Impact of Advancing Age on Frequency Tuning of Ocular Vestibular Evoked Myogenic Potential

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This Dissertation is submitted as part of fulfillment
for the Degree of Master of Science in Audiology to
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Certificate

This is to certify that this dissertation entitled **“Impact of Advancing Age on Frequency Tuning of Ocular Vestibular Evoked Myogenic Potential. ”** is a bonafide work in fulfillment for the degree of Master of Science (Audiology) of the student with Registration No. 13AUD008. This has been carried out under the guidance of a faculty of this institute and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

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Declaration

This dissertation entitled **“Impact of Advancing Age on Frequency Tuning of Ocular Vestibular Evoked Myogenic Potential’’** is the result of my own study under the guidance of Mr. Niraj Kumar Singh, Lecturer in Audiology, Department of Audiology, All India Institute of Speech and Hearing, Mysore, and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

Mysore, **Register No: 13AUD008**

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Dedication

This dissertation is dedicated to all those who are a part of fulfillment of my dissertation.

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The task of the excellent teacher is to stimulate "apparently ordinary" people to unusual effort. The tough problem is not in identifying winners: it is in making winners out of ordinary people. - K. Patricia Cross

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Abstract

Normal aging is mostly associated with global decline in almost all aspects. While aging affects ocular vestibular evoked myogenic potentials (oVEMP) by reducing the amplitudes and prolonging the latencies, its interaction with oVEMP responses at other frequencies has sparingly been explored. Therefore the present study aimed at investigating the impact of advancing age on the frequency tuning of oVEMP. The oVEMPs were recorded from 50 healthy individuals divided under five age groups (20-30, 30-40, 40-50, 50-60, & > 60 years) for tone-burst frequencies of 250, 500, 750, 1000, 1500 and 2000 Hz. The results revealed significantly lower response rates for age groups > 50 years than all the other groups at almost all the frequencies ($p < 0.05$). Although there was a trend towards lower peak-to-peak amplitudes in age groups > 50 years, the differences were not significant ($p > 0.05$). Further, the frequency tuning was obtained at 500 Hz or 750 Hz in majority of individuals < 60 years and at ≥ 1000 Hz in most of the individuals > 60 years. The proportion of ears showing frequency tuning at ≥ 1000 Hz was significantly higher in the > 60 years age groups than < 60 years ($p < 0.05$). Thus, there was a significant shift in frequency tuning of oVEMP from 500 Hz or 750 Hz in younger and middle-aged adults to ≥ 1000 Hz in older adults, especially above 60 years of age. Since the shift in frequency tuning to ≥ 1000 Hz is popularly used for identification of Meniere's disease, it is suggested that age-related correction should be used for diagnosis of Meniere's disease.

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Chapter 1

Introduction

Human being are blessed with extraordinarily tuned senses. This makes it possible for the human kind to cope effectively with the environment by utilizing the capability in processing the incoming sensory input. In fact, the human senses have been artistically crafted in a careful manner by the nature to effortlessly encode required information from the pool of surrounding information. Unfortunately, advancing age brings a systematic reduction in the effectiveness of this capability of unstrained processing of the sensory systems (Harman, 2003; Zimmermann et al., 2007; Crane, Devries, Safdar, Hamadeh, & Tarnopolsky, 2009).

Normal aging is mostly associated with global decline in almost all aspects. Human aging has been shown to involve changes at molecular, biochemical and also cellular level across all the body systems (Harman, 2003). Changes in the skin, epithelial membrane, skeletal muscles, bones, joints and tissues have also been reported (Harman, 2003; Zimmermann et al., 2007; Crane, Devries, Safdar, Hamadeh, & Tarnopolsky, 2009). Our system of balance is a congregation of a number of the above mentioned parts and therefore would be likewise affected by the aging process.

From the day of birth, an individual is highly reliant on his/her sense of balance for well being and survival. The vestibular system is a major contributor to the maintenance of an individual’s balance, mostly during head and body acceleration. The semicircular canals are involved in maintaining balance during angular acceleration, where as the otolith organs contribute to balance sustenance during linear acceleration (Honrubia et al., 1997; Desmond, 2011).

As in any organ, age-related degenerative changes are also shown in the vestibular system. These changes have been identified from the end organs of the vestibular system to its central nuclei (Johnsson, 1972; Bergstrom, 1973). Previous studies have shown steady decline in the vestibular hair cell counts and densities with advancing age (Merchant, Velazquez-Villasenor, Tsuji, Glynn, Wall, & Rauch, 2000). Similar data was also presented for Scarpa's ganglion neuronal counts (Merchant, Velazquez-Villasenor, Tsuji, Glynn, Wall, & Rauch, 2000) and vestibular neurons in the vestibular brainstem (Tang, Lopez, & Baloh, 2001-2002).

The advances in technology pr ovided the clinicians an opportunity to assess function of semicircular canals and the otolith organs. The functioning of the semicircular canals can be accessed through a wide range of tests that include bithermal caloric irrigation (Capps, Preciado, Paparella, & Hoppe, 1973), rotational chair test (Palomar-Asenjo, Boleas-Aguirre, Sanchez-Ferrandiz, & Fernandez, 2006) and head impulse test (Aw, Fetter, Cremer, Kalberg, & Halmagyi, 2001). However, only a few tests help in the evaluation of the functional integrity of the otolith organs. One such test is vestibular evoked myogenic potential (VEMP). VEMP is a clinical tool that helps explore the functional integrity of the otolith organs and reflexes that involve them as a of their primary components (Colebatch & Halmagyi, 1992; Colebatch, Halmagyi, & Skuse, 1994).

VEMP is a biphasic potential which can be evoked by air-conducted (Ferber-Viart, Dubreuil, & Duclaux, 1999; Welgampola, & Colebatch, 2005; Honaker, & Samy, 2007; Mudduwa, Kara, Whelan, & Banerjee, 2010), bone-conducted (Basta, Todt, & Ernst, 2005; Iwasaki et al., 2008; Chihara, Iwasaki, Fujimoto, Ushio, Yamasobaa, & Murofushi, 2009; Tseng, Wang, & Young, 2012) or galvanic stimulation (Cunha, Labanca, Tavares, & Goncalves, 2014). This myogenic potential may be recorded from various locations in the body and based on the recording site, the target generation site differs. The primary recording site is the sternocleidomastoid (SCM) muscle along the cervical spine (Colebatch & Halmagyi, 1992). The VEMP elicited from the SCM muscle is called Cervical VEMP (cVEMP). VEMP can also be recorded from the inferior extraocular muscles of the eye (Rosengren, Todd, & Clebatch, 2005), in which case it is referred as Ocular VEMP (oVEMP). In addition to being easier to perform and being less taxing on the patient than cVEMP, oVEMP also provides information regarding the vestibulo-ocular reflex (VOR) pathway (Rosengren et al., 2005; Todd, Rosengren, Aw, & Colebatch, 2007; Chihara et al., 2009; Welgampola, Migliaccio, Myrie, Minor, & Carey, 2009), which makes it a complementary test rather than supplementary test to cVEMP. Since oVEMP has contributions from most of the afore mentioned areas that are affected by the process of aging, it is likely that oVEMP could also be affected by the process of aging and hence diagnosis needs to be made only after comparisons with the age-related norms.

Age-related changes for oVEMP are well-documented in the literature. Piker, Jacobson, McCaslin and Hood (2011) reported absent oVEMPs in 25% of otologically and neurologically intact normal subjects over the age of 60 years. Similar alterations to various oVEMP parameters were also reported after the age of 60 years in other studies (Nguyen, Welgampola, & Carey, 2010; Tseng, Chou, & Young, 2010). Therefore, aging plays an important role in the determination of the norms to which a pathological finding should be compared.

1.1. Need for the study

oVEMPs were shown to be clinically useful in the diagnosis of several peripheral vestibular pathologies like Meniere's disease (Winters, Campschroer, Grolman & Klis, 2011; Kartner & Gurkov, 2012; Winters, Berg, Grolman, & Klis, 2012; Zuniga, Janky, Schubert, & Carey, 2012; Zuniga, Janky, Nguyen, Welgampola, & Carey, 2013; Lin, Wang, & Young, 2013; Jerin, Berman, Krause, Ertl-Wagner, & Gurkav 2014), superior canal dehiscence (Rosengren, Halmagyi, & Colebatch, 2008; Welgampola, Myrie, Minor, & Carey, 2008; Manzari, Burgess, McGarvie, & Curthoys, 2012; Janky, Nguyen, Zuniga, Welgampola, & Carey, 2013), and vestibular neuronitis (Chiarovano, Zamith, Vidal, & Waele, 2011; Taylor, Bradshaw, Halmagyi, & Welgampola, 2011; Adamec et al., 2013). Meniere's disease is one among the most explored pathologies using oVEMP. The parameters like latency, amplitude and assymetry ratio have been shown to be useful in diagnosis of Meniere's disease by the above studies. However, the finding in these parameters do not apply only to Meniere's disease, rather they are common to several other pathologies like benign paroxysmal positional vertigo (BPPV) (Akkuzu, Akkuzu, & Ozluoglu, 2006; Yang, Kim, Lee, & Lee, 2008; Korres, Gkoritsa, Giannakakou-Razelou, Yiotakis, Riga, & Nikolpoulos, 2010; Singh, & Barman, 2015), vestibular neuritis (Chiarovanoet al., 2011; Taylor et al., 2011; Adamec et al, 2013) and labyrinthitis (Murofushi, Halmagyi , Yavor , & Colebatch, 1996; Moon, Lee, Park, & Lee, 2012). Frequency tuning is the most recent addition to this list of parameters that are reported to be useful in the diagnosis of Meniere's disease.

Frequency tuning or the tuned frequency refers to the frequency at which largest oVEMP responses are obtained (Sandhu, Low, Rea, & Saunders, 2012). Healthy individuals have shown the presence of frequency tuning at 500 Hz (Singh & Barman, 2013, 2014), 1000 Hz (Lewis, Mustain, Xu, Eby, & Zhou, 2010; Taylor et al., 2011) or between 400 and 800 Hz (Todd, Cody, & Banks, 2000). The frequency tuning is found to be shifted to higher frequencies in patients with Meniere's disease and the best frequency for such patients was reported to be 1000 Hz or higher frequencies (Sandhu et al., 2012; Winters et al., 2012). This shift in frequency tuning from 500 Hz in normals to 1000 Hz or higher frequencies in Meniere's disease was explained on the basis of changes in the stiffness characteristics of the utricular membrane causing a change in the resonance frequency of the utricle (Sandhu et al., 2012).

The prevalence of Meniere's disease is known to increase with increasing age (Alexander, & Harrid, 2010). The disease was reported to be most commonly affecting the individuals in their fifth and sixth decade of life (Alexander, & Harris, 2010). Infact, the mean age of individuals with Meniere's disease was found to be above 50 years in a number of studies (Niedecker, Pfaltz, Malefi, & Benz, 1981; Shojaku et al., 2009;

Alexander & Harris, 2010). Likewise, the age range above 50 years have also been shown to involve age related degenerative changes in the vestibular system (Johnson 1972; Walther and Westhofen, 2007). These studies reporded neural degeneration as well as reduction in volume and number of otoconia in the utricular macula. Reduction in the number of otoconia and the consequent reduction in the volume of macula would potentially affect the resonant frequency by virtue of altering the balance between mass and stiffness characteristics within the utricle. Therefore there would be a likelihood of a change in resonant frequency and a consequent change in frequency tuning could take place due to aging. Nonetheless, such a phenomenon has sparingly been explored previously in healthy individuals.

Piker, Jacobson, Burkard, McCaslin and Hood (2013) explored the effect of advancing age on frequency tuning of oVEMP. They considered 39 individuals, who were divided into three groups of 13 subjects each as young adults (18-39 years), middle aged adults (40-59 years) and old adults (≥ 60 years group). They reported a significant shift in the frequency tuning towards higher frequencies with increasing age. Although they showed significant effect of age on frequency tuning of oVEMP, they did so using a small sample size which would give only a few data points under each of the age groups. Additionally, the study also included wider and unevenly distributed age ranges which may result in erroneous conclusions. This substantiates the need for more research for evaluating the effect of advancing age on frequency tuning of oVEMP by including larger participant number, spacing the groups evenly and using smaller age spans for each group.

