EFFECTS OF AGE ON THE FREQUENCY TUNING
OF THE CVEMP AND OVEMP

By

Erin Gillikin Piker

Dissertation

Submitted to the Faculty of the
Graduate School of Vanderbilt University
in partial fulfillment of the requirements
for the degree of
DOCTOR OF PHILOSPHY
in
Hearing and Speech Sciences

May, 2012

Nashville, Tennessee

Approved:

Professor Gary P. Jacobson

Professor Devin L. McCaslin

Professor Linda J. Hood

Professor Robert F. Burkard

AKNOWLEDGEMENTS

This work would not have been possible without the financial support of the Vanderbilt Institute for Clinical and Translational Research. I am grateful to all those whom I have had the pleasure to work during my time at Vanderbilt. I want to thank each member of my Dissertation Committee who have been supportive of this project and helped me tremendously in completing it. I am especially indebted to Dr. Gary P. Jacobson who, as my teacher and mentor, has not only provided me extensive personal and professional guidance but has taught me more than I could ever give him credit for here. He has shown me, by his example, what a good scientist, clinician, and a good person should be.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS ii

LIST OF TABLES v

LIST OF FIGURES vi

Chapter

I. INTRODUCTION 1

- cVEMP and oVEMP 1
- Effects of Aging on the VEMP 3
- Frequency Tuning of the VEMP 4
- Altered Frequency Tuning due to Pathology of the Inner Ear 5
- Purpose 8

II. METHODS 10

- Subjects 10
- Screening 10
- Procedures 11
- Statistical Analysis 14

III. RESULTS 16

- cVEMP 16
  - cVEMP Descriptives 16
  - cVEMP Amplitude Main Effects 18
  - cVEMP Amplitude Interaction Effects 21
    - Age Group x Frequency 21
    - Frequency x Level 22
    - Age Group x Level 24
  - cVEMP Frequency Tuning 25
- Contralateral oVEMP 29
  - Contralateral oVEMP Descriptives 29
  - Contralateral oVEMP Amplitude Main Effects 31
  - Contralateral oVEMP Amplitude Interaction Effects 34
    - Age Group x Frequency 34
    - Frequency x Level 36
    - Age Group x Level 38

Contralateral oVEMP Frequency Tuning 39
Ipsilateral oVEMP 43
Ipsilateral oVEMP Descriptives 43
Ipsilateral oVEMP Amplitude Main Effects..44
Ipsilateral oVEMP Frequency Tuning..45
cVEMP and oVEMP Latency..47
cVEMP Latency..47
oVEMP Latency..48
Frequency Tuning Test-Retest Reliability...49
Frequency Tuning Inter-Ear Differences...50
Middle Ear Power Analysis (MEPA) 52

IV. DISCUSSION...54

Frequency Tuning of the VEMP..54
Effects of Age on the Resonant Frequency of the VEMP 56
Other Age Effects 60
Effects of Stimulus Frequency on the Latency of the VEMP....................60

V. CONCLUSION...64

Appendix

A. CVEMP AMPLITUDE MEANS AND STANDARD DEVIATIONS...................66
B. CONTRALATERAL OVEMP MEANS AND STANDARD DEVIATIONS........68

REFERENCES 70

LIST OF TABLES

Table Page

1. cVEMP Response Rates Across Stimulus Frequencies 18

2. Frequencies with the greatest cVEMP peak-to-peak amplitude 26

3. Contralateral oVEMP Response Rates Across Stimulus Frequencies......................31

4. Frequencies with the greatest contralateral oVEMP peak-to-peak amplitude..........40

5. Ipsilateral oVEMP Response Rates Across Stimulus Frequencies...........................44

6. Mean (SD) peak-to-peak amplitude of the oVEMP and cVEMP at 127 and 122 dB pSPL during the test and re-test sessions 50

7. Mean (SD) Interaural Amplitude Asymmetries (IAA) 51

8. Mean (SD) peak-to-peak amplitude of the oVEMP and cVEMP at 127 and 122 dB pSPL from the right and left ears 52

LIST OF FIGURES

Figure Page

1. The effect of stimulus frequency on the VEMP. The individual VEMP waveforms from all 39 subjects are in the left column and the corresponding grand average waveforms are in the right column. Stimulus level was 127 dB pSPL. (a) cVEMP waveforms. (b) oVEMP waveforms. 17,30

2. Effects of age, stimulus level, and stimulus frequency on the peak-to-peak amplitude of the cVEMP. (a) The main effect of Age Group is shown. Data is collapsed between stimulus level and stimulus frequency. (b) The main effect of stimulus Level is shown. Data is collapsed between age groups and frequencies. (c) The main effect of stimulus Frequency is shown. Data is collapsed between age groups and stimulus levels 20

3. The peak-to-peak amplitude of the cVEMP as a function of stimulus frequency for each age group. (a) The interaction effect of Age group x Frequency at stimulus intensity level of 127 dB pSPL. (b) The interaction effect of Age group x Frequency at stimulus intensity level of 122 dB pSPL. (c) The interaction effect of Age group x Frequency at stimulus intensity level of 117 dB pSPL 22

4. The peak-to-peak amplitude of the cVEMP as a function of stimulus level at each stimulus frequency. (a) The interaction effect of Frequency x Level in the young adult group. (b) The interaction effect of Frequency x Level in the middle age group. (c) The interaction effect of Frequency x Level in the old adult group...23

5. The input/output (I/O) function for the cVEMP shown for each age group 25

6. cVEMP frequency tuning curves from all 39 subjects based on normalized peak amplitudes. The stimulus level was 127 dB pSPL 28

7. cVEMP frequency tuning curves from mean normalized peak amplitudes at 127 dB pSPL. (a) Tuning curves from all 3 age groups. (b) Tuning curves from the young adult and old adult groups only.......................................29

8. Effects of age, stimulus level, and stimulus frequency on the peak-to-peak amplitude of the oVEMP. (a) The main effect of Age Group is shown. Data is collapsed between stimulus level and stimulus frequency. (b) The main effect of stimulus Level is shown. Data is collapsed between age groups and frequencies. (c) The main effect of stimulus Frequency is shown. Data is collapsed between age groups and stimulus levels 33

9. The peak-to-peak amplitude of the contralateral oVEMP as a function of stimulus frequency for each age group. (a) The interaction effect of Age group x Frequency at stimulus intensity level of 127 dB pSPL. (b) The interaction effect of Age group x Frequency at stimulus intensity level of 122 dB pSPL. (c) The interaction effect of Age group x Frequency at stimulus intensity level of 117 dB pSPL..35

10. The peak-to-peak amplitude of the contralateral oVEMP as a function of stimulus level at each stimulus frequency. (a) The interaction effect of Frequency x Level in the young adult group. (b) The interaction effect of Frequency x Level in the middle age group. (c) The interaction effect of Frequency x Level in the old adult group..37

11. The input/output (I/O) function for the contralateral oVEMP shown for each age group..39

12. Contralateral oVEMP frequency tuning curves from all 39 subjects based on normalized peak amplitudes. The stimulus level was 127 dB pSPL..42

13. Contralateral oVEMP frequency tuning curves from mean normalized peak amplitudes at 127 dB pSPL. (a) Tuning curves from all 3 age groups. (b) Tuning curves from the young adult and old adult groups only 43

14. Ipsilateral oVEMP frequency tuning curves based on normalized peak amplitudes at 127 dB pSPL..46

15. Ipsilateral oVEMP frequency tuning curves from mean normalized peak amplitudes at 127 dB pSPL. Mean data is collapsed across age groups..47

16. Mean latency of the first positive peak of the cVEMP (P13) as a function of stimulus frequency. Mean data was collapsed across Age Group and Level 48

17. Mean latency of the first negative peak for both the contralateral and ipsilateral oVEMP as a function of frequency. Mean data was collapsed across Age Group and Level for the contralateral oVEMP and across Age Group for the ipsilateral oVEMP..49

18. Screenshot of the frequency-reflectance profile created by the MEPA3 from an individual subject. The upper panel shows power reflectance (%) as a function of frequency, for the left and right ear separately. The lower power shows the power transmittance (dB) as a

function of frequency. The shaded region represents the normative values provided by the manufacturer 53

CHAPTER I

INTRODUCTION

cVEMP and oVEMP

When an acoustical stimulus of sufficiently high intensity is presented to an ear, a series of reflexes are triggered that include short latency sound-evoked activations, and, sound-evoked inhibitions, of electromyographic (EMG) activity. These sound evoked muscle reflexes are also known as “sonomotor” reflexes and consist of a receptor end organ, an afferent pathway, central connections, an efferent pathway, and an end muscle. High intensity auditory signals not only stimulate the cochlea but also activate the vestibular system and can evoke short latency sound evoked muscle reflexes in the anterior neck muscles and extraocular muscles, and elsewhere. This sonomotor response can easily be recorded with surface electrodes placed either on the sternocleidomastoid muscle (SCM) or in proximity to the inferior oblique (extraocular) muscle (Colebatch and Halmagyi 1992; Rosengren, McAngus Todd et al. 2005; Todd, Rosengren et al. 2007). These evoked responses are referred to as vestibular evoked myogenic potentials (VEMP). A VEMP recorded from the SCM is traditionally referred to as the cervical VEMP (or “cVEMP”) and a VEMP recorded from surface electrodes placed beneath the eyes has been termed the ocular VEMP (or “oVEMP”).

The end organ of the cVEMP is the saccule (Colebatch and Halmagyi 1992; Colebatch, Halmagyi et al. 1994; McCue and Guinan 1994). Electrical activity from the saccule is routed through the inferior vestibular nerve to the medial, lateral, or inferior vestibular nuclei. The efferent limb of the reflex begins when the electrical activity is routed from the vestibular nucleus through the medial or lateral vestibulospinal tract to the spinal accessory nucleus of cranial nerve XI and finally to the motor neurons of the SCM resulting in an inhibition/relaxation of the muscle from its contracted state (for review see Rosengren et al, 2010).

The end organ origin of the oVEMP in response to air conduction stimuli is being debated in the most contemporary literature and is either the saccule, utricle, or both (Curthoys 2010; Halmagyi and Carey 2010; Manzari, Burgess et al. 2010; Murofushi, Wakayama et al. 2010; Rosengren, Welgampola et al. 2010; Govender, Rosengren et al. 2011). However the strongest contemporary evidence supports the utricle as being the end organ responsible for the oVEMP in response to both vibratory and acoustical stimulation (Curthoys 2010; Manzari, Burgess et al. 2010; Manzari, Tedesco et al. 2010; Murofushi, Wakayama et al. 2010; Taylor, Wijewardene et al. 2010; Govender, Rosengren et al. 2011; Lin and Young 2011; Valko, Hegemann et al. 2011). First, recent studies in guinea pigs have confirmed that both otolith end organs respond to acoustic stimulation (Curthoys and Vulovic 2010). Second, there is considerable evidence suggesting that the inferior oblique is the end muscle responsible for the oVEMP (Rosengren, McAngus Todd et al. 2005; Iwasaki, McGarvie et al. 2007; Todd, Rosengren et al. 2007; Welgampola, Migliaccio et al. 2009). The utricle has strong connections to the extraocular muscles, whereas the saccule does not. Third, several reports have been published illustrating different results from the cVEMP and oVEMP in humans with known vestibular lesions. These studies support the contention that the cVEMP and oVEMP have different peripheral origins and are measuring two different pathways.

We contend that the peripheral end organ of the oVEMP is the utricle, thus the afferent pathway of the oVEMP is the superior vestibular nerve. The central pathway for the oVEMP is a bilateral pathway mediated by the vestibulo-ocular reflex (VOR) to the vestibular nucleus (i.e. superior vestibular nerve to vestibular nucleus). The efferent pathway begins at the medial longitudinal fasciculus (MLF). The MLF routes electrical activity through cranial nerve III which innervates four of the six extraocular muscles.

Effects of Aging on the VEMP

Both the cVEMP and oVEMP can be recorded across the lifespan, but the responses are often reduced and/or absent in older individuals. Age-related changes in both the cVEMP and oVEMP are well-documented and appear to be very similar for both responses (Welgampola and Colebatch 2001; Ochi and Ohashi 2003; Su, Huang et al. 2004; Zapala and Brey 2004; Basta, Todt et al. 2007; Brantberg, Granath et al. 2007; Iwasaki, Smulders et al. 2008; Nguyen, Welgampola et al. 2010; Tseng, Chou et al. 2010). The most consistent finding is a decrease in peak-to-peak amplitude and increased threshold with increasing age. Reportedly up to 40% of otologically and neurologically intact subjects over the age of 60 years do not produce a cVEMP response in response to an air conduction tone burst at 500 Hz (Su, Huang et al. 2004). Absent oVEMPs in response to the same stimulus have been reported in 25% of normal subjects over 60 years of age (Piker, Jacobson et al. 2011). Aging has an effect on more routinely used vestibular function tests, such as caloric testing, but a dramatic decline with aging is not a prominent feature of all measures. For example, bilaterally absent caloric responses are uncommonly observed in elderly patients while we often see (e.g. up to 40% of the time) a complete absence of cVEMP responses in elderly patients (e.g. Su et al, 2004). The absence of VEMP responses in elderly patients may be due to an impairment occurring anywhere along the VEMP pathway, or may be due to the limited stimulus levels and/or type of stimulus used (i.e. acoustic stimulation vs a more natural vestibular stimulus such as head decelerations/accelerations). Whereas the presence of impairment along the VEMP reflex pathway is difficult to study non-invasively, we can examine the stimulus used to elicit the VEMP response. Further, there appears to be frequency "tuning" in the vestibular system with certain acoustic frequencies eliciting larger amplitude VEMPs, at least in young healthy individuals (McCue and Guinan 1995; Sheykholeslami, Habiby Kermany et al. 2001; Welgampola and Colebatch 2001; Akin, Murnane et al. 2003; Rauch, Zhou et al. 2004; Node, Seo et al. 2005; Lin, Timmer et al. 2006; Timmer, Zhou et al. 2006; Chihara, Iwasaki et al. 2009; Todd, Rosengren et al. 2009; Todd, Rosengren et al. 2009; Donnellan, Wei et al. 2010; Lewis, Mustain et al. 2010; Park, Lee et al. 2010; Murnane, Akin et al. 2011; Zhang, Govender et al. 2011; Winters, Berg et al. 2012).

Frequency Tuning of the VEMP

Several investigators have assessed the effectiveness of different acoustic stimuli for evoking VEMPs with supramaximal stimulation (i.e. between 120 and 130 dB pSPL). Welgampola & Colebatch (2001) measured cVEMP responses using tone bursts at 100 Hz increments between 200 Hz and 1000 Hz. They observed the largest cVEMP amplitude in response to tone bursts between 600 Hz and 1000 Hz, with a mean ~700 Hz

(Welgampola and Colebatch 2001). Other human studies have shown very similar results with the maximum cVEMP recorded in response to a tone burst between 500 and 1000 Hz (Murofushi, Matsuzaki et al. 1999; Akin, Murnane et al. 2003; Node, Seo et al. 2005; Lin, Timmer et al. 2006; Timmer, Zhou et al. 2006; Park, Lee et al. 2010). The finding that mid-frequency tone burst stimuli yield larger VEMP responses at lower threshold levels has been viewed as evidence of frequency "tuning" in the vestibular system.

The oVEMP has also demonstrated frequency tuning to air conduction tone bursts. Several investigators have recorded the oVEMP in response to air conducted tone bursts with peak amplitude elicited by tone bursts between 400 and 1000 Hz (Todd, Cody et al. 2000; Chihara, Iwasaki et al. 2007; Chihara, Iwasaki et al. 2009; Lewis, Mustain et al. 2010; Park, Lee et al. 2010). Park et al (2010) measured oVEMP responses to frequencies of 250, 500, 1000, and 2000 Hz in 20 normal subjects. Mean amplitudes were similar across the four frequencies (3.0 µV, 5.7 µV, 5.7 µV, and 3.2 µV, respectively) but an analysis of variance revealed that amplitudes at 500 and 1000 Hz were significantly larger than those at 250 and 2000 Hz. Similarly, Lewis et al (2010) reported the greatest oVEMP amplitude at 1000 Hz for 8 out of their 12 subjects. In summary, most studies have reported very similar frequency tuning between the cVEMP and oVEMP. The notion that changes in the saccule and utricle, and consequent changes in their resonant frequency, could alter the frequency tuning of the VEMP has also been investigated.

Altered Frequency Tuning Due to Pathology of the Inner Ear

Meniere’s disease (MD) is associated with pathologic changes in the saccule. Histopathologic studies in human temporal bones have shown that MD patients often present with cochleosaccular hydrops (Fraysse, Alonso et al. 1980; Okuno and Sando 1987; Rauch, Merchant et al. 1989; Yazawa and Kitahara 1990; Sperling, Paparella et al. 1993; Merchant, Adams et al. 2005; Morita, Kariya et al. 2009). Cochleosaccular hydrops causes an expansion in the saccule membrane as the saccule is distended. This can disrupt the electrical resonance of the hair cells and cause the saccule to become thinner and stiffer. Horner & Rydmarker (1991) reported that prolonged cochleosaccular hydrops injures the saccular hair cells causing a loss of kinocilia and stereocilia (Horner and Rydmarker 1991). Several investigators hypothesized that these saccular changes result in either a complete loss of frequency tuning or an increase in the resonant frequency of the saccule.

cVEMP tuning curves in MD patients have been reported to be broader (i.e. less frequency specific) and more tuned to higher frequencies compared to normal subjects (Rauch, Zhou et al. 2004; Node, Seo et al. 2005; Lin, Timmer et al. 2006; Timmer, Zhou et al. 2006). Using tone burst stimuli at 250, 500, and 1000 Hz, Rauch et al (2004) observed the lowest cVEMP threshold (i.e. best sensitivity of the system) in normal controls in response to a 500 Hz tone burst. In the MD patients there was no clear “tuning curve” (i.e. there was no increased sensitivity at any frequency). Rauch et al (2004) hypothesized that the frequency tuning in MD patients had been either shifted to another frequency that was not tested or had simply been lost. Additionally, the unaffected ears of MD patients produced results that were more similar to the affected ears (i.e. reduced amplitudes and a loss or a shift of tuning) than to normal controls. The authors stated that this finding may indicate the beginnings of bilateral MD. Node et al (2005), who also examined frequency tuning of the cVEMP in patients with MD, reported a best frequency between 500 Hz and 1000 Hz in 35 of the 36 normal control ears. The mode tone burst frequency was 500 Hz. The best frequency was reportedly between 700 Hz and 1000 Hz in MD patients. They attributed this slight shift to a higher best frequency in patients with MD to the changes in the morphologic features of the saccule due to cochleosaccular hydrops.

