UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING AND THE ENVIRONMENT

INSTITUTE OF SOUND AND VIBRATION RESEARCH

Objective Vestibular Testing for Balance Function

by

Faten Saeed Obeidat

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ABSTRACT

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Objective Vestibular Testing for Balance Function

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Objective testing of balance function is crucial for clinical applications that diagnose and monitor the progression of balance disorders. In recent years, a number of new objective testing methods have been developed, including cervical and ocular vestibular evoked myogenic potentials (VEMPs) and the video head impulse test (vHIT). However, the clinical application of these new methods is still being studied. Although methods are often described as objective, in many cases the interpretation of data still relies upon subjective visual interpretation, and so results are highly dependent upon clinical expertise. A statistical analysis of test results can reduce the need for subjective (visual) interpretation. In addition, novel stimulation paradigms have the potential to improve measurement methods by reducing test time or increasing sensitivity to vestibular disorders. The current thesis has two main objectives: 1) to improve objective testing methods through improved stimulation and analysis of responses and 2) to apply the new methods to clinical populations and to compare them with other recently developed objective test approaches.

The key findings of this thesis are:

- Responses can be recorded from the sternocleidomastoid (SCM) muscle with 500 Hz tone-bursts at high stimulation rates, but not in many subjects. The optimal trade-off between recording time and response detection for the majority of subjects appears to be a rate of 10 Hz.
- The onset of the stimulus generates the cVEMP response, so increasing tone-burst durations at the same peak level has little effect on the VEMP.
- cVEMP responses can be objectively detected by using statistical approaches, such as a Hoteling's T^2 test, at significantly lower thresholds than those obtained through subjective inspection by experienced audiologists. Statistical testing is a sensitive and efficient method that could replace subjective estimates for detecting the presence of cVEMP responses. This study was the first to objectively estimate the frequency-tuning curve of the saccule using statistical approaches in both healthy subjects and Ménière's disease (MD) patients.
- Electrocochleography (ECochG) and cVEMP tests failed to pick up some cases that fulfilled American Academy of Otolaryngology–Head and Neck Surgery (AAO-HNS) criteria for definite MD. However, ECochG was more sensitive to the disease when patients were symptomatic during test recording.
- Although a statistical analysis was not performed for the cochlear implant (CI) study, due to the small sample size, this preliminary study highlighted the importance of evaluating the function of the otolith organs prior to implantation, as the otoliths appear more affected by implantation than the semi-circular canals. However, larger clinical studies would be necessary to confirm these findings.

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Declaration of authorship

I, Faten Obeidat, declare that the thesis entitled "Objective vestibular testing for balance function" and the work presented in the thesis are both my own and have been generated by me as the result of my own original research.
I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as:
    - Obeidat, F., and Bell, S. (2016). Vestibular Evoked Myogenic Potential Responses to Amplitude-Modulated Tones. Poster presented at the British Society of Audiology annual conference, United Kingdom, 25th – 27th April 2016.

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- Obeidat, F. S., & Bell, S. L. (2019). Comparing the sensitivity and specificity of cervical vestibular evoked myogenic potential and electrocochleography in the diagnosis of Ménière's patients. *International Journal of Audiology* (in press).
- Obeidat, F. S., & Bell, S. L. (2019). An exploration of vestibular function pre and post unilateral cochlear implantation. *Cochlear Implants International* (under review).
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Signed…….....

Date……

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List of abbreviations

AAO-HNS	: American Academy of Otolaryngology Head and Neck surgery
ABR	: Auditory Brainstem Response
AC	: Air Conduction
ACS	: Air Conducted Sound
AEP	: Auditory Evoked Potentials
AM	: Amplitude Modulation
AP	: Action potential
AR	: Asymmetry Ratio
ART	: Acoustic Reflex Threshold
ASSR	: Auditory Steady-State Response
Avg	: Average
AVN	: Acute Vestibular Neuritis
BC	: Bone Conduction
BCV	: Bone Conducted Vibration
BM	: Basilar Membrane
BPPV	: Benign Paroxysmal Positional Vertigo
CF	: Carrier Frequency
CHL	: Conductive Hearing Loss
CI	: Cochlear Implant
CM	: Cochlear Microphonic
cVEMP	: Cervical Vestibular Evoked Myogenic Potential
dB	: Decibel
dB A	: A-weighted decibels
dB LAS	: A-weighted sound level with a slow time constant
dB LEQ (A)	: A-weighted Equivalent Continuous Sound Level
dB nHL	: Decibels above Normal Hearing Level

List of abbreviations

dB p.e. SPL	: Decibel Peak Equivalent Sound Pressure Level
dB SPL	: Decibel Sound Pressure Level
ECochG	: Electrocochleography
EMG	: Electromyography
ENG	: Electronystagmography
ET	: Extra-Tympanic
FFT	: Fast Fourier Transform
FPR	: False Positive Rate
F_{SP}	: F value at a single point. A statistical method was developed by Elberling and Don (1984) for objective evaluation of the quality of averaged auditory brainstem responses.
FM	: Frequency Modulation
HIT	: Head Impulse Test
HT^2	: Hotelling's T^2 test
IHCs	: Inner Hair Cells
LA	: Left Anterior
LARP	: Left Anterior-Right Posterior
LL	: Left Lateral
LLpK (peak)	: True peak level of the input signal in dB
LP	: Left Posterior
L	: Left
M	: Mean
MASTER	: Multiple Auditory Steady-State Responses
MD	: Ménière's disease
MF	: Modulation Frequency
ms	: Millisecond
mV	: Millivolt
µs	: Microsecond
N	: Negative
n	: Sample size

oVEMP	: Ocular Vestibular Evoked Myogenic Potential
P	: Positive
PTA	: Pure Tone Audiometry
RA	: Right Anterior
RALP	: Right Anterior-Left Posterior
RL	: Right Lateral
RP	: Right Posterior
R	: Right
RWA	: Round Window Approach
S	: Second
SCCs	: Semi-Circular Canals
SCD	: Superior Canal Dehiscence
SCM	: Sternocleidomastoid
SD	: Standard deviation
SE	: Standard error
SNHL	: Sensorineural Hearing Loss
SNR	: Signal-to-Noise Ratio
SP	: Summating Potential
S-VEMP	: Steady State VEMP
TT	: Trans-tympanic
USVN	: Unilateral Superior Vestibular Neuritis
uV	: Microvolt
V	: Volt
VCR	: Vestibulo-Collic Reflex
VEMP	: Vestibular Evoked Myogenic Potential
vHIT	: Video Head Impulse Test
VM	: Vestibular Migraine
VN	: Vestibular Neuritis
VNG	: Videonystagmography
VOR	: Vestibulo-Ocular Reflex
VPM	: Vibrotactile Perception Meter
VRBQ	: Vestibular Rehabilitation Benefit Questionnaire

Chapter 1 : Introduction

1.1 Motivation for the research

Vestibular evoked myogenic potentials (VEMPs) are short-latency myogenic potentials elicited by stimulating the ear with high-level air-conducted sound (ACS), bone-conducted vibration (BCV), forehead taps or electrical stimulation, and can be recorded by placing surface electrodes over muscles (Rosengren et al., 2010; Young, 2013). The term myogenic is used to indicate the origin of the response from the averaged electrical activity of the muscle; this is confirmed by the observation that the response does not occur during muscle relaxation (Jacobson & Shepard, 2008; Rosengren et al., 2010). Afferent neurons of the otolith organs activate reflexive electromyographic (EMG) activity of the cervical and ocular muscles, which can be recorded using surface electrodes (Kantner and Gürkov, 2012).

VEMPs recorded from the contracted sternocleidomastoid (SCM) muscle are referred to as cervical VEMPs (cVEMPs), while those recorded from the eye muscles are ocular VEMPs (oVEMPs) (Piker et al., 2013; Young, 2013). Stimulation of the saccular afferents with a high-level of either ACS or BCV causes EMG activity of the contracted SCM muscle as a manifestation of the vestibulo-collic reflex (VCR), in normal human subjects (Rosengren et al., 2010). cVEMP is widely used in clinical practice as an objective technique for measuring the function of otolith organs, predominately the saccule (Kantner and Gürkov, 2012). Activation of utricular afferents by either ACS or BCV results in extraocular muscle activity as a manifestation of the vestibulo-ocular reflex (VOR) in normal human subjects (Rosengren et al., 2010). Although it is still unclear whether the utricle or the saccule is responsible for oVEMP and cVEMP responses, there is clinical agreement that cVEMPs predominantly reflect saccular function, while it is likely that oVEMPs are mainly elicited in response to utricular activation. These two new electrophysiological methods can be clinically helpful in providing diagnostic information regarding the function of the otolith organs.

Tuning investigations have established that the VEMP response is, to an extent, frequency specific, and low frequency tones (such as 400-500 Hz tone-bursts) evoke a peak response (largest amplitude and lowest threshold) compared to mid-

frequency tones such as 1 KHz and 2 kHz tone-bursts (Marimuthu and Harun, 2016). Thus, low frequency tone-bursts are generally used to evoke a VEMP response. For ACS-evoked VEMPs, clicks and tone-bursts are the most widely used stimuli. Click stimuli have a broad spectral content with rapid onset, so they may not be optimal for frequency specific stimulation, as a large area on the basilar membrane (BM) is activated with a broad range of frequencies (Katz et al., 2015). Compared to click stimuli, tone-bursts are narrow spectrum stimuli. Such stimuli are intended to produce energy at a single characteristic frequency on the BM, with little distribution of energy at other frequencies. However, tone-burst stimuli have a brief onset, which may cause a spectral splatter of acoustic energy to other unwanted frequencies. As a result, the response may not be representative of the characteristic frequency. Despite this, a short stimulus is needed to elicit a VEMP, so spectral splatter of the stimulus cannot be completely avoided. Recently, it has been reported that VEMPs can also be evoked using amplitude modulated (AM) tones, which are commonly used in Auditory Steady-State Response (ASSR) as regularly-repeating stimuli. In the research literature to date, only two studies have demonstrated that a VEMP from the SCM muscle can be obtained at 500 Hz and modulated at different frequencies (Bell et al., 2010; Oliveira et al., 2014). VEMPs evoked by AM tones are referred to as steady-state VEMPs (S-VEMPs). The motivation behind using this method lies in the fact that an AM VEMP is more frequency-specific than the tone-bursts commonly used in standard VEMP. However, there is insufficient normative data for VEMPs evoked by AM tones and more research is needed on this topic.

Since there is no standard for VEMP testing, there is no agreement among experts on the best stimulus parameters for eliciting a robust VEMP response (Eleftheriadou and Koudadounarakis, 2011). It has been reported that VEMPs are affected by the duration of the stimulus (Meyer et al., 2015; Singh et al., 2014). A few studies have investigated the effects of different rise/fall times and plateau durations of 500 Hz tone-burst stimuli on the amplitude and the latency of cVEMP response in AC mode (Cheng & Murofushi, 2001a, 2001b; Marimuthu & Harun, 2016; Singh et al., 2014b). Singh et al. (2014b) found that a 2 ms rise/fall time and 1 ms plateau duration yielded the largest amplitude of cVEMP response, while others have suggested a two-cycle rise/fall and no plateau (Welgampola & Colebatch, 2005; Wuyts et al, 2007; Young, 2006). Cheng and Murofushi (2001a, 2001b) concluded that a 1 ms rise/fall time and a 2 ms plateau duration would elicit the best possible cVEMP response to 500 Hz tone-burst stimuli. In a recent systematic review, Meyer at al. (2015) concluded that this was preferred for the clinical recording of cVEMP

in AC mode. In addition, previous studies have also reported that the rate at which the stimulus is delivered affects the VEMP response (Carnaúba et al., 2013; van Tilburg, 2016). Many studies have used a repetition rate of 5 Hz to evoke AC VEMP for different purposes (Akin et al., 2003; Govender et al., 2011; Park et al., 2010; Piker et al., 2013; Todd et al., 2000; Young, 2006). A few studies have explored the effects of different repetition rates on cVEMP in AC mode using tone-burst stimuli (Carnaúba et al., 2013; van Tilburg, 2016). Carnaúba et al. (2013) reported that a repetition rate of 10.2 Hz would elicit a robust cVEMP response for 500 Hz tone-burst stimuli. However, Van Tilburg et al. (2016) concluded that a rate of 5 Hz yielded the best VEMP response. Previous studies have not explored the effect of very high repetition rates on the cVEMP response. High repetition rates require a shorter recording time than low repetition rates for the same number of averages, so using high rates to evoke cVEMP could potentially lessen fatigue and the need for the subject to maintain neck tension.

Previous studies have subjectively explored the effects of rate and duration on ACS VEMPs by measuring peak-to-peak amplitude and peak latency. None have used objective (statistical) methods to assess the quality of responses. The conventional approach for evaluating the presence of VEMP responses is visual inspection by the audiologist of two or more runs to identify replications of significant peak and trough components in the waveform. Different criteria have been used in studies reported in the literature to visually judge the presence of a VEMP response. This visual evaluation is problematic when the signal-to-noise ratio (SNR) is poor due to a small response relative to the physiological background noise. Thus, caution is required when visually identifying the presence and absence of the VEMP response clinically. To the author's knowledge, objective detection of VEMP responses using standard statistical evoked response detection methods has not been performed. To estimate the SNR of other evoked responses, several statistical approaches have been explored and used for automated detection of evoked potential responses: Elberling and Don (1984) proposed the F_{sp} (F at a single point) statistic as an objective estimate of response quality that increases with SNR. Lv et al. (2007) proposed a bootstrap approach, which was used to determine whether the value of F_{sp} indicated a significant response. More recently, the Hotelling's T^2 test (HT2) (Hotelling, 1931) was used by Chesnaye et al. (2018) to objectively detect auditory brainstem response (ABR).

Similarly, objective measures to evaluate the tuning curve of the saccule in patients with Ménière's disease (MD) have not yet been explored. As the afferents from the

saccular macula give rise to the cVEMP response, a distended saccule can result in an alteration in the mechanics of its motion, which causes an alteration in the frequency tuning of the saccule (Rauch et al., 2004). Previous studies on healthy subjects have reported that cVEMP showed frequency tuning, with the greatest sensitivity (lowest threshold and highest amplitude) occurring at 500 Hz (Akin et al., 2003; Murofushi et al., 1999; Park et al., 2010; Rauch et al., 2004; Todd et al., 2009). Others have suggested that tone-bursts at 700 Hz (Welgampola and Colebatch, 2001), between 500 Hz and 700 Hz (Node et al., 2005), or between 300 Hz and 350 Hz (Todd et al., 2000) would elicit a robust cVEMP response. These discrepancies in the tuning frequency might be related to either differences in the stimulus duration of the tone-bursts used for stimulation or variability in the criteria used to visually judge the presence of a cVEMP response. Several studies have suggested that the saccular tuning curve for MD-affected ears has either been lost or shifted to higher frequencies (Rauch et al., 2004; Timmer et al., 2006).

Electrocochleography (ECochG) has been used for over 45 years as an objective measurement of the electrical potentials produced by the cochlea and the auditory nerve in the inner ear for diagnosis and/or monitoring of endolymphatic hydrops (Eggermont, 2017). However, its clinical application remains a subject of debate among researchers, because its sensitivity has been found to vary from 20 % to 92 % in different studies (Al-momani et al., 2009; Campbell et al., 1992; Chung et al., 2004). Therefore, the clinical utility of ECochG to diagnose MD is still questionable.

At present, there is no standard method to diagnose MD, signalling a need to reach an international consensus regarding its diagnosis. There is some evidence in the literature suggesting that cVEMPs and ECochG could be useful in the clinical monitoring of MD; however, their clinical utility for diagnosis of the disease is still limited (Ciorba et al., 2017). Assessment of the power of VEMP and ECochG to diagnose MD has received little attention in the literature. A study by Lamounier et al. (2017) found that the ability of both tests to correctly rule out the disease was high (specificity), but the ability to identify the disease varied from low to moderate (sensitivity). From the available data in the literature, none of the evoked potential tests could be considered a gold standard for MD diagnosis; currently, these tests can only be used in a complementary fashion to support the clinical diagnosis (Ciorba et al., 2017).

In addition, little attention has been given to separately examining the effects of cochlear implant (CI) surgery on each sensory organ of the balance system pre- and post-operatively. More specifically, new objective testing methods, such as VEMP

and the video head impulse test (vHIT), which separately measure the effect of CI surgery on different components of the vestibular system (otolith organs and the semi-circular canals), are rarely covered in the literature. Prior to starting this project, to the researcher's knowledge, previous studies had not assessed the function of the entire vestibular system before and after implantation using both VEMP and vHIT. However, over the time span of the present research (between 2014 and 2018), two studies have been published on this topic (Janky and Givens, 2015; Maheu et al., 2017). Maheu et al. (2017) found that 75% of patients showed a complete loss of cervical and ocular VEMP responses in the implanted ear following surgery, while the function of the semi-circular canals (SCCs) was not affected by CI surgery. However, these authors did not mention any of the stimulus parameters that were used to elicit VEMP, which could affect its validity to determine the effect of CI surgery on the balance function of CI users. Janky and Givens (2015) reported that the rate of vestibular loss in the SCCs and the otolith organ's function was higher in children with CI compared to healthy children. However, their study did not evaluate vestibular function prior to implantation; therefore, the effect of CI surgery on balance function cannot be evaluated, as deafness is associated with vestibular dysfunction in some aetiologies.

The current research project was motivated by the possibility of exploring new approaches to stimulation and analysis to improve the measurement of cervical VEMP. Novel stimulation paradigms could improve the frequency specificity of cVEMP responses by exploring how they adapt to long stimulus duration as well as investigating the mechanism that generates cVEMP responses. Such approaches could also improve the quality of cVEMP responses and reduce recording times by objectively quantifying the response quality as a function of rate and exploring the adaptation of cVEMP to high repetition rates. Automated response detection was explored to improve the clinical use of cervical VEMP by eliminating the high variability of visual assessments of test results and increasing the sensitivity of response detection. The current research was also motivated by the opportunity to explore the clinical applications of the testing approaches developed in this research and to compare them with other recently developed objective testing methods, such as ECochG and vHIT, to assess the diagnostic potential of these methods in clinical populations. Based on the gap in the literature regarding the stimuli used to elicit and detect cVEMP responses, the broad aims of this research project are as follows:

1) To explore whether new stimulation and analysis approaches could improve the acquisition of cVEMPs.

2) To compare the diagnostic potential of the new approaches developed in this thesis with that of other recently developed test approaches in clinical populations.

1.2 Structure of the thesis

Chapter 2: Literature review

This chapter provides a general background on the anatomy and physiology of the vestibular system, followed by a section covering the newly developed objective testing techniques for investigating labyrinth function, including VEMP and vHIT. A critical analysis of the standard parameters used in the literature for eliciting VEMP responses is also carried out. The rest of the chapter provides an overview of MD, the use of VEMP in the diagnosis or monitoring of the cochleosaccular hydrops of MD, an overview of CI and the use of vestibular testing to discover the effect of CI on balance function. The research aims are identified at the end of this chapter.

Chapter 3: VEMP in response to AM tones (Experiment 1)

This chapter reports an experiment that investigated VEMP responses from SCM muscle to AM tones over a range of modulation frequencies and compared thresholds of S-VEMP and standard cVEMP in healthy subjects, with the aim of providing a more frequency-specific stimulus to elicit VEMP response than the tone-bursts commonly used in standard VEMP elicitation.

Chapter 4: Effects of changes in the stimulus repetition rate and plateau duration on cVEMP in response to tone-bursts (Experiments 2 & 3)

This chapter reports the experimental findings of two separate experiments that investigated the effect of changes in 1) repetition rates and 2) plateau durations on the amplitude, latency and quality of cVEMP responses, using 500 Hz tone-burst stimuli at a fixed tone-burst level for otologically normal subjects. The motivation behind this work was to improve the quality of cVEMP response by exploring what generates cVEMP and how cVEMP adapts to high repetition rates. By doing so, it was possible to identify possible reasons for the lack of S-VEMP responses in the majority of participants in Experiment 1. This present study is the first to objectively quantify response quality as a function of stimulus rate and duration. This study also examined the effect of very high repetition rates on the cVEMP

response, by exploring whether increasing the rates could reduce recording time but still obtain 100 % detection of responses.

Chapter 5: A comparison of objective and subjective detection of cVEMP responses (Experiment 4)

This chapter reports an experiment on the use of a new, objective analysis of cVEMP responses. This study compared the sensitivity (threshold estimate) of objective (statistical) approaches and subjective estimates by experienced observers for the cVEMP threshold. It further evaluates and compares the detection time of the statistical objective measures when detecting cVEMP responses. The motivation behind this work was to detect cVEMP responses objectively, to eliminate the high variability of visual judgements and to increase the sensitivity of response detection.

Chapter 6: Comparing objective cVEMP-tuning curves with ECochG for the diagnosis of Ménière's disease (Experiment 5)

This chapter reports an experiment on the use of an objective approach to measure the frequency-tuning curve of the saccule in healthy subjects and compare the data to those from MD patients. This work was the first to measure the saccular tuning curve objectively (using statistical approaches). This study also compared the sensitivity and specificity of objective saccular tuning curves with ECochG to detect MD.

Chapter 7: Clinical evaluation of vestibular function in unilateral cochlear implant candidates (Experiment 6)

This chapter reports an experiment on the effects of CI surgery on vestibular function in adult patients with bilaterally severe to profound sensorineural hearing loss (SNHL) who had undergone unilateral implantation. More specifically, the saccule, utricle, and the three SCC functions were separately assessed before and after surgery, using cVEMP to ACS, oVEMP to vibration with a mini-shaker, and vHIT, respectively. One limitation of the study was the small sample size, as only seven CI candidates were recruited for the investigation. Therefore, statistical analysis could not be performed, and a case-by-case approach with CI recipients was used instead.

Chapter 8: Summary/conclusions and further research

This chapter presents the main conclusions of this research project. Possible areas for further research are also proposed.

1.3 Original contributions to knowledge

Although several studies across the literature have attempted to find the optimal stimulus parameters for eliciting a robust cVEMP response, there is still a knowledge gap regarding what triggers a cVEMP response, how cVEMP adapts to high and low repetition rates, and the optimal stimulus duration and rate of stimulation to elicit a robust cVEMP response. The present study aimed to address these shortcomings in the literature by exploring novel stimulation and analysis approaches to improve cVEMP measurements.

The first finding is a low prevalence of S-VEMPs compared to cVEMPs (Experiment 1). The lack of response could be attributed to the stimulus used for eliciting the cVEMP response; standard cVEMP was evoked by short tone-bursts, and S-VEMP was evoked by a continuous AM tone. It appears that the response to a long duration AM tone is not predicted from the response to a short tone-burst. Moreover, the threshold of S-VEMP is higher than that of cVEMP, so the need to avoid breaching noise exposure limits was a restriction in this study. Thus, the current research project furthers understanding of what triggers cVEMP responses and how cVEMP adapts with repetition rates.

From Experiment 2 on stimulus rate, it was found that responses to 500 Hz tone-bursts at high rates could be recorded from the SCM muscle, but only in a few subjects. This may reflect variation in the distribution of muscle fibre types (slow and fast twitch) across subjects. Recording at very high rates might reduce recording times, but a high rate recording was not possible in the majority of subjects. Evoking cVEMP using 1, 5, and 10 Hz repetition rates produced the maximum response rate and the highest F_{sp}. The rate of 10 Hz required a considerably shorter time for recording compared to 1 and 5 Hz rates but still gave 100% response detection, so this appears to be the optimal trade-off between recording time and response detection for the majority of subjects. These findings have been published as a letter to the editor in the Journal of Clinical Neurophysiology in Practice (Obeidat and Bell, 2018).

