify the magnitude, synchrony, and non-linearity from the sacculo-collic reflex. This study advances our overall understanding of age-related changes in the vestibular system. Further utility of AMcVEMP in clinical populations will advance our comprehension of vestibular pathophysiology. Furthermore, linking vestibular non-linearity with functional balance may facilitate the development of strategies to mitigate the risk of falls among older adults.

Chapter I.

Introduction

The aging population is a significant health concern today, and it is expected that by 2050, one out of every five individuals in the US will be over the age of 65 (Duggan et al., 2015). As individuals grow older, the risk of falling increases. Falls are a leading cause of both fatal and non-fatal injuries among people aged 65 years and older (Houry et al., 2016). The detrimental impact of falls on quality of life is well-documented (see for review, Schoene et al., 2019). The high prevalence of falls, their adverse effects on quality of life, and the substantial costs associated with falls in older adults pose a burden on our healthcare system. As the proportion of the aging population continues to rise, the number of falls and their associated costs are projected to increase significantly (Houry et al., 2016). However, it's important to note that falls are not an inevitable consequence of aging. They can be prevented by identifying risk factors and taking appropriate precautions to mitigate those risks.

Vestibular impairment stands out as a significant and independent risk factor contributing to falls among elderly individuals. Similar to other bodily processes, the vestibular system undergoes a gradual decline in both structure and function as a result of aging. Age-related anatomical and physiological changes manifest within the vestibular system, starting from the peripheral end organs (Bergström, 1973; Richter, 1980) extending to the vestibular structures within the brainstem and cerebellum (Andersen et al., 2003; Torvik et al., 1986), and continuing through thalamic projections to the vestibular cortex (Brandt et al., 2005). Changes in these structures might result in a reduced ability to sense head movement and regulate body control while maintaining balance, thereby increasing the risk of falls among older adults.

To effectively assess the vestibular system in older adults, it is crucial to identify the risk factors for falls. However, despite significant advancements in vestibular system assessment over the past 30 years, our understanding of vestibular functioning and the ability to assess its various mechanisms remains limited. Consequently, there is often a lack of specific diagnosis for individuals, particularly older adults, who seek medical care for dizziness (Wassermann et al., 2022). This knowledge gap highlights the need for further research on age-related changes in the vestibular system.

To address these knowledge gaps, our project aims to utilize an amplitude-modulated stimulus to record cervical vestibular-evoked myogenic potentials (cVEMPs) across different age groups. This novel approach allows us to gain insights into the ongoing activity of the sacculo-collic pathways, The approach allows for detailed examination of the vestibular (sacculo-collic reflex) function and can tap vestibular mechanisms (non-linearity and synchrony) that cannot be assessed using other existing methods. By conducting this study, we aim to enhance our understanding of vestibular physiology and uncover unexplored aspects of vestibular functioning in the aging population. The outcomes of our research have the potential to pave the way for improvements in vestibular diagnostic, and development of strategies that might mitigate the risk for falls among older adults.

Chapter II.

Review of Literature

Age related changes in the vestibular system

Age related changes in the vestibular system occur both in the periphery as well as in the central vestibular structures. In each ear, the peripheral vestibular apparatus constitutes of the 5 sensory end organs: the utricle, saccule and the 3 semicircular canals oriented perpendicular from each other. The semicircular canals code the motion for the angular acceleration while the translational or linear head acceleration is coded by the otolith organs. Age related deteriorating changes occur in each of these vestibular end organs as well as the central structures. The central structures constitute the vestibular nucleus, thalamic connections and the vestibular cortex. Below we discuss the age-related anatomical changes in the peripheral and the central vestibular structures.

Aging process of otoconia

The otolith organs: the utricle and the saccules are gravity sensors and code linear translations. The otoconia particles are the microstructures in the otoliths that cause shearing actions of the otolith hair cells and help in sensing translational head movements. Studies have shown significant reduction in the volume and density of otoconia, as an effects of aging in the macula of otolith organs in humans (Igarashi et al., 1993; Walther et al., 2008) and animals (Suzuki et al., 1997). Degenerative and morphological changes in the otoconia particles begin in the periphery of the otoliths (Rosenhall, 1973). Further dislocation of the dysmorphic otoconia particles occur from the saccule to the cochlea (Gussen, 1980) and from the utricle to the semicircular canals (Parnes & McClure, 1992;

Buckingham, 1999). Dislocation of the otoconia from the utricle to the semi-circular canals are suggested to be the cause of BPPV (Parnes & McClure, 1992; Buckingham, 1999). The incidence of BPPV have shown to be higher in elderly individuals (Balatsouras et al., 2018). The prevalence of BPPV increases with age, making elderly individuals more susceptible to falls. The incidence of BPPV is 7 times higher than those in the age range of 15-40 years (Liu et al., 2017; Von Brevern et al., 2007). Significant degradation in the otoconia density as an effects of aging is attributed to age related metabolic changes.

Suzuki et al. (1997) found significant decrease in the proportions of globular substances: precursor of otoconia in the aged mice suggesting reduced production of otoconia in aging population (Suzuki et al., 1997). Reduced production can cause changes in the density of otoconia resulting into its dislocation (Suzuki et al., 1997). Another possible cause of degeneration of otoconia as an effect of aging, could be the dysregulation of ionic components surrounding the otoconia: calcium dysregulation. Reports of altered vestibular functioning in individuals with osteoporosis strengthens the hypothesis of calcium dysregulation and its effects on vestibular functioning (Singh et al., 2018; Vibert et al., 2008). Osteoporosis and osteopenia are a disorder where the intestinal absorption of calcium is altered thereby, resulting in calcium dysregulation. Otoconia particles are basically calcium carbonate crystals, thus calcium dysregulation can cause otoconia degeneration. Osteoporosis or osteopenia are highly common in older adults and osteoporosis has been shown to have higher association with BPPV.

Whatever the cause of otoconia degeneration would be, dislocation of otoconia particles from the utricle and saccule mean a reduction in the mass component of the otoliths. Reduction in the mass component is inversely proportional to the resonant frequency of the system, therefore, changes in the density and volume of otoconia in the saccule and utricle are likely to increase the resonant frequency or frequency tuning of otolith organs. These changes are reflected in the vestibular assessment methods in older adults. Various studies utilizing cVEMP and oVEMP: that can assess the function of otoliths have shown an upward shift in the frequency tuning of saccule and utricle as an effect of aging for an air conduction stimulus. Recent study by Jha et al. (2022) made observations strengthening, the hypothesis that age related shift in the tuning of VEMPs might be emanating from the inner ear changes.

Aging and Hair cells

Histopathological studies have shown degenerative changes in the vestibular hair cells (Lopez-Escamez et al., 2005; Rauch et al., 2001; Richter, 1980; Rosenhall, 1973). Merchant et al. (2000) reported that the hair cell proportion in those with greater than 70 years decreased by 21% in the utricle, 24% in saccule and 40% in ampulla of the semi-circular canals. Decline in the hair cell count in the vestibular end organ have been reported by other studies has well (Lopez-Escamez et al., 2005; Rosenhall, 1973). The degeneration rate occurs faster for the type I hair cells compared to the type II hair cells (Anniko et al., 1984; Rauch et al., 2004a; Velázquez-Villaseñor et al., 2000). Anniko et al. (1994) hypothesized that type I hair cells have reached a higher degree of specialization. He further suggested that the morphologic decline to type I hair cells as an effect of aging, might be occurring due to actin based metabolic dysfunction. He also reported pathological changes in the calyx nerve endings which might be causing disproportionate loss of type I irregular afferents compared to type II afferents.

The type I hair cell have shown to phase lock up to the frequency of 1500 Hz for vibrations (Curthoys & Grant, 2015). Type I hair cells/ irregular neurons in the otoliths are shown to be activated by high frequency (500 Hz) lower levels of AC and BC stimulation (Curthoys et al., 2019). It has been shown that the irregular neurons in the semi-circular canals are only activated at very high levels. These differences in the larger sensitivity of type I hair cells in the otoliths but not for semi-circular canals, and type I hair cells susceptibility towards age related decline might be a plausible explanation of significant age-related decline seen on otolith examining tests (cVEMP and oVEMP) in middle aged adults but not on VOR examining tests (vHIT and Rotary chair examination). These are discussed in more details in the upcoming sections. Clinard et al. (2022a) recorded AMcVEMP and reported non-linearity in the form of harmonic distortions from young healthy adults (Clinard et al., 2022a). They attributed that the non-linear distortion of AMcVEMP might be coming from the type I hair cells preserving high degree of neural synchrony. Disproportional loss in the type I hair cell as an effects of aging might show poor neural synchrony in aging population and might also lead to absent AMcVEMP distortion products.

Aging and Scarpa ganglion neurons (Vestibular nerve)

Scarpa's ganglion neurons are bipolar neurons of the vestibular nerve with their cell bodies located in the vestibular nerve ganglions. The ganglion can be divided in its superior and inferior part. The superior part provides innervations from the utricle and the anterior semi-circular canal, and the inferior part contains nerve fibers from the posterior SCC and the saccule. Several studies have reported age related reduction in the number of Scarpa ganglion neurons (e.g. Park et al., 2001; Richter, 1980). Park et al. (2001) reported

20% decline in the number of neurons in individuals with increasing age after 4th decade. Richter reported Scarpa ganglion being roughly constant until mid-age with abrupt decline after that. Similar reports by Rosenhall, (1973) suggested that loss of sensory neurons begins at 40 years and follows a linear degeneration post that (Rosenhall, 1973). Moriyama et al. (2007) reported overall reduction in the transverse area of the vestibular nerve as an effect of aging (Moriyama et al., 2007). Valazquez et al., reported that the superior part of the ganglion is more affected than the inferior part. cVEMP and oVEMP assess the integrity of inferior and superior vestibular nerve respectively and studies have shown lower response rates for oVEMPs compared to cVEMPs in older adults (Velázquez-Villaseñor et al., 2000).

Aging and Vestibular nucleus

The vestibular nucleus is the junction in the brainstem where most afferent fibers from the vestibular hair cells terminate. The vestibular nucleus is a complex structure also receiving afferent and efferent connections from the cerebellum and the autonomic nervous system. The vestibular nucleus is divided into four major nuclear bodies: lateral, medial, superior and spinal. Age related neuronal loss have been reported reaching upto 40% loss in individuals beyond 75 years (Bergström, 1973). Age related accumulation of lipofuscin has been reported in the vestibular nuclei that could be fastening the process of neuronal apoptosis/cell death (Alvarez et al., 2000; Lopez-Escamez et al., 2005) observing up to 3% loss of neuron per decade beyond 40 years.

The histopathological age-related deterioration in the vestibular structures is well co-related with the available vestibular tests. The following section discusses the effects of aging on various vestibular tests.

Vestibular assessments in aging individuals

In the last 30 years, advancement in the field of vestibular science has made it possible to assess each of the vestibular end organs and the central vestibular structures. While the caloric, rotary chair examination and video head impulse test provides information about the functioning of the semicircular canals for different frequencies of head movements, the vestibular evoked myogenic potentials can assess the functioning of otolith organs namely oVEMP which assess utriculo-ocular pathways and cVEMP that assess sacculo-collic pathways. Nystagmographic examination helps in differentiating peripheral from central vestibular lesion (Kattah et al., 2009, 2020; Mantokoudis et al., 2021; Saber Tehrani et al., 2014).

Aging and Nystagmography

Nystagmographic examinations can act as a window to the brainstem and cerebellum and provide topographical information about the site of diagnostic lesions. Nystagmographic examination can differentially diagnose between peripheral vs central vestibular pathologies (Kattah et al., 2009; Kattah et al., 2020; Mantokoudis et al., 2015; Sankalia et al., 2021) and have been used in clinics since more than 50 years. Age related decline on nystagmographic examinations have been reported in the oculomotor, Dix Hall Pike, and caloric testing. Irving and colleagues reported age related decline in all the parameters of saccadic examination beyond 86 years of age. Peltch et al. (2006) reported that elderly individuals beyond 80 years exhibit slower initiation of saccade, more directional errors and slower reaction times. Some smooth pursuit and optokinetic studies have also reported reduction in gain in those greater than 70 years (Kanayama et al., 1994; Kato et al., 1995; Sharpe & Sylvester, 1978). Hajiaf et al. (2000), suggested presence of non-symptomatic vestibular anomaly and higher variability in terms of their responses, in elderly individuals. They emphasized a need for normative data for older adults to assess individuals with vestibular illnesses.

Aging and Semicircular canal assessments

Caloric examination that utilizes thermal stimulation to evoke vestibular responses have shown inconsistent results across age. While some studies showed increased slow phase velocity with increasing age (Jacobson et al., 2012; Maes et al., 2010), other studies have shown no effects of aging on warm and cool stimulation (Zapala et al., 2008; Peterka et al., 1990), and some studies have shown greater variability in both young and old adults (Davidson et al., 1988; Jacobson et al., 2018). One of the peculiar phenomenon seen with increasing age is failure to perceive sensation of movement during caloric examination (Chiarovano et al., 2016; Jacobson et al., 2018; Piker et al., 2020). Chiaravaon et al. (2016) described this as disassociated vestibular neglect: failing to perceive vertigo despite robust nystagmus post caloric stimulation. Vestibular neglects are reported in 1/4th of the patients and this behavior is predictive with age (Jacobson et al., 2018) and these individuals demonstrate greater postural instability (Piker et al., 2020). Failing to perceive vestibular stimulation may hinder detecting small bodily movements and make necessary corrections in body posture in daily bodily activities putting elderly individuals at higher risk for falls.

Rotary chair assesses the functioning of the semi-circular canals for low to mid frequency of head movements. Most clinical available instruments assess the functioning of lateral canals. Sinusoidal harmonic accelerations and step testing have been utilized to examine the effects of age on the functioning of lateral semicircular canals. Significant decrease in the slow phase velocity beyond 70 years have been reported (Wall et al., 1984;

Peterka et al., 1990). Paige (1994) observed that the age difference was see only for the lowest frequency of movements . At higher frequency of movements, they did not find any significant difference for the VOR gain. Similar observation was reported form a longitudinal examination of rotary chair examination over the period of 5 years in individuals beyond 75 years (Enrietto et al., 1999).

Video Head Impulse Test can assess the functioning of all the six semi-circular canals for high frequency head movements. The VOR gain that is the ratio of eye velocity to head velocity remains stable until the age of 70 years for head impulses less than 200 degrees/second (Li et al., 2015) and until 80 years with less than 160 degrees/second (McGarvie et al., 2015). Aging adults demonstrate significantly larger compensatory saccades compared to young adults (Anson et al., 2016) possibly due to cerebellar disinhibition (Walker & Zee, 2005) that might make older adults vulnerable for falls.

Otolith functioning using VEMPs

Otolith functioning can be assessed by vestibular evoked myogenic potentials (VEMPs). The potentials recorded from the cervical muscle: sternocleidomastoid, are called the cVEMP which assesses the integrity of sacculo-collic pathways. The responses recorded from the ocular muscles: inferior oblique assesses the integrity of the utriculo-ocular pathways. VEMPs are non-invasive tests and are generated by stimulating the otolith organ with an intense air conduction or bone conduction stimuli. VEMPs are robust, replicable, reliable, and unambiguous waveforms that are utilized clinically to assess the functioning of saccule and inferior vestibular nerve using cVEMP and utricle and superior vestibular nerve using oVEMP.

Transient VEMPs

VEMPs have typically been elicited using transient clicks or tone bursts. Transient evoked VEMPs have shown to be clinically useful in patients with various vestibular pathologies, including central vestibular disorders (Heide et al., 2010; Ito et al., 2001; Kim et al., 2014) and peripheral vestibular disorders (Brantberg et al., 2007; Rauch et al., 2004a; Taylor et al., 2012; Winters et al., 2011).

Peripheral Vestibular lesions and VEMPs

VEMPs have shown to be sensitive in patients with a peripheral vestibular lesion. There occurs an upward shift in the tuning frequency of VEMPs in patients with Meniere’s disease (Rauch et al., 2004a; Singh & Barman, 2016; Taylor et al., 2012; Winters et al., 2011). Utilizing the ratio of VEMP amplitudes at 1000 Hz and 500 Hz has shown to be useful in discerning Meniere’s disease (Maxwell et al., 2017; Singh & Barman, 2016). VEMPs have shown to have higher sensitivity and specificity in diagnosing superior semicircular dehiscence (Ward et al., 2017). VEMP thresholds have been shown to be highly reduced in patients with SSCD. The presence of response at 4kHz for VEMPs indicates SSCD. VEMPS has also been useful in demonstrating repaired dehiscence: AC and BC VEMP thresholds return to normal after canal plugging (Welgampola et al., 2008). As cVEMPs and oVEMPs assess the integrity of different branches of vestibular nerve, their conjunction can be useful in diagnosis of vestibular neuritis too. VEMPs have also shown to be clinically useful in monitoring recovery from vestibular neuritis as well. Following vestibular neuritis, complete absence of vestibular symptoms with absent VEMPs might be due to vestibular compensation but present re-emergence of VEMP after vestibular neuritis indicates recovery (Ochi et al., 2006).

Central vestibular disorders and VEMPs

VEMPs can only be useful in diagnosing central vestibular abnormality in cases with optimal peripheral system. Among the central structures, the cVEMP are usually absent when the lesion involves the vestibular nuclei, spinal accessory nucleus or if the lesion is in the areas of medial longitudinal fasciculus. VEMPs has also shown to be absent in patients with vestibular schwannoma (Taylor et al., 2015; Ushio et al., 2009). Presence of cVEMP and absence of oVEMP is indicative of a lesion above the vestibular nucleus. For eg. the probability of absent cVEMP is lower than the probability of absent cVEMP in patients with internuclear ophthalmoplegia (Kim et al., 2014). VEMPs have also shown to be absent in patients with initial stages of infarcts (Kim et al., 2014).

Aging, transient VEMPs and limitations

As with other vestibular end organs, significant age-related decline in the functioning of otolith organs have been shown using vestibular evoked myogenic potentials (Brantberg et al., 2007; Nguyen et al., 2010; Ochi & Ohashi, 2003; Piker et al., 2013; Singh & Firdose, 2018, 2021; Su et al., 2004; Welgampola & Colebatch, 2001; Jha et al., 2022). As age increases, the VEMP amplitude reduces, the threshold or the stimulus level needed to elicit VEMPs increases, and the response rates tend to decrease with increasing age (Piker et al., 2015). Another more robust phenomenon seen with increasing age is the upward shift in the tuning frequency of both cVEMP and oVEMPs (Piker et al., 2013; Singh & Firdose, 2018, 2021; Jha et al., 2022). Evidence suggests that 750 or 100 Hz could be a better stimulus to elicit VEMPs in older adults due to the stiffening changes and shift in the resonant frequency of inner ear. Thus, diagnosing Meniere's disease using inter frequency amplitude ratio for VEMPs, needs to be done with caution by taking the age factor into consideration.

While VEMPs are useful in diagnosing both peripheral as well as central vestibular pathologies when complemented with other vestibular tests, it gives very limited information about the pathophysiology of most vestibular disorders barring superior semicircular canal dehiscence. Moreover, despite the prominence of vestibular disorders in older adults relative to younger individuals, conventional transient cVEMPs have shown to have insubstantial utility in assessing older adults where responses are frequently absent (Piker et al., 2015). Conventional cVEMPs yield reliable onset response but do not give any information about the sustained/ongoing activity of the sacculo-collic pathways. Sustained responses are responses from the system to a periodically changing stimulus.

These responses have been reported for other sensory processes : Vision, somatosensory and auditory system. We will be discussing steady state response for the auditory system in the upcoming sections.

Auditory Steady state Sustained responses

The steady state responses for the auditory system were in its inception stage in the early 1960s and 70s (Geisler, 1960; Campbell et al., 1977). The first detailed description of ASSR came from Galambos and colleagues' work in 1981. The authors, recorded MLR using tone bursts at repetition rates between 3.3/sec to 55/sec in adults with normal hearing and a deaf child (Galambos et al., 1981). The authors found that for a repetition rate of 40/sec, overlapping positive and negative peaks occurred after every 25 msec interval. The authors observed similar response occurred for a 50/sec repetition rate repeating itself every 20 msec but the amplitude was reduced. They found that the amplitude was most robust at 40 Hz repetition rate. They also found that a 40 Hz response was absent in a deaf patient suggesting auditory origin of the response. They also reported that 40 Hz response had robust amplitude even at threshold level of stimulation, the findings that encouraged future research in utilizing 40 Hz responses in objective detection of hearing thresholds in difficult to test population group. Further, several investigators reported limitation of 40 Hz responses in pediatric population in terms of lower response amplitude (Stapells et al., 1988; Suzuki & Kobayashi, 1984) and the effects of sleep or anaesthesia (Cohen et al., 1991) on it. Later studies reported that a stimulus of >70 Hz repetition rate can overcome these limitations (Rance et al., 1995; Rickards et al., 1994).

ASSR stimulus

ASSR can be elicited using different types of stimuli that can be majorly divided into broadband and frequency specific stimuli. The broad band signal are the clicks, (used by Galambos et al., 1981) or noise which constitutes large range of frequencies. The other stimuli that are more frequency specific are amplitude modulated tones, and frequency modulated tones. Amplitude modulated tones are the most utilized stimuli to generate ASSR (Picton et al., 2003). Amplitude modulated tones are created when a primary sinusoid (carrier frequency) is modulated by secondary lower frequency sinusoid: modulation frequency (Lins et al., 1995). The amplitude of the carrier frequency changes over time (at the rate of modulation frequency). The degree of change in its amplitude is called the modulation depth. 100% modulation depth indicates that the amplitude of the CF is changing from maximum to zero in each cycle. If we look at the spectral content of a 100% modulated tones, we will see energy at the carrier frequency and its side bins (CF±MF). The stimulus is described in the method section in detail. Stimulus for ASSR have been presented using both air (D'haenens et al., 2008; Herdman & Stapells, 2003) and bone conduction (Small et al., 2007; Small & Stapells, 2006) modality and have shown to measure frequency specific hearing sensitivity reliably (D'haenens et al., 2008).

Utility of ASSRs

One of the major advantages of ASSR is in objectively measuring hearing thresholds in difficult to test populations. ASSRs studies have claimed to provide frequency specific and place (tonotopic) specific hearing threshold reliably (Herdman et al., 2002). Moreover, ASSR measures uses objective algorithms to detect presence or absence of response making the threshold estimate a more objective measure compared to transient (ABR) responses that is usually done through visual detection.

Nonlinearity

ASSR have shown to demonstrate non-linearity from the cochlea in the form of present responses at the harmonics of the modulation frequencies (Cebulla et al., 2006; Lins et al., 1995). The presence of harmonics in ASSR is as an outcome of cochlear compression and hair cell rectification (Lins et al., 1995; Oxenham et al., 2004). A recent study by Bidelman & Bhagat (2020) utilized amplitude modulated tones and reported non-linearity in sustained responses from the scalp recordings (brainstem) in the form of robust harmonic distortion products (Bidelman & Bhagat, 2020). The authors reported that the strength of ASSR non-linearity was directly correlated with the speech in noise perception scores. Individuals with reduced ASSR non-linearity showed poor speech in noise performance. The authors also recorded OAEs in the same group of participants and reported some similarity and difference in the cochlear non-linearity (measured through OAEs) and the brainstem non-linearity (measured using ASSR). ASSR non-linearity can thus provide additional information than obtained from cochlear non-linearity.

Phase coherence and neural synchrony

Auditory steady-state responses (ASSRs) can assess the functional integrity of auditory circuits and provide neural synchronization index (Lins et al., 1995; Regan 1982) across frequencies (Lins et al., 1995; Regan 1982). In ASSR, while, the carrier frequency provides information about the place specificity of the cochlea and auditory structures, the modulation frequency is the frequency at which the EEG is synchronized to fire. The synchrony of the auditory structure is measured using phase coherence that ranges from 0 to 1. A value closer to 1 indicates that the response is clustered at a constant phase angle of the stimulus. A value closer to zero indicates that response distribution is not locked to the phase of the stimulus suggesting poor neural synchrony. The phase coherence values decrease with lowering intensity levels. Utility of ASSR phase based measured have been clinically informative in patients with schizophrenia (Brenner et al., 2009; Kwon et al., 1999; O'donnell et al., 2013) and in comatose recovery patients (Binder et al., 2017). Patients with auditory neuropathy have shown to have poor ASSR thresholds compared to behavior thresholds suggesting ASSR could be a good tool identifying poor phase synchrony with intact detection ability in those with auditory neuropathy. In my readings, I could not find any study that utilized AM tones to record ASSR in patients with auditory neuropathy. ASSR phase coherence can provide a quantification of neural synchrony in individuals with auditory neuropathy that could be useful in predicting the benefits from implants or amplification in these rare population groups.