Winters et al (2012) examined the possible differences in oVEMP frequency tuning for healthy controls and patients with MD using tone burst stimuli at 250, 500, and 1000 Hz. They reported findings similar to Rauch et al (2004) stating the best frequency for healthy controls was 500 Hz and the best frequency for MD patients was 1000 Hz. Their data actually showed no statistical difference in the amplitudes between 500 and 1000 Hz in the healthy control group, though threshold at 500 Hz was significantly better than that at 1000 Hz (105 dB vs 109 dB). They did not report any statistically significant differences between the 3 frequencies in the MD group, but stated that the largest amplitude and lowest thresholds in the MD group were observed at 1000 Hz (Winters, Berg et al. 2012).

There are several confounds in these studies that should be addressed. First, typically only 3 frequencies are assessed, such as 250, 500 and 1000 Hz. Welgampola & Colebatch (2001) reported frequency tuning around 700 Hz in normal subjects, a frequency that falls directly between the presumed “normal” 500 Hz and the MD altered frequency tuning at 1000 Hz. Animal studies have shown minor differences in the tuning across vestibular nerve fibers, even in the same ear. It is possible that these differences in frequency tuning are normal variants. This may explain why the unaffected ears in the MD patients showed a similar tuning curve pattern to that of the affected ear. In other words, either the frequency tuning was occurring at a higher frequency than normal controls or a lack of a clear tuning curve altogether was a normal variant for that subject. A more accurate measure of the cVEMP frequency tuning in MD patients may be possible by plotting tuning curves using tone bursts at smaller frequency intervals. cVEMPs recorded at multiple frequencies are required to accurately measure the tuning of the response.

An additional confound with these studies was that age was not considered. A consistent observation in older adults is the considerable variation in the amount of change in the vestibular system that occurs with age (Schuknecht 1965; Johnsson 1971; Rosenhall 1973; Ross, Peacor et al. 1976; Igarashi, Saito et al. 1993; Tang, Lopez et al. 2001; Jang, Hwang et al. 2006; Walther and Westhofen 2007). Some elderly individuals retain an almost normal vestibular system while others at the same age show extensive degeneration. It is not known if these well-documented neuroanatomic age-related changes occurring in the otolith organs result in altered frequency tuning, similar to that reported in patients with MD. In the report by Rauch and colleagues (2004), the control group ranged in age from 21 – 52 years, the mean age was not reported. The MD group was older and ranged in age from 21 – 77 years. Similarly, in the report by Winter et al, (2012) the control group ranged in age from 23-52 years with a mean age of 30. The age range of the MD group was from 33 – 76 year with a mean age of 56 years.

Purpose

In studies examining younger adults, tone burst frequencies yielding the largest VEMP amplitude and smallest VEMP threshold occur in response to stimulation between 500 and 1000 Hz (Murofushi, Matsuzaki et al. 1999; Akin, Murnane et al. 2003; Node, Seo et al. 2005; Lin, Timmer et al. 2006; Timmer, Zhou et al. 2006; Park, Lee et al. 2010). For this reason 500 Hz has been the most commonly used frequency to record a VEMP. In an aged vestibular system it is possible that the same 500 Hz auditory stimulus might not generate enough force to evoke the sonomotor response. The result would be an absent VEMP. It is not known if the changes in the aging vestibular system also alter the frequency tuning of the system, as has been suggested for pathological vestibular systems (e.g. Meniere's Disease; Rauch et al, 2004). If this is true we may need to alter the stimulus parameters to accommodate aging populations. Using an age adjusted optimal stimulus protocol for recording the VEMP might have the effect of improving the recordability of the response and accordingly improve the overall sensitivity of the diagnostic test battery for the identification of vestibular impairments.

The purpose of the present investigation was to define for young, middle age, and elderly subjects the best frequency(cies) to record both the cVEMP and the oVEMP. Further, it is the objective of this study to describe age related changes in the "tuning" of these two sonomotor responses.

CHAPTER II

METHODS

Subjects

Thirty-nine subjects met inclusion criteria and participated in the main portion of this investigation (mean age 46.3 ± 15.7 years; range = 22 – 78 years; 15 males). Subjects were equally divided into 3 age groups of 13 subjects each: Age Group 1/Young Adult (18 - 39 years), Age Group 2/Middle Age (40 – 59 years), and Age Group 3/Old Adult (≥ 60 years). Data was obtained from one ear of the 39 participants (left ear of the odd-numbered participants and right ear of the even-numbered participants) yielding data from 20 left ears and 19 right ears. Five from the initial 39 subjects were recruited to participate in two smaller sub-studies examining the test-retest reliability and the inter-ear symmetry of VEMP frequency tuning. Data was obtained from these 5 subjects from both ears and on 2 separate test sessions.

Screening

To assess hearing sensitivity and middle-ear status, air-conduction threshold testing was conducted at 250, 500, 1000, 2000, 4000, and 8000 Hz and bone-conduction threshold audiometry was conducted at 500, 1000, 2000, and 4000 Hz. Tympanometry and ipsilateral auditory reflex testing at 1000 Hz was also completed. Additionally, we screened for the presence of an oVEMP in both ears using a stimulus consisting of 500 Hz, 750 Hz, and 1000 Hz tone bursts presented randomly.

To be enrolled in this investigation, subjects could not present with a conductive hearing loss or an asymmetry between ears of 15 dB or greater at any frequency. One subject did not meet these criteria and was excluded during the screening process. Four subjects did not produce an oVEMP response during the screening process and were excluded. Subjects with sensorineural hearing loss that presented with a positive Metz test were included. The Metz test measures the difference between acoustic reflex threshold and pure-tone threshold and is used to evaluate recruitment. Reflexes occur at reduced sensation levels in ears with cochlear hearing loss (positive Metz test) but are elevated or absent in ears with 8th nerve lesions (Metz 1952). In a normal ear the acoustic reflex threshold is between 70-105 dB above the pure tone hearing threshold level (Jerger, Jerger et al. 1972). In subjects with hearing loss, a difference less than 60 dB between pure tone threshold in HL and the acoustic reflex threshold is considered a positive Metz test (Jerger, Jerger et al. 1972) and supports a cochlear origin of the hearing impairment. Since we know VEMP responses are not affected by sensorineural hearing impairment we did not want to exclude subjects with cochlear hearing loss. For this reason 3 subjects with sensorineural hearing loss and a positive Metz test were included. Additional exclusion criteria for all participants included complaints of dizziness or imbalance, known otologic disease, neurologic disease, conductive hearing loss, or known disease affecting the cervical vertebrae or spinal cord.

Procedures

Subjects were placed in semi-recumbent position in a comfortable reclining chair for the cVEMP and were sitting upright for the oVEMP. Disposable silver/silver-chloride electrodes were used. To record the cVEMP, subjects were asked to lift their heads off the headrest and turn their heads away from the ear that was being stimulated. To record the oVEMP subjects were instructed to direct their gaze at a visual target at a vertical elevation of ~30 degrees. Subjects maintained these positions during data collection and were asked to rest while data collection was paused. Subjects were given as many rest periods as they needed. All recordings were replicated a minimum of one time so that repeatability of the data could be assessed.

A 1-channel cVEMP recording was made with the non-inverting input placed on the sternocleidomastoid muscle midway between the insertion at the mastoid and the sternum ipsilateral to the side of stimulus presentation. The inverting electrode was placed on the chin. The ground electrode was placed at Fpz. Individual electrode impedances were ≤ 10 kOhms and interelectrode impedances were ≤ 5 kOhms. Ongoing EMG in the SCM was monitored visually using a second evoked potential machine that contained an EMG feedback system (Interacoustics, Denmark) in an attempt to ensure that subjects were generating a consistent and adequate amount of tonic background EMG activity between 50 and 200 µV.

Two-channel oVEMP recordings were made with the non-inverting (active) electrodes placed infraorbitally at midline as close as possible to the lower margin of the lower eyelid of both the ipsilateral and contralateral eye (relative to the ear being stimulated). The inverting (reference) electrodes were placed 2-3 cm inferior to the active electrodes. The ground electrode was placed at Fpz. Individual electrode impedances were ≤ 10 kOhms and interelectrode impedances were ≤ 5 kOhms.

The stimuli for both the cVEMP and oVEMP were presented monoaurally through Etymotic ER-3A insert earphones. A single stimulus was used consisting of 125, 250, 500, 750, 1000, 1500, and 2000Hz Blackman-gated tone bursts with a 2ms rise/fall and 2ms plateau. Each stimulus block (i.e. each run) consisted of the 7 test frequencies presented in a randomized sequence at a rate of 5.1/second. Each run lasted ~30 seconds, with ~30 second rest periods between runs, and each stimulus was presented approximately ~150 times. The tone bursts were presented at three stimulus levels, 127 dB pSPL, 122 dB pSPL, and 117 dB pSPL, which were also randomized in their order of presentation.

Artifact rejection was used off-line for the oVEMP recordings in an attempt to eliminate eye blinks. The bioelectrical activity was amplified and analog filtered (5 – 500 Hz) with a commercially produced multi-channel neurophysiological amplifier (Neuroscan Synamp, Herndon, VA). For each single record the electromyographic activity was digitized (at a rate of 5000 Hz) and recorded as a continuous one-channel recording on a commercially available electrophysiological recording system (Neuroscan, Herndon, VA). A second channel contained unique triggers associated with the 7 stimulus frequencies. Data was off-line epoched into segments of EMG associated with stimulus onset. The data for each frequency was signal averaged separately. Following signal averaging the latencies of the prominent peaks were recorded as well as their peak-to-peak amplitudes.

To account for the possibility that the differences in VEMP frequency tuning between subjects were due to differences in the transmission of the air-conduction stimuli through the middle ear, the status of the middle ear was assessed using the Mimosa Acoustics HearID middle ear power analyzer (MEPA3; Mimosa Acoustics, Champaign, IL). The MEPA3 can be used to assess the status of the middle ear and provides a measurement of how the middle ear filters the sound it receives. In other words, when sound is presented to the ear, some of the sound is absorbed by the middle ear and some of the sound is reflected from the ear drum, and this varies by frequency. Power reflectance is defined as the percentage of reflected power to incidental (total) power. Power absorption is the percentage of the absorbed power to the incident power, and mirrors the results of power reflectance. Power transmittance is the power absorption converted to a decibel scale.

MEPA measurements were made on each subject either before or after the VEMP recordings. The MEPA3 system was calibrated prior to each recording session using a four-chamber coupler (model: CC4-V) in accordance with manufacture guidelines. A probe tip (Etymotics ER10C) was used to deliver sound into the external ear canal. An in-the-ear pressure calibration with the probe in the subject's ear was performed on each subject, in each ear, prior to the MEPA measurement. The stimuli consisted of a chirp stimulus set to 60 dB SPL presented over a measurement time period of 1 second. The MEPA measurement was made a minimum of 2 times in each ear.

Statistical Analysis

For each subject, VEMPs were derived separately for the 7 frequencies and at the 3 different stimulus levels. Each VEMP was repeated and the average of the two runs was used. Thus, each subject yielded 21 cVEMPs and 21 contralateral and ipsilateral oVEMPs.

A present cVEMP was defined as an initial positive polarity peak (i.e. occurring at ~ 15 ms) followed by a subsequent negative polarity (i.e. occurring at ~25 ms). All responses were repeated and the two runs were grand averaged. The first positive polarity peak of the averaged run was labeled as P1 and the following negative peak labeled N1. P1 absolute latency and P1-N1 peak-to-peak amplitudes were measured and tabulated. An absent response was assigned an amplitude value of 0 µV and the latency value was considered missing data.

A present oVEMP was defined as an initial negative peak (i.e. occurring at ~10 ms) with a subsequent positive peak (i.e. occurring at ~15 ms). All responses were repeated and the two runs were grand averaged. The first negative peak of the averaged run was labeled as N1 and the following positive peak as P1. N1 absolute latency and N1-P1 peak-to-peak amplitude was measured and tabulated. An absent response was assigned an amplitude value of 0 µV and the latency value was considered missing data.

The data were analyzed using SPSS version 20.0 (SPSS, Inc., Chicago, IL). An analysis of variance (ANOVA) was used to assess the effect of age group, stimulus level, and stimulus frequency on the amplitude and latency of the VEMP. A post hoc analysis of multiple comparisons was conducted, when appropriate, using a Tukey test. When significant interaction effects were present, simple main effects tests were performed.

CHAPTER III

RESULTS

cVEMP

cVEMP Descriptives

Figure 1a shows the individual (left column) and grand average (right column) cVEMP waveforms in response to 127 dB pSPL stimuli at 125, 250, 500, 750, 1000, 1500, and 2000 Hz. The cVEMP peak-to-peak amplitude means ± standard deviations (SD) for each frequency, for each intensity level, and for all 3 age groups are shown in Appendix 1. The largest average peak-to-peak amplitude was obtained at 750 Hz.

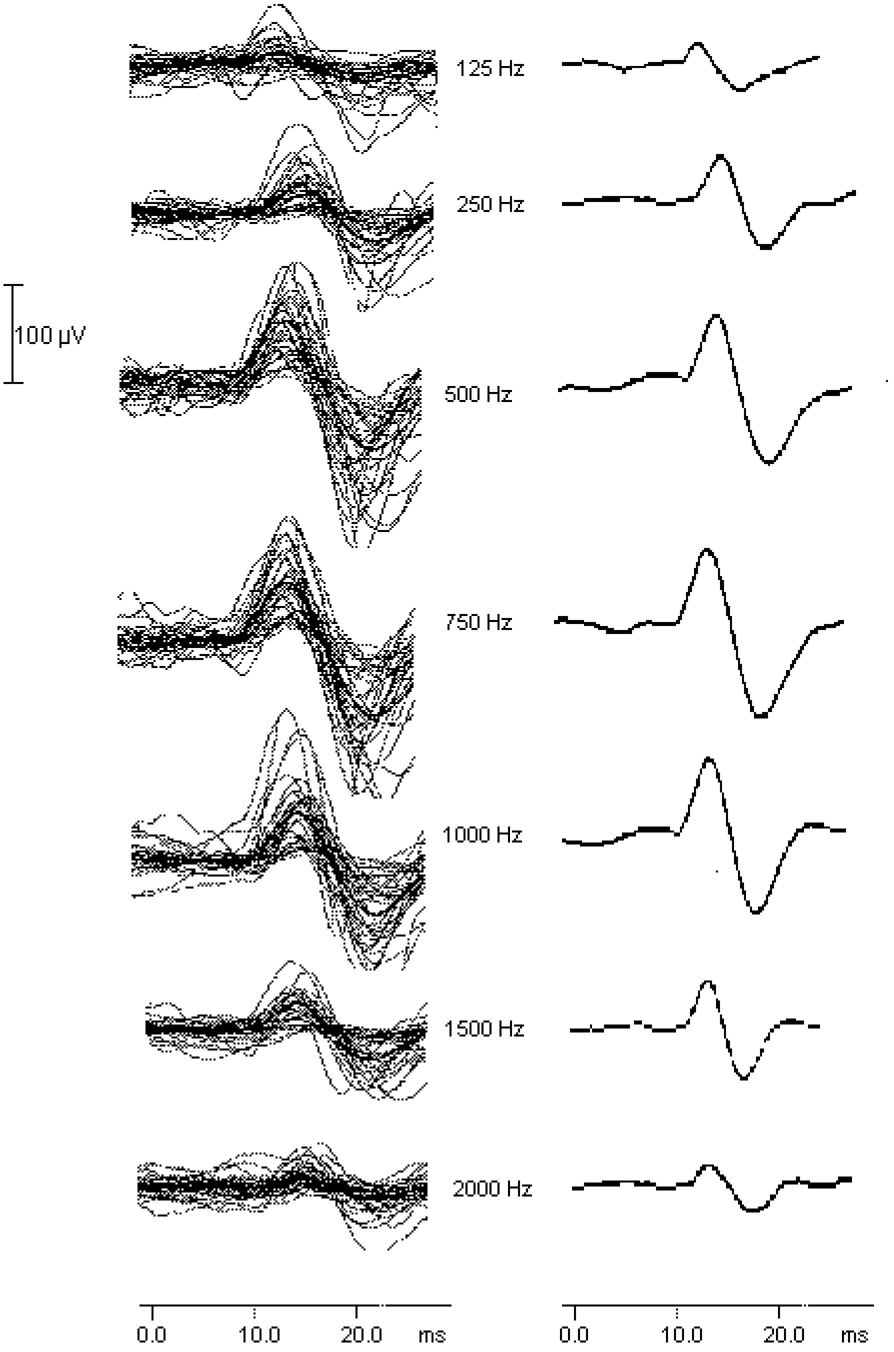

Figure 1a: The effect of stimulus frequency on the cVEMP. The individual cVEMP waveforms from all 39 subjects (including those responses classified as "absent" and entered into the database with an amplitude value of 0 µV) are in the left column and the corresponding grand average waveforms are in the right column. Stimulus level was 127 dB pSPL

Table 1 shows the cVEMP response frequency of occurrence for each age group at each stimulus level across stimulus frequency. At the maximum stimulus intensity (127 dB pSPL), the cVEMP response rate was highest at 750 and 1000 Hz (39 of 39 subjects, 100%). The response rate decreased slightly at 500 Hz (38 of 39 subjects, 97%), and continued to fall at the lowest and highest frequencies. The response rate tended to decrease as age increased and as stimulus intensity level decreased.

Table 1. cVEMP response rates across stimulus frequencies

		125 Hz	**250 Hz**	**500 Hz**	**750 Hz**	**1000 Hz**	**1500 Hz**	**2000 Hz**
127 dB pSPL	Young Adult (n = 13)	69%	92%	100%	100%	100%	100%	46%
	Middle Age (n = 13)	38%	92%	100%	100%	100%	85%	38%
	Old Adult (n = 13)	23%	46%	92%	100%	100%	53%	31%
122 dB pSPL	Young Adult (n = 13)	23%	62%	100%	100%	100%	85%	31%
	Middle Age (n = 13)	8%	61%	100%	100%	100%	61%	8%
	Old Adult (n = 13)	0%	8%	54%	61%	54%	23%	8%
117 dB pSPL	Young Adult (n = 13)	15%	54%	85%	92%	77%	31%	0%
	Middle Age (n = 13)	0%	15%	69%	61%	69%	0%	0%
	Old Adult (n = 13)	0%	0%	15%	23%	23%	8%	0%

cVEMP Amplitude Main Effects

The peak-to-peak amplitude of the cVEMP varied with stimulus frequency, stimulus level, and age group. A 3 x 3 x 7 univariate ANOVA was conducted examining Age Group x Level x Frequency with mean cVEMP peak-to-peak amplitude as the dependent variable. There were significant main effects for Age Group (F = 111.6, df = 2, p < .001; see Figure 2a), stimulus Level (F = 102.5, df = 2, p < .001; see Figure 2b), and stimulus Frequency (F = 36.0, df = 6, p < .001; see Figure 2c). cVEMP amplitude decreased with increasing age and decreasing stimulus intensity. The effects of frequency on cVEMP amplitude were more complex. cVEMP amplitude was greater at the mid frequencies than at the highest and lowest frequencies.