From Experiment 3 on stimulus duration, it was found that the majority of subjects had responses to short plateau durations, whereas only a small number of subjects had responses to long plateau durations. This might be attributed to either SCM muscle fatigue or the impact of stapedial reflex at long plateau durations, for the majority of people. No significant latency shift occurred when plateau durations were increased, keeping the peak level and the ramp duration constant. These findings show that evoking cVEMP using shorter plateau durations elicits a high incidence, with the largest amplitude and highest quality, although it reduces frequency specificity. In addition, the onset of the stimulus appeared to generate the cVEMP response. As an ASSR stimulus can be considered as a long tone-burst, this may explain the low response rate of S-VEMP in Experiment 1.

The current research project also explored objective methods for the detection of cVEMP response; this is a novel approach, which has not been covered in the existing literature. In experiment 4, significant variability was seen between subjective estimates of cVEMP thresholds. Objective analysis methods have the potential to reduce the variability of measurement in threshold estimates, compared to subjective analysis. Objective detection with the HT^2 test was more sensitive than subjective analysis in detecting cVEMP responses. This study also found that the measurement time of cVEMP was considerably reduced with the HT^2 test, as it can detect the cVEMP response more quickly, needing less than five seconds at high stimulus intensities (109 and 106 A-weighted decibels (dB A)) which could lessen neck fatigue for patients during repeated clinical measurements. These findings show that using the HT^2 test as an objective test for the purposes of detecting the presence or absence of cVEMP responses is likely to be valuable in clinical settings and could replace subjective inspection of the response, for many applications.

Furthermore, the current research project explored the use of objective measures, such as the HT^2 test, to objectively estimate saccular frequency-tuning curves in healthy volunteers and MD patients. From experiment 5, objective saccular tuning curves in volunteers showed the strongest responses at 500 Hz; this is broadly consistent with the data obtained from subjective estimates of response tuning found in previous studies. cVEMP tuning curves were affected by the presence of MD and showed flatter tuning than for the control group. Thus, this finding shows that objective analysis methods are able to measure saccular tuning curves, which had not been previously reported in the existing literature. It was also found that both amplitude and threshold measures showed cVEMP frequency tuning with the

strongest response at 500 Hz. Similarly, both measures showed changes in both ears for bilateral MD patients, with more alteration in the most-affected ear. However, cVEMP amplitudes showed more variance than threshold measurements, and thus threshold appears to be the best measure to use to discriminate the groups. In this research project, it was also found that cVEMP tuning curves assessed using objective statistical analysis methods to define threshold are not very sensitive to long-term MD. VEMP sensitivity may increase in the acute phase of the disease, but even then does not appear high. ECochG has higher sensitivity than cVEMP in the diagnosis of Ménière's patients, but the ECochG SP/AP amplitude ratio measure is not perfect for the diagnosis of MD. ECochG sensitivity increased to 89% during a symptomatic period, compared to 33% for cVEMP. However, ECochG can be difficult to schedule during symptomatic periods. For identified MD cases, both cVEMP and ECochG showed high specificity. Hence, these findings indicated that ECochG is superior to cVEMP in clinical use. In addition, high sound levels and a long test duration are concerns in cVEMP testing.

Finally, the effects of unilateral CI surgery on each sensory organ of the balance system were explored separately in deaf adults pre- and post-operatively. Otolith organ (saccule and utricle) function was generally affected by implantation, whereas the function of the SCCs was not affected. A possible correlation was observed between postoperative changes in VEMP responses and short-term dizziness, although the sample size was low. These findings may have important implications clinically. For example, the present project highlights the importance of performing cervical and ocular VEMP prior to implantation, which could predict the risk of balance dysfunction following CI surgery, and, therefore, provide early vestibular therapy to at-risk deaf patients. Furthermore, with the increasing prevalence of bilateral implantation, it is crucial to quantify the potential risks of vestibular damage following CI surgery and inform patients about the possibility of bilateral vestibular dysfunction after CI surgery. However, a further study with a larger sample of patients is needed to confirm the present findings.

Parts of the present research project have been published as a research paper and reported at a number of international auditory research conferences, which were listed in the Declaration of Authorship form (on page xxi).

Chapter 2 : Literature review

2.1 Chapter overview

To provide the reader with a general background on the vestibular system, this chapter first introduces the anatomy and physiology of the five neural structures of the vestibular system, including the otolith organs (i.e., the utricle and saccule) and three SCCs (section 2.2). Newly developed methods (including vHIT and cervical and ocular VEMPs) for the diagnosis of vestibular end organ function are then described (section 2.3). The clinical applications of these new objective testing methods in two selected patient groups (MD and CI) are then discussed (section 2.4). An overview of MD is provided in section 2.4.1, together with the use of cervical VEMP and ECochG testing in the diagnosis or monitoring of the cochleosaccular hydrops present in MD. An overview of CIs and the use of vestibular testing to discover their effects on balance function are discussed (section 2.4.2). At the end of this chapter, the research objectives are identified (section 2.5) to address the gaps in the literature and to extend the existing research.

2.2 Anatomy and physiology of the peripheral vestibular system

As shown in Figure 2.1, the cochlea, which comprises the sensory organ of hearing, is located alongside the vestibular labyrinth. The ducts in the membranous labyrinth allow the endolymph to flow between the cochlea and the vestibular labyrinth (Furman et al., 2010). The perilymphatic spaces between the vestibular labyrinth and the cochlea are contiguous, and they share a common blood supply (Furman et al., 2010). Because of their proximity, it is not surprising that disorders affecting the vestibular system often also affect the cochlea, causing dizziness (vertigo and loss of balance), which is often accompanied by hearing problems and/or tinnitus (ringing sound in the ear) (Furman et al., 2010).

The vestibular labyrinthine space includes five neural structures. Three SCCs (i.e., horizontal, posterior, and superior) are connected to two membranous sacs called

the utricle and saccule, which are often referred to as the otolith organs (Jacobson & Shepard, 2008) (Figure 2.1). The maculae consist of the neurosensory epithelium of the otolith organs, whereas the cristae ampullarae consist of the sensory epithelium of the SCCs (Furman et al., 2010).The maculae of the otolith organs in the membranous labyrinth of the saccule and utricle are responsible for detecting linear acceleration (i.e., change in velocity in a straight-line direction) with respect to gravity as well as changes in head position (i.e., tilting right or left, backward or forward) (Jacobson & Shepard, 2008). The saccule is sensitive to vertical acceleration (e.g., riding in an elevator) and the utricle is sensitive to horizontal acceleration (e.g., riding in a car), and this is related to the way that they are placed within the vestibular apparatus (Jacobson & Shepard, 2008). The cristae ampullarae of the SCCs are responsible for angular acceleration (i.e., simultaneous change in velocity and direction) of the head along a specific plane (Jacobson & Shepard, 2008). The three SCCs are oriented orthogonally to each other, which means there is an angle of about 90 degrees between pairs. Each canal in the temporal bone has a contralateral coplanar pair; the horizontal SCC shapes the coplanar pair, while the superior (anterior) canals, with the contralateral posterior canals, form a coplanar mate: right anterior-left posterior (RALP) and left anterior-right posterior (LARP) (Jacobson & Shepard, 2008). Further details are provided in Appendix A.

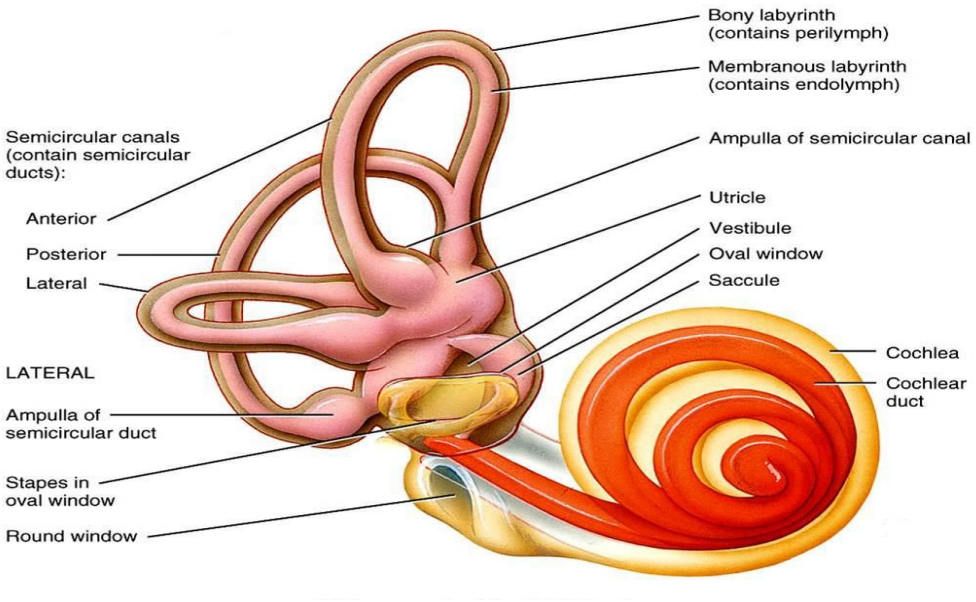

Figure 2. 1: The peripheral vestibular apparatus, including three SCCs, horizontal, posterior and superior, and the two otolith organs (utricle and saccule) are located adjacent to the cochlea in the inner ear. Modified from Tortora and Derrickson (2013) with consent from Wiley Publications.

2.3 Assessing vestibular end organ function

Vertigo or dizziness, nystagmus, ataxia, and nausea are considered manifestations of human vestibular dysfunction, which emanate from various sites in the central nervous system (Brandt & Strupp, 2005). Based on the patient's history, a complete neurological examination must be carried out either to establish the reason for vestibular dysfunction or to obtain a differential diagnosis (Brandt & Strupp, 2005). The vestibular end organs are evaluated using several vestibular tests, which are often applied in testing batteries, such as caloric testing, positional testing, and head impulse tests (HIT), which are used to test the function of horizontal SCCs (Wuyts et al., 2007). Recently, cervical and ocular VEMP tests were developed to evaluate the function of the otolith organs through ACS or BCV (Wang et al., 2010). In addition, the vHIT was recently developed based on the clinical HIT to objectively investigate the VOR of the horizontal SCCs. The vHIT was later expanded to investigate the function of each of the vertical SCCs (anterior and posterior) (Halmagyi & Curthoys, 1988; MacDougall et al., 2013). These newly developed methods for the diagnosis of otolith organs and SCCs, combined with the widely used standard tests, may provide a comprehensive means for investigating labyrinth function. The focus of this chapter is on the newest additions to the standard vestibular testing battery: vHIT, cVEMP, and oVEMP, which are used to objectively test vestibular end organ function, which includes the otolith organs and the three SCCs.

2.3.1 The video head impulse test (vHIT)

Evaluation of the semi-circular canal function

In 1988, the HIT, also called the Halmagyi test, was developed by Halmagyi and Curthoys as a practical test for identifying chronic peripheral vestibular loss and distinguishing the sides of lesions (Kaplan & Slovik, 2005). HIT was developed to diagnose the VOR of each SCC individually at high frequencies (Jorns-Häderli et al., 2007). This test relies on moving the patient's head abruptly to one side 20 degrees from the midline position in the angular-horizontal plane. During this test, the patient must focus on a midline target, which is usually the examiner's nose (Kaplan & Slovik, 2005). In healthy patients, this focus leads to correct eye movement in the direction opposite the movement of the head and with identical velocity (Kaplan & Slovik, 2005). However, patients with peripheral hypofunction

cannot maintain a fixation on the target when the head is turned toward the side of the lesion, which means that they generate a compensatory catch-up saccade in the direction opposite the head movement in order to return their eyes to the target (Kaplan & Slovik, 2005). According to Beynon et al. (1998), the HIT has poor sensitivity in detecting mild to moderate vestibular hypofunction although it has good sensitivity and specificity in detecting severe vestibular loss. In the HIT, the detection of overt saccades is subjective, and it depends on whether the observer notes the corrective saccade following the head movement. Hence, although overt saccades that occur at the end of the head rotation can be detected the HIT cannot detect covert saccades that occur during head movement (Weber et al., 2009).

Curthoys (2012) suggests that the scleral search coil method is an optimal approach for recording eye movements such as corrective saccades. This method consists of a soft contact lens with a coil of wire attached to the head (Curthoys, 2012). The current is inducted into the coil when the magnetic field has been applied to track the subject's eye movement (Curthoys, 2012). However, the apparatus used to track eye movements is time consuming, expensive, and uncomfortable for the patient. This method is therefore not suitable for routine clinical testing (MacDougall et al., 2009; MacDougall et al., 2013).

The HIT of Halmagyi and Curthoys was further developed to improve its objectivity, where video pupil tracking is used to investigate eye movement along the vertical and horizontal planes (Ulmer and Chays, 2005). This method was developed into a system consisting of lightweight goggles that contain a small and high-speed digital video camera that tracks the subject's eye movements and a gyroscope that tracks the angular acceleration of the head (MacDougall et al., 2009) (Figure 2.2). Head impulses can be applied in the three planes of the SCCs. In each plane, a pair of canals is tested; the lateral SCCs, RALP canals, or LARP canals. During a vHIT, the patient must maintain fixation on the target. If the patient exhibits an abnormal compensatory eye movement, the VOR gain is abnormal, and a catch-up saccade may emerge. The function of the VOR is to stabilize images on the retina during head movement by the generation of eye movements that are equal and opposite to head movements, thus preserving the image in the centre of the visual field (Alhabib & Saliba, 2017). For instance, turning the head to the right excites neurons in the right vestibular nucleus and causes reflexive eye movements to the left. In the absence of such reflexive eye movements (i.e. in patients with vestibular loss), the visual targets would shift widely, mainly during head movements and visual orientation would be difficult or impossible (Alhabib & Saliba, 2017). The gain of

the VOR is defined as the ratio of compensatory eye velocity to head impulse velocity, which is around one in healthy subjects (Alhabib & Saliba, 2017). VOR gains which are significantly less than one indicate reduced SCC function (Curthoys & Manzari, 2017).

The vHIT uses a video camera and an accelerometer to measure eye movement and acceleration during the head movement; thus, it is objective. As a result, vHIT can detect both overt and covert saccades (Weber et al., 2009). Covert saccades that occur during head rotation are extremely fast and impossible to detect with the naked eye, so false negatives can occur during clinical HITs (Weber et al., 2009). Indeed, they can entirely conceal even a complete vestibular hypofunction (Weber et al., 2009). Thus, the vHIT has better sensitivity and specificity than the clinical bedside HIT (Zellhuber et al., 2013). The vHIT shows covert saccades during the head rotation as well as overt saccades at the end of the head rotation (Weber et al., 2009). Regarding partial vestibular deficit, the evaluation of VOR via vHIT is dependent on the level of acceleration. Higher levels of acceleration are needed to reveal VOR dysfunction (Beynon et al., 1998). The head acceleration should be sufficient to drive one canal in a pair into inhibition, so the measurement will be primarily testing the VOR arising from the other canal in effective isolation (Alhabib & Saliba, 2017). It has been suggested that a vHIT is equivalent to scleral search coils in detecting dysfunction in vertical and horizontal SCCs (Curthoys, 2012; MacDougall et al., 2009; MacDougall et al., 2013). However, pupil video tracking (vHIT) is easier to perform in a clinical setting than scleral search coils. Moreover, it is a non-invasive test for the individual evaluation of all SCC dysfunctions (MacDougall et al., 2013).

Previous studies compared the caloric test, which is used to assess the function of the lateral (horizontal) SCCs (see Appendix B), in conjunction with clinical HITs or vHITs in detecting vestibular disorders (Bartolomeo et al., 2014; Bell et al., 2015; Jorns-Häderli et al., 2007; Perez & Rama-Lopez, 2003). In a clinical HIT, Perez & Rama-Lopez (2003) obtained a specificity of 91 % and sensitivity of 45 % in a large number of dizzy patients, including 23 patients with vestibular neuritis (VN), when the canal paresis in the caloric test was more than 42.5 %. They found that canal paresis above 42.5 % was consistent with an abnormal HIT. This lack of sensitivity was recently reported in another study by Bartolomeo et al. (2014) in which a vHIT obtained better sensitivity and specificity. In a study of 29 patients with VN, these authors found that a sensitivity of 86.7 % and a specificity of 100 % were obtained

when canal paresis was above 40 %. They also found 100 % sensitivity and specificity of a vHIT could be obtained relative to a caloric deficit of more than 62.5 % (Bartolomeo et al., 2014). The results of their study suggested that the vHIT lacks sensitivity compared to caloric testing, but it is considered a fast, convenient, and specific test for detecting vestibular disorders in VN. In addition, Bell et al. (2015) found that a vHIT was insensitive to detecting peripheral vestibular disorders which had already been detected using a caloric canal test. With reference to the manufacturer's suggestion that an abnormal VOR gain was below 0.8, of 14 dizzy patients with significant canal paresis (>20 %), only 4 patients showed a significant loss in either canal when a vHIT was applied; Hence, the vHIT's sensitivity and specificity were 29 % and 94 %, respectively. Overall, a vHIT is likely to be insensitive in detecting moderate vestibular lesions compared with caloric testing although they may be used to test different frequencies.

The evaluation of the reliability and sensitivity of new vestibular testing methods in detecting vestibular deficits is a frequent problem, as there is no widely accepted gold standard for diagnosing vestibular disorders (Bell et al., 2015). Several authors have suggested that a vHIT yields information that is complementary to caloric testing and is therefore likely to be a useful addition to the vestibular testing battery. The reason is that caloric testing of the lateral SCCs is conducted at very low frequencies of 0.002–0.004 Hz (Perez & Rama-Lopez, 2003) and 0.006 Hz (McGarvie et al., 2015), whereas the vHIT tests each vertical and lateral SCC at high frequencies roughly equivalent to 2.5 Hz (McGarvie et al., 2015).

Since the vHIT is new, the published data on normative VOR gain values for lateral and vertical SCCs are still sparse. The manufacturer of the ICS Impulse System software suggests that a VOR gain above 0.8 is normal in lateral canals and above 0.65 in vertical canals (GN Otometrics, 2011), although it is not clear on which peer-reviewed research journals these normative VOR values are based (Bell et al., 2015). MacDougall et al. (2009) measured the average VOR gains in lateral SCCs, using both the vHIT and scleral search coil approaches across eight healthy subjects and eight patients with known vestibular disorders. They found that the normal VOR gain in lateral SCCs was 0.68 or higher. Curthoys et al. (2010) found that the normal VOR gain in lateral canals ranged between 0.7 and 1.1. Bell et al. (2015) redefined the normal VOR gain in lateral canals as between 0.83 and 1.21 based on 30 asymptomatic healthy subjects. A previous research study (Faten, 2014) using the GN Otometrics vHIT system on 20 healthy subjects found that the normal VOR gain ranged between 0.96 and 1.04 in lateral canals and between 1.21

and 1.34 in vertical canals. Bell et al. (2015) found that the sensitivity of the vHIT in identifying peripheral vestibular disorders depended on the lower limit of normal VOR gain. Further research is needed to establish normative data on VOR gain in all canals in a large number of normal human subjects.

Because only a few previous studies have used vHIT to evaluate the vestibular function of all SCCs in CI patients (Maheu et al., 2017; Melvin et al., 2009; Migliaccio et al., 2005), this area will be explored in depth in Chapter 7.

Figure 2. 2: The ICS Impulse-Video Head Impulse Test (vHIT) system.

2.3.2 Vestibular-evoked myogenic potentials (VEMPs)

This section is focused on the evaluation of the otolith organ function.

2.3.2.1 Background

From an evolutionary point of view, the cochlea has been considered a late development within the membranous labyrinth in humans (Ferber-Viart et al., 1999; Todd et al., 2000). In fish and amphibians, in the absence of a cochlea, the saccule is thought to be responsive to sound (Fay & Popper, 1999; Popper et al., 1982).

Some authors speculate that in humans, the saccule has preserved a primitive acoustic sensitivity although it has a specific role in the balance function (i.e. sensing gravity and linear acceleration) (McCue & Guinan, 1997; Todd et al., 2000). Based on anatomical studies on temporal bones, the saccular macula is known to be directly located underneath the footplate of the stapes, which could cause it to be stimulated by loud sounds (Rauch et al., 1989). However, it is still unknown whether high sound pressure elicits a vestigial acoustic response or the endolymphatic compression generates a mechanical response by the vestibular hair cells (Zhou & Cox, 2004).

In 1935, Von Bekesy was the first to report that high intensity sound stimuli elicited head movements, which he suggested was the result of vestibular stimulation (Welgampola & Colebatch, 2005). Bickford et al. (1964) reported an inion response to loud sounds, which was present in deaf patients with normal vestibular end organ function but absent in deaf patients with vestibular dysfunction. This finding indicated that that the response originates in the vestibular system and is not affected by cochlear dysfunction; thus, it could be performed in patients with deafness (Jacobson & Shepard, 2008). Recently, a myogenic response was first recorded by Colebatch, Halmagyi et al. using surface electrodes over the contracted SCM muscle following vestibular stimulation by high-level AC click stimuli in normal human subjects (Colebatch and Halmagyi, 1992; Colebatch et al., 1994). These responses were labelled click-evoked VCR, which is thought to primarily measure the saccular function (Rosengren & Kingma, 2013). Based on this finding, a reliable method was established for measuring click-evoked myogenic potential (Colebatch et al., 1994). Other researchers have described these responses as VEMPs, as they are myogenic potentials elicited by the stimulation of the vestibular end organs (Zhou & Cox, 2004). The term myogenic is used to indicate that the origin of the response is in the averaged electrical activity of the muscle, which was confirmed because the response was observed to be abolished during muscle relaxation (Jacobson & Shepard, 2008; Rosengren et al., 2010). Recently, similar myogenic responses have been recorded using other muscles. In previous research, recording was performed by placing surface electrodes beneath the eye muscle (the inferior oblique muscle) (Piker et al, 2013; Rosengren et al, 2010; Todd et al, 2007). To differentiate them from the conventional VEMPs, the recording of VEMP from the contracted SCM muscle is referred to as cVEMP, whereas VEMP recorded from the eye muscles is termed oVEMP (Piker et al., 2013; Young, 2013). Like cVEMP, which is a form of the VCR, oVEMP is a myogenic reflex of the extraocular muscles, which is a manifestation of a VOR (Rosengren and Kingma, 2013).

Cervical and ocular VEMPs are otolith-dominated responses. However, in the clinical condition of superior SCC dehiscence, superior SCC receptors are also activated by ACS and BCV and they enhance the otolith-dominated VEMP responses (Curthoys et al., 2018). After dehiscence, the previously unresponsive canal afferent neurons in the canal receptors can be stimulated by a sound or vibration, resulting in abnormally large amplitudes and low thresholds of VEMPs (Curthoys & Grant, 2015). The authors suggested that opening the bony wall of the canal would cause increased fluid displacement in the canal. Hence, the canal afferent neurons that could not be stimulated in the sealed labyrinth could now be stimulated after dehiscence, thus contributing to the VEMP response (Curthoys & Grant, 2015).

2.3.2.2 Cervical VEMPs

cVEMP is described as an inhibitory short-latency myogenic response that is recorded by the ipsilateral SCM muscle in response to loud sounds (Rosengren et al., 2010). The response consists of an early positive-negative component that arises 13–23 ms after a stimulus is presented (P13-N23 or P1-N1) and then a later component at 34–44 ms (N34-P44 or N3-P4). The first wave complex (P13-N23) is thought to be specifically vestibular dependent, whereas the later wave complex (N34-P44) is thought to originate in the cochlear afferents and is only present in about 60 % of healthy people, which limits its clinical usefulness (Colebatch et al., 1994; Eleftheriadou & Koudounarakis, 2011). Figure 2.3 shows a typical cVEMP to ACS with an initial positive deflection, P (or P13), arising at around 13 ms and a subsequent peak, N1 (or N23), arising around 23 ms after stimulus presentation. The positive potential (P13) of the cVEMP was indicated to be inhibitory in the tonically contracted SCM muscles (Iwasaki et al., 2008).