1.2. Aim of the study

The present study aimed to investigate the effect of advancing age on frequency tuning of oVEMP in healthy individuals.

1.3. Objectives

1. To study the ear differences, if any, in the response rate of oVEMP in each age group of healthy individuals.
2. To evaluate the effect of frequency of tone-burst on the response rate of oVEMP in each age group of healthy individuals.
3. To evaluate the effect of age on response rate of oVEMP in healthy individuals.
4. To study the ear differences, if any, in peak-to-peak amplitude of oVEMP in each age group of healthy individuals.
5. To evaluate the effect of frequency of tone-burst on peak-to-peak amplitude of oVEMP in each age group of healthy individuals.
6. To evaluate the effect of age on peak-to-peak amplitude of oVEMP in healthy individuals.
7. To evaluate the effect of age on the frequency tuning of oVEMP in healthy individuals.

1.4. Hypothesis

The study began with the following Null hypothesis:

1. There is no significant ear difference in response rate of oVEMP in each age group of healthy individuals.
2. There is no significant effect of frequency of tone-burst on the response rate of oVEMP in each age group of healthy individuals.
3. There is no significant effect of age on response rate of oVEMP in healthy individuals.
4. There is no significant ear difference in peak-to-peak amplitude of oVEMP in each age group of healthy individuals.
5. There is no significant effect of frequency of tone-burst on peak-to-peak amplitude of oVEMP in each age group of healthy individuals.
6. There is no significant effect of age on peak-to-peak amplitude of oVEMP in healthy individuals.
7. There is no significant effect of age on the frequency tuning of oVEMP in healthy individuals.

Chapter 2

Literature Review

Clinical examination is an important component for the assessment and successful rehabilitation of patients with vestibular dysfunction. Until the evolution of recent technology, there were only a few clinical tools to check the functional integrity of the vestibular system, most of which were based around the assessment of vestibulo-ocular reflex (VOR) mediated by the horizontal semicircular canal. Advances in technology in the recent past have been of great help for the assessment of otolith organs. One of the techniques which evolved to clinical use in the 1990s and which is meant for the assessment of functional integrity of the otolith organs and reflexes that involve them is VEMP (Colebatch & Halmagyi, 1992; Colebatch et al., 1994). .

VEMP is a biphasic potential which is reported to be evoked by high intensity air-conducted (Ferber-Viart et al., 1999; Welgampola, & Colebatch, 2005; Honaker, & Samy, 2007; Mudduwa et al., 2010), bone-conducted (Basta et al., 2005; Iwasaki et. al., 2008; Chihara et al., 2009; Tseng et al., 2012) or galvanic stimulation (Cunha et al., 2014). This myogenic potential can be recorded from various locations in the body and based on the recording site, the target generation site differs. The primary recording site is the sternocleidomastoid (SCM) muscle along the cervical spine (Colebatch & Halmagyi, 1992). The VEMP elicited from the SCM muscle is called cVEMP. It is a well established, reliable and valuable clinical test for assessment of saccular function (Colebatch & Halmagyi, 1992; Colebatch et al., 1994). VEMP can also be recorded from the inferior extra-ocular muscles of the eye, in which case it is referred as oVEMP. The ocular VEMPs are optimally recorded from the extra-ocular muscles slightly below the eye contralateral to the ear of acoustic stimulation (Iwasaki et al., 2008). This response arises from the otolith-ocular reflex and the studies have confirmed that this is not a blink response (Rosengren et al., 2005; Todd et al., 2007; Chihara et al., 2009; Welgampola et al., 2009). Previous research suggested that the oVEMP response at the level of the extra-ocular muscles is an excitatory response owing to the presence of an initial negative peak in the response waveform (Todd et al., 2007).

For the successful and reliable recording of oVEMP, upward gazing was reported to be mandatory because the inferior extra-ocular muscles are best activated when the eyes are in supero-medial gaze (Rosengren et al., 2005; Chihara, Iwasaki, Ushio, & Murofushi, 2007; Govender, Rosengren, & Colebatch, 2009; Wang, Jaw, &Young, 2009; Welgampola et al., 2009). The neuronal pathway from utricle to the inferior extra-ocular muscles of the eye, which traverses via the superior vestibular nerve, vestibular nuclei, medial longitudinal fasciculus, contralateral oculomotor nuclei and ocular nerves, was described by Rosengren, Welgampola and Colebatch (2010).

Once recorded, the of oVEMP is marked by the presence of an initial negative peak at around 10-12 ms and a subsequent positive peak at about 15-20 ms, by virtue of which it is mainly referred as a biphasic response (Rosengren et al., 2005; Todd et al., 2007; Rosengren, Jombik, Halmagyi, & Colebatch 2009). Although other peaks (such as n2, p2, and n3) are also reported to occur in oVEMP recordings of healthy individuals

(Iwasaki, Smulders, Burgess, McGarvie, Macdougall, & Halmagyi, 2008; McElhinney, O'Beirne, Lin, & Hornibrook , 2010), they have less often been explored and reported.

As in any other evoked potential, oVEMP response characteristics largely depend on various stimulus and recording parameters. The effects of a number of subject related parameters such as gender (Piker et al, 2011), gaze elevation angle **(**Iwasaki et al., 2008; Welgampola et al., 2009; Murmane, Akin, Kelly, & Byrd, 2011; Rosengren, Govender, & Colebatch, 2011), and body position (Govender et al., 2009) on the air-conduction-evoked oVEMP have been examined and found to affect its various response parameters. In addition to the changes in subject related parameters, the effect of stimulus related parameters such as stimulus type (Cheng et al., 2010 & Rosengren, Govender, Colebatch, 2011), stimulus mode (Rosengren et al., 2005; Cheng, Chen, Wang , & Young, 2010; Rosengren et al., 2011), presentation type (Wang et al., 2009; Kim & Ban, 2012), rise/fall and plateau times (Cheng, Wu, & Lee, 2012; Omprakash & Singh, 2013) and stimulus intensity (Rosengren et al., 2010; Murnane et al., 2011) on the air-conduction-evoked oVEMP have also been examined. Tone-bursts were shown to produce larger amplitudes and better thresholds than clicks (Chihara et al., 2007; Rosengren et al., 2011) and therefore the modern day’s oVEMP are mainly recorded using tone-bursts.

Stimulus frequency is an important stimulus related factor whose impact on oVEMP has been studied by several groups of researchers (Chihara et al., 2007; Chihara et al., 2009; Rosengren, & Colebatch, 2009; Park, Lee, Shin, Lee, & Park, 2010; Murnane et al., 2011; Todd, Piker, 2012; Sandhu, et al., 2012; Taylor et al., 2011; Zhang, Govender, & Colebatch, 2012; Singh & Barman, 2013, 2014). Although extensive research has been conducted in the past to study the effect of stimulus frequency of tone-bursts on oVEMP, there have been some casting disagreements among results of the previous studies.

2.1. Effect of tone-burst frequency on oVEMP

It has been well documented in various studies that low frequency stimuli result in better oVEMP responses when compared to high frequency stimuli. Welgampola, Rosengren, Halmagyi and Colebatch (2003) obtained responses to 250, 500, 1000 and 2000 Hz bone-conducted tone-bursts from 10 healthy subjects in the age range of 24 to 52 years. They reported that oVEMP responses for 250 Hz had highest amplitude and lowest threshold and these parameters worsened (amplitudes reduced & thresholds became higher) for subsequent increase in the tone-burst frequency. However, this study used a small sample size considering it was a normative study.

Chihara et al., (2009) reported frequency tuning properties of oVEMP in 12 healthy individuals in the age range of 26-40 years using air-conduction and bone -conduction vibration of 250, 500, and 1000 Hz short tone- bursts (rise fall time = 1 ms; plateau time = 2 ms). Results demonstrated that the best frequency of the oVEMPs to air-conducted sound and bone-conducted vibration were 500 and 250 Hz respectively. The latencies of nl decreased as the stimulus frequency increased for both modes of stimulation. However this study also drew conclusions based on the findings from a small sample size. Further, they did not evaluate the effect of higher frequencies (> 1000 Hz) on oVEMP parameters.

Similar results for only air-conducted tone-bursts were also obtained by several other studies later (Murnane et al., 2011; Singh & Barman, 2013, 2014).

The best frequency (frequency tuning peak) of oVEMP have also been reported to occur at 1000 Hz by some studies (Lewis et al., 2010; Taylor et al., 2011) and between 400 and 800 Hz by one of the studies (Todd et al., 2007). Although these differences could be logically attributed to differences in some of the major stimulus related differences such as use of larger stimulus duration and differences in unit of calibration, the fact remains that there is a lack of consensus regarding the best frequency for air-conduction oVEMP recording.

2.2. Effect of age on oVEMP

Age related changes are not only evident in the auditory system but also seen in vestibular system. As age increases beyond 60 years, the various sub-sections within the vestibular system were shown to undergo changes (Johnson 1972; Walther & Westhofen, 2007). oVEMP being the response from one such sub-section (mainly utricle), several studies have evaluated the age-related changes in the response parameters of oVEMP (Nyugen et al., 2010; Tseng et al., 2010; Piker et al., 2011). While some studies showed an effect of advancing age on amplitude as well as latency measures, others revealed the impact only on the amplitude of oVEMP.

Nyugen et al (2010) obtained oVEMP from 53 healthy individuals in the age range of 20-70 years and studied the effect of age on oVEMP for three different stimuli- clicks, 500Hz tone-burst and 500 Hz bone-conduction vibration. They observed significant reduction in peak-to-peak amplitude after the age of 50 years. However, there was no significant effect of age on latencies and asymmetry ratio for all the three types of stimuli.

Tseng et al (2010) evaluated 70 subjects in the age range of 24 to 76 years who were divided into 6 groups (each age group corresponded to a decade) and obtained oVEMP for bone-conduction vibration using 500 Hz tone-bursts. They observed 100% response rates in the age groups between 20 years and 59 years and subsequent reduction after 60 years. Further, they also reported significant prolongation of the latencies after 60 years of age. In their study, the amplitude reduction was noticed after the age of 40 years.

Piker et al (2011) recorded ipsilateral as well as contralateral oVEMP for 500 Hz tone-burst at 95 dB nHL. Age-related differences in oVEMP latencies, amplitudes, interaural amplitude asymmetries and thresholds were studied in 100 ears in the age range of 8-88 years. Subjects were divided into three groups - below 18 years, 18-49 years, and above 50 years. The amplitude of the responses significantly decreased and the thresholds significantly increased with increasing age, with the greatest age effects occurring in subjects who were 50 years or older. Response rate was reported to be 100% in subjects less than 50 years of age while 77% for subjects who were above 50 years of age.

The effect of age on oVEMP parameters shows a general agreement regarding decrease in amplitude with increasing age. However, there is lack of consensus regarding the impact of aging on the latency measures of oVEMP.

2.3. Effect of age on frequency tuning on oVEMP

Stimulus and subject related factors such as age and frequency tuning can potentially interact. This potential interaction was studied by Piker et al (2013). They considered 39 individuals, who were divided into three groups of 13 subjects each as young adults (18-39 years), middle aged adults (40-59 years) and old adults (≥ 60 years group). They observed dominance of 500 Hz as the best frequency in majority of young adults and reported a shift in this dominance towards higher frequencies in the middle-aged adults and older adults. Although they showed significant effect of age on frequency tuning of oVEMP, this study considered a small sample size which would give only a few data points under each of the age groups. It also included wider and unevenly distributed age ranges which may result in erroneous conclusions.

The above studies show the inconsistencies present in literature regarding the frequency tuning of oVEMP and scarcity of studies on the impact of age on frequency tuning of oVEMP. The only study using a congregation of these two factors to study their combined impact on best frequency was marred by severe deficits in sample size and affected by the uneven distribution of age groups. This highlights the need for more structured and detailed study design in future explorations of these factors.