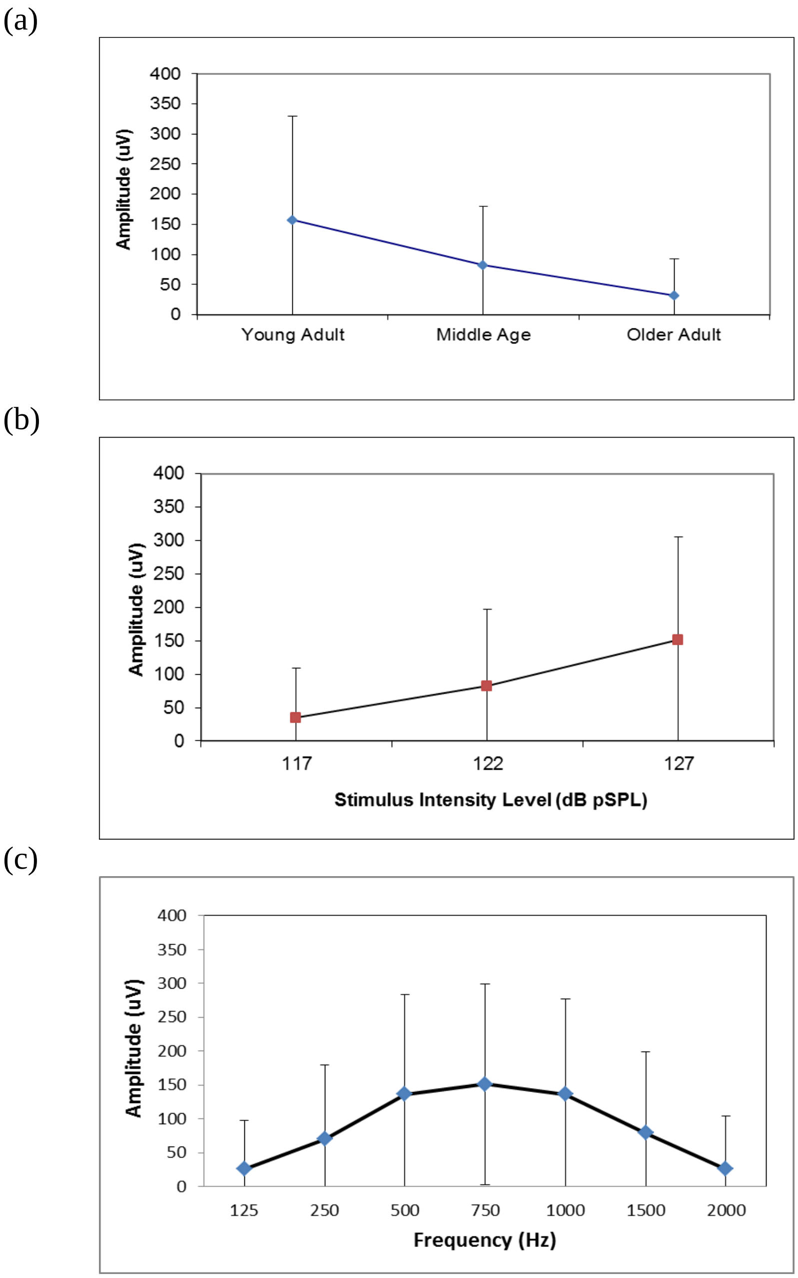

Figure 2: Effects of age, stimulus level, and stimulus frequency on the peak-to-peak amplitude of the cVEMP. (a) The main effect of Age Group is shown. Data is collapsed between stimulus level and stimulus frequency. (b) The main effect of stimulus Level is shown. Data is collapsed between age groups and frequencies. (c) The main effect of stimulus Frequency is shown. Data is collapsed between age groups and stimulus levels.

Post hoc Tukey tests revealed that the mean amplitude for the young adult age group was significantly larger than that for both the middle age and older adult age groups. The mean amplitude for the middle age group was significantly larger than that of the older adult group. There were also significant differences in amplitude between all possible pairs of stimulus intensity levels. Stimulus frequencies at 500, 750, and 1000 Hz produced significantly larger amplitudes than 125, 250, 1500, and 2000 Hz. However, no significant differences in mean amplitude were observed between 500, 750, and 1000 Hz. cVEMP amplitude for 125 Hz was not statistically different from the amplitude for the 2000 Hz (most likely due to the number of absent responses at those frequencies). cVEMP amplitudes at 250 Hz and 1500 Hz were not significantly different from each other, but did show significantly larger amplitudes than 125 Hz and 2000 Hz.

cVEMP Amplitude Interaction Effects

Age Group X Frequency

There was a significant interaction between the effects of Age Group and Frequency on the amplitude of the cVEMP ($F = 4.1$, $df = 12$, $p < .001$; see Figure 3), indicating that the change in cVEMP amplitude as a function of frequency was dependent on age group. Simple main effects analysis showed that cVEMP amplitude was significantly more affected by frequency in the young adult group ($p < .01$, all intensity levels) and middle age group ($p < .01$, all intensity levels) with no significant differences between frequencies in the older adult group (127 dB pSPL: $p = .139$; 122 dB pSPL: $p = .531$; 117 dB pSPL3: $p = .999$). There may be a “flattening” or loss of frequency tuning in the older adult age group. Alternatively, the lower response rate in the older adults (see Table 1) may have contributed to this interaction.

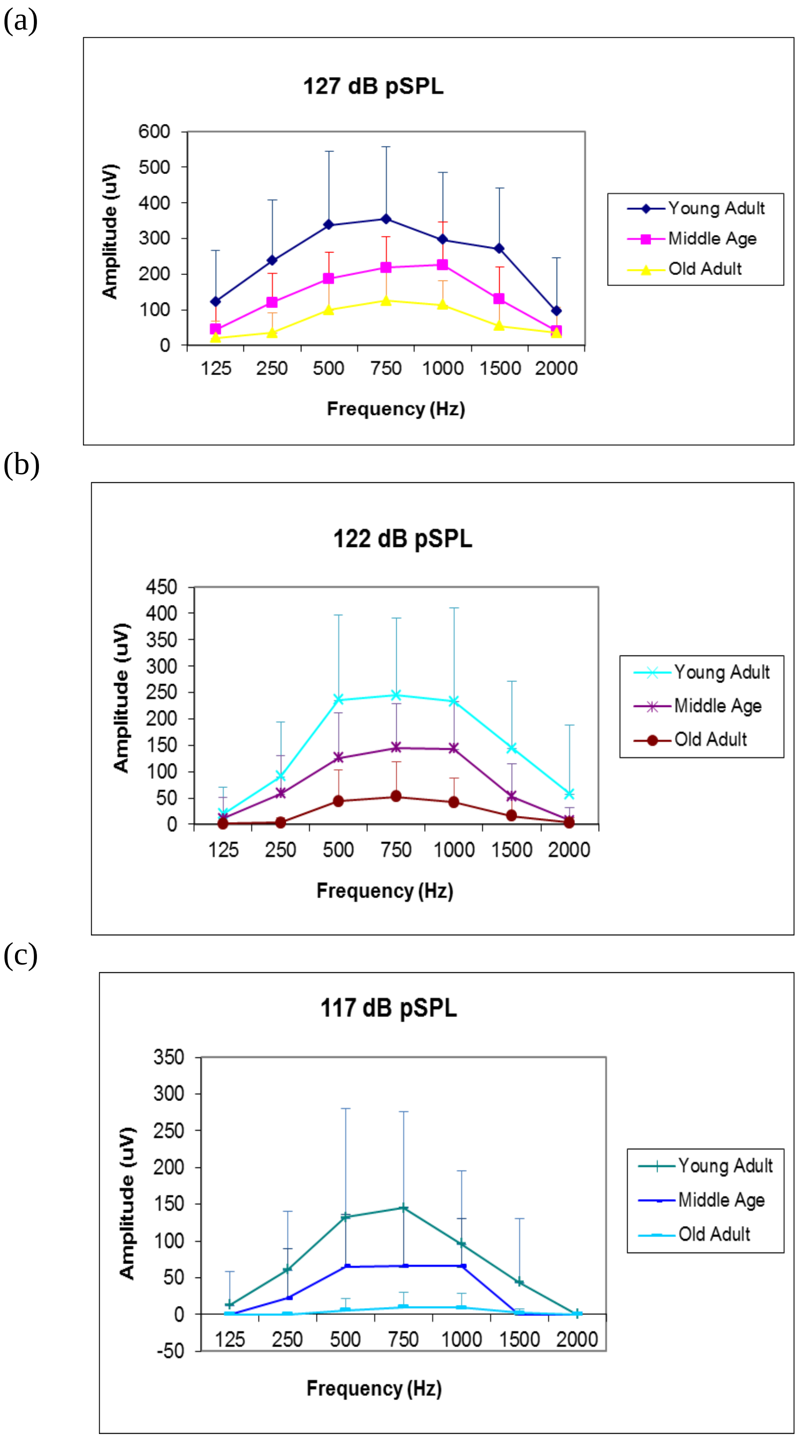

Figure 3: The peak-to-peak amplitude of the cVEMP as a function of stimulus frequency for each age group. The interaction effect of Age group x Frequency at: (a) stimulus intensity level of 127 dB pSPL, (b) stimulus intensity level of 122 dB pSPL, and (c) stimulus intensity level of 117 dB pSPL

Frequency X Level

There was a significant interaction between the effects of Frequency and Level on the amplitude of the cVEMP (F = 2.22, df = 12, p = .01; see Figure 4), indicating that the change in cVEMP amplitude as a function of stimulus frequency was dependent on stimulus level.

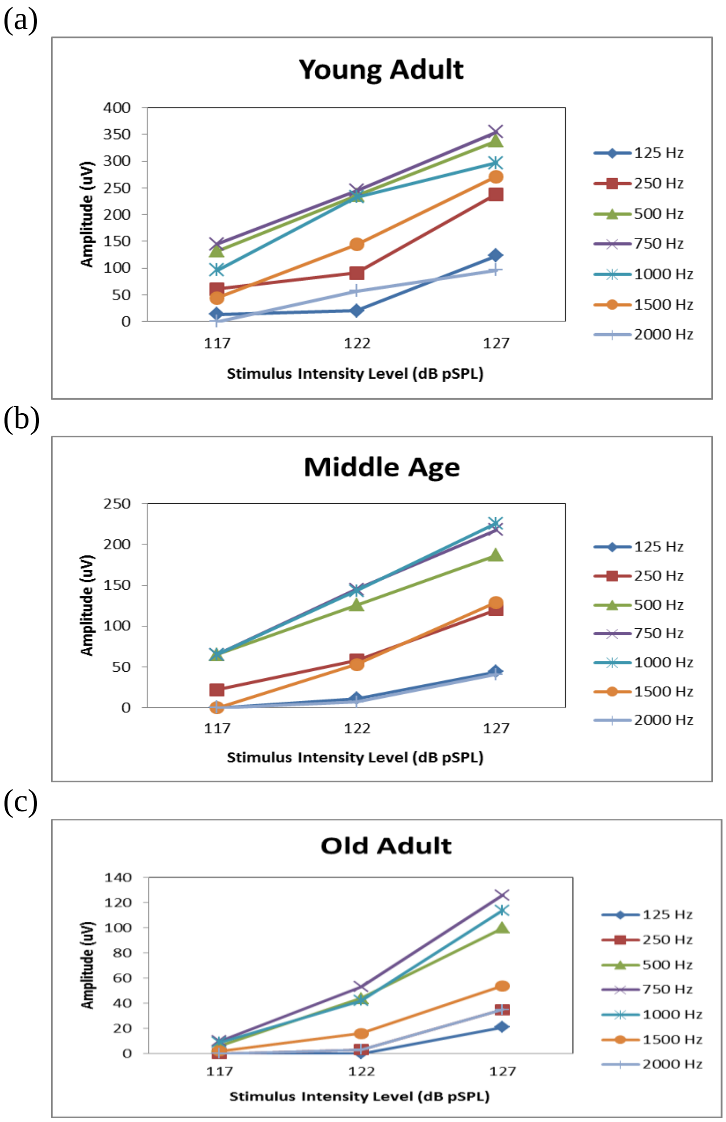

Figure 4: The peak-to-peak amplitude of the cVEMP as a function of stimulus level at each stimulus frequency. (a) The interaction effect of Frequency x Level in the young adult group. (b) The interaction effect of Frequency x Level in the middle age group. (c) The interaction effect of Frequency x Level in the old adult group

Simple main effects analysis showed that for each Age Group, cVEMP amplitude was significantly more affected by frequency for the intensities 127 dB pSPL (p <.01) and 122 dB pSPL (young adult: p < .01; middle age: p < .001; old adult: p = .01). There were no significant differences between frequencies at 117 dB pSPL (young adult: p = .079; middle age: p = .13; old adult: p = .995). The interaction effect seen at 117 dB pSPL may be due to the low response rates at that intensity level (see Table 1). In other words, at least for the 2 highest stimulus intensity levels, there is no difference in frequency tuning between stimulus levels. At the lowest stimulus intensity level there did not appear to be a best frequency (i.e. the frequency plot appeared flat). This occurred more than likely because we were below cVEMP threshold for many subjects (i.e. there were no data points at the highest and lowest frequencies). As shown in Figure 4, there was greater variation in the amplitude of the cVEMP across frequencies at 127 and 122 dB pSPL compared to 117 dB pSPL. At 117 dB pSPL the amplitude of the cVEMP tended to be smaller at all frequencies. This effect is best illustrated in the older adult group (Figure 4c).

Age Group X Level

The Age Group x Level interaction was significant (F = 7.58, df = 4, p < .001), indicating that the change in cVEMP amplitude as a function of stimulus level was dependent on age group. Simple main effects analysis showed that cVEMP amplitude was significantly affected by stimulus intensity level for all age groups (p < .001). Figure 5 displays the input/out (I/O) functions.

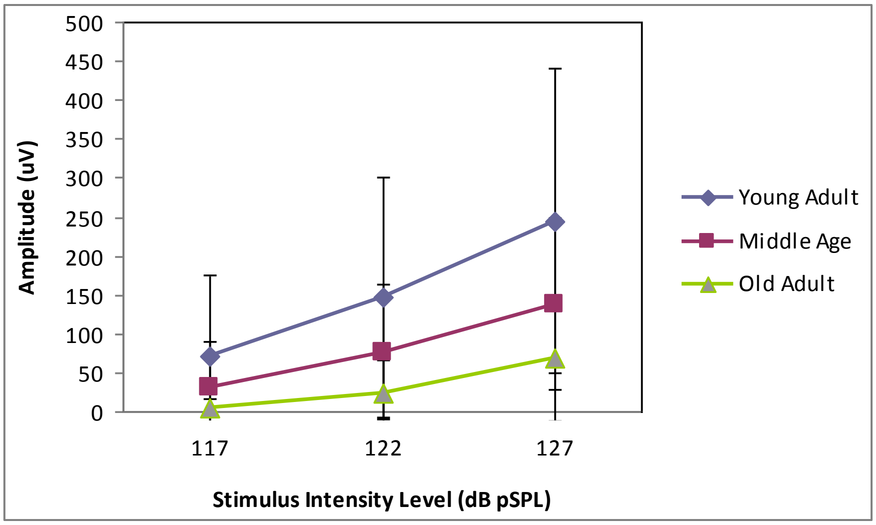

Figure 5: The input/output (I/O) function for the cVEMP shown for each age group

The slope of the I/O function was steeper for the younger age group. For the young adult group, the cVEMP amplitude grew an average of 87.5 uV per 5 dB increase in stimulus intensity level (i.e. mean of 70.6 uV at 117 dB pSPL, mean of 147.2 uV at 122 dB pSPL, and a mean of 245 uV at 127 dB pSPL). The cVEMP amplitude grew an average of 53.5 uV for the middle age group and 32.5 uV per 5 dB increase in stimulus intensity level for the old adult group. In other words, there was a greater increase in amplitude with increasing stimulus level for the young adult group compared to the other two groups.

cVEMP Frequency "Tuning"

The frequency resulting in the largest response amplitude (i.e. "best" frequency) for each individual subject is shown in Table 2.

Table 2. Frequencies with the greatest cVEMP peak-to-peak amplitude (i.e. “best” frequency), with the corresponding amplitude, from each individual subject. The mean peak amplitude for each age group and for the entire cohort is also shown. Stimulus intensity level was 127 dB pSPL.

Subject	Age Group	Best Frequency (Hz)	Peak Amplitude (μV)
1	1	500	390.3
2	1	500	410.9
3	1	500	613.6
4	1	500	583.6
5	1	750	824.3
6	1	750	107.4
7	1	750	278.8
8	1	750	140.7
9	1	750	279.1
10	1	750	364.6
11	1	750	147.7
12	1	1000	344.7
13	1	1000	275.1
Age Group 1 Average			**366.22 ± 206.2**
14	2	500	280.9
15	2	500	230.7
16	2	500	120.1
17	2	500	354.4
18	2	750	182.4
19	2	750	273.4
20	2	750	208.6
21	2	750	59.6
22	2	1000	221.1
23	2	1000	288.6
24	2	1000	531.9
25	2	1000	241.8
26	2	1500	175.2
Age Group 2 Average			**243.7 ± 115.2**
27	3	750	134.8
28	3	750	88.9
29	3	750	144.6
30	3	750	257.4
31	3	750	316.8
32	3	750	239.7
33	3	750	93.3
34	3	750	106.6
35	3	1000	107.0
36	3	1000	64.0
37	3	1000	158.2
38	3	1000	98.5

39	3	1000	134.8
Age Group 3 Average			**149.58 ± 75.7**
Total Average			**253.18 ± 165.8**

The best frequency tended to increase with increasing age. For example 85% of the youngest age group showed greatest amplitudes at either 500 or 750 Hz with only 15% showing the best amplitude at 1000 Hz. In contrast to this, no one in the oldest age group demonstrated a best amplitude at 500 Hz. However, 62% in the oldest age group showed the greatest amplitude at 750 Hz and 38% at 1000 Hz. The best frequency for the middle age group was evenly split at 31%, 31%, and 31% for 500, 750, and 1000 Hz with 1 subject (7%) at 1500 Hz.

To qualitatively examine the frequency tuning of the cVEMP, frequency tuning curves were constructed by plotting the peak-to-peak cVEMP amplitude as a function of stimulus frequency for each subject. Given the variability in amplitude, tuning curves were graphed based on normalized amplitudes at each frequency (f) expressed as a ratio of the largest measured amplitude (at fmax Hz). Thus the normalized amplitude for a given frequency was equal to amplitude(f)/amplitude(fmax) and expressed on a scale from 0 – 1.0. The tuning curves for individual subjects are show in Figure 6. The frequency tuning of the cVEMP is very broad and varies greatly between subjects. As Figure 6 shows, the frequency tuning peaks between 500 and 1000 Hz.