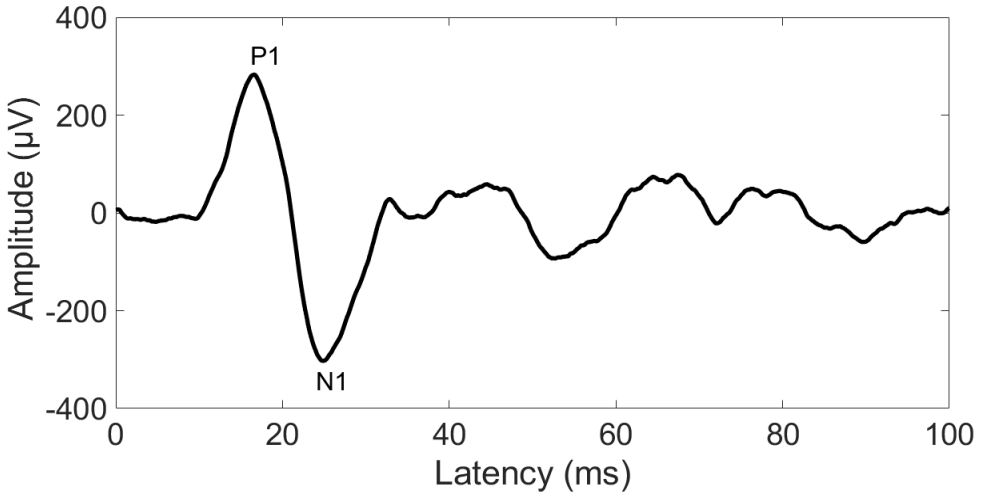

Figure 2. 3: Example of a typical biphasic clear cVEMP response to ACS, consisting of a positive-negative component that arises at 13-23 ms after stimulus presentation (P13-N23 or P1-N1). The P13 indicates the positive (inhibitory) myogenic potential that is recorded over the tensed SCM muscles (ipsilateral to the sound stimulus).

Based on evidence elucidating otolith-spinal neural projections in cats (Kushiro et al., 1999; Suzuki et al., 1969; Uchino et al., 2005), the proposed projection from the saccular macula to the SCM muscle in humans is shown in Figure 2.4. Sound stimulates the saccule and then crosses the afferent inferior vestibular nerve to reach the brain stem vestibular nuclei. From there, impulses are sent to the SCM muscle through the descending medial vestibule spinal tract, the spinal accessory nucleus, and the spinal accessory nerve (X1), resulting in the inhibition or relaxation of the muscle (Kushiro et al., 1999; Uchino et al., 1997; Uchino et al., 2005). Because the stimulation of the saccule can deliver a strong inhibitory response in the ipsilateral SCM muscle, the contraction of the SCM muscle is required when cVEMP is recorded (Curthoys, 2010).

Animal studies on the vestibular projections to neck muscles showed that the saccular afferents had only an ipsilateral projection to the SCM neck muscles, whereas afferents from the utricular macula (and possibly the SCCs) both shared this projection and had an additional excitatory projection to the contralateral SCM muscle (Fukushima et al., 1979; Kushiro et al., 1999). Welgampola and Colebatch (2001) found that in some normal subjects (about 40 %), an inverted peak (n12-p20) was evident in the response to loud sounds by the contralateral SCM muscle. Based on the evidence from animal studies, this crossed response is thought to be produced by the spread of the stimulus to other vestibular organs, most probably the utricle (Rosengren et al., 2010). Regarding vibration cVEMP (conducted through

bone: forehead taps or BC tone burst), previous clinical studies found that all patients with superior vestibular neuritis (SVN) (but preserved inferior vestibular nerve) showed normal cVEMP responses on the affected side, suggesting they originate in the inferior vestibular nerve and saccule (Manzari et al., 2010; Iwasaki et al., 2009). In contrast, other studies found that vibration-evoked cVEMP was abnormal in patients with SVN, suggesting that the superior nerve and utricle may play a significant role in the VCR (Brantberg et al., 2004; Govender et al., 2011). Thus, this issue remains unresolved; however, there is clinical agreement that cVEMPs are predominantly mediated through the inferior vestibular nerve, probably because the saccular receptors as saccular neurons have a strong projection to the neck muscles and a weak projection to the extra-ocular muscles (Curthoys, 2010). The overall consensus regarding the origin of these effects is based on evidence of the otolith-spinal neural projections, which was found in research using the localised electrical stimulation of vestibular nerve branches in anaesthetised cats (Uchino et al., 2005). These authors reported that approximately 30 % of the extraocular muscle motoneurons responded to saccular nerve activation, whereas 100 % of the neck muscle motoneurons responded. Hence, the neural projection from the utricular macula to the neck muscles must be of minor importance in generating the p13 of a cVEMP, which is an indicator of ipsilateral saccular function. A review of the evidence obtained in animal and clinical studies which elaborated the specificity of the saccular macula to acoustic and vibratory stimulation is provided in Appendix C.

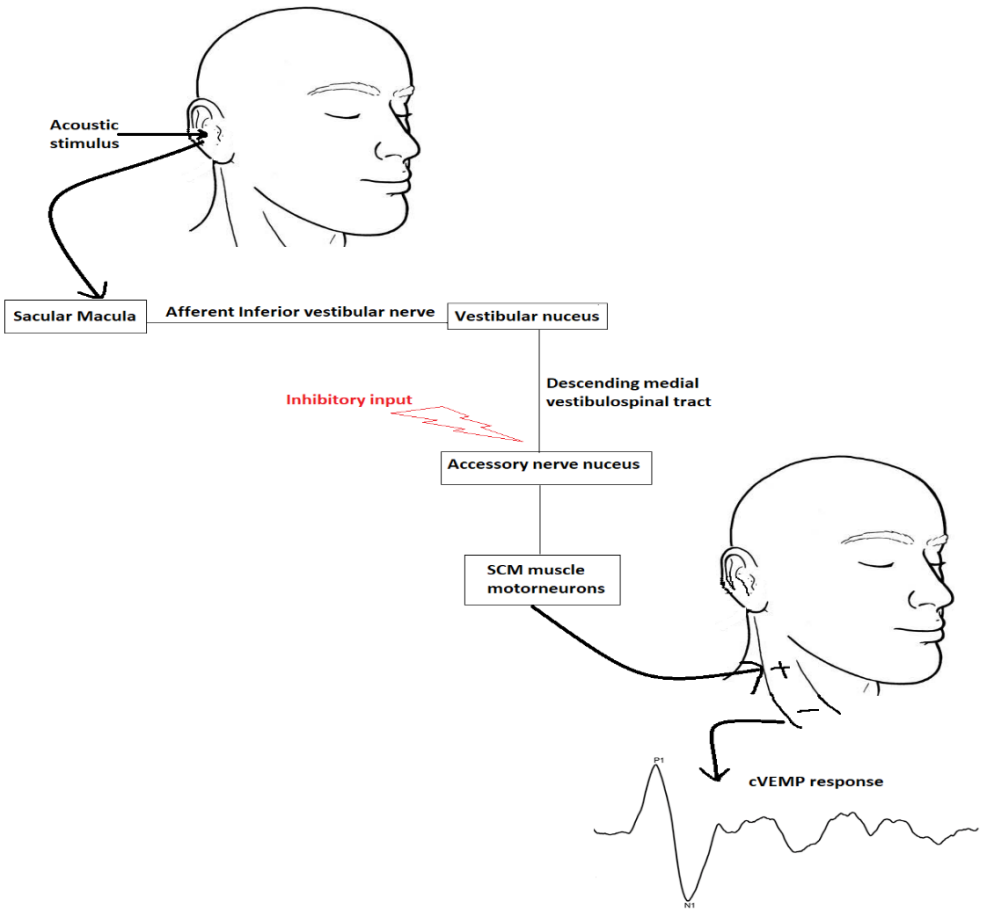

Figure 2. 4: The proposed neural pathway of cVEMP, which was established by Uchino et al. (2005) and others, reflects sacculo-collic reflexes in response to stimulation. Sound stimulates the saccular macula, which transmits the electrical activity through the ipsilateral inferior vestibular nerve to the brainstem vestibular nuclei from which the descending medial vestibulospinal tract conveys an inhibitory signal to the cranial spinal accessory nerve, resulting in the inhibition or relaxation of the ipsilateral SCM muscle.

2.3.2.3 Ocular VEMPs

Unlike cVEMP, oVEMPs were added only recently to the neuro-otologic test battery as a technique for assessing the otolith ocular function (Kantner & Gürkov, 2012). Todd et al. (2003) were the first to elicit short-latency VEMP potentials in response to BC tone bursts throughout the scalp. Rosengren et al. (2005) found that these evoked potentials were best recorded visually and suggested that the extraocular muscles (predominantly inferior oblique muscles) were their source (Kantner & Gürkov, 2012). The myogenic origin of the potentials was confirmed by Todd et al., who found that the simultaneous short-latency extraocular potentials preceded the eye movements, indicating that the source of the recorded potentials could not be generated by the latter (Todd et al., 2007). Chihara et al. (2009) provided evidence

that the oVEMP response originates in the extraocular muscle activated through the VOR pathway by showing that in patients with facial palsy, profound deafness, or exenteration of the eyeball with the preservation of the extraocular muscles, oVEMPs were preserved and unaffected by cochlear or facial nerve impairment.

Figure 2.5 shows the proposed projection from the utricular macula to the extraocular muscles. The afferent from the utricular macula flows mainly in the superior vestibular nerve and the synapses on the excitatory neurons in the vestibular nuclei, which are projected to the contralateral oculomotor neurons (III), controlling the extraocular muscles (i.e., the inferior oblique muscle) (Curthoys, 2010; Rosengren et al., 2010). This projection is based on evidence elucidating otolith-ocular neural projections in cats (Kushiro et al., 1999; Suzuki et al., 1969; Uchino et al., 2005).

The stimulation of the utriclular macula leads to the activation of the inferior oblique and the inferior rectus muscle of the contralateral eye. The superior rectus or superior oblique of the ipsilateral eye is also activated (Suzuki et al., 1969). oVEMP responses are best recorded below the eye contralateral to the stimulated ear and measured in the up-gaze position (Chihara et al., 2007). In normal subjects, oVEMP responses can also be recorded below the ipsilateral eye. However, when oVEMP potentials are recorded from the ipsilateral side of stimulation, the responses are characterised by lower response rates and reduced amplitudes in the up-gaze position compared to the contralateral responses (Govender et al., 2009). The maximal amplitude of the oVEMP response can be achieved in the maximal up-gaze position (Govender et al., 2009). The impact of the gaze position on the oVEMP response has been attributed to the inferior oblique muscle (Rosengren et al., 2010). In oVEMP potentials recorded in the inferior electrodes site, the response amplitudes are particularly high in the up-gaze position, where the most likely source is the EMG of extraocular muscles (the inferior oblique muscles). By contrast, the response in the neutral gaze position may be difficult to interpret because it may represent the summation of the responses of all tonically-active muscles (Rosengren et al., 2005; Todd et al., 2007). Thus, because the amplitude of the response increases in the up-gaze position, oVEMP is affected by the direction of the gaze and the use of the same angle of elevation (Rosengren et al., 2010). However, compared with cVEMP, there is no need to contract the neck muscle during oVEMP recording. Hence, oVEMP can be recorded in the elderly, children, and patients with spinal arthritis (involving the degeneration of tissues

and bones in the neck) (Chihara et al., 2009). Nevertheless, the different pathways of cVEMP and oVEMP can be clinically complementary, which suggests that oVEMP has an ascending pathway from the vestibular nucleus, whereas the cVEMP includes a descending pathway to the SCM muscles (Welgampola & Colebatch, 2005). cVEMP is an inhibitory EMG response measured over the SCM muscle ipsilateral to the stimulated side, whereas oVEMP is an excitatory EMG response produced by the inferior oblique muscle contralateral to the stimulated side (Zuniga & Janky, 2013).

Moreover, oVEMP can be evoked by either ACS or BCV. Elicited by acoustic stimulation (either clicks or tone bursts), oVEMPs were first described by Todd et al. (2007). In BCV, it can be either applied on the mastoid or the forehead. Tseng et al. (2012) found that forehead tapping stimulated the otolith afferents bilaterally, while mastoid tapping produced larger amplitudes and earlier latencies, which could be due to the short path to the vestibule. Mastoid tapping can be conducted using a classical bone vibrator B71, which has a low maximum output and thus is not loud enough to elicit oVEMP responses (Iwasaki et al, 2008). A larger vibratory output to evoke oVEMP is achieved using a mini-shaker, such as the Bruel and Kjer 4810, which, compared to the classical bone conductor, yields a more reliable and consistent response and a higher response prevalence (Iwasaki et al., 2008).

Compared to acoustic stimulation, BCVs have been found to produce a higher response prevalence and larger amplitudes in normal healthy subjects (Cheng et al., 2009; Wang et al., 2010). In patients with conductive hearing loss (CHL), oVEMPs to acoustic stimulation are usually absent, whereas oVEMPs to BCVs are usually present (Wang et al., 2010). Thus, in patients with the absence of oVEMP to ACS, CHL should be excluded, because it could be the reason for failure to elicit a potential (Kantner and Gürkov, 2012). Stimulation of the utricle using ACS causes excitation of the contralateral inferior oblique muscle and ipsilateral superior rectus muscles as well as the inhibition of the contralateral superior oblique muscle. BCV leads to the bilateral inhibition of the inferior oblique muscles and excitation of superior oblique muscles, consequently eliciting bilateral oVEMP responses in both eyes simultaneously (Eleftheriadou & Koudounarakis, 2011). In both methods, the EMG activity in the inferior oblique muscle can be recorded by placing electrodes on the skin beneath each eye: the oVEMP active electrodes are placed 1–2 cm below the eyes (Eleftheriadou & Koudounarakis, 2011). Reference electrodes should be placed nearby and about 2-3 cm lower on the cheek to reject any muscle activity from distant sources (Rosengren et al., 2010).

In contrast to cVEMP, oVEMP usually shows a negative peak with a latency of about 10 ms (N10 or N1) (Rosengren et al., 2010). A typical oVEMP response to a vibratory stimulus (e.g., a mini-shaker) is shown in Figure 2.6. The response comprises an initial negative deflection, N1 (or N10), arising at around 10 ms after stimulus presentation and a subsequent peak, P1 (or P15), arising at around 15 ms. Unlike the P13 of the cVEMP, which is a positive (inhibitory) potential that indicates the inhibition of the activated SCM muscles, the early negative (excitatory) component (N10) of oVEMP is a negative potential, which indicates the excitation of the extraocular muscles (Iwasaki et al., 2008).

Several previous studies suggested that oVEMP originated in the utricular macula, because N10 was found to be reduced or absent in patients with SVN (e.g., Manzari et al., 2010). Animal studies on vestibular nerve branches in anaesthetised guinea pigs showed that all afferents from the utricular macula and the anterior and horizontal SCCs were toward the large superior vestibular nerve in addition to a small bundle of fibres in the hook region of the saccular macula. However, most nerve fibres from the "shank" saccular macula combined with afferents from the posterior SCC toward the inferior vestibular nerve (Uzun et al., 2007; Uzun-Coruhlu et al., 2007). All patients with SVN who had preserved the inferior vestibular nerve, which was shown by the presence of P13-N23 cVEMP to ACS and BCV, had absent or reduced oVEMP N10 to both ACS and BCV (Iwasaki et al., 2009; Manzari et al., 2010). Despite vestibular projections from the utricular macula to the neck muscles (Fukushima et al., 1979; Kushiro et al., 1999), SVN did not noticeably affect the P13-N23 component of the cVEMP in either ACS or BCV but reduced or abolished the crossed N10 component of the oVEMP. However, patients with inferior VN showed reduced or abolished ipsilateral cVEMP P13-N23, and the oVEMP N10 was unaffected (Manzari et al., 2012). This outcome strongly implies that N10 of the oVEMPs originates in the contralateral utricular macula, and the cVEMP originates in the saccular macula (Govender et al., 2015; Manzari et al., 2010). As originally proposed by Curthoys (2010), oVEMP reflects the contralateral utricular function and cVEMP reflects the ipsilateral saccular function (Curthoys, 2010). Thus, oVEMP and cVEMP are independent measures that can be used to differentiate the functions of the saccular and utricular macula not by measuring the stimulus selectivity in either of the two maculae but by measuring the responses, which are determined by the differential neural projections of the utricular instead of the saccular neural information conveyed to various muscle groups (Curthoys et al., 2018). In other words, the specificity of the vestibular system to acoustical and

vibratory stimulation is motor-related rather than sensory-related (Curthoys, 2010). However, Todd et al. thought that oVEMPs produced by head translations in the horizontal plane from a mini-shaker are likely to arise predominantly from the utricle (Todd et al., 2008). Thus, the origin of oVEMPs is still being debated in the literature (see Todd, 2014, for a review).

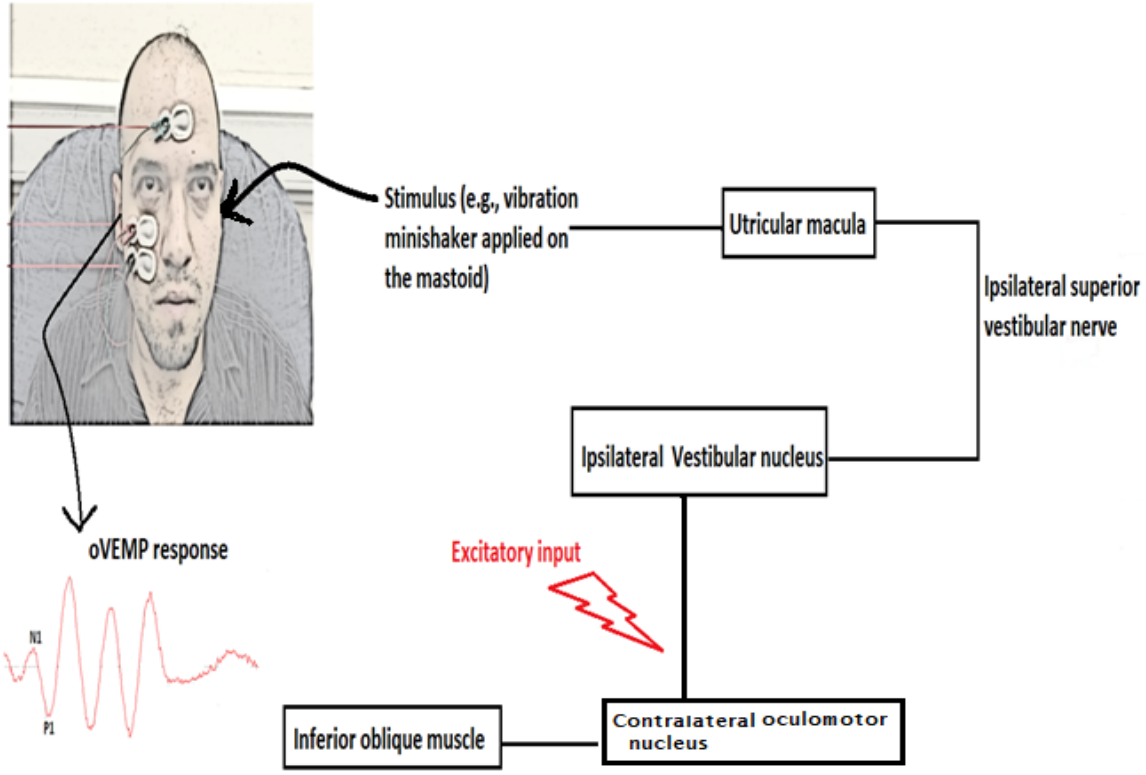

Figure 2. 5: The proposed neural pathway of oVEMP**,** established by Uchino et al. (2005) and others**,** reflects utriculo-ocular reflexes in response to vibration stimulation. BCV stimulates the utricular macula, which transmits the electrical activity through the ipsilateral superior vestibular nerve to the brainstem ipsilateral vestibular nuclei in which signals cross to the contralateral medial longitudinal fasciculus, oculomotor nuclei, and oculomotor neuron, resulting in the excitation of the extraocular muscles (i.e., the inferior oblique muscle) contralateral to the stimulated side.

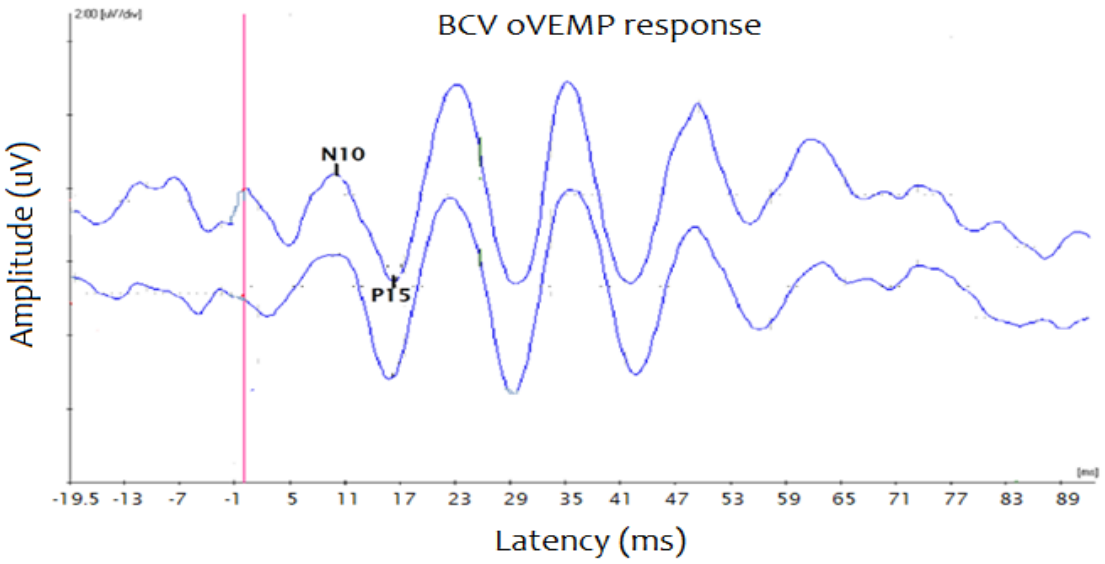

Figure 2. 6: Typical oVEMP responses to a vibratory stimulus (i.e., a mini-shaker). A biphasic potential with an initial negative deflection, N1 (or N10), and a subsequent peak, P1 (or P15). The early negative component (N10) of oVEMP is an excitatory myogenic potential recorded over the extraocular muscles contralateral to the stimulated ear. The vertical line displays the stimulus onset. This example was taken from the current research.

2.3.2.4 VEMP stimulation parameters

A. Stimulus type

VEMP can be evoked by either click (0.1 ms in duration) or short duration tone-burst stimuli for AC; however, the normative data will differ (Deepak et al., 2013). In previous animal studies, it was found that acoustically responsive afferents in the vestibular nerve were the most responsive to low frequencies in the range of 500–1,000 Hz. However, there were slight or no responses to acoustic stimuli above 3,000 Hz (McCue & Guinan, 1994; Murofushi et al, 1995). Similarly, the VEMP responses in normal humans exhibited optimal sensitivity to low frequencies at 300–500 Hz (Todd et al., 2000), 500 Hz (Rauch et al, 2004), and 700 Hz (Welgampola and Colebatch, 2001). Therefore, a tone burst may be a more efficient stimulus compared with a broadband click-induced VEMP (Rosengren et al., 2010). The stimulus intensity required to elicit a VEMP response is higher in clicks than in tone bursts, at 95-100 decibels above normal hearing level (dB nHL) (equivalent to 140-145 decibels peak equivalent sound pressure level (dB p.e. SPL), which would be considered at the safe limits and which patients could find intense and

uncomfortable (Welgampola & Colebatch, 2005). Short tone bursts of about 120 dB p.e. SPL are required to evoke a VEMP response (Welgampola & Colebatch, 2005). Because the VEMP response to tone bursts has a lower threshold than clicks have, it can be elicited by low presentation levels of the stimuli (Welgampola & Colebatch, 2005). At the same peak SPL, a tone burst is louder than a click (as it contains more energy), so it is more clinically appropriate than a click in eliciting VEMP responses, which can be evoked at the lowest possible stimulus intensity (Rauch et al., 2004).