Chapter 3

Method

3.1. Participants

Fifty healthy volunteers (25 males & 25 females) in the age range of 20-80 years were selected as participants. They were equally divided into 5 age groups [Group I: 20-30 years (mean age = 25.6, Standard deviation = 2.9), Group II: 30-40 years (mean age = 34.7, Standard deviation = 2.1), Group III: 40-50 years (mean age = 45.6, Standard deviation = 3.5) , Group IV:50-60 years (mean age = 56.7, Standard deviation = 3.1), Group V: >60 years (mean age = 70.5, Standard deviation = 6.2)], each covering a span of 10 years except for the fifth group which covered a span of 20 years. Care was taken to include equal number of participants above and below the mid-point of each of these groups in order to have even spread of age within each group. Also, there were equal number of male and female participants within each age group in order to overcome the gender effect, if any. Prior to the testing, each participant was explained regarding the experiment and subsequently a written consent for participation was obtained. The written consent form is enclosed as Appendix A. None of the participants were paid for their participation in this study.

Pure-tone audiometry, speech audiometry, immittance evaluation, and auditory brainstem response testing were done to rule out conductive pathology and any retro-cochlear pathology. All the participants showed a fair-to-good agreement between pure-tone average and speech recognition thresholds. Those showing only ‘A’ type tympanograms were included in the study. The subjects with complaint or history of vertigo, dizziness, light headedness, and/or imbalance were excluded from the study. The study also excluded those with high blood pressure and diabetes, as they were shown to have deleterious impact on the oVEMP responses (Ghosh & Sinha, 2012). All the participants had UCL of 100 dB HL or more.

3.2. Test environment

Pure-tone audiometry and speech audiometry were carried out in a double room situation. Immittance evaluation, auditory brainstem response recording and oVEMP were administered in a single room situation. All the rooms were sound treated, complying with American National Standards Institute guidelines (ANSIS 3.1 1991) for permissible ambient noise levels. They were also well illuminated and air-conditioned.

3.3. Instrumentation

A calibrated two channel Grason-Stadler Incorporated (GSI-61) diagnostic audiometer with TDH 39 supra-aural headphones housed in MX41/AR ear cushions was used for air-conduction threshold estimation and speech audiometry. The same audiometer with Radioear B-71 bone vibrator was used for bone-conduction threshold estimation. A calibrated GSI-tympstar clinical immittance device was used for tympanometry and reflexometry. Biologic Navigator Pro evoked potential system version 7.0.0 with impedance matched Etymotic ER-3A insert earphones was used to record and analyze auditory brainstem responses and oVEMP.

3.4. Procedure

A detailed structured case history was taken before the commencement of audiological and vestibular work-up. This included questions related to complaints of conductive hearing loss, vestibular pathologies and neurological disorders. For this, the Otoneurological proforma (unpublished) developed by Department of ENT, AIISH, was used. The questionnaire is shown in Appendix B.

The audiological evaluation included pure-tone audiometry, speech audiometry, immittance evaluation and auditory brainstem response recording. The vestibular evaluation included subjective vestibular assessments like Romberg test, Fukuda stepping test, Tandem gate test and Past pointing test (Finger-to-nose test) in order to screen out any vestibular pathology.

3.4.1. Pure-tone audiometry

Pure-tone thresholds were obtained using modified Hughson and Westlake procedure (Carhart & Jerger, 1959) at octave frequencies from 250 Hz to 8000 Hz for air-conduction and 250 Hz to 4000 Hz for bone-conduction stimulation. The thresholds were also obtained at mid-octave frequencies in case a difference exceeding 20 dB HL between the two adjacent octave frequencies.

3.4.2. *Speech audiometry*

The bracketing method was adopted for obtaining speech recognition thresholds. For this, the spondee word lists in the participants’ native language were used. The speech identification scores were obtained using phonetically balanced word lists at levels recommended for each test (mostly 40 dB above the SRT) in participants’ native language. The uncomfortable level was obtained for speech using the ascending trial.

3.4.3. *Immittance evaluation*

Immittance evaluation incorporated tympanometry and reflexometry. The participants were seated comfortably and were instructed to avoid swallowing or any other head movements during the entire testing. Immittance evaluation was carried out with a probe tone frequency of 226 Hz at 85 dB SPL. Tympanogram was obtained by changing the pressure within the ear canal from +200 to -400 daPa by using a pump speed of 50 daPa/s. Ipsilateral and contralateral acoustic reflexes thresholds were measured for 500 Hz, 1000 Hz and 2000 Hz using the above mentioned probe tone frequency.

3.4.4. *Auditory brainstem responses (ABR)*

Conventional two-channel ABRs was recorded for 11.1 Hz and 90.1 Hz repetition rates for click stimuli to rule out retrocochlear pathology. For the recording of ABRs, the participants were seated comfortably in a reclining chair and were instructed to relax while the test was being performed. The skin area used for electrode placement was scrubbed with a commercially available skin preparing gel to reduce electrode impedance. The disc type gold plated electrodes with wire length of 1.5 meters were placed at Cz (non-inverting electrode), M1 and M2 (inverting electrode) and Fpz (ground electrode) as per international 10/20 system for electrode placement (Jasper, 1958). The electrodes were secured in place using surgical plaster. The absolute and inter-electrode impedence were maintained below 5 kΩ and 2 kΩ respectively. The stimulus intensity used was 90 dB nHL. An epoch of 10 ms, which also included a pre-stimulus recording of 2 ms, was used and 2000 sweeps were averaged per recording. The responses were band-pass filtered between 1 Hz and 1000 Hz and amplified by a factor of 100000.

3.4.5. *Romberg test*

The Romberg test was performed with the patient standing with their feet together, initially with their eyes open and later closed condition. They were asked to outstretch their hands forward so that they stayed apart by chest width and parallel to the ground. Presence of significant sway during eyes closed condition or inability to stay balanced after closing eyes were considered abnormal responses.

3.4.6. *Fukuda stepping test*

The subjects were asked to close their eyes and hold their arms out stretched directly in front (similar position to Romberg test). They were instructed to march at a place for 50 steps at the rate of about 1 step per second. Deviation greater than 45^{o} on either side and/or distance of greater than 1m from the original starting point was considered abnormal (Harit & Singh, 2012).

3.4.7. *Tandem gate test*

The participants were instructed to place one foot in front of the other making sure that, with each step, the heel of one foot was directly in front of the toes of the other foot. They were instructed to walk forward as fast as possible on an imaginary straight line for about 5 meters. Presence of significant imbalance (stretching out of hands to maintain balance) or falling during the task was considered an abnormal response.

3.4.8. *Finger-to-nose-test (Past pointing test)*

The subject was asked to touch his/her nose tip and clinician's finger tip alternately with his/her index finger. The distance and direction of the clinician's finger was constantly changed. The presence of tremors or undershooting/overshooting of target was considered an abnormal responses on the past pointing test.

All the above tests (audiological & vestibular assessments) were done for the fulfilment of the subject selection criteria. For the fulfilment of the aim of the present study, oVEMPs were obtained from the participants of the present study.

3.4.9. *Recording of oVEMP*

oVEMPs were recorded from both ears of all the participants. For recording oVEMP, participants were instructed to sit in an upright position. A commercially available skin preparing gel was used to scrub the electrode sites and gold plated electrodes (wire length = 1.5 m) were placed with the help of a commercially available conduction paste and surgical plaster. The non-inverting electrode was placed 1 cm below the centre of the lower eye lid, the inverting electrode 2 cm below the non-inverting electrode and ground electrode on the lower forehead. This electrode placement is similar to those used previously (Rosenberg et al, 2005; Chihara et al, 2009; Singh & Barman, 2013, 2014, 2015). The absolute and inter-electrode impedance were maintained below 5 kΩ and 2 kΩ respectively. The contralateral stimulation was achieved through the use of default Etymotic ER-3A insert earphones of the Biologic Navigator Pro evoked potential system. During the recording, the participants were required to fix their gaze at a point kept constant for every participant at an angle of 30°. Further, the participants were also instructed to avoid any movements of head, neck or jaw to avoid adulteration of responses through muscle artifacts.

The ocular VEMPs were recorded using monaural mode of stimulation using alternating polarity short tone-bursts of 250 Hz, 500 Hz, 750 Hz, 1000 Hz, 1500 Hz and 2000 Hz. The intensity was kept constant at 125 dB SPL. The stimuli were ramped using Blackman gating with rise/fall and plateau times of 2 ms and 1 ms respectively and were presented at a repetition rate of 5.1 Hz (Singh et al., 2013). The responses were band-pass filtered between 1 Hz and 1000 Hz (Wang, Jaw, & Young, 2013) and amplified by a factor of 30000. An epoch time of 70 ms, inclusive of pre-stimulus recording of 10 ms, was used and 200 sweeps were averaged per recording. Monoaural stimulation with contralateral eye recordings was employed for recording of oVEMPs. The number of subjects tested with ascending and descending order of frequencies was counterbalanced in order to avoid order effect. Further equal number of participants were tested with right ear first and left ear first in order to overcome the order effects for the ears, if any.

3.5. Response analysis

Two independent experienced audiologists analyzed the responses. The oVEMPs were analyzed along the major parameters of peak-to-peak amplitude and response rate. A ‘present oVEMP’ was defined as an initial negative peak occurring at about 10 ms (8-12 ms) with a subsequent positive peak occurring at about 15 ms (14-18 ms). Conversely, oVEMPs were deemed absent when the biphasic waveform was not observed. The frequency with the largest peak-to-peak amplitude of oVEMP was termnd as "best frequency". This way of deciding frequency tuning (best frequency) is similar to all the previous studies (Piker et al., 2013; Singh & Barman, 2013).

3.6. Statistical analysis

A free public domain software namely SSP (Smith's Statistical Package) was used for Equality of tests of proportion. The descriptive statistical analyses, subsequent statistical procedures and plotting of graphs was done using a commercially available statistical tool- Statistical Package for Social Sciences (SPSS) version 17.0. The following statistical analyses were used.

1. Shapiro Wilk's test of normality was used to check whether the data is normally distributed. Due to non-normal distribution of the data in several age groups and at several frequencies, the non-parametric statistical procedures were subsequently used.
2. McNemar test was used for within group comparisons of the response rate between ears at each frequency and also response rates between frequencies.
3. Friedman's test was used for investigating differences in peak-to-peak amplitude among frequencies in each ear.
4. Statistical analysis using Wilcoxon signed rank test was done for within group between ears comparison of peak-to-peak amplitude. This was also done for pair-

wise comparison of peak-to-peak amplitude between frequencies in the instance of a significant difference found in Friedman's test.

5. The data was subjected to statistical evaluation using Kruskal Wallis test for comparison of peak-to-peak amplitude between groups. Mann-Whitney U test was done when ever Kruskal Wallis test revealed a significant difference among age groups.
6. Equality of test for proportions was used for comparison of response rates between the groups. The same procedure was also used for between groups comparison of proportion of individuals with frequency tuning at a particular frequency.

Chapter 4

Results

The present study was designed with an aim to investigate the effect of age on frequency tuning properties of oVEMP. In order to achieve the aim, oVEMP responses were acquired from both ears of 50 individuals, with 10 individuals in each of the 5 age groups (Group I: 20- 30 years, Group II: 30-40 years, Group III: 40-50 years, Group IV: 50 to 60 years & Group V: > 60 years). Figure 4.1 shows the representative waveforms of oVEMP across the octave and mid-octave frequencies from 250 Hz to 2000 Hz from one individual in each age group.

