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Effects of age and middle ear resonance on the frequency tuning of the cVEMP

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Effects of Age and Middle Ear Resonance on the Frequency Tuning of the cVEMP

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Abstract

Objective: The purpose of this study was to assess age-related changes in the frequency tuning of the cervical vestibular evoked myogenic potential (cVEMP) and determine the optimal air conduction tone-burst stimulus frequency to elicit a cVEMP in the young, middle age, and older adult populations. Additionally, we performed wideband acoustic immittance measures to better delineate whether observed changes in frequency tuning properties of the cVEMP across the lifespan emanate from changes in the middle ear transfer function or from the otolith end organs.

Design: A cross-sectional study design included 98 healthy participants divided into 3 age groups of at least 29 participants in each: young adult (20 - 39 years), middle age adult (40 - 59 years), and older adult (≥ 60 years). Screening measures included otoscopy, tympanometry, and case history rule out a conductive hearing loss or vestibular dysfunction. cVEMPs were elicited using air conduction tone-bursts stimuli at 500 Hz, 750 Hz, and 1000 Hz presented at a 125 dB pSPL. Electromyographic (EMG) target ranges were established to ensure consistent and adequate generation of tonic muscle activity and average EMG was recorded to normalize the cVEMP response amplitudes. Wideband acoustic immittance measures were performed on each subject to assess the resonance frequency of the middle ear.

Results: No significant differences in mean middle ear resonant frequency were observed across age groups. The normalized cVEMP means and adjusted means with the middle ear resonant frequency as covariate were similar, however, all cVEMP analyses were performed on the adjusted means to control for individual differences of middle ear resonant frequency. There was a significant main effect of age group and cVEMP normalized response amplitude. Normalized cVEMP amplitudes for the younger adult and middle age adult groups to a 500 Hz and 750 Hz tone-burst stimuli were significantly larger compared to the older adult group. No significant differences in normalized cVEMP amplitude were observed at 1000 Hz across the three age groups. There was also a significant interaction of age group and stimulus frequency in the older adult group only. In the older adult group, the 1000 Hz tone-burst stimulus elicited the largest normalized cVEMP amplitude compared to both 500 Hz and 750 Hz, however, no significant differences in normalized amplitude between 500 Hz and 750 Hz were observed.

Conclusions: The observed shift in frequency tuning of the cVEMP in normal, healthy, older adults suggests the best stimulus frequency to elicit a response may be higher than the 500 Hz tone burst stimulus utilized clinically. The frequency tuning of the cVEMP in older adults may be attributed to peripheral vestibular system degeneration or saccular changes in the aging adult population.

I. Introduction

Cervical vestibular-evoked myogenic potentials (cVEMPs)

Vestibular-evoked myogenic potentials (VEMPs) are neurophysiological assessments used to determine the functionality of the otolith end organs of the peripheral vestibular system. VEMP testing requires a high intensity auditory stimulus, via air or bone conduction, to stimulate the vestibular end organs, resulting in an activation or inhibition of muscle activity. The electromyographic (EMG) activity of the muscle following the presentation of the stimuli is recorded via surface electrode. The amplitude, latency, threshold, and interaural differences of the evoked responses are recorded and interpreted in various ways to assess vestibular function. Cervical vestibular-evoked myogenic potentials (cVEMP) are recorded from the sternocleidomastoid muscles (SCM) and measures of the integrity of the saccule and its afferent pathway through the inferior vestibular nerve (Colebatch and Halmagyi 1992; McCue and Guinan 1994). Ocular vestibular-evoked myogenic potentials (oVEMP) are recorded from surface electrodes placed beneath the eyes and are used to test the function of the utricle and its afferent pathway through the superior vestibular nerve (Todd et al. 2007). This manuscript will focus mostly on the cVEMP. The cVEMP can be recorded across the lifespan, but age-related changes in responses have been widely acknowledged.

Effects of age on the cVEMP

Age-related changes in both the cVEMP and oVEMP are well-documented and appear to be similar in regards to response amplitude decreases, threshold increases, and response rate decreases with age (Ochi and Ohashi 2003; Su et al. 2004; Zapala and Brey 2004; Basta et al. 2007; Brantberg et al. 2007; Iwasaki et al. 2008; Nguyen et al. 2010;

Piker et. al., 2013). Ochi and Ohashi (2003) looked at age-related changes in cVEMPs using 60 healthy subjects that did not report any history of dizziness, imbalance, or known vestibular pathologies. The investigators found a significant correlation between age and both the stimulus threshold and amplitude of the cVEMP, suggesting decreased activity in the vestibular neural pathway rather than changes in muscle tone. The results show the relationship of an increase in age, the threshold of the cVEMP increases and the p1-n1 amplitude of the cVEMP response decreases.

Piker et al. (2015) retrospective review looked at cases of patients that underwent vestibular assessments presenting with normal vestibular test results (i.e., normal caloric and rotary chair testing) that also underwent cVEMP and/or oVEMP testing. Patients with a history of conductive hearing loss and unilaterally abnormal VEMP results were excluded from the analysis due to results secondary to the affected conductive mechanism of sound transmission or possible vestibular pathology. A total of 895 patients met criteria for cVEMPs. As expected, the analysis showed cVEMP responses decreased in amplitude as we age. The study also observed that cVEMPs elicited by a 500 Hz tone burst air conduction stimuli are bilaterally absent in a large percentage of older adult patients who had otherwise normal vestibular and auditory testing for their age.

Although there are known age-related physiological and anatomical changes in the otolith end organs occur due to aging, an absent cVEMP in response to an air conduction stimulus may indicate a decrease in the sensitivity of the end organ to sound pressure but not necessarily to linear acceleration, and bilaterally absent cVEMPs as isolated findings have unknown clinical significance. This observation is supported by findings showing a smaller age effect on VEMPs elicited with bone conduction or mechanical taps, which produce linear acceleration (Rosengren et al. 2011). For these reasons, a traditional 500 Hz air conduction tone burst stimulus presented at approximately 125 dB pSPL, which is the most common stimulus used to elicit cVEMPs, may not be an ideal stimulus to elicit a cVEMP in an older adult.

Unfortunately, increasing the stimulus intensity for purposes of eliciting a VEMP is contraindicated due to the potential for cochlear damage. However, altering the stimulus, either by using mechanical stimulation or bone conduction or by utilizing a different frequency air conduction stimulus, may increase the likelihood of eliciting a response in an older adult.

Frequency tuning of the cVEMP

Several investigators have assessed the effectiveness of different acoustic stimuli frequencies in evoking cVEMP responses. Welgampola and Colebatch (2001a) measured cVEMP responses to air conduction frequencies of 250, 500, 1000, and 2000 Hz in normal subjects using a sample size of 12. Responses were largest at 500 Hz for 7 subjects and largest at 1000 Hz for 5 subjects. In a different study, the same researchers were the first to use air conduction tone burst stimuli at interoctave frequencies to elicit cVEMP responses and measured cVEMPs between 200 Hz and 1000 Hz in ascending 100 Hz increments. The largest cVEMP response amplitudes were elicited from tone bursts between 600 Hz and 1000 Hz, with a mean of approximately 700 Hz (Welgampola and Colebatch 2001a).