Although tone bursts have a narrow spectral content, they have a brief onset, which may cause spectral splatter, producing responses from unwanted frequencies and thus reducing the frequency specificity of the VEMP. Hence, tone bursts may not be the best stimulus in eliciting VEMPs. Nonetheless, because the VEMP is an onset response, a brief and abrupt stimulus is needed. Thus, the spectral splatter of the stimulus cannot be completely avoided. Because VEMPs show tuning properties, a more frequency-specific stimulus with less spectral splatter is needed. The frequency specificity of a sound depends on the property of the stimulus. The spectral resolution is directly proportional to the duration of the stimulus. The frequency representation is enhanced as a function of stimulus duration in which resolution = 1/time, where the resolution of frequencies is measured in Hz, and time is measured in seconds (Rance, 2008). In other words, there is generally a trade-off between the stimulus duration and the frequency-specificity. Recent studies have reported that VEMP can also be evoked using AM tones that are used in ASSR as regularly repeated stimuli. In the research literature to date, only two studies have established that VEMP from the SCM muscle could be obtained at the frequency of 500 Hz amplitude modulated at different frequencies, and the properties of these responses were consistent with a saccular origin (Bell et al., 2010; Oliveira et al., 2014). However, further clinical research is required to confirm the possibility of recording VEMP to AM tones, which has been adopted to improve the frequency specificity of VEMP. However, Bell et al. (2010) compared the thresholds of VEMPs in response to 500 Hz tone bursts and 500 Hz modulated tones and found that the modulated VEMP produced a significantly higher threshold, by about 3.7dB LEQ (A) (A-weighted Equivalent Continuous Sound Level) than the standard VEMP. This finding may indicate the limited use of AM tones to evoke VEMP in clinical testing (Bell et al., 2010), although further clinical research is needed to confirm this finding. Experiment 1 is presented in Chapter 3, which aims to explore the VEMP response from the SCM muscle to AM sounds in a range of modulation rates. Thus, the following research questions are posed:

RQ1: What is the optimal modulation rate for AM VEMP?

RQ2: Is the threshold of steady VEMP significantly higher than the threshold of standard VEMP?

B. Stimulus repetition rate

Because there is no established standard for VEMP testing, there has been no agreement among experts regarding the optimal stimulus parameters for eliciting a VEMP response (Eleftheriadou & Koudadounarakis, 2011). However, some studies in the literature have attempted to find the best stimulus parameters in eliciting a robust VEMP response. Previous findings suggested a stimulus rate of 5 Hz for tone bursts and clicks to be optimal in recording VEMP responses (Wu & Morufushi, 1999). A few previous studies evaluated the effects of different repetition rates on VEMP responses using tone-burst stimuli (Carnaúba et al., 2013) and click sounds (Wu & Murofushi, 1999). Although previous studies (Bogle et al., 2015; Carnaúba et al., 2013; Singh et al., 2014a) explored the effects of different repetition rates of tone-burst stimuli on cervical and ocular VEMPs evoked by ACS by measuring peak-to-peak amplitude and the latency of each peak, the quality of the VEMP response was only measured in one study on oVEMP using objective approaches (Singh et al., 2014a). Because most previous estimates were subjective, considerable variations between observers may have occurred regarding the presence of VEMP responses to a given stimulus. Thus, exploring objective and automated methods for identifying the presence of VEMP response may reduce the reliance on subjective interpretations of study results. Several statistical methods have been explored for the automated identification of the responses of evoked potentials, such as the F_{sp} statistic proposed by Elberling and Don (1984) as an objective estimate of response quality, which increases with SNR. By using the F_{sp}, an objective measure of quality can be obtained. Such objective statistical approaches have not been previously used with VEMP. In addition, none of the previous studies explored the effects of high repetition rates of more than 40.8 Hz on VEMP responses. High repetition rates require shorter recording times than low rates do, so evoking VEMP using high rates may lesson neck tension and fatigue. Chapter 4 presents Experiment 2, which aims at studying the effects of changes in stimulus repetition rates on the quality and amplitude of cVEMP responses, using low and high rates. Thus, the following research questions are posed:

RQ3: What is the effect of the tone-burst stimulation rate on the detection of cVEMP response?

RQ4: What is the effect of the tone-burst repetition (stimulation) rate on the quality and amplitude of VEMP response?

RQ5: What is the optimal repetition rate for the clinical recording of cVEMP?

C. Stimulus duration

A few previous studies investigated the effects of different rise and fall times and plateau durations on VEMP responses triggered by short tone bursts. Their results indicated that that using shorter plateau durations produced higher amplitudes of cVEMP responses, compared to longer plateau durations (Cheng & Murofushi, 2001b, 2001a; Marimuthu & Harun, 2016; Singh et al., 2014b). The optimal stimulus duration of tone bursts was found to be a 1-ms rise/fall and a 2-ms plateau (Cheng & Murofushi, 2001b & 2001a). Other results suggested a two-cycle rise/fall and no plateau (Marimuthu & Harun, 2016). However, the combination of 1-ms rise/fall time and 2-ms plateau duration is preferred by most clinicians and thus is suggested for clinical use (Meyer et al., 2015). However, because longer plateau durations reduce spectral splatter, they are more frequency specific in eliciting VEMP response. No previous studies measured the effects of plateau durations longer than 10 ms on cVEMP or oVEMP response. In addition, previous studies investigated the effects of different durations of tone-burst stimuli on cVEMP by subjectively measuring peak-to-peak amplitude and the latency of each peak. However, no previous study has used objective methods to assess the quality of the responses. Furthermore, there is a gap in the knowledge about the origin of the VEMP response according to different ramp and plateau durations. Exploring the generation of VEMP could be clinically important in identifying the optimal ramp and plateau durations of a tone-burst stimulus that elicits responses. In Chapter 4, these responses are addressed in Experiment 3, which is aimed to explore the effects of stimulus duration on the quality and amplitude of cVEMP response using plateau duration longer than 10 ms and to determine the causes of the cVEMP response. Thus, the following research questions are posed:

RQ6: What are the effects of the stimulus duration of tone-burst sounds on the quality and amplitude of cVEMP response?

RQ7: Do varying plateau durations (maintaining the same ramp time) affect the detection of cVEMP response?

RQ8: What generates or causes cVEMP?

2.4 Clinical applications of objective balance testing

Cervical and ocular VEMP and vHIT are still relatively new measurement techniques although their clinical utility in the common peripheral vestibular disorders encountered in otology clinics has already been established (Basta et al., 2008; Migliaccio et al., 2005; Rauch et al., 2004; Weber & Rosengren, 2015; Xu et al., 2015). These objective tests provide complementary information about all the vestibular end organs (otolith organs and all SCCs) in the inner ear. The ECochG test provides an objective measure of the electrical potentials produced in the inner ear by the acoustic stimulation of the cochlea. This test is often used to determine whether there is an excessive amount of endolymphatic pressure, which can cause the symptoms of vertigo, tinnitus, hearing loss, and ear pressure, which could be indications of definite ear pathologies, such as MD. This section is focused on discussing the clinical evidence of these electrophysiological measurement techniques, based on two selected patient groups: MD and CI. These patient groups were selected because to the best of the author's knowledge, they have the highest clinical relevance in otology clinics.

2.4.1 Ménière's disease (MD)

2.4.1.1 Overview

In 1861, Prosper Ménière described a new disease that affected the inner ear, which was characterised by episodic spells of vertigo lasting several minutes and was associated with tinnitus, ear fullness, and fluctuating hearing loss (Stapleton & Mills, 2008). It is believed to be caused by an abnormal accumulation of endolymph fluid termed endolymphatic hydrops most often in the cochlear duct and the sacculus followed by the utricle and the SCCs, respectively based on evidence from human temporal bones studies (Merchant et al., 2005), although hydrops alone does not explain the mechanism underlying all the clinical symptoms of the disease, including the progression of hearing loss and the frequency of vertigo attacks (Rauch et al., 1989). Chapter 6 (section 6.1) provides a detailed overview of the disease.

2.4.1.2 Diagnosing MD

At present, there is no widely accepted gold standard in vestibular testing for the diagnosis of MD. Thus, there is a need to reach an international consensus of MD diagnosis. Unfortunately, other vestibular disorders mimic MD (e.g. vestibular migraine [VM]; see Appendix D). However, the disease is differentiated from other vestibular disorders by its three clinical symptoms: episodic attacks of vertigo, low to medium frequency cochlear loss, and fluctuating aural symptoms (tinnitus, hearing, and sensation of ear pressure) (de Waele et al., 1999; Rosengren et al., 2010). Cochlear symptoms typically last from hours to weeks, whereas acute spinning vertigo typically lasts for at least 20 minutes. However, it usually persists for hours, followed by mild imbalance for a day or two (Furman et al., 2010).

In 1995, the American Academy of Otolaryngology Head and Neck surgery (AAO-HNS) developed a set of criteria to help in the diagnosis of MD, which are widely used. Based on these criteria, patients are clinically classified as having definite MD if they have had at least two definite episodic spontaneous vertigo attacks for at least 20 minutes, documented hearing loss on at least one occasion, and the presence of tinnitus or ear pressure in the affected ear. The disease is classified as probable if one definite episodic vertigo attack has occurred with documented hearing loss on at least one occasion with the presence of tinnitus or ear pressure. The disease is also considered possible if one episodic vertigo attack has occurred without documented hearing loss or if there is SNHL (fixed or fluctuating) associated with disequilibrium without episodic vertigo attacks. The diagnosis of MD can be difficult, especially if vestibular symptoms occur in isolation (i.e., vestibular MD) (de Waele et al., 1990). Therefore, in addition to the patient's history, a physical examination must be conducted to identify the disease. Some evidence in the literature suggests that cVEMPs and ECochG could be useful in the clinical monitoring of MD; however, their clinical utility in the diagnosis of the disease is still limited (Ciorba et al., 2017).

2.4.1.3 VEMPs with MD

Because the otolith end organs are anatomically close to the cochlea, it is assumed that their functional loss may occur immediately after cochlear dysfunction. Okuno and Sando (1987) examined 22 human temporal bones from dead patients with MD and reported that all temporal bones showed cochlear hydrops, saccular hydrops in 86.5 %, utricular hydrops in 50 %, and SCCs hydrops in 36.4 % (Kim et al., 2013). Evidence suggests that saccular neurons have a strong projection to the

neck muscles and a weak projection to the extraocular muscles (Uchino et al., 2005). Because cVEMP responses to AC stimulation are primarily considered to be saccular in origin, a distended saccule can cause alterations in the mechanics of its motion, which might lead to alterations in the frequency tuning of the cVEMP response in MD subjects (Rauch et al., 2004). Based on this evidence, cVEMP may help in the diagnosis or monitoring of the cochleosaccular hydrops in MD (Rauch et al., 2004; Rosengren et al., 2010). Several studies have confirmed that the cVEMP tuning curve, which is a plot of the cVEMP threshold as a function of frequency, occurs at 500 Hz in healthy subjects with the best response and the lowest threshold (Akin et al., 2003; Park et al., 2010; Piker et al., 2013; Rauch et al., 2004; Timmer et al., 2006). Compared with normal subjects, the affected ear in unilateral MD subjects showed a shift upward from 500 to 1000 Hz with a rise in the thresholds of all frequencies (Rauch et al., 2004; Timmer et al., 2006). The unaffected ear of MD subjects also showed alterations in cVEMP tuning and a threshold shift comparable with normal subjects; however, the reason was not identified. It is believed that these may be early signs of the development of bilateral MD, which was suggested by Rauch et al. (2004). Flattening of the cVEMP threshold response curve was found in normal subjects with aged above 60 years (Janky & Shepard 2009; Piker et al., 2013; Piker, 2012). This effect could limit the clinical utility of using the cVEMP threshold response curve in the diagnosis of MD patients over 60 years old. The problem with cVEMP threshold measurements is the subjective identification of the response thresholds. The problem of variability in subjective estimates of response thresholds has been identified in other evoked responses, such as the ABR (see Chapter 5 for details). Several statistical approaches have been explored and used for the objective detection of evoked potentials, such as ABR and ASSR. A brief overview of these approaches is provided in Chapter 5. However, to date, such statistical methods have not been well tested for the measurement of cVEMP threshold curves. An objective analysis may reduce the measurement variability in threshold estimates compared to subjective analysis, because the interpretation of the presence of a waveform by the observer is not required in objective statistical response detection. This is tested in Experiment 4 (Chapter 5), which aims to explore the use of the objective analysis of cVEMP thresholds to improve the measurement of cVEMP thresholds and therefore reduce reliance on subjective interpretative approaches. Thus, the following research questions are posed:

RQ9: Can the cVEMP response be detected objectively using statistical approaches?

RQ10: How do subjective and objective estimates of the cVEMP threshold compare?

In Chapter 6, Experiment 5 addresses the additional aim of exploring the application of the statistical methods used to measure the saccular frequency-tuning curves in patients with MD. Thus, the following research question is also posed:

RQ11: How does objective VEMP frequency-tuning of the saccule compare in subjects with normal hearing and patients with MD?

2.4.1.4 ECochG with MD

ECochG has been widely used as an objective clinical tool for the diagnosis of cochlear hydrops. EcochG response is composed of three basic potentials: the action potential (AP), the summating potential (SP), and the cochlear microphonic (CM) (Ferraro & Durrant, 2006; Wuyts et al., 1997). The AP, which is also referred to as the whole nerve or compound action potential, is the summation of the action potentials of the spiral ganglion and auditory cochlear nerve. The CM is an alternating current voltage, which is generated predominantly by the outer hair cells in the cochlea. The SP is a constant direct current component, which is thought to reflect the excess time displacement of the basilar membrane (BM) toward the scala tympani in response to the asymmetrical vibration of the BM at high intensities (Wuyts et al., 1997). Currently, the CM is thought to be useful in the differential diagnosis of inner ear and auditory nerve diseases, whereas the magnitude of the AP is measured in relation to that SP amplitude and the latter is thought to increase in ears with hydrops due to the distention of the BM towards the scala tympani. At present, it is widely accepted that elevation of the SP amplitude compared with the AP amplitude may be a positive indicator of endolymphatic hydrops in patients with suspected MD. For details on the history of EcochG, see Eggermont (2017).

ECochG has long been used as an objective clinical test for the diagnosis and/or monitoring of MD by the identification of endolymphatic hydrops, which suggests a cause and effect relationship between them (Shepard, 2015). The previous assumption that all patients with endolymphatic hydrops are diagnosed with MD has been rejected by the evidence from temporal bone studies (Merchant et al., 2005). These findings confirm the close correlation between MD symptoms and hydrops and therefore show the difficulty of using ECochG to identify the presence

of MD. As a result, ECochG is considered useful only in conjunction with the patient's clinical findings, audiogram, and historical information (Shepard, 2015).

In addition, the sensitivity and specificity of using ECochG to identify MD using click sound have been found to vary from 20–95 % (Campbell et al., 1992) and 71–96 % (Chung et al., 2004) to 84–92 % (Al-Momani et al., 2009). This variation may be related to the use of different clinical criteria to determine an abnormal SP/AP ratio, different stimulation methods (a brief overview of the recording techniques is presented in Appendix E), sample sizes, recording parameters, and electrode locations (Devaiah et al., 2003). Retrospective studies showed that the sensitivity of ECochG in detecting MD was considerably improved by adding the SP/AP area under the curve ratio measurement to the protocol, in addition to the conventional SP/AP amplitude ratio (Al-momani et al., 2009; Devaiah et al., 2003). The previous studies compared the accuracy of detecting MD using both the conventional SP/AP amplitude ratio and the SP/AP area ratio. In their study of 138 patients with MD, Devaiah et al. (2003), reported that 14 % showed possible MD (as defined by AAO-HNS, 1995), and eight met all the exclusion criteria. Of these 8 patients, 50 % had an abnormal SP/AP amplitude ratio, whereas most (7 patients) had an abnormal SP/AP area ratio. However, the sample size of this study was limited; the findings, therefore, may not be considered robust evidence. In the study by Al-momani et al. (2009), 178 patients were divided into two groups: an MD group and a non-MD group. The diagnosis of MD was based on MD signs and symptoms, including aural fullness and/or dizziness, and/or tinnitus associated with subjective fluctuating, low-frequency hearing loss. In this study, the 92% sensitivity of ECochG in the diagnosis of MD was significantly higher compared with previous studies by Campbell et al. (1992) and Chung et al. (2004), who reported sensitivities of 20 % and 71 %, respectively. However, at 84 %, the specificity was slightly lower compared with earlier studies, which were closer to 95 % and 96 %, respectively (Chung et al., 2004; Margolis et al., 1995). These findings confirmed that measuring the SP/AP area under the curve ratio improved the diagnostic protocol and reduced the testing time for patients with MD (Al-momani et al., 2009). Al-Momani et al. (2009) concluded that using only ECochG to diagnose MD was adequate with specificity and sensitivity values of 84 % and 92 %, respectively.

In contrast, Kim et al. (2005) found that ECochG lacked sensitivity in identifying MD, and concluded that it should not be used to diagnose its presence or absence. These authors performed ECochG using a tympanic membrane electrode on 97

patients with MD (definite n = 60, probable n = 5, possible n = 32) according to the AAO-HNS guidelines. The ECochG tests showed that one in three patients with definite MD had normal SP/AP amplitude ratios (the normal ratio was less than 40 %). In addition, there was no significant difference in ECochG results between the definite and non-definite MD subjects in their study. This result could be related to the use of different normal SP/AP ratios in considering the abnormality. Using higher SP/AP ratios increased the specificity of ECochG in identifying endolymphatic hydrops with decreasing sensitivity, whereas using low SP/AP ratios improved the sensitivity of ECochG with decreasing test specificity (Kim et al., 2005). In the literature, different recording techniques and analytical methods have been used to detect MD. However, no consensus has been reached regarding the optimal clinical method, which remains a subject of debate among researchers.

Few studies in the literature have assessed the efficacy of VEMP and ECochG in diagnosing MD. Lamounier et al. (2017) found that the capability of both tests to identify healthy cases was high (specificity), but their capability in identifying the disease varied from low to moderate (sensitivity). Based on the literature, none of the potential tests could be considered the gold standard for MD diagnosis. Currently, these tests could only be used to complement a clinical diagnosis (Ciorba et al., 2017). In Chapter 6, Experiment 5 is aimed to evaluate the sensitivity and specificity of cVEMP and ECochG in the diagnosis of MD, based on the clinical diagnosis according to the 1995 AAO-HNS criteria. Thus, the following research question is posed:

RQ12: What is the diagnostic power of ECochG and cVEMP in the diagnosis of MD compared to a diagnosis based on the AAO-HNS criteria (i.e. the hypothetical "gold standard")?

2.4.2 Cochlear implant (CI) and its effects on the vestibular system

A CI is a surgically-implanted electronic prosthesis that bypasses the damaged ear. It is inserted directly into the inner ear to stimulate the auditory nerve. It is widely used to initiate or restore hearing in patients with bilateral deafness who are not able to benefit from conventional hearing aids. However, fitting the CI poses risks to the contiguous organs, especially the vestibule (Jacot et al., 2009). In fact, 23–100 % of cases were found to have vestibular dysfunction following implantation (Robard et al., 2015). Histopathological analyses of the inner ear in human and

animal temporal bones after implantation showed that this surgery could damage the peripheral vestibular end organs (Hanzel et al., 2006; Tien & Linthicum, 2002). One hypothesis regarding vestibular impairment following implantation is that the surgical insertion of CI electrodes into the inner ear causes direct trauma to the vestibular receptors (Tien & Linthicum, 2002; Todt et al., 2008). Within the vestibular apparatus, the saccule was found to be the most affected receptor, followed by the utricle and the SCCs (Tien & Linthicum, 2002). It was suggested that when the cochleostomy opening to insert the CI electrodes is closer to the saccular macula, the utricular and SCC functions usually remain undamaged because of their distance from the cochleostomy site (Basta et al., 2008; Tien & Linthicum, 2002). Thus, the saccule is the most commonly affected vestibular organ compared with the utricle and the SCCs.

Generally, the otolith organs are evaluated by VEMP. However, further research is needed to confirm the relationships between the stimulus used (ACS, BCV, and different frequencies), the response measured (cVEMP or oVEMP), and the end organ that gives the response.

To date, the measured rate of saccular dysfunction using cVEMP testing which has been reported after CI surgery is between 21–100 % (Basta et al., 2008; Jin et al., 2006; Krause et al., 2010; Licameli et al., 2009; Melvin et al., 2009; Robard et al., 2015; Todt et al., 2008). However, not all CI patients suffer from post-operative dizziness (Buchman et al., 2004; Fina et al., 2003; Katsiari et al., 2013). Thus, there is a lack of correlation between vestibular test outcomes and subjective symptoms following implantation in CI recipients (see Chapter 7 for a detailed explanation). The evaluation of the risk of the impairment of the utricle has received little attention in the literature. Xu et al. (2014) reported that 82 % of children with clear preoperative AC oVEMP responses showed utricular dysfunction in the implanted ear following surgery. However, it is still debatable whether oVEMP to acoustical stimulation is a saccular response or a utricular response. SCCs are the vestibular organs that are the least affected by implantation (Handzel et al., 2006; Tien & Linthicum, 2002). To date, very few studies have evaluated the function of all SCCs before and after implantation using vHIT and 3D HIT. Migliaccio et al. (2005) reported that only one subject (9 %) had a significant decrease in VOR gain in all SCCs on the side of the implant and experienced transient vertigo following CI surgery. Melvin et al. (2009) reported that only 1 of 28 patients (3.6 %) showed a significant drop in the vestibular function of all three SCCs (from normal, to severe,

to profound vestibular dysfunction). However, Maheu et al. (2017) did not report any loss of function in any patient when using the vHIT, which suggested that the function of the SCCs was not affected by CI surgery. Thus, further studies are needed to evaluate the function of vertical SCCs before and after CI surgery in order to confirm the slight reduction in canal function after implantation which was reported in the previous two studies (Melvin et al., 2009; Migliaccio et al., 2005). A detailed overview of the effects of CI surgery on each sensory organ of the vestibular system is presented in Chapter 7.

Because of the recent increase in bilateral implantation, the comprehensive assessment of all vestibular end organs before and after implantation is critical. Only one recent study evaluated the function of each of the five sensory vestibular organs separately using VEMP and vHIT (Maheu et al., 2017). The findings showed that 75 % (3 of 4) of CI recipients showed the complete loss of cervical and ocular VEMP responses in the implanted ear post-operatively, but no patient reported a change in SCC function. However, in-depth research is required to confirm this finding by measuring the function of all vestibular organs separately before and after implantation. In Chapter 7, these functions are addressed in Experiment 6, which aims to study the risks of balance dysfunction following implantation by using VEMP and vHIT vestibular measurements in adults before and after implantation. Thus, the following research question is posed:

RQ13: Does cochlear implantation affect vestibular function in adult humans?

2.5 Research objectives

The aims of this research are as follows:

Experiment 1 aims to explore the VEMP response from the SCM muscle to AM sounds in a range of modulation rates (see Chapter 3).

Experiment 2 aims to explore the generation cVEMP responses and the adaptation of cVEMP to low and high repetition rates (see Chapter 4).

Experiments 3 and 4 aim to detect the cVEMP response objectively using statistical approaches and to explore the use of these approaches to objectively estimate the frequency-tuning curve of the saccule in both healthy subjects and MD patients (see Chapter 5 and 6).

Experiment 5 aims to determine the risks of balance dysfunction following cochlear implantation by using VEMP and vHIT vestibular measurements in adults before and after implantation (see Chapter 7).

These studies should improve understanding of 1) the generation of VEMP responses, 2) optimal stimulations for measuring responses and 3) clinical applications of objective testing assessment methods.