ASSR Response analyses

ASSR waveforms are usually analyzed in the frequency domain as compared to traditional time domain analyses (subjective peak picking) of amplitude and latency measures for AEPs. The interesting part of ASSR is that the stimulus contains energy at the carrier frequencies and at its side bins (CF±MF). There is no energy at the MFs in the stimulus, however the response contains energy at the modulation frequencies and harmonics. The ASSR analyses utilizes algorithms to measure presence or absence of responses with a significant degree of statistical accuracy. ASSR analyses are usually based on amplitude and phase-based measures.

Amplitude

The response energy (signal strength) is calculated from the FFT bin of the modulation frequency. The spectral width of the FFT bin is based on the stimulus duration. For e.g., an amplitude modulated signal of duration 1024 msec will have an FFT bin of 0.97 Hz (1/1.024). Sometimes, in addition to containing the response energy from the FFT bin of the modulation frequency, the bin might also contain energy at that frequency due to some noise sources (physiological, electrical artefact) that might probably overestimate/underestimate the true amplitude/response strength.

SNR

To measure Signal to Noise Ratio (SNR) the response energy (signal strength) is measured from the FFT bin of the MFs and the noise are estimated from the adjacent (background) side bins. The F test statistics permit this comparison at a significant level. For e.g. F statistic for comparing signal at MF to noise form 10 adjacent bins, reaches a significance value when the SNR is greater than 6.13 dB (John et al., 1998). The true ASSR is relatively stable in amplitude and its timing of response, therefore, averaging the responses reduce the overall noise levels, and improves signal to noise ratio.

Phase based measures

The ASSR response is expected to have a delay compared to the stimulus. The difference in the onset of the stimulus to the onset of the response is called the phase delay. If the onset of the response is occurring at the same phase angle of the stimulus each time (sweep), the response is said to have phase locked to the stimulus or have higher phase coherence values. Phase coherence values range from 0 to 1 and value closer to 1 means that the response is occurring at the same phase angle for each sweep (John & Picton, 2000). A circular polar plot shows the vector measure of the ASSR, where the length of the line from the origin gives the amplitude and the angle gives the phase of the response.

ASSR artifacts & Steady state response from the vestibular system

In ASSR because the stimulus and response overlap in time, the stimulus energy can alias to the modulation frequency energy and can be misinterpreted as a response. Several studies have reported artefacts of unknown origin at high presentation levels for AC and BC stimuli while recording ASSR (Gorga et al., 2004; Small & Stapells, 2004). Gorga and colleagues reported present ASSR responses at 20-22 dB below the level of equipment limit in patients who could not hear ASSR tones (behaviorally) even at maximum level of the equipment. Similar findings were reported by Small & Stapells (2004) where the authors utilized different A/D conversion rates and utilized alternating polarity to reduce the stimulus artefacts. Small and Stapells, reported that the alternating polarity and A/D conversion rate that is not a multiple of the carrier frequency (e.g. If CF = 500 Hz, A/D rate 1225 and not 1000) and is at least twice more than the maximum frequency content in the signal, can reduce the aliasing errors but not completely eliminate it. The authors suggested that the spurious ASSRs (not accounted for stimulus artefacts) they observed might be having physiological origin that might be non-auditory. A possible non-auditory contribution could be of vestibular origin (Rance, 2008)

Rance (2008) commented on the possibility of evoking vestibular responses while recording ASSR. The ASSR recordings is believed to involve rectification of sensory hair cells in the cochlea, is typically captured through surface electrodes placed on the scalp. It was proposed that certain ASSRs, unrelated to stimulus artifacts, may originate from the vestibular system, particularly when high-intensity stimuli at frequencies of 500 Hz and 1000 Hz are used, leading to the generation of myogenic responses. These vestibular-evoked myogenic responses, with a magnitude of approximately 100 microvolts, could potentially introduce contamination when recording ASSR due to volume conduction. With the motivation to explore if VEMP could be a possible source of ASSR contamination, Bell et al. (2010) recorded cVEMP using amplitude modulated signals (Amplitude modulated cVEMP: AMcVEMP). This was the first available report of recording AMcVEMPs (Bell et al., 2010).

Amplitude Modulated Cervical Vestibular Evoked Myogenic Potentials

Early Works

Bell et al. (2010) utilized a 500 Hz career frequency (CF) amplitude modulated (100%) using 5, 39, 59, 78, 98 and 122 Hz and presented the stimulus using air conduction modality at 96 dBA (LEQ). Not all participants had present responses at these levels (Bell et al., 2010). The authors reported robust amplitude for a broad range of modulation frequencies (39 to 122 Hz) recorded from sternocleidomastoid muscle. The maximum amplitude was seen at 39 Hz with an SNR being maximum at 78 Hz. The authors also found the AMcVEMP response to scale up with the increase in the EMG: consistent with myogenic responses. With a motivation to explore the role of vestibular response as a possible source of contamination while recording ASSR, the authors not only found that VEMPs could be a possible source of artefact during ASSR recording, but also laid foundation for recording vestibular responses with a novel (amplitude modulated) stimulus that has the potential to assess the sustained activity of sacculo-collic pathways.

Few years later, De Olivera et al. (2014) expanded on the work done by Bell et al. (2010). They studied the effects of career frequencies and modulation frequencies on AMcVEMP (Oliveira et al., 2014). Olivera and colleagues utilized three carrier frequencies: 250, 500 and 1000 Hz and modulation MFs between 20 and 80 Hz: 20, 37, 40, 43, 70, 77, and 80 Hz and presented them using air conducted modality. The authors found reliable and robust AMcVEMP responses for all three carrier frequencies with 500 CF eliciting largest amplitudes. Modulation frequencies 37, 40 and 43 Hz elicited the most robust response concurrent with the findings from Bell et al. (2010). The authors also reported presence of response in individual with profound sensorineural hearing loss eliminating any notions about contributions of auditory responses while recording AMcVEMP. The early works of Bell et al., De Olivera and colleagues, showed that AMcVEMP not only shares the basic characteristics of transient cVEMPs (direct relation with EMG and present in those with profound hearing loss) but can also provide advantageous sustained response information from the vestibular system. However, there were limited number of studies utilizing AMcVEMP, until recently.

Air conducted AMcVEMP concerns and limitations

Despite the advantage of AMcVEMP to provide information about the sustained activity, the major concerns with using air conducted AMcVEMP could be of noise exposure. Both the previous studies have utilized air conduction modality to record AMcVEMP (Bell et al., 2010; Oliveira et al., 2014). For a stimulus of 1024 msec (duration for AMcVEMP stimulus), being presented 132 times, the maximum safe level for presentation is 108 dBA (NIOSH recommendation for a stimulus of 132 sweeps*1.024s = 2min 16 sec < 2 min 22 seconds). A more conservative stimulus level would be 107 dBA (allowable duration, 2 min 59 sec).

Data from Romero et al. (2021: Completed PhD Thesis), showed transient AC responses were not being reliably detected in healthy young individuals for intensity below 110 dB pSPL (90dBA) for 500 Hz tone bursts. For a tone burst of 500 Hz, the values in pSPL are 21 dB higher than values in dBA (Bell et al., 2010). AMcVEMP thresholds are elicited at 3.7 dBA higher compared to 500 Hz TB (Bell et al., 2010), thus, we speculate that we may not be able to reliably detect AMcVEMP below 94 dBA. Olivera et al. (2014) presented their AMcVEMP stimulus at 95 dBA but do not provide details for response rates at that intensity. Bell et al. (2010) presented stimuli at 96 dBA but, not all of their participants had present responses at 96 dBA. Further increasing the level of the stimulus to 100 dBA elicited reliable AMcVEMP detection in all participants (Bell et al., 2010). Thus, in our extrapolated conclusion, the level at which AMcVEMP can reliably be elicited using air conduction modality and yet be kept safe would be between 100 dBA (Bell et al., 2010), and the maximum level that can be presented for the duration is 107 dBA. The range of presentation levels for AMcVEMP here is very small and doesn't provide enough room to study various nuances of AMcVEMP responses. Moreover, AC VEMPs are often absent in older adults (Piker et al., 2015), in addition to inner ear changes, complete absence of VEMPs in older adults could also be due to the air conduction stimulation not being intense enough. Resorting to BC stimulus for eliciting AMcVEMP could be a possible alternative.

Bone conduction vs Air conduction AMcVEMP

Resorting to bone conduction stimulus could be an alternative way to record AMcVEMP. Bone conducted stimulus delivery can elicit VEMP at lower levels compared to AC stimulation. Patterson et al. (2021) presented stimulus using air conduction and bone conduction modality and recorded transient cVEMPs among individuals across different age groups. They reported similar response rates for air conduction stimuli at 125 dBSPL and bone conduction stimuli at 75 dBnHL (105 dBSPL). Moreover, the response rates for transient cVEMPs are higher for bone conducted VEMPs as they are not affected by conductive hearing loss (Mahdi et al., 2013). McNerny and Burkard, (2011) showed that BC stimulus elicits significantly larger cVEMP amplitudes compared to AC tone bursts at equivalent intensity levels (dBSPL/dBFL). At intensities beyond 105 dBFL/dBSPL, the amplitude for BC stimulation was approximately 1.5 times to 2.5 times larger (Mcnerney & Burkard, 2011) compared to AC VEMPs. In the same study, participants having absent responses for AC stimulation showed present responses at 20 dB lover levels for BC stimuli. BC stimuli is likely to increase the presence of responses rates among older adults in whom air conduction stimuli are frequently absent. Speculating based on the findings of transient cVEMPs, bone conducted AMcVEMP might be elicited at lower levels. Recently, studies have utilized bone conduction stimulation at 500 Hz to elicit AMcVEMP and have reported AMcVEMP responses from 45 dBHL to 65 dBHL (96 to 106 dBpFL) in healthy young adults without causing any discomfort to the participants. The BC AMcVEMP stimulus might not be putting the cochlea at risk for noise exposure.

AMcVEMP using bone conducted stimuli

Clinard et al. (2020) utilized bone conduction (BC) stimulation and systematically examined the effects of EMG activation in eliciting AMcVEMP for a 500 Hz carrier frequency modulated at 37 Hz. In young, healthy females, the authors found that the AMcVEMP amplitude increases with increasing EMG. The measures of AMcVEMP response: SNR, phase coherence, and EMG corrected amplitudes reach their maximum values at 30 µV EMG activation (Clinard et al., 2020). The authors suggested that higher levels of EMG activation are not needed to elicit robust AMcVEMP. The authors also reported about the asymmetry of AMcVEMP responses across different measures. Interaural asymmetry ratio is the most clinically used measure for diagnosis of unilateral vestibular pathologies. The authors reported smaller range for interaural asymmetry for SNR and phase coherence compared to amplitude measures, alluding SNR and phase coherence to be a more sensitive and specific measures than amplitude measure, in the diagnosis of unilateral vestibular pathologies. Future studies, utilizing AMcVEMP phase coherence and SNR measure in patients with known vestibular pathology could help in generalizing the findings from young female adults. The authors also provided some evidence of vestibular non-linearity in the form of harmonic distortion consistent with the non-linear rectification of otolith hair cells. The findings from the study opened areas for further explorations.

Vestibular non-linearity

As with the auditory system, vestibular hair cells have also shown non-linear behaviors in animals in the form of rectification (Levin & Holt, 2012), distortion products (Jaramillo et al., 1988) and vestibular micro-phonics (Eatock, et al., 1987). Studies from the bullfrog saccule have shown inwardly rectifying currents in saccular hair cells. Jaramilo et al. (1994) reported exertion of force at the vestibular hair cell at inter-modulated distortion products (2f1-f2, 2f2-f1) in addition to the harmonics (2f1, 3f1). Evidence of non-linearity in the form of half wave rectification and polarity reversals have also been reported from otoliths and semi-circular canals. The presence of non-linearity in animal models is attributed to the gating compliance of the vestibular hair cells. Similar mechanisms may exist in humans' as well, however, due to the limitations in available methodologies to assess human vestibular system, no non-invasive method has reported non-linear behavior in human vestibular end organs until recently. <u>Clinard et al. (2020) was the first study to demonstrate vestibular non-linearity in the form of rectification from human vestibular end organs</u>. Further studies from their lab delineate this aspect in detail.

AMcVEMP & Vestibular non-linearity in humans

Recently, Clinard et al. (2022) provided a detailed spectral analysis of AMcVEMP responses for a range of modulation frequencies and at their harmonics from 7 Hz to 407 Hz (Clinard et al., 2022). All their participants (young healthy) had robust responses at the modulation frequencies and the harmonics of modulation frequencies consistent with saccular hair cell rectification. The authors attributed generation of responses at the harmonics to be emanating from the irregular hair cells surrounding the striola. The authors further discussed that the dense calyx around the type I hair cells might be contributing to the high degree of neural synchrony seen in the form of high degree of phase coherence for AMcVEMP responses. The study illustrated the first non-invasive means to study vestibular non-linearity for a range of modulation frequency in humans. The outcome from the methods used in the study can bridge our understanding between animal studies of otoliths non-linearity and vestibular non-linearity in humans. Aging studies with similar methodologies will help in determine any non-linear changes in the otolith functioning as an effect of aging. Aging has shown to cause a faster decline in the type I hair cells compared to the type II hair cells. AMcVEMP harmonic distortion is postulated to be occurring from the type I hair cells (Clinard et al., 2022). Thus, aging is likely to alter the non-linear characteristic of AMcVEMP seen in young adults.

AMcVEMP Temporal modulation transfer function

Recently, Lawlor et al., 2022 reported AMcVEMP temporal modulation transfer function in healthy young adults using a 500 Hz career frequency for amplitude, signal to noise ratio and phase coherence measures (Lawlor et al., 2022). The authors reported differences in the TMTF shape across different measures. The amplitude TMTF had a sharp peak (29 – 37 Hz), while signal-to-noise ratio (17 to 127 Hz) and phase coherence TMTFs (17 to 143 Hz) had broader shapes with plateaus across a range of modulation frequencies. The narrower TMTF for the amplitude measure peaked at the 37 Hz modulation frequency. The modelling study by Wit and Kingma, (2006) showed that the spectral content of motor unit action potential from the surface electrodes over the sternocleidomastoid muscle peaks around 40 Hz. Wit and Kingma’s spectral analyses of MUAP of was from the muscle contraction before the stimulus was presented (Wit & Kingma, 2006). Similar findings were reported in the detailed MUAP contribution in VEMP measurements (Lütkenhöner & Basel, 2011). The AMcVEMP analyses in Lawlor et al., 2022’s paper was based on the ASSR based analyses for the amplitude, SNR and phase, discussed previously. The amplitude was calculated from the FFT bin of the MFs. The amplitude in the FFT bin of 37 Hz might have higher contributions from the MUAP action potentials centered around 40 Hz. This might explain the peaking of amplitude measures of AMcVEMP TMTF around 40 Hz modulation frequencies. The AMcVEMP TMTF for the SNR measure had a broader response range where the amplitude was measured at the FFT bin of modulation frequency, and the noise was measured from the bins surrounding the FFT bin. Broader TMTF for the SNR and phase coherence measures supports the understanding that human otolith organs are capable of coding translational head acceleration for slower as well as faster head movements to maintain postural stability. The authors established the frequency limit of AMcVEMP TMTF to be from 11 Hz to 287 Hz in young healthy adults.

Objective and specific aims

AMcVEMP is a highly reliable technique (Clinard et al., 2021) with potentials to advance our understanding of vestibular physiology. So far, AMcVEMP assessed the integrity of sacculo-collic pathway in young healthy adults. The AMcVEMP responses share the same basic properties as that of transient cVEMPs that is, AMcVEMP is seen in individuals with profound hearing loss and the amplitude of AMcVEMP increases with increase in EMG. Compared to transient cVEMP responses, AMcVEMP responses can provide additional information: phase coherence, non-linearity of vestibular system and the range and shape of AMcVEMP temporal modulation transfer function. Assessing changes in the characteristics of AMcVEMP as an effects of aging could advance our overall understanding of the aging vestibular system.

Previously we discussed that aging causes structural and functional changes in the entire vestibular system including the sacculo-collic pathways. As AMcVEMP assess the sacculo-collic pathways: starting from the vestibular end organ (saccule) to the vestibular nucleus, to the spinal accessory nucleus and then the innervations to the sternocleidomastoid muscle, age related changes at any point in these junctions are likely to affect AMcVEMP. As AMcVEMP is postulated to be originating from the type I vestibular hair cells (Clinard et al., 2022a) and age-related decline results in disproportionate loss of type I hair cells, AMcVEMP might show altered findings in aging populations.

The overall aim of the current study is to study the effects of aging on the AMcVEMP temporal modulation transfer function (TMTF) with the following specific aims.

1. Examine the effects of age on AMcVEMP temporal modulation transfer function for amplitude, SNR and phase coherence.
2. Determine the limit and shape of AMcVEMP for various metrics.
3. Characterize non-linear harmonic distortion for AMcVEMP in each age groups.

Chapter III:

Method

Methodology:

Participants

A total of 49 participants were included in this study. The participants were divided into three age groups: young (20-39 years), middle-age (40-59 years), and older (>60 years) adults. All the participants included in the current had a negative history of middle ear pathology and had a normal middle ear functioning which was confirmed using pure tone audiometry and immittance evaluation. Even though the proposed study utilized a bone conduction stimulus, participants with an air-bone gap of greater than 10 dB at any frequency were excluded from the study due to the possible history/presence of otologic pathology. Participants with a history of balance complaints, otologic, or neurological illness were excluded from the study. The subjective questionnaire described by Furman and Cass, (2010) was utilized to exclude any participants with a history of vestibular or neurological complaints.

Stimuli

We utilized amplitude modulated (AM) tones with a carrier frequency (CF) of 500 Hz. The carrier frequency was 100% modulated with 10 different modulation frequencies (MF): 11, 17, 23, 37, 53, 79, 113, 173, 263, 397 Hz. The modulation frequencies covered the range of AMcVEMP in young adults (Lawlor et al., 2022), and based on our preliminary investigation, we could assume that these might encompass middle age and older adults' range of frequencies. The amplitude modulated tone was generated in MATLAB and was presented using bone B81 at the mastoid. The order of presentation of the modulated tone was randomized for each participant to eliminate any effect of order. The duration of the tone was 1024 msec. The stimuli were delivered to the mastoid, 3 cm posterior and 2 cm superior to the external auditory meatus (Rosengren, 2005), at 130.24 dBpFL using Radioear B81 bone vibrator. The bone conduction stimuli will be calibrated with a Larson Davis AMC493B artificial mastoid, 6cc coupler and 4-5 N weight using a Larson Davis sound level meter. The amplitude modulated stimulus contained the energy at the carrier frequency and at its adjacent side bins. For example, an amplitude modulated stimulus with CF 500 Hz and MF: 37 Hz contained energy at the carrier frequency: 500 Hz and at its adjacent side bins: 500 ± 37: 463 and 537 Hz (See Figure 2.1).

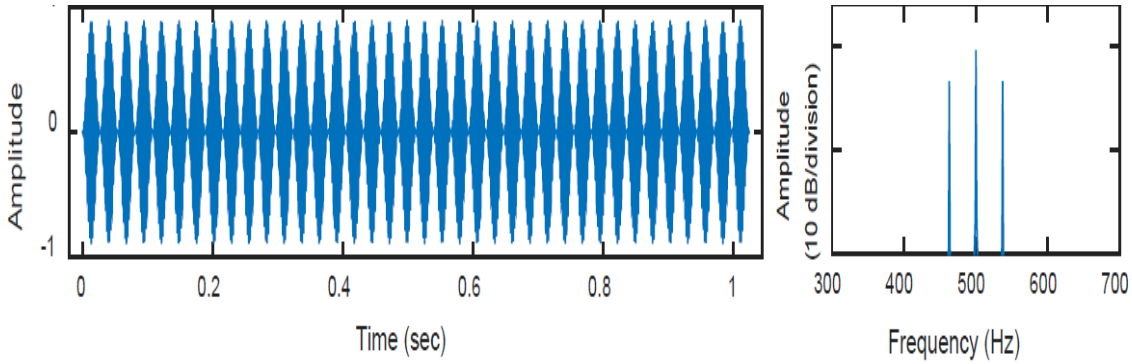

Figure 2.1: Amplitude modulated tone with a CF:500 Hz, 100% modulated using 37 Hz. The left panel shows the waveform of the stimulus in the time domain, the right panel shows the spectral content of the AM stimulus which has energy at the CFs and at its adjacent side bins. The intensity of the adjacent side bins is 3 dB lower than the energy at the CF.

Carrier frequency: 500 Hz

A carrier frequency of 500 Hz was chosen to achieve the objectives of the study. Previous studies on AMcVEMP have all used 500 Hz as its carrier frequency. This allows better comparison. A 250 Hz CF could have been another option for our carrier frequency, however the commonly used B81 transducers are limited in terms of their maximum output. A newly available bone conduction transducers could allow for utilizing 250 CF at higher levels. It may be possible that 250 Hz or even frequencies lower than 250 Hz (eg. 100 Hz) could be a better vibratory stimulus compared to 500 Hz to elicit AMcVEMP. Todd et al., 2009 showed that the otoliths are tuned for lower frequency tone bursts (100 Hz) for vibratory stimuli. Comparing the effects of carrier frequencies for amplitude modulated cVEMP would be a very interesting endeavor but these objectives were beyond the scope of this project and therefore we chose to use 500 Hz carrier frequency that have been used before.

Modulation frequencies

The modulation frequencies: 11, 17, 23, 37, 53, 79, 113, 173, 263, 397 Hz were precisely chosen using coherent sampling for a duration of 1024 msec. Coherent sampling allowed the number of cycles to fit into the predefined sampling window. For example, the 37 Hz MF was actually 37.10938, which completed 540 cycles within the sampling rate of 20,000. Using 37.10938 instead of 37 Hz allowed the stimulus to complete a full cycle of modulation without clipping. Similarly, other modulation frequencies were also chosen using coherent sampling, approximated to their closest whole numbers. For instance, 53 Hz was actually 53.60355. The modulation frequencies were logarithmically spaced (MF2\MF1 ≈ 1.5) and fell within the limits of the AMcVEMP temporal modulation transfer function established for young adults (Lawlor et al., 2022).

Why BC stimulus?

Bone conducted stimulus delivery can elicit VEMP at lower levels compared to air conducted (AC) stimulation (McNerny & Burkard., 2011; Mahdi et al., 2013; Patterson et al., 2021), thereby reducing the risk of noise exposure. Also, response rates are higher for BC stimulus (Mahdi et al. 2013) probably because the thresholds for BC stimulus are lower. Moreover, BC stimulus elicits significantly larger cVEMP amplitudes compared to AC tone bursts at equivalent intensity levels: dBSPL/dBFL (McNerny & Burkard., 2011)

Recording:

The stimulus was delivered using the Neuroscan Stim2 system and the response was acquired using Curry software. The recordings were made using a single channel: the ground electrode was placed at the Fpz, the non-inverting electrode at the belly of the ipsilateral sternocleidomastoid, and the inverting electrode at the sternoclavicular junction. The ear of stimulation was randomly chosen. A 5-3000 Hz band pass filter was used, and the recording for each modulation frequency consisted of 132 sweeps lasting approximately 2.75 minutes. The inter-stimulus duration was 1233.33 msec.

The participant was asked to turn their head away from the muscle of recording and maintain muscle tension generating 50 µV, while the stimuli were being delivered. Electromyographic (EMG) monitoring was performed throughout each recording. Participants viewed a live, real-time bar graph and were instructed to maintain the muscle tension adequate enough to reach the target level of 50 microvolt, considered sufficient to generate the AMcVEMP response (Clinard et al., 2020). EMG data was passed from Curry software to MATLAB to enable this monitoring; this monitoring interface used custom MATLAB programming. Prior to beginning the first recording, participants practiced reaching the EMG target to become familiar with activating their sternocleidomastoid muscle adequately. Figure 2.2 provided a schematic of the AMcVEMP procedure used in the proposed study.