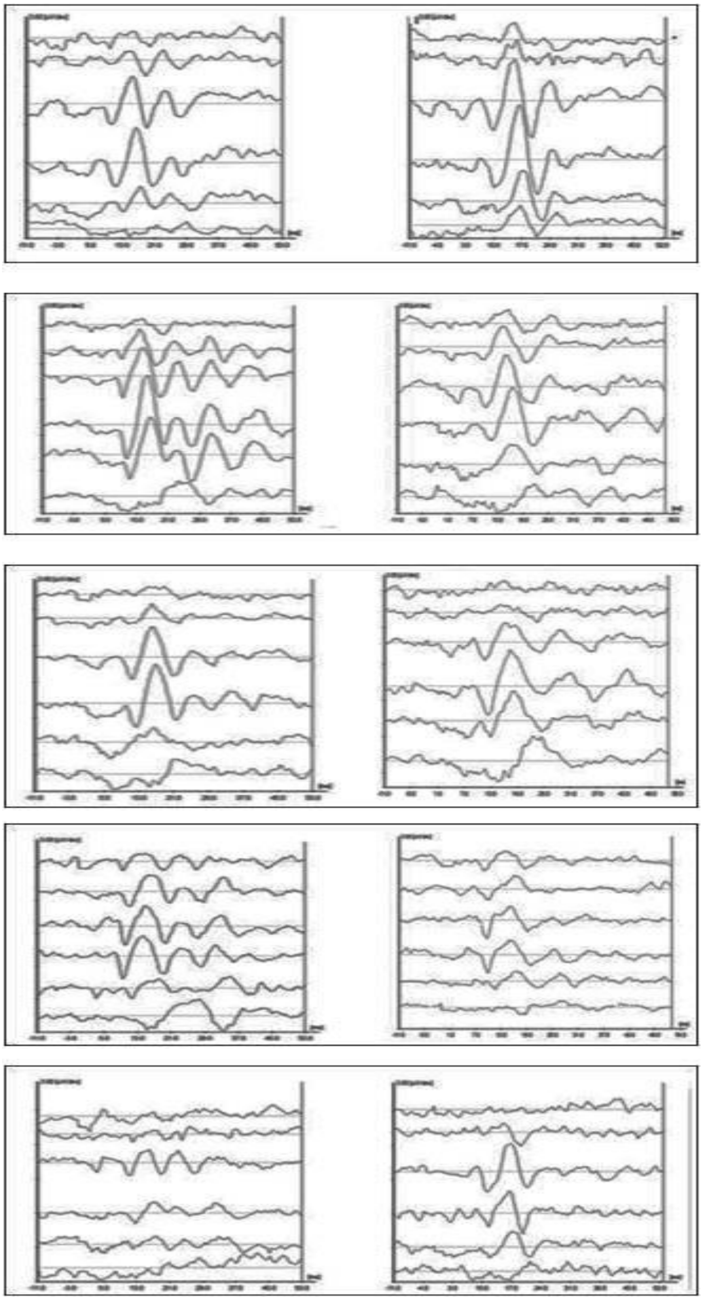

Figure 4.1: The representative oVEMP waveforms of obtained at octave and mid-octave frequencies from both ears of one individual in each of the above mentioned age groups with Group I (20-30 years) displayed in the top-most panel and Group V (> 60 years) displayed in the lowermost panel.

The acquired responses were analysed for response rates, peak-to-peak amplitude and frequency tuning. In order to test whether the data is normally distributed, Shapiro-Wilk's test of normality was used and the results showed non-normal distribution for all the frequencies in all the age groups. Therefore non-parametric statistical procedures were used for further statistical analyses. The results of the study are discussed below under the above mentioned parameters.

4.1. The ear differences in response rate of oVEMP

Response rate was defined as the percentage of ears in which the responses were present at a particular frequency. Table 4.1.1 displays the response rates of oVEMP across frequencies in each of the age groups for both right and left ears. The response rates were compared between the ears in the same group (within group) using McNemar test. The results revealed no significant difference in the response rates between the ears at any frequency in any of the age groups. Table 4.1.1 also displays the outcome of McNemar test for between ears comparison of response rate at each frequency within each of the age groups. At certain frequencies, the dichotomy of data was not found due to presence of responses in 100% of individuals at least in one ear. Since dichotomous data is a major assumption of McNemar test (McNemar 1947), the between ears comparison at these frequencies could not be performed and these are represented as 'CNP' in Table 4.1.2.

Table 4.1.1: The outcome of McNemar test for within group between ears comparison at each frequency

Age (in years)	250 Hz			500 Hz			750 Hz			1000 Hz			1500 Hz			2000 Hz		
	Right RR	Left RR	$\chi^2(1)$	Right RR	Left RR	$\chi^2(1)$	Right RR	Left RR	$\chi^2(1)$	Right RR	Left RR	$\chi^2(1)$	Right RR	Left RR	$\chi^2(1)$	Right RR	Left RR	$\chi^2(1)$
20-30	90	90	10	90	90	10	80	80	10	70	80	5.83	50	60	6.66	40	40	3.40
30-40	90	80	4.44	90	90	0.12	100	100	CNP	90	80	3.40	60	60	3.40	40	50	1.66
40-50	80	80	1.40	90	80	4.44	90	70	2.59	80	70	5.83	50	40	1.66	30	10	0.47
50-60	50	50	3.60	50	40	1.66	50	50	0.40	40	60	4.44	40	40	3.40	10	10	0.12
> 60	30	60	2.85	50	60	6.66	50	60	6.66	40	50	6.66	20	50	2.50	10	20	0.27

Note: ‘RR’- Response rates in %; ‘CNP’- could not be performed.

Thus, the Null hypothesis (H_0) that there is no significant difference in response rates of oVEMP between the ears in each group of healthy individuals is accepted.

4.2. The effect of frequency of tone-burst on response rates of oVEMP

The above statistical analyses (McNemar test) for comparison of repetition rates between the ears revealed no significant ear effect on response rate of oVEMP at any frequency in any age group. Therefore the data of response rates for the ears were combined in each group and are shown in Table 4.2.1.

Table 4.2.1: *Response rates of oVEMP across frequencies in each of the age groups*

Age (in years)	250 Hz		500 Hz		750 Hz		1000 Hz		1500 Hz		2000 Hz	
	N	RR	N	RR	N	RR	N	RR	N	RR	N	RR
20-30	18	90	18	90	16	80	15	75	11	55	8	40
30-40	17	85	18	90	20	100	17	85	12	60	9	45
40-50	16	80	17	85	16	80	15	75	9	45	4	20
50-60	10	50	9	45	10	50	10	50	5	25	2	10
>60	9	45	11	55	11	55	9	45	7	35	3	15

Note: ‘RR’- response rate in %; ‘N’- number of ears with presence of oVEMP.

In order to investigate the effect of frequency on response rate of oVEMP, within group between frequencies comparison of response rates was done using McNemar test. The results revealed that response rates at 1500 Hz and 2000 Hz were significantly lower than those at 500 Hz and 750 Hz in all the age groups, with few exceptions. The results of McNemar test for within group between frequencies comparisons are shown in Tables 4.2.1, 4.2.2, 4.2.3, 4.2.4, 4.2.5 and 4.2.6 for age groups I, II, III, IV and V respectively. The response rates could not be compared between certain frequency pairs due to non-dichotomous data. These comparisons have been mentioned as ‘CNP’ in these tables.

Table 4.2.2: *Outcome of McNemar test for within group between frequencies comparison of response rates in the age group of 20-30 years (Group I)*

Frequency (in Hz)	500	750	1000	1500	2000
250	20.00	8.88	6.66	2.71*	1.48*
500		8.88	6.66	6.01*	1.48**
750			15.00	6.11	3.33**
1000				8.14	4.44*
1500					10.90

Note: ‘*’- $p < 0.05$; ‘**’- $p < 0.01$ & ‘***’-$p < 0.001$.

Table 4.2.3: *Outcome of McNemar test for within group between frequencies comparison of response rates in the age group of 30-40 years (Group II)*

Frequency (in Hz)	500	750	1000	1500	2000
250	12.59	CNP	0.93	5.29*	2.88**
500		CNP	0.39	3.33*	1.81**
750			CNP	CNP	CNP
1000				5.29	2.88**
1500					10.90

Note: ‘*’- $p < 0.05$; ‘**’- $p < 0.01$; ‘***’-$p < 0.001$ & ‘CNP’- could not be performed.

Table 4.2.4: *Outcome of McNemar test for within group between frequencies comparison of response rates in the age group of 40-50 years (Group III)*

Frequency (in Hz)	500	750	1000	1500	2000
250	14.11	9.45	1.66	0.80*	1.25***
500		14.11	3.26	2.88**	0.88***
750			1.66	4.09*	1.25***
1000				5.45*	1.66**
1500					6.11

Note: ‘*’- $p < 0.05$; ‘**’- $p < 0.01$ & ‘***’-$p < 0.001$.

Table 4.2.5: *Outcome of McNemar test for within group between frequencies comparison of response rates in the age group of 50-60 years (Group IV)*

Frequency (in Hz)	500	750	1000	1500	2000
250	5.05	12.80	12.80	13.33	2.22**
500		9.89	9.89	9.73	2.71*
750			12.80	13.33	2.22**
1000				13.33	2.22**
1500					0.33*

Note: ‘*’- $p < 0.05$; ‘**’- $p < 0.01$ & ‘***’-$p < 0.001$.

Table 4.2.6: *Outcome of McNemar test for within group between frequencies comparison of response rates in the age group of >60 years (Group V)*

Frequency (in Hz)	500	750	1000	1500	2000
250	13.38	13.38	7.10	7.21	4.31*
500		20.00	13.38	8.81	2.88**
750			13.38	8.81	2.88**
1000				7.21	0.66
1500					1.55

Note: ‘*’- $p < 0.05$; ‘**’- $p < 0.01$ & ‘***’-$p < 0.001$.

Thus, the Null hypothesis (H_0) that there is no significant effect of frequency of tone-burst on response rates of oVEMP is rejected. Hence the Alternative hypothesis (H_1) is that there is a significant effect of frequency of tone-burst on response rates of oVEMP.

4.3. Effect of age on response rate of oVEMP

Equality of test for proportions was used for between groups comparison of response rates of oVEMP. The results revealed that the age groups upto 50 years demonstrated significantly higher response rates across the frequencies than the age groups above 50 years of age ($p < 0.05$). Further, there was no significant difference in response rates between the age groups of 50-60 years and > 60 years ($p > 0.05$). Also, there was no significant difference in response rates between the groups till 50 years (20-30 years, 30-40 years & 40-50 years) ($p > 0.05$). Tables 4.3.1, 4.3.2, 4.3.3, 4.3.4, 4.3.5 and 4.3.6 show the outcomes of the Equality of test for proportions for proportions between groups comparison of response rates at 250 Hz, 500 Hz, 750 Hz, 1000 Hz, 1500 Hz and 2000 Hz respectively. Figure 4.3.1 displays the response rates across different age groups for different stimulus frequencies.

Table 4.3.1: *Outcome of Equality of test for proportions for between groups comparison of response rates at 250 Hz*

Age (in years)	30-40	40-50	50-60	> 60
20-30	0.47	0.88	2.76***	3.03***
30-40		0.41	2.36*	2.65**
40-50			1.98*	2.28*
50-60				0.31

Note: ‘*’- $p < 0.05$; ‘**’- $p < 0.01$ & ‘***’-$p < 0.001$.

Table 4.3.2: *Outcome of Equality of test for proportions for between groups comparison of response rates at 500 Hz*

Age (in years)	30-40	40-50	50-60	> 60
20-30	0.00	0.47	3.03**	2.47*
30-40		0.47	3.03**	2.47*
40-50			2.65*	2.07*
50-60				0.63

Note: ‘*’- $p < 0.05$ & ‘**’- $p < 0.01$.

Table 4.3.3: *Outcome of Equality of test for proportions for between groups comparison of response rates at 750 Hz*

Age (in years)	30-40	40-50	50-60	> 60
20-30	2.10*	0.00	1.98*	1.68
30-40		2.10*	3.65***	3.40***
40-50			1.98*	1.68
50-60				0.31

Note:‘*’- $p < 0.05$ & ‘***’-$p < 0.001$.

Table 4.3.4: *Outcome of Equality of test for proportions for between groups comparison of response rates at 1000 Hz*

Age (in years)	30-40	40-50	50-60	> 60
20-30	0.79	0.00	1.63	1.93
30-40		0.79	2.36*	2.65**
40-50			1.63	1.93
50-60				0.31

Note: ‘*’- $p < 0.05$ & ‘**’- $p < 0.01$.

Table 4.3.5: *Outcome of Equality of test for proportions for between groups comparison of response rates at 1500 Hz*

Age (in years)	30-40	40-50	50-60	> 60
20-30	0.31	0.63	1.93	1.27
30-40		0.94	2.23*	1.58
40-50			1.32	0.64
50-60				0.69

Note: ‘*’- $p < 0.05$.