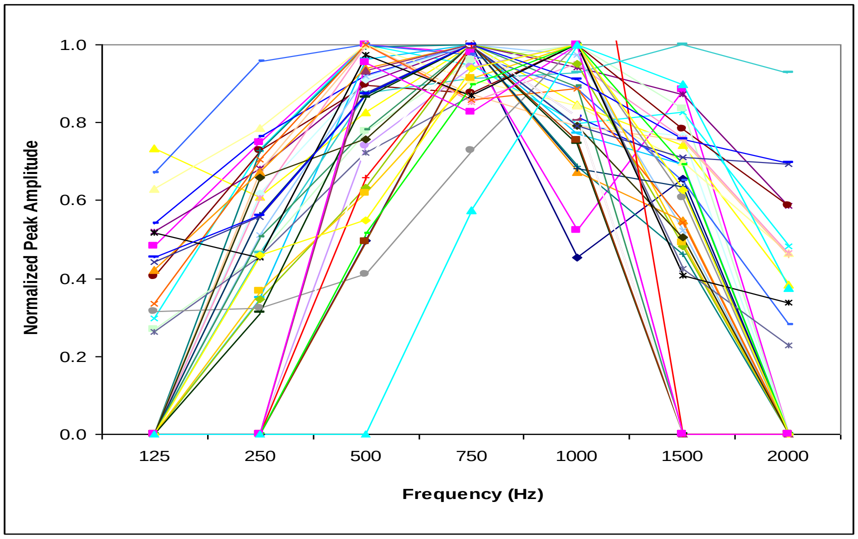

Figure 6: cVEMP frequency tuning curves from all 39 subjects based on normalized peak amplitudes. The stimulus level was 127 dB pSPL

Frequency tuning curves based on the mean normalized peak amplitudes from each age group are show in Figure 7. Figure 7(b) highlights the youngest and oldest age groups. In the young adult group, the peak of the tuning curve is at 750 Hz, whereas in the old adult group the tuning curve peaks at 1000 Hz. The frequency "tuning" shifted to a slightly higher frequency in the old adult group.

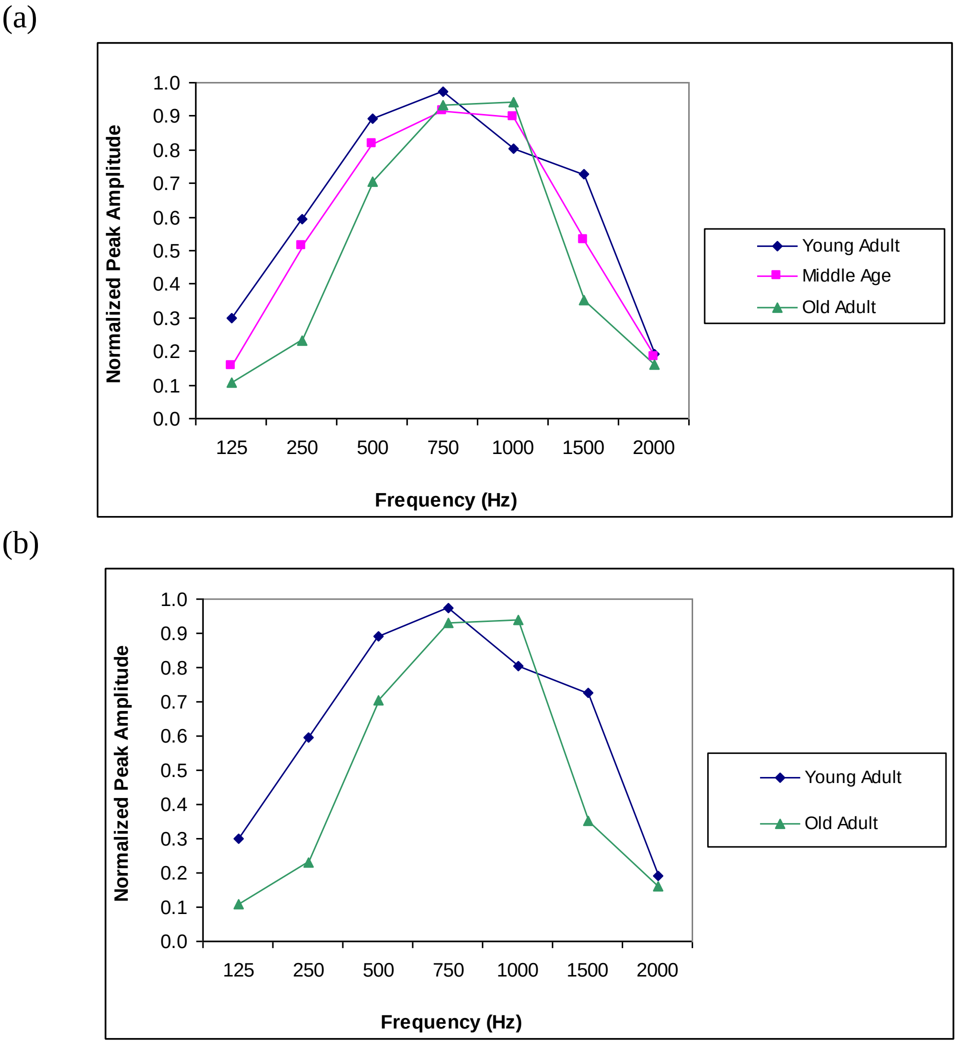

Figure 7: cVEMP frequency tuning curves from mean normalized peak amplitudes at 127 dB pSPL. (a) Tuning curves from all 3 age groups. (b) Tuning curves from the young adult and old adult groups only

Contralateral oVEMP

Contralateral oVEMP Descriptives

Figure 1b shows the individual (left column) and grand average (right column) oVEMP waveforms in response to 127 dB pSPL stimuli at 125, 250, 500, 750, 1000, 1500, and 2000 Hz. The oVEMP peak-to-peak amplitude means ± standard deviations (SD) for each frequency, for each intensity level, and for all 3 age groups are shown in

Appendix 2. As with the cVEMP, the largest average peak-to-peak amplitude was obtained at 750 Hz.

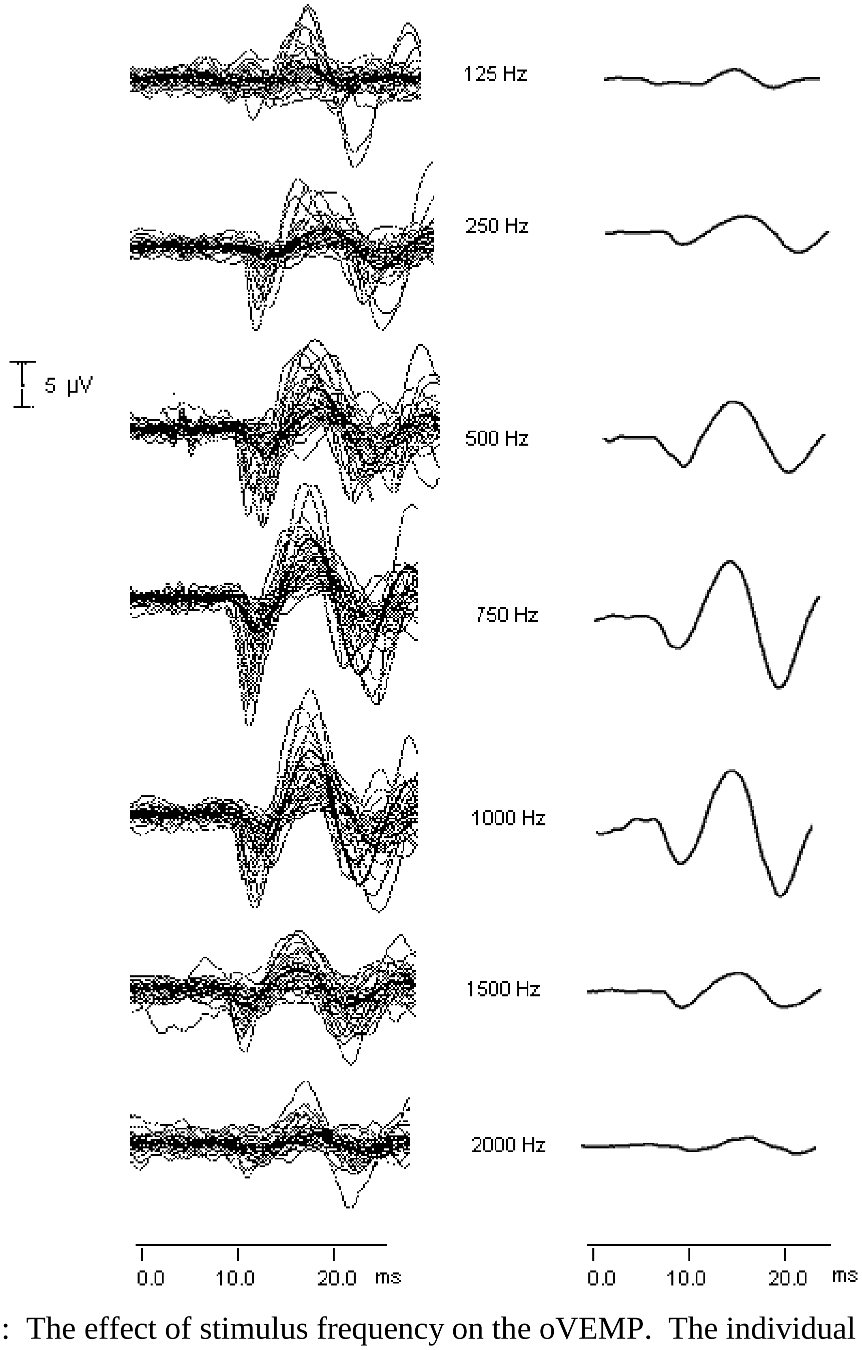

Figure 1b: The effect of stimulus frequency on the oVEMP. The individual oVEMP waveforms from all 39 subjects (including those responses classified as “absent” and entered into the database with an amplitude value of 0 µV) are in the left column and the corresponding grand average waveforms are in the right column. Stimulus level was 127 dB pSPL

Table 3 shows the oVEMP response frequency of occurrence for each age group at each stimulus level across stimulus frequency. At the maximum stimulus intensity (127 dB pSPL), the oVEMP response rate was highest at 750 and 1000 Hz (39 of 39 subjects, 100%). The response rate decreased slightly to 95% (37 of 39 subjects) at 500 Hz and continued to fall at the lowest and highest frequencies. The response rate tended to decrease as age increased and as stimulus intensity level decreased.

Table 3. Contralateral oVEMP response rates across stimulus frequencies.

		125 Hz	**250 Hz**	**500 Hz**	**750 Hz**	**1000 Hz**	**1500 Hz**	**2000 Hz**
127 dB pSPL	Young Adult (n = 13)	46%	69%	92%	100%	100%	69%	31%
	Middle Age (n = 13)	15%	54%	100%	100%	100%	46%	31%
	Old Adult (n = 13)	0%	23%	92%	100%	100%	31%	8%
122 dB pSPL	Young Adult (n = 13)	23%	46%	85%	85%	85%	31%	0%
	Middle Age (n = 13)	15%	15%	69%	77%	77%	8%	8%
	Old Adult (n = 13)	0%	0%	8%	23%	15%	8%	0%
117 dB pSPL	Young Adult (n = 13)	8%	8%	38%	23%	23%	0%	0%
	Middle Age (n = 13)	0%	8%	15%	15%	15%	0%	0%
	Old Adult (n = 13)	0%	0%	0%	8%	0%	0%	0%

Contralateral oVEMP Amplitude Main Effects

The peak-to-peak amplitude of the oVEMP varied with stimulus frequency, stimulus level, and age group. A 3 x 3 x 7 univariate ANOVA was conducted examining Age Group x Level x Frequency with oVEMP peak-to-peak amplitude as the dependent variable. There were significant main effects for Age Group (F = 38.1, df = 2, p < .001; see Figure 8a), Level (F = 121.1, df = 2, p < .001; see Figure 8b), and Frequency (F = 33.7, df = 6, p < .001; see Figure 8c). oVEMP amplitude decreased with increasing age and decreasing stimulus intensity. As with the cVEMP, the effects of frequency on the contralateral oVEMP amplitude were more complex. As with the cVEMP, it appears that oVEMP amplitude was greater at the mid frequencies than at the highest and lowest frequencies.

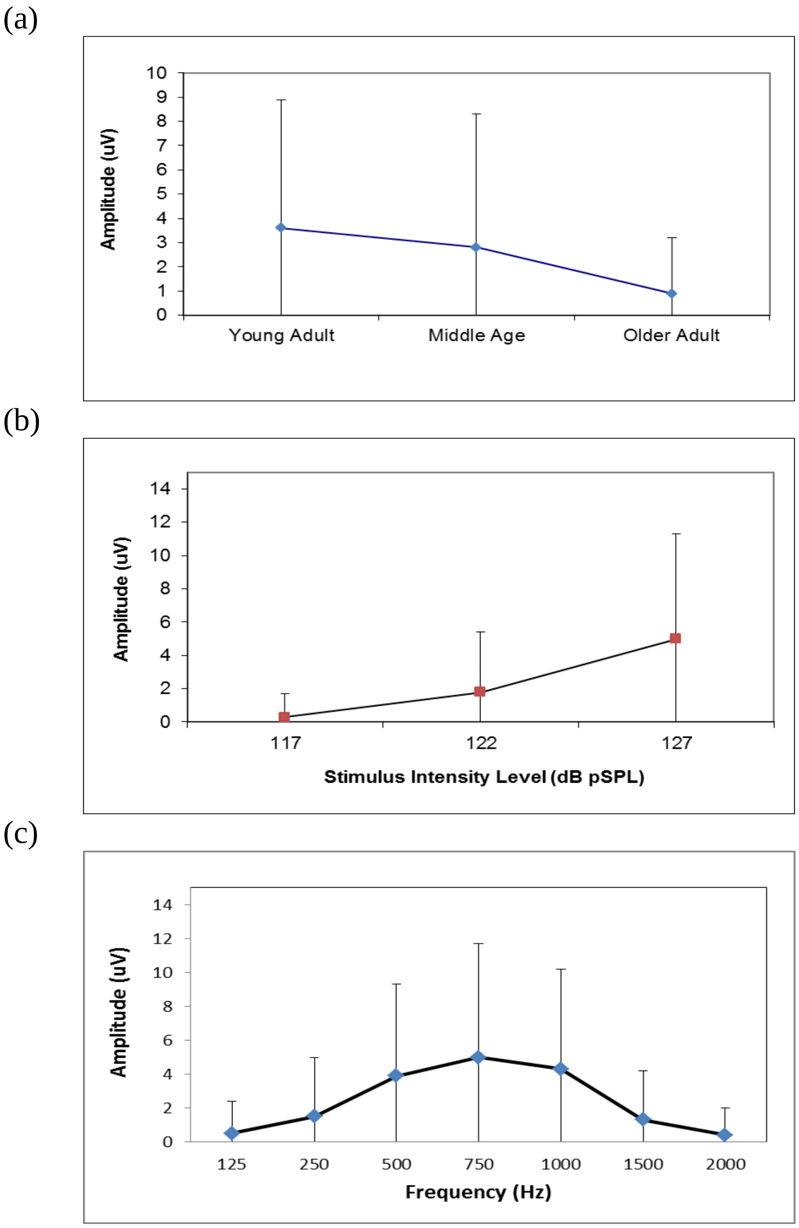

Figure 8: Effects of age, stimulus level, and stimulus frequency on the peak-to-peak amplitude of the oVEMP. (a) The main effect of Age Group is shown. Data is collapsed between stimulus level and stimulus frequency. (b) The main effect of stimulus Level is shown. Data is collapsed between age groups and frequencies. (c) The main effect of stimulus Frequency is shown. Data is collapsed between age groups and stimulus levels

Post hoc Tukey tests showed that the mean oVEMP amplitude for the young adult and middle age groups were significantly larger than for the old adult group. Unlike the cVEMP, the young and middle age adults were not significantly different from each other. As with the cVEMP, there were significant differences in amplitude between all possible pairs of stimulus intensity levels. Stimulus frequencies 500, 750, and 1000 Hz produced significantly larger oVEMP amplitudes than 125, 250, 1500, and 2000 Hz. No significant differences in mean amplitude were observed between 500, 750, and 1000 Hz. oVEMP amplitudes at125, 250, 1500, and 2000 Hz were also not significantly different from each other.

Contralateral oVEMP Amplitude Interaction Effects

Age Group X Frequency

There was a significant interaction between the effects of Age Group and Frequency on the amplitude of the contralateral oVEMP (F = 2.58, df = 12, p = .002; See Figure 9), indicating that the magnitude of change in oVEMP amplitude as a function of frequency was dependent on age group. Simple main effects analysis showed that at the greatest stimulus intensity (i.e. 127 dB pSPL) oVEMP amplitude was significantly affected by frequency in all Age Groups (young adult: p < .001; middle age: p < .001; old adult: p = .005). At 122 dB pSPL the oVEMP amplitude was significantly more effected by frequency in the two younger age groups (p < .01) with no significant differences between frequencies in the old adult group (p = .902). At 117 dB pSPL, the oVEMP amplitude was significantly affected by frequency only in the young adult group (p = .006) with no significant differences between frequencies in middle age group (p = .187) or old adult group (p = 1.0).