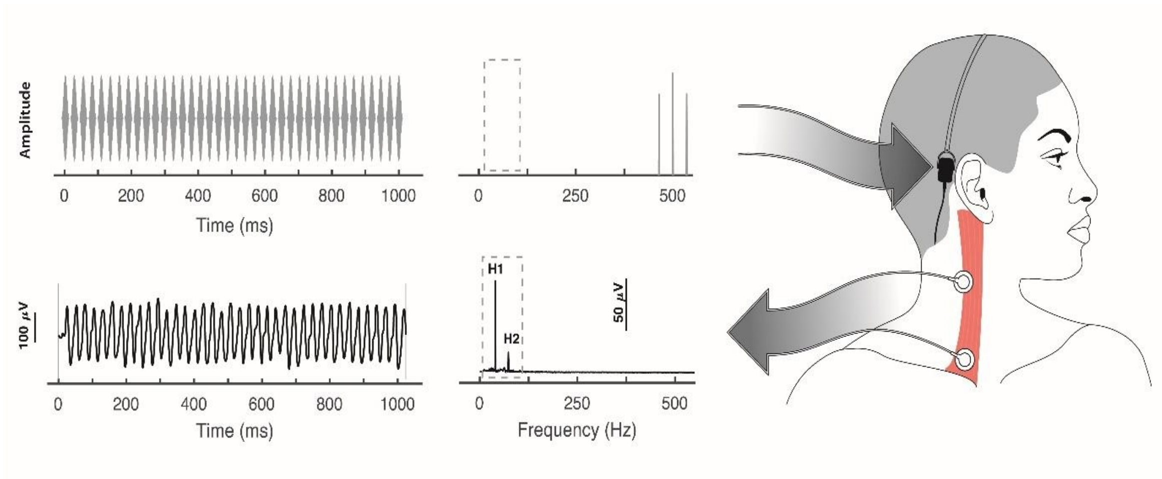

Figure 2.2. Schematic of AMcVEMP procedure. In the figure, career frequency of 500 Hz and modulation frequency of 37 Hz are used as examples to illustrate the AMcVEMP procedure to be used in the proposed study. An amplitude modulated tone is delivered to the ear via a bone vibrator. The AM tone (stimulus) contains energy at carrier frequency and sidebands (537 and 463Hz): 500 Hz ± modulation frequency, but the response contains energy at the modulation frequency (37 Hz) and its harmonics(74 Hz) .

Controlling for Artefacts

We ensured that there was no presence of stimulus artefacts. We used a sampling rate of 20,000 that ensured that there was no aliasing of the carrier frequency to the modulation frequency (Small & Stapells, 2004). Our stimulus did not contain any energy at the modulation frequency or at its harmonics, while the response was measured at the modulation frequencies and at its harmonics. Our control recordings, in which the B81 was separated from the skull, elicited no response for AMcVEMP, confirming the absence of stimulus artifacts in our recordings. Additionally, AMcVEMP at with no head turn (0 µV EMG) resulted in no responses, suggesting that the response was physiological and not artifact based.

AMcVEMP Analyses:

The AMcVEMP recordings were analyzed for amplitude, SNR and phase coherence measures at all modulation frequencies and their harmonics (H2 through H4). Established ASSR analysis methods (Dobie and Wilson, 1989; John et al., 1998) were utilized.

For amplitude and SNR analyses, the AMcVEMP sweep averaged waveform for each participant was subjected to Fast Fourier Transform (FFT). The amplitude (energy) was extracted from the FFT bin corresponding to the modulation frequency and its harmonics (H2 to H4). Each bin represented a width of 0.97 Hz (based on the 1/1024 ms stimulus). To estimate noise, the amplitudes of FFT bins surrounding the modulation frequency were averaged over a range of ±5 Hz. Signal-to-noise ratios were calculated using these amplitude and noise estimates. Objective response detection was determined using the SNR as an F-ratio with 2,10 degrees of freedom (Dobie and Wilson, 1989). The F-test statistics allowed comparison at a significant level. For example, a significance value was reached when the SNR exceeded 6.13 dB (Clinard et al., 2022), indicating significantly larger signal at modulation frequency compared to 10 adjacent frequencies.

Regarding phase coherence, the AMcVEMP response was expected to exhibit a delay compared to the stimulus. The difference between the stimulus onset and the response onset was referred to as the phase delay. If the response consistently occurred at the same phase angle of the stimulus for each sweep, it was considered to be perfectly phase locked, or to have higher phase coherence values. Phase coherence values ranged from 0 to 1, with values closer to 1 indicating that the response occurred at the same phase angle for each sweep. Circular plot was used, and the vector measure (representing the amplitude) of the AMcVEMP, was the line's length from the origin, and the phase angle represented the phase of the response. The Rayleigh test was employed with an alpha level of 0.05 for statistical response detection (Fisher, 1993).

Statistical analysis:

Specific aim 1: Effects of age on AMcVEMP TMTF

In order to investigate how age affects AMcVEMP TMTF (Temporal Modulation Transfer Function), a mixed factorial repeated measures ANOVA was conducted. The analysis included three groups as between factors and ten modulation frequencies as within-group factors. The main effects of groups and modulation frequencies, as well as the interactions between groups and modulation frequencies, were examined. Further, in each age group, one-way RMANOVA was administered, and Tukey’s HSD test was used to identify specific significant differences between pairs. Spearman’s correlation was also administered to assess any significant correlation between age as a continuous variable vs AMcVEMP measures: Amplitude, SNR and phase coherence.

Specific aim 2: Determine the limit and shape of AMcVEMP.

The limit of AMcVEMP TMTF have been reported in three ways: 1. Based on the average response rates, for SNR and phase coherence. 2. Based on the modulation frequencies at which the response rate is greater than 75%. The limit based on 75% response rate is reported for both SNR and phase coherence. 3. Finally, modulation gain was calculated using the formula: Modulation Gain (dB) = 20 Log (10) of (PC/depth of AM), and the limit of AMcVEMP TMTF was also determined using the averaged modulation gains in each groups of participants. We also report the modulation frequency for which the average modulation gain was greater than -10 dB.

Specific Aim 3: Characterize non-linear harmonic distortions in AMcVEMP

To characterize non-linearity, we reported the amplitude, SNR and phase coherence TMTF (Temporal Modulation Transfer Function) across harmonics H1 through H4. We also reported the response rates across harmonics based on the criteria of SNR > 6.13 dB and phase coherence > 0.155. Additionally, we calculated the total harmonic response by summing the values from H2 through H4 for amplitude, SNR, and phase coherence measures. Subsequently, we examined the effects of age on the total harmonic response using mixed factorial repeated measures ANOVA. Further one-way RMANOVA was performed for each age group and Tukey's HSD post hoc analyses used to compare difference between groups.

Chapter IV

Results

The specific aims of this study were to

1. Examine the effects of age on AMcVEMP temporal modulation transfer function for amplitude, SNR and phase coherence.
2. Determine the limit and shape of AMcVEMP for various metrics.
3. Characterize non-linear harmonic distortion for AMcVEMP in each age groups.

We are reporting results from a total of 49 participants divided in three groups: Young (N =16; age range: 20-39 years), Mid-age (N = 17; age range: 40-59 years) and older adults (N = 16; age range: 60-75 years). The age distribution of participants in each group is shown in figure 4.1 and table 4.1.

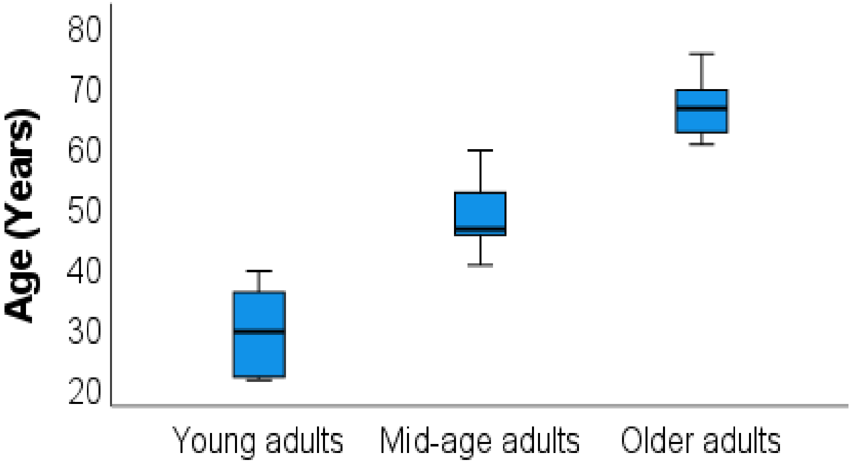

Figure 4.1. Box plots showing distribution of age in each groups. The thick center line in the box plot represents the median. The box represents the 25th to 75th percentile of the data. The whiskers show the 95% CI.

Table 4.1. Descriptive statistics for age distribution of participants

	Mean	Median	SD	range
Young (N = 16)	28.9	29	7.3	20-39
Mid-age (N = 17)	48.3	46	5.9	40-59
Older (N = 16)	66.1	66	4.2	60-75

Note: N = number of participants, SD = Standard deviations

AMcVEMP response waveforms and analyses:

AMcVEMP was recorded from surface electrodes using 10 different stimuli. Overall, the AMcVEMP waveforms showed a clear demarcation between onset, sustained and offset portion of the response (figure 4.2) consistent with what is seen for auditory amplitude-modulated responses.

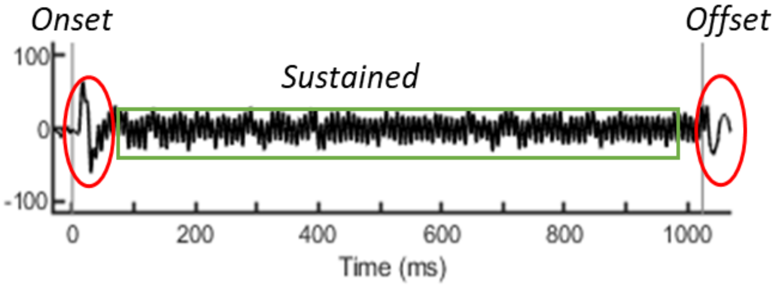

Figure 4.2 AMcVEMP showing onset, sustained and offset response at 113 Hz modulation frequency. We see an onset, sustained and offset portion in the response just as seen with auditory amplitude modulated responses. The sustained portion of the response has the same periodicity as that of the modulation frequency.

Grand averaged waveforms

We recorded AMcVEMP in three age groups. Figure 4.3 shows the grand averaged AMcVEMP waveforms of all the participants for each age groups (16 young, 17 mid-age and 16 older adults). The AMcVEMP waveform follows the same periodicity of the modulation frequency. This periodicity can be seen very clearly (see figure 4.3) upto 79 Hz modulation frequency. There occurs a clear demarcation between the positive peaked onset, prolonged sustained response and a negative peaked offset at 79 Hz and above. The demarcation between onset, sustained and offset portions can be seen clearly in the averaged waveforms for the young and midage-adults. For the older adults, this differentiation is difficult to view in the averaged waveforms as some participants in the older group didn’t have robust AMcVEMP. Figure 4.4,4.5 and 4.6 shows responses for 23-

113 Hz modulation frequencies, from a representative young, midage and an older adult. The response is shown in the time domain, frequency domain, and using polar plot. In the time domain waveform, the onset, sustained and offset portions of the response can be seen. The frequency domain (FFT of the time domain waveform) provides information about the responses at the modulation frequencies and their harmonics. The polar plot gives information about the phase coherence.

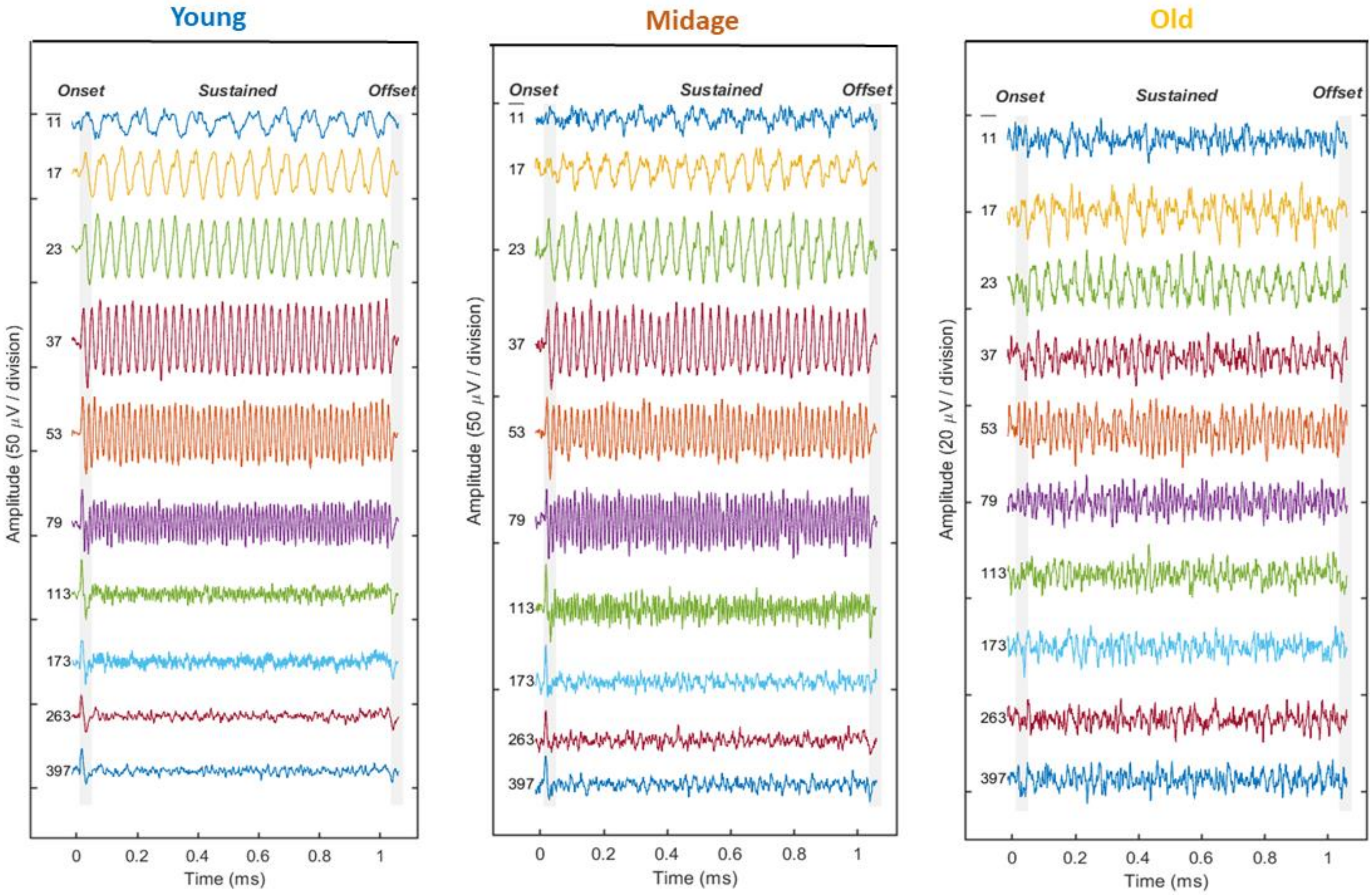

Figure 4.3. Grand averaged AMcVEMP response for three age groups (left:young (n=16), center:midage (n=17), right:older adults (n=16). In each column, the AMcVEMP waveforms across modulation frequencies (11,17,23,37,53,79,113,173,263,397) are plotted one below the other. Each panel demostrates onset, sustained and offset marking. The onset, sustained and the offset demarcation are more clearer for the young and midage adult

Young adults' representative response:

The AMcVEMP waveform from a 22-year participant (figure 4.4) shows good periodicity for lower modulation frequency (23, 37 and 53 Hz) and clear differentiation between onset, sustained and offset response for the higher modulation frequencies (e.g., 79 and 113 Hz). The FFT shows robust non-linear harmonic distortion from H2 through H4. The polar plot shows phase coherence values >0.97.

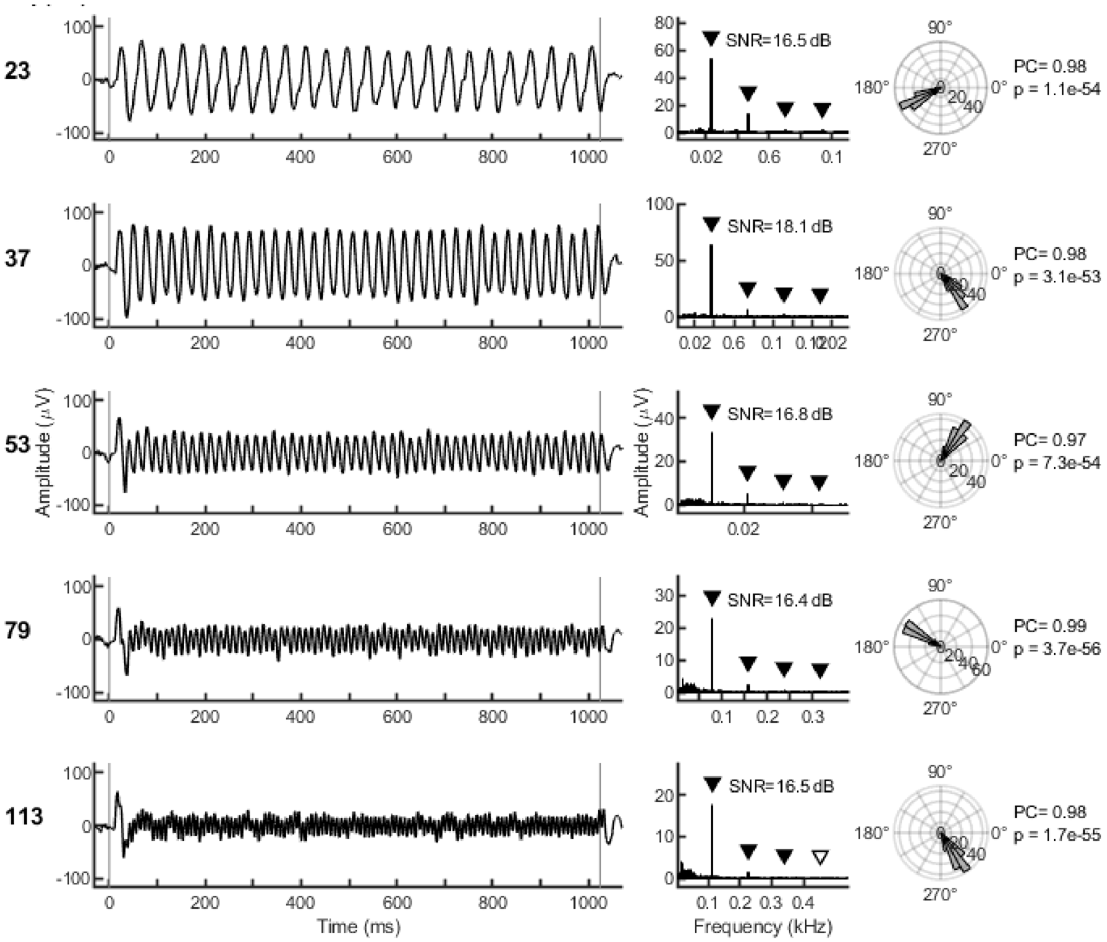

Figure 4.4. Representative AMcVEMP responses from a 22years young adult at 5 modulation frequencies organized in rows. Left column: AMcVEMP waveforms in time domain, Middle columns: FFT of the waveforms in the left column; filled triangles represent present responses and open triangles represent absent responses. The SNR is shown in each panel. Present responses can be seen at the MF, 2*MF, 3*MF and 4*MF (see filled triangles) for 23-79 Hz. Right column: Phase coherence, corresponding p values shown in polar histogram.

Mid-age adults' representative response

Representative AMcVEMP waveforms (23-113 Hz) from a 44 years midage participant is shown in the figure 4.5 The time domain waveform appears to have lesser periodicity and reduced amplitude compared to the young adult (figure 4.4). FFT shows robust non-linear harmonics only at H1 and H2 from 23-79 Hz, while the younger adults had robust responses upto H4 (see figure 4.4). The polar plot shows high phase coherence values > 0.8 (23-79 Hz). The PC values are lower than what is seen for the young adult.

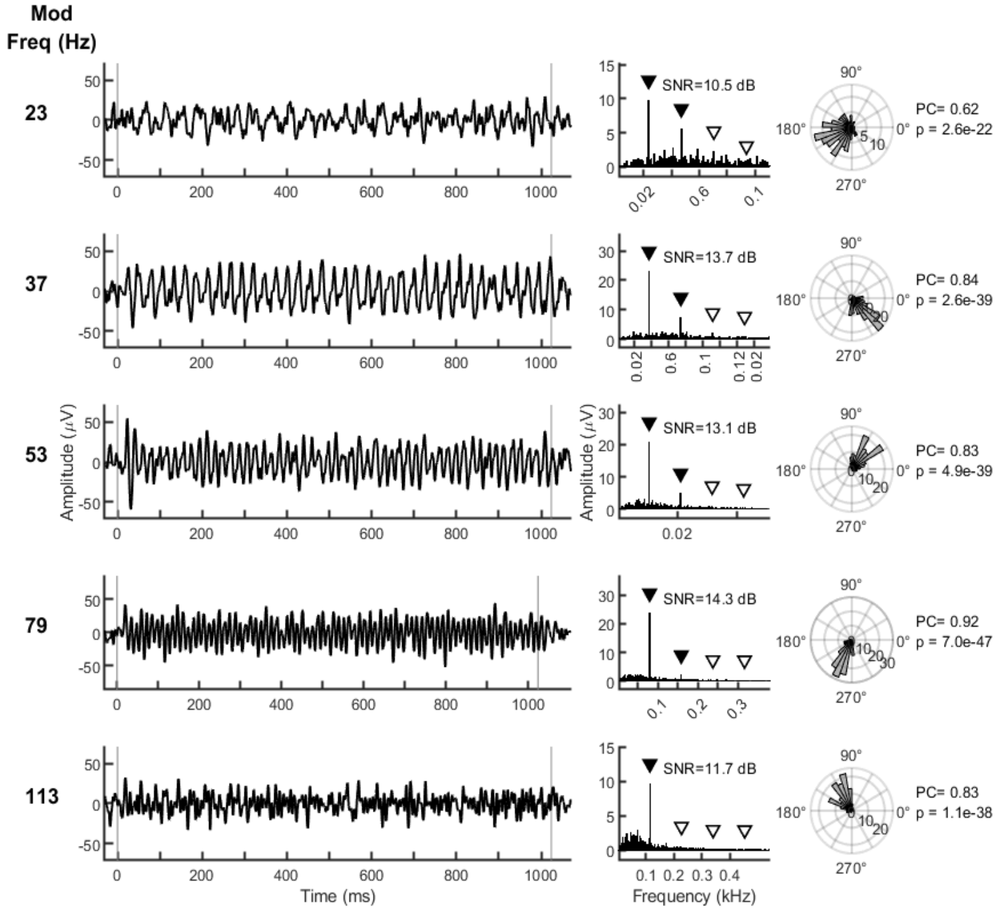

Figure 4.5. Responses from a 44-year midage adult at 5 Modulation frequencies organized in rows. Left column: AMcVEMP waveforms in time domain, Middle columns: FFT of the waveforms in the left column; filled triangles represent present responses and open triangles represent absent responses. The SNR is shown in each panel. Present responses can be seen at the MF, and 2*MF (see filled triangles) for 23-79 Hz. Right column: Phase coherence and corresponding p values shown in each corresponding polar histogram.

Older adults' representative response

Representative AMcVEMP waveforms (23-113 Hz) from a 63-year older adult is shown in figure 4.6. The time domain waveform appears to have lesser periodicity and lowered amplitude compared to what is seen for the young adult (figure 4.3). FFT shows responses only at H1 and H2 at 53, and 79 Hz opposed to H1 through H4 responses seen in young adults: indicating reduced non-linearity in older adults. The polar plot shows moderate phase coherence values around 0.4 excpet at 79 Hz that has a PC of 0.93. The PC values across modulation frequencies are lower than what is seen for the young and the midage adult.

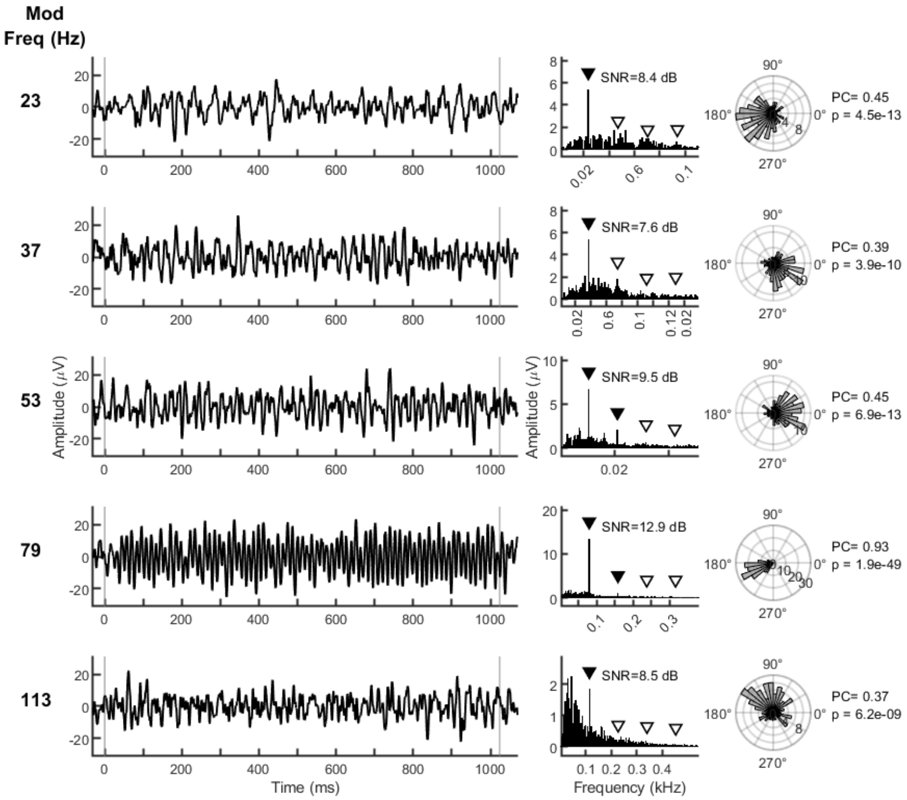

Figure 4.6. Representative AMcVEMP responses from a 63-year older adult at 5 Modulation frequencies organized in rows. Left column: AMcVEMP waveforms in time domain, Middle columns: FFT of the waveforms in the left column; filled triangles represent present responses and open triangles represent absent responses. The SNR is shown in each panel. Present responses can be seen at the MF, and 2*MF (see filled triangles) only at 53 and 79 Hz. At other modulation frequencies the responses is not seen at the harmonics. Right column: Phase coherence and corresponding p values shown in each corresponding polar histogram.