Table 4.3.6: *Outcome of Equality of test for proportions for between groups comparison of response rates at 2000 Hz*

Age (in years)	30-40	40-50	50-60	> 60
20-30	0.31	1.38	2.19*	1.77
30-40		1.68	2.47*	2.07*
40-50			0.88	0.41
50-60				0.47

Note: ‘*’- $p < 0.05$.

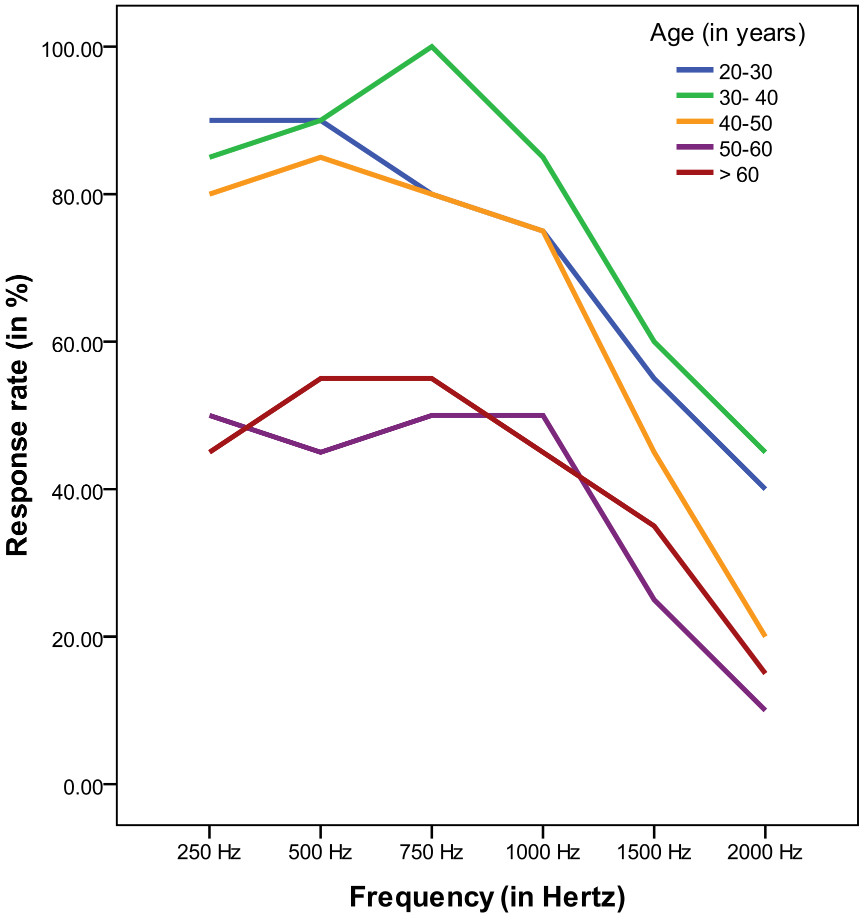

Figure 4.3.1: Response rates of oVEMP across frequencies in different age groups.

Thus, the Null hypothesis (H_0) that there is no significant effect of age on response rates of oVEMP is rejected. Hence the Alternative hypothesis (H_1) is that there is a significant effect of age on response rates of oVEMP.

4.4. The ear differences in peak-to-peak amplitude of oVEMP

The peak-to-peak amplitude of oVEMP of the right and left ear were obtained from all the participants and the data was subjected to descriptive statistics. Both ears appeared to portray similar values of peak-to-peak amplitude. The outcomes of descriptive statistics are shown in Tables 4.4.1 and 4.4.2 for right and left ears respectively.

Table 4.4.1: Mean, Median and standard deviation of peak-to-peak amplitude of oVEMP obtained from right ears of individuals in each age group

Age (in years	250 Hz			500 Hz			750 Hz			1000 Hz			1500 Hz			2000 Hz		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
20-30	1.91	1.66	0.64	3.05	2.69	1.20	4.80	5.77	3.10	4.00	4.12	1.77	2.53	2.14	1.13	1.36	1.36	0.52
30-40	1.84	1.92	0.93	2.63	2.41	1.39	3.66	3.31	2.39	2.58	2.78	2.57	2.23	1.31	1.78	1.55	1.42	0.59
40-50	2.00	1.81	0.69	2.08	2.20	0.58	2.80	2.56	1.44	1.62	1.54	0.83	1.99	1.59	0.80	1.22	1.22	0.37
50-60	2.99	2.96	0.72	2.48	2.35	1.73	4.52	5.37	1.86	3.35	3.77	1.27	2.25	2.29	0.57	2.38	2.38	CNP
>60	2.12	2.04	0.58	2.60	2.69	1.18	3.02	2.44	1.64	3.73	3.49	2.37	2.61	2.61	0.41	1.25	1.25	CNP

Note: 'SD'- standard deviation; 'CNP'- could not be performed.

Table 4.4.2: Mean, Median and standard deviation of peak-to-peak amplitude of oVEMP obtained from left ears of individuals in each age group

Age (in years	250 Hz			500 Hz			750 Hz			1000 Hz			1500 Hz			2000 Hz		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
20-30	1.70	1.71	0.69	3.37	2.29	2.25	4.62	4.53	3.29	4.07	4.10	2.97	2.17	2.18	1.34	2.23	2.20	0.73
30-40	2.43	2.09	0.88	3.56	2.50	2.33	5.35	3.29	5.15	4.07	4.35	2.30	3.19	3.42	1.56	1.96	2.14	0.57
40-50	2.03	2.10	0.76	2.57	2.42	1.25	4.30	3.99	3.27	2.53	2.69	1.94	2.14	1.86	1.39	2.35	2.35	CNP
50-60	1.85	2.14	0.48	2.18	2.32	1.02	4.00	4.12	1.15	3.32	3.36	1.20	2.21	2.09	0.67	0.95	0.95	CNP
>60	2.44	2.13	1.13	3.09	2.64	1.41	3.69	2.97	1.82	3.36	2.23	3.02	2.19	1.83	1.39	2.50	2.50	0.43

Note: 'SD'- standard deviation; 'CNP'- could not be performed.

In order to examine the statistical significance of the above mentioned observations from descriptive statistics, a Wilcoxon signed rank test was done to investigate the ear differences in the peak-to-peak amplitude of oVEMP. The results revealed no significant difference between the two ears at any frequency in any age group. Table 4.4.3 displays the outcome of Wilcoxon signed rank test for between ears comparison of peak-to-peak amplitude of oVEMP in each age group.

Table 4.4.3: Outcome of Wilcoxon signed rank test for between ears comparison of peak-to-peak amplitude of oVEMP in each age group

Frequency (in Hz)	20-30 years		30-40 years		40-50 years		50-60 years		>60 years	
	$\chi^2(1)$	*p*-value	$\chi^2(1)$	*p*-value	$\chi^2(1)$	*p*-value	$\chi^2(1)$	*p*-value	$\chi^2(1)$	*p*-value
250	0.00	1.00	2.20	0.02	0.00	1.00	1.46	0.14	1.34	0.18
500	0.28	0.77	1.78	0.07	0.84	0.40	1.60	0.10	1.21	0.22
750	0.16	0.86	0.53	0.59	0.67	0.49	0.53	0.59	0.94	0.34
1000	0.50	0.61	1.52	0.12	1.35	0.17	0.00	1.00	0.00	1.00
1500	0.67	0.50	1.82	0.06	0.53	0.59	1.34	0.18	0.44	0.65
2000	1.60	0.10	1.06	0.28	CNP	CNP	CNP	CNP	CNP	CNP

Note: 'SD'- standard deviation; 'CNP'- could not be performed.

Thus, the Null hypothesis (H_0) that there is no significant ear difference in peak-to-peak amplitude of oVEMP in each age group of healthy individuals is accepted.

4.5. The effect of frequency of tone-burst on peak-to-peak amplitude of oVEMP

The above statistical analyses revealed no significant ear effect on peak-to-peak amplitude of oVEMP at any frequency in any age group. Therefore the data of peak-to-peak amplitude for the ears were combined in each group and are shown in Table 4.5.1.

Table 4.5.1: *Mean, standard deviation median and variance of peak-to-peak amplitude of oVEMP in each age group*

Age (in years)	**250 Hz**				**500 Hz**				**750 Hz**				**1000 Hz**				**1500 Hz**				**2000 Hz**			
	Mean	Median	SD	Variance	Mean	Median	SD	Variance	Mean	Median	SD	Variance	Mean	Median	SD	Variance	Mean	Median	SD	Variance	Mean	Median	SD	Variance
20-30	1.81	1.68	0.65	0.4	3.21	2.42	1.75	3.0	4.71	4.62	3.09	9.5	4.04	4.12	2.40	5.7	2.33	2.14	1.20	1.4	1.79	1.62	0.74	0.5
30-40	2.21	1.92	0.93	0.8	3.10	2.45	1.92	3.6	4.51	3.29	4.00	16.	3.28	2.78	2.03	4.1	2.71	2.78	1.67	2.8	1.78	2.08	0.58	0.3
40-50	2.01	1.87	0.70	0.4	2.31	2.20	0.96	0.9	3.46	2.64	2.45	6.0	2.05	1.80	1.48	2.1	2.06	1.77	1.03	1.0	1.50	1.40	0.64	0.4
50-60	2.42	2.23	0.83	0.7	2.35	2.35	1.38	1.9	4.26	4.44	1.48	2.2	3.33	3.68	1.16	1.3	2.23	2.15	0.58	0.3	1.66	1.66	1.01	1.0
>60	2.33	2.04	0.95	0.9	2.87	2.69	1.27	1.6	3.38	2.66	1.69	2.8	3.53	2.26	2.59	6.7	2.31	2.17	1.17	1.3	2.05	2.20	0.78	0.6

Note: ‘Hz’- Hertz; ‘SD’ - standard deviation.

A Friedman's test was done to compare the peak-to-peak amplitude among frequencies. The results revealed significant differences among frequencies [$\chi^2(3) = 0.00$, $p < 0.05$]. The frequency of 250 Hz and 2000 Hz were not used due to low response rates ($N \leq 5$) at these frequencies. Since there was a significant difference among the frequencies, further pair-wise analysis using Wilcoxon signed rank test was done. The results revealed that the amplitude at 1500 Hz was significantly smaller than the amplitude at 750 Hz in all the age groups, whereas it was significantly smaller than those at 1000 Hz in the age groups upto 40 years. The results of Wilcoxon signed rank test for within group between frequencies comparison are shown in the Tables 4.5.2, 4.5.3, 4.5.4, 4.5.5 and 4.5.6 for the age groups I, II, III, IV and V respectively.

Table 4.5.2: *Outcome of Wilcoxon signed rank test for within group between frequencies comparison of peak-to-peak amplitude in the age group of 20-30 years (Group I)*

Frequency (in Hz)	750	1000	1500
500	2.22*	1.36	2.40*
750		3.03**	2.93**
1000			2.93**

Note: ''- p<0.05, '**'- p<0.01 & '***'-p<0.001.*

Table 4.5.3: *Outcome of Wilcoxon signed rank test for within group between frequencies comparison of peak-to-peak amplitude in the age group of 30-40 years (Group II).*

Frequency (in Hz)	750	1000	1500
500	2.10*	0.99	1.88
750		3.00**	3.05**
1000			2.93**

Note: ''- p<0.05, '**'- p<0.01 & '***'-p<0.001.*

Table 4.5.4: *Outcome of Wilcoxon signed rank test for within group between frequencies comparison of peak-to-peak amplitude in the age group of 40-50 years (Group III).*

Frequency (in Hz)	750	1000	1500
500	1.96*	1.28	1.26
750		3.18**	2.66**
1000			0.17

Note: ''- p<0.05, '**'- p<0.01 & '***'-p<0.001.*

Table 4.5.5: *Outcome of Wilcoxon signed rank test for within group between frequencies comparison of peak-to-peak amplitude in the age group of 50-60 years (Group IV).*

Frequency (in Hz)	750	1000	1500
500	2.52*	1.40	0.00
750		2.07*	2.24*
1000			1.68

Note: ''- $p<0.05$, '**'- $p<0.01$ & '***'-$p<0.001$.*

Table 4.5.6: *Outcome of Wilcoxon signed rank test for within group between frequencies comparison of peak-to-peak amplitude in the age group of >60 years (Group V).*

Frequency (in Hz)	750	1000	1500
500	1.37	0.88	1.85
750		0.17	2.19*
1000			1.78

Note: ''- $p<0.05$, '**'- $p<0.01$ & '***'-$p<0.001$.*

Thus, the Null hypothesis (H_0) that there is no significant effect of frequency of tone-burst on peak-to-peak amplitude of oVEMP is rejected. Hence the Alternative hypothesis (H_1) is that there is a significant effect of frequency on peak-to-peak amplitude of oVEMP.