Many studies on human subjects have offered similar results with the maximum cVEMP amplitude occurring in response to a tone burst between 500 Hz and 1000 Hz

(Murofushi et al. 1999; Akin et al. 2003; Node et al. 2005; Lin et al. 2006; Timmer et al. 2006; Piker et al. 2013; Wei et al. 2013). Clinically, a 500 Hz stimulus is most often used as most studies assessing young, healthy, adults showed larger amplitudes at this 500 Hz when compared to 250 Hz, 750 Hz, and 1000 Hz. However, several investigators have shown that pathologic changes can alter the frequency tuning of the VEMP.

Altered frequency tuning of the cVEMP

Altered tuning due to pathology of the inner ear

The first studies that examined the altered frequency tuning of the cVEMP were conducted in patients with Meniere's disease (Rauch et al. 2004; Node et al. 2005; Lin et al. 2006; Timmer et al. 2006; Zhu et al. 2014). Rauch et al. (2004) measured cVEMP responses to a broadband click stimulus and tone burst stimuli at 250, 500, 1000, 2000, and 4000 Hz. Frequency tuning of cVEMP responses in the control group was observed to be elicited by a 500 Hz tone burst stimulus, yielding the lowest threshold and largest response amplitude compared to all other stimuli tested. They also observed a shift in frequency tuning of the cVEMP response to a 1000 Hz tone burst stimuli in the affected Meniere's disease ears, eliciting the lowest thresholds and largest amplitude responses. They hypothesized that the shift in tuning to a higher frequency in the Meniere's disease patients was due to morphological changes in the saccule secondary to endolymphatic hydrops. However, it should be noted that the control group ranged in age from 21 to 52 years whereas the group of subjects with unilateral Meniere's disease ranged from 21 to 77 years. The mean age for the two groups in this study was not disclosed and it is not clear if the control group and Meniere's disease group were age matched. Follow-up studies looking at cVEMP frequency tuning in Meniere's disease patients reported similar findings that patients with Meniere's disease showed a shift in frequency tuning to 1000 Hz or 750 Hz from 500 Hz (Timmer et al. 2006; Sandhu et al. 2012; Salviz et al. 2015). However, age was not well controlled for in these studies. If the change in frequency tuning is presumed to be due to morphologic changes in the saccule, then the age-related morphologic changes in the saccule may also result in the change in frequency tuning.

Altered tuning due to age

Aging can result in a significant loss of hair cells in the vestibular system and changes in mass of the saccule and utricle, which are hypothesized to contribute to the changes in frequency tuning observed in these vestibular end organs. Frequency tuning has been observed in the vestibular system, meaning certain acoustic frequencies evoked larger amplitude responses of the cVEMPs at lower thresholds.

Piker et al. (2013) studied both cVEMP and oVEMP responses elicited by different tone burst stimuli across three different age groups in the adult population (group 1: 18-39 years old, group 2: 40-59 years old, group 3: 60 years and older). The researchers found stimulus frequencies 500 Hz, 750 Hz, and 1000 Hz produced significantly larger VEMP amplitudes than 125 Hz, 250 Hz, 1500 Hz, and 2000 Hz, however, no significant differences were observed between mean amplitudes obtained in response to 500 Hz, 750 Hz, and 1000 Hz tone bursts.

Several significant differences were observed for the mean cVEMP response amplitudes between the different age groups. The young adult group mean cVEMP response amplitude was significantly larger than the mean response amplitudes for both the middle age and older adult groups. The mean cVEMP response amplitude for the middle age group was significantly larger than the mean response amplitude of the older adult group. These findings suggest that the amplitude of the cVEMP decreases with increasing age. The cVEMP amplitude appeared to decrease with increasing age.

Piker et al. (2013) also observed greater response rates and larger amplitudes elicited by the 500 Hz tone burst stimulus in some individuals and some individuals showed greater responses at the other two frequencies tested. However, there was a tendency for subjects in the older adult group, those over 60 years of age, to produce larger amplitude VEMP responses in response to 750 and 1000 Hz tone burst stimuli. The sample size in the Piker et al, (2013) was small, with 13 participants in each of the three age groups, and no significant age-related changes in frequency tuning were observed. However, it was proposed that a change in the stimulus frequency from 500 Hz to 750 Hz or 1000 Hz might increase the response amplitude and response rate in older adults. There is evidence to suggest significant age-related effects of the tuning in the VEMP. It is noteworthy that each of these studies utilized an air conduction tone burst. Thus, it is not clear whether the observed changes emanate from the otolith end organs themselves or whether changes in frequency tuning are due, at least in part, to age related changes in the middle ear transfer function.

Middle ear transfer function

Middle ear transfer function in normal adults

In adults with normal aural anatomical structures of the outer ear, acoustic stimuli are funneled from the pinna and the external ear canal to the middle ear system. The middle ear system possesses two major functions in the process of sound transmission:

impedance matching from acoustic stimulus in the environment to the fluid filled inner ear and acting as a band-pass filter.

One of the main functions of the middle ear system, impedance matching, provides approximately 30 dB of gain at the resonant frequency of the middle ear in humans. The impedance matching of tympanic membrane and middle ear structures recover the approximately 30 dB of sound attenuated by the fluid filled system. The three mechanisms in middle ear impedance matching are areal ratio, tympanic membrane buckling, and the lever action of the ossicular chain.

The middle ear system acts as a band-pass filter for acoustic stimuli above and below the resonant frequency of the middle ear, which is estimated to be between 800 to 1200 Hz (Bekesy, 1941; Moller, 1960) and provides the most gain for acoustic stimuli at the resonant frequency. The middle ear resonant frequency is the frequency at which the opposing spring and mass elements of the middle ear structures negate each other, and the admittance is zero. The components of mass and stiffness can affect sound transmission through the middle ear if one is more dominant. In a more mass dominated middle ear system, sound transmission will be poorer for stimuli above 1200 Hz, whereas, a more stiffness dominated middle ear system, sound transmission will be poorer for stimuli below 1200 Hz. An increased stiffness of the middle ear system results in an increase in resonant frequency and can be seen in patients with middle ear pathologies such as otosclerosis. A decreased stiffness of the middle results in a decrease in resonant frequency and can be seen in patients with middle ear pathologies such as ossicular discontinuity (Shanks, J. E. 1984).