Specific aim 1: Effects of age on AMcVEMP Temporal Modulation Transfer Function

The effects of age were examined for the AMcVEMP amplitude (uncorrected and EMG corrected), signal to noise ratio (SNR), and phase coherence measures. Mixed factorial RMANOVA (with age groups as between factor and modulation frequencies as within factor) was performed to examine the overall effects of age group, modulation frequencies and any interaction effects between age group and modulation frequencies. Follow up, post hoc analyses were also performed to determine significant differences between age groups. Further one-way RMANOVA was administered in each group to examine effects of modulation frequencies and subsequently Tukey's HSD post hoc analyses were performed to examine any differences between pairs (modulation frequencies) in each age group. Finally, spearman's correlation has been reported between age (as a continuous variable) and amplitude, SNR and phase coherence. Overall, AMcVEMP was elicited for a wide range of modulation frequencies: 11-263 for young adults. The AMcVEMP range was reduced for midage, and older adults. None of the participants elicited AMcVEMP at 397 Hz**.**

Amplitude

Effects of age on uncorrected amplitude

The uncorrected amplitude showed inter-subject variability in each groups. On average (see figure 4. 7 and Appendix table A.1), the younger adults had larger amplitude compared to mid-age and older adults. The mixed factorial RMANOVA, (with group as between factor and modulation frequencies as within factor) showed a significant overall effects of age-group ($F = 9.2_{(2,46)}$, $p < 0.001$, $\eta 2 = 0.29$). Post hoc analyses (Tukey's HSD) examined the difference between groups (see table 4.2) and revealed that young adults had significantly larger amplitude than mid-age and older adults from 11-263 Hz modulation frequencies. No significant difference in amplitude was found between midage, and older adults.

4.2 Post hoc results for Amplitude (p values in the table).

MFs (Hz)	11	17	23	37	53	79	113	173	263	397
Y-M	<.001*	.002*	.031*	.006*	.005*	.04*	.086	<.001*	.015*	.15
Y-O	<.001*	<.001*	.002*	.001*	<.001*	.006*	<.001*	<.001*	.005*	.89
M-O	.502	.871	.27	.197	.24	.422	.054	.239	.206	.16

Note: * mark indicates significant results. MFs-Modulation frequencies (Hz), Y-young, M-Midage, O-Old

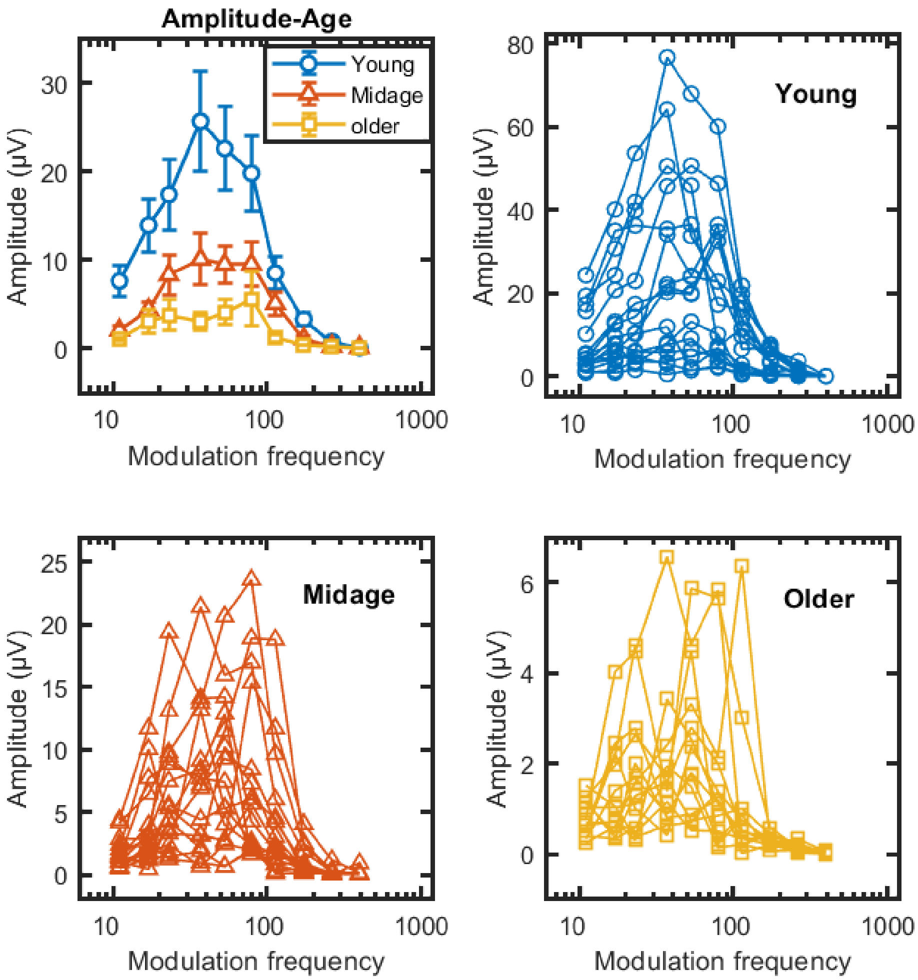

Figure 4.7 The amplitude (mean ± SE for three groups and individual data in each age group) of AMcVEMP across modulation frequencies for the three groups.

Effects of modulation frequencies on uncorrected amplitude in each age groups

Mixed factorial RMANOVA also revealed, a significant effect of modulation frequencies (F = 26.3 $_{(9)}$, $p < 0.001$, η2 = 0.36). Figure 4.7 shows the amplitude of AMcVEMP across modulation frequencies for the three groups. Further, we performed a one-way RMANOVA (within factor: modulation frequencies) in each age group and subsequent post hoc analyses to identify any differences between pairs of modulation frequencies. Across the modulation frequencies, <u>for the young adults, 37, 53 and 79 Hz had significantly larger amplitudes</u> than the rest of the modulation frequencies, with 37 and 53 being significantly larger than 79 Hz. The modulation frequencies 263 and 397 had significantly smaller amplitudes compared to the rest of the modulation frequencies ($p<0.05$). <u>For the mid-age adults</u>, 23-79 Hz were significantly larger than the other frequencies, with no statistically significant differences among each other. Further, the 17 and 113 Hz had significantly larger amplitudes compared to 11, 173, 263 and 397 Hz, with the latter set having statistically smallest amplitudes ($p<0.05$). <u>For the older adults</u>, 79 Hz had the largest amplitude followed by 53 Hz, but there was no statistically significant difference for the amplitudes across the modulation frequencies (17-79 Hz). Further, 113-397 had statistically the least amplitude among older adults.

It is important to note that although there was an observed significant interaction between age-groups and modulation frequencies (F = 5.36 $_{(2,18)}$, $p < 0.001$, η2 = 0.19), these effects were not very meaningful. The interaction between age-groups and modulation frequencies could potentially be attributed to inadequate responses at higher modulation frequencies (specifically 397 Hz) across all three age groups.

Effects of age on EMG

AMcVEMP is a myogenic potential, and scales with the amount of EMG activation. Participants in all three groups were instructed to maintain a constant muscle tension of approximately 50 µV. The mean data for EMG activation for all groups across conditions is shown in Appendix table A.2. RMANOVA with 3 groups across 10 modulation frequencies revealed no significant effect of age-groups ($F_{(2,46)}$ = .87, p = 0.23) or modulation frequencies for EMG ($F_{(2,9)}$ = 0.114, p = 0.893). Figure 4.8 shows EMG activation across modulation frequencies for each group. We do see individual variability for averaged EMG across participants, but there is no overall significant group difference. Overall, the individual EMG activation across participants ranged from 40-65µV.

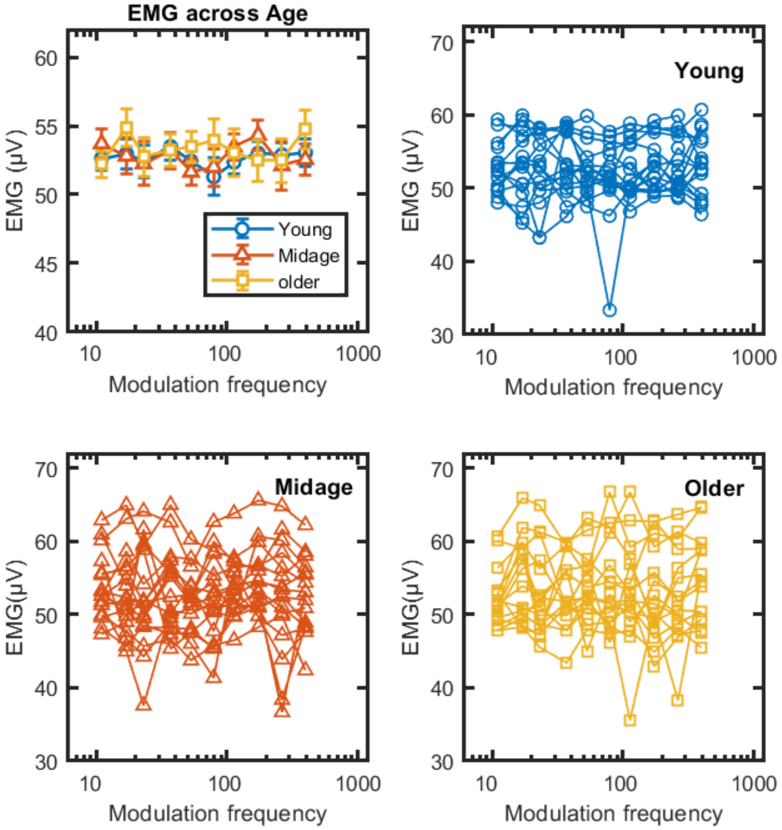

Figure 4.8 EMG activation across modulation frequencies for each group. Mean ± SE for three groups and individual data in each age group.

Effects of age on Corrected Amplitude

Corrected amplitude for AMcVEMP was calculated by dividing the individuals' uncorrected AMcVEMP amplitude by their averaged EMG activation. Corrected amplitude was calculated and analyzed to account if EMG variability across the participants might have led to the seen AMcVEMP amplitude difference across groups. The mean data for corrected AMcVEMP amplitude is presented in appendix table A.3. The effects of age on the corrected amplitude (RMANOVA and post hoc analyses) were similar to the effects of age seen for the uncorrected amplitude (see appendix table A.4). There was no difference for the effects of age between corrected and uncorrected amplitude. Previous studies on AMcVEMP TMTF have utilized only the uncorrected amplitude for their analyses. Thus, for better comparison with previous studies, we utilized uncorrected amplitude for further analyses.

Signal to Noise Ratio

Effects of age on SNR

For the SNR, inter-subject variability was seen across all the three groups. The mean data showed that younger adults had the best SNR, followed by mid-age adults, while older adults had the poorest SNR (see appendix table A.5). Further, mixed factorial RMANOVA showed a significant effect of age group on SNR ($F = 15.33_{(2,46)}$, $p < 0.001$, $\eta 2 = 0.4$). Subsequent post hoc analyses (see table 4.3) revealed that young adults had higher SNR compared to mid-age adults at the extreme modulation frequencies (11-23 and 173-263 Hz), but not for the modulation frequencies in between 37-113 Hz. The older adults had significantly poorer SNR compared to young adults across all modulation frequencies. Furthermore, older adults had significantly poorer SNR compared to mid-age adults at the mid modulation frequencies (23-113 Hz). Figure 4.9 shows the SNR across modulation frequencies for each groups.

Table 4.3. Post hoc results for SNR (p values in the table)

MFs	11	17	23	37	53	79	113	173	263	397
Y-M	.007*	.016*	.040*	.055	.104	.085	.219	.009*	.007*	.279
Y-O	<.001*	<.001*	<.001*	<.001*	<.001*	<.001*	<.001*	<.001*	.002*	.854
M-O	.067	.093	.001*	.004*	.022*	<.001*	.003*	.062	.650	.369

Note: * mark indicates significant results. MFs-Modulation frequencies (Hz), Y-young, M-Midage, O-Old

Effects of modulation frequencies on SNR in each age groups

There was also a significant overall effect of modulation frequencies ($F = 61.1_{(2,9)}$, $p < 0.001$, $\eta 2 = 0.57$). Overall, the young adults had robust SNR (>6.13 dB) from 11 – 263 Hz while the average AMcVEMP TMTF reduced as an effect of aging (see figure 4.9), ranging from 17 to 173 Hz for midage adults and only 53 Hz for the older adults.

RMANOVA ran in each age group revealed significant effect of modulation frequency in each age group. Further, post hoc analyses using Tukey's HSD were run to determine the pair of modulation frequency that differed from each other. Among the young adults, 37-79 Hz had significantly largest SNR compared to other modulation frequencies, followed by 23 and 113 Hz. There was no difference between 37-79 Hz. Further, 17 and 173 Hz had lower SNR than 23-113 but were statistically larger than 11 and 263 Hz.

For the mid-age adults as well, 79 Hz had the statistically highest SNR, followed by 23, 37, 53 and 113 Hz, with no statistical difference between them but higher than the rest. Further, 17 and 173 had larger SNR than 11, 293 and 397 Hz. The older adults had significantly larger SNR at 53 Hz compared to 11-37 and 113- 397 Hz.

A significant interaction between age group and modulation frequency was also observed, ($F = 4.1_{(2,9)}$, $p < 0.001$, $\eta2 = 0.15$). The effect of age group was more pronounced for the mid-modulation frequencies and not on the extreme low (11 Hz) and high (263 and 397 Hz) modulation frequency that had very poor response for majority of young and most midage and older adults.

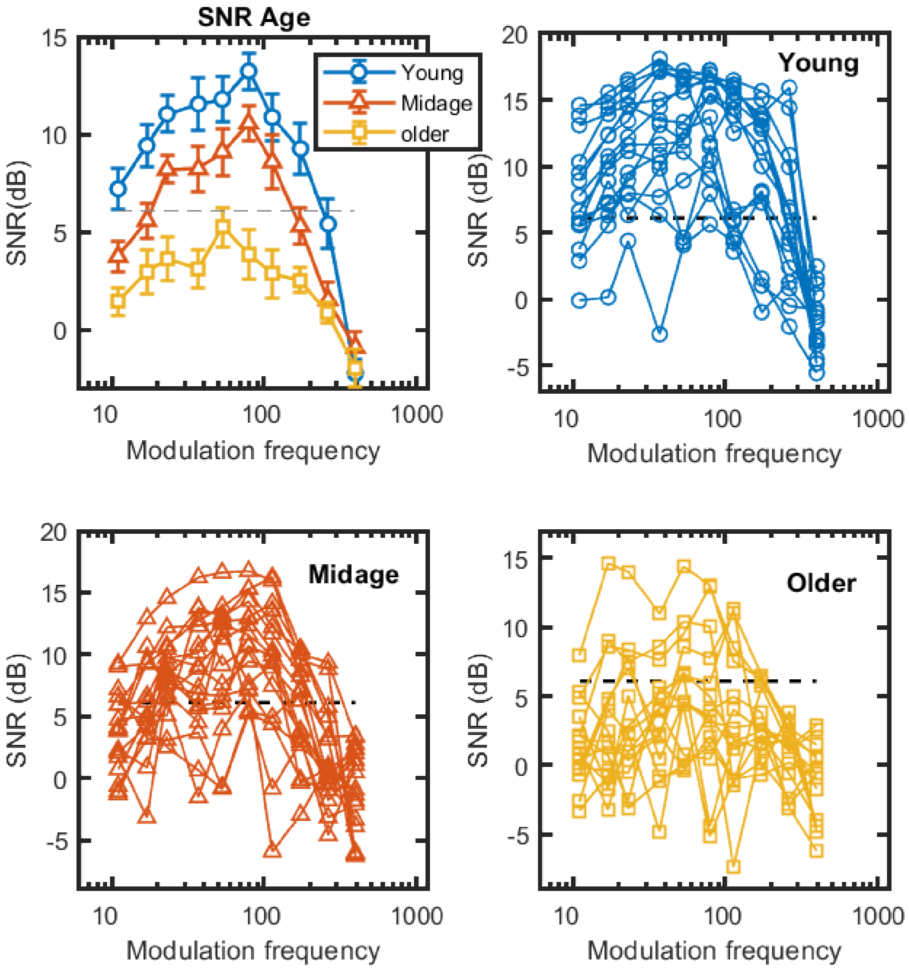

Figure 4.9. SNR across modulation frequencies for three groups. Mean ± SE for three groups and individual data in each age group. Dotted line (each section) in grey is SNR of 6.13 dB beyond which the SNR yields significant value.

Phase Coherence

Effects of age on Phase coherence (PC)

Phase coherence (PC) was calculated across modulation frequencies and a PC value of greater than 0.155 yielded significant p value based on Rayleigh's criterion for circular distribution. PC data also showed inter-subject variability (see figure 4.10). The mean data for PC across modulation frequencies for three groups are shown appendix table A.6. Mixed RMANOVA with three age-groups and 10 modulation frequencies revealed a significant effect of age group on the PC ($F_{(2,46)} = 13.43$, $p < 0.001$, $\eta 2 = 0.37$). Post-hoc analyses (see table 4.4) showed that young adults had larger PC compared to mid-age adults at 11, 17 and 37 Hz and 173-263 Hz, but not at mid modulation frequencies 53-113 Hz. The young adults had significantly higher phase coherence compared to older ($p < 0.001$) adults at all modulation frequencies. Older adults had significantly smaller PC compared to mid-age adults at mid MFs (23-173 Hz). Figure 4.10 shows the phase coherence data across modulation frequencies for the three groups.

Table 4.4. Post hoc analyses results for PC (p values shown in the table)

	11	17	23	37	53	79	113	173	263	397
Y-M	.010*	.010*	.059	.045*	.104	.051	.270	.002*	.011*	.368
Y-O	<.001*	<.001*	<.001*	<.001*	<.001*	<.001*	<.001*	<.001*	.001*	.976
M-O	.289	.184	.014*	.006*	.009*	.002*	.007*	.050*	.406	.384

Note: * mark indicates significant results. MFs-MFs, Y-young, M-Midage, O-Old

Effects of modulation frequencies *on PC in each age groups*

There was also a significant effect of modulation frequency ($F = 47.08_{(2,9)}$, $p < 0.001$, $\eta 2 = 0.505$) for all three groups. For the young adults across MFs, the PC values were > 0.16 across MFs 11-263 Hz. Further, 79 Hz had significantly largest PC, followed by 23, 37, 53 and 113 with no statistical difference among 23, 37 and 113 Hz. Furthermore, 17 and 173 Hz had larger phase coherence than 11 and 263 Hz. For the mid-age adults, average phase coherence > 0.16 was recorded from 11 to 173 Hz, with the statistically largest PC being at 79 Hz and 53 Hz, followed by 23, 37 and 113 Hz with no statistically significant difference among themselves. The MFs 17 and 173 had larger PC compared to 11, 263 and 397 Hz. The AMcVEMP TMTF range reduced for the mid-age adults compared to young adults. Further, among the older adults, significant PC was observed from 17 to 113 Hz MFs with no statistical difference among themselves. Overall, the phase coherence TMTF got flatter and its range reduced as an effect of aging.

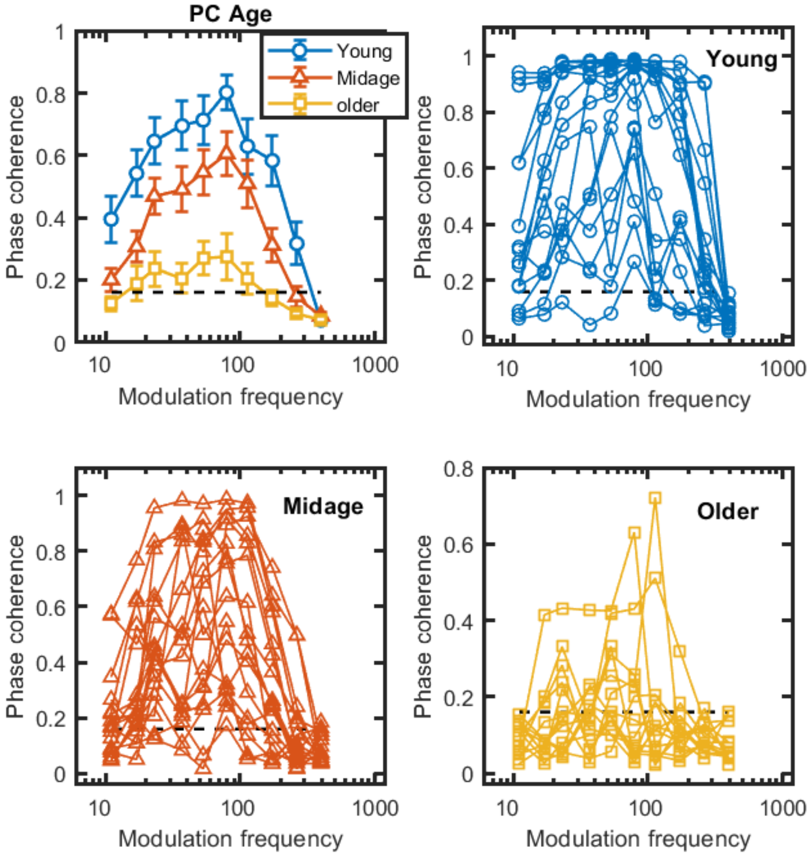

Figure 4.10. Phase coherence across modulation frequencies for three groups. Mean± SE data and individual data for each group has been shown. Dotted line in grey is PC of 0.155 beyond which the PC was significant based on Rayleigh's Criterion.

A significant interaction between age group and modulation frequency was also observed, ($F = 4.7_{(2,9)}$, $p < 0.001$, $\eta 2 = 0.17$). The effect of age group was more pronounced for the mid-modulation frequencies and not on the extreme low (11 Hz) and high (263 and 397 Hz) modulation frequency that had very poor response for majority of young and most midage and older adults.

Effects of age (as a continuous variable) on measures of AMcVEMP

We calculated spearman's correlation between age as the continuous variable and various AMcVEMP measures: amplitude, SNR and phase coherence. There was a significant negative correlation between age and amplitude, age and SNR, and age and phase coherence at all MFs except 397 Hz. (See Table 4.5). Table 4.5 plots the spearman's correlation between age and AMcVEMP measures.

Table 4.5**.** Spearman's correlation between age and the various AMcVEMP measures

MFs	Age vs amplitude	Age vs SNR	Age vs PC
11	$r_s = -0.709, p < 0.001$	$r_s = -0.639, p < 0.001$	$r_s = -0.607, p < 0.001$
17	$r_s = -0.641, p < 0.001$	$r_s = -0.603, p < 0.001$	$r_s = -0.627, p < 0.001$
23	$r_s = -0.651, p < 0.001$	$r_s = -0.681, p < 0.001$	$r_s = -0.625, p < 0.001$
37	$r_s = -0.679, p < 0.001$	$r_s = -0.687, p < 0.001$	$r_s = -0.686, p < 0.001$
53	$r_s = -0.585, p < 0.001$	$r_s = -0.561, p < 0.001$	$r_s = -0.593, p < 0.001$
79	$r_s = -0.693, p < 0.001$	$r_s = -0.744, p < 0.001$	$r_s = -0.722, p < 0.001$
113	$r_s = -0.572, p < 0.001$	$r_s = -0.626, p < 0.001$	$r_s = -0.600, p < 0.001$
173	$r_s = -0.612, p < 0.001$	$r_s = -0.609, p < 0.001$	$r_s = -0.612, p < 0.001$
263	$r_s = -0.490, p < 0.001$	$r_s = -0.470, p < 0.001$	$r_s = -0.544, p < 0.001$
397	$r_s = -0.050, p = 0.73$	$r_s = -0.066, p < 0.652$	$r_s = -0.015, p < 0.918$

Note: r_s = spearman's correlation coefficient, MFs-Modulation frequencies. No significant correlation seen at age and measures of AMcVEMP (amplitude, SNR and PC) at 397 Hz.