Chapter 3 : VEMP in response to AM Tones

3.1 Chapter overview

In the research literature to date only two studies have used AM tones to evoke cVEMPs (Bell et al., 2010; Oliveira et al., 2014). The motivation behind using AM tone for evoking VEMP lies in the fact that AM tone is more frequency specific than the tone-bursts that are commonly used in standard VEMP. VEMPs evoked by steady-state AM tones are termed as S-VEMPs in previous studies. Bell et al. (2010) measured VEMP responses from the SCM muscle in response to a frequency of 500 Hz, with 100 % amplitude modulation, modulated at different frequencies (5, 39, 59, 78, 98 and 122 Hz) (Bell et al., 2010). They found that the highest SNR (18.9) was obtained at a 78 Hz modulation frequency, whereas the greatest amplitude was observed at a modulation rate of 39 Hz. Oliveira et al. (2014) investigated the tuning of S-VEMP responses to 250 Hz, 500 Hz and 1 KHz carrier frequencies, modulated at 20, 37, 40, 43, 70, 77 and 80 Hz. They found the optimal modulation frequency for different carrier frequencies in addition to 500 Hz. In their study, all these carrier frequencies showed statistically higher amplitude at modulation frequencies between 37-43 Hz responses. However, there is insufficient normative data for cVEMP evoked by AM tones. More research is therefore required to confirm the optimal modulation rate for VEMP. Only one study (Bell et al., 2010) has compared thresholds of S-VEMPs and standard VEMPs (frequency 500 Hz 1:2:1cycle tone pips). This showed that a 500 Hz-modulated frequency at 78 Hz produced a significantly higher threshold (by about 3.7dB LEQ (A)) than standard VEMP. This finding may limit the use of AM tones to evoke VEMP in clinical testing (Bell et al., 2010), although more research is needed to confirm this finding. As VEMP is a large amplitude muscle response to loud sounds, sounds should be loud enough to evoke a clear VEMP, but overexposure to noise may cause damage to the cochlea (Rosengren et al., 2010). However, a possibility may be to test a number of frequencies simultaneously with the S-VEMP approach. This may potentially be useful in an early diagnosis of MD by measuring the saccular tuning curves for subjects with the disease. (Rauch et al., 2004). Using a regularly-repeating stimulus such as an AM tone is more frequency-specific than the 1:2:1 tone pips that are commonly used for standard VEMP measurement. However, individual waves in the

VEMP response cannot be seen when viewed in the frequency domain by using the S-VEMP approach, so standard VEMP may be more appropriate for some diagnostic purposes. (Bell et al., 2010).

Therefore, the present study aimed to investigate VEMP responses from the SCM muscle to AM tones over a range of modulation frequencies and compare thresholds of S-VEMP and standard cVEMP. This chapter presents the results of one experiment with two parts: 1a) VEMP in response to AM tone and 1b) threshold measurements of standard cVEMP and S-VEMP.

Research questions:

- What is the optimal modulation rate for AM VEMP?
- Is the threshold of S-VEMP significantly higher than the threshold of standard VEMP?

Aims

The aims of this project were as follows:

- To explore VEMP responses from the SCM muscle to AM sounds over a range of modulation rates.
- To find the optimal modulation rate for S-VEMP.
- To compare the stimulus thresholds for standard cVEMP with the stimulus response for S-VEMP.

3.2 Methods

The experimental protocol for this study was approved by the Human Experiment Safety and Ethics Committee (ERGO: 17544) of the University of Southampton before the research commenced.

3.2.1 Participants

For experiment 1a, 45 potential subjects were recruited. They had a mean age of 30.25 years, and a range of 25-48 years. However, of the total, only six subjects were included in the study, as the majority of subjects did not show responses on S-VEMP. Modulated VEMP could not be recorded in the majority of potential volunteers, so the power of the study was low. This study required approximately 17 adult subjects to get a power >82 %. The required sample size was determined with the use of data from a previous study on AM VEMP (Bell et al., 2010), assuming

that the mean (m) difference for 10 subjects (19 ears) is 16.6 dB LEQ (A) and standard deviation (SD) difference is 22.2 dB LEQ (A) for two modulation rates (39 Hz and 59 Hz) of 500 Hz carrier tone. For 17 subjects, the difference between the two modulation rates would be detected with a power of 82 %.

For experiment 1b, 12 normal adult subjects were recruited, with a mean age of 32.45 years, and an age range of 25–49 years. This experiment required approximately 10 adult subjects to get a power >80 %. The required sample size (n = 10) was determined using data from a previous study on finding the threshold of standard VEMP and S-VEMP (Bell et al., 2010), assuming the following amplitudes in terms of dB A:

Mean (standard VEMP): 85.73 Mean = (modulated VEMP):87.92

SD (standard VEMP): 5.39 SD = (modulated VEMP):3.35

For 10 subjects (19 ears), a mean difference of 2.18 dB A and SD difference of 2.03 dB A in thresholds of standard VEMP and modulated VEMP are required to obtain a power of 84 %.

For experiments 1a and 1b, all subjects gave informed consent to participate and had pure-tone thresholds better than 20 dB HL at frequencies between 250 Hz and 8 KHz. Subjects who had hearing problems, balance problems, neck/back stiffness or neck/back pain were excluded from the study.

3.2.2 Stimuli

For experiment 1a, VEMP responses from the SCM muscle were measured using 500 Hz carrier frequency 100 % modulated at 15, 25, 35, 45, 55, 65, 75, 85, 95, and 105 Hz, for a fixed stimulation level of 111 dB A (122.1 dB p.e. SPL). The total duration for 10 recordings was 50 s, with 5 s for each recording. The order of presentation of modulation frequencies was randomised among subjects. Subjects who did not have VEMP responses to AM tones at a modulation frequency of 75 Hz were excluded from the study. The eight-hour or daily personal exposure level in dB A for 10 conditions was 75.1 dB A, which was within the conservative limit of ‘usual’ exposure defined by the ISVR’s Human Experiment Safety and Ethics Committee by means of an equivalent eight-hour noise dose of 76 dB A.

For experiment 1b, subjects who had S-VEMP responses in the first part of experiment 1 were included in the second part, and subjects who did not have standard VEMP responses were excluded. Threshold measurements were compared for standard cVEMP using a 500 Hz 1:2:1 (one cycle rise/fall and two cycles plateau) tone-burst stimulus and for S-VEMP using a 500 Hz tone modulated at 75 Hz (the highest mean SNR was obtained for 75 Hz modulation, so this modulation frequency was chosen for threshold measurements of S-VEMP). For standard cVEMP, the stimulus was presented at a rate of 5 Hz (epoch length 0.2 s). Each recording was 150 repeats of an 8 ms tone pip, and total duration for each recording was 30 s. The stimulus level was decreased in 4 dB steps from 103 dB A (123.4 dB p.e. SPL). For modulated VEMP, the total duration for each recording was 5s. The stimulus level was decreased in 3 dB steps from 111 dB A (122.1 dB p.e. SPL). To avoid excessive noise exposure and SCM muscle fatigue, the recordings of thresholds were performed on two separate days for standard VEMP and S-VEMP.

For both parts (1a and 1b), stimuli were presented to the subjects using insert earphones (Etymotic ER-3A). The calibration of the stimuli was carried out through a Brüel and Kjar (B&K) type 2260 sound level meter (SLM), attached to an occluded ear-canal simulator type 4157 (IEC-711coupler). Responses were recorded ipsilaterally from the right SCM muscle. It was not possible to record data from both ears to obtain bilateral VEMP responses, since a long recording period would exceed the maximum allowable amount for a typical exposure and could cause muscle fatigue. Thus, to minimise the measurement time and reduce the risk of muscle fatigue, it was decided to measure the response only from one ear (right) of each subject.

3.2.3 Apparatus

The equipment used in this study to deliver the stimuli for cVEMP measurement was Cambridge Electronic Device's CED 1401 data acquisition system and CED 'signal' software (http://ced.co.uk/). A sampling rate (input and output) of 10 KHz was used. The output from the Digital to Analogue Converters (DAC) port was routed through a headphone amplifier (OBH-21) to control the intensity of the stimulus. Amplification of the signals was performed using an isolated amplifier (CED 1902) with a 1-3000 Hz bandpass filter and 1000 gain.

3.2.4 cVEMP recording

VEMPs from the SCM muscle were recorded ipsilaterally while subjects were seated upright on a chair with their chin turned over the contralateral shoulder to tense the SCM muscle. The electromyographic (EMG) activity of the SCM muscle was recorded using surface electrodes placed on the muscle (as shown in Figure 3.1 below): active on the belly of the ipsilateral SCM muscle, and reference on the upper sternum of the test side. A ground electrode was placed on the lower forehead. The impedance of the electrodes was kept below 10 KΩ. The EMG activity of the SCM muscle was visually monitored on an oscilloscope and kept between 80 and 100 mV, to ensure tension of the muscle.

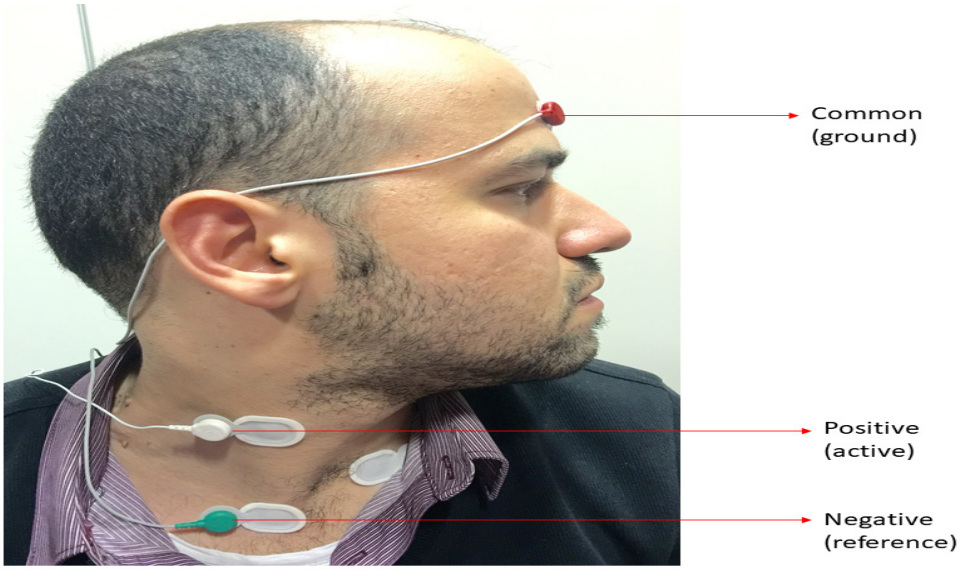

Figure 3.1: Positions of the electrodes in cVEMP recording.

3.2.5 Response analysis

For standard VEMP, the stimulus level was decreased in 4 dB steps from 103 dB A, and the threshold was determined as the lowest stimulus level that produced a clear and significant response using F_{SP}>3. Thus, the presence of the response was defined via F_{SP}> 3 (Elberling & Don, 1984). This was completed by calculating the ratio between the estimated level of the VEMP signal and the averaged background noise to estimate the SNR, as defined in Equation 3.1 below, to confirm the cVEMP response:

$$Fsp = N \times \frac{VAR\ (Signal)}{VAR\ (Single\ point)} \qquad (3.1)$$

where VAR indicates variance (squared standard deviation) and N is the number of epochs or sweeps. The variance, *VAR (Signal)* was obtained from the coherent averaging of the signals to obtain the variance within the averaged signal after the beginning of the stimulus over a defined time window, and *VAR (single point)* was found from the ensemble of N signals prior to averaging. Therefore, *VAR (signal)* represents the signal whereas the *VAR (single point)* represents the averaged background noise.

The single point was arbitrarily chosen prior to averaging, based on the assumption that the variance of the electroencephalography (EEG) can be assumed to be fixed over the interval between stimuli (Lv et al., 2007). The time window used for analysis was 40 ms (single point at 20 ms). Hence, the resulting statistic is called F for a single point (F_{sp}).

For S-VEMP, the stimulus level was decreased in 3 dB increments from 111 dB A, and the threshold was determined as the lowest stimulus level that produced a clear and significant response using a SNR > 2 dB, as defined in Equation 3.2 below. Thus, the presence of a response was confirmed by a SNR > 2 dB (amplitude at the modulation frequency in relation to the average amplitude in 10 FFT (Fast Fourier Transform) bins spaced at 1 Hz (in a 1 s window) on either side of the modulation rate):

$$\frac{A\,(index)}{\sum_{n=1}^{n=10}[A(index-n)+A(index+n)]} \quad (3.2)$$

where A (index) refers to the FFT amplitude corresponding to the modulation rate, and n is the modulation rate spaced at 1 Hz in 10 FFT bins on either side of the modulation rate.

3.3 Results

Experiment 1a: VEMP in response to AM tone

Including those recruited participants who were eventually screened out, 45 subjects participated in this study. However, of the total, only six subjects were included in the study, as the majority of subjects did not show responses to S-VEMP. As normality was not rejected, a repeated-measures ANOVA has been used to analyse the results of this study. Two analyses were carried out for amplitude and SNR of the modulated cVEMP (or S-VEMP). The independent factor was the

modulation frequency (n = 10). To be cautious, a Greenhouse-Geisser correction was applied for sphericity.

Figure 3.2 shows the amplitude of S-VEMP responses at different rates and suggests that no modulation frequency evoked a significantly higher amplitude than the others. A repeated-measures ANOVA with Greenhouse-Geisser corrected values showed a non-significant effect of modulation rate on VEMP amplitude F (1.98) =2.37, p=0.144.

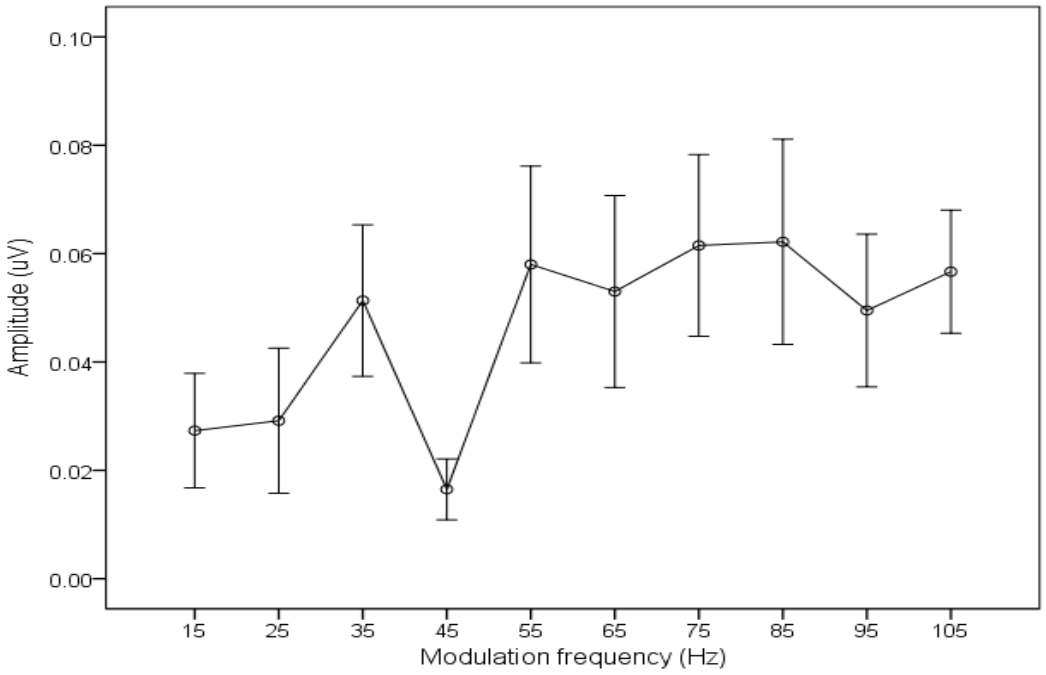

Figure 3.2: Amplitude of S-VEMP responses to 500 Hz carrier frequency 100 % modulated at different rates. Error bars represent ±1 standard error (SE) of the mean.

Figure 3.3 shows the SNR of S-VEMP responses to a 500 Hz carrier frequency 100 % modulated at different frequencies. A repeated-measures ANOVA with Greenhouse-Geisser corrected values showed a significant effect of modulation rate on the SNR of VEMP response F (2.42) =3.73, p<0.05. Post hoc paired t-tests with Bonferroni correction were carried out to identify which modulation frequencies showed statistically significant differences from one another. The results showed that the SNR of the 500 Hz carrier frequency modulated at 75 Hz was significantly higher than those of 15, 25, 35, 45, 55, 65, and 95 Hz (p < 0.05). The greatest mean SNR of 8.5 dB was obtained for 75 Hz modulation. Thus, this modulation frequency (75 Hz) was chosen for subsequent threshold measurements of S-VEMP.

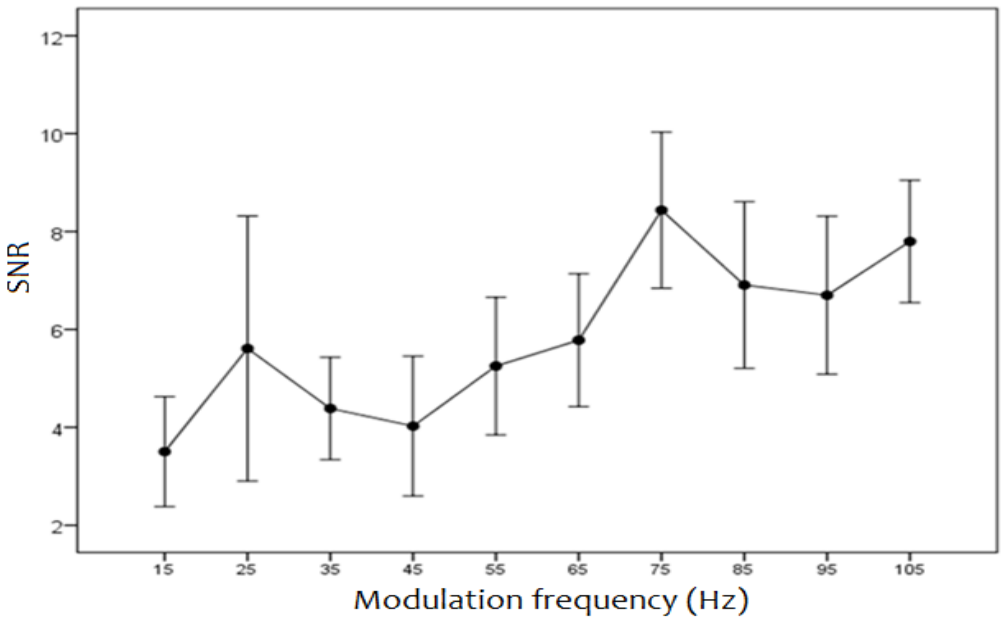

Figure 3.3: SNRs of S-VEMP responses to a 500 Hz carrier frequency, 100 % modulated at different frequencies. Error bars represent ±1 SE of the mean.

Experiment 1b: Threshold measurements of standard cVEMP and S-VEMP

Thresholds of standard VEMP and S-VEMP were compared for six subjects with S-VEMP response. In dB A, thresholds of S-VEMP were 100 (*SD*=7.50) and 93 (*SD*=3.29) for standard VEMP. As normality was not rejected ($p > 0.05$), a paired-measures *t*-test was conducted to analyse the data. The *t*- test showed that the thresholds of standard VEMP and S-VEMP were not statistically significantly different ($p > 0.05$); t (5) = 1.68, $p = 0.154$, two-tailed (Figure 3.4). The trend of a higher S-VEMP threshold is consistent with earlier work (Bell et al., 2010), although the sample is fairly small, so may lack statistical power to detect the difference in effects that might be evident with a larger group of subjects.

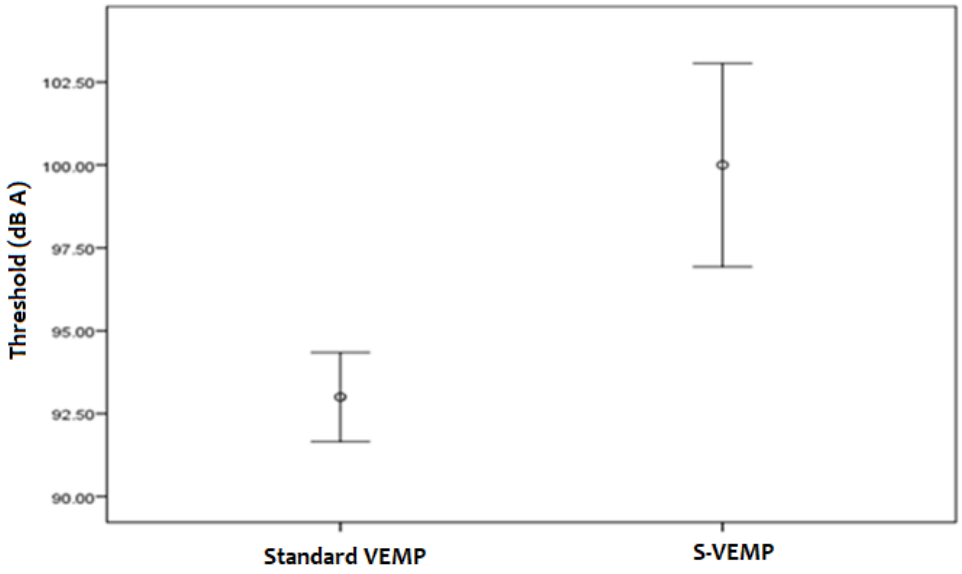

Figure 3.4: Threshold measurements of standard cVEMPs and S-VEMP modulated 75 Hz. Error bars represent ±1 SE of the mean.

3.4 Discussion

Experiment 1a: VEMP in response to AM tone

Of the initial 45 individuals who had volunteered to participate, only six subjects were included in the study. This is because the majority of volunteers did not show S-VEMP responses, although they did show standard VEMP responses. This problem in recording S-VEMP responses among subjects has not been reported in previous studies (Bell et al., 2010; Oliveira et al., 2014). The lack of S-VEMP response might be related to using different stimuli: standard VEMP was evoked by short tone-bursts with 8 ms duration, and S-VEMP was evoked by a continuous AM tone. The presence of responses to short tone-bursts, in combination with a lack of responses to AM tone, might suggest that VEMP may be triggered by a peak level. However, it is still unknown exactly what characteristic of the stimulus triggers the VEMP response; it could be either the peak level of the tone (e.g., in a series of short tone-bursts), or energy within the stimulus (e.g., in a continuous tone). More research is required in order to confirm what generates VEMP. In addition, the noise exposure limits for studies in ISVR are conservative. Thus in this study the exposure was kept within a 'usual' exposure limit of 76 dB A for 8 hours equivalent. If the sound was louder, then this might increase the response rate of S-VEMP, but this was not considered ethical.

For the current study, in order to make sure that the system was working properly, a simulation of the S-VEMP test procedure was conducted, as shown in Figure 3.5 below. The output from the DAC port of the CED 1401 data acquisition system (A) was routed through a signal rectifier to generate a half-wave rectified signal (B). Thee rectified signal was then filtered using an Icemo Dual Variable Filter (a 300 Hz low bandpass filter) (C). The filtered signal was routed to the input port of the CED system and produced a clear VEMP response to an AM signal, with its highest amplitude at the modulation frequency of 75 Hz (D).