Researchers have utilized multifrequency tympanometry to investigate the normal middle ear resonant frequency in the adult population and a majority of the data revealed comparable means. Colletti et al. (1977) examined normal middle ear resonant frequency by performing multifrequency tympanometry using a sweep of frequencies ranging from 226 Hz to 2000 Hz in 1/6 octave steps. Resonant frequency was determined at the lowest probe tone frequency at which the minimum value of in the notch was inferior to the susceptance (B) at the positive pressure tail. Findings in the normal healthy control group had middle ear resonant frequencies ranging from 900 to 1250 Hz, with the median middle ear resonant frequency occurring at 1120 Hz. Similar findings of middle ear resonant frequency were found by other investigators also utilizing multi-frequency tympanometry. Shanks, J. E. (1984) found normal middle ear resonant frequency ranged from 800 to 1200 Hz. Valvik et al. (1994) found a mean resonant frequency of approximately 1000 Hz with a range of 1049 Hz ± 261 Hz. Although the findings of normal middle ear resonant frequency were relatively consistent across studies, factors of age on the middle ear resonant frequency were not well controlled for, as the studies were performed on primarily young normal, healthy subjects.

Clinical diagnostic assessment of middle ear function

In current clinical protocols, the most common diagnostic acoustic immittance assessment in the adult population uses a single probe tone frequency at 226 Hz and pressure sweep to assess the function of the middle ear system. Terkildsen and Thomsen (1959) created the first commercial acoustic immittance instrument using a low-frequency probe tone stimulus (220 Hz). A low-frequency probe tone was selected for acoustic immittance measurements to avoid high-frequency artifacts, electrical noise (lower frequency noise), and less calibration error given the small size of the ear canal. While other probe-tone frequency assessments were created afterwards, a majority of the early clinical research of acoustic immittance testing was conducted using a low-frequency probe tone stimulus. The significant amount of research published using a 220 or 226 Hz probe tone continues to influence clinical protocols and diagnostic assessments of middle ear function.

Wideband acoustic immittance

Wideband acoustic immittance (WAI) testing assesses middle ear system function and was developed with the intention of providing improved diagnostic information for neonates and detection of conductive/middle ear pathologies compared to conventional immittance testing (Margolis et al. 1999; Feeney et al. 2003; Sanford et al. 2009; Shahnaz et al. 2009; Beers et al. 2010; Hunter et al. 2010; Ellison et al. 2012). WAI uses a wideband click stimulus that generates tympanometric measurements for frequencies between 226 Hz and 8000 Hz. WAI protocols yield results for 226 Hz probe tone immittance, 1000 Hz probe tone immittance, averaged wideband immittance, immittance at subject’s resonant frequency, and absorbance as a function of frequency.

Concerns for variability of wideband absorbance measures were investigated by Vander Werff et al. (2007) between adult and infant age groups using test-retest measures. Researchers assessed concerns for variability of wideband absorbance measures and performed same day test-retest measures on adult subjects. The evidence indicated adequate reliability and replication of wideband absorbance measures testing for same day test-retest measures in the adult population.

Middle ear changes in the aging adult

The properties of the middle ear transfer function in typical adults barring conductive pathologies is crucial to understanding the effects of aging on the sound transmission process. The research of the functional middle ear system changes from neonate to adulthood are widely known and accepted, however, various studies have produced inconsistent findings regarding middle ear system changes with increasing age in adulthood. The inconclusive findings may result from equipment and assessment limitations of single frequency or multifrequency tympanometry measurements as well as the different procedures researchers use to estimate resonant frequency. The middle ear system continues to change in older adults and researchers found that adults 48 to 90 years old have lower static admittance and larger ear canal volume compared to the middle ear system of younger adults (Wiley et al., 1996). The decrease in middle ear compliance observed in the older adult population may alter the sound transmission process through the middle ear due to changes in the impedance components.

It is unknown whether the aging process in adulthood affects the sound transmission properties of the human middle ear. The tympanic membrane and middle ear structures have been shown to undergo structural changes at older adult ages (Ruah et al., 1991). Feeney & Sanford (2004) found that older adults had lower reflectance values than younger adults at mid-frequency range and higher reflectance at higher frequencies than the young group. This was interpreted to suggest that the wideband reflectance pattern for the young adult group was more stiffness dominated than that of the old group (Feeney et al., 2014). A recent investigation utilizing wideband tympanometry reported an observed conductive component in the aging middle ear system (Williams et. al., 2016), possibly indicative of middle ear system stiffening. An increase in stiffness of the middle ear system would result in an increase in resonant frequency.

Minimal differences have been observed between the young and older adult populations when using 226 Hz probe tone immittance testing, but significant middle ear age effects have been observed when using WAI (Williams et. al., 2016). The combination of the results from the WAI test protocol provides clinicians with more comprehensive information regarding middle ear functioning, improved diagnostic data for specific middle ear pathologies, and creates the potential to identify previously unknown age-related effects in the middle ear. Acoustic energy presented between 600 and 1340 Hz is generally enhanced due to middle ear resonance (Colletti, 1977; Shanks 1984; Valvik et. al., 1994). The gain occurring at the resonance frequency of the middle ear, may be indicative of the findings of the best VEMP frequency tuning between 500 and 1000 Hz, however, middle ear resonance frequency and VEMP frequency tuning have, to date, not been investigated together.

There is a need for efficient vestibular diagnostic tests that identify clinically meaningful vestibular impairments in older patients. An understanding of the contribution of the middle ear system may further our understanding of air conduction stimuli eliciting the cVEMP and the frequency tuning of the response. The purpose of the current study was to examine age-related changes in the frequency tuning of the cVEMP while controlling for middle ear resonant frequency. Based upon the current research, we hypothesize an air conduction tone burst stimulus frequency higher than 500 Hz will elicit a larger cVEMP response amplitude and greater response rate in older adults. We suspect that the middle ear system stiffens with age resulting in increased resonant frequencies, which may affect cVEMP responses elicited by air conduction tone bursts in older adults.

II. Methods

Participants

We recruited participants from 3 age groups to replicate the findings from Piker et al. (2013), but in a larger, more statistically robust sample size. The age groups are characterized by the same age ranges utilized by Piker et al. (2013), and are as follows: young adults ages 18 - 39 years, middle age adults ages 40 - 59 years, and older adults ages 60 years and older. A statistical power analysis was performed for sample size estimation, based on data from Piker et al. 2013, comparing the amplitude of the cVEMP elicited using 500 Hz, 750 Hz, and 1000 Hz tone bursts in the oldest age group. The effect size in that study was 0.34, which was calculated using Cohen's d. With an alpha = .05 and power = 0.80, the projected sample size needed with this effect size (GPower 3.1) is approximately 29 participants per group. At least 29 participants per age group were recruited to replicate the frequency tuning findings of the cVEMP responses in the Piker et al. (2013) study.