The scatter plot between AMcVEMP measures: Amplitude, SNR and Phase coherence and age for 37, 53 and 79 Hz modulation frequency is shown in figure 4.11. Close observation of the data showed that the decline in the amplitude becomes more pronounced beyond the age of 50 (Figure 4.11). The decline in SNR was steady (relative to amplitude), with robust SNRs seen in some individuals even greater than 60 years of age especially for 79 Hz. Further for the phase coherence, some of the older participants also exhibited moderate phase coherence (greater than 0.5) until the age of 60 years for 79 Hz. The decline in amplitude is steeper compared to SNR and phase coherence as an effect of aging.

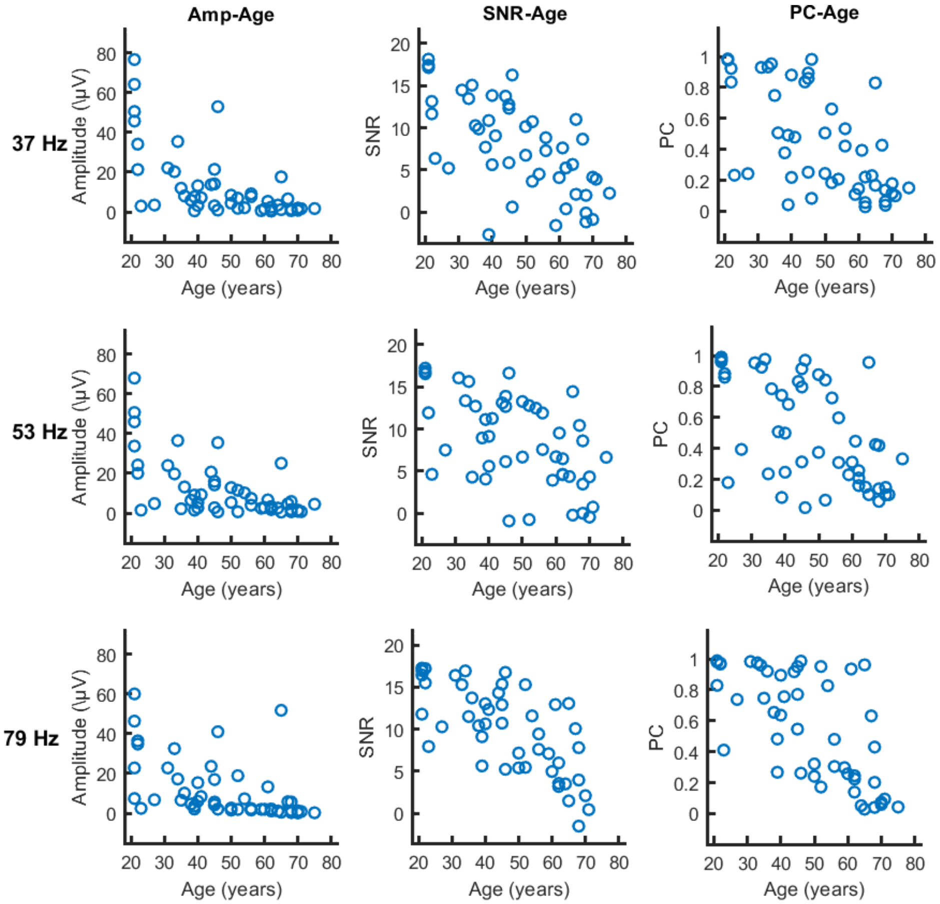

Figure 4.11 scatter plot between AMcVEMP measures (in columns) and age for three modulation frequencies (in rows: 37,53 and 79 Hz). Column1: Amplitude vs age, Column 2: SNR vs Age, Column 3: Phase Coherence vs age.

Summary of results for specific aim 1.

Young adults had significantly larger AMcVEMP amplitude compared to mid-age and older adults, while there was no significant difference in amplitude between mid-age and older adults. The average amplitude also varied across different modulation frequencies, with 37 and 53 Hz having the largest amplitudes for young adults and midage adults, while 79 Hz had the largest amplitude for older adults. As an effect of aging, the AMcVEMP TMTF range was reduced. There was no significant difference in EMG activation across age groups, indicating that the difference in amplitude was not due to muscle tension. The corrected amplitude showed a similar effect of age as that seen for the uncorrected amplitude.

For the SNR measure, the young adults had significantly better SNR, compared to mid-age adults, and older adults. The older adults had significantly poorer SNR compared to mid-age adults only for the mid modulation frequencies (23-113 Hz). The young adults had robust SNR (>6.13 dB) from 11 – 263 Hz. The average AMcVEMP TMTF reduced as an effect of aging, ranging from 17 to 173 Hz for midage and only 53 Hz for older adults.

The young adults have higher PC compared to midage and older adults, with responses being most robust across MFs 11-263 Hz for young adults and 11-173 Hz for mid-age and older adults. The TMTF range reduced as an effect of aging, with mid-age and older adults having a flatter phase coherence TMTF compared to young adults. Overall, the study showed that the AMcVEMP amplitude, SNR and PC TMTF decreased as an effect of aging. The effects of age were different for different measures with pronounced deterioration in amplitude compared to SNR and phase coherence.

Specific aim 2: Determine the upper limit and shape of AMcVEMP TMTF in three age groups.

The second purpose of the study was to determine the limit of AMcVEMP in each of the three groups. We determined the limit of AMcVEMP based on SNR, phase coherence and modulation gain. Determining the limit of AMcVEMP TMTF gives us the understanding of the modulation frequencies that could elicit AMcVEMP responses in each age group. So further, we also calculated the shape of the AMcVEMP TMTF. To report shape, we normalized the response of each participants to their best modulation frequency. We plotted the average normalized value for different metrics for each groups. Further, we have also reported the best modulation frequency for amplitude, SNR and phase coherence.

Limit of AMcVEMP TMTF

Based on SNR

Limit of AMcVEMP based on group average SNR data.

Based on the SNR criterion of >6.13 dB for present response, we observed that, on average, the young adults had significantly robust SNR from 11-263 Hz modulation frequencies (see figure 4.9). The range and the upper limit of the AMcVEMP TMTF reduced as an effect of aging. On average, the mid-age adults had robust SNR from 17-173 Hz while for the older adults, on average, only the 53 Hz modulation frequency elicited SNR > 6.13 dB.

Response rate at each modulation frequency

We also observed variability for presence of responses (SNR > 6.13 dB) across modulation frequencies in each of the three age groups. The number of participants having present responses in each of the three groups at each of the modulation frequency is represented in table 4.6. Among the young adults, all the participants had present responses at 79 Hz, while the response rates for 17 to 173 Hz were above 75%. For the mid-age adults, the response rate was highest at 79 Hz (82%), while the average lower (17 Hz) and upper (173 Hz) MFs had a response rate of 41 and 53% respectively. For the older adults: 53 Hz had maximum response rates (44%). Further, all the participants in the young and mid-age group had present responses at least at one modulation frequencies, while only 9/16 (56%) of older adults had present response at least at one MFs.

Table 4.6. Percentage of participants having SNR >6.13 dB in each age group.

MFs	11	17	23	37	53	79	113	173	263	397
Y(n =16)	11	**14**	**15**	**14**	**14**	**16**	**12**	**12**	7	0
M (n = 17)	6	7	**14**	**13**	**14**	**14**	12	9	3	0
O (n = 16)	1	3	5	4	7	5	4	4	0	0

Note: MFs-Modulation frequencies (Hz), Y-young, M-Midage, O-Old, n – total number of participants, The bolded values amount to >75% response rates

Defining limit of AMcVEMP TMTF (>75% responses)

We can conclude that about 75% (> 3/4th) of young adults may have present AMcVEMP responses based on SNR for 17-113 Hz, while the range for 75% response rate, got narrower for mid-age adults: 23-79 Hz. For the older adults, the response rates were highest at 53Hz (44%). We fail to define the limit of AMcVEMP for older adults. We further try to establish the AMcVEMP TMTF limit based on phase coherence criterion.

Based on Phase coherence (PC)

Grouped average AMcVEMP TMTF limit

According to Rayleigh's criterion of circular distribution, a phase coherence value greater than 0.155 yield significant values, and thus, 0.155 was considered to be the criterion for present AMcVEMP response based on the phase coherence. On average, young adults exhibited present responses over a wide range:11-263 Hz (see figure 4.5). The TMTF became flatter, and the range reduced with aging. Specifically, mid-age adults had an average present response from 11-173 Hz, while older adults had an average response ranging from 17-113 Hz.

Response rates at modulation frequencies

In terms of response rates based on PC, there were variabilities within each age group, see table 4.7. For young adults, the response rate was 100% at 79 Hz, while it was 81% and 69% at the 11 Hz (average lowest MF) and 263 Hz (average uppermost MF), respectively. Among the mid-age group, all participants had present responses at 79 Hz, while at the average lower (11 Hz) and average upper (173 Hz) AMcVEMP limit, the response rate was 53% and 65%, respectively. For the older adult group, the maximum response rate was about 56% at 53 Hz. Furthermore, at the 17 Hz (average lower) and 113 Hz (average upper) AMcVEMP limit, the response rates were 38% and 44%, respectively.

Table 4.7 . No. of participants having PC >0.155 in each age group

	11	17	23	37	53	79	113	173	263	397
Y (n =16)	**13**	**14**	**15**	**15**	**15**	**16**	**13**	**13**	11	1
M (n = 17)	9	**13**	**15**	**15**	**15**	**16**	**15**	11	5	1
O (n = 16)	2	6	8	8	9	8	7	4	1	0

Note: MFs-Modulation frequencies, Y-young, M-Midage, O-Old. The values, in bold have >75% response rates

Defining limit >75% response rates

Regarding the number of modulation frequencies eliciting present responses based on PC, all young adults had present responses at least at 7 modulation frequencies with greater than 75% response rate for 11-173 Hz . For mid-age adults, >75% of participants had present responses on at least 6 MFs (17-113 Hz). Among older adults, about 50% of participants had present responses on at least 4 MFs and all participants had present responses on at least at 1 MF. We fail to provide a limit AMcVEMP TMTF for older adults as no modulation frequency elicited response rates greater than 75%.

AMcVEMP TMTF limit based on Modulation Gain

The modulation gain was computed using the formula : Modulation Gain (dB) = 20 Log $_{(10)}$ of (PC/depth of AM). In the auditory literature, criterion point of -3 dB and -10dB have been considered. Figure 4.12 shows the AMcVEMP modulation gain TMTF data (mean +SE and individual data) across modulation frequencies for each group.

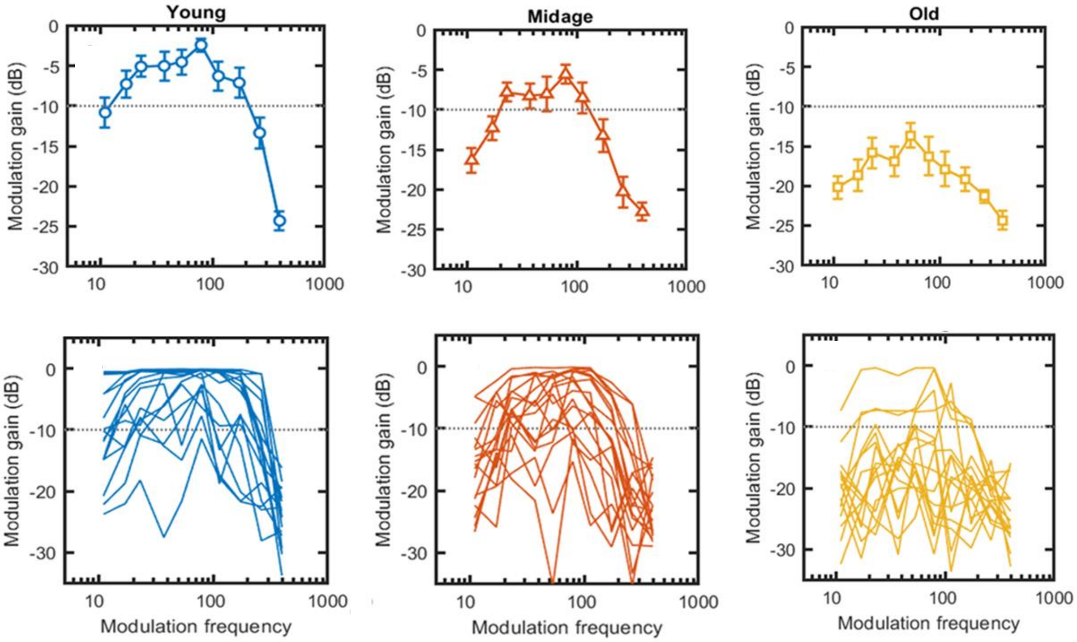

Figure 4.12 AMcVEMP modulation gain TMTF data: Upper row shows mean + SE data. The lower row shows the individual data. Column represents age groups: Left: Young, Middle: Column, Right: Old. The dotted line is the cutoff criterion of -10 dB modulation gain.

The average range of AMcVEMP modulation gain TMTF for the cutoff of -10 dB was <u>11-173 Hz for young adults</u>, <u>23-113 Hz for mid-age adults</u>, and only a few participants in the older adult group had modulation gain higher than -10 dB. Among the young adults, the peak was observed at 79 Hz for most participants, with an average modulation gain of -2.4 dB. Furthermore, the majority of young adults, demonstrated a modulation gain higher than -3 dB for the modulation frequencies of 11-173 Hz. For the mid-age adults, there was a higher inter-subject variability, with a peak (average response) seen at 79 Hz, having an average modulation gain of -5.57 dB. Among the older adults, few participants (5/16) had a modulation gain of higher than -10 dB, with an average modulation gain of -14 dB peaking at 53 Hz.

As we are unable to define the range of AMcVEMP TMTF for older adults, we wanted to identify the best modulation frequency that could elicit most robust AMcVEMP responses in older adults. This will aid in choosing the appropriate stimuli to elicit AMcVEMP in older adults. We report the best modulation frequency that elicited most robust responses across the metrics for each group.

Best Modulation frequency to elicit AMcVEMP in each age group.

The best modulation frequency that elicited largest AMcVEMP response for different measures have been plotted in figure 4.13. For this analysis, only participants with an SNR greater than 6.13 dB at least at one modulation frequency were considered, resulting in a total of 16, 17, and 9 participants in the young, mid-age, and older adult groups, respectively.

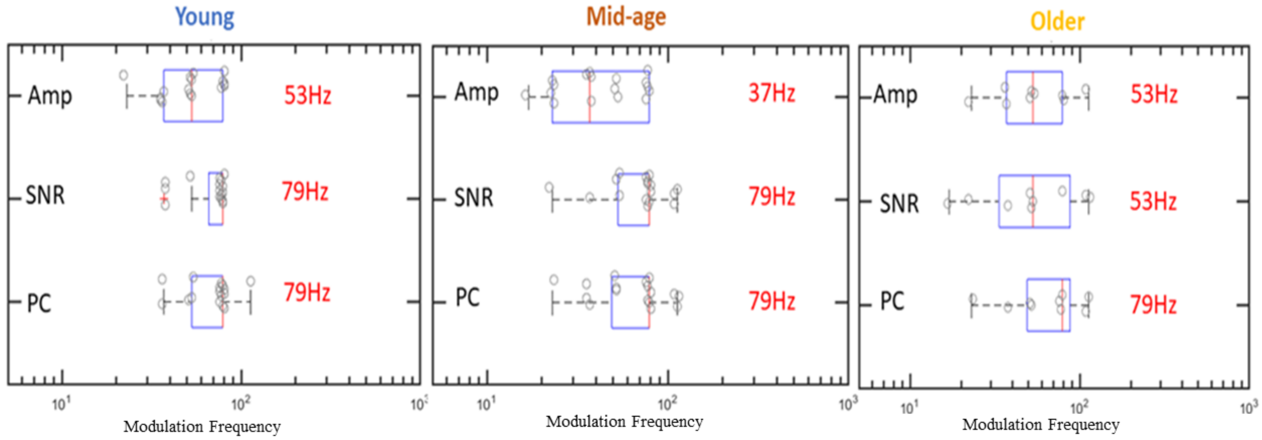

Figure 4.13 Boxplots showing best modulation frequency eliciting most robust AMcVEMP, for each group for each measure. The circles represent individual participants at the corresponding modulation frequency eliciting largest responses for Amplitude (Amp), SNR, PC and MSC. The red line in each box plot is the median values. The median values for each metric are written beside the boxplot. The blue box shows 25th to 75th percentile. The whiskers plot shows the range.

For young adults, the largest amplitude was observed in the range of 23-79 Hz, with a median value of 53 Hz. The participants had the best SNR in the range of 37-79 Hz, with 75% having the best SNR at 79 Hz. The phase coherence measure had the best values in the range of 37-113 Hz, with 63% having the best PC at 79 Hz.

For mid-age adults, the amplitude measure had the best response in the range of 17-79 Hz, with the median of 37 Hz. The best SNR ranged from 23-113 Hz, with 53% of participants having the best SNR at 79 Hz. The best PC across participants followed the same 23-113 Hz range, with the median value being at 79 Hz.

PC older adults, the median best modulation frequency for amplitude, SNR and PC, were 53, 53, and 79 respectively. The range for these measures was 23-113 Hz. In summary, the best modulation frequency for amplitude measure was between 37-53 Hz for each age group. The SNR had best modulation frequency as 79 Hz for young and midage adults with a downward shift to 53 Hz for older adults. The PC was best elicited at 79 Hz for each group. <u>Overall, the best modulation frequency to elicit AMcVEMP was between 37-79 Hz for each age group for each measures</u>.

Shape of AMcVEMP across different measures

To compare the shape of the AMcVEMP TMTF across measures, normalized response metrics were compared. Normalized values were obtained by dividing each participant's individual values (at MFs) by the maximum value (across MFs) for that measure. This was done for all participants in all three groups. The average normalized curve for each measure for each group is plotted in figure 4.14.

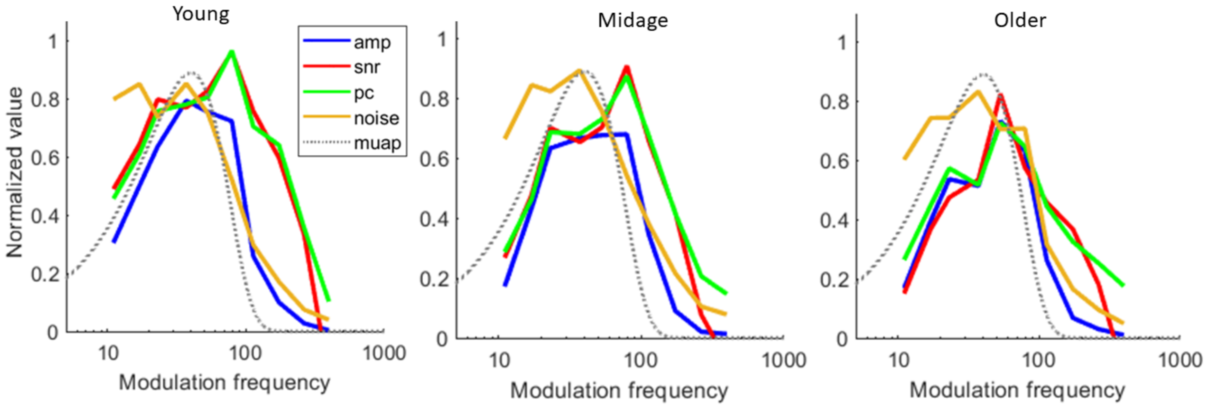

Figure 4.14 Averaged Normalized TMTF of AMcVEMP for different metrics: amp, snr, pc, noise. The dotted curve is the spectrum of the modeled SCM muap shape for young adults (Wit & Kingma, 2006). The maximum value that could be obtained is 1.

For young adults, the PC and SNR, curves on average had a peak at 79 Hz and had robust responses across a range of MFs. The normalized amplitude TMTF had a slightly broader shape with a peak at 37 Hz. The normalized AMcVEMP amplitude was not very robust beyond 113 Hz. The average amplitude TMTF for young adults appeared to have the similar shape as the muap curve consistent with previously reported in Lawlor et al., 2022. Additionally, the noise TMTF had a shape, with a peak coinciding at 37 Hz, overlapping with that of the amplitude and muap, with some additional energy in the lower frequencies (11-23 Hz), appearing like a low pass filter. For mid-age adults, the shape of the PC and SNR peaked around 79 Hz, while the shape of the amplitude TMTF becomes flatter and has the highest values in the range of 23 to 79 Hz. The noise TMTF shape follows a similar pattern as that of MUAP with little more energy in 17 and 23 Hz. For older adults, the shape of the TMTF across all measures including amplitude peaks around 79 Hz with the exception of normalized noise TMTF that peaks at 37 Hz. The average values of normalized TMTF in all groups were less than one. A lower averaged normalized value means more variability in terms of best modulation frequency.

Summary Results for specific aim 2.

Based on SNR, (>75% response rates), the TMTF limit was 17-173 Hz for young adults and 23-79 Hz for midage adults. Further, a higher proportion of participants had present responses based on PC measure compared to SNR. For PC, the TMTF limit based on >75% response rates, was 11-173 for young and 17-113 for midage adults For the modulation gain, the average range for the cutoff of -10 dB was 11-173 Hz for young adults, 23-113 Hz for mid-age adults. Fewer (5/16) participants in the older adult had more than -10dB modulation gain. We could not determine the limit of AMcVEMP for older adults as no modulation frequency elicited more than 75% response rates, or higher than -10 dB modulation gain among older adults. Further, the best modulation frequency that elicited most robust AMcVEMP responses were between 37-79 Hz for each age group including the older adults. The shape of the AMcVEMP normalized SNR, and PC showed a peak at 79 Hz in each age group. For the normalized amplitude, the peak seen at 37 Hz for young adults shifted to 79 Hz among older adults.

Specific aim 3: Characterize non-linear harmonic distortion in AMcVEMP.

To characterize non-linearity, we are reporting amplitude, SNR and Phase coherence TMTF across harmonics H1 through H4. We further report the response rates across harmonics for SNR >6.13 dB and phase coherence >0.155. Finally we calculated the total harmonic response (sum of values from H2 through H4), for amplitude, SNR and phase coherence and examined the effects of age on it.

Amplitude TMTF across harmonics:

Previous studies have shown that AMcVEMP responses can be present at the harmonics of the modulation frequencies. Although AMcVEMP TMTF across harmonics (H1 though H4) have been reported for young adults, there is no information for mid-age and older adults. Figure 4.15 shows the amplitude across harmonics.

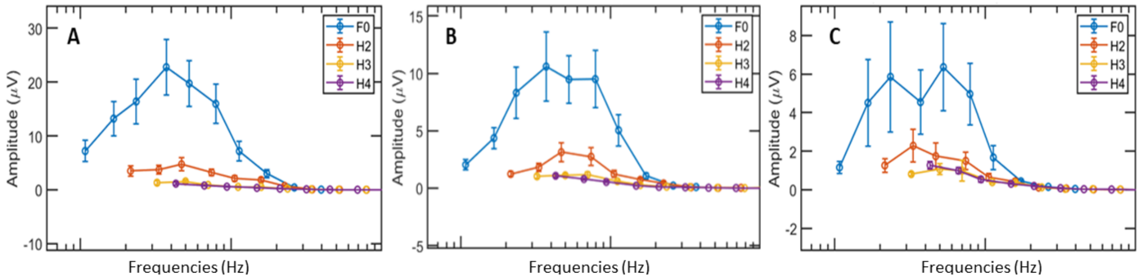

Figure 4.15 Amplitude (Average ±1 SE) TMTF for H1 through H4. A. young, B. Midage, C. Older adults

Our observations revealed that for the young adults (figure 4.15 A) first harmonic amplitude was highest in the 30-40 Hz range, which is consistent with previous findings. For middle-aged adults (figure 4.15 B), the first harmonic TMTF was broader and plateaued with average maximum amplitude observed in the 35-80 Hz range. For older adults, the amplitude at H1 was low, and the TMTF appeared flatter (figure 4.15 C). Additionally, in all the groups, for H2 TMTF, the modulation frequency peak, coincided with the peaked frequency regions observed for H1**.** The average amplitude for H3 and H4 were very low in all three groups.