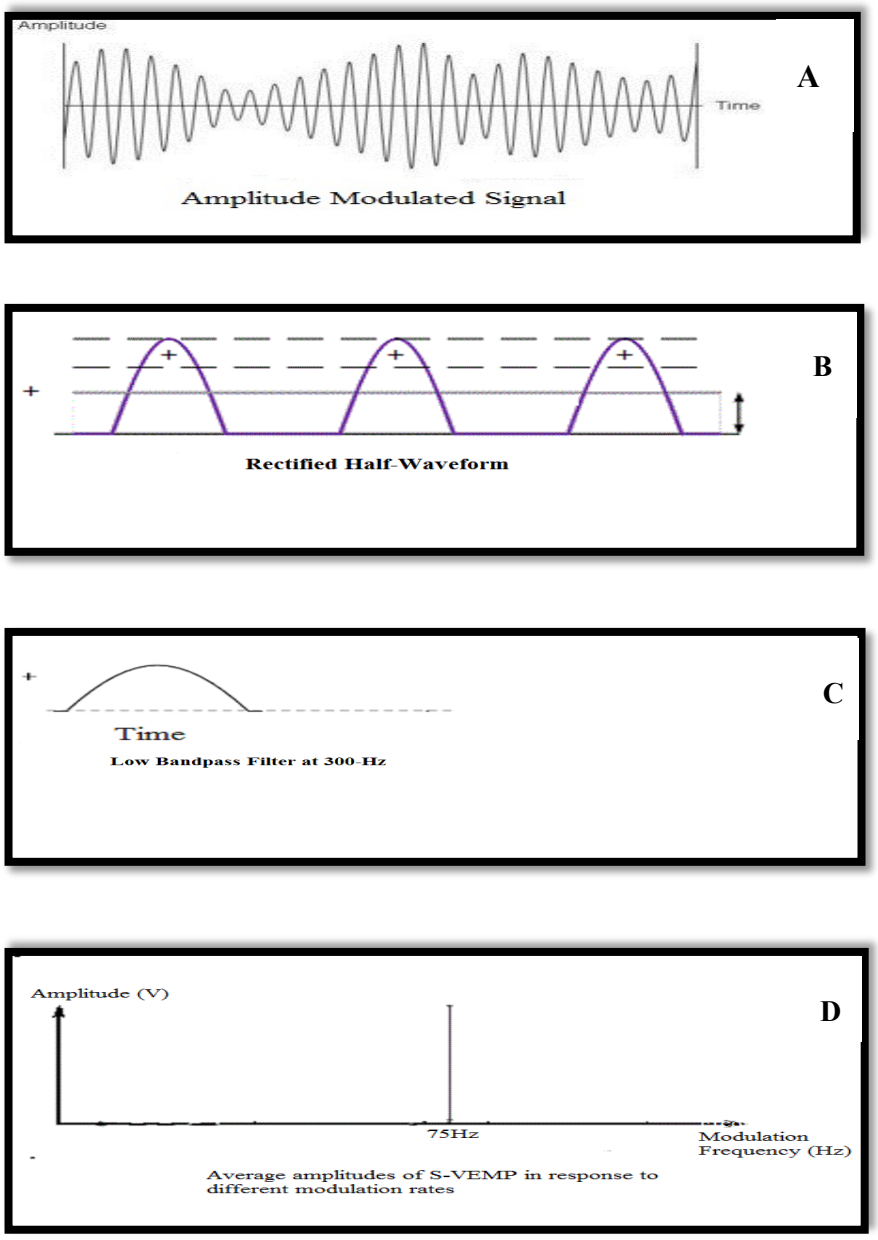

Figure 3.5: Simulation of the S-VEMP test procedure. A: Amplitude modulated signal. B: Half-wave rectified signal. C: a 300 Hz low bandpass filter. D: a clear VEMP response to an AM signal modulated at 75 Hz.

In addition, the presence of an S-VEMP response was double-checked using another system, the Biologic MASTER™, which is similar to systems that previous studies have used to demonstrate VEMP responses to AM tones (Bell et al., 2010). Three subjects showed responses to S-VEMP on this system, whereas two of them did not show responses on the CED 1401 system used in the current study. However, those two subjects showed a response at a level of 103 dB A (113.8 dB p.e. SPL) when the number of sweeps (epochs or time windows) in the spectrum was 16 for one subject and 8 for the other subject. Therefore, the total number of epochs for the two subjects should be at least 256 and 128 epochs, respectively, in order to obtain a response on the CED equipment. The current study used five epochs for each recording on the CED equipment, and each recording lasted 5s. In contrast, Bell et al. (2010) used 12 epochs with a 55s duration for each recording, using the Biologic MASTER system. Possibly, subjects who did not show responses to AM tone on the CED system may need more averaging of the repeated sweeps in order to get higher SNR. However, trying a recording for a similar time at the same level (103 dB A) on the CED equipment to see if a response could be measured was not possible, as a long recording would exceed the maximum allowable amount for a typical noise exposure and might cause muscle fatigue.

The current study reviewed data for VEMP responses evoked by AM tone and it was found that the availability of such data was limited, as it is mentioned in only two studies (Bell et al., 2010; Oliveira et al., 2014). The finding of the current study regarding the optimal modulation rate for evoking S-VEMP is similar to that observed in a previous study (Bell et al., 2010), which showed higher SNR response (18.9) to a high modulation frequency of 78 Hz, although the higher amplitude was obtained at 39 Hz. In contrast, Oliveira et al. (2014) found better amplitude responses of S-VEMP at low modulation frequencies of 37, 40, and 43 Hz. In the latter study, 250 Hz, 500 Hz, and1 KHz carrier frequencies were tested, whereas in the current study and earlier study (2010), only one carrier frequency, 500 Hz, was tested. This discrepancy in the results among studies might be related to using different methods to define the presence of the response of S-VEMP; the current study and Bell et al. (2010) used a similar method to confirm the response. Bell et al. (2010) defined the response by using the FFT to calculate the bin amplitude of the modulation rate in relation to the average amplitude in 64-FFT bins spaced at 0.61 Hz on either side of the modulation rate. The current study defined the response by using the FFT to calculate the bin amplitude of the modulation rate in relation to the average amplitude in 10-FFT bins spaced at 1 Hz (in a 1-s window)

on either side of the modulation rate. Therefore, the presence of the response was defined as a SNR > 6 dB (2010) and a SNR >2 (current study). In contrast, Oliveira et al. (2014) used a magnitude-squared coherence method with an estimate of the ratio of signal power to total power.

Experiment 1b: Threshold measurements of standard VEMP and S-VEMP

Threshold values of standard VEMP and S-VEMP modulated at 75 Hz were compared for six subjects with S-VEMP response. The mean thresholds of S-VEMP were higher by 7 dB A than the mean thresholds for standard VEMP. A paired-measures *t*-test showed that thresholds of standard VEMP and S-VEMP were not statistically significantly different. There is a trend for the data to be significant, but there might be insufficient power to detect the effects that might be evident with a larger group of subjects. The current study reviewed data from studies which compared the thresholds of S-VEMP with those of standard VEMP and found that the availability of this kind of data was limited. Such data is mentioned in only one study, which found that thresholds of a 500 Hz modulated frequency at 78 Hz were significantly higher by approximately 3.7 dB LEQ (A) than those for standard VEMPs (Bell et al., 2010). Overall, the trend of a higher S-VEMP threshold is consistent with earlier work, although the sample is fairly small in this study.

The power of the current study was calculated using IBM SPSS Sample Power (*t*-test for paired samples), taking the mean and SD difference for six subjects, m=0.21 and *SD*=0.22 for two modulation rates (85 Hz and 95 Hz) of a 500 Hz carrier tone. For the six subjects, the difference between two modulation rates was detected with a low power of 50 %. Therefore, for the current study, there was insufficient power to identify any difference in effects that might be evident with a larger group of subjects, although an issue for the study was the low number of subjects with S-VEMPs.

3.4.1 Main Findings

- The majority of potential volunteers did not show responses on S-VEMP. Only six subjects were included in the study, so the power of the study was low, at 50 %.
- Responses at 75 Hz produced the highest SNR, although the sample size was low and the effect of modulation rate on amplitude was not significant.

- For subjects with S-VEMP response, mean thresholds for standard VEMP response were lower by 7 dB than the thresholds for S-VEMP response. However, the thresholds were not statistically significantly different.
- Staying within ISVR noise exposure limits, it is hard to record S-VEMP; although not conclusive, it looks likely that this is because S-VEMP thresholds are higher than those of cVEMP, which been shown in another study.

3.5 Conclusions

This study demonstrated that responses from the SCM muscle to 500 Hz amplitude modulated tones can be recorded, but not in many subjects. The majority of subjects did not show S-VEMP responses, although they did show standard VEMP responses. The prevalence of S-VEMPs appears lower than that of standard VEMPs. It appears that the response to a long duration AM tone cannot be predicted from the response to a short tone-burst. In addition, S-VEMP thresholds are higher than those of cVEMP, so the noise exposure limits are a limitation of the study. More research is required in order to confirm what generates VEMP with different rates and stimulus durations. Replication of the study using a larger group of subjects to increase the power of the study might be a desirable direction for future research. However, noise exposure and the low incidence of S-VEMPs are issues.

The original plan from this chapter was explored by investigating VEMP to AM tone (called S-VEMP). However, this plan did not work for the majority of people, so it was decided to explore effects of repetition rates and plateau durations in more detail in the next study (Chapter 4).

Chapter 4 : Effects of changes in the stimulus repetition rate and plateau duration on cVEMP in response to tone-bursts

4.1 Chapter overview

In the previous experiment measuring the response of cVEMP to AM tones, the majority of subjects did not show S-VEMP responses, although they did show standard cVEMP responses. The prevalence of S-VEMPs appears lower than that of standard VEMPs at acceptable experimental test levels. The lack of response could be attributed to the stimulus used for eliciting the VEMP response: standard VEMP is evoked by short tone-bursts, whereas S-VEMP is evoked by a continuous AM tone. It appears that a long duration AM tone does not elicit VEMPs as effectively as short tone-bursts. However, it is still unknown exactly what characteristics of the stimulus trigger the cVEMP response. Thus, the current experiment explores how cVEMP adapts with repetition rate and stimulus duration.

This chapter presents the experimental findings of two separate experiments, finding the effect of changes in 1) repetition rates and 2) plateau durations on the amplitude, latency and quality of cVEMP responses, using 500 Hz tone-burst stimuli for normal subjects. By exploring what generates cVEMP responses, and how cVEMP adapts with low and high repetition rates, it was possible to establish the likely reason for the lack of S-VEMP responses in the majority of participants in Experiment 1.

4.2 Experiment 2: The effect of changes in stimulus repetition rate on cVEMP response parameters

4.2.1 Introduction

There is no agreed standard for VEMP testing. Previous studies have used a 5 Hz repetition rate to evoke AC cVEMP, for different purposes (Akin et al., 2003; Govender et al., 2011; Park et al., 2010; Piker et al., 2013; Todd et al., 2000; Young, 2006). A few studies have explored the effects of different repetition rates on cVEMP in AC mode, using tone-burst stimuli (Carnaúba et al., 2013; van Tilburg et al, 2016) and click sounds (Brantberg & Fransson, 2001; Wu & Murofushi, 1999). Wu and Murofushi (1999) investigated the optimal stimulation rate for eliciting cVEMP with click stimuli. Latency and peak-to-peak amplitude of cVEMP for five click stimulation rates (1, 5, 10, 15 and 20 Hz) were analysed and calculated. They found that the rates of 1, 5, and 10 Hz produced 100 % response rate. Although 1 and 5 Hz rates produced the highest amplitude, they concluded that a 5 Hz repetition rate is preferable for the clinical use of cVEMP elicited by click stimuli, since it requires a shorter recording time than a 1 Hz rate. Brantberg and Fransson (2001) investigated the optimal stimulation rate for eliciting cVEMP with click stimuli. Latency and peak-to-peak amplitude of cVEMP for five click stimulation rates (4, 6, 8, and 20 Hz) were analysed and calculated. The authors found that at a 4 Hz rate a response was present in all of the 23 subjects, and that this rate produced higher amplitudes than all the other rates, with no significant difference in latency between these rates. Thus, they concluded that 4 Hz is the best rate for clinical recording of cVEMP elicited by click sounds.

A study by Carnaúba et al. (2013) evaluated the effect of repetition rates (5.1, 10.2, 20.4 and 40.8 Hz) on AC cVEMP using 500 Hz tone-burst stimuli. They observed a progressive decline in amplitude with increased repetition rates. They found that 5.1 and 10.2 Hz repetition rates produced higher amplitudes than all the other rates, with no significant difference in amplitude between these rates. Therefore, they recommended 10.2 Hz as the best rate for clinical recording of cVEMP, as it requires a shorter recording time and consequently reduces the patient's discomfort, produces better cVEMP morphology and shows a higher reliability than the 5.1 Hz rate. Recently, van Tilburg et al. (2016) assessed the effect of two rates, 5 and 13

Hz, on the threshold and amplitude of cVEMP, using 500, 750, and 1000 Hz tone-burst stimuli. They found that the rate of 5 Hz produced significantly larger cVEMP amplitudes than the 13 Hz rate for 500 and 750 Hz tone-burst stimuli, with no significant difference in threshold. However, these authors recommended the 13 Hz rate for the clinical recording of cVEMP, as the duration of muscle contraction required is about three times shorter than for the 5 Hz rate. No previous studies have explored the effect of very high repetition rates on the cVEMP response.

For oVEMP, a few studies have also explored the optimal stimulation rate for eliciting oVEMP in AC mode using tone-burst stimuli (Bogle et al., 2015; Singh et al., 2014a) and concluded that 5 Hz is the best repetition rate for clinical recording of oVEMP in this context. Few studies have investigated the optimal stimulation rate of VEMP using BCV. Sheykholeslami et al. (2001) reported that 10 Hz was the optimal repetition rate for clinical recording of BC cVEMP, whereas Chang at al. (2010) found that 20 Hz was an optimal repetition rate for the clinical recording of BC-evoked oVEMP and cVEMP.

Although these previous studies subjectively investigated the effect of different repetition rates of tone-burst stimuli on cervical and ocular VEMPs evoked by ACS by measuring peak-to-peak amplitude and the latency of each peak, the objective measurement of response quality, which can be measured by using objective statistical methods (e.g. the F_{sp} statistic), was only measured in one study on oVEMP (Singh et al., 2014a). No previous studies have explored the effect of different repetition rates of tone-burst stimuli on the quality of cVEMP in AC mode using objective metrics, which is a novel contribution of the present study. Previous work (Carnaúba et al., 2013; van Tilburg et al., 2016; Wu & Murofushi, 1999) looked at parameters of the cVEMP such as amplitude, latency or threshold as a function of rate. However, these studies did not directly measure response quality. For a given cVEMP response, the response can contain high or low noise. Indeed, a potential problem with subjective estimates of amplitude or latency is that noise and response can be confused, and thus introduce measurement variability. By using F_{sp}, an objective measure of quality is obtained. By combining this with bootstrapping, a p value (probability of obtaining significant test results if the null hypothesis is true) can be added to the F_{sp} of the response, so determining if the response is statistically different from noise or not. Such objective response metrics as a function of rate

have not been used before with cVEMP. van Tilburg et al. (2016) only looked at two rates and did not map the rate function over many rates. Moreover, they did not use objective methods to determine when a response was present. Wu & Murofushi (1999) did look at several rates, but only measured amplitude and latency subjectively, and they did not measure quality objectively. Whilst Brantberg & Fransson (2001) attempted to objectively indicate the presence of a response for the cVEMP, they did not use statistical metrics of quality that can be compared across rates, such as the F_{sp}, and their method for response detection is not a standard one for evoked response detection. They also only explored a limited set of rates. The approach of bootstrapping the F_{sp} to obtain a p value should be much more statistically robust than the method used by Brantberg and Fransson.

As high repetition rates require a shorter recording time than low repetition rates for the same number of averages, using high rates in evoking cVEMP could potentially lessen fatigue and the need to maintain neck tension for the subject. At high rates, more epochs can be recorded in a short period of time. Thus, increasing the recording time for high repetition rates might increase the number of averages of multiple epochs, thereby improving the quality of the cVEMP response. Therefore, the current study aimed to investigate the effect of low and high repetition rates (1-100 Hz) on the quality (using F_{sp} values) and peak-to-peak amplitude of cVEMP responses. This study also aimed to clarify whether the repetition rates commonly used in the clinical recording of cVEMP truly optimise the response.

4.2.2 Aims

The aims of this project were as follows:

- To explore the VEMP response from the SCM muscle to ACS 500 Hz tone-bursts over a range of repetition rates;
- To determine how cVEMP adapts to repetition rates; and
- To identify the optimal repetition rate with the maximum amplitude and quality for clinical recording of AC cVEMP evoked by 500 Hz tone-bursts.

4.2.3 Research questions:

- What is the effect of the tone-burst stimulation rate on detection of cVEMP response?
- What is the effect of the tone-burst repetition (stimulation) rate on the quality, amplitude, and peak latency of cVEMP response?
- What is the optimal repetition rate for clinical recording of cVEMP?

4.2.4 Methods

The methods used in the current research on cVEMP were approved by the Human Experiment Safety and Ethics Committee (ERGO: 20543) of the University of Southampton before the research commenced. The present study was conducted using human subjects with normal hearing and vestibular function, and it investigated the effect of changes in stimulus repetition rates on cVEMP response parameters (F_{sp} value, peak-to-peak amplitude, and peak latency).

4.2.4.1 Participants

The current study included 18 healthy subjects in the age range of 25-48 years (mean age = 30.25 years). Based on a paired-sample t-test in IBM SPSS SamplePower 3, this study required approximately 18 adult (aged 18 years or older) subjects to obtain a power > 81 %. The required sample size (N = 18) was determined using data from a previous study on ocular VEMP (Singh et al., 2014a), which found that the mean difference in amplitude for 52 subjects was 4.6 uV and SD of the difference was 6.4 uV, with a significance level of 0.05 (alpha, two tails) for two repetition rates (3.1 and 15.1 Hz) of 500 Hz tone-bursts.

Subjects aged 18 years or over were recruited for the study. Subjects who were determined to have hearing problems, balance problems, or neck/back stiffness or pain (using a self-report questionnaire) were excluded from the study. Further details regarding the inclusion/exclusion criteria used to recruit healthy subjects in this experiment are as described in Chapter 3, section 3.2.1. Recording data from both ears separately to obtain bilateral VEMP responses was not undertaken, since a long

recording period would exceed the maximum allowable length for a typical exposure and could cause muscle fatigue. Thus, to minimise the measurement time and reduce the risk of muscle fatigue, it was decided to measure the response only from one ear (right ear) of each subject.

4.2.4.2 Stimuli

The stimuli were administered using insert earphones (Etymotic ER-3A). The calibration of the stimuli was carried out with a Brüel and Kjar (B&K) type 2260 SLM, attached to the ear simulator (IEC-711). VEMP responses from SCM muscle were measured using 500 Hz 1:2:1 tone-bursts over a range of repetition rates, namely: 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, and 100 Hz, for a fixed stimulation level of 119.2 dB p.e. SPL (according to the International Organization for Standardization [ISO] 389-6 2007) for peSPL measurement. The number of epochs was fixed (N=150) for all rates, so the duration for each recording reduced with rate. At high repetition rates, 150 epochs take less time for recording compared to low repetition rates. Table 4.1 shows the recording time in seconds, dB LEQ (A) level and dB peak sound level for each repetition rate. The order of presentation of repetition rates was randomised among subjects. The maximum sound exposure for any subject was within the limit of usual exposure defined by the ISVR's Human Experiment Safety and Ethics Committee, which is less than an equivalent of 8-hour noise dose of 76 dB A.

Acoustic reflex thresholds (ARTs) were measured for some subjects with and without high-rate cVEMP (8 and 7 subjects, respectively), as it was expected that people with high-rate cVEMP may have abnormally elevated or absent ART. The stapedial reflex (also known as the acoustic reflex) refers to the involuntary reflex contraction of the middle ear muscles in response to a high-intensity sound stimulation (Katz, 2009). The ART is defined as the softest sound which can elicit the reflex contraction (Katz, 2009). ART was measured ipsilaterally using the Grason Stadler GSI Tympstar Middle Ear Analyzer, with a probe frequency of 226 Hz. For ipsilateral testing, the stimulus of 500 Hz pure tone was presented in the right ear, where the measurements were performed. The ipsilateral (uncrossed) pathway of the stapedial reflex contains both sensory and motor parts (Katz, 2009, p 190). The sensory part of the stapedial reflex goes from the stimulated cochlea in the inner ear through the vestibulocochlear nerve (8th nerve) to the ipsilateral cochlear nucleus (Katz, 2009, p 190). The motor

part of the stapedial reflex goes from the cochlear nucleus to the ipsilateral superior olivary complex, from which motor neurons of the ipsilateral facial nerve (7th nerve) stimulate the stapedial reflex (Katz, 2009, p 190).

For acoustic reflex, the intensity (in HL) of sound was initially presented at 75 dB HL and varied in 1 dB steps to find a reflex response. The ART was measured by incrementally increasing the intensity of sound by a 1 dB step until the reflex response occurred. For a reflex response, the minimum compliance change must be 0.02 ml or greater (Grason-Stadler Instruments- GSI Tympstar Manual V1 Rev C, 2011, p 4-26). If the compliance change of a reflex response is equal to the minimum change of 0.02 ml or greater, the stimulus is presented a second time at the same level to obtain a repeatable reflex response (see the example in Figure 4.1). For the current study, the minimum compliance change was 0.02 ml (deflection ≥ 0.02 ml), stimulus intensity started at 75 dB HL and the maximum stimulus level did not exceed 95 dB HL. The reflex threshold is defined as the softest sound that can elicit the reflex contraction with a compliance change of at least of 0.02 ml on at least two of three trials.

Table 4.1: The recording time (in seconds), dB LEQ (A) level and dB peak sound level for all repetition rates.

Repetition rate (Hz)	Recording time (in seconds)	A-weighted sound level (dB LEQ (A))	Sound level in dB p.e. SPL
1	150	90	119.2
5	30	95.4	119.2
10	15	98.1	119.2
15	10	99.9	119.2
20	7.5	101.1	119.2
25	6	102	119.2
30	5	102.9	119.2
35	4.29	102.9	119.2
40	3.75	104	119.2
45	3.33	104.5	119.2
50	3	104.8	119.2
60	2.50	105.6	119.2
70	2.14	106	119.2
80	1.88	106.5	119.2
90	1.66	106.7	119.2
100	1.50	107	119.2

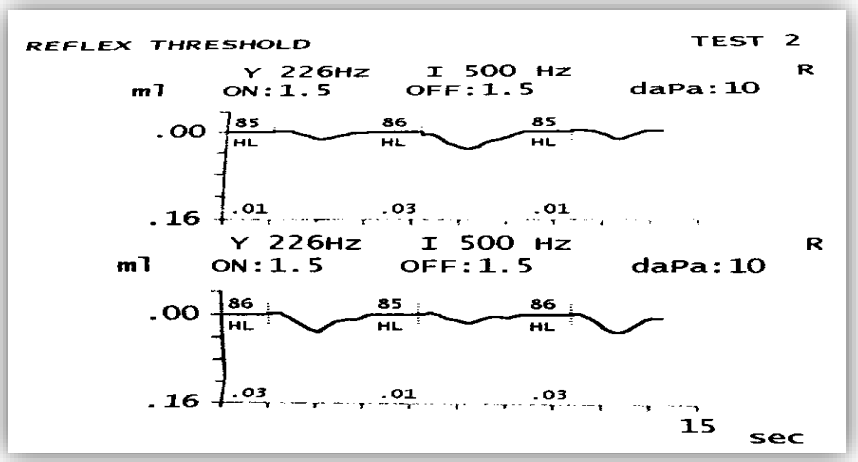

Figure 4.1: Example of stapedial reflex response (right ear) obtained from Grason Stadler GSI Tympstar Middle Ear Analyzer. The result shows that ART is established at 86 dB HL for a normal ear (deflection ≥ 0.02 ml). The compliance change is reported in ml units and recorded within 1.5 s.

4.2.4.3 Apparatus

The equipment setups (apparatus) used with subjects to conduct this experiment were identical to those used in Experiment 1, as described in section 3.2.3.

4.2.4.4 Procedure

The optimal repetition rate was measured for a 500 Hz tone-burst stimulus in response to cVEMP for normal hearing subjects. Participants underwent cVEMP stimulation, using 500 Hz 1:2:1(cycles) tone-bursts over a range of repetition rates. The EMG activity of the SCM muscle was visually monitored by the investigator, using a digital real-time oscilloscope (TDS 210). Variable loud sounds of 500 Hz 1:2:1 tone-bursts over a range of repetition rates were applied, at 119.2 dB p.e. SPL. In this section, all procedures used with subjects in this experiment to conduct cVEMP measurements were identical to those used in Experiment 1, as described in section 3.2.4.