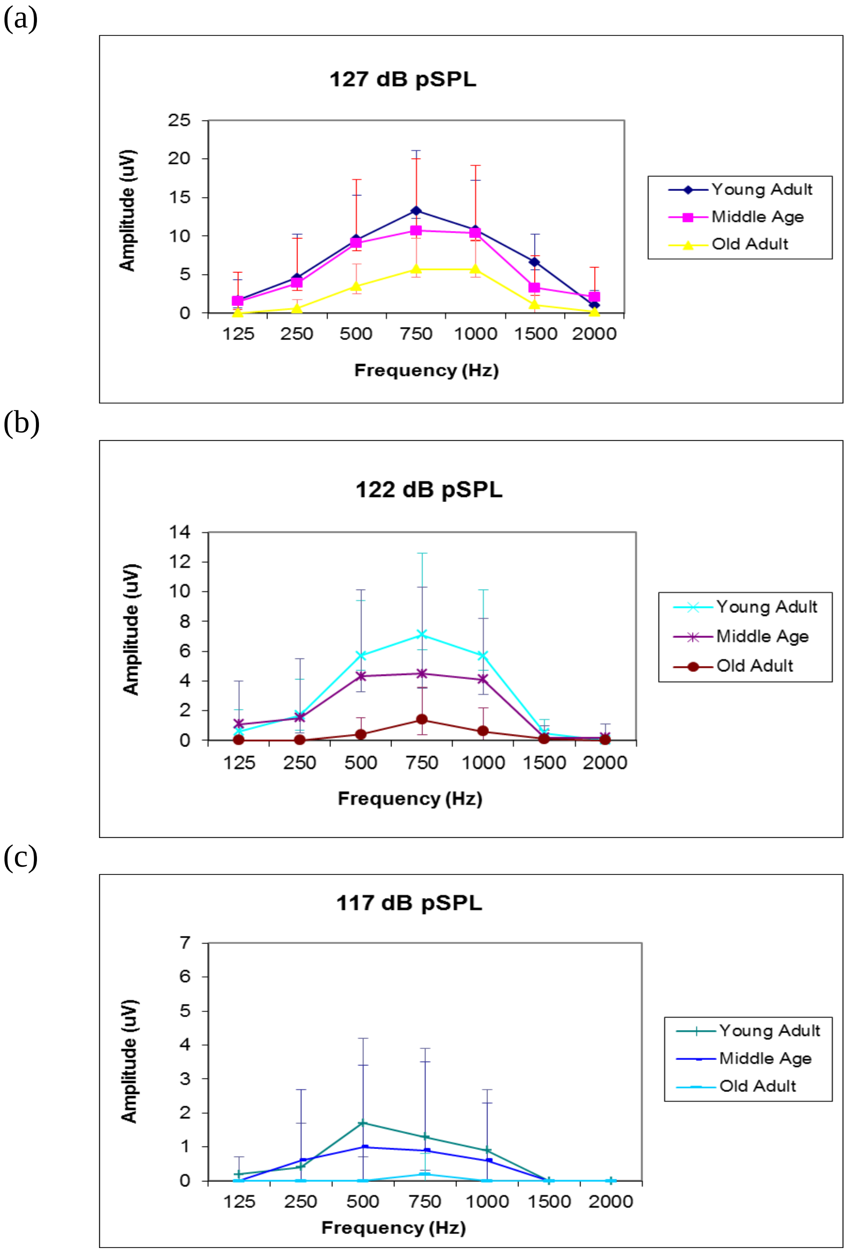

Figure 9: The peak-to-peak amplitude of the contralateral oVEMP as a function of stimulus frequency for each age group. (a) The interaction effect of Age group x Frequency at stimulus intensity level of 127 dB pSPL. (b) The interaction effect of Age group x Frequency at stimulus intensity level of 122 dB pSPL. (c) The interaction effect of Age group x Frequency at stimulus intensity level of 117 dB pSPL

In contrast to the cVEMP, at the highest intensity levels we observed similar frequency tuning for all 3 age groups. The low oVEMP response rate observed often in the older adults at the lesser intensity levels (see Table 3) may have contributed to the Age x Frequency interaction.

Frequency X Level

There was a significant interaction between the effects of Frequency and Level on the amplitude of the oVEMP (F = 8.85, df = 12, $p < .001$; see Figure 10), indicating that the change in oVEMP amplitude as a function of frequency was dependent on stimulus level.

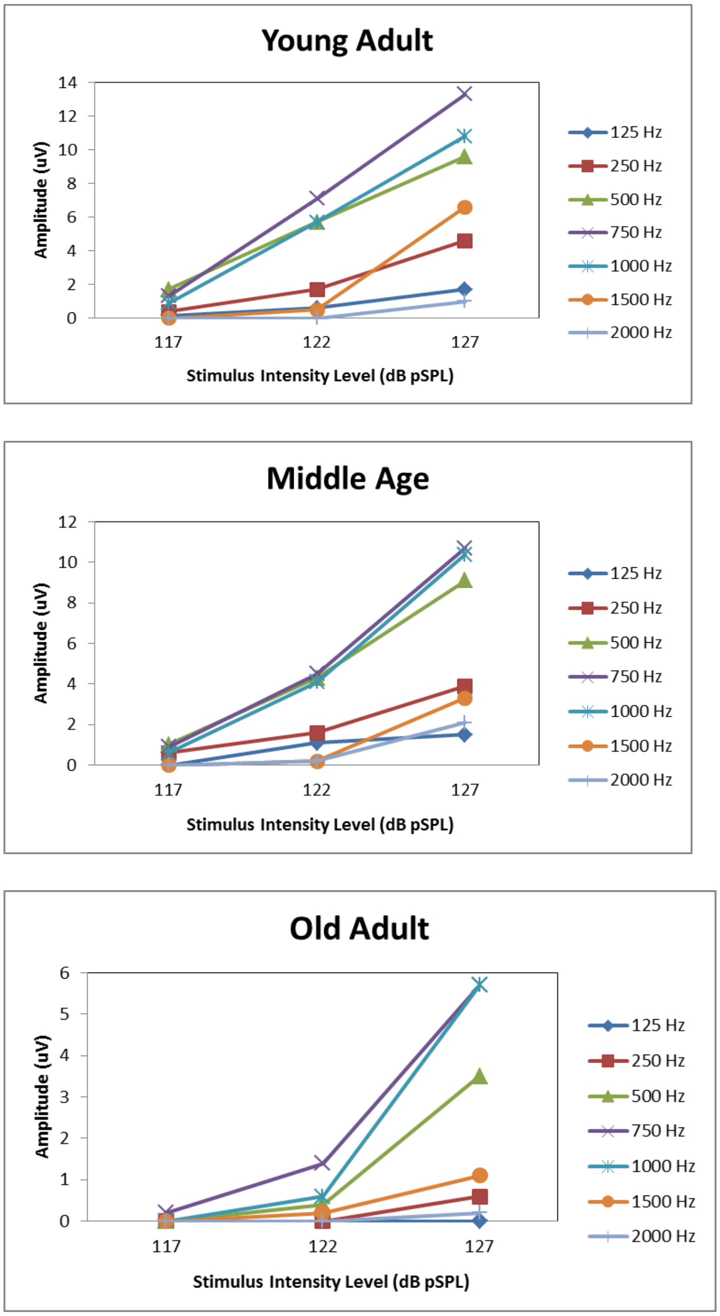

Figure 10: The peak-to-peak amplitude of the contralateral oVEMP as a function of stimulus level at each stimulus frequency. (a) The interaction effect of Frequency x Level in the young adult group. (b) The interaction effect of Frequency x Level in the middle age group. (c) The interaction effect of Frequency x Level in the old adult group

Simple main effects analysis showed that contralateral oVEMP amplitude was significantly more affected by frequency at 127 dB pSPL ($p < .01$), for all age groups, and at 122 dB pSPL for the 2 younger age groups (young adult: p < .01; middle age: p = .034). There were no significant differences between frequencies at 117 dB pSPL (young adult: p = .87; middle age: p = .993; old adult: p = 1.0). The interaction effect seen at the lowest stimulus intensity level (i.e. 117 dB pSPL) may be due to the low response rates at that stimulus level (see Table 3). In other words, at least for the 2 younger age groups, at the highest intensity levels there is no difference in frequency tuning between levels. At the lowest stimulus intensity level there may be a loss of frequency tuning, but more than likely there is no tuning because we were below oVEMP threshold for a many subjects. As shown in Figure 10, there were greater differences in the amplitude of the oVEMP across frequencies at 127 and 122 dB pSPL compared to 117 dB pSPL.

Age Group X Level

The Age Group x Level interaction was significant (F = 7.16, df = 4, p < .001), indicating that the change in oVEMP amplitude as a function of stimulus level was dependent on age group. Simple main effects analysis showed that oVEMP amplitude was significantly affected by stimulus intensity level for all age groups (p < .001). Figure 11 displays the I/O function.

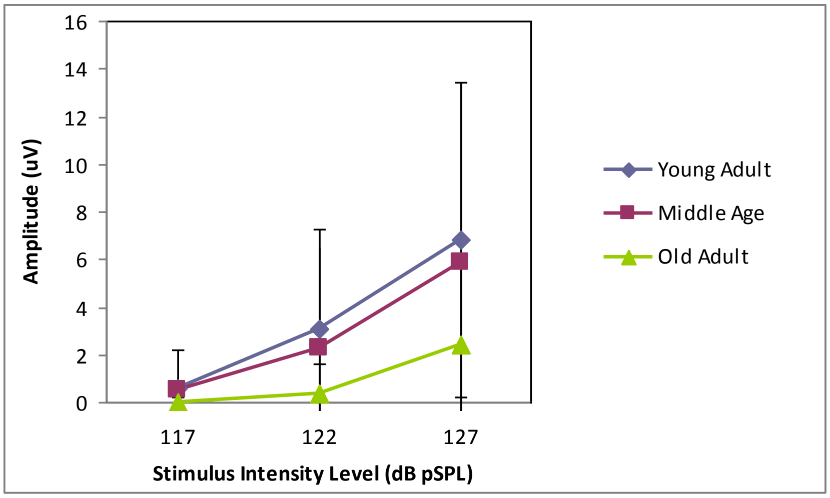

Figure 11: The input/output (I/O) function for the contralateral oVEMP shown for each age group

The slope of the I/O function was steeper for the young adult and middle age compared to the old adult group. The oVEMP amplitude grew 2.7 - 3.1 uV per 5 dB increase in stimulus intensity level for the young adult and middle age groups. The oVEMP amplitude only grew an average and 1.2 uV per 5 dB increase in stimulus intensity level for the old adult group. In other words, there was less of an increase in amplitude with increasing stimulus level for the old adult group compared to the other two groups.

Contralateral oVEMP Frequency “Tuning”

The frequency resulting in the largest response amplitude (i.e. “best” frequency) for each individual subject is shown in Table 4. The best frequency tended to increase with increasing age. For example 92% of subjects in the young adult age group showed greatest amplitudes at either 500 or 750 Hz with only a single subject showing the best amplitude at 1000 Hz. In contrast to this, no one in the old adult age group had the best amplitude at 500 Hz. However, 38% of subjects in the old adult group showed the greatest amplitude at 750 Hz and 62% at 1000 Hz. The best frequency for the middle age group was 31%, 38%, and 31% for 500, 750, and 1000 Hz, respectively.

Table 4. Frequencies with the greatest contralateral oVEMP peak-to-peak amplitude (i.e. “best” frequency), with the corresponding amplitude, from each individual subject. The mean peak amplitude for each age group and for the entire cohort is also shown. Stimulus intensity level was 127 dB pSPL

Subject	**Age Group**	**Best Frequency (Hz)**	**Peak Amplitude (μV)**
1	1	500	16.1
2	1	500	10.1
3	1	500	5.8
4	1	750	14.4
5	1	750	11.4
6	1	750	24.4
7	1	750	8.7
8	1	750	23.9
9	1	750	20.6
10	1	750	9.7
11	1	750	21
12	1	750	4.2
13	1	1000	21.8
Age Group 1 Average			**14.77 ± 7.0**
14	2	500	12.8
15	2	500	20.8
16	2	500	9.1
17	2	500	16.6
18	2	750	37.7
19	2	750	3.7
20	2	750	6.2
21	2	750	10.6
22	2	750	5.1
23	2	1000	9.3
24	2	1000	8.4
25	2	1000	11.7
26	2	1000	11.2
Age Group 2 Average			**12.55 ± 8.84**
27	3	750	7.1
28	3	750	10.7
29	3	750	1.9
30	3	750	4.1

31	3	750	12.4
32	3	1000	14.8
33	3	1000	5.5
34	3	1000	3.4
35	3	1000	3.6
36	3	1000	8.7
37	3	1000	9.3
38	3	1000	4.4
39	3	1000	5.7
Age Group 3 Average			**7.05 ± 3.89**
Total Average			**11.46 ± 7.47**

To qualitatively examine the frequency tuning of the contralateral oVEMP, frequency tuning curves were constructed by plotting the peak-to-peak oVEMP amplitude as a function of stimulus frequency for each subject. Just as with the cVEMPs, tuning curves were graphed based on normalized amplitudes (i.e. normalized on a scale from 0 – 1.0 with respect to the peak amplitude of the best frequency for that individual subject). The tuning curves for individual subjects are show in Figure 12.

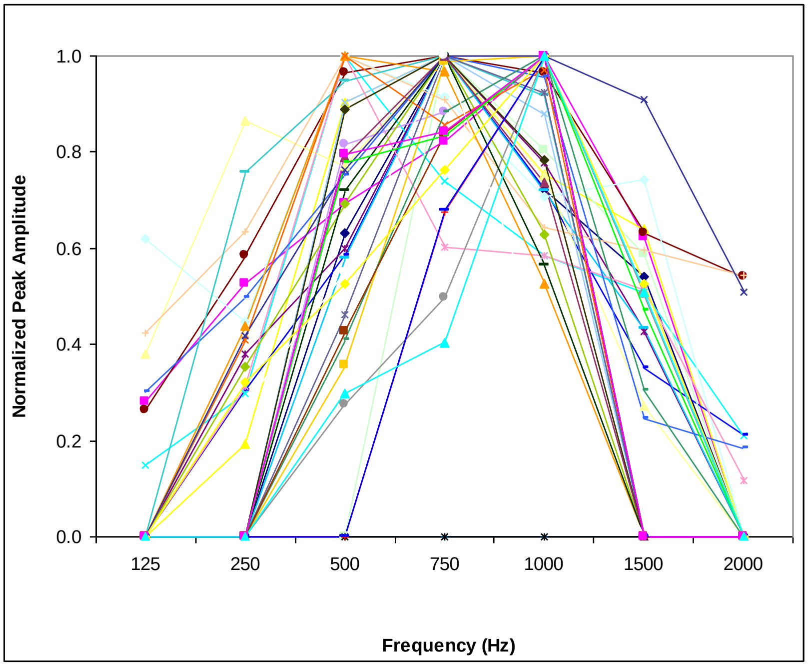

Figure 12: Contralateral oVEMP frequency tuning curves from all 39 subjects based on normalized peak amplitudes. The stimulus level was 127 dB pSPL

The frequency tuning of the contralateral oVEMP (Figure 12) is qualitatively sharper than that of the cVEMP (Figure 6). As Figure 12 shows, the frequency tuning curves peaked between 500 and 1000 Hz and amplitude values were reduced significantly at the lower and higher frequencies.

Frequency tuning curves based on the mean normalized peak amplitudes from each age group are show in Figure 13. Figure 13(b) highlights the youngest and oldest age groups. In the young adult group, the peak of the tuning curve is at 750 Hz, whereas in the old adult group the tuning curve peaks at 1000 Hz. As with the cVEMP, the best frequency shifted to a slightly higher frequency in the oldest age group.

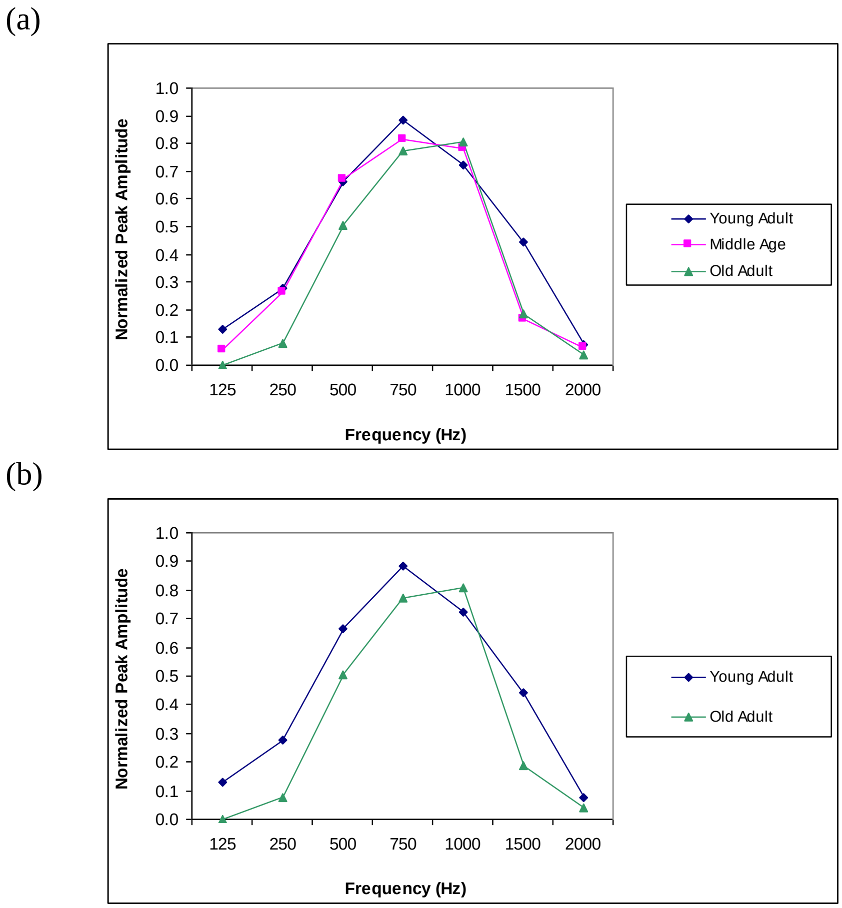

Figure 13: Contralateral oVEMP frequency tuning curves from mean normalized peak amplitudes at 127 dB pSPL. (a) Tuning curves from all 3 age groups. (b) Tuning curves from the young adult and old adult groups only

Ipsilateral oVEMP

Ipsilateral oVEMP Descriptives

The response rate of the ipsilateral oVEMP was markedly reduced compared to the contralateral oVEMP. At 127 dB pSPL, 21/39 (54%) of subjects generated an oVEMP response beneath the ipsilateral eye for at least one frequency. Of those 21, 4 (19%) showed the greatest amplitude at 500 Hz, 12 (57%) showed the greatest amplitude at 750 Hz, and 5 (24%) showed the greatest amplitude at 1000 Hz. Only 12/39 subjects had an ipsilateral oVEMP response at 122 dB pSPL, and only 1/39 had a response at 117 dB pSPL. Table 5 shows the response frequency of occurrence for each age group across stimulus frequency at a stimulus level of 127 dB pSPL. The ipsilateral oVEMP response rate was highest at 500, 750 and 1000 Hz. The response rate tended to decrease as age increased.

Table 5. Ipsilateral oVEMP response rates across stimulus frequencies. Stimulus level was 127 dB pSPL

		125 Hz	**250 Hz**	**500 Hz**	**750 Hz**	**1000 Hz**	**1500 Hz**	**2000 Hz**
127 dB pSPL	Young Adult (n = 13)	0%	31%	69%	77%	62%	31%	0%
	Middle Age (n = 13)	8%	8%	38%	31%	38%	8%	0%
	Old Adult (n = 13)	0%	0%	8%	15%	23%	0%	0%

Ipsilateral oVEMP Amplitude Main Effects

A 3 x 7 univariate ANOVA was conducted examining Age Group x Frequency with ipsilateral oVEMP peak-to-peak amplitude as the dependent variable. Due to the lack of ipsilateral responses at the lower intensity levels, stimulus level was not included in the statistical analysis. Only the data from 127 dB pSPL was analyzed. There were significant main effects for Age Group (F = 4.39, df = 2, p = .013) and Frequency (F = 6.42, df = 6, p < .001). The interaction of Age Group x Frequency was not significant (p = .776).