Middle age and older adult participants were recruited from a registry of faculty and staff in the James Madison University community via an email request and informational flyers posted on campus seeking individuals in the defined age groups for this research study interested in participating in clinical research. The participants in the young adult age group were recruited from students enrolled in the Communication Sciences and Disorders program at James Madison University. The participants were compensated a small sum for their participation in the research study. The written research protocol, risks, and benefits of the research study were administered and thoroughly explained to participants. All participants included in the study indicated understanding of the research protocol and provided informed consent to the methods. Human subject approval for the proposed study has been obtained by the James Madison University Institutional Review Board (IRB #17-0302).

Inclusion/Exclusion Criteria

All participants met the inclusion criteria to participate in our investigation. Upon receiving informed consent from participants, a brief verbal audiologic case history was taken by the researchers and screening protocol was conducted to assess for anything that would preclude subjects from participating in the study. All participants included in the study were adults over the age of 18 without persistent complaints of dizziness, unsteadiness, and certain disorders and diseases affecting physiologic function detailed in the exclusion criteria.

Exclusion criteria include individuals that present with any of the following: known permanent or current transient conductive hearing loss, history of middle ear pathology, current middle ear dysfunction, neurological disease, balance disorders, or complaints of dizziness or unsteadiness. Participants that reported a permanent conductive pathology, a longstanding history of conductive hearing loss, or current middle ear dysfunction were excluded from participating in this study. Conductive pathologies exclude participants from participating due to the mechanism of the middle ear system that affects the transmission of the sound pressure level of the tone burst stimulus of the VEMP to the vestibular end organs of the utricle and saccule. Welgampola and Colebatch (2001a) found that subjects with varying degrees of sensorineural hearing loss, including profound sensorineural hearing losses, elicited present VEMP responses to both tone-burst and click stimuli. Given these findings, participants with any degree of sensorineural hearing independent of co-occurring conditions mentioned in the exclusion criteria were not precluded from participating in this investigation.

Screening

To rule out participants with significant symptoms of any vestibular or balance dysfunction, the Dizziness Questionnaire (Furman, J. and Cass, S., 1996) was administered and thoroughly reviewed with each participant. To rule out conductive hearing loss and middle ear pathologies, all subjects underwent otoscopy and immittance testing using a 226 Hz probe (Interacoustics Titan). If a participant with Type A tympanometric tracing was suspected to have a conductive hearing loss during vestibular test procedures, a pure tone air and bone conduction audiologic evaluation would be performed (GSI AudioStar).

Procedures

Participants were asked to remain still and quiet for the duration of each WAI procedure. After completing otoscopy, an appropriately sized Sanibel eartip was placed on the end of the probe and then inserted into the ear canal of the participant. A hermetic seal of the eartip was required to initiate wideband absorbance under tympanometric peak pressure (WBT), but was not necessary for wideband absorbance under ambient pressure (WBA).

The stimuli for both WBA and WBT were presented monaurally through the probe on the Interacoustic Titan attached with a Sanibel eartip. The wideband stimulus click was presented at 100 dB peak equivalent SPL (65 dB nHL) at a rate of 21.5 Hz. During WBT, a simultaneous pressure sweep from +200 daPa to -400 daPa occurred at a pump speed of 200 daPa per second and absorbance was measured at tympanometric peak pressure. During WBA, absorbance was measured at ambient pressure.

Data was collected on Interacoustic Otoaccess software using wideband absorbance under ambient pressure (WBA) and wideband absorbance under tympanometric peak pressure (WBT) research protocols. For each ear tested, protocols yielded results for 226 Hz probe tone immittance, 1000 Hz probe tone immittance, wideband stimulus immittance, absorbance at tympanometric peak pressure, and absorbance at ambient pressure. Data values collected from the wideband immittance protocol were equivalent ear canal volume (ECV), compliance at 226 Hz and 1000 Hz, tympanometric peak pressure, tympanometric gradient, resonant frequency, and absorbance percentages at 250, 500, 1000, 2000, 4000, and 8000 Hz (at ambient and tympanometric peak pressure). The Interacoustics Titan analyzes the susceptance (B) component of the tympanogram to calculate the resonant frequency of the middle ear. The resonant frequency of the middle ear is determined at the lowest frequency at which susceptance (B) is 0 mmho at peak pressure. The susceptance (B) component of the tympanogram should equal 0 mmho when the stiffness elements of the middle, which produce positive mmho, and the mass elements of the middle ear, which produce negative mmho, contribute equally and essentially cancel each other out.

Participants were placed in a semi-recumbent position in a comfortable reclining chair for cVEMP testing. Single-use, disposable silver/silver-chloride Ambu electrodes were used. To record the cVEMP, participants were instructed to lift the head off the headrest and turn the head away from the ear being stimulated. Participants were instructed to sustain the described head positioning long enough to acquire 80 samples at a rate of 5/second for each cVEMP recording. Participants were given rest periods in between each cVEMP recording and as needed if fatigue is observed. All recordings were replicated a minimum of one time to ensure repeatability of the cVEMP response obtained.

The (active) non-inverting input of the cVEMP was placed on the sternocleidomastoid muscle (SCM) midway between the insertion at the mastoid and the sternum ipsilateral to the side of stimulus presentation. The inverting (reference) electrode was placed on the manubrium of the sternum. The ground electrode was placed on the forehead at Fpz. All electrode impedances were measured prior to testing. Electrode impedance testing was performed prior to recording cVEMP responses to ensure individual electrode impedances were less than 10 kOhms and interelectrode impedances were less than 5 kOhms. Ongoing EMG in the SCM was closely monitored using a second surface electrode placed on the SCM, adjacent to the non-inverting electrode, in an attempt to ensure that subjects were generating a consistent and adequate amount of tonic background EMG activity between 50 and 200 μV (Akin et al. 2004). The Otometrics Chartr EP 200 measured the pre-stimulus activation of the SCM muscle to determine the EMG activity level for each sweep of the cVEMP response. The EMG activity level was measured during the 100 msec interval prior to the onset of the stimulus throughout the entire recording of the cVEMP response. When the EMG activity was greater than 200 μV or less than 50 μV, the protocol in the software was set to automatically reject those sweeps and were excluded from the cVEMP response waveform. Additionally, the participants were given a handheld EMG monitor in an effort to ensure sufficient and constant EMG activity was elicited. The three indicator lights (high, good, and low) on the handheld EMG monitor were explained to participants and were instructed to maintain good EMG activity reflected as a “green” light on the monitor. The average EMG activity for each cVEMP response was recorded to later be used to calculate normalized cVEMP amplitudes.