After that stage, ARTs were measured for subjects with and without high-rate cVEMP (8 and 7 subjects, respectively). For ipsilateral testing, the stimulus of 500 Hz pure tone was presented in the right ear, where the measurements were performed. The participant sat on a chair in a double-walled sound booth and was asked to abstain from speaking, swallowing and unnecessary movement during testing.

4.2.4.5 Response analysis

Response quality was assessed using the F_{sp}. Although an F_{sp} threshold can be defined for a group to indicate the presence of a response, the significance of a given F_{sp} value can vary across individuals, as the degrees of freedom of subject data can vary (Lv et al., 2007). For ABR, Elberling & Don (1984) have estimated the critical (95 %) value for F_{sp}. For VEMP recordings, the degree of freedom of the signal and noise (or the critical (95 %) cut-off value of F_{sp}) are not known. Response presence was therefore objectively determined from bootstrapping techniques based on random resampling of the data, to indicate whether the F_{sp} of a given recording was significantly different from that of random noise (Lv et al., 2007).

This method is based on randomly resampling the original data to produce *p*-values. First, the normal F_{sp} values obtained from the array of coherent epochs were calculated (epochs were aligned by the stimulus timing) (as explained in Chapter 3, section 3.2.5). This was completed by calculating the ratio between the estimated level of the VEMP signal and the averaged background noise, as defined in Equation 3.1 in Chapter 3, to confirm the cVEMP response. The single point was arbitrarily chosen in the current study. The time window used for analysis of each stimulus was varied for low and high repetition rates: 40 ms (single point at 20 ms) for 1-25 Hz repetition rates, 20 ms (single point at 10 ms) for 30-50 Hz rates, and 10 ms (single point at 5 ms) for 60-100 Hz rates and the responses to 150 stimuli were averaged for each run.

After calculating the array of coherent epochs to obtain the F_{sp} value, the bootstrap test was applied by selecting random starting points throughout the original recorded signal to generate an ensemble of non-coherent epochs with lengths similar to the raw signal. Non-coherent epochs are epochs with random starting points that are not aligned with the stimulus timing (Lv et al., 2007). From the new array of non-coherent epochs, the F_{sp} value was again calculated ($F_{sp}B$ (i)). This bootstrap process was repeated 500 times, and a bootstrap distribution of $F_{sp}B$ was obtained. $F_{sp}B$ (i) indicates the F_{sp} of the bootstrap sample, as defined in Equation 4.3:

$$FspB\ (i) = N \times \frac{var\ (SIG\ R)}{var\ (SP\ R)} \quad (4.3)$$

where i is the number of repetitions of the bootstrap process (i varies from 1 to 500), N is the number of epochs (N=150), var (SIG_R) is the mean of the random starting points for all epochs and var (SP_R) is the variance at random starting points for each epoch.

As the random samples are not coherent with the stimulation, this technique produces an estimate of the null distribution of F_{sp}, which would be anticipated if there was no stimulation present. The F_{sp} values of the cVEMP response (from the original data) were compared to the F_{sp} values of all the bootstrap arrays. A p value was determined from the proportion of bootstrap values that exceeded the F_{sp} of the coherent average. For a real cVEMP response, the F_{sp} of the VEMP should be significantly higher than most of the random F_{sp}Bs. For example if 1 % of bootstrap

values are greater than the true F_{sp} value, then this would produce p=0.01. A cut-off of p<0.05 was used to determine the presence of a response. The bootstrap values of F_{sp} were obtained for all recordings for all subjects using MATLAB software.

Figure 4.2 below shows the bootstrap distribution of $F_{sp}B$ (i) for one subject. In this example, the 95 % critical value for F_{sp} after bootstrapping with 500 repeats was calculated (approximately 1.71). The critical value is a number on the test distribution corresponding to a certain significance level which the test statistics should exceed to reject the null hypothesis. The value of F_{sp} from the original data was compared to the critical value of the 95 % confidence interval (corresponding to a false-positive rate of 5 %) of the bootstrap distribution of $F_{sp}B$ (i). If the F_{sp} value (X_1 in Figure 4.2) was higher than the critical value of F_{sp} after bootstrapping (p<0.05), the null hypothesis of no response was rejected and the F_{sp} value was considered to be statistically significant and, consequently the VEMP response had been detected. However, if the F_{sp} value (X_2 in Figure 4.2) was lower than the critical value of F_{sp} after bootstrapping (p>0.05), the null hypothesis of no response was accepted, and it was considered that a significant VEMP response had not been detected.

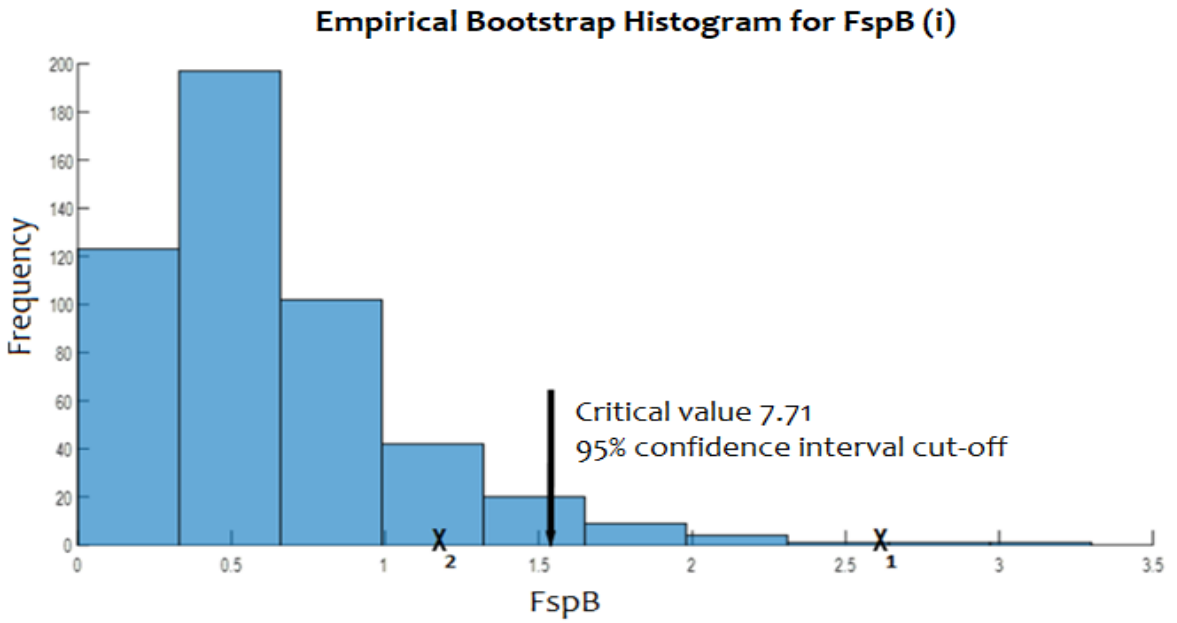

Figure 4.2: Bootstrap distribution of $F_{sp}B$ (i) for one subject. X_1 and X_2 indicate examples of F_{sp} values obtained from coherent averages. The red reference line shows the 95 % critical value for F_{sp} after bootstrapping with 500 repeats. The confidence interval cut-off values determine the boundaries of test statistics (corresponding to a significance level of 0.05) that result in rejecting or not rejecting the null hypothesis. The F_{sp} value from the original data should be above the cut-off value to reject the null hypothesis. Example F_{sp} value X_1 provides confirmation of a significant cVEMP response with 95 % confidence (with 5 % false positive rate), but example F_{sp} value X_2 does not provide evidence of a statistically significant cVEMP response; thus, the response was not detected.

4.2.4.1 Pilot Study

Piloting was performed to determine the existence of cVEMP response for 500 Hz 1:2:1 tone-bursts presented at 119.2 dB p.e. SPL across different repetition rates. The peak-to-peak amplitude and the F_{sp} values to ipsilateral stimulation were measured in 3 ears at all 16 repetition rates (1, 5, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, and 100 Hz). Ipsilateral cVEMP responses to repetition rates were found in the three tested ears. However, two of the ears were devoid of response at repetition rates beyond 50 Hz.

4.2.5 Results

4.2.5.1 Objective detection of cVEMP using a bootstrap technique

From bootstrap analysis, a *p*-value was obtained for all repetition rates for 18 subjects, using MATLAB software. Figure 4.3 shows the number of subjects with a significant cVEMP response ($p < 0.05$) as a function of rate. The cVEMP was successfully recorded at all 16 repetition rates for the study; however, the number of subjects showing a presence of cVEMP varied from rate to rate. The response rate had a general trend of reduction with increasing repetition rate of the stimulus beyond 10 Hz. The response rate was 100% at repetition rates of 1, 5 and 10 Hz; and decreased with increasing the repetition rates further.

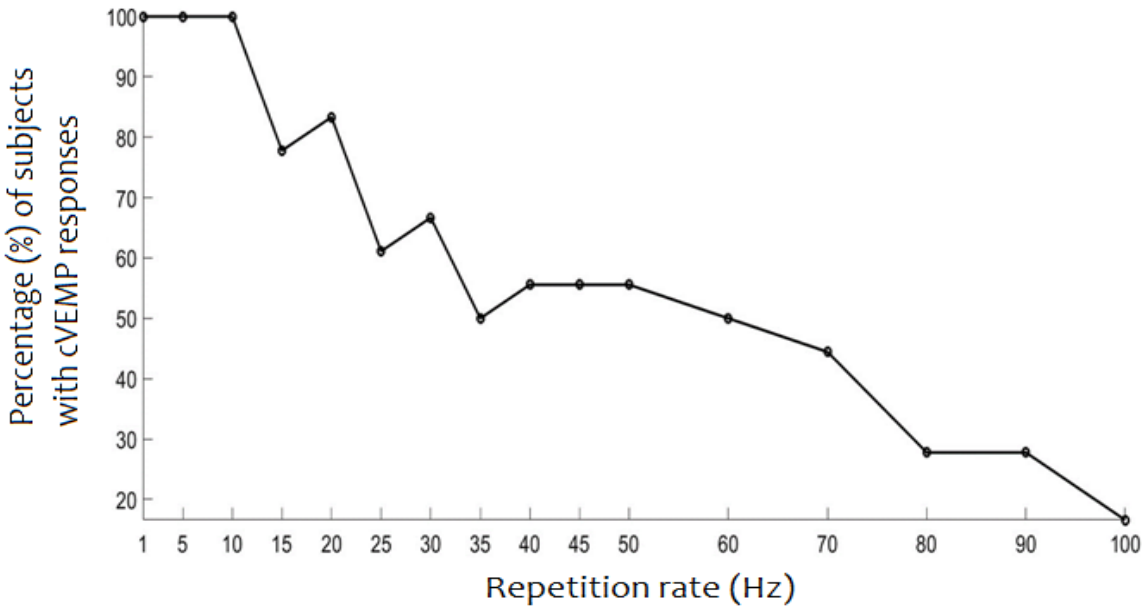

Figure 4.3: Percentage of subjects with a cVEMP response as a function of repetition rates.

The grand average waveforms of cVEMP were recorded from all of the participants, and the results are presented in Figure 4.4. In the current study, the clarity of cVEMP waveforms deteriorated as repetition rate was increased above 35 Hz. One reason for waveform distortion of cVEMP at high repetition rates was response overlap. At high rates, the duration of cVEMP response is greater than that of the stimulus

interval, producing overlap (superposition) between stimuli: each new stimulus occurs before the previous one is over; hence, the second stimulus is partially added to the first, creating difficulty in finding the significant peaks in the cVEMP waveform. Therefore, the duration of the stimulus should be greater than the duration of the response to a stimulus.

For low rate cVEMPs, the response was recorded in the first 30 ms after stimulus presentation, with an early positive polarity peak arising at about 13 ms and a negative polarity peak arising at about 23 ms (P13-N23 or P1-N1) (Akin et al., 2004; Eleftheriadou & Koudounarakis, 2011). At high rates (more than 35 Hz), the epoch (frame) length became shorter than 30 ms, which explains the loss in the waveform positive or negative polarity components. The rates of 40, 45, and 50 Hz showed the first positive peak of the waveform, whereas the end negative component disappeared as the frame length became shorter than 30 ms. Thus, the epoch length was not long enough to complete the negative polarity component, while rates of 60, 70, 80, 90, and 100 Hz showed loss in both positive and negative components (Figure 4.4). The stimulus occurred after 8 ms for 500 Hz 1:2:1 tone-burst, so for these rates, the frame length became shorter than the time needed for the first positive peak component to occur. Thus, waveform distortion at high stimulus rates caused difficulty in waveform identification (p13-n23) of VEMP responses, which may restrict the clinical usefulness of these rates. An example of typical subject cVEMP waveforms across repetition rates is shown in Figure 4.5.

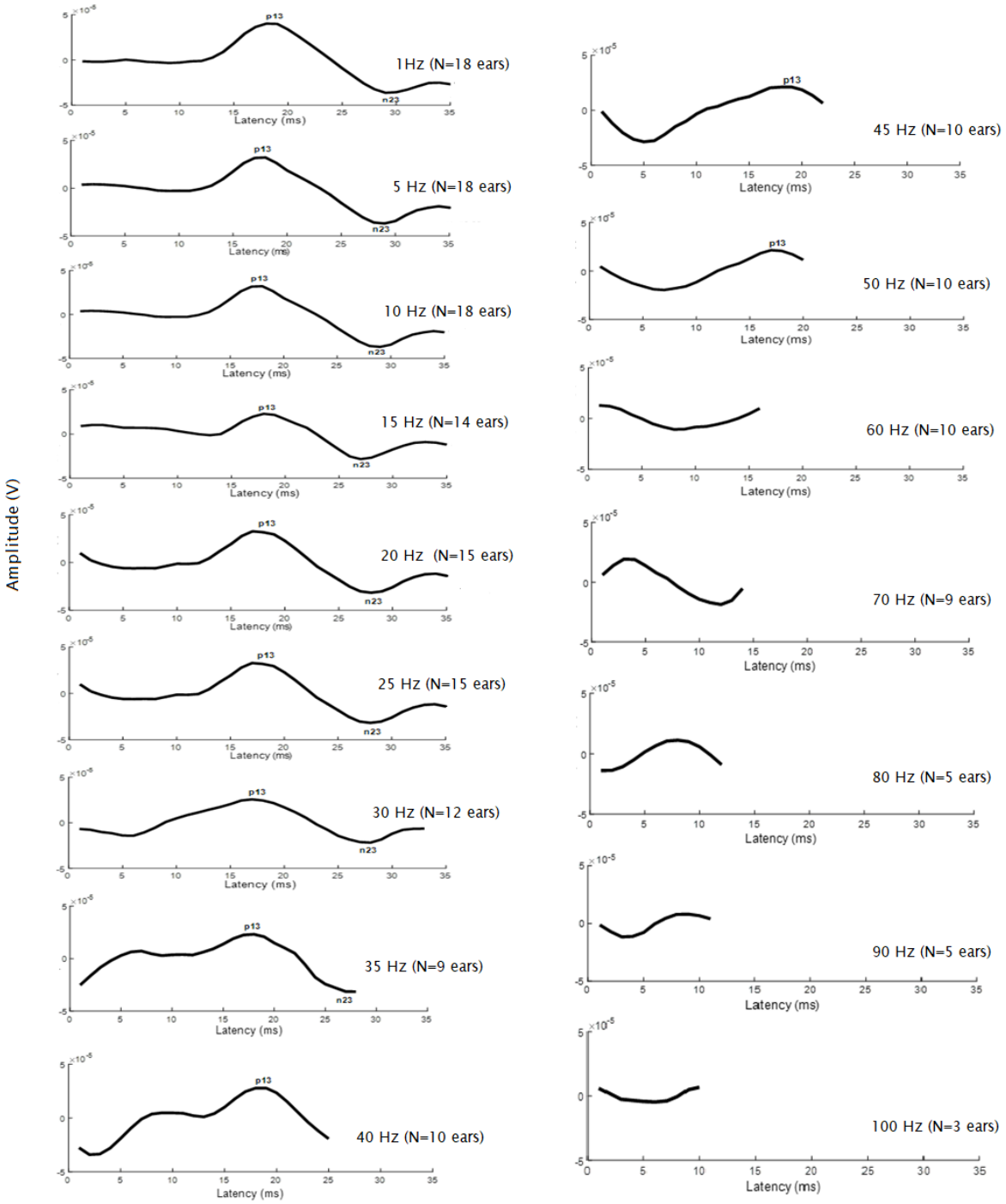

Figure 4.4: Grand average waveforms of cVEMP across repetition rates. In the images, the upward peak (positive, p13) and downward peak (negative, n23) represent the actual cVEMP waveform response. N is the number of subjects with positive cVEMP responses at each repetition rate.

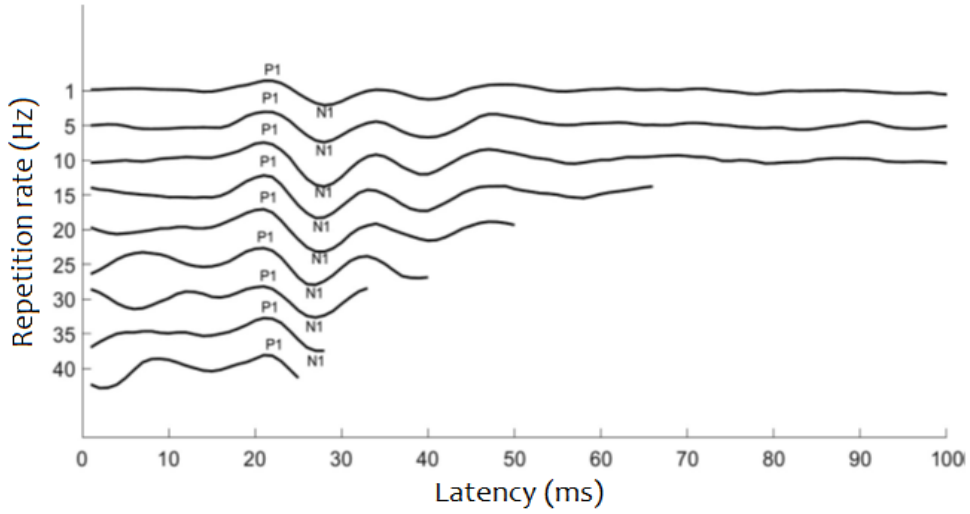

Figure 4.5: The waveforms of cVEMP across repetition rates for a typical subject. In the image, the upward peak (positive, P1) and downward peak (negative, N1) of the VEMPs are labelled.

With regard to the *p*-values produced by the bootstrap analysis, most subjects did not show cVEMP responses at repetition rates above 60 Hz. Therefore, these rates were not included in the F_{sp}, peak-to-peak amplitude, and peak latency analysis. Rates of 45 and 50 Hz were not included in the analysis due to difficulty in finding the significant peaks resulting from the overlap between stimuli at high rates.

4.2.5.2 F_{sp} values of cVEMP response as a function of repetition rates

The quality of VEMP responses was measured using the F_{sp} values obtained for all repetition rates using MATLAB software. The F_{sp} values for cVEMP were measured at 119.2 dB p.e. SPL for 18 ears. Shapiro-Wilk testing revealed that normality was rejected ($p < 0.05$). Therefore, a non-parametric Friedman's test was used to analyse the data. F_{sp} values of the non-responsive ears at the lower repetition rates were set as one for missing data (the average F_{sp} value when no response is present in the data would be 1. It would be a random distribution with some values below and above 1, but the mean should be 1). Figure 4.6 shows the variation in mean cVEMP F_{sp} with repetition rate. The results showed a significant effect of repetition rates on the F_{sp} value [χ^2 (10) = 96.143, $p < 0.001$]. Wilcoxon signed-rank testing was conducted for multiple comparisons to identify which repetition rates were significantly different from one another in respect to the F_{sp} value. The results revealed that there was no significant difference between 1, 5 and 10 Hz ($p > 0.05$). These three rates produced significantly higher F_{sp} values than all of the other rates

(p< 0.05; Figure 4.6). A rate of 20 Hz produced a significantly higher F_{sp} than rates of 25, 30, 35, and 40 Hz did, whereas a rate of 25 Hz produced a significantly lower F_{sp} than rates of 30, and 40 Hz did. There was a general trend toward a decrease in the F_{sp} value with an increase in the repetition rate. Nevertheless, repetition rates of 20, 30 and 40 Hz produced higher F_{sp} values, respectively, than 15, 25 and 35 Hz did. This could be a superposition effect at some rates where peaks cancel troughs.

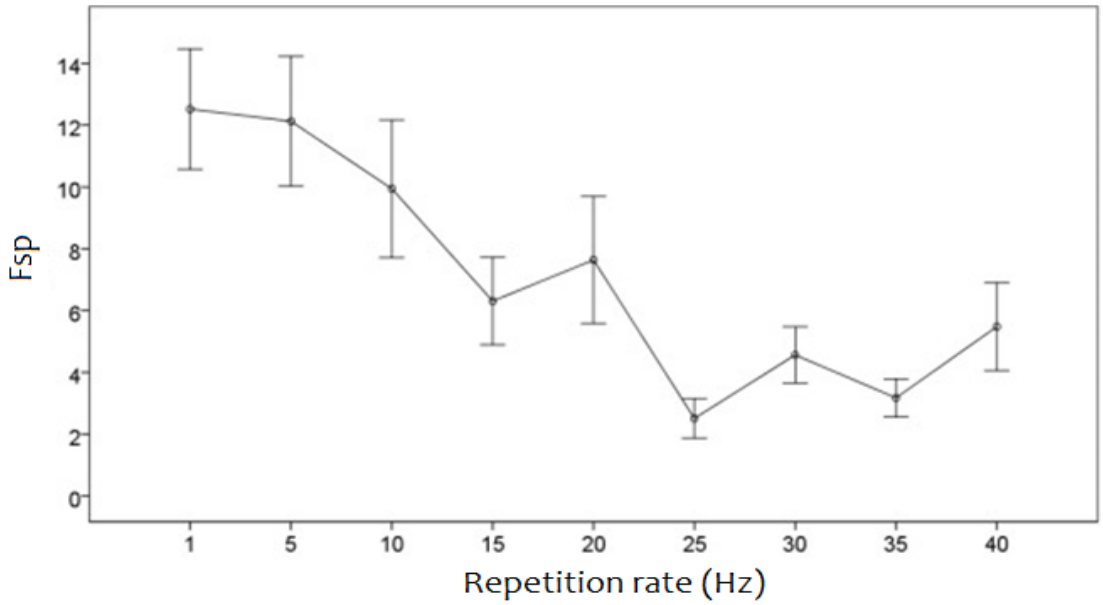

Figure 4.6: F_{SP} values of cVEMP as a function of repetition rate. Error bars represent ± 1 SE of the mean. The F_{sp} values of the non-responsive ears were set to unity for missing data (on average this is the F_{sp} obtained when no response is present in data).

4.2.5.3 Peak-to-peak amplitude of cVEMP as a function of repetition rate

The peak-to-peak amplitudes for the p13 and n23 waves of cVEMP at 119.2 dB p.e. SPL were subjectively determined (by visual inspection) for a total of 18 ears. As normality was rejected , a non-parametric Friedman test was used to investigate the effect of varying repetition rates on peak-to-peak amplitude. The amplitude values of recordings of the non-responsive ears were set to zero for missing data (if no response is present in the data then there should be no measurable amplitude; hence, it was set to zero). It can be seen in Figure 4.7 that there was a significant effect of repetition rates on the peak-to-peak amplitude [χ^2 (8) = 22.710, p <0.001].

To identify which repetition rates were significantly different from one another in peak-to-peak amplitude, Wilcoxon signed-rank testing was conducted for multiple comparisons. The results revealed that the rates of 1 Hz produced significantly higher amplitudes than repetition rates of 25, 30, 35 and 40 Hz. The Wilcoxon signed-rank test also revealed that the rate of 5 and 10 Hz produced significantly higher amplitudes than repetition rates of 35 and 40 Hz.