SNR TMTF across harmonics:

SNR exhibited varying trends compared to amplitude, across modulation frequencies in each group. Among young adults (see figure 4.16 A), the SNR TMTF had a plateaued response from 17 to 113 Hz at H1 and from 23 to 113 Hz at H2. TMTF had a peak energy at 79 Hz for H1, but for the H2 the TMTF was flat with no observed peak. In contrast the middle age had a narrower TMTF and among older adults, for H1, on average, only 53 Hz elicited an SNR greater than 6.13 dB. In H2-H4, participants in both middle-aged and older adults, average data, did not exhibit an SNR greater than 6.13 dB on any of the frequencies. However, there was individual variability seen in both midage and older adult groups.

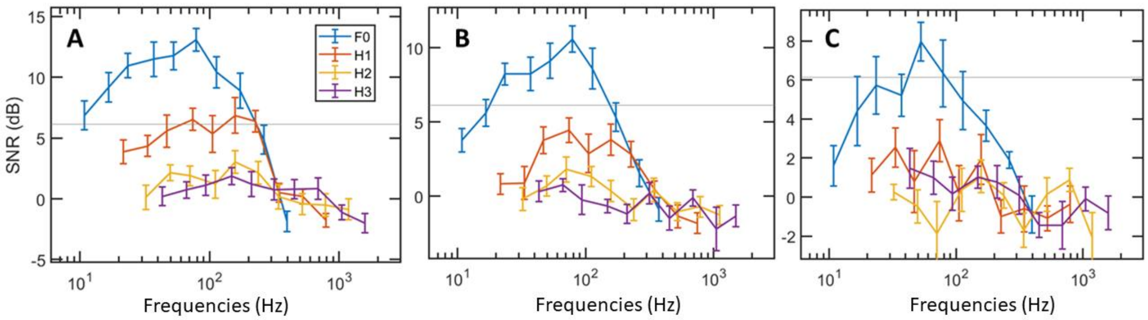

Figure 4.16 SNR TMTF (Average ±1 SE) TMTF for H1 through H4. A. young, B. Midage, C. Older adults. Dotted lines indicate criterion for SNR: 6.13 dB.

Phase coherence TMTF across harmonics:

Phase coherence exhibited a very distinct pattern compared to amplitude and SNR TMTFs across harmonics. Figure 4.17 shows the average data for phase coherence TMTF for H1 through H4 across modulation for three groups. In the case of young adults, at H1, the PC had a broader TMTF (11-263) with a peak at 79 Hz (figure 4.17 A). Further, despite the lower response at H2, the criterion point of 0.155 was surpassed for modulation frequencies ranging from 11 to 113 Hz. For H3, the young adults had PC > 0.155 at 53 and 79 Hz.

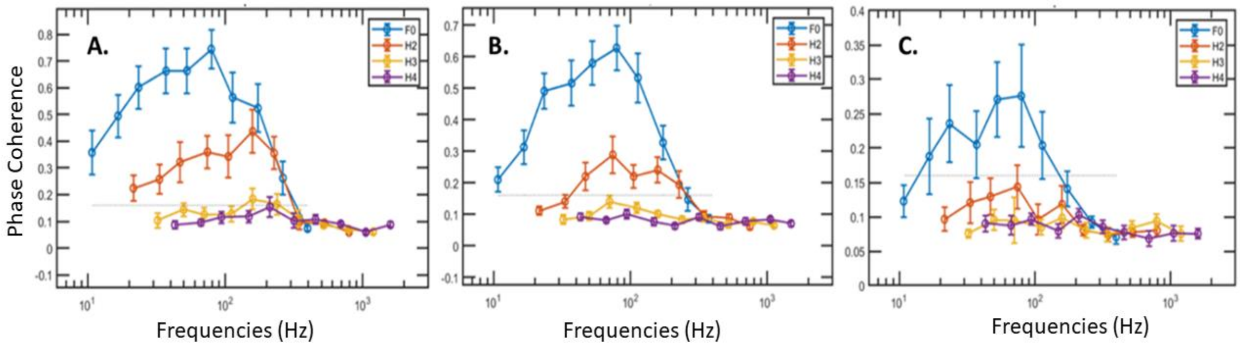

Figure 4.17 Phase Coherence TMTF (Average ±1 SE) TMTF for H1 through H4. A. young, B. Midage, C. Older adults. Dotted lines indicate criterion for PC: 0.16

As for mid-age adults (figure 4.17 B), the H1 peak response occurred at 79 Hz with robust response for a broad range. At H2, the response exceeded the criterion point for a reduced range (23-113 Hz). The average H3 responses were lower than criterion point across all the modulation frequencies. For older older adults, on average the PC values were low at H1 but surpassed the criterion point from 17 to 113 Hz. However, on average, there were no responses observed at H2. It's worth noting that the PC values at H2 for young adults were comparable to the PC values at H1 for older adults.

Response rates across harmonics

Response rates at harmonics are being reported based on SNR (>6.13 dB) and PC (>0.16) criterion.

Based on Signal to Noise ratio

Response rates (% of participants exhibiting present responses: SNR > 6.13 dB) were calculated for all three groups at the modulation frequencies and their harmonics H2 through H4 (Figure 4.18). Among all the groups, all young and middle-aged adults and 9 out of 16 older adults exhibited SNR>6.13 dB at least at one modulation frequency.

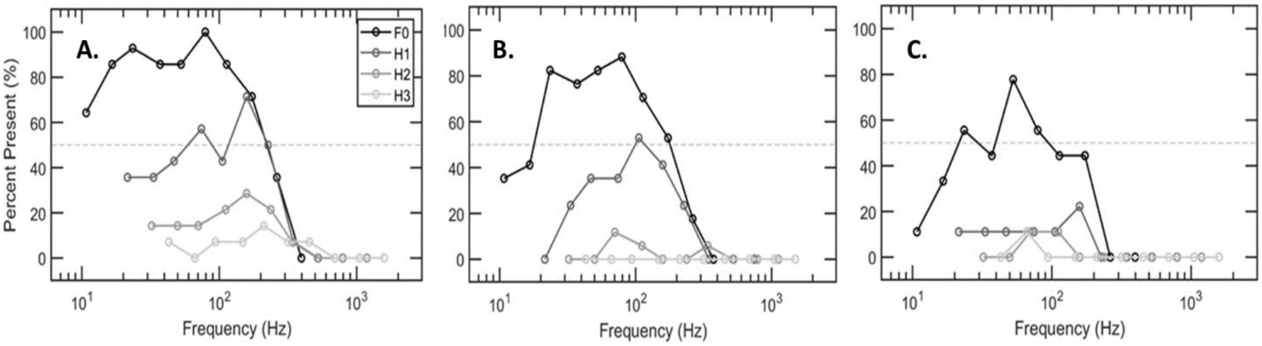

Figure 4.18 A. Response rates based on SNR > 6.13 dB for young (A), midage (B) and older (C) adults for H1 through H4 across modulation frequencies. Dotted line indicates 50% response rate.

For young adults (Figure 4.18 A), response rates were >80% for six stimulus conditions (17-113 Hz), with 100% response rate at 79 Hz. Furthermore, for H2, about 50% of young adults exhibited present responses for 4 stimuli (37-113). Further the response rate at H3 was about 20% for 37-79 Hz. For middle-aged adults, response rates at the modulation frequencies were about 80% for 23-79 Hz, (figure 4.18 B). For H2, the response rate was greater than 50% only for the 53 Hz stimulus. The response rate for H3 and H4 was very low for middle-aged adults. Among the older adults (figure 4.18 C), for

H1, no stimulus elicited a response rate of greater than 80%. The highest response rate was 77% of (9/16) at 53 Hz and negligible responses at H2 through H4. Overall, the response rates at the modulation frequencies and their harmonics decreased as an effect of aging indicating loss of non-linearity with increasing age.

Based on Phase coherence > 0.155

Response rates (% of participants exhibiting present responses: PC > 0.155) were calculated for all three groups at the modulation frequencies and their harmonics H2 through H4 (Figure 4.19). For these analyses, participants having PC > 0.155 at least at one modulation frequency were considered. Among all the groups, all young, middle-aged and older adults exhibited PC > 0.155 at least at one modulation frequency.

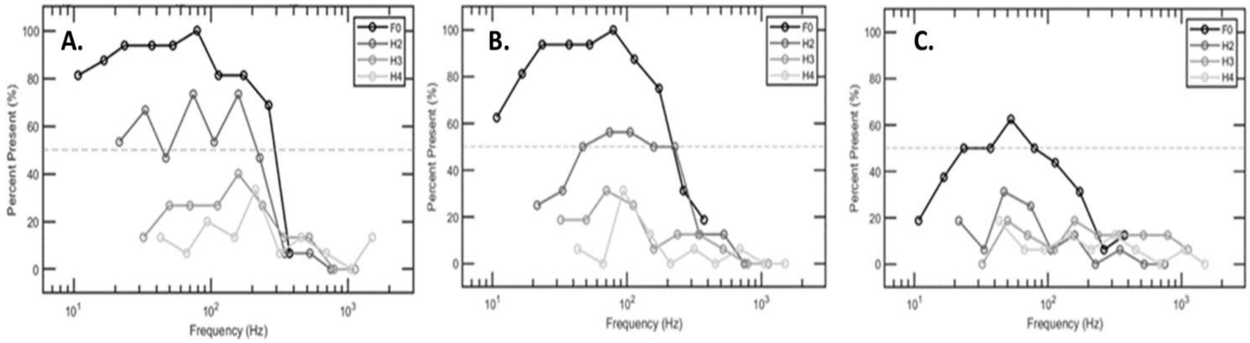

Figure 4.19 A. Response rates based on PC > 0.155 for young (A), midage (B) and older (C) adults for H1 through H4 across modulation frequencies. Dotted line indicates 50% response rate

For young adults (Figure 4.19 A), response rates were about 80% for 8 stimulus conditions (11-173Hz), with 100% response rate at 79 Hz. Furthermore, for H2, response rates were greater than or about 50% for 7 stimuli (11-113 Hz). The response rate for H3 was about 25% for 5 stimuli (17-79 Hz). For middle-aged adults, response rates at the modulation frequencies were about 80% for 6 stimuli (17-113 Hz), with the 100% response rate observed at 79 Hz (figure 4.16 B). For H2, the response rate was greater than 50% for 5 stimuli (23-113 Hz). The response rate for H3 was overall low around 20% for 11-37 Hz. For older adults (figure 4.19 C), no stimuli, elicited any response greater than 80%. Response rate greater than 50% was elicited at 23-79 Hz. For H2, the response was seen in 25% (4 participants) at 23 Hz. The response rates at H3 through H4 were lower than 20%.

Total Harmonic Response:

Total harmonic response was calculated as the sum of values from H2 through H4 for each metric (amplitude, SNR and PC). E.g., Total Harmonic of amplitude = Sum (ampH2+ampH3+ampH4). This was done at all the modulation frequencies.

THR amplitude:

Total harmonic response for amplitude on average showed maximum THR at 11-23 Hz for young 23 Hz for midage adults and 17 Hz for older adults. Mean data for THR for amplitude is given in Appendix 1 table A.7. On further analyses, a significant effect of age-group and modulation frequency on the total harmonic response for amplitude was observed in the study (F = 5.05 (2,46), $p < 0.01$, $\eta 2 = 0.18$) and (F = 50.353 (2,9), $p < 0.001$, $\eta 2 = 0.52$,) respectively. However, there was no significant interaction between groups and modulation frequencies (F = 2.19 (2,9), $p < 0.056$, $\eta 2 = 0.08$). Post hoc analyses (see table 4.8) showed that young adults had a larger total harmonic amplitude for 11-113 Hz compared to older adults. There was no significant difference between young and midage and between midage and older adults.

Table 4.8 Post hoc analyses for Total harmonic response for amplitude, p values

MFs	11	17	23	37	53	79	113	173	263	397
Y-M	.14	.15	1	.99	.09	.01	.24	.99	.73	.72
Y-O	.034*	.05*	.04*	.08	.007*	.001*	.004*	.77	1	1
M-O	1	.15	.44	.03	.91	1	1	1	1	.77

Note: *mark indicates significance, MFs-Modulation frequencies, Y-young, M-midage, O-older adults

Overall, the total THR amplitude data showed individual variabilities across participants in each group. On average, across modulation frequencies within the group, the young adults had largest total harmonic amplitude. We saw a total harmonic response of 6 µV on average at 11,17,23 Hz for young adults. Statistically, the largest THR amplitude was noted at 11-23 Hz for young, at 23 Hz for mid-age and 17 Hz for older adults. Overall, the total harmonic amplitude decreased with increasing modulation frequencies for all the groups with no statistically significant differences between 113-397 Hz. Figure 4.20 plots total harmonic response for amplitude across modulation frequencies for three groups.

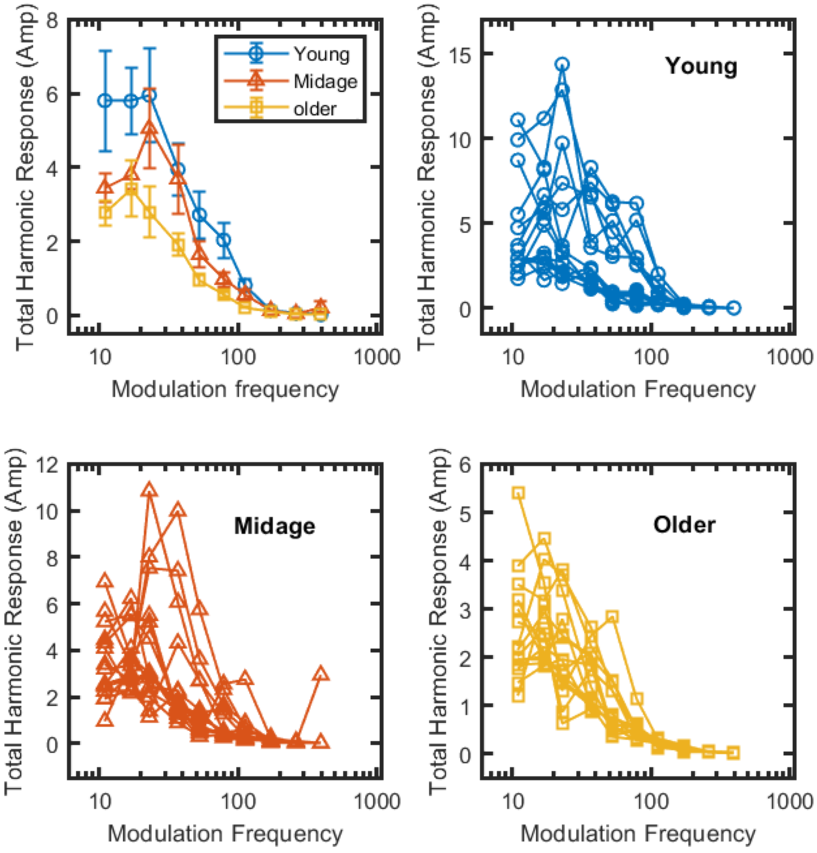

Figure 4.20 total harmonic response (THR) for amplitude across modulation frequencies for three groups. Mean ±SE and individual data presented for each groups.

Total Harmonic Response: Signal to Noise Ratio

THR for SNR was measured as sum of SNR for H2 through H4 and was done for all the modulation frequencies. Table in appendix 1 A.8 gives the mean data for THR SNR across modulation frequencies for three groups.

Further, RMANOVA revealed a statistically significant effect of age-group on the total harmonic response for SNR (F = 6.59 (2,46), $p < 0.003$, $\eta 2 = 0.22$). Additionally, there was a significant effect of modulation frequency (F = 17.43 (2,9), $p < 0.001$, $\eta 2 = 0.27$) across all three groups. Furthermore, there was a significant interaction between age-groups and modulation frequencies (F = 4.58 (2,9), $p < 0.001$, $\eta 2 = 0.16$). Post hoc analyses (see table 4.9) showed significantly larger total harmonic SNR for young compared to older adults for 23-113 Hz. There was no statistically significant difference between young and midage and midage and older adults.

Table 4.9 Post hoc analyses showing significance (p) values, for Total harmonic response SNR (group comparison)

MFs	11	17	23	37	53	79	113	173	263	397
Y-M	.16	.18	.57	.2	.01	.009	.07	.448	1	1
Y-O	.10	.09	.03*	.02*	002*	.001*	.002*	.19	1	.42
M-O	1	1	.57	.9	.9	.9	.577	1	.57	.18

Note: *mark indicates significance, MFs-Modulation frequencies, Y-young, M-midage, O-older adults

When considering the response at modulation frequencies within each age-group, the results showed distinct patterns. Figure 4.21 shows the Total Harmonics response for SNR for three groups across modulation frequencies. There was inter-subject variability seen in all the three groups. However, on average, among the young group, the total harmonic SNR was maximized in the frequency range of 23-79 Hz, with a consistently high response from 11-113 Hz. In contrast, average midage adults showed maximal total harmonic SNR at 23 and 37 Hz, with a decreasing response as the modulation frequency increased. Finally, older adults showed an average total harmonic SNR of around 4 dB and remained flat across modulation frequencies.

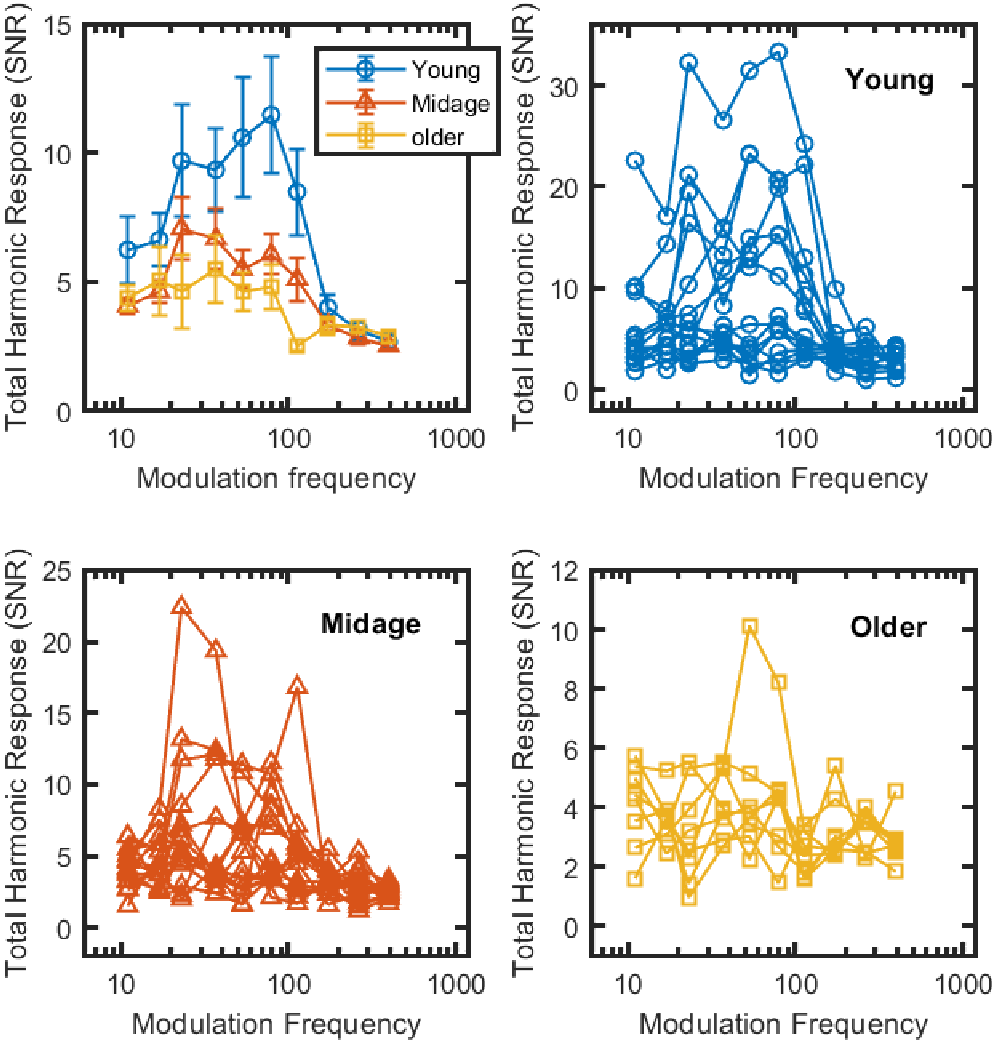

Figure 4.21 Total harmonic response for signal to noise ratio across modulation frequencies for three groups. Left: mean ±SE data, Right: individual data points.

Total Harmonic response: Phase coherence

We also measured THR for phase coherence. We summed up the phase coherence values from H2 through H4. We did this at all the modulation frequencies for all the participants in each groups. The findings across groups showed a distinct pattern; maximized pc seen at 79 Hz for young, and 23-37 Hz for midage adults, and flat for older adults. Table A.9 in the appendix section shows the mean data for the THR PC across modulation frequencies for three groups.

Further analyses revealed a significant effect of age-group on the total harmonic response for phase coherence (F = 4.16 (2,46), $p < 0.022$, $\eta 2 = 0.153$). Additionally, there was a significant effect of modulation frequency (F = 16.7 (2,9), $p < 0.001$, $\eta 2 = 0.27$) for all three groups, with a significant interaction between groups and modulation frequencies (F = 3.43 (2,9), $p < 0.004$, $\eta 2 = 0.13$). Further investigation through post hoc analyses (see table 4.10) indicated that young adults demonstrated significantly larger total harmonic phase coherence than older adults for modulation frequencies between 37-113 Hz. No significant difference between young and midage and midage and older adults was observed.

Table 4.10 Post hoc analyses showing significance (p) values, for Total harmonic response PC (group comparison)

MFs	11	17	23	37	53	79	113	173	263	397
Y-M	.50	.15	0.11	0.1	.15	.21	.11	1	1	1
Y-O	.13	.08	.19	.045*	.018*	.006*	.003*	.99	1	.635
M-O	1	1	.40	.254	0.66	.453	.48	1	1	.21

Note: *mark indicates significance, MFs-Modulation frequencies, Y-young, M-midage, O-older adults

There were inter-subject variability seen for participants in all the three groups, however on average, the total harmonic phase coherence was highest in young adults at

53-79 Hz, with robust average responses between 17-113 Hz (see figure 4.22). In midage adults, the 23-37 Hz range showed the greatest total harmonic response for phase coherence, followed by 53-113 Hz. In contrast, the responses of older adults were consistently low across all modulation frequencies. Figure 4.22 shows the Total harmonic response for Phase coherence across modulation groups for three groups.

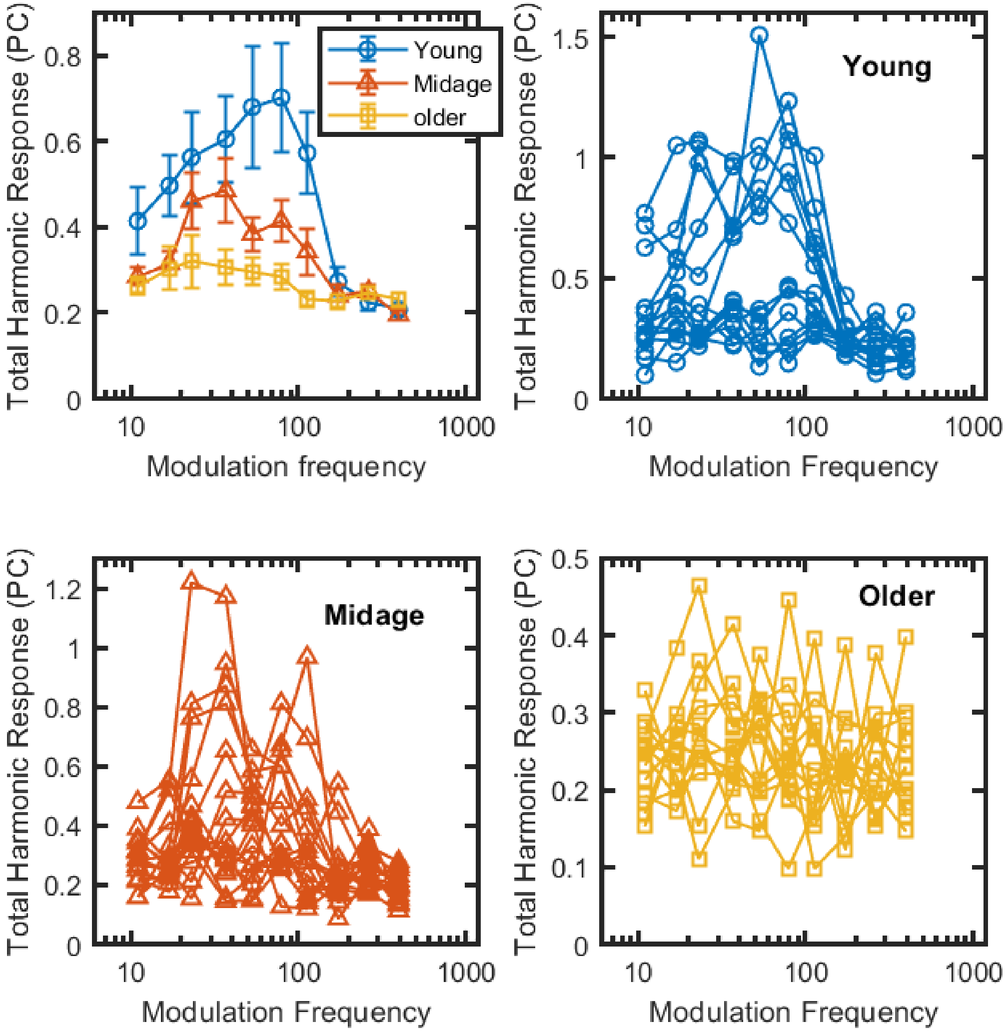

Figure 4.22 total harmonic response for Phase coherence across modulation frequencies for three groups. Left: mean ±SE data, Right: individual data points.