Post hoc Tukey tests showed that the mean amplitude for the young adult group was significantly larger than both the middle age and old adult groups. The middle age and old adult groups were not significantly different from each other. As with the contralateral oVEMP, responses for 500, 750, and 1000 Hz resulted in significantly larger amplitudes than the other 4 frequencies. No significant differences in mean amplitude were observed between 500, 750, and 1000 Hz. Frequencies 125, 250, 1500, and 2000 Hz were not significantly different from each other.

Ipsilateral oVEMP Frequency Tuning

To qualitatively examine the frequency tuning of the ipsilateral oVEMP, frequency tuning curves were constructed by plotting the peak-to-peak amplitude as a function of stimulus frequency for the 21 subjects with present ipsilateral oVEMP responses. Tuning curves were graphed based on normalized amplitudes. The tuning curves for individual subjects are show in Figure 14.

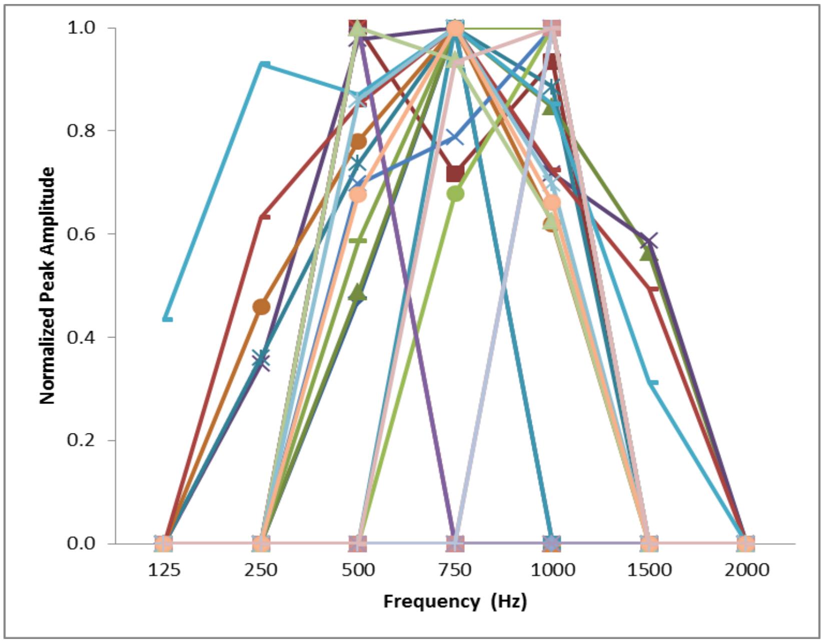

Figure 14: Ipsilateral oVEMP frequency tuning curves based on normalized peak amplitudes at 127 dB pSPL

The frequency tuning of the ipsilateral oVEMP is qualitatively sharper than the contralateral oVEMP. This is most likely a result of the marked decline in response rate for frequencies below 500 Hz and above 1000 Hz (i.e. amplitude = 0 uV for most responses at those frequencies). Frequency tuning curves based on the mean normalized peak amplitudes are shown in Figure 15. The tuning curve peaks at 750 Hz. Given the low response rates, frequency tuning curves between age groups were not compared.

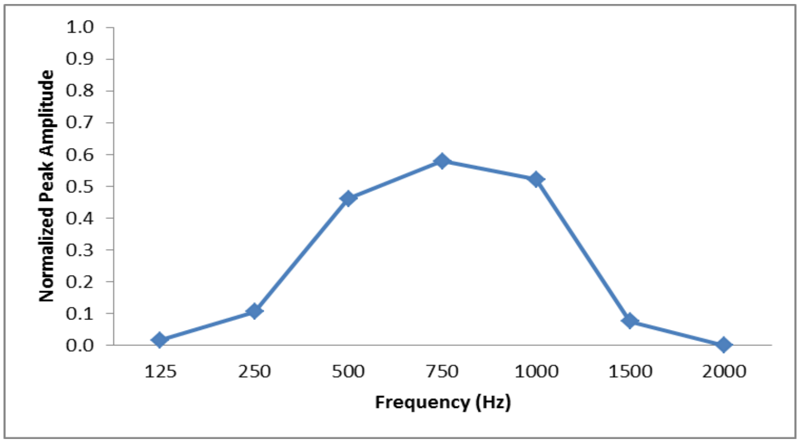

Figure 15: Ipsilateral oVEMP frequency tuning curves from mean normalized peak amplitudes at 127 dB pSPL. Mean data is collapsed across age groups

cVEMP and oVEMP Latency

cVEMP Latency

A 3 x 3 x 7 univariate ANOVA was conducted examining Age Group x Level x Frequency with mean P13 latency of the cVEMP as the dependent variable. The main effects of Age Group (F = 1.25, df = 2, p = .286) and stimulus Level (F = 1.18, df = 2, p = .306) were not significant. The main effect of stimulus Frequency was significant (F = 9.71, df = 6, p < .001). Since there was no main effect of Age Group or Level, data was collapsed across Age Group and Level to show the mean latencies and standard deviations across frequencies. Results are shown in Figure 16.

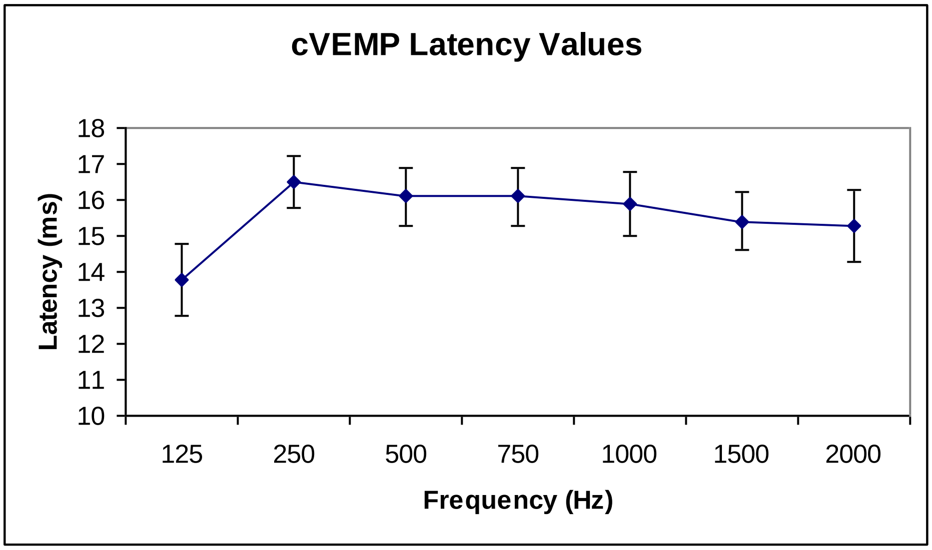

Figure 16: Mean latency of the first positive peak of the cVEMP (P13) as a function of stimulus frequency. Mean data was collapsed across Age Group and Level.

Post hoc Tukey tests showed that the mean latency at 125 Hz (13.8 ms) was significantly shorter than the other 6 frequencies. Additionally, the mean latencies at 250, 500, 750, and 1000 Hz (range: 15.9 – 16.5 ms) were significantly longer than the mean latencies at 1500, and 2000 Hz (15.4 ms and 15.3 ms, respectively). With the exception of 125 Hz, cVEMP latency decreased as the stimulus frequency increased.

oVEMP Latency

The same effect of stimulus frequency on latency was not seen in the oVEMP. The latency of the contralateral oVEMP ranged from 11.6 – 12.3 ms across frequencies. The oVEMP latency values are shown in Figure 17.

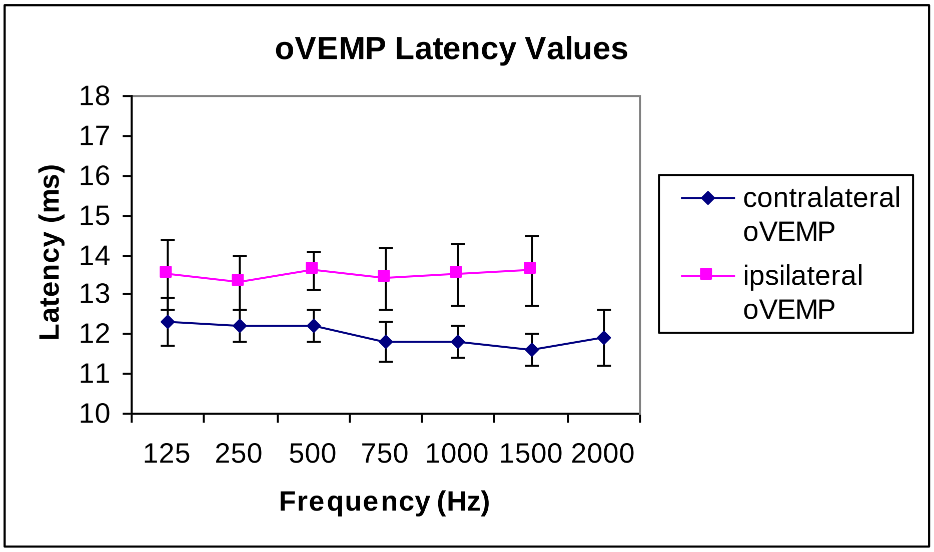

Figure 17: Mean latency of the first negative peak for both the contralateral and ipsilateral oVEMP as a function of frequency. Mean data was collapsed across Age Group and Level for the contralateral oVEMP and across Age Group for the ipsilateral oVEMP

There was no significant main effect for Age Group (F = .231, df = 2, p = .995), stimulus Level (F = 5.42, df = 2, p = .89), or stimulus Frequency (F = 2.63, df = 6, p = .99). The ipsilateral oVEMP data from the 127 dB pSPL stimulus level were also analyzed to assess the effects of age and frequency (due to the low response prevalence, the effects of stimulus level were not analyzed for the ipsilateral oVEMP). The latency of the ipsilateral oVEMP ranged from 13.3 – 13.6 ms across frequencies. There was no significant main effect for Age Group (F = 1.46, df = 2, p = .243) or Frequency (F = 1.33, df = 5, p = .930). No subject produced an ipsilateral oVEMP in response to 2000 Hz. The mean latency of the ipsilateral oVEMP was longer than the contralateral oVEMP, and this was consistent across frequencies (see Figure 17).

Frequency Tuning Test-Retest Reliability

To examine the test-retest reliability of VEMP frequency tuning, cVEMPs and oVEMPs were recorded at 2 stimulus intensities, 127 and 122 dB pSPL , from 5 subjects on two separate occasions averaging 30 days (sd 7 days) apart. Pearson product correlation coefficients were calculated between the test and retest sessions. There was a moderate to strong correlation (i.e. range of r = .5 - .9) between the test and re-test session for each VEMP condition at each frequency. Table 6 shows the mean peak-to-peak amplitude for the test session and the re-test session.

Table 6. Mean (SD) peak-to-peak amplitude of the oVEMP and cVEMP at 127 and 122 dB pSPL during the test and re-test sessions of 5 subjects ranging in age from 25 – 50 years. The greatest amplitude for each VEMP condition is in **bold**

VEMP Condition	**Test Session**							**Re-test Session**						
	125 Hz	250 Hz	500 Hz	750 Hz	1000 Hz	1500 HZ	2000 Hz	125 Hz	250 Hz	500 Hz	750 Hz	1000 Hz	1500 HZ	2000 Hz
oVEMP 127 dB	0.9 (1.3)	6.4 (4.7)	11.9 (4.6)	**12.4 (6.2)**	12.1 (6.8)	6.4 (5.7)	2.8 (5.3)	0.4 (.9)	4.4 (5.0)	9.72 (3.8)	**12.3 (7.7)**	10.5 (6.0)	6.04 (4.5)	2.42 (5.4)
oVEMP 122 dB	0 (0)	1.6 (2.1)	5.6 (3.9)	5.3 (3.9)	**5.7 (4.3)**	1.5 (2.2)	0 (0)	0 (0)	1.3 (1.8)	4.6 (2.2)	5.7 (3.1)	**5.9 (3.2)**	1.8 (1.9)	0 (0)
cVEMP 127 dB	119.8 (101.6)	192.2 (91.7)	247.1 (83.9)	**254.6 (89.5)**	226.5 (150)	180.2 (61.1)	27.6 (61.8)	95.1 (79.6)	139.6 (53.4)	224.4 (76.8)	**238.8 (96.7)**	196 (79.7)	179.9 (60.3)	22.9 (51.3)
cVEMP 122 dB	0 (0)	43.8 (64.8)	**162.2 (70.6)**	160.4 (75.2)	150 (104.7)	52.3 (77.6)	0 (0)	0 (0)	37.9 (52.7)	159.8 (67.7)	**180.5 (53.6)**	153.7 (62.6)	73.3 (75.3)	0 (0)

The “best” frequency (highlighted in **bold** in Table 6) was always 500, 750 or 1000 Hz and was consistent between the test and re-test sessions, with the exception of the cVEMP at 122 dB pSPL where the “best” frequency changed from 500 Hz during the test session to 750 Hz during the re-test session. Overall, frequency tuning was found to be reliable across time.

Frequency Tuning Inter-Ear Differences

The possibility of a difference in frequency tuning between ears was examined in 5 subjects. In these 5 subjects, cVEMP and oVEMP responses were recorded at 2 stimulus intensities, 127 and 122 dB pSPL, in both the right and left ears. The interaural amplitude asymmetry (IAA) values between the right and left ears are shown in Table 7.

Table 7. Mean (SD) interaural amplitude asymmetries (IAA). Values are given as a percentage (%). In conditions where the mean is based on a single subject no SD is given

VEMP Condition	125 Hz	250 Hz	500 Hz	750 Hz	1000 Hz	1500 HZ	2000 Hz
oVEMP 127 dB	18 (14)	15 (05)	13 (08)	17 (.09)	21 (06)	13 .14)	27
oVEMP 122 dB	0	17 (21)	15 (07)	21 (09)	20 (09)	20	0
cVEMP 127 dB	08 (08)	8 (09)	9 (07)	13 (08)	8 (07)	13 (18)	22
cVEMP 122 dB	0	10 (09)	15 (08)	13 (07)	12 (09)	9	0

For the 3 best frequencies (500, 750, and 1000 Hz), the upper limit of IAA (i.e. mean + 2 SD) ranged from 29% – 39% for the oVEMP and from 22 – 31% for the cVEMP. VEMP responses were symmetrical (i.e. these IAA values are consistent with those reported previously in the literature; e.g. (Zapala and Brey 2004; Chihara, Iwasaki et al. 2007). Table 8 shows the mean peak-to-peak amplitude from each stimulus frequency for the right and left ears in each VEMP condition. The mean data show that although the frequencies resulting in the greatest VEMP amplitudes for both ears were always 500, 750, and 1000 Hz, the “best” frequency (highlighted in **bold** in Table 8) was often different between ears.

Table 8. Mean (SD) peak-to-peak amplitude of the oVEMP and cVEMP at 127 and 122 dB pSPL from the right and left ears of 5 subjects ranging in age from 25 – 50 years. The greatest amplitude for each VEMP condition is in **bold.**

VEMP Condition	**Right Ear**							**Left Ear**						
	125 Hz	250 Hz	500 Hz	750 Hz	1000 Hz	1500 HZ	2000 Hz	125 Hz	250 Hz	500 Hz	750 Hz	1000 Hz	1500 HZ	2000 Hz
oVEMP 127 dB	0.5 (1.2)	6.3 (4.6)	12 (4.6)	**12.4 (6.3)**	12 (6.8)	6.3 (5.6)	2.9 (5.3)	0.2 (.5)	7.9 (6.5)	**11.6 (6.5)**	11 (4.4)	10.5 (4.3)	6.2 (4.8)	1.4 (3.1)
oVEMP 122 dB	0 (0)	1.6 (2.1)	5.6 (3.9)	5.3 (3.9)	**5.7 (4.3)**	1.5 (2.2)	0 (0)	0 (0)	2.3 (3.3)	**6.3 (4.3)**	5.6 (3.9)	4.9 (1.8)	1.1 (1.5)	0 (0)
cVEMP 127 dB	95.1 (79.6)	139.6 (53.4)	224.4 (76.8)	**238.8 (96.7)**	196 (79.7)	179.9 (60.3)	22.9 (51.3)	81.1 (54.9)	142.5 (75.1)	**236.2 (101.5)**	224.1 (130.8)	208 (109.6)	149.1 (85.3)	40.3 (58.4)
cVEMP 122 dB	0 (0)	37.9 (52.7)	159.8 (67.7)	**180.5 (53.6)**	153.7 (62.6)	73.3 (75.3)	0 (0)	0 (0)	30.8 (42.2)	167.3 (64.8)	**195.8 (117.5)**	184.2 (115.2)	86.2 (98.6)	0 (0)

Middle Ear Power Analysis (MEPA)

Qualitatively, middle ear power reflectance varied as a function of frequency. Higher power reflectance values were observed below 1000 Hz and above 5000 Hz. At the lower and higher frequencies a larger proportion of the incident power was reflected back out through the ear canal. Overall results were a U-shaped curve that approximates the middle ear transfer function (see Figure 18).

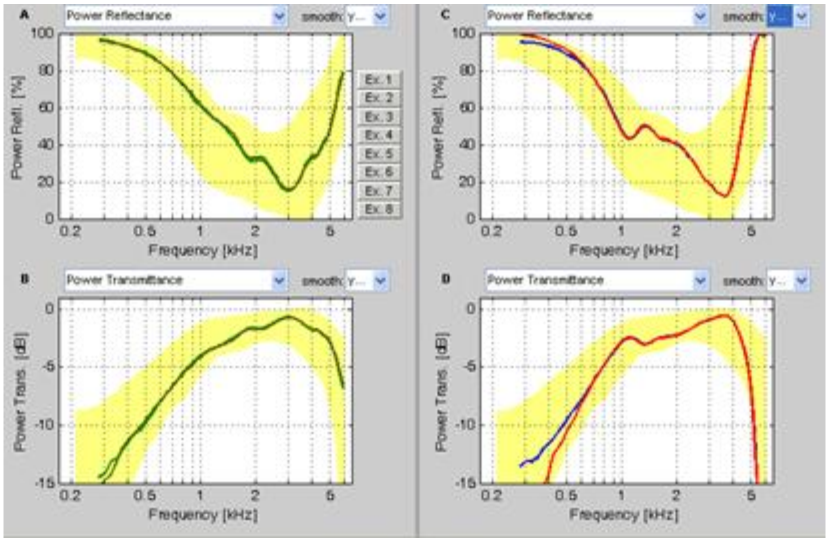

Figure 18: Screenshot of the frequency-reflectance profile created by the MEPA3 from an individual subject. The upper panel shows power reflectance (%) as a function of frequency, for the left and right ear separately. The lower power shows the power transmittance (dB) as a function of frequency. The shaded region represents the normative values provided by the manufacturer

These results were similar to previous investigations examining middle ear power reflectance in adults without middle ear pathology (e.g. (Feeney and Sanford 2004; Allen, Jeng et al. 2005) and were consistent across age groups. In other words, the tendency for the best VEMP frequency in older adults to be at a higher frequency was independent of the middle ear transfer function.