4.6. The effect of age on peak-to-peak amplitude of oVEMP

The peak-to-peak amplitude was observed to be largest for younger age groups and reduced subsequently with advancing age. In order to examine the statistical significance of these observations, a Kruskal Wallis test was done for comparison of peak-to-peak amplitude among age groups. The results revealed no significant difference in the peak-to-peak amplitude among the age groups at 250 Hz [$\chi^2(4) = 4.42$, $p > 0.05$], 500 Hz [$\chi^2(4) = 3.00$, $p > 0.05$], 750 Hz [$\chi^2(4) = 2.32$, $p > 0.05$], 1000 Hz [$\chi^2(4) = 8.43$, $p > 0.05$], 1500 Hz [$\chi^2(4) = 1.12$, $p > 0.05$] and 2000 Hz [$\chi^2(4) = 1.50$, $p > 0.05$]. Since there was no significant difference in peak-to-peak amplitude of oVEMP among the age groups at any frequency, further pair-wise analysis (Mann- Whitney U test) was not done.

Hence, the Null hypothesis (H_0) that there is no significant effect of age on peak-to-peak amplitude of oVEMP is accepted.

4.7. The effect of age on the frequency tuning of oVEMP

The frequency resulting in the largest response amplitude was termed as “best frequency” or frequency tuning. Table 4.7.1 provides the percentage of ears with frequency tuning at various frequencies in each age group. The best frequency was observed mainly at 500 Hz, 750 Hz and 1000 Hz, with occasional prevalence of frequency tuning at frequencies above 1000 Hz. There was no individual who demonstrated frequency tuning at 250 Hz. The largest percentage of ears in each group showed frequency tuning at 750 Hz, except the age group of > 60 years. In this age group, the largest percentage of ears demonstrated frequency tuning at 1000 Hz or higher frequencies. Figure 4.7.1 displays the percentage of ears with frequency tuning at each frequency. It should be noted that the number of ears showing presence of response was different among age groups. Since frequency tuning was obtained only from those ears in whom responses were present, the percentages for some of the groups are similar despite the differences in the number of ears with frequency tuning at a particular frequency.

Table 4.7.1: *Percentage of ears with frequency tuning at a particular frequency in each age group*

Age (in years)	N	500 Hz		750 Hz		≥ 1000 Hz	
		N^1	N^1%	N’	N^1%	N$^{’}$	N^1 %
20-30	18	8	44.44%	10	55.55.%	0	0%
30-40	20	6	30%	12	60%	2	10%
40-50	18	8	44.44%	8	44.44%	2	11.11%
50-60	12	1	8.33%	9	75%	2	16.66%
>60	11	3	27.27%	2	18.18%	6	54.54%

Note: ‘N’- number of ears with presence of oVEMP atleast at one frequency; ‘N^1’ – number of ears with frequency tuning at a particular frequency; ‘N^1%$^{’}$ – proportion (percentage) of ears with frequency tuning at a particular frequency out of the ‘N’.

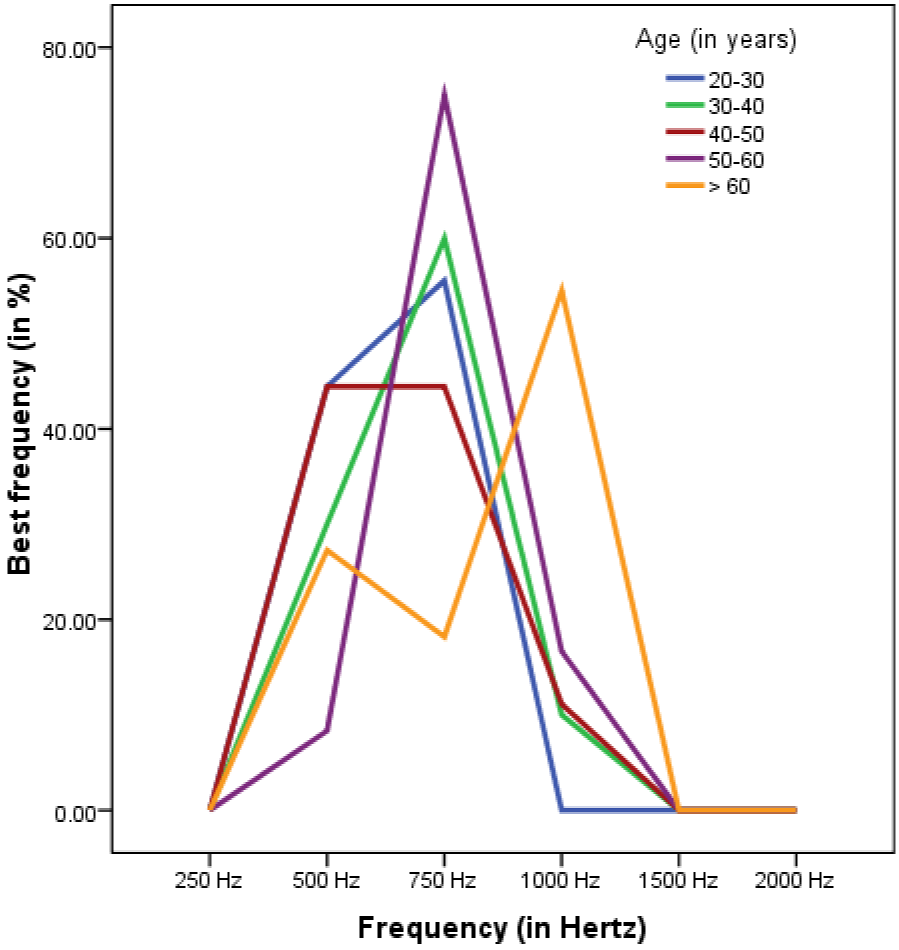

Figure 4.7.1: Proportion of ears in each age group showing frequency tuning (best frequency) at various frequencies.

As can be seen from Table 4.7.1, the percentage of individuals with frequency tuning at different frequencies was different. In order to examine if these differences were statistically significant, Equality of test for proportions was used for between groups comparison of these proportions at each frequency. The proportion of ears with frequency tuning at 1000 Hz or higher frequencies was significantly higher in the age group > 60 years than all the other age groups ($p < 0.05$). There was no significant difference in proportion of ears with frequency tuning at any other frequency between the groups. The results of Equality of test for proportions are shown in Tables 4.7.2, 4.7.3, 4.7.4 for between groups comparison of proportion of ears with frequency tuning at 500 Hz, 750 Hz and 1000 Hz respectively.

Table 4.7.2: *The outcome of Equality of test for proportions for between groups comparison of proportion of ears with frequency tuning at 500 Hz*

Age (in years)	30-40	40-50	50-60	> 60
20-30	0.92	0.00	2.11*	0.92
30-40		0.92	1.43	0.16
40-50			2.11*	0.92
50-60				1.19

Note: ''- $p < 0.05$.*

Table 4.7.3: *The outcome of Equality of test for proportions for between groups comparison of proportion of ears with frequency tuning at 750 Hz*

Age (in years)	30-40	40-50	50-60	> 60
20-30	0.27	0.66	1.08	1.98*
30-40		0.95	0.86	2.23*
40-50			1.65	1.44
50-60				2.72***

Note: ''- p<0.05& '***'-p<0.001.*

Table 4.7.4: *The outcome of Equality of test for proportions for between groups comparison of proportion of ears with frequency tuning at >1000 Hz*

Age (in years)	30-40	40-50	50-60	> 60
20-30	1.37	1.45	1.79	3.51***
30-40		0.11	0.55	2.71***
40-50			0.43	2.53*
50-60				1.90

Note: ''- p<0.05 & '***'-p<0.001.*

Thus, the Null hypothesis (H_0) that there is no significant effect of age on the frequency tuning of oVEMP is rejected. Hence the Alternative hypothesis (H_1) is that there is a significant effect of age on the frequency tuning of oVEMP.

Overall, the results indicate that there is a reduction in the response rate at all the frequencies in the age groups above 50 years. There was also a trend towards reduction in the peak-to-peak amplitude of oVEMP with age, however it was statistically not significant. Frequency tuning was found at 500 Hz or 750 Hz in majority of individuals below 60 years of age and was shifted to 1000 Hz or higher frequencies in majority of individuals above 60 years of age.

Chapter 5

Discussion

The purpose of this study was to investigate the effect of advancing age on frequency tuning of oVEMP in healthy individuals. To fulfil the purpose of the present study, the contralateral oVEMPs were recorded from 50 individuals with normal audio-vestibular system. Response rates, peak-to-peak amplitude and the frequency tuning of oVEMPs were considered as the major parameters across which the ear differences, effect of frequency and aging effect of were assessed.

5.1. Ear differences in response rate and peak-to-peak amplitude of oVEMP

The results of within group between ears comparison of response rate and peak-to-peak amplitude of oVEMP revealed no significant ear effect on both these parameters of oVEMP at any frequency in any age group. These findings could be attributed to the relative symmetry between the ears which has been demonstrated by the findings of ≤ 20 % asymmetry ratio in healthy individuals by a number of studies (Khalil & Kabarity, 2011; Murnane et al., 2011; Piker et al., 2011; Rosengram et al., 2011; Winters et al., 2011; Chihara, Iwasaki, Murofushi, Yagi, Inoue, Fujimotoet al., 2012; Iwasaki et al., 2013; Singh & Barman, 2014, 2015; Singh et al., 2014).

5.2. Effect of frequency of tone-burst on response rate of oVEMP

The response rates at each of the frequencies were compared against those at other frequencies in each age group. The results revealed that response rates at 1500 Hz and 2000 Hz were significantly lower than those at 500 Hz and 750 Hz in most of the age groups. This is in agreement with the previous studies which also reported reduction in response prevalence for higher frequencies (Murnane et al., 2011; Sandhu et al., 2012). Higher response rates for low to mid frequencies might be attributed to low frequency resonance of the otolith organs (Todd et al., 2007). Additionally, some amount of contribution to this could also be made by the middle ear resonance which is likely to enhance the energy between 600 Hz to 1340 Hz (Colletti, 1977; Shanks, 1984; Valvik, Johnsen & Laukli, 1994). Since the higher energy is associated with higher amplitude of oVEMP (Murnane et al., 2011), the higher amplitude could make some of the low amplitude responses that are hidden within the EMG noise more visible and thereby result in higher response rate.

5.3. Effect of age on response rate of oVEMP

When the effect of age on response rate of oVEMP was analysed, the results revealed that the age groups upto 50 years of age had significantly higher response rates across the frequencies than the age groups above 50 years of age. Further, there was no difference in response rates between the age groups of 50-60 years and > 60 years. Also there was no significant difference in response rates between the age groups till 50 years of age (20-30 years, 30-40 years, & 40-50 years). These results are in agreement with those of previous studies (Piker et al., 2013; Asal, 2014). Asal (2014) reported that oVEMPs were present contralaterally in 88% of the healthy participants (44 of 50 ears) and the percentage of individuals with presence of oVEMP decreased with age to 60% in the oldest age group (>55 years).

The reduction in the response rate with advancing age could be attributed to the age related degenerative changes which have been identified from the end organs of the vestibular system to its central nuclei (Johnsson, 1972; Bergstrom, 1973). Previous studies have shown steady decline in the vestibular hair cell counts and densities with advancing age (Merchant et al., 2000). Similar data was also presented for Scarpa's ganglion neuronal counts (Merchant et al., 2000) and vestibular neurons in the vestibular brainstem (Tang et al., 2001-2002). Thus, the well-documented neuro-anatomical and physiological changes due to aging in the peripheral and central vestibular system may explain the commonly reported decrease in the response rate of responses acquired from the vestibular system (Rosenhall, 1973), oVEMP in the case of the present study.