The stimuli for the cVEMP were presented monaurally through Etymotic ER-3A insert earphones. The stimuli consisted of 500 Hz, 750 Hz, and 1000 Hz Blackman-gated tone bursts with a 2 ms rise/fall and 0 ms plateau. In an attempt to avoid latency effects due to signal duration, the duration of each frequency was the same at 4 ms. The tone bursts were randomized in their order of presentation and presented at a rate of 5.1/second. Stimulus levels were calibrated and were presented at 125 dB pSPL at all frequencies. The bioelectrical activity was amplified and analog filtered (5 – 500 Hz) with a commercially produced neurophysiological amplifier (GN Otometrics, Tasstrup, Denmark). For each single record the electromyographic activity was digitized (at a rate of 5000 Hz) and recorded on a commercially available electrophysiological recording system (GN Otometrics, Tasstrup, Denmark). Following signal averaging, the latencies of the prominent peaks were recorded as well as their peak- to-peak amplitudes. cVEMP response rates for each ear were tabulated.

Statistical analyses

The data was analyzed using SPSS version 26.0 (SPSS, Inc., Chicago, IL). A repeated measures analysis of covariance (ANCOVA) with a 3 by 3 design with a between-subjects factors of age group (young adult, middle age, and older adult age groups) and a within-subjects factors of stimulus frequency (500 Hz, 750 Hz, and 1000 Hz tone burst). The dependent variable was the cVEMP normalized amplitude. The normalized cVEMP amplitudes were calculated by dividing the peak-to-peak cVEMP amplitude by the EMG amplitude for each response. The covariate was middle ear resonant frequency. Greenhouse-Geisser values were used for the ANCOVA analysis of the data. Pairwise comparisons using post hoc Tukey tests were utilized when significant main effects were observed with the ANCOVA analysis.

III. Results

Ninety-eight subjects met the previously defined inclusion criteria and participated in this study. The subjects were divided into 3 defined age groups: young adult group (n = 39; mean age with standard deviation 20.41 ± 0.498 years; range 20 - 21 years; 1 male, 38 females), middle age group (n = 29; mean age with standard deviation 50.14 ± 6.157 years ; range 40 - 58 years; 2 males, 27 females), and older adult age group (n = 30; mean age with standard deviation 66.03 ± 4.731 years; range 60 - 79 years; 14 males, 16 females). See Table 1 for subject demographics for each of the three age groups. Data were obtained from both ears of all 98 participants, however, data were analyzed from one ear. Prior to statistical analysis of results, an independent samples t-test was run comparing dependent values between the right and left ears. Results indicated no significant differences between data obtained from the ears. Left ear data for all subjects were chosen at random for statistical analysis.

Table 1: Subject demographics for each of the three age groups

	Age Group		
	Young Adult	Middle Age Adult	Older Adult
Sample Size	39	29	30
Mean Age	20.14 (0.50)	50.14 (6.16)	66.03 (4.71)
Minimum Age	20	40	60
Maximum Age	21	58	79
Females	38	27	16
Males	1	2	14

Middle Ear Resonant Frequency

One-way ANOVA showed no statistically significant effects of age group on middle ear resonant frequency ($F(2, 93) = .187$, $p = .830$, partial $\eta^2 = .004$). The middle ear resonant frequency differed between participants and is used as a covariate in the following analyses concerning the effects of frequency and age on air-conducted cVEMP.

Table 2: Middle ear resonant frequency (Hz) means across age groups.

	Age Group		
	Young Adult	Middle Age Adult	Older Adult
Mean RF (Hz)	791.44	844.21	792.57
Standard Deviation	156.98	105.72	78.39
Standard Error	25.14	19.63	14.31
Minimum	346	679	654
Maximum	1121	1100	987

cVEMP Raw Amplitude

Table 3: cVEMP raw amplitude means (standard deviation) p1-n1 peak-to-peak amplitudes across frequencies measured in µV for each age group.

	Age Group								
	Young Adult			Middle Age Adult			Older Adult		
Frequency (Hz)	500	750	1000	500	750	1000	500	750	1000
Means (SD)	260.35 (111.19)	250.14 (119.08)	256.14 (102.43)	171.32 (75.61)	169.10 (80.39)	193.32 (83.17)	133.27 (74.85)	134.91 (60.36)	156.45 (83.63)

Table 3 depicts the mean cVEMP raw amplitudes across all three age groups. The statistical analysis was only performed on normalized amplitudes to account for differences in tonic EMG activity level produced for each response. In the young adult group, the cVEMP elicited by the 500 Hz tone burst stimulus yielded the largest mean amplitude response. However, in the middle age and older adult groups, the 1000 Hz tone burst stimulus elicited the largest mean amplitude response. The peak-to-peak cVEMP amplitudes were divided by the EMG amplitudes for each recording to calculate the normalized amplitudes. Normalizing the cVEMP amplitudes reduces the intersubject variability of EMG activity levels that may influence the amplitude of the raw cVEMP response.

cVEMP Electromyography (EMG) Activity

The normalized amplitude of the cVEMP was calculated for each subject for each individual cVEMP frequency assessed to account for the variability of EMG activity produced. The normalized amplitude value is expressed as cVEMP amplitude (µV) divided by EMG activity (µV). The statistical analyses performed on the normalized cVEMP amplitude was used to determine the effects across age groups and stimulus frequencies on the cVEMP amplitude, while controlling for EMG activity produced by each individual subject.

Table 4: cVEMP EMG activity means (standard deviation) measured in µV for each stimulus frequency (Hz) for each of the three age groups tested

	Age Group								
	Young Adult			Middle Age Adult			Older Adult		
Frequency (Hz)	500	750	1000	500	750	1000	500	750	1000
Mean EMG (µV)	96.10 (33.0)	88.05 (22.6)	92.8 (21.9)	70.3 (12.8)	76.4 (12.0)	84.3 (21.9)	86.29 (28.7)	95.6 (38.7)	86.4 (22.8)

There was a statistically significant interaction between the stimulus frequency and age group on EMG generated during the cVEMP recordings ($F(4,184) = 3.898$, $p = .005$, partial $\eta_2 = .078$).

Specifically, there was a statistically significant difference in EMG between age groups for both the 500 Hz stimulus ($F(2, 92) = 7.290$, $p = .001$, partial $\eta_2 = .137$) and 750 Hz stimulus ($F(2, 95) = 4.311$, $p = .016$, partial $\eta_2 = .083$). Post hoc comparisons using Tukey tests showed that at 500 Hz, EMG was statistically significantly smaller in the middle age group (70.3 μV) compared to the younger age group (96.1 μV; $p =.001$). At 750 Hz, EMG was statistically significantly smaller in the middle age group (76.4 μV) compared to the older age group (95.6 μV; $p = .015$). No statistically significant differences in EMG were observed between age groups for the 1000 Hz stimulus condition ($F(2, 95) = 1.644$, $p = .199$, partial $\eta_2 = .033$).

No statistically significant effects of stimulus frequency on EMG were observed in the young age group ($F(2, 76) = 1.897$, $p = .061$, partial $\eta_2 = .048$) or old age group ($F(2, 54) = 1.433$, $p = .215$, partial $\eta_2 = .013$). There was a statistically significant effect of stimulus frequency on EMG for the middle age group ($F(2, 54) = 7.670$, $p = .003$, partial $\eta_2 = .221$). Follow-up analyses using pairwise comparisons showed that EMG in the middle age group was statistically significantly smaller at 500 Hz compared to 1000 Hz ($p = .007$).