CHAPTER IV

DISCUSSION

Frequency Tuning of the VEMP

The purpose of this investigation was to characterize the frequency tuning of the cVEMP and oVEMP and to examine the effects of age on the tuning of the response. Previous studies that have used air conduction tone bursts have reported frequency specific tuning in both the cVEMP and oVEMP of young normal adults centered around 500 Hz. Our findings slightly disagree with this. Although there is an effect of stimulus frequency on the response rate and VEMP amplitude, this effect is not very frequency specific. In fact, air conduction tone bursts of 500, 750, and 1000 Hz all produced repeatable VEMP responses at amplitudes that did not significantly differ from one another. Both response rate and amplitude decreased at frequencies above and below these mid frequencies. One reason for the discrepancy in our findings compared to others (i.e. we did not find specific frequency tuning) may be due to our stimulus. First, we used seven different frequencies to measure the frequency dynamics of the cVEMP and oVEMP. Second, we developed a stimulus consisting of all 7 different tone burst frequencies. The amplitude of the response depends on muscle tone (at least for the cVEMP) and repeated examination may cause muscular fatigue (especially in aged individuals). Thus, we reasoned that the best way to compare multiple frequencies would be to present them all during the same run. In doing this we showed that VEMP amplitude and response rates for 500, 750, and 1000 Hz tone bursts were not different from one another. Further, the “best” frequency (i.e. frequency resulting in the largest response amplitude) was always 500, 750, or 1000 Hz, but the best frequency often differed between ears. Given that there was no single “best” stimulus frequency but instead a range of best frequencies, it may be a misnomer to describe the VEMP as being “tuned.” Additionally, though it is clear why the auditory system requires fine tuning, it is not clear what benefit fine tuning would provide the vestibular system.

The response to sound from vestibular neurons is very different than that of cochlear neurons. Cochlear neurons respond to a broader frequency range and at much lower thresholds (i.e. softer intensity levels) compared to the vestibular neurons, which respond to a very restricted range of frequencies at greater thresholds. Single 8th nerve fibers demonstrate very fine frequency tuning, which is vital to the function of our auditory system. The basilar membrane (BM) varies in thickness and elasticity with the base being relatively narrow and stiff allowing it to respond best to high frequencies, while at the apex the BM is wider and more flexible and responds best to low frequencies. Each point on the BM shows greater displacement to a certain frequency, called its characteristic frequency (CF). While the cochlea has both passive (i.e. BM) and active (i.e. outer hair cells) processes contributing to its fine frequency tuning, the vestibular system’s frequency “tuning” is a by-product of the system’s resonant frequency.

The frequency tuning of the otolith end organs has been attributed to the elastic and inertial properties of the end organs, the mechanical resonance of individual stereocilia, and/or the electrical tuning of the hair cells (Fernandez and Goldberg 1976; Young, Fernandez et al. 1977; Crawford and Fettiplace 1981; Holton and Hudspeth 1983;

Fettiplace and Fuchs 1999; Welgampola and Colebatch 2001). The physical mass of the saccule and utricle and the way they are attached to the temporal bone contribute to their inertial properties and passive resonance. In other words, a smaller, and more rigidly attached (i.e. stiffer) end organ should resonate at a higher frequency than a larger and more mobile end organ. Stereocilia also behave as mechanical resonators with the resonant frequency being a function of the stereocilia's stiffness and mass. Thus shorter and more stiff stereocilia should resonate at a higher frequency (Fettiplace and Fuchs 1999). The electrical tuning of a hair cell is determined by the number of potassium (K^+) channels (Fettiplace and Fuchs 1999; Steinacker 2004). The cell's resonant frequency is correlated with the number of K^+ channels, with more channels resulting in a higher resonant frequency (Fettiplace and Fuchs 1999; Steinacker 2004). This would enable a stimulus with a frequency corresponding to the resonant frequency of the end organ, stereocilia, and/or hair cells to produce the largest signal in the cell. Thus, the term "resonant frequency" may be a more accurate description than "frequency tuning" when describing the effects of stimulus frequency on the vestibular system.

Effects of Age on the Resonant Frequency of the VEMP

Consistent with the findings of previous investigators, we observed an effect of age on the amplitude of both the cVEMP and oVEMP. The tonic EMG of the SCM was held relatively constant across subjects (i.e. between 50 - 200 µV), but VEMP amplitude still significantly decreased with increasing age. The effect of age was more pronounced for the cVEMP where amplitudes from both the middle age and older adult groups were significantly smaller compared to the young adult group, whereas for the oVEMP amplitudes were significantly smaller in the older adult group only. What was more interesting, however, was the Age Group x Frequency interaction effect. cVEMP amplitude was more affected by frequency in the young adult and middle age group, with no significant differences between frequencies in the older adult group. These results suggest a “flattening” or loss of frequency tuning with no best frequency in the older adult group. In contrast, oVEMP amplitude was affected by frequency in all age groups at least at the greatest stimulus intensity level.

We hypothesized that damage to the saccule or utricle, as a result of aging, would alter the resonant frequency of the end organ thus altering the frequency tuning characteristics of the vestibular system. In the auditory system, the BM is sharply tuned in young healthy subjects. The better the physiological condition of the cochlea, the sharper the tuning (Moore 1998). We posited that the better the physiological condition of the saccule and utricle, the sharper the vestibular frequency “tuning”. Our results showed an Age Group x Frequency interaction for the cVEMP with a loss of best frequency for adults 60 years of age and older. This interaction was not observed for the oVEMP. However, older adults showed a slightly higher “best” frequency compared to young adults for both the cVEMP and oVEMP. Thus, aging does appear to affect the frequency tuning of the VEMP and the effect is greater on the cVEMP than the oVEMP.

Significant hair cell and neuronal loss with increasing age has been reported for the saccule, with lesser effects for the utricle. Johnsson (1971) examined human temporal bones from 150 patients ranging in age from newborns to 97 years and observed a marked degeneration in the saccular macula and with only a moderate degeneration in the utricular macula after the age of 60 years (Johnsson 1971). Richter (1980) observed a decrease in the density of hair cells in the sensory epithelium of the saccule of individuals over the age of 50 years, with relatively little decrease in the utricle (Richter 1980). In a series of case studies, Schuknecht et al (1965) observed extensive age-related degeneration with a 50% loss of hair cells in the macula of the saccule of both animal and human temporal bones (Schuknecht 1965). As with Johnson (1971) and Richter (1980), the utricle appeared relatively undamaged. Merchant et al (2000) reported a highly significant age-related decline in the density of Type I and Type II hair cells in all the peripheral vestibular end organs, including both the saccule and utricle (Merchant, Velazquez-Villasenor et al. 2000). Rosenhall (1973) also found age-related affects in both otolith end organs with a 24% decrease in the hair cell density of the saccule and a 21% decrease in the utricle (Rosenhall 1973).

The volume and number of otoconia in the macula of both the utricle and saccule in elderly individuals is also reduced compared to younger adults. Again, the effects of age appear to be greater for the saccule. Igarashi et al (1993) examined temporal bones in both children and elderly adults and reported a ratio of utricular otoconia volume between young and elderly at 100:42 and a ratio of 100:21 for saccular otoconia (i.e. greater age-related loss of saccular otoconia with age; (Igarashi, Saito et al. 1993). Ross et al (1976) examined the otoconia from the saccule and utricle across varying ages and reported a decrease in number and a “hollowed-out” appearance in the saccular otoconia of older subjects. These findings were less pronounced in the utricular otoconia of older subjects (Ross, Peacor et al. 1976). In addition to the reduction of otoconia with age, distinct changes in the shape of otoconia have been shown in aged individuals (Johnsson and Hawkins 1972; Igarashi, Saito et al. 1993; Jang, Hwang et al. 2006). “Giant” otoconia have been observed in the otolith organs of older rats, presumably due to a loss of controlled inhibition of mineralization (Jang, Hwang et al. 2006). These changes in the shape and number of otoconia in the otoliths may result in a smaller otolith mass. Lower mass results in less force translating the kinocilia so we would expect poorer threshold levels. Additionally, a change in otolith mass may alter the resonant frequency of the end organ, thereby altering the frequency tuning characteristics of the VEMPs of the older subjects in this study.

If the air conduction cVEMP and oVEMP originate from two different end organs (i.e. saccule and utricle, respectively) we may expect to see some differences in the frequency tuning of the two VEMP responses. The utricle is larger (i.e. larger in mass) and less anchored (i.e. less rigid) to the temporal bone than is the saccule. Thus, if it is the elastic and inertial properties of the end organ alone that contribute to the frequency tuning of the VEMP we would expect a lower best frequency for the oVEMP compared to the cVEMP. The resonant frequency characteristics of the cVEMP and oVEMP in this investigation were very similar and consistent with previous investigations reporting similar frequency tuning for the cVEMP and oVEMP in response to air conduction stimuli (Park, Lee et al. 2010). However, if the frequency tuning of the VEMP is also related to mechanical resonance of individual stereocilia and the electrical tuning of the hair cells then we would expect to see a change in tuning in individuals with this type of end organ damage. We cannot deduce from these findings if the older adults in our study had degenerative changes in the otolith organs. However, the greater effects of age on the cVEMP compared to the oVEMP are consistent with structural investigations showing a greater effect of age on the saccule compared to the utricle.

Other Age Effects

The effects of stimulus level on the amplitude of the VEMP response differed by age group. Previous studies have shown that both cVEMP and oVEMP amplitude increase with increases in stimulus intensity level (e.g. Colebatch, 1994; Ochi et al, 2001; Akin et al 2003; Murnane et al, 2011). Our findings show that this increase is not uniform across age groups as the slope of the I/O function was steeper for the younger age group. Again, this age effect is more pronounced for the cVEMP where the I/O function was shallower for both the middle age and old age groups, whereas this effect was only evident for the old age group in the oVEMP. The finding that amplitude grows more with increasing stimuli for younger subjects may be related to hair cell and neuronal loss reported with increasing age. In a young healthy adult, the consequence of increasing the stimulus intensity level is a greater translation of otoliths, a greater transduction at the hair cell level, and a greater population of neurons fire. The result is a larger amplitude evoked potential. In an older individual where the number of otoliths, hair cells, and neurons has decreased, an increase in stimulus intensity does not have the same effect as there are less available neurons to recruit.

Effects of stimulus Frequency on the Latency of the VEMP

The effects of stimulus frequency on the latency of the VEMP differed between the cVEMP and oVEMP. The cVEMP and oVEMP latency values reported in this investigation were both independent of age group and stimulus level. However, even with stimulus gating (i.e. stimulus rise/fall time) held constant, frequency was found to have an effect on the P1 latency of the cVEMP, but not the N1 latency of the oVEMP. Previous investigators have shown that cVEMPs elicited by AC clicks occur earlier than cVEMPs elicited by mid-frequency tone bursts (~13 ms vs ~15 ms; e.g. Colebatch & Halmagyi, 1992; Akin et al 2003; Janky and Shepard 2009). The mean P1 latency of the cVEMP elicited by the 125 Hz stimulus in this investigation (i.e. mean 13.8 ms) is more consistent with cVEMP latency values elicited by click stimuli. It is possible that our 125 Hz stimulus was more akin to a transient than a low frequency tone burst, resulting in such an early latency cVEMP response. Our findings showed that the mean latencies for 125 Hz (i.e. possibly a click stimulus) were significantly shorter compared to the other 6 stimulus frequencies. These findings were consistent across subjects. For example, 16 subjects produced a cVEMP at 125 Hz and of those 16, 14 showed a latency value 2-3 ms shorter than the other test frequencies.

Akin et al (2003) reported that cVEMP latency, when stimulation duration was held constant, was independent of tone burst frequency; however a closer look at their data showed that this was only true for frequencies 250 – 1000 Hz. The cVEMP latency at 1500 Hz was found to be significantly shorter (Akin, Murnane et al. 2003). Those findings are very consistent with ours showing that the mean latencies for 1500 and 2000

Hz (i.e. 15.4 and 15.3 ms, respectively) were significantly shorter compared to 250 – 1000 Hz (i.e. range: 15.9 – 16.5 ms). The high frequency stimuli of 1500 and 2000 Hz may also have acted more "click-like" compared to the low and mid frequency stimuli. There was no affect of stimulus frequency on cVEMP latency between 250, 500, 750, and 1000 Hz.

In contrast to the cVEMP, the mean latency of the oVEMP did not significantly decrease as frequency increased (i.e. mean N1 latency ranged from 11.6 – 12.3 across frequencies). The frequency of the oVEMP at 125 Hz was also not significantly different from the other frequencies. Eight subjects produced an oVEMP at 125 Hz and of those 8, 7 showed a latency value similar to the other test frequencies. Similarly, Zhang et al (2011) reported no significant effect of frequency on N1 latency values for frequencies 100 – 1200 Hz (Zhang, Govender et al. 2011). Murnane et al (2011) reported no significant differences in N1 latency between 500, 1000, and 2000 Hz and Winters et al (2012) reported no significant differences in oVEMP latency at the frequencies of 250, 500, and 1000 Hz (Murnane, Akin et al. 2011; Winters, Berg et al. 2012). Additionally, Rosengren et al (2011) reported that the first negative peak of the oVEMP in response to AC clicks occurred at approximately the same latency as AC 500 Hz tone bursts (Rosengren, Govender et al. 2011). Overall, these results suggest that the frequency of the acoustic stimulus has more of an effect on cVEMP latency than oVEMP latency.

The reason why stimulus type (AC click vs AC tone burst) and stimulus frequency affect the latency of the cVEMP but not the oVEMP is not understood. Neither VEMP response is cochlear in origin. One possible reason may have to do with the fact that the saccule and utricle attach differently to the temporal bone. Recent studies using high-resolution x-ray microtomography have shown that the saccule is closely attached to a curved bony surface while the utricle is delicately suspended by cells attached to a flexible membrane (Uzun-Coruhlu, Curthoys et al. 2007; Curthoys, Uzun-Coruhlu et al. 2009). Uzun-Coruhlu et al (2007) described the utricular macula as "essentially floating on fluid". Given these morphological differences it can be reasonably hypothesized that an acoustic stimulus would deflect the saccular and utricular macula differently. The utricle is partly affixed and mostly free-floating in endolymph and is thus mass dominated. If the input signal is a click, or noise burst, the system will respond only to the low frequency energy in the signal, which may explain the low response rate in this study for the oVEMP at 125 Hz (i.e. the click-like 125 Hz tone burst contained little low frequency energy). The utricle will respond normally but at a greater amplitude to low and mid frequency tone bursts. The result would be similar oVEMP latencies across stimuli. It may be that the cVEMP occurs at a shorter latency for clicks and high frequency tone bursts (i.e. 1500 and 2000 Hz) because the saccule is firmly attached to the temporal bone allowing the system to be stiffness dominated. Thus, the stiffer saccule is responding to the high frequency energy of a click stimulus.

Although the reasons for the different effects of stimulus frequency on the latency of the cVEMP and oVEMP are speculation, the finding that acoustic stimulation has more of an effect on cVEMP latency than oVEMP latency adds to the growing knowledge that the origins of the cVEMP and oVEMP in response to air conduction differ from one another. Further studies are needed to both confirm these findings and further examine the differences in the effects of stimulus frequency between the latency of the cVEMP and the latency of the oVEMP.

CHAPTER V

CONCLUSION

Whether it is due to the electrical resonance of the hair cells or due to the mass-spring damping properties of the otolith end organs, the frequency tuning of the vestibular system is presumably influenced by the resonance characteristics of the saccule and utricle. The finding that there was no significant difference in VEMP amplitude evoked by 500 Hz, 750 Hz and 1000 Hz tone bursts suggests that there is no true frequency "tuning" in the vestibular system, but in fact a range of best frequencies that may be used to evoke the VEMP response.

Our results suggest that aging had an effect on the resonant frequency characteristics of the saccule and utricle as shown by a change in the frequency tuning of both the cVEMP and oVEMP. Accordingly, for elderly patients 500 Hz may not be the ideal frequency to elicit VEMPs. In fact, for some older subjects in this investigation the VEMP was only present using tone burst stimuli of 750 and 1000 Hz. For this reason in cases where the VEMP response is absent at 500 Hz we recommend using a stimulus frequency of 750 or 1000 Hz.

In summary, we conclude the following: 1) the frequency "tuning" of the cVEMP and oVEMP is very broad and may, or may not, be inappropriately interpreted as evidence of true frequency tuning, 2) the "best" frequency (i.e. frequency resulting in the largest VEMP amplitude) was found to be reliable across time, but often differed between ears, 3) aging had an effect on the frequency tuning curves of the VEMP with results showing either a loss of tuning or tuning to a slightly higher frequency, 4) VEMP amplitude grows more with increasing stimulus intensity for younger subjects compared to older subjects, 5) the aging effects observed in this study (i.e. loss of frequency tuning, shallower I/O functions) were more pronounced for the cVEMP than the oVEMP, and 6) stimulus frequency has an effect of the latency of the cVEMP but not the oVEMP.