Summary of Results for specific aim 3.

The first harmonic (H1) had the maximum amplitude in the 30-40 Hz range for young adults, plateaued with a broad range of 35-80 Hz for middle-aged adults, and had a reduced and flatter response for older adults. The frequency at which peak amplitude occurred for H2 was consistent with H1 in all groups. SNR showed varying trends across modulation frequencies in each group. For the young adults, the SNR TMTF had a plateaued response from 17 to 113 Hz at H1 and from 23 to 113 Hz at H2. The range of modulation frequencies at which the response was robust, reduced with increasing age. For PC, young adults had a broader TMTF with a peak at 79 Hz for H1 and H2, while middle-aged adults had a peak response at 79 Hz for H1 and at 37 Hz for H2. Older adults had low PC values at H1 and no response at H2. It is noteworthy that the PC values at H2 for young adults were comparable to the PC values at H1 for older adults.

For response rates, the results showed that all young and middle-aged adults and 9 out of 16 older adults exhibited SNR > 6.13 dB at least at one modulation frequency. For young adults' response rates for SNR at H1 was about 80% for six stimulus conditions (17-113 Hz). For middle-aged adults, response rates were about 80% for 23-79 Hz. For older adults, no modulation frequency elicited response greater than 80%. The range of modulation frequencies eliciting responses from H2 through H4 reduced with age.

Response rates based on PC showed that all participants in each groups exhibited PC > 0.16 at least at one modulation frequency. For young adults and midage adults, response rates were >80% for six stimulus conditions, for older adults, modulation frequencies 23-79 Hz elicited a response rate greater than 50% with the peak response seen at 53 Hz. The range of modulation frequencies eliciting responses from H2 through H4 reduced with age.

For the total harmonic response, the results showed a significant effect of age-group and modulation frequency on the THR for amplitude, SNR and PC. Overall, for the total harmonic response, the amplitude and SNR and PC measures showed a very distinct pattern. The THR amplitude TMTF had maximum values in frequencies less than or around 23 Hz, but for the phase coherence and SNR, the maximum values were seen above 23 Hz that is around 79 Hz.

Chapter V.

Discussion

Underlying physiology for AMcVEMP

The underlying physiological mechanism for generation of AMcVEMP could be attributed to the rectification process occurring in vestibular (saccular) hair cells (see for review, Eatock & Songer, 2011). During the saccular transduction, when the hair bundle of the saccule tilts towards the kinocilium, the resulting voltage changes are significantly larger than those caused by displacements in the opposite direction (Soto et al., 2002). This directional sensitivity of the hair cells essentially acts as a rectifier, transducing signals for unidirectional input. When combined with a low-pass filter, the rectifier functions as an envelope detector (Lütkenhöner, 2019a) and follows the pattern of that of the stimulus envelope as seen in AMcVEMP. The envelope following responses generated at the saccular hair cells are reflected at the level of the SCM muscle in the form of AMcVEMP. Theoretical models for VEMP generation have demonstrated that motor unit action potentials in the SCM follow the envelope of an ongoing stochastic signal, exhibiting a similar pattern to the stimulus envelope (Lütkenhöner, 2019a). These envelope following responses, referred to as VEMPcorr by Lütkenhöner (2019), is similar to what we are calling as AMcVEMP.

AMcVEMP shows a high degree of synchrony.

The AMcVEMP responses in our study exhibited a remarkably high degree of synchrony, with a phase coherence (PC) greater than 0.95, particularly in our young adult participants. This finding aligns with a similar observation reported by Lawlor et al. in 2022, where the average AMcVEMP PC for young adults exceeded 0.91 (Lawlor et al., 2022). Interestingly, these observations indicate that AMcVEMP phase locking is substantially higher compared to the auditory system.

Animal studies have also demonstrated that vestibular nerve fibers exhibit greater phase locking and more precise synchronization compared to cochlear nerve fibers (Curthoys et al., 2019; Palmer & Russell, 1986). Moreover, recordings from irregular vestibular afferents have shown a higher degree of synchrony (Curthoys et al., 2019; McCue & Guinan, 1994) and saccular synaptic transmission has been found to be faster than cochlear afferent transmission (McCue & Guinan, 1994). This higher synchrony observed in AMcVEMP responses may be attributed to the type I vestibular hair cells, which are surrounded by calyceal synapses (Curthoys & Grant, 2015). The specialized hair cell-synapse calyceal arrangement in type I hair cells enhances the speed of both quantal and non-quantal transmission, facilitating impulses to the irregular vestibular afferents (Songer & Eatock, 2013). The minimal response latency of non-quantal synaptic transmission in type I hair cells leads to faster synaptic transmission between hair cells and vestibular afferents, which may contribute to the higher synchrony observed in AMcVEMP recordings (McCue & Guinan, 1994; Songer & Eatock, 2013). Furthermore, irregular vestibular afferents exhibit higher conduction velocities (Eatock & Songer, 2011), which likely contribute to the higher synchrony observed in our AMcVEMP recordings from the sternocleidomastoid muscle.

In our AMcVEMP recordings, we utilized a carrier frequency of 500 Hz, resulting in robust responses. This choice of carrier frequency is consistent with other studies investigating air-conducted and bone-conducted AMcVEMP (Bell et al., 2010; Lawlor et al., 2022; Oliveira et al., 2014). Additionally, studies on cats' afferents have indicated that irregular vestibular afferents are tuned to frequencies below 1000 Hz (McCue & Guinan, 1994), suggesting that the irregular vestibular afferents may convey rectified signals (for a carrier frequency below 1000 Hz) from the saccule to the SCM, contributing to the generation of AMcVEMP responses with a higher degree of synchrony.

Effects of age on AMcVEMP

Our study demonstrated a significant effect of aging on the AMcVEMP across modulation frequencies. With increasing age, there occurred reduction in the AMcVEMP amplitude, SNR, and PC. The AMcVEMP temporal modulation transfer function (TMTF) range also reduced with increasing age.

Age-related degenerative changes

The decline in AMcVEMP responses with increasing age, can be attributed to age-related degenerative changes that occur in the entire vestibular system, and particularly the sacculo-collic reflex pathway. These degenerative changes can manifest from the peripheral end organs to the central vestibular structures, as indicated by previous studies (Richter, 1980; Smith, 2016; Velázquez-Villaseñor et al., 2000; Rauch et al., 2001). Notably, these studies have shown a reduction in the proportion of vestibular hair cells (Richter, 1980) and neuronal cell counts in Scarpa's ganglion (Richter, 1980; Velázquez-Villaseñor et al., 2000; Rauch et al., 2001), as well as alterations in the neurochemical balance within the vestibular nuclei and brainstem (Smith, 2016).

These structural, functional, and neurochemical degenerative changes likely compromise the sacculo-collic reflexes, leading to a reduction in AMcVEMP amplitude, SNR, and phase coherence. Additionally, diminished range of the temporal modulation transfer function may also result from age-related degenerative changes occurring at various points along the sacculo-collic pathways. In the previous section, we proposed that the generation of AMcVEMP originates from type I hair cells. Thus, specifically, age-related synaptic loss in calyceal innervations and type I hair cells (Wan et al., 2019) could also be responsible for the diminished transmission between hair cells and synapses, resulting in reduced AMcVEMP responses as an effect of aging. The study conducted by Wan et al. (2019) on aging mice provides supporting evidence for this hypothesis by demonstrating a specific loss of type I hair cells in the otoliths during the early stages of aging.

Effects of age are less pronounced for phase coherence compared to SNR

In our study, we observed that the impact of age on phase coherence of AMcVEMP was less pronounced compared to SNR. When considering older adults, we observed a 100% response rate using the PC criterion ($PC > 0.155$), while the response rate using the SNR criterion ($SNR > 6.13$ dB) was 56%. The SNR and PC measures obtained from AMcVEMP responses potentially reflect firing rates and synchronization index, respectively, of irregular vestibular afferents. Previous recordings from individual irregular afferents have shown that even with lower levels of vestibular stimulation, it is still possible to achieve a higher synchronization index (McCue & Guinan, 1994). On the other hand, achieving an adequate firing rate requires a higher level of stimulation. Based on these findings, we propose that when subtle vestibular damage exists or when the stimulation reaching the vestibular system is limited, synchronization measures may not be significantly affected. In such conditions, measures of synchrony (PC) may remain relatively stable, while measures of firing rate (SNR) may be poorer, necessitating higher levels of stimulation to elicit a response. Interestingly, in our study, we observed moderate phase coherence but poorer signal-to-noise ratios (SNRs) in our older adult participants. This observation suggests that measures of synchrony are less affected by age-related damage. It implies that even in the presence of age-related changes in the vestibular system, the ability of irregular afferents to synchronize their activity may be relatively preserved compared to measures reflecting firing rates (SNR). We observed that the AMcVEMP could be elicited for a range of modulation frequency.

The AMcVEMP responses elicited across a range of modulation frequencies support the idea that human otolith organs can encode both slower and faster translational head accelerations to ensure postural stability. In a study by Curthoys et al. (2019), it was concluded that irregular afferents with higher phase locking ability would exhibit faster responses to head acceleration, potentially serving as jerk detectors.

Effects of age on Limit of AMcVEMP TMTF

One of the objectives of our study was to examine the effects of age on the limit and shape of AMcVEMP TMTF. The use of AMcVEMP as a tool for assessing vestibular function is relatively new, and thus far, research has primarily focused on young and healthy adults. In contrast, temporal modulation transfer functions have been extensively studied in the auditory system, encompassing both young and aging populations (Anderson & Karawani, 2020; Kale & Heinz, 2012; Purcell et al., 2004). In the following paragraphs, we aim to elucidate the effects of age on AMcVEMP TMTF limit and shape by drawing comparisons with findings from the auditory literature.

AMcVEMP is relatively new.

Amplitude-modulated stimuli have been widely used in the study of the auditory system, whereas AMcVEMP (used to study vestibular function) is a relatively new technique. In fact, the initial study on AMcVEMP (Bell et al., 2010) was not primarily focused on investigating the vestibular system but rather aimed to assess any possible contaminating artifacts from vestibular responses during the recording of ASSR. In contrast, amplitude-modulated auditory responses have been employed for decades in studying auditory detection (Picton, John, Purcell, et al., 2003) , speech perception (Mepani et al., 2021), and auditory physiology (Encina-Llamas et al., 2019). Temporal modulation transfer functions (TMTFs) for the auditory system have been extensively studied using behavioral (He et al., 2008) and electrophysiological techniques (see for review, Anderson & Karawani, 2020) in both young and aging populations, while this is the first study to report AMcVEMP in aging population. When comparing AMcVEMP TMTF with auditory TMTF, some similarities and differences emerge.

AMcVEMP TMTF limit: young adults

In our study, for the young adults, the average AMcVEMP responses remained above -10dB modulation gain for modulation frequencies between 11-173 Hz. The AMcVEMP TMTF for other measures (SNR and PC) also yielded similar TMTF limit. Thus, the appearance of the AMcVEMP TMTF is more like a broad band-pass filter. For the auditory system, however, the appearance of TMTF is more like a low pass filter. Among young adults, the auditory sensitivity to the envelope of the amplitude-modulated signal remains relatively constant as long as the modulation frequency is below approximately 100 Hz. Beyond this point, auditory sensitivity gradually decreases, resembling a low-pass filter (Bacon & Viemester, 1983). Young listeners have robust auditory responses for amplitude modulation (25% depth) up to frequencies of about 500 Hz using surface electrodes on the scalp (Purcell et al., 2004). The AMcVEMP TMTF exhibits a slightly different pattern. The upper limit of AMcVEMP TMTF for young adults is lot lower compared to auditory TMTF upper limits (>500 Hz). The upper limit of AMcVEMP TMTF for young adults observed in our study is 263 Hz as none of the participants in any of the groups elicited responses at 397 Hz modulation frequency. The finding is consistent with the previous reports on AMcVEMP TMTFs upper limit (287 Hz) in young adults (Lawlor et al., 2022b); slight differences could be attributed to the different age groups and different modulation frequencies used in this study.

Effects of age on AMcVEMP TMTF limit

The effects of aging on auditory TMTF versus AMcVEMP TMTF differ significantly. For auditory TMTF, the effect of age is not significant for modulation frequencies below approximately 80 Hz (Grose et al., 2009; Leigh-Paffenroth et al., 2006; Purcell et al., 2004). In contrast, the effects of aging on AMcVEMP TMTF are observed across a broad range of modulation frequencies, including frequencies below 80 Hz. Since auditory responses to 20-Hz and 40-Hz rates are primarily driven by cortical generators, while responses to higher rates involve brainstem generators (Herdman & Stapells, 2003), auditory literature suggest that aging affects brainstem, but not cortical phase locking for the auditory responses. However, for AMcVEMP, the effects of aging are seen across a range of modulation frequencies, possibly because lower frequency TMTFs in AMcVEMP do not involve cortical generators but may instead have contributions from motor units in the sternocleidomastoid muscle. Further, only the upper limit auditory TMTF reduces as an effect of aging (Purcell et al., 2004) while for AMcVEMP TMTF, aging reduces the bandwidth for both the upper as well as lower limit. The average middle-aged adults had a TMTF limit of 23-113 Hz compared to young adults 11-173 Hz (see figure 4.12). The variations in the impacts of aging on the AMcVEMP TMTF compared to auditory TMTF may be due to the distinct ways in which the two sensory processes function, and the distinct ways in which they are recorded.

Shape of AMcVEMP TMTF

The shape of the AMcVEMP TMTF appeared more like a band pass filter compared to the low pass filter seen for auditory responses (Purcell et al., 2004). The temporal modulation transfer function (TMTF) varied across three different groups and across three measures. Specifically, the SNR and phase coherence TMTF exhibited a broader shape compared to the amplitude TMTF. In young adults, the amplitude TMTF (see figure 4.7) showed a peak at 37 Hz, whereas the SNR(see figure 4.9) and PC TMTF (see figure 4.10) had a peak at around 79 Hz in young adults. This finding aligns with previous studies that have also reported a similar peaked amplitude at around 37 Hz and SNR peaks at 79 Hz in young adults (Bell et al., 2010; Lawlor et al., 2022; Oliveira et al., 2014). Further, the normalized TMTFs for young adults also showed similar findings (See figure 4.14); amplitude peaking around 37 Hz while SNR and PC peaked at 79 Hz.

Amplitude TMTF peaks around 37 Hz among young adults

The peaking of the amplitude temporal modulation transfer function (TMTF) at 37 Hz in young adults may be attributed to contributions from motor units in the sternocleidomastoid (SCM) muscle, which exhibit a spectral peak around 40 Hz. Wit and Kingma (2006) conducted a study where they analyzed the modeled response of motor unit action potentials (MUAPs) recorded from surface electrodes over the SCM muscle. They found that these MUAPs had a spectral peak around 40 Hz (Wit & Kingma, 2006). The presence of energy maxima at approximately 40 Hz from the motor units in the SCM muscle could be contributing to the observed peak in the amplitude TMTF in our study.

In our study, the amplitude analysis was based on the fast Fourier transform (FFT) bin of the modulation frequencies. The peak amplitude observed at 37 Hz may reflect the energy contributions from motor unit action potentials, which have a spectral peak around 40 Hz. Additionally, the noise TMTF in our study also exhibited a peak at around 37 Hz (see figure A.1 in the appendix 2), which could also be influenced by the motor unit action potentials. During our analysis, the noise was estimated within a range of ±5 Hz around the modulation frequency bin. The higher levels of noise in these bins suggest contributions from motor unit action potentials. Furthermore, the shape of the amplitude TMTF in young adults overlapped with the modeled responses of motor unit action potentials in previous studies (Lawlor et al., 2022) as well as in our current study (see figure 4.14). Additionally, theoretical models proposed by Lutkenhoner suggest that AMcVEMP (referred to as VEMPcorr in their study) can be considered as a band-pass filtered representation of motor unit action potentials (Lütkenhöner, 2019).

Effects of age on the Amplitude TMTF peak

In our study, the average normalized AMcVEMP TMTF for amplitude showed a flattened response (37-79 Hz) in middle-aged adults and an upward peak shift (to 79 Hz) among older adults compared to 37 Hz peak seen in young adults (see figure 4.14). This study is the first to investigate the AMcVEMP TMTF in the aging population, and our explanation for these age-related changes is based on speculative reasoning. Previous modeled studies from EMG surface electrodes were administered in young adults, and currently, there is no information available about the spectral content of SCM EMG from middle-age and older adults. A study examining the age-related changes in the spectral content of the activated SCM would be an interesting endeavor. Nonetheless, there is some information on the changes in the viscoelastic properties of the SCM muscles as a result of aging and these changes may contribute to changes in the AMcVEMP responses measured as an effect of aging.

For instance, Kocur and colleagues reported increased stiffness (28%) and reduced elasticity (53%) of SCM in older adults (Kocur et al., 2017, 2019). These age-related elastic and stiffening changes in the SCM might also alter the characteristics of motor units in the aging population. Since stiffness is directly linked to the resonance frequency of a system, the increased stiffness in the SCM could lead to a higher resonance frequency and result in an upward shift in the modulation frequency resulting in a more robust responses at a higher modulation frequency as a consequence of aging.

It is important to note that the upward shift in modulation frequency observed in our study should not be confused with the upward shifts in the frequency tuning of conventional toneburst VEMPs (Jha, et al., 2022; Piker et al., 2013). In our study, the upward shift was observed in the modulation frequency, whereas conventional VEMPs demonstrate an upward shift in the frequency tuning of tone bursts, which is similar to the carrier frequencies used in our study. Although these phenomena may appear different, comparing the two based on viscoelastic properties of SCM is warranted. The shifts in the tuning of conventional VEMPs have been attributed to stiffening changes in the inner ear due to aging (Singh et al., 2021; Jha et al., 2022). Nevertheless, it is also possible that the upward shift in conventional VEMPs observed in older adults may be influenced by changes at the muscles and not solely by inner ear alterations.

Shape of SNR and PC TMTF

In our study, we found that the average signal-to-noise ratio (SNR) among young adults was around 10 dB for a wide range of modulation frequencies (17-173 Hz). This value is slightly lower compared to previous studies by Bell et al. and Lawlor et al., where the average SNR was around 15 dB. However, it's important to note that both of these studies had a younger group of participants compared to the young adults used in our study (20-39 years). Despite the overall average difference, most of our younger young adults (20-30 years), also exhibited an SNR above 15 dB, which is consistent with previous findings.

The young adults, SNR (see figure 4.9) and PC TMTF (see figure 4.10) appeared more like a broad band pass filter compared to the appearance of narrow bandpass filter for amplitude TMTF (see figure 4.7). Among the young adults, the peak of the amplitude TMTF was found to be at 37 Hz, while the highest signal-to-noise ratio (SNR) occurred at 79 Hz, which aligns with the findings of Bell et al. and Lawlor et al. Additionally, our study revealed that the noise TMTF reached its peak at 37 Hz and decreased as the modulation frequencies increased (see appendix 2, figure A.1). It is important to note that despite the largest amplitude occurring at 37 Hz, the higher levels of noise at 37 Hz could have resulted in a better SNR around 79 Hz, where the amplitude response remained robust, but the noise was significantly lower. Additionally, we also observed the average highest phase coherence values at 79 Hz. Highest SNR and PC at 79 Hz is significant because 80 Hz is the most commonly used test frequency for auditory steady-state responses (ASSR) and have demonstrated to have large SNR and phase coherence (Picton et al., 2003). Previous auditory studies have attributed these larger responses at 79 Hz to brainstem generators, and similar brainstem generators may exist for vestibular (AMcVEMP) responses as well.

Further when comparing AMcVEMP with ASSR, in rarest of the rare case, one could argue that the amplitude modulation AMcVEMP might contribute to artifacts when recording ASSR at very high levels in tense patients. Notably, Clinard et al. (2020) reported that even a moderate low EMG level of 30μV is sufficient to elicit AMcVEMP (Clinard et al., 2020a). Therefore, it is plausible that some contamination from the vestibular system may occur during ASSR recordings if the participant's muscles are tensed. To address this potential issue, it would be advantageous to conduct individual recordings of both ASSR and AMcVEMP, while also performing simultaneous recordings of both measures. This approach would provide a clearer understanding of any possible contributions of AMcVEMP during ASSR recordings, helping to untangle the effects of vestibular contamination during recording of ASSR.

Best modulation frequency:

Our study found that the AMcVEMP can be elicited across a range of modulation frequencies. However, we observed that the best modulation frequency, which produced the most robust AMcVEMP response in terms of amplitude, signal-to-noise ratio (SNR), and phase coherence, fell within the range of 37-79 Hz (as depicted in Figure 4.13). These findings suggest that when assessing any participant, there is a higher likelihood of observing a response when using modulation frequencies within this optimal range. These frequencies, between 37-79 Hz, may be the most effective for obtaining significant AMcVEMP responses.

In clinical assessments of auditory function, the auditory steady-state response (ASSR) is administered at the modulation frequencies that elicit the most robust ASSR, typically around 80 Hz (as 40 Hz is affected by sleep). Although ASSR can be obtained across a range of modulation frequencies (Purcell et al., 2004), clinical practice often focuses on the modulation frequencies around 80 Hz. Similarly, for studying vestibular function in clinical populations using AMcVEMP, it might be time-efficient to employ the best modulation frequencies within the range of 37-79 Hz. However, it is essential to note that AMcVEMP is a relatively new technique, and there is limited information available regarding its application in clinical populations. Moreover, the optimal modulation frequencies in different clinical populations may differ from what is observed in healthy adults, depending on the specific underlying pathophysiology being studied.

Vestibular non-linearities

Another objective of our study was to examine the non-linear harmonic distortion in AMcVEMP across three different age groups. In our study, we analyzed measures including amplitude, signal-to-noise ratio (SNR), and phase coherence (PC) across harmonics (H2 through H4). We also reported, response rates based on SNR >6.13 and PC >0.155 at each harmonics of each modulation frequency. Further we used a novel approach to characterize harmonics by calculating the total harmonic response.

Total Harmonic Response:

In this study, we employed a novel method to characterize harmonics by calculating the total harmonic response, which is the sum of values from H2 through H4 for each metric (amplitude, SNR, and phase coherence). Previously, a measure called, total harmonic distortion (THD) have been utilized to report non-linearity from both the auditory (Bidelman & Bhagat, 2020; Jeng et al., 2009) as well as AMcVEMP responses (Clinard et al., 2022b). The formula for THD ={ ($\sqrt{H_2^2 + H_3^2 + H_4^2}$)/H1}*100.

While this formula appears to be effective in calculating THD as a percentage, when the responses at modulation frequencies (H1) are significant and robust, it presents a challenge when the responses at the H1 are insignificant or absent. Using this formula, lower values at H1, would result in higher THD values, leading to potentially misleading findings. For instance, let's consider the following scenario: at 79 Hz, the H1 through H4 values are (12, 7, 5, 1), resulting in a THD of approximately 72%. However, at 397 Hz, if the H1 through H4 values are (0.8, 1.2, 0.9, 1), the THD would be >200%. In this example, even though the response at 397 Hz is absent (H1 through H4 values), it yields a lot higher THD compared to 79 Hz.

To address this issue, Clinard and colleagues, characterized non-linearity in young adults by reporting THD only for modulation frequencies that elicited a response rate of at least 50% at H2 (Clinard et al., 2022; Lawlor et al., 2022). However, in the current study, this analysis was not feasible as the response rates at H2 were lower than 50% for all modulation frequencies among older adults and most modulation frequencies in middle aged adults. Therefore, we resorted to a new method to characterize non-linearity: total harmonic response.