5.4. Effect of frequency of peak-to-peak amplitude of oVEMP

In each of the age groups, the peak-to-peak amplitude were compared between the frequencies. The comparison of peak-to-peak amplitude between the frequencies revealed maximum amplitude at 500 Hz and 750 Hz and a subsequent reduction in the amplitudes on either side of these frequencies thereafter. These findings conform with the findings of the previous studies (Todd et al, 2007; Park et al, 2010; Sandhu et al, 2012), who also reported reduction in the peak-to-peak amplitude of oVEMP with increasing frequencies above 1000 Hz and decreasing below 500 Hz. The findings of the present study are however in disagreement with some of the other studies reported previously in this context (Lewis, Mustain, Xu, Eby, & Zhou, 2010; Taylor et al., 2011; Zhang et al., 2012), all of which reported largest amplitude at 1000 Hz for oVEMP in majority of their subjects.

The differences from the findings of Lewis et al (2010) might be attributed to the use of 10 ms plateau time and 1 ms rise/fall time by these authors as against the use of 0 ms plateau time and 2 ms rise/fall time in the present study. Longer plateau time in Lewis et al (2010) would have caused greater difference in the energy between 500 Hz and 1000 Hz in their study than the present study, thereby showing larger amplitudes at 1000 Hz than 500 Hz in their study as opposed to the other way round in the present study. In addition to the differences in stimulus parameters observed between the two set of studies, the differences might also have been brought about by the large difference in sample size [12 subjects in Lewis et al (2010) as against 50 subjects in the present study].

The differences from Taylor et al (2011) might be attributed to the use of a different unit of intensity level of the stimulus [dB nHL in the study by Taylor et al (2011) as opposed to dB peSPL in the present study]. The dB nHL values across different frequencies considers the middle ear properties in the calibration which results in an increased sound pressure level at mid frequencies. This does not happen when the unit is dB peSPL. These differences would result in variation in the total energy reaching the inner ear for dB nHL than when dB peSPL is used. Energy reaching the inner ear is an important variable which affects the utricular response (Murnane 2011) and therefore Taylor et al (2011) obtained largest amplitude at 1000 Hz.

The differences from the findings of Zhang et al (2012) could be attributed to the differences in the mode of stimulation. They studied the effect of frequencies on the peak-to-peak amplitude through bone-conduction mode of stimulation as against the use of air-conduction mode in the present study. For a bone-conduction mode, the stimulus bypasses the external ear and middle ear and directly stimulates the inner ear (Bekesy, 1954), whereas in an air-conduction mode the stimulus travels through the external ear via the middle ear to the inner ear (Zwislocki, 1975). The resonance characteristics, of especially the middle ear, are concentrated in the low to mid frequencies which might also play a role in enhancing the energy at these frequencies and thereby the amplitude when using air-conduction mode of stimulus presentation.

The findings of maximum amplitude at 500 Hz or 750 Hz have been explained previously on the basis of electrical resonance of the otolothic steriocilia (Welgampola & Colebatch, 2005) and mechanical resonance caused by mass-spring properties within the otolith organs (Todd et al 2000, 2007). The latter explanation has received more support from the studies on the pathologies which cause changes in the finding of largest amplitude from 500 Hz in normals to 1000 Hz in these pathologies (Sandhu et al., 2012). The resonance frequency for human otolith organs has been reported to be in the vicinity of 500 Hz (Hudetz, 1970; Goldberg & Fernandes, 1975).Therefore the oVEMPs show the finding of maximum amplitude at 500 Hz with slight variations in some of the individuals probably causing it to be present at 750 Hz in these healthy individuals.

5.5. Effect of age on peak-to-peak amplitude of oVEMP

The results of the present study revealed no significant effect of age on peak-to-peak amplitude of oVEMP, although the age groups above 50 years demonstrated a trend towards lower amplitude than those below 50 years. The finding of no significant difference in peak-to-peak amplitude of oVEMP between the age groups is in disagreement with most of the previous studies who showed significant reduction in amplitude and significant increase in threshold due to aging (Iwasaki et al., 2008a, 2008b; Nguyen et al., 2010; Tseng et al., 2010; Piker et al., 2013). The finding of no significant effect of age on the peak-to-peak amplitude of oVEMP could be attributed to high variability which is proved by the presence of high values of standard deviation, which was more than 50% of the mean (occasionally almost equal to the mean) and also high values of variance in the obtained data across the age groups. A larger data pool might have been effective in reducing the variance in peak-to-peak amplitude in each of the age groups which might have yielded a clearer picture of the age effects on this parameter. This might be looked at in the future studies.

Although the present study did not reveal any significant difference in the peak-to-peak amplitude between the age groups at any of the frequencies, there was a trend towards higher amplitude in the younger age groups (≤ 50 years) than the older (> 50 years). This could be attributed to the changes in the labyrinthine neural epithelia with advancing age (Rosenhall 1973; Richter 1980) and also age related degenerative changes which has been identified from the end organs of the vestibular system to its central nuclei (Johnsson, 1972; Bergstrom, 1973). The studies have shown a steady decline in the vestibular hair cell counts and densities with advancing age (Merchant et al., 2000). Similar data was also presented for Scarpa’s ganglion neuronal counts (Merchant et al., 2000) and vestibular neurons in the vestibular brainstem (Tang et al., 2001-2002).

However, these changes probably were to a lesser extent which prevented the differences from being significant.

5.6. Frequency tuning of oVEMP

The results of the present study showed that frequency tuning was obtained at 500 Hz or 750 Hz in majority of the individuals in all the age groups until 60 years of age. This is in consonance with those reported previously in this regard (Todd et al., 2007; Winters et al., 2011; Sandhu et al.,2012; Singh & Barman, 2013, 2014). However, these findings are in dissonance with the findings of some of the other studies (Lewis et al., 2010; Taylor et al., 2011; Zhang et al., 2012). The differences from these studies could be attributed to the use of longer plateau times and smaller sample size in the study by Lewis et al (2010), use of dB nHL rather than dB peSPL for stimulus calibration in the study by Taylor et al (2011) and use of bone-conduction mode of stimulation rather than air-conduction mode in the study by Zhang et al (2012), as explained above.

The finding of frequency tuning at 500 Hz or 750 Hz in majority of individuals could be explained on the basis of the second order mechanical system that consists of the components of mass and stiffness which was proposed to explain a similar finding for cVEMP by Todd et al (2001) and later for oVEMP in 2009 by the same authors. The utricle could be considered a second order mechanical system with the mass being contributed by the utricular macula and stiffness by the otolithic membrane of the utricle. As in any mechanical system, the interaction between the mass and stiffness components tends to nullify each other’s impact which causes the structure to resonate at this frequency (Vanhuyse, Creten & Van Camp, 1975; Popelka & Winter, 2013). This characteristic frequency for the otolith organs has been reported to lie in the low-to-mid frequency region (Hudetz, 1970; Goldberg & Fernandes, 1975), in the vicinity of 500 Hz. This could therefore be contributing to the finding of peak in the frequency tuning curve of oVEMP at 500 Hz or 750 Hz in these individuals. Further, there could also be a contribution from the resonance properties of the middle ear which could be altering the energy reaching the utricle in the range of frequencies between 600 Hz and 1340 Hz (Colletti, 1977; Shanks, 1984; Valvik et al., 1994), when using air conduction mode of stimulation. Therefore probably a combination of resonance properties of the middle ear and the otolith organs might be attributed to cause the peak of the frequency tuning curve at 500 Hz or 750 Hz in the majority of the individuals in the present study.

5.7. Effect of age on frequency tuning of oVEMP

The effect of aging on frequency tuning of oVEMP was also explored in the present study. Out of all the frequencies used in the present study, 500 Hz, 750 Hz and 1000 Hz had larger response amplitude than the rest of the frequencies in each individual participant. In the age groups upto 60 years, the frequency tuning was found to be at 500 Hz or 750 Hz in 91.1% of the individuals and at 1000 Hz in 8.82 % of individuals. The frequency tuning at 1000 Hz was in a significantly higher proportion of individuals in the age group of > 60 years (54.5%) than the age groups ≤ 60 years (8.82%). The only previous study, to the best of our knowledge, compared frequency tuning between only three age groups [young adults (18-39 years), middle-aged adults (40-59 years), & older adults (≥ 60 years)]. Although they did report of higher incidence of frequency tuning at 1000 Hz in the older adults, a direct comparison with the present study would be difficult owing to the differences in the way the groups were formed in the present study and that by Piker et al (2013). If the data of the present study is reallocated in the way it was considered by Piker et al (2013), the results of the present are in consonance with their's study. They found frequency tuning in the older age group to be around 62% at 1000 Hz which was nearly similar to the 54.5% in the present study. The best frequencies according to Piker et al (2013) for the middle age group were evenly split between 500 Hz (in 31%), 750 Hz (in 38%), and 1000 Hz (in 31%). The findings in the present study are also in agreement with their study for this age range, with slight differences in the proportion of ears with frequency tuning at 750 Hz and 1000 Hz. In the current study, the best frequencies for the middle age group was 500 Hz (in 30%), 750 Hz (in 56.6%), and 1000 Hz (in 13.3%). These slight differences might be attributed to the differences in the sample size between the studies [13 in Piker et al (2013) as against 40 in the middle-aged group of the present study]. For the young adults, the majority of participants (nearly 92%) showed the best amplitudes at either 500 Hz or 750 Hz with only one participant showing the best frequency at 1000 Hz in the study by Piker et al (2013). In the present study also, the frequency tuning for the young adults was either at 500 Hz or 750 Hz in majority of individuals (nearly 96%) with only two participants showing the best frequency at 1000 Hz. Thus in general, there seems to be agreement in the findings of age and frequency interaction between present study and those of Piker et al (2013).

The frequency tuning of the vestibular end organs has been attributed to the inertial and elastic properties of these end organs, the mechanical resonance of the stereocilia, and/or the electrical tuning of the hair cells (Fernandez & Goldberg, 1976; Crawford &

Fettiplace, 1981; Holton & Hudspeth, 1983; Fettiplace & Fuchs, 1999). However as stated earlier, the utility of the second order mechanical model in explaining frequency tuning of oVEMP has received wider acceptance (Todd et al., 2001,2009). The resonant frequency is a function of the mass and stiffness characteristics of a system (Vanhuyse et al., 1975; Popelka & Winter, 2013), which arise out of the utricular macula and the utricular membrane, respectively in case of utricle. The resonant frequency is directly proportional to the square root of stiffness and inversely proportional to the square root of mass (Vanhuyse et al., 1975; Popelka & Winter, 2013)This means that smaller the mass of the macula and/or higher the stiffness of the utricular membrane, higher will be the resonance frequency. This perspective, along with the understanding of age related changes in the utricle, could be helpful in understanding why the frequency tuning shifts to higher frequencies in older adults. Previous studies have shown that the mass of the utricular macula reduces as a result of degeneration associated with aging (Johnson 1972; Walther & Westhofen, 2007). Since the resonance frequency is inversely proportional to the square root of mass (Vanhuyse et al., 1975; Popelka & Winter, 2013), the reduction in the mass of the utricular macula would cause an increase in the resonance frequency of the utricle, which is largely accepted as the generator end organ for oVEMP. This would therefore enhance the amplitude in the higher frequencies in older adults and reduce the amplitude in the lower frequencies where the resonance was occurring in the younger age groups. Thus, the changes in aging-associated anatomy in the utricular macula could be attributed to the shift the frequency tuning from 500 Hz or 750 Hz in individuals below 60 years of age to ≥ 1000 Hz in individuals > 60 years of age.

Chapter 6

Summery and Conclusion

Ocular VEMP is an excitatory muscle potential which can be recorded from the inferior extraocular muscles using the surface electrodes placed on the skin overlying the inferior oblique muscle (Todd et al., 2007). It is characterized by an initial negative peak at 10–12 ms (n10 or n1) and a subsequent positive peak at 15-20 ms (p15 or p1) post stimulus onset (Rosengren et al., 2005; Walther et al., 2011). In addition to being easier to perform and being less taxing on the patient than cVEMP, oVEMP also provides information regarding the vestibulo-ocular reflex (VOR) pathway (Welgampola et al., 2009), which makes it a complementary test rather than supplementary test to cVEMP.