APPENDIX

A. CVEMP AMPLITUDE MEANS AND STANDARD DEVIATIONS

Age Group	Level (dB pSPL)	Frequency (Hz)	Mean Amplitude (uV)	SD
Young Adult	127	125	123.60	143.40
		250	238.06	170.52
		500	338.05	206.19
		750	355.82	202.83
		1000	297.14	188.38
		1500	271.27	170.47
		2000	96.39	151.38
	122	125	20.65	50.45
		250	91.86	103.56
		500	236.37	161.19
		750	245.58	146.53
		1000	233.76	178.37
		1500	144.75	128.65
		2000	57.38	131.47
	117	125	13.40	46.44
		250	61.05	80.94
		500	132.98	148.16
		750	145.25	131.23
		1000	96.93	99.54
		1500	44.97	86.71
		2000	.00	.00
Middle Age	127	125	44.87	76.81
		250	120.80	82.11
		500	187.00	74.46
		750	218.32	87.75
		1000	226.08	121.29
		1500	129.23	92.00
		2000	41.04	61.76

	122	125	11.57	41.74
		250	58.06	73.21
		500	126.04	86.34
		750	145.86	84.80
		1000	143.54	90.45
		1500	53.28	61.08
		2000	7.03	25.37
	117	125	.00	.00
		250	22.38	67.86
		500	65.29	71.75
		750	65.56	80.95
		1000	65.75	65.43
		1500	.00	.00
		2000	.00	.00
Old Adult	127	125	21.66	46.85
		250	35.99	57.22
		500	100.87	86.85
		750	126.70	87.27
		1000	114.81	68.88
		1500	54.53	75.85
		2000	35.28	72.40
	122	125	.00	.00
		250	2.89	10.42
		500	44.32	59.83
		750	53.18	66.95
		1000	42.23	45.80
		1500	16.31	33.43
		2000	2.99	10.78
	117	125	.00	.00
		250	.00	.00
		500	6.20	15.61
		750	9.99	19.69
		1000	9.54	19.27
		1500	1.84	6.65
		2000	.00	.00

B. CONTRALATERAL OVEMP MEANS AND STANDARD DEVIATIONS

Age Group	Level (dB pSPL)	Frequency (Hz)	Mean Amplitude (uV)	SD
Young Adult	127	125	1.72	2.68
		250	4.69	5.61
		500	9.63	5.77
		750	13.33	7.82
		1000	10.85	6.48
		1500	6.60	3.78
		2000	1.00	1.94
	122	125	.63	1.49
		250	1.71	2.41
		500	5.70	3.72
		750	7.13	5.58
		1000	5.71	4.48
		1500	.50	.92
		2000	.00	.00
	117	125	.15	.51
		250	.38	1.32
		500	1.74	2.51
		750	1.32	2.59
		1000	.93	1.85
		1500	.00	.00
		2000	.00	.00
Middle Age	127	125	1.54	3.80
		250	3.97	5.83
		500	9.13	8.29
		750	10.76	9.31
		1000	10.42	8.86
		1500	3.37	4.29
		2000	2.16	3.96
	122	125	1.10	2.90
		250	1.59	4.05
		500	4.30	5.89

		750	4.54	5.89
		1000	4.14	4.11
		1500	.22	.80
		2000	.25	.91
	117	125	.00	.00
		250	.59	2.13
		500	1.00	2.45
		750	.97	2.63
		1000	.66	1.68
		1500	.00	.00
		2000	.00	.00
Old Adult	127	125	.00	.00
		250	.66	1.29
		500	3.56	2.94
		750	5.71	4.08
		1000	5.70	3.85
		1500	1.11	1.91
		2000	.21	.77
	122	125	.00	.00
		250	.00	.00
		500	.43	1.10
		750	1.39	2.23
		1000	.63	1.61
		1500	.19	.69
		2000	.00	.00
	117	125	.00	.00
		250	.00	.00
		500	.00	.00
		750	.16	.61
		1000	.00	.00
		1500	.00	.00
		2000	.00	.00

REFERENCES

Akin, F. W., O. D. Murnane, et al. (2003). "The effects of click and tone-burst stimulus parameters on the vestibular evoked myogenic potential (VEMP)." J Am Acad Audiol **14**(9): 500-509; quiz 534-505.

Allen, J. B., P. S. Jeng, et al. (2005). "Evaluation of human middle ear function via an acoustic power assessment." J Rehabil Res Dev **42**(4 Suppl 2): 63-78.

Basta, D., I. Todt, et al. (2007). "Characterization of age-related changes in vestibular evoked myogenic potentials." J Vestib Res **17**(2-3): 93-98.

Brantberg, K., K. Granath, et al. (2007). "Age-related changes in vestibular evoked myogenic potentials." Audiol Neurootol **12**(4): 247-253.

Chihara, Y., S. Iwasaki, et al. (2009). "Frequency tuning properties of ocular vestibular evoked myogenic potentials." Neuroreport **20**(16): 1491-1495.

Chihara, Y., S. Iwasaki, et al. (2007). "Vestibular-evoked extraocular potentials by air-conducted sound: another clinical test for vestibular function." Clin Neurophysiol **118**(12): 2745-2751.

Colebatch, J. G. and G. M. Halmagyi (1992). "Vestibular evoked potentials in human neck muscles before and after unilateral vestibular deafferentation." Neurology **42**(8): 1635-1636.

Colebatch, J. G., G. M. Halmagyi, et al. (1994). "Myogenic potentials generated by a click-evoked vestibulocollic reflex." J Neurol Neurosurg Psychiatry **57**(2): 190-197.

Crawford, A. C. and R. Fettiplace (1981). "An electrical tuning mechanism in turtle cochlear hair cells." J Physiol **312**: 377-412.

Curthoys, I. S. (2010). "A critical review of the neurophysiological evidence underlying clinical vestibular testing using sound, vibration and galvanic stimuli." Clin Neurophysiol **121**(2): 132-144.

Curthoys, I. S., H. Uzun-Coruhlu, et al. (2009). "The configuration and attachment of the utricular and saccular maculae to the temporal bone. New evidence from microtomography-CT studies of the membranous labyrinth." Ann N Y Acad Sci **1164**: 13-18.

Curthoys, I. S. and V. Vulovic (2010). "Vestibular primary afferent responses to sound and vibration in the guinea pig." Exp Brain Res.

Donnellan, K., W. Wei, et al. (2010). "Frequency tuning of bone-conducted tone burst-evoked myogenic potentials recorded from extraocular muscles (BOVEMP) in normal human subjects." Laryngoscope **120**(12): 2555-2560.

Feeney, M. P. and C. A. Sanford (2004). "Age effects in the human middle ear: wideband acoustical measures." J Acoust Soc Am **116**(6): 3546-3558.

Fernandez, C. and J. M. Goldberg (1976). "Physiology of peripheral neurons innervating otolith organs of the squirrel monkey. III. Response dynamics." J Neurophysiol **39**(5): 996-1008.

Fettiplace, R. and P. A. Fuchs (1999). "Mechanisms of hair cell tuning." Annu Rev Physiol **61**: 809-834.

Fraysse, B. G., A. Alonso, et al. (1980). "Meniere's disease and endolymphatic hydrops: clinical-histopathological correlations." Ann Otol Rhinol Laryngol Suppl **89**(6 Pt 3): 2-22.

Govender, S., S. M. Rosengren, et al. (2011). "Vestibular neuritis has selective effects on air- and bone-conducted cervical and ocular vestibular evoked myogenic potentials." Clin Neurophysiol.

Halmagyi, G. M. and J. P. Carey (2010). "Vestibular evoked myogenic potentials - we live in interesting times." Clin Neurophysiol **121**(5): 631-633.

Holton, T. and A. J. Hudspeth (1983). "A micromechanical contribution to cochlear tuning and tonotopic organization." Science **222**(4623): 508-510.

Horner, K. C. and S. Rydmarker (1991). "The vestibular epithelia in experimental hydrops." Scanning Microsc **5**(3): 755-765.

Igarashi, M., R. Saito, et al. (1993). "Otoconia in young and elderly persons: a temporal bone study." Acta Otolaryngol Suppl **504**: 26-29.

Iwasaki, S., L. A. McGarvie, et al. (2007). "Head taps evoke a crossed vestibulo-ocular reflex." Neurology **68**(15): 1227-1229.

Iwasaki, S., Y. E. Smulders, et al. (2008). "Ocular vestibular evoked myogenic potentials to bone conducted vibration of the midline forehead at Fz in healthy subjects." Clin Neurophysiol **119**(9): 2135-2147.

Jang, Y. S., C. H. Hwang, et al. (2006). "Age-related changes on the morphology of the otoconia." Laryngoscope **116**(6): 996-1001.

Janky, K. L. and N. Shepard (2009). "Vestibular evoked myogenic potential (VEMP) testing: normative threshold response curves and effects of age." J Am Acad Audiol **20**(8): 514-522.

Jerger, J., S. Jerger, et al. (1972). "Studies in impedance audiometry. I. Normal and sensorineural ears." Arch Otolaryngol **96**(6): 513-523.

Johnsson, L. G. (1971). "Degenerative changes and anomalies of the vestibular system in man." Laryngoscope **81**(10): 1682-1694.

Johnsson, L. G. and J. E. Hawkins, Jr. (1972). "Sensory and neural degeneration with aging, as seen in microdissections of the human inner ear." Ann Otol Rhinol Laryngol **81**(2): 179-193.

Lewis, A., W. Mustain, et al. (2010). "Frequency tuning in the tone burst-evoked myogenic potentials in extraocular muscles in normal human subjects." J Otolaryngol Head Neck Surg **39**(5): 491-497.

Lin, K. Y. and Y. H. Young (2011). "Correlation between subjective visual horizontal test and ocular vestibular-evoked myogenic potential test." Acta Otolaryngol **131**(2): 149-155.

Lin, M. Y., F. C. Timmer, et al. (2006). "Vestibular evoked myogenic potentials (VEMP) can detect asymptomatic saccular hydrops." Laryngoscope **116**(6): 987-992.

Manzari, L., A. M. Burgess, et al. (2010). "Dissociation between cVEMP and oVEMP responses: different vestibular origins of each VEMP?" Eur Arch Otorhinolaryngol **267**(9): 1487-1489.

Manzari, L., A. Tedesco, et al. (2010). "Ocular vestibular-evoked myogenic potentials to bone-conducted vibration in superior vestibular neuritis show utricular function." Otolaryngol Head Neck Surg **143**(2): 274-280.

McCue, M. P. and J. J. Guinan, Jr. (1994). "Acoustically responsive fibers in the vestibular nerve of the cat." J Neurosci **14**(10): 6058-6070.

McCue, M. P. and J. J. Guinan, Jr. (1995). "Spontaneous activity and frequency selectivity of acoustically responsive vestibular afferents in the cat." J Neurophysiol **74**(4): 1563-1572.

Merchant, S. N., J. C. Adams, et al. (2005). "Pathophysiology of Meniere's syndrome: are symptoms caused by endolymphatic hydrops?" Otol Neurotol **26**(1): 74-81.

Merchant, S. N., L. Velazquez-Villasenor, et al. (2000). "Temporal bone studies of the human peripheral vestibular system. Normative vestibular hair cell data." Ann Otol Rhinol Laryngol Suppl **181**: 3-13.

Metz, O. (1952). "Threshold of reflex contractions of muscles of middle ear and recruitment of loudness." AMA Arch Otolaryngol **55**(5): 536-543.

Moore, B. (1998). Cochlear Hearing Loss. London, Whurr Publishers Ltd.

Morita, N., S. Kariya, et al. (2009). "Membranous labyrinth volumes in normal ears and Meniere disease: a three-dimensional reconstruction study." Laryngoscope **119**(11): 2216-2220.

Murnane, O. D., F. W. Akin, et al. (2011). "Effects of stimulus and recording parameters on the air conduction ocular vestibular evoked myogenic potential." J Am Acad Audiol **22**(7): 469-480.

Murofushi, T., M. Matsuzaki, et al. (1999). "Short tone burst-evoked myogenic potentials on the sternocleidomastoid muscle: are these potentials also of vestibular origin?" Arch Otolaryngol Head Neck Surg **125**(6): 660-664.

Murofushi, T., K. Wakayama, et al. (2010). "oVEMP to air-conducted tones reflects functions of different vestibular populations from cVEMP?" Eur Arch Otorhinolaryngol **267**(6): 995-996.

Nguyen, K. D., M. S. Welgampola, et al. (2010). "Test-retest reliability and age-related characteristics of the ocular and cervical vestibular evoked myogenic potential tests." Otol Neurotol **31**(5): 793-802.

Node, M., T. Seo, et al. (2005). "Frequency dynamics shift of vestibular evoked myogenic potentials in patients with endolymphatic hydrops." Otol Neurotol **26**(6): 1208-1213.

Ochi, K. and T. Ohashi (2003). "Age-related changes in the vestibular-evoked myogenic potentials." Otolaryngol Head Neck Surg **129**(6): 655-659.

Okuno, T. and I. Sando (1987). "Localization, frequency, and severity of endolymphatic hydrops and the pathology of the labyrinthine membrane in Meniere's disease." Ann Otol Rhinol Laryngol **96**(4): 438-445.

Park, H. J., I. S. Lee, et al. (2010). "Frequency-tuning characteristics of cervical and ocular vestibular evoked myogenic potentials induced by air-conducted tone bursts." Clin Neurophysiol **121**(1): 85-89.

Piker, E. G., G. P. Jacobson, et al. (2011). "Normal characteristics of the ocular vestibular evoked myogenic potential." J Am Acad Audiol **22**(4): 222-230.

Rauch, S. D., S. N. Merchant, et al. (1989). "Meniere's syndrome and endolymphatic hydrops. Double-blind temporal bone study." Ann Otol Rhinol Laryngol **98**(11): 873-883.

Rauch, S. D., G. Zhou, et al. (2004). "Vestibular evoked myogenic potentials show altered tuning in patients with Meniere's disease." Otol Neurotol **25**(3): 333-338.

Richter, E. (1980). "Quantitative study of human Scarpa's ganglion and vestibular sensory epithelia." Acta Otolaryngol **90**(3-4): 199-208.

Rosengren, S. M., S. Govender, et al. (2011). "Ocular and cervical vestibular evoked myogenic potentials produced by air- and bone-conducted stimuli: comparative properties and effects of age." Clin Neurophysiol **122**(11): 2282-2289.

Rosengren, S. M., N. P. McAngus Todd, et al. (2005). "Vestibular-evoked extraocular potentials produced by stimulation with bone-conducted sound." Clin Neurophysiol **116**(8): 1938-1948.

Rosengren, S. M., M. S. Welgampola, et al. (2010). "Vestibular evoked myogenic potentials: past, present and future." Clin Neurophysiol **121**(5): 636-651.

Rosenhall, U. (1973). "Degenerative patterns in the aging human vestibular neuro-epithelia." Acta Otolaryngol **76**(2): 208-220.

Ross, M. D., D. Peacor, et al. (1976). "Observations on normal and degenerating human otoconia." Ann Otol Rhinol Laryngol **85**(3 pt 1): 310-326.

Schuknecht, H. F. I., Makota; Gacek, Richard R. (1965). "The Pathological Types of Cochleo-Saccular Degeneration." Acta Otolaryngol **59**: 154 - 170.

Sheykholeslami, K., M. Habiby Kermany, et al. (2001). "Frequency sensitivity range of the saccule to bone-conducted stimuli measured by vestibular evoked myogenic potentials." Hear Res **160**(1-2): 58-62.

Sperling, N. M., M. M. Paparella, et al. (1993). "Symptomatic versus asymptomatic endolymphatic hydrops: a histopathologic comparison." Laryngoscope **103**(3): 277-285.

Steinacker, A. (2004). Sensory Processing and Ionic Currents in Vestibular Hair Cells. The Vestibular System. S. Highstein, R. Fay and A. Popper, Springer New York. **19:** 202-234.

Su, H. C., T. W. Huang, et al. (2004). "Aging effect on vestibular evoked myogenic potential." Otol Neurotol **25**(6): 977-980.

Tang, Y., I. Lopez, et al. (2001). "Age-related change of the neuronal number in the human medial vestibular nucleus: a stereological investigation." J Vestib Res **11**(6): 357-363.

Taylor, R. L., A. A. Wijewardene, et al. (2010). "The vestibular evoked-potential profile of Meniere's disease." Clin Neurophysiol.

Timmer, F. C., G. Zhou, et al. (2006). "Vestibular evoked myogenic potential (VEMP) in patients with Meniere's disease with drop attacks." Laryngoscope **116**(5): 776-779.

Todd, N. P., F. W. Cody, et al. (2000). "A saccular origin of frequency tuning in myogenic vestibular evoked potentials?: implications for human responses to loud sounds." Hear Res **141**(1-2): 180-188.

Todd, N. P., S. M. Rosengren, et al. (2007). "Ocular vestibular evoked myogenic potentials (OVEMPs) produced by air- and bone-conducted sound." Clin Neurophysiol **118**(2): 381-390.

Todd, N. P., S. M. Rosengren, et al. (2009). "A utricular origin of frequency tuning to low-frequency vibration in the human vestibular system?" Neurosci Lett **451**(3): 175-180.

Todd, N. P., S. M. Rosengren, et al. (2009). "Low-frequency tuning in the human vestibular-ocular projection is determined by both peripheral and central mechanisms." Neurosci Lett **458**(1): 43-47.

Tseng, C. L., C. H. Chou, et al. (2010). "Aging effect on the ocular vestibular-evoked myogenic potentials." Otol Neurotol **31**(6): 959-963.

Uzun-Coruhlu, H., I. S. Curthoys, et al. (2007). "Attachment of the utricular and saccular maculae to the temporal bone." Hear Res **233**(1-2): 77-85.

Valko, Y., S. C. Hegemann, et al. (2011). "Relative diagnostic value of ocular vestibular evoked potentials and the subjective visual vertical during tilt and eccentric rotation." Clin Neurophysiol **122**(2): 398-404.

Walther, L. E. and M. Westhofen (2007). "Presbyvertigo-aging of otoconia and vestibular sensory cells." J Vestib Res **17**(2-3): 89-92.

Welgampola, M. S. and J. G. Colebatch (2001). "Characteristics of tone burst-evoked myogenic potentials in the sternocleidomastoid muscles." Otol Neurotol **22**(6): 796-802.

Welgampola, M. S. and J. G. Colebatch (2001). "Vestibulocollic reflexes: normal values and the effect of age." Clin Neurophysiol **112**(11): 1971-1979.

Welgampola, M. S., A. A. Migliaccio, et al. (2009). "The human sound-evoked vestibulo-ocular reflex and its electromyographic correlate." Clin Neurophysiol **120**(1): 158-166.

Winters, S. M., I. T. Berg, et al. (2012). "Ocular vestibular evoked myogenic potentials: frequency tuning to air-conducted acoustic stimuli in healthy subjects and Meniere's disease." Audiol Neurootol **17**(1): 12-19.

Yazawa, Y. and M. Kitahara (1990). "Bilateral endolymphatic hydrops in Meniere's disease: review of temporal bone autopsies." Ann Otol Rhinol Laryngol **99**(7 Pt 1): 524-528.

Young, E. D., C. Fernandez, et al. (1977). "Responses of squirrel monkey vestibular neurons to audio-frequency sound and head vibration." Acta Otolaryngol **84**(5-6): 352-360.

Zapala, D. A. and R. H. Brey (2004). "Clinical experience with the vestibular evoked myogenic potential." J Am Acad Audiol **15**(3): 198-215.

Zhang, A. S., S. Govender, et al. (2011). "Tuning of the ocular vestibular evoked myogenic potential (oVEMP) to AC sound shows two separate peaks." Exp Brain Res **213**(1): 111-116.