AMcVEMP responses at the harmonics:

AMcVEMP responses at the harmonics can be hypothesized to be occurring due to the mechanical movement of hair cells and subsequent distortions produced in these responses. Measure of non-linearity through motility of isolated vestibular hair cells have been seen to be more pronounced for the type I hair cells (Zenner et al., 1990; Takumida et al., 1994). The saccular hair cells directional sensitivity allows the hair cells to essentially act as a rectifier, and the mechanical distortions at the hair cells result in responses at the harmonics of the modulation frequency.

Non-linearities in the vestibular system have been extensively studied in animal models (Jaramillo & Hudspeth, 1993; Roongthumskul & Bozovic, 2015; Zenner et al., 1990; Levin & Holt, 2012); non-linearities in the form of rectification (Levin & Holt, 2012) and distortion products (Jaramillo et al., 1993) from animal models have also been reported. For e.g, In a study by Jaramilo et al., they stimulated the hair cells in a bullfrog's saccule and observed responses not only at the stimulating frequency but also at the second harmonic in all the hair cells they investigated (Jaramillo et al., 1993). AMcVEMP responses align with the rectification process in the saccule, and responses at the harmonics reflect the non-linear distortions. Currently AMcVEMP represents the only non-invasive method to assess non-linearities from the human vestibular system. Utilizing and building upon the practical approach to measure the non-linearities from the human vestibular hair cells could significantly enhance our understanding of vestibular function in both healthy and clinical populations.

Reduced vestibular non-linearities may have perceptual and functional impact.

Our study found that younger adults had higher total harmonic responses across all metrics, but these responses decreased with increasing age. Additionally, middle-aged and older adults showed reduced AMcVEMP response rates at the harmonics. These findings suggest that age-related degenerative changes in type I hair cells, calyceal synapses, irregular nerve fibers, and throughout the sacculo-collic reflexes, may contribute to the reduced responses at the harmonics. The age-related reduction in non-linearities in AMcVEMP responses might have a similar impact as the observed age-related reduction in auditory non-linearities. The reduction in auditory non-linearity has been linked to negative perceptual and functional outcomes, such as increased sensitivity to loudness and difficulties in speech perception, especially in noisy environments. (Abdala et al., 2021; Bidelman & Bhagat, 2020). The changes in the non-linearity of the vestibular system could also negatively affect one’s functional balance and put them at risk for falls. However, the relationship between the non-linear vestibular changes and their impact on the functional balance is not yet fully understood. It is important to investigate whether similar (to the auditory system) functional and perceptual consequences exist due to negative changes in the non-linearity of the vestibular system.

Previous research has shown age-related changes in perceptual sensitivity to vestibular stimulation, and have also indicated an increased risk of falls among older adults (Chiarovano et al., 2016; Jacobson et al., 2018; Piker et al., 2020). For e.g., Piker et al. (2020) reported that a higher proportion of older adults fail to perceive movement during caloric stimulation, and these individuals also exhibit poor performance on functional measures, indicating a potential increased risk of falls. While we do not claim that changes in the observed non-linearities in AMcVEMP are the sole cause of vestibular perceptual and functional deficits in older adults, they could be one of the contributing factors and exploring the link between AMcVEMP and functional balance is important.

Previous studies investigating the relationship between conventional cVEMPs, and functional outcomes have yielded mixed results. Anson et al. (2017, 2019a, and 2019b) did not observe a significant association between otolith function, as measured by conventional VEMPs, and gait (Anson et al., 2017, 2019a, 2019b). However, other studies by some overlapping authors have reported an association between otolith function assessed through conventional cVEMPs and functional balance (Agrawal et al., 2013; Gandhi et al., 2021). We propose that AMcVEMP is mediated by a different mechanism than conventional cVEMPs; While AMcVEMP reflects saccular rectification and vestibular non-linearity, there is no evidence of conventional cVEMPs doing the same. Our hypothesis suggests that AMcVEMP may originate from the type I hair cells in the saccule and is mediated by the irregular afferents that connect to the sternocleidomastoid muscle. Physiological recordings from monkeys have demonstrated that these irregular afferents are specialized in detecting natural head movements. (Schneider et al., 2015). As a result, AMcVEMP measures (SNR and PC), mediated through irregular afferents, may provide more detailed information about the connection between vestibular physiology and functional balance beyond mere associations or dissociations observed with conventional VEMPs. Linking vestibular non-linearity, such as AMcVEMP, to functional balance could enhance our understanding of the vestibular system and open up avenues for further research to mitigate the risk of falls among older adults and patients with dizziness. Additionally, incorporating AMcVEMP into clinical assessments can enhance the standard of vestibular diagnostics.

Comparing Novel AMcVEMPs with conventional cVEMPs:

The use of AMcVEMP to evaluate vestibular functions is relatively new, and therefore it is essential that we compare it with the widely used conventional cVEMPs to understand its effectiveness.

Novel AMcVEMP vs conventional cVEMP: Basic properties

So far AMcVEMP have only been administered in young adults (Bell et al., 2010; Clinard et al., 2020a, 2022; Lawlor et al., 2022; Oliveira et al., 2014) and this is the first study to report effects of aging on the AMcVEMP TMTF. Despite the limited work with AMcVEMP we have learnt that the fundamental properties of AMcVEMP are similar to the conventionally used transient cVEMPs. First, AMcVEMP responses are not affected by the condition of cochlear function (Oliveira et al., 2014), meaning they can be observed in individuals with sensorineural hearing loss. Second, the magnitude of AMcVEMP increases with higher EMG activity (Bell et al., 2010; Clinard et al., 2020a) and intensity.

<u>There are also notable differences between conventional cVEMP and AMcVEMP</u>. The primary distinction lies in the stimuli used. AMcVEMP employs sustained amplitude-modulated tones instead of tone bursts used in conventional cVEMP. By using sustained amplitude-modulated tones, AMcVEMP allows for additional analyses compared to transient cVEMP (Clinard et al., 2020, 2022; Lawlor et al., 2022). While transient cVEMP provides an onset biphasic response and traditionally relies on amplitude and latency-based analyses (for review see. Rosengren et al., 2019) without providing any information about the sustained activity of the sacculo-collic reflexes, AMcVEMP waveforms exhibit clear distinctions between onset, sustained, and offset responses (see figure 4.2). Moreover, AMcVEMP provides more than just amplitude and latency-based analyses. It enables the examination of AMcVEMP magnitude, synchrony, and non-linearity within the sacculo-collic reflex pathway. <u>Therefore, although AMcVEMP shares similarities with conventional VEMPs, it also offers the ability to assess vestibular mechanisms such as synchrony and non-linearity, which cannot be studied using the conventional cVEMPs.</u>

Novel AMcVEMP vs conventional cVEMP: Effects of age

The effects of age on the sacculo-collic reflexes have been well documented using conventional VEMPs: reduced response rates, decreased amplitude, elevated thresholds, and an upward shift in the frequency tuning (Brantberg et al., 2007; Ochi & Ohashi, 2003; Piker et al., 2013; Singh et al., 2021; Jha et al., 2022). However, when it comes to evaluating older adults with dizziness, the usefulness of conventional VEMPs is quite limited (Piker et al., 2015). This is because conventional cVEMPs primarily rely on amplitude-based analyses and are more frequently absent in older adults. The absence of cVEMPs in older adults can occur due to various reasons and does not provide any pathophysiologic information. Further, the overall limitation in the assessment of vestibular system, results in higher proportion of unspecific diagnosis among dizziness patients (Wassermann et al., 2022). Therefore, considering the high prevalence of dizziness among older individuals and the overall limited capabilities of current assessment methods, it is crucial to enhance the standard of vestibular diagnostics, and one potential aid in this regard is AMcVEMP.

Our study demonstrated that AMcVEMP responses exhibit differences compared to conventional cVEMPs, allowing for more detailed analysis of measures such as amplitude, signal-to-noise ratio (SNR), and phase coherence. We observed varying effects of aging on different AMcVEMP measures. While conventional cVEMPs are often absent in older adults, AMcVEMP response rates for middle-aged and older individuals are higher when considering synchrony measures. By applying specific synchrony criteria (PC > 0.155), all participants, including older adults, showed present responses at least at one modulation frequency, resulting in 100% response rates. Although the response rates for SNR measures (56%) are comparable to conventional cVEMPs, the novel AMcVEMP provide objective and quantifiable measures of magnitude and synchrony and could be a more effective tool for assessing older adults and clinical population.

Clinical Implications:

Using AMcVEMP in clinical populations can improve our understanding of the underlying mechanisms of various vestibular disorders. For example, Meniere's Disease (MD) currently lacks a reliable biomarker for diagnosis. In the last couple of decades, the frequency tuning of conventional VEMPs has been proposed as a biomarker for MD diagnosis (Rauch et al., 2004b; Singh et al., 2023) . Conventional tone burst VEMPs have shown an upward shift in the frequency tuning of VEMP, attributed to stiffening changes caused by endolymphatic hydrops. However, an upward shift in the frequency tuning of VEMP is also observed in aging population (Jha et al., 2022; Singh et al., 2021; Piker et al., 2013), and the current biomarkers would often lead to false positive diagnosis of MD, especially in midage and older adults. AMcVEMP measures may offer an advantage in studying the tuning characteristics in MD. The carrier stimulus used in AMcVEMP is more frequency specific compared to conventional tone bursts. The carrier frequency of AMcVEMP can provide information about frequency-specific tuning changes, while the modulation frequency may indicate alterations in the mechanical transduction properties associated with MD.

Furthermore, it would be interesting to utilize AMcVEMP to study patients with auditory neuropathy spectrum disorders (ANSD) and myasthenia gravis (MG). ANSD patients may experience a higher prevalence of vestibular abnormalities due to the close proximity between the auditory and vestibular nerves (Sheykholeslami et al., 2009). Conventional vestibular tests may also reveal abnormal findings (e.g., absent vestibular reflexes) in ANSD patients (Chen et al., 2021). Adding AMcVEMP to the test battery will allow for the examination of synchrony and non-linearity measures within the vestibular pathways, providing a more comprehensive assessment in ANSD cases that may involve vestibular dyssynchrony. In the case of MG, repetitive vestibular evoked myogenic potential (rVEMP) stimulation has been introduced to aid in the diagnosis of this autoimmune condition affecting neuromuscular transmission. MG patients typically exhibit a decrementing response to repetitive nerve stimulation, and the repetitive VEMP amplitude decreases with an increasing number of sweeps (Wirth et al., 2020). It would be interesting to see how MG affects AMcVEMP across various modulation frequencies and sweeps. Assessing AMcVEMP TMTF in MG might provide deeper understanding of pathophysiology and severity of the condition.

AMcVEMP can also be useful in pediatric vestibular assessments. AMcVEMP provides an objective approach to examine vestibular functions. Simultaneous ASSR and AMcVEMP recordings can be beneficial, particularly in pediatric populations. This combined approach might facilitate efficient examination of auditory and vestibular functions in children with congenital sensorineural hearing loss, a population that often experiences coexisting vestibular impairment. Vestibular assessments in pediatric population have gained tremendous interest in the last decade. Simultaneous AMcVEMP and ASSR provide objective analyses for both vestibular and auditory responses that can aid interpretation, even for less experienced examiners in a time efficient manner.

Finally, the non-linear changes observed in the AMcVEMP reflex due to aging may have functional and perceptual implications. Currently, AMcVEMP is the only non-invasive tool to assess non-linearities from the sacculo-collic pathways and linking electrophysiological findings with functional balance assessment will open avenues for improving overall vestibular rehabilitation and the standard of care for older adults.

Limitations:

Origin of AMcVEMP response.

One of the question that was frequently asked during our conference presentations was about the primary source of the AMcVEMP response, whether it is auditory, myogenic, or vestibular? Firstly, our assertion is that AMcVEMP responses are not auditory but are of vestibular origin. AMcVEMP responses are present in individuals with hearing loss (Oliveira et al., 2014). Additionally, the AMcVEMP response amplitudes and phase coherence values are much larger than conventional ASSRs, and these responses scale with EMG, which is consistent with VEMP responses. Secondly, as far as the response is to have myogenic origin, we posit that while AMcVEMP responses might have contributions from the motor units of the SCM, they are not exclusively non-vestibular; Rather, the AMcVEMP vestibular reflex are initiated at the level of otoliths and are recorded at the level of the SCM. Our research has demonstrated that despite the adequate muscle tension required to generate AMcVEMP, responses that were present at higher intensity levels were absent at lower intensity levels (unpublished data presented at the American Balance Society conference in 2021). This suggests that eliciting AMcVEMP responses requires a sufficiently intense signal or vestibular stimulation to activate the AMcVEMP reflex, and mere muscular contraction is insufficient to elicit AMcVEMP. Consequently, we posit that the origin of the AMcVEMP response lies within the vestibular hair cells, and the reflex initiated at the saccule is subsequently reflected at the sternocleidomastoid muscle (SCM).

Carrier frequency

The current study utilized a 500 Hz carrier frequency to elicit bone conducted AMcVEMPs. Even though, a previous study examining effects of carrier frequencies to elicit AMcVEMP showed 500 Hz to be the best carrier frequency to elicit air-conducted AMcVEMP (Oliveira et al., 2014b), we think that 500 Hz carrier frequency may not be the best stimulus to elicit bone conducted AMcVEMP. It may be possible that 250 Hz or even frequencies lower than 250 Hz (eg. 100 Hz) could be a better vibratory stimulus compared to 500 Hz to elicit AMcVEMP. Todd et al., (2009) showed that the otoliths are tuned for lower frequency tone bursts (100 Hz) for vibratory stimuli. Comparing the effects of carrier frequencies for amplitude modulated cVEMP would be a very interesting endeavor but these objectives were beyond the scope of this project and therefore we chose to use 500 Hz carrier frequency that have been used before.

Age groups

This the first study to report AMcVEMP TMTF across three different age groups (young, midage and older adults), spanning across two decades. Our sample size is not large enough to divide the participants into age groups based on decade. We suggest that future studies consider dividing groups into narrower age ranges, such as across a single decade which would enable parsing out a more detailed effects of aging.

Conclusions

AMcVEMP responses exhibit characteristics consistent with saccular rectification, mimicking the stimulus envelope. Compared to conventional cVEMPs, AMcVEMP waveforms display a distinct separation between the onset, sustained, and offset responses, allowing for more detailed analysis for amplitude, SNR and phase coherence. As an effect of aging, there is a reduction in AMcVEMP amplitude, signal-to-noise ratio (SNR), and phase coherence. The effect of age on phase coherence measures is less pronounced compared to amplitude and SNR. The shape of the AMcVEMP TMTF resembles a bandpass filter and the range of TMTF reduces with increasing age. The optimal modulation frequency for eliciting AMcVEMP responses falls within the range of 37-79 Hz for all the three groups. AMcVEMP non-linear harmonic responses reflect non-linearity from the sacculo-collic pathways. As age increases, there is a decrease in non-linearity. The reduced non-linearity (response rates at harmonics) in middle-aged and older adults may have negative perceptual and functional implications that require further exploration. Utilizing AMcVEMP in clinical populations has the potential to enhance our understanding of vestibular pathophysiology.

Appendix 1

Table A.1 Mean amplitude (SD) at the modulation frequencies for three groups

Groups	ModFreq (Hz)	11	17	23	37	53	79	113	173	263	397
Young		7.61 (7.4)	13.91 (12.4)	17.37 (16.5)	25.72 (23.3)	22.59 (19.7)	19.78 (17.7)	8.45 (7.71)	3.3 (2.82)	.74 (.98)	.03 (.02)
Midage		2.04 (1.8)	4.36 (3.8)	8.31 (9.4)	10.05 (12.4)	9.48 (8.8)	9.50 (10.66)	5.04 (5.77)	1.07 (1.1)	.22 (.29)	.10 (.20)
Older		1.00 (0.8)	2.99 (5.5)	3.75 (7.09)	3.03 (4.24)	4.1 (5.89)	5.56 (7.1)	1.16 (1.58)	.35 (.22)	.13 (.07)	.04 (.02)

Note: Mean (SD) values represented in the table across modulation frequencies for three groups (in rows)

Table A.2 Mean EMG (SD) at the modulation frequencies for three groups

Groups	ModFreq (Hz)	11	17	23	37	53	79	113	173	263	397
Young		52.63 (.81)	52.98 (1.10)	52.44 (1.18)	53.49 (1.03)	52.43 (.82)	51.29 (1.37)	52.35 (0.899)	53.01 (0.88)	52.89 (0.88)	53.05 (1.04)
Midage		53.71 (1.05)	52.80 (1.34)	52.24 (1.60)	53.21 (1.32)	51.63 (1.0)	51.99 (1.36)	53.46 (.96)	54.33 (1.07)	52.09 (1.78)	52.55 (1.16)
Older		52.20 (.93)	54.90 (1.35)	52.74 (1.40)	53.21 (1.19)	53.47 (1.16)	53.99 (1.49)	53.03 (1.72)	52.48 (1.52)	52.46 (1.57)	54.74 (1.43)

Note: Mean (SD) values represented in the table across modulation frequencies for three groups (in rows)

Table A.3 Mean Corrected Amplitude (SD) at the modulation frequencies for three groups

Groups	ModFreq (Hz)	11	17	23	37	53	79	113	173	263	397
Young		.154 (.15)	.285 (.25)	.35 (.32)	.513 (.44)	.457 (.38)	.424 (.38)	.172 (.15)	.067 (.055)	.015 (.02)	.001 (.0004)
Midage		.037 (.04)	.082 (.06)	.151 (.14)	.188 (.20)	.184 (.16)	.176 (.18)	.093 (.10)	.020 (.02)	.004 (.004)	.002 (.004)
Older		.020 (.02)	.058 (.11)	.073 (.14)	.059 (.08)	.080 (.11)	.114 (.26)	.021 (.026)	.007 (.004)	.002 (.001)	.001 (.002)

Note: Mean (SD) values represented in the table across modulation frequencies for three groups (in rows)

Table A.4 Post hoc analyses results for corrected amplitude (p values in the table).

Groups	ModFreq (Hz)	11	17	23	37	53	79	113	173	263	397
Young		<.001*	<.001*	.013*	.002*	.003*	.017*	.046*	<.001*	.009*	.146
Midage		<.001*	<.001*	.001*	<.001*	<.001*	.004*	<.001*	<.001*	.004*	.935
Older		.571	.678	.318	.205	.240	.536	.064	.292	.700	.170

Note: * mark indicates significant results.

Table A.5 Mean SNR (SD) at the modulation frequencies for three groups

Groups	ModFreq (Hz)	11	17	23	37	53	79	113	173	263	397
Young		7.23	9.44	11.06	11.57	11.82	13.26	10.9	9.28	5.42	-2.20
		(4.3)	(4.58)	(3.85)	(5.51)	(4.68)	(3.7)	(4.95)	(5.3)	(5.28)	(2.9)
Midage		3.77	5.61	8.23	8.25	9.13	10.59	8.6	5.33	1.54	-.90
		(3.3)	(3.86)	(3.05)	(4.85)	(5.08)	(3.7)	(5.85)	(4.1)	(3.8)	(3.3)
Older		1.45	2.99	3.64	3.14	5.23	3.86	2.87	2.56	0.91	-1.98
		(2.9)	(4.6)	(4.52)	(4.02)	(4.16)	(5.32)	(4.9)	(2.54)	(2.1)	(3.1)

Note: Mean (SD) values represented in the table across modulation frequencies for three groups (in rows)

Table A.6 Mean PC (SD) at the modulation frequencies for three groups

Groups	ModFreq (Hz)	11	17	23	37	53	79	113	173	263	397
Young		.395	.542	.646	.695	.714	.803	.63	.583	.316	.070
		(.30)	(.54)	(.30)	(.32)	(.32)	(.23)	(.37)	(.33)	(.28)	(.03)
Midage		.20	.306	.469	.491	.547	.606	.510	.311	.146	.084
		(.16)	(.30)	(.24)	(.30)	(.31)	(.29)	(.32)	(.22)	(.14)	(.04)
Older		.123	.188	.235	.205	.269	.276	.204	.141	.092	.071
		(.09)	(.22)	(.23)	(.20)	(.22)	(.30)	(.20)	(.10)	(.03)	(.04)

Note: Mean (SD) values represented in the table across modulation frequencies for three groups (in rows)

Table A.7 Mean Data (SD) for total harmonic response (amplitude)

Groups	ModFreq (Hz)	11	17	23	37	53	79	113	173	263	397
Young		5.79 (5.26)	5.94 (5.94)	5.81 (4.95)	4.21 (2.93)	2.94 (2.58)	2.29 (2.1)	.89 (.74)	.13 (.08)	.03 (.03)	.02 (.004)
Midage		3.50 (1.55)	3.94 (3.94)	4.93 (4.33)	3.57 (3.74)	1.59 (1.42)	.94 (.78)	.54 (.6)	.12 (.06)	.02 (.01)	.19 (.07)
Older		2.77 (1.36)	3.43 (3.43)	2.79 (2.88)	1.90 (1.24)	.96 (.63)	.55 (.28)	.21 (.06)	.10 (.04)	.009 (.008)	.02 (.008)

Note: Mean (SD) values represented in the table across modulation frequencies for three groups (in rows)

Table A.8 Mean Data (SD) for total harmonic response (SNR)

Groups	ModFreq (Hz)	11	17	23	37	53	79	113	173	263	397
Young		6.16 (5.11)	6.62 (3.95)	9.73 (8.44)	9.76 (6.45)	11.39 (9.51)	12.05 (9.09)	8.44 (6.51)	3.91 (1.81)	3.04 (1.4)	2.60 (.94)
Midage		4.0 (1.31)	4.56 (1.65)	6.96 (4.95)	6.45 (4.73)	5.30 (3.01)	5.89 (3.14)	4.92 (3.46)	3.26 (.87)	2.73 (1.09)	2.48 (.49)
Older		3.75 (1.44)	4.14 (3.27)	4.05 (3.45)	4.66 (3.26)	3.87 (1.96)	4.32 (2.24)	2.94 (.96)	2.95 (.97)	3.26 (.68)	3.05 (1.08)

Note: Mean (SD) values represented in the table across modulation frequencies for three groups (in rows

Table A.9 Mean data (SD) for Total harmonic response (Phase coherence)

Groups	ModFreq (Hz)	11	17	23	37	53	79	113	173	263	397
Young		.41 (.30)	.48 (.28)	.54 (.42)	.58 (.4)	.65 (.56)	.67 (.51)	.55 (.37)	.26 (.13)	.23 (.07)	.20 (.05)
Midage		.31 (.13)	.33 (.13)	.49 (.29)	.49 (.30)	.41 (.18)	.45 (.25)	.36 (.22)	.23 (.10)	.24 (.06)	.19 (.04)
Older		.26 (.08)	.30 (.21)	.32 (.25)	.31 (.16)	.30 (.13)	.28 (.13)	.23 (.07)	.22 (.06)	.24 (.06)	.23 (.06)

Appendix 2.

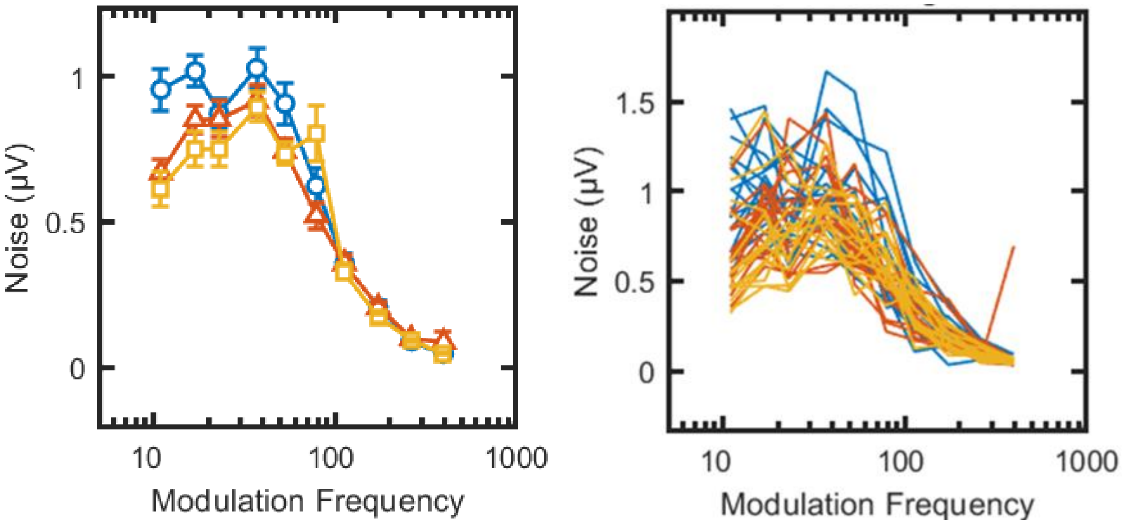

Figure A.1 Noise across modulation frequencies for three groups: separate lines (young-blue, midage-brown, old-yellow). Left: Mean and SE data. Right: Individual data of the participants across modulation frequency for 3 groups.

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