Several oVEMP parameters have been found to be useful in the diagnosis of vestibular pathologies. The diagnosis of a pathology that has enhanced the utility of oVEMP is Meniere's disease (Sandhu et al., 2012; Winters et al., 2012; Jerin et al., 2014). While there is considerable overlap between Meniere's disease and other vestibular pathologies when using latency, amplitude or asymmetry ratio of oVEMP (Moon et al., 2012; Taylor et al., 2011; Singh & Barman, 2015), shift in frequency tuning to ≥ 1000 Hz has been recently reported to be unique to Meniere's disease (Taylor et al., 2011). The reason behind the shift in frequency tuning in Meniere's disease is believed to be caused by the changes in the balance between mass and stiffness components (increase in stiffness) within the utricle owing to accumulation of excessive endolymph (Winters et al., 2011; Sandhu et al; 2012). The balance between these components could also be potentially tilted due to aging, age-related reduction in the mass of macula has been shown by previous studies (Johnson 1972; Walther & Westhofen, 2007). Resonance frequency being inversely proportional to the square root of mass (Vanhuyse et al., 1975; Popelka & Winter, 2013), there might be a likelihood of the shift in frequency tuning peak towards higher frequencies in older individuals. This was indeed shown in a study by Piker et al (2013) who demonstrated frequency tuning at 1000 Hz in older adults (≥ 60 years). However, the study used only a small sample size (N = 39) in the age range of 18-88 years and distributed them into three groups such that only 13 individuals formed each group and the age spans of these groups were very wide. Therefore there is a need for studies with better control of these parameters. Thus, the present aimed at investigating the effect of aging on the frequency tuning of oVEMP.

To fulfill the aim and objective of the present study, 50 healthy individuals were grouped under five age groups (Group I: 20 to 30 years; Group II: 30 to 40 years; Group III: 40 to 50 years; Group IV: 50 to 60years and Group V: > 60 years), each covering a span of 10 years except for the fifth group which covered a span of 20 years. The responses were obtained using air-conduction alternating tone bursts of 250 Hz, 500 Hz, 750 Hz, 1000 Hz, 1500 Hz and 2000 Hz (rise/fall time of 2 ms & plateau of 1 ms; intensity of 125 dB pe SPL). The repetition rate of the tone-burst presentation was set to 5.1 Hz. The responses were recorded using surface electrodes with the non-inverting electrode placed 1 cm below the centre of the lower eyelid of the contralateral eye, inverting electrode 2 cm below non-inverting and ground on the forehead. The recorded myogenic activity was band-pass filtered between 1 and 1000 Hz and was amplified by a factor of 30000. The analysis window was set to 64 ms, which included a pre-stimulus baseline recording of 10 ms. A total of 200 sweeps were taken for obtaining the averaged oVEMP waveforms.

The response rate, peak-to-peak amplitude and frequency tuning were obtained from both ears of each participant. A McNemar test was used for within group between ears comparisons of the response rate at each frequency and also between frequencies. Friedman’s test was used for investigating the differences in peak-to-peak amplitude among frequencies in each ear. Wilcoxon signed rank test was done for within group comparison of peak-to-peak amplitude between ears and also between frequencies. Kruskal Wallis test was done for comparison of peak-to-peak amplitude between the groups. The between groups comparison of response rates and the between groups comparison of proportion of individuals with frequency tuning at a particular frequency was done using Equality of test for proportions.

The response rate and peak-to-peak amplitude of oVEMP revealed no significant ear effect at any frequency in any age group. These findings could be attributed to the relative symmetry between the ears which has been demonstrated by the findings of ≤ 20% asymmetry ratio of oVEMP in healthy individuals by a number of studies (Chihara et al., 2012; Singh & Barman, 2015).

The response rates at 1500 Hz and 2000 Hz were found to be significantly lower than those at 500 Hz and 750 Hz in almost all the age groups. Higher response rates in the low to mid frequencies might be attributed to low frequency resonance of the otolith organs (Todd et al., 2009).

Further, the results revealed that the age groups upto 50 years had significantly higher response rates across the frequencies than the age groups above 50 years. There was no significant difference in response rates between the age groups 50-60 years and > 60 years. Also there was no significant difference in response rates between the groups till 50 years of age (20-30 years, 30-40 years, & 40-50 years). The reduction in the response rate after 50 years could be attributed to the age related degenerative changes which has been identified from the end organs of the vestibular system to its central nuclei (Johnsson, 1972; Bergstrom, 1973). These include a steady decline in the vestibular hair cell counts and densities with advancing age (Merchant et al., 2000), reduction in the Scarpa's ganglion neuronal counts (Merchant et al., 2000) and reduction in the numbers of vestibular neurons in the vestibular brainstem (Tang et al., 2001-2002).

The comparison of peak-to-peak amplitude between the frequencies revealed maximum amplitude at 500 Hz and 750 Hz and a subsequent reduction in the amplitudes on either side of the frequency thereafter. The finding of maximum amplitude at 500 Hz or 750 Hz can be explained on the basis of electrical resonance (Welgampola & Colebatch, 2005) and mechanical resonance caused by mass-spring properties within the otolith organs (Todd et al 2001, 2009). The mass-spring model appears better suited to explain these findings as this can also explain the changes caused by certain pathologies.

The results of present study revealed no significant effect of age on peak-to-peak amplitude of oVEMP, although the age groups above 50 years demonstrated a trend towards lower amplitude than those below 50 years. In the current study, an age effect on the peak-to-peak amplitude was not seen. This could be attributed to high variability in the data which was substantiated by the presence of high values of variance in the obtained data for peak-to-peak amplitudes across the age groups.

For the frequency tuning, the best frequency was observed mainly at 500 Hz, 750 Hz and 1000 Hz with occasional prevalence of frequency tuning at frequencies above 1000 Hz, in all age groups, except the age group of > 60 years. In this age group, (> 60 years) the largest percentage of ears demonstrated frequency tuning at 1000 Hz or higher frequencies. These findings could be attributed to age related degenerative changes in the vestibular system after 50 years (Johnson 1972; Walther & Westhofen, 2007). These studies reported reduction in the number of otoconia and the consequent reduction in the mass of the utricular macula which would potentially affect the resonant frequency by virtue of altering the balance between mass and stiffness characteristics within the utricle. Therefore there would be a likelihood of a change in resonant frequency and a consequent change in frequency tuning could take place due to aging.

Thus to conclude, the response rates and peak-to-peak amplitude were independent of the ear being stimulated. Stimulus frequency of tone-burst had an effect on the response rates and peak-to-peak amplitude of oVEMP, with highest response rates and largest amplitudes coinciding at 500 Hz or 750 Hz. There was a significant decline in the response rate across the frequencies with advance of age beyond 50 years. The peak-to-peak amplitude of oVEMP showed a trend towards decrement with increase in age beyond 50 years, although it was statistically not significant. Aging had an effect on frequency tuning of oVEMP, with frequency tuning shifting to 1000 Hz or higher frequencies in majority of individuals over 60 years of age as opposed to 500 Hz or 750 Hz below it.

Frequency tuning has been shown to be useful in the diagnosis of some peripheral vestibular pathologies like Meniere’s disease and superior canal dehiscence (Sandhu et al., 2012; Taylor et al., 2011). However since Meniere’s disease is more prevalent in the older age groups, contribution of advancing age to frequency selectivity cannot be completely eliminated. Hence the present study provides age appropriate norms for obtaining frequency tuning which can be used as a correction factor for an appropriate diagnosis in patients with Meniere’s disease. Also, it provides information on electrophysiological correlates of the anatomical and physiological changes within the utricle due to aging.

The outcome of the study were limited by the use of a smaller sample size in each group (N=10). Further, the study did not account for middle ear transfer function. This was because a previous study done by Piker et al (2013) did showed no significant relation between the frequency tuning of oVEMP and the middle ear transfer function. Using similar methodology and considering more number of participants, the effect of age on the peak-to-peak amplitude and frequency tuning could be considered by future studies.

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Appendix A

Informed Consent Form

Information regarding the study

I HusnaFirdose, student [MSc-Audiology], am doing my masters dissertation on the topic entitled **"Impact of advancing age on Ocular vestibular evoked myogenic potentials in healthy individuals"** under the guidance of Mr.Niraj Kumar Singh, Lecturer in Audiology. The evaluations used in the study are meant for assessing the organ of hearing as well as balance and outcomes will provide information about the statoacousticalwell being of a person. The procedures used during the study are not harmful in anyway and are approved by the governing health agencies all over the world.

Signature

(HusnaFirdose)

To be filled by the participant

I (Name, age and gender) have been explained about the procedure, its benefits and outcomes. I am also aware of the consequences (if any) and that the information disclosed will be used for publications. I am willing to participate in the study on a non-payment basis.

Left hand thumb impression/Signature

Appendix B

Neuro – Otological (Proforma)

Name: **Occupation:** **Date:**

O.P Number: **SEX: M/F** **Age:**

Address: **BP:**

Patient to tick only

1. VERTIGO:

a. Swaying	( )	
b. Light headed	( )	
c. Turning	( ) R	( ) L
d. Falling	( ) R	( ) L
e. Falling	( ) F	( ) B
f. Blackouts	( )	
g. Uncertainty	( )	
h. Unconsciousness	( )	

2.VEGETATIVE SYMPTOMS:

a. Sweating	( )
b. Nausea	( )
c. Retching	( )
d. Vomiting	( )
e. Collapse	( )

3. TRIGGER MECHANISM:

a. Motion Sickness ( )

b. Turing of the head ( )

c. Bending down ( )

d. Standing up ( )

e. Gazing ( ) R ( ) L

4. DURATION OF SYMPTOMS:

a. Hours ( )

b. Days ( )

c. Weeks ( )

d. Months ( )

e. Years ( )

f. Decades ( )

5. DURATION OF SINGLE ATTACK AND FREQUENCY:

	D	**F**
a. Few Seconds	**( )**	**( )**
b. Minutes	( )	( )
c. Hours	( )	( )
d. Days	( )	( )
e. Weeks	( )	( )
f. Months	( )	( )

g. Long continuous ( ) ( )

h. Long but off and on ( ) ( )

6. SMELL DISORDERS:

a. Anosmia ( )

b. Parosmia ( )

7. EYE DISORDERS:

a. Blurring

b. Double vision

smooth jerky

c. Feeling of objects Moving ( ) ( ) ( )

e. Blindness ( ) ( ) R ( ) L

8. EAR SYMPTOMS:

a. Tinnitus ( ) R ( ) L ( )

b. Deafness ( ) R ( ) L ( )

c. Fullness ( ) R ( ) L ( )

d. Post OP ( ) R ( ) L ( )

9. TASTE DISORDERS:

a. Ageusia ( )

b. Parageusia ( )

10. TRIGEMINAL NEVE AFFECTATION:

Right ( )

Left ( )

11. FACIAL PARALYSIS:

Peripheral ( ) R ( ) L ( )

Central ( ) R ( ) L ( )

12. HEAD AND NECK TRAUMA:

a. Vehicular acceleration ( )

b. Occupational acceleration ( )

c. Sport Injury ( )

d. Domestic acceleration (**)**

13. NEUROLOGICAL DISORDERS:

14. CARDIO VASCULAR DISORDERS:

a. Hypertension ( )

b. Hypotension ( )

c. Arterio Sclerosis ( )

d. Myocardial ischaemia ( )

e. Status after infarct ( )

15. DIABETES MELLITUS:

16. KIDNEY DISORDERS:

17. DRUGS PROLONGED USE:

a. Alcohol ( )

b. Nicotin ( )

c. Caffeine ( )

d. Salicylates ( )

e. Steptomycin ( )
f. Gentamycin ( )
g. Contraceptives ( )
h. Antivertiguions ( )
i. Others ( )

18. KNOWN ALLERGY:

19. PROGRESS OF SYMPTOMS:

a. Unchanged ( )
b. Slightly better ( )
c. Much better ( )
d. Slightly Worse ( )
e. Much Worse ( )

20.OTHERS:

1. LOCAL EXAMINATION:

(R) (L) (Positive - Findings only)

a. Ear
b. Nose
c. Tongue
d. Larynx
e. PNS
f. VI Nerve
g. Palate

h. Gag