Given the variability of average EMG activity level for responses at different stimulus frequencies and age groups, all statistical analyses are performed on the normalized amplitude.

cVEMP Normalized Amplitude

Table 5: cVEMP normalized amplitude means and adjusted means (standard deviation/error) in unspecified units for each stimulus frequency (Hz) for each of the three age groups tested									
	Age Group								
	Young Adult			Middle Age Adult			Older Adult		
Frequency (Hz)	500	750	1000	500	750	1000	500	750	1000
Means (SD)	2.49 (1.13)	2.33 (.82)	2.41 (.98)	2.37 (1.30)	2.18 (1.01)	2.40 (1.29)	1.60 (.78)	1.53 (.82)	2.31 (1.03)
Adjusted Means (SE)	2.49 (.17)	2.33 (.14)	2.41 (.16)	2.37 (.21)	2.18 (.17)	2.41 (.19)	1.59 (.20)	1.53 (.16)	2.08 (.19)

When assessed by inspection of a boxplot, several outliers were detected for the normalized cVEMP amplitude. Inspection of their values did not reveal them to be extreme and they were kept in the analysis. Normalized cVEMP amplitude was not normally distributed for 4 out of the 9 combinations of frequency and age groups, as assessed by Shapiro-Wilk's test. Specifically, 500 Hz in the young group ($p = .001$), 750 Hz in the old age group ($p = .004$), 1000 Hz in the young age group ($p = .026$), and 1000 Hz in the old age group ($p = .008$). When assessed by inspection of histograms, the 4 categories were mildly positively skewed. We ran the data in its original form and again as transformed data using a logarithmic transformation ($\log_{10}$). There were no meaningful differences or changes in statistical conclusions, so only the data in its original form are presented.

Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction ($\chi^2(2) = 11.419$, df = 2, $p = .003$). Greenhouse-Geisser corrections are reported for all analyses.

The adjusted means were calculated by utilizing a covariate to control for any potential effects of a secondary continuous variable that may affect the main and interaction effects. The adjusted means of the normalized cVEMP response amplitudes reflect corrected mean estimates by removing effects of a secondary confounding variable that may influence the dependent and independent variables. The middle ear resonant frequencies were used as the covariate in the analysis. The adjusted means of the normalized cVEMP response amplitude were obtained following the repeated measures ANCOVA analysis.

There was a statistically significant interaction between the stimulus frequency and age group on cVEMP normalized amplitude ($F(4,182) = 2.914$, $p = .0123$, partial $\eta^2 = .060$). There was a statistically significant difference in cVEMP amplitude between age groups for both the 500 Hz stimulus ($F(2, 92) = 5.599$, $p = .005$, partial $\eta^2 = .109$) and 750 Hz stimulus ($F(2, 91) = 7.183$, $p = .001$, partial $\eta^2 = .136$). Post hoc comparisons using Tukey tests showed that at 500 Hz, normalized amplitude was statistically significantly greater in both the younger age group (2.49; $p = .002$) and middle age group (2.37; $p = .021$) compared to the older age group (1.59). Similarly, at 750 Hz normalized amplitudes were statistically significantly greater in both the younger age group (2.33; $p < .001$) and middle age group (2.18; $p = .007$) compared to the older age group (1.53). No statistically significant differences in normalized amplitudes were observed between age groups for the 1000 Hz elicited cVEMP ($F(2, 92) = .957$, $p = .388$, partial $\eta^2 = .020$).

No statistically significant effects of stimulus frequency were observed in the young age group ($F(2, 74) = 2.601$, $p = .081$, partial $\eta^2 = .066$) or the middle age group ($F(2, 50) = .323$, $p = .698$, partial $\eta^2 = .013$). There was a statistically significant effect of stimulus frequency on cVEMP normalized amplitude for the older age group ($F(2, 58) = 3.816$, $p < .001$, partial $\eta_2 = .364$). Follow-up analyses showed that cVEMP normalized amplitude in the older group was not statistically significantly different between 500 Hz (1.59) and 750 Hz (1.53), but amplitude was significantly greater at 1000 Hz (2.08) compared to both 500 Hz ($p = .002$) and 750 Hz ($p < .001$).

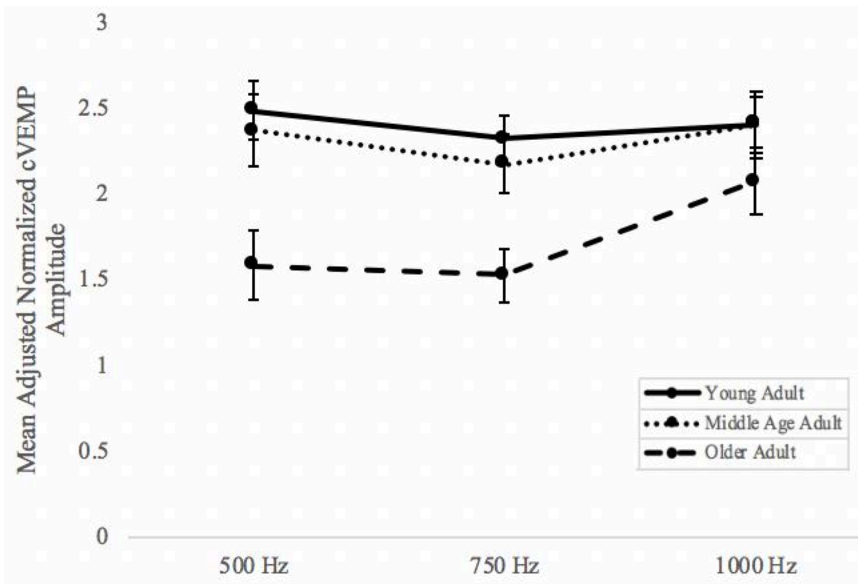

Figure 1. Line graph showing the mean$_{adj}$ normalized cVEMP amplitude with standard error bars as a function of stimulus frequency for each of the three age groups. Each connecting line represents an age group where solid line = young age, dotted line = middle age, and dashed line = old age.

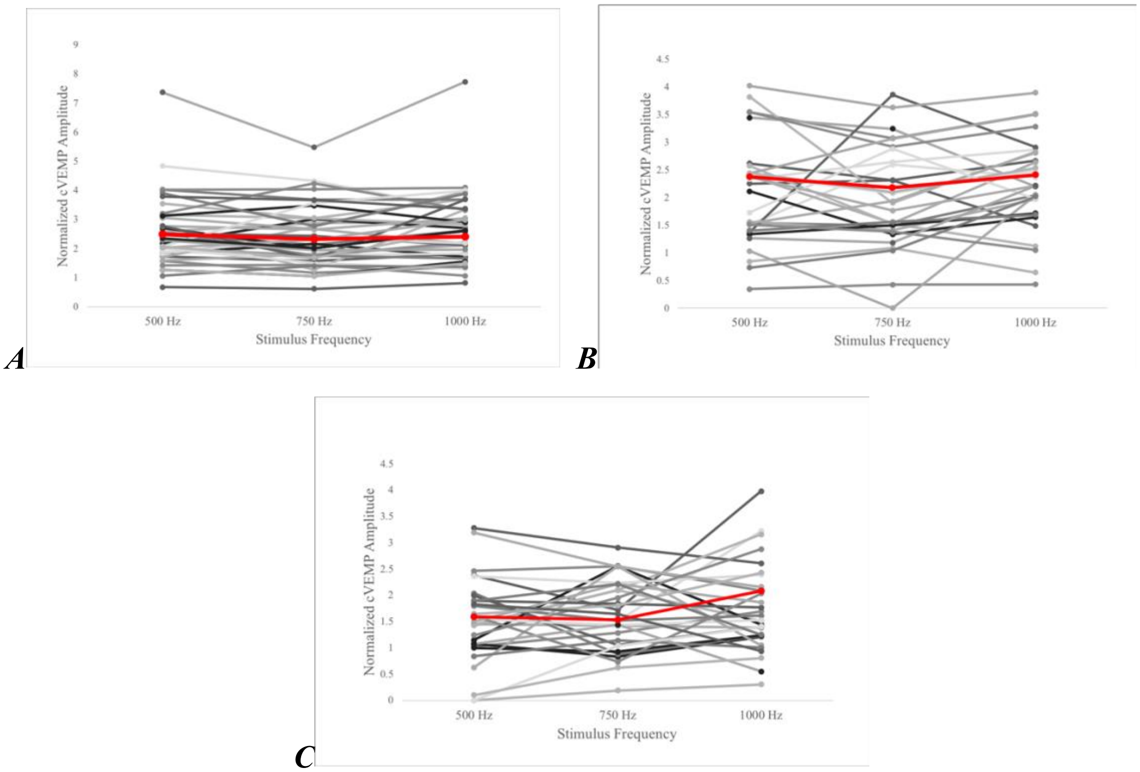

Figure 2. Line graph showing the mean_{adj} normalized cVEMP amplitude for as a function of stimulus frequency for each age group. Each connecting line represents an individual subject where grey = each participant and red = adjusted mean of age group. Figure 2a. young adult group. Figure 2b. middle age adult group. Figure 2c. older adult group**.**

cVEMP Response Rate

Table 6 depicts the response rate for the cVEMP across stimulus frequencies for each age group; response rate defined as percentage of subjects that produced present responses for each age group. For the young adult group, a cVEMP response was elicited 100% across all three frequencies tested. In the middle age group, a cVEMP response was elicited 100% at 500 Hz and 1000 Hz, however, in response to the 750 Hz tone burst, a cVEMP response was only elicited 97% (i.e., 28 of 29 subjects). In the older adult group, a cVEMP response was elicited at 100% at 750 Hz and 1000 Hz, however, in response to the 500 Hz tone burst, a cVEMP response was only elicited 93% (i.e., 28 of 30 subjects).

Table 6: cVEMP response rate for each stimulus frequency (Hz) for each of the three age groups tested									
	Age Group								
	Young Adult			Middle Age Adult			Older Adult		
Frequency (Hz)	500	750	1000	500	750	1000	500	750	1000
Response Rate	100%	100%	100%	100%	97%	100%	93%	100%	100%

IV. Discussion

The purpose of the current study was to examine the age-related effects of the frequency tuning of the cVEMP while controlling for middle ear resonant frequency. Initial research suggested that a tone-burst acoustic stimulus between 500 - 1000 Hz as the frequency range that elicited “best” cVEMP responses, with 500 Hz tone-burst considered the stimulus frequency that produced the “best” responses, defined as the largest response amplitude. Age-related changes in cVEMP responses are widely known and research supports that due to the cVEMP frequency tuning observed with increasing age that 500 Hz may not be the optimal stimulus. Our findings provide supporting evidence that for some age groups that 500 Hz does not always elicit the largest cVEMP response amplitude and that 1000 Hz may be the optimal stimulus in certain age populations. Previous research provided evidence of age-related frequency tuning of the cVEMP to a higher stimulus frequency in older adults, however, the anatomical and physiological factors contributing to the tuning require further investigation. Researchers found evidence of otolith end organ and peripheral vestibular system degeneration in the aging population (Johnsson 1971; Ross et al 1976). The utility of the cVEMP is to clinically assess the integrity of the saccule and the afferent vestibular nerve fiber projections. The observed cVEMP frequency tuning (i.e., decreased response amplitude, decreased response rate) in older adults may be the result of the age-related degeneration of the otolith end organ and afferent vestibular pathway.

Middle Ear Resonant Frequency

Changes in resonant frequency in the aging middle ear have been widely varied and inconclusive across studies. Our study yielded measurements of middle ear resonant frequency across three different age groups to examine possible changes related to aging and possible effects on the frequency tuning of the cVEMP. Based upon previous research, our hypothesis was that age-related anatomical changes of the middle ear would result in a lower resonant frequency, which would contribute to the frequency tuning of the cVEMP in the aging population. The middle ear resonant frequency means of the young adult, middle age adult group, and older adult group young adult group, 791 Hz ± 157 Hz, 844 Hz ± 261 Hz, and 793 Hz ± 78 Hz respectively, did not significantly differ and align with the current literature that normal middle ear resonant frequencies ranges from approximately 600 - 1340 Hz (Colletti et al. 1977; Shank et al. 1984; Valvik et al. 1994) and middle ear resonant frequencies are not significantly different between age groups (Williams et al. 2016). The middle ear resonant frequency values were used as a covariate in our analysis of the cVEMP normalized amplitudes to control for any contributions related to the middle ear on the cVEMP response.

cVEMP Electromyography (EMG) Activity

The cVEMP assesses the saccule and the inferior vestibular nerve afferent pathways through to the central vestibular nucleus by measuring the inhibition of the contracted sternocleidomastoid (SCM) muscle. Due to limitations of equipment, previous cVEMP frequency tuning studies were unable to record average EMG activity produced for each response resulting in all analysis to be conducted on the raw amplitude of the response. Vanspauwen et al. (2006) conducted attempted to control for amount of SCM muscle contraction by having subjects turn heads to apply pressure to a blood pressure cuff. This method was found to reduce variability and provided investigators with an indirect measurement of muscle contraction, however improvements in technology allow for EMG activity to be more accurately measured to determine a more exact normalized cVEMP amplitude to be calculated.

Our study used equipment with a real-time EMG activity feedback monitor for subjects and provided the average EMG activity level of all the accepted sweeps comprising the response. Sweeps were automatically discarded from the responses that did not fall within the EMG target level range of 50 to 200 uV to ensure adequate muscle contraction was achieved for each response. Akin et al. (2011) found the peak-to-peak cVEMP amplitudes increased as a function of increased EMG activity target level in both young adult and older adult groups. Investigators also attempted to limit differences in EMG activity level across individuals by setting a EMG target ranges, but still found older adults produced significantly smaller and more variable EMG activity during recordings compared to younger adults. The reduced cVEMP amplitude response in the older adult population suggests age-related changes in the vestibular system and age-related changes in the sternocleidomastoid muscle.

cVEMP Normalized Amplitude

In our analysis of the cVEMP normalized amplitudes, we accounted for the amount of EMG activity produced by each individual subject during each response and utilized the adjusted means with the covariate as the individual middle ear resonant frequency. Given the amount of variability of the EMG activity, even within target ranges, for each individual response, the normalized amplitude responses using the middle ear resonant frequency at a covariate were utilized in the data analysis.

The findings from our study yielded results that show the frequency tuning of the cVEMP shifts to a higher frequency in the older adult group and suggest that a frequency besides the clinically used 500 Hz tone-burst stimulus might be more effective in eliciting a cVEMP response. Specifically, we observed smaller response amplitudes in the older adult group compared to the young adult and middle age adult group at 500 Hz and 750 Hz tone-burst stimuli. However, no significant age differences were observed in response to the 1000 Hz stimuli between the three age groups. Within the young adult and middle age adult groups, there was no significant difference in cVEMP normalized response amplitudes between the 500 Hz, 750 Hz, and 1000 Hz stimulus frequencies. For the older adult group, the normalized cVEMP response was greatest in response to a 1000 Hz tone-burst air conduction stimulus compared to both the 500 Hz and 750 Hz stimuli. Our findings suggest that the vestibular system is more broadly tuning in the young adult and middle age adult populations as there was no clear optimal stimulus frequency of the ones utilized in this investigation. However, there is a significant shift in frequency tuning for the older group where 1000 Hz yielded larger responses.

The findings of broader frequency tuning of the cVEMP at the two younger age groups compared to the shift in tuning observed in the older adult group differs from previous findings in tuning curves. Examining the difference in threshold tuning curves across different age groups, Janky and Shepard (2009) found sharper tuning curves in the younger adult groups and a more broader tuning curve with increasing age, however, no significant differences in mean amplitude were observed between age groups at any frequencies tested. Our findings of a sharper tuning of the cVEMP in the older adult group and significantly decreased response amplitude may be attributed to eliciting the cVEMP response at suprathreshold, whereas Janky and Shepard (2009) obtained cVEMP responses at threshold. Threshold cVEMP assessment is rarely performed clinically given that threshold cVEMP responses do not provide significant diagnostic information, except in cases of suspected superior canal dehiscence (SCD). Suprathreshold cVEMP testing elicits a larger amplitude response and is clinically preferred due to visual detection of the response waveform.

Todd et al. (2009) found the largest cVEMP responses were elicited by air conduction tone burst stimuli between 400 and 800 Hz and observed saccular resonance at 600 Hz. Researchers concluded that the resonance was due to contributions from the saccule, ionic currents of hair cells as well as the afferent nerve fiber projections from the saccule. This suggests these multiple factors each contribute to the observed frequency tuning of the cVEMP. The observed resonance in this study align with previous research that found cVEMP amplitudes were largest in response to air conduction tone burst stimuli between 500 Hz and 1000 Hz. Young normal, healthy adults were the primary subjects in these studies and age-related changes in these structures may affect the frequency tuning of the cVEMP.

The otolith end organ response may be affected by degeneration that occurs with increasing age. Schuknect et al. (1965) examined the different pathological types of cochleo-saccular degeneration based on temporal bone studies, including age-related changes. Findings indicated degeneration of the saccule, severe degeneration of the macula, and significant loss of hair cells in an older adult. Igarashi et al. (1993) found elderly adults had significantly less otoconia in the macula of the saccule than the young children, indicating severe reduction of otoconia. Reductions in hair cells in the saccule have been observed in older adults (Rosenhall, 1973). The age-related degeneration observed in the saccule has been suggested to reduce the excitability of the vestibular system, which may explain the shift in the frequency tuning of the cVEMP in older adults.

cVEMP Response Rate

The response amplitude is an important characteristic in determining the “best” frequency to elicit a cVEMP response in different age populations, however, response rate is a crucial consideration. Previous studies (Piker et al. 2013; Piker et al. 2015) found that a proportion of the older adult population did not produce a cVEMP response in the absence of any vestibular dysfunction, normal results for all other assessments in vestibular test battery, and no underlying conductive or neurological pathologies. While having a larger amplitude cVEMP responses are ideal for allowing clinicians to analyze via visual detection, a present response is better than an absent response. In our study, 100% of the subjects in the young adult population were able to produce a cVEMP response elicited by all three stimulus frequencies tested. Marginal decreases in response rates in the middle age and older adult group were observed at certain frequencies. Consistent with Piker et al. (2013), a proportion of the older adult age group had absent cVEMP responses to the clinically utilized 500 Hz tone-burst stimulus and higher response rates to the two other stimulus frequencies assessed in our investigation. Comparing the cVEMP response rate for all subjects between the three frequencies, only the 1000 Hz tone burst stimulus elicited a present cVEMP response in all subjects across all three age populations. cVEMP response rate is an important factor in improving the recordability of the response and accordingly improve the overall sensitivity of the cVEMP to accurately detect vestibular impairments.

V. Conclusion

The observed shift in frequency tuning of the cVEMP in older adults suggests the best stimulus frequency to elicit a response may be higher than the 500 Hz tone burst stimulus utilized clinically. A 1000 Hz tone burst stimulus may to be a better stimulus frequency to elicit a cVEMP response for individuals 60 years and older. There are currently no established normative data values on the cVEMP elicited by a 1000 Hz tone burst stimulus and further supporting research is needed prior to definitively recommending 1000 Hz as the default stimulus frequency in clinical cVEMP protocols for individuals 60 years and older. There is more evidence supporting the 500 Hz tone burst stimulus and normative data values have been widely established for this frequency, therefore, it remains the more clinically valuable stimulus for the young adult and middle age adult populations. Future studies should establish normative data for cVEMPs elicited at frequencies other than 500 Hz, including 750 Hz and 1000 Hz, in a large age-stratified sample.

Further research is required to determine the cause of the frequency tuning shift that occurs in the aging adult population and to better shape clinical protocols for more accurate information regarding vestibular function. Obtaining conclusive evidence to support the origin of the mechanism contributing to the frequency tuning of the cVEMP will allow clinicians to gather valuable diagnostic results to guide accurate recommendations and referrals.

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