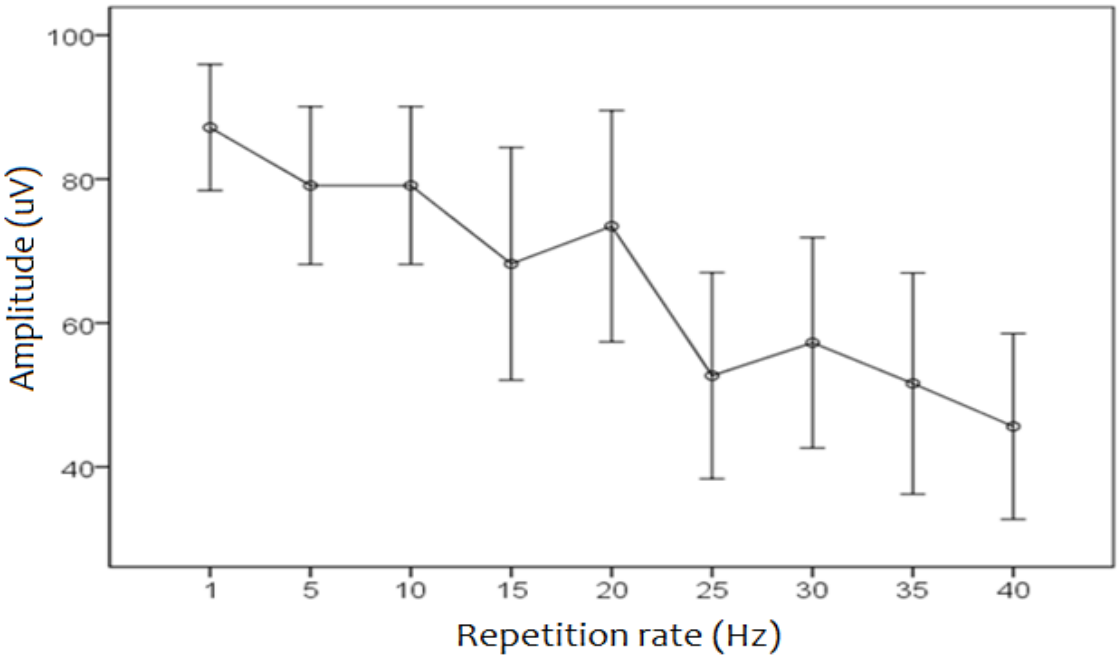

Figure 4.7: Peak-to-peak amplitude of cVEMP as a function of repetition rate. Error bars represent ±1 SE of the mean. The amplitude values of recordings that did not show a significant response on bootstrap analysis were set to zero for the analysis of amplitude values.

4.2.5.4 Variations of peak-to-peak amplitude of cVEMP between subjects as a function of repetition rate

Figure 4.8 shows the peak-to-peak amplitude of cVEMP across repetition rates for each subject. For most subjects, the amplitude of cVEMP response tended to drop with an increasing repetition rate beyond 10 Hz. Repetition rates of 1, 5 and 10 Hz produced higher amplitudes than all other rates in the majority of subjects. It is clear that some subjects have consistently larger VEMPs than other subjects.

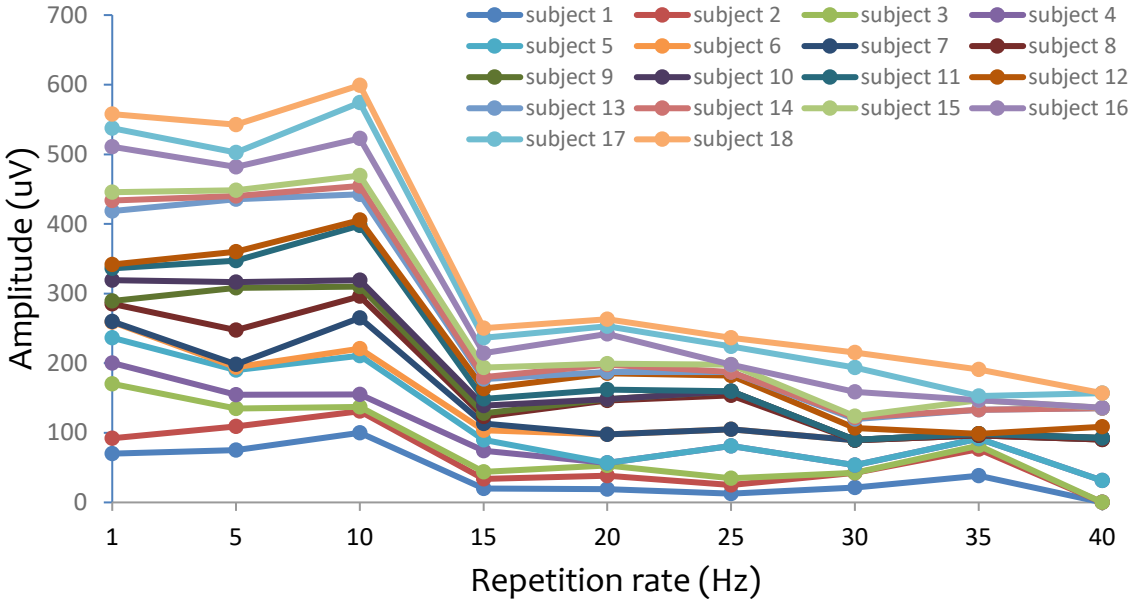

Figure 4.8: Peak-to-peak amplitude of cVEMP as a function of repetition rates for all 18 subjects.

4.2.5.5 VEMP amplitude at a 5 Hz repetition rate between subjects with and without high-rate VEMPs

The peak-to-peak amplitudes of cVEMPs at a 5 Hz repetition rate were compared between subjects with and without responses at high rates (Figure 4.9). Testing of normality was carried out on data using the one-sample Shapiro-Wilk test because the sample size was 18 (n < 50). As normality was not rejected (p > 0.05), a parametric independent-measures t-test was used to analyse the data. A paired t-test showed that the amplitude of cVEMP responses at 5 Hz rate for subjects with responses at high rates was not statistically significantly higher than those with no high-rate VEMPs (p > 0.05), t (16) = .322, p = .752, two-tailed. Thus, it seems not to be the case that subjects who had a high rate VEMP responses simply had larger VEMPs.

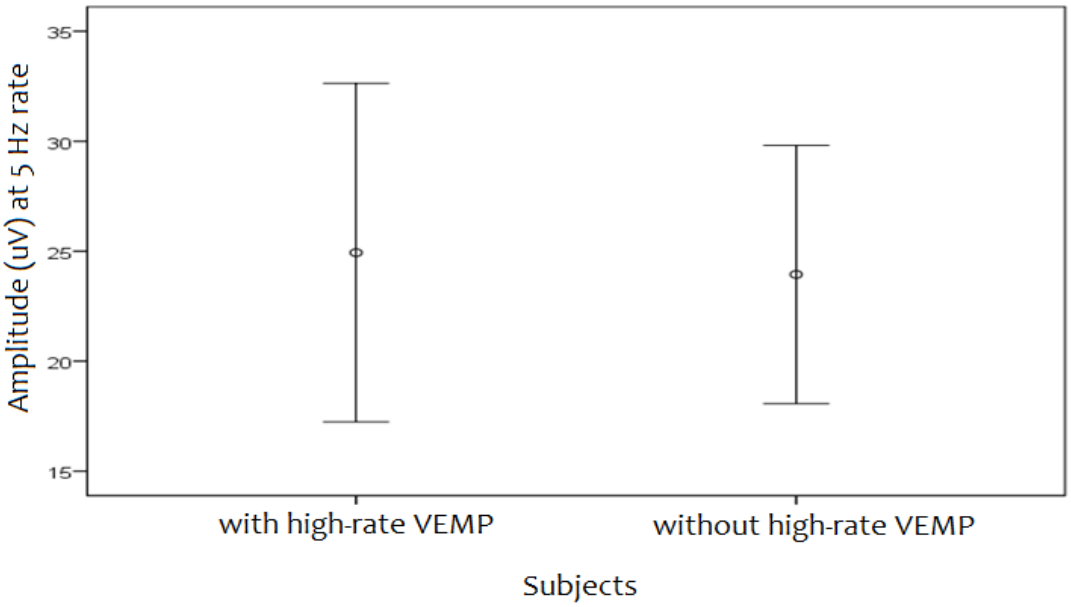

Figure 4.9: Comparison of VEMP amplitude at a 5 Hz repetition rate for subjects with and without high-rate VEMPs. Error bars represent ±1 SE of the mean.

4.2.5.6 Peak latencies of cVEMP as a function of repetition rates

A One-Way Repeated Measures ANOVA was conducted and it was found that the mean peak latencies of cVEMP did not differ significantly between repetition rates, p13 [F (3.069, 81.250) =1.108, p = .369].

4.2.5.7 ART measurement for subjects with and without high-rate VEMPs

Measurement of ipsilateral ART for the right ear was conducted for 8 subjects with and 7 subjects without high-rate cVEMP, as it was expected that people with high-rate cVEMP may have abnormally elevated or absent ART. Testing of normality was carried out on the data using the one-sample Shapiro-Wilk test, as the sample size was 15 ($n < 50$). From the results, it was found that normality was rejected ($p < 0.05$). Therefore, the Mann-Whitney test was used to analyse the data. From the results it was noted that there was a significant difference in ART for subjects with (m=87.7, SD=4.09) and without (m=83.2, SD=3.7) high-rate cVEMP: ($U = 8.500$, $p = .023$). Figure 4.10 below shows means with SE mean error bars of ART for subjects with and without high-rate cVEMP. Looking at the error bars only in Figure 4.10, it appears that there may be a fairly large difference in the ARTs between the two groups, i.e. subjects with high-rate cVEMP had significantly higher ARTs compared to subjects

without. However, errors bars are based on the assumption that the data is normally distributed (when data is not normally distributed, it may convey misleading information). When data is non-normal (e.g. skewed, or has outliers as is the case for this data), then a more accurate representation of the data is given by box plots, which show the median and interquartile range of data. The data is therefore also presented in Figure 4.11 using boxplots.

Looking at Figure 4.11, the ARTs of subjects without high-rate cVEMP appeare negatively skewed (the data contain higher frequency of low values) and the positive skew was reduced by a logarithmic transform. However, the ARTs of subjects with high-rate cVEMP, which were approximately normally distributed, became negatively skewed following the logarithmic transform. As the sample comes from populations with non-Gaussian distribution, this may better reflect the distribution of the data. For completeness, figures with both error bars and box plots are shown below.

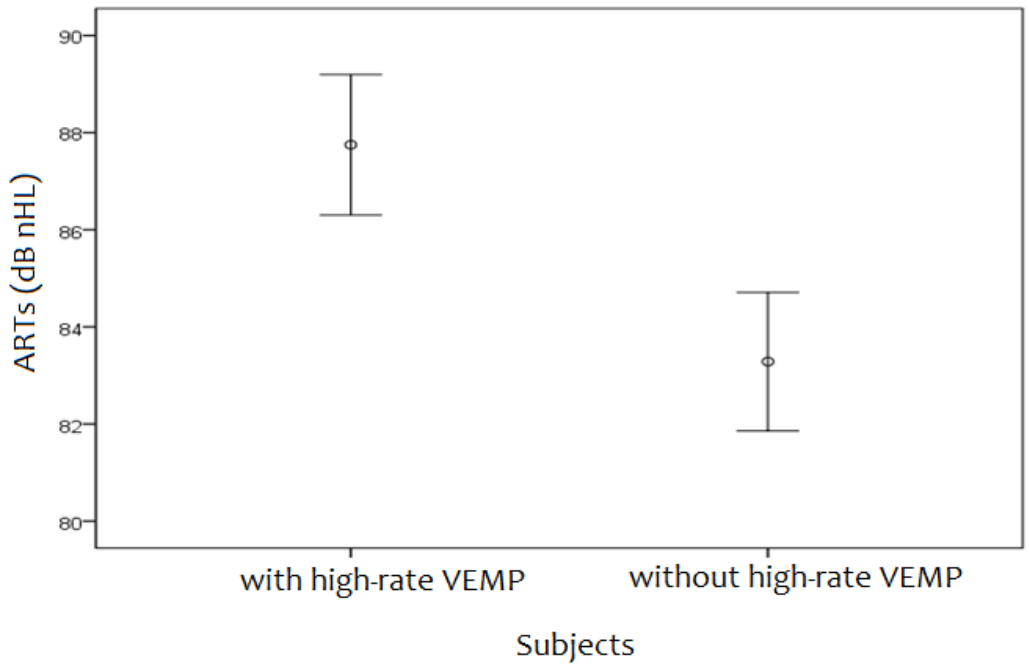

Figure 4.10: Ipsilateral acoustic reflex measurement (in dB HL) of 500 Hz pure-tone stimulus for 8 subjects with and 7 subjects without high-rate cVEMP. Error bars represent ±1 SE of the mean.

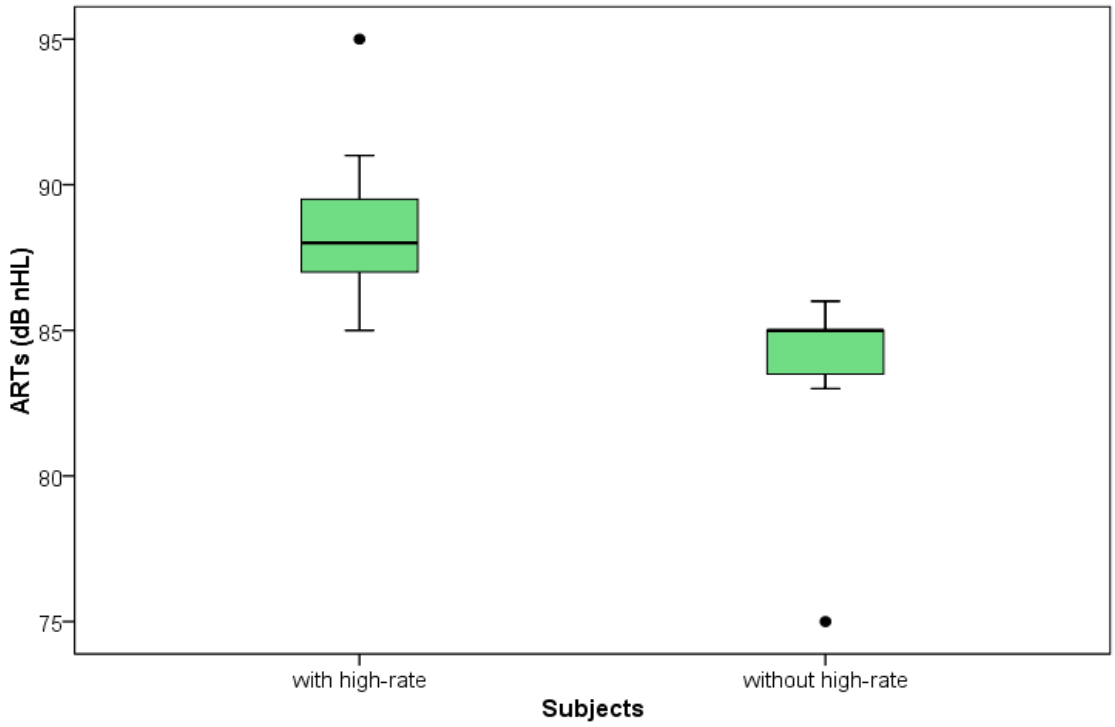

Figure 4.11: Box plots of ipsilateral acoustic reflex measurement (in dB HL) of 500 Hz tone-burst for 8 subjects with and 7 subjects without high-rate cVEMP. Each box represents the two middle quartiles (end of boxes, known as interquartile range), separated by median (horizontal line) and the lowest and highest values (horizontal line at the end of whiskers). The circles indicate outliers, which are values that lie between 1.5 and three times the interquartile range below the first quartile or above the third quartile.

4.2.6 Discussion

The motivation behind this work was to improve the quality of cVEMP response by exploring how the cVEMP adapts to high repetition rates. The response rate was 100 % for rates up to 10 Hz and decreased progressively for higher rates. There was a trend of progressive decrease in amplitude and F_{sp} value with rate. Rates up to 10 Hz produced higher quality than all others. Rates up to 10 Hz produced higher amplitude of cVEMPs than 35 and 40 Hz rates. Carnaúba et al. (2013) evaluated the effect of rates on amplitude and reported a progressive decrease in amplitude with rate similar to that of the current study. The reduction in the response rate and amplitude of cVEMP response following repetition rate increases can probably be attributed to the refractory period of the SCM muscle fibres. The refractory period is

defined as the time after a neuron fires or a muscle fibre contracts during which a test stimulus cannot evoke a response (McComas et al., 2006, p. 130). The mean firing rates of human neck muscles' motor units are normally between 10 and 15 spikes per second (Schomacher et al., 2012). Stimulus rates up to 15 Hz are within the physiological responding time of the SCM muscle fibres, whereas higher stimulation rates will not be.

In the present study, it was expected that subjects with high-rate VEMP response might have larger cVEMP response at low rates compared with subjects without high-rate cVEMP. This is because having a larger cVEMP response at low repetition rates could be the reason for the continued responses at high repetition rates. Therefore, the amplitude of cVEMP at a 5 Hz repetition rate was compared between subjects with and without high-rate cVEMP. It appeared from the results that the amplitudes of cVEMP responses for subjects with responses at high rates were not statistically higher than for those without high-rate VEMPs responses.Therefore, subjects with high rate cVEMPs did not have larger cVEMPs at low rates compared with subjects without high-rate cVEMPs.

It was also expected that people with high-rate cVEMP may have abnormally elevated or absent ART. ART threshold measurement was conducted for subjects with and without high-rate VEMPs. It can be concluded that the ART for subjects with high-rate cVEMP was not absent or abnormally elevated, as the mean ART for both groups was within the normal range of ART, which usually occurs around 75.5-90.5 dB HL (equivalent to 85-100 dB SPL). However, people with high-rate cVEMP reported significantly higher ART than people without. Thus, this could be one of the reasons for the reduction in the response rate for most subjects with lower ART at high rates.

Singh et al. (2014a) attributed the decline in the amplitude of the responses with increasing repetition rates to the effect of the stapedial reflex. In normal hearing subjects, the average cVEMP threshold to tone-bursts (250-2000 Hz) is in the range of 100-120 dB p.e. SPL (equivalent to 90-95 dB nHL; Akin et al., 2003), whereas the ART usually occurs at around 85-100 dB SPL for pure tone stimuli (equivalent to 100-105 dB p.e. SPL; Katz, 2009, p. 195). As the stapedial reflex occurs at a lower threshold than VEMP, any VEMP recording will be influenced by the reflex response

(Singh et al., 2014a). However, the rate of stimulation may have different effects on the thresholds and amplitudes of the acoustic reflex.

Rawool (1995) investigated the effect of click repetition rates (50, 100, 150, 200, 250 and 300 Hz) on the threshold and amplitude of the acoustic reflex for 16 healthy subjects and found that high repetition rates showed higher acoustic reflex amplitudes and lower thresholds than lower rates. In this study, the intensity (85 dB SPL) and duration (1.5 s) of the stimuli were constant at all repetition rates, whereas the number of clicks for high rates was higher than that for low rates. Thus, the total energy for high repetition rates is higher than that for low rates, which may explain the decrease in ART and increase in amplitude of the reflex response with increasing repetition rates (Rawool, 1995).

The same might be relevant for the effect of the stapedius reflex on repetition rates of the 500 Hz tone-burst-evoked VEMP. In the current study, repetition rates presented with the same number of epochs or averages (150 frames) and with constant intensity (119.2 dB p.e. SPL), but they had different durations and rates of stimulation. The stimuli with higher stimulation rates and shorter durations were found to be louder than stimuli with lower rates and longer durations (Figure 4.12 below). This is because the total energy of the stimulus increases momentarily due to faster firing of muscle fibres at high repetition rates. This might be the reason for the decreasing amplitude and response rate for the majority of subjects in the current study at higher repetition rates. The reduction in the level of stimulus, particularly at high repetition rates, could decrease the effect of reflex habituation; however, more studies are required to confirm this. Nevertheless, the effect of stapedial reflex on the low rates used in the current study (1-50 Hz) was not considered in any of the previous studies. Therefore, more studies are needed to investigate the effect of acoustic reflex on the thresholds of low repetition rates.

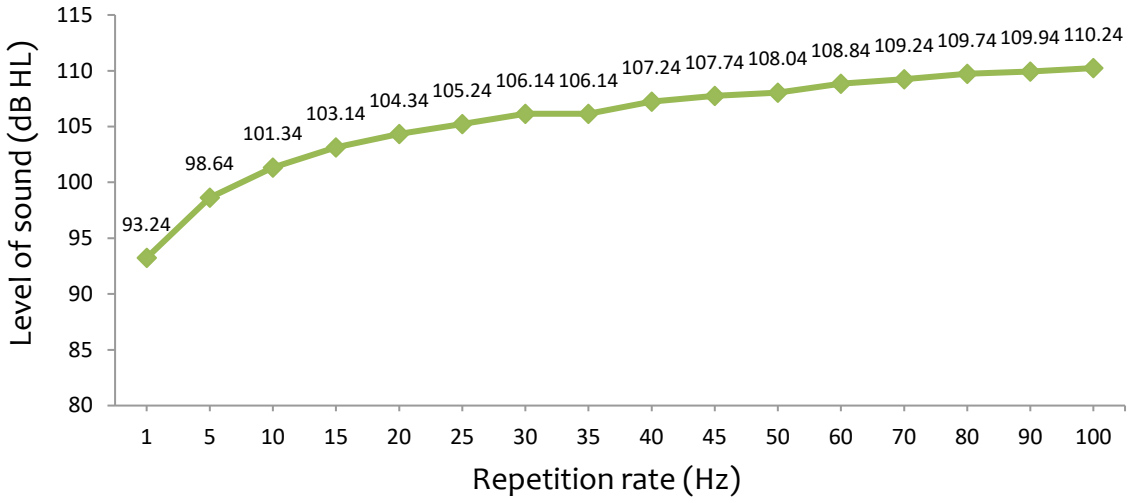

Figure 4.12: Levels of a stimulus sound (in dB HL) at each repetition rate used in the current study. The stimulus levels for high and low rates used for eliciting the cVEMP response were above the normal range of ART, which usually occurs around 75.5-90.5 dB HL (equivalent to 85-100 dB SPL). Therefore, all cVEMP recordings may have been influenced by the reflex response in the present study.

Skeletal muscles consist of varying percentages of fast-twitch and slow-twitch muscle fibres (Hall, 2016). Slow-twitch fibres are structured for endurance whereas fast-twitch fibres can fire more rapidly but fatigue more quickly. Some humans have more fast-twitch than slow twitch fibres, whereas others have more slow-twitch fibres (Hall, 2016, p. 1090). This factor might explain why in the current study a few people showed responses at very high repetition rates, but the majority did not.

The results of the current experiment may seem similar to those of the previous experiment measuring cVEMP in response to AM tones (Chapter 3). However, the stimuli used in the two studies were different; one employed 500 Hz tone AM at different rates, while the other used 500 Hz tone-bursts at different repetition rates. Although the stimuli were different in the two studies, recording VEMP at high rates may simulate S-VEMP. In the previous study, of the initial total of 45 people who had volunteered to participate, only 6 subjects were included in the study. This was because the majority of volunteers did not show S-VEMP responses. Piloting was performed to determine the existence of VEMP to 500 Hz modulated at 75 Hz, and only six subjects reported responses. Thus, the response rate was only 13.3% in the previous study, while in the current study, there was only a 38% response rate to

500 Hz tone-bursts using high repetition rates (70 and 80 Hz). The reason for differences in the percentage could be related to the different sample sizes, as 45 participants were recruited in the previous study and 18 were included in the present study. Still, both percentages are considered low.

4.2.7 Conclusions

This study has demonstrated that responses can be recorded from the SCM muscle to 500 Hz tone-bursts at high repetition rates, but only in a few subjects. Recording at very high rates might reduce recording times, but this is not possible for the majority of subjects. The prevalence of cVEMP decreased with rate, with only few subjects showing high rate cVEMP response. This may reflect variation in the distribution of muscle fibres types (slow and fast twitch) across subjects. Evoking cVEMP using 1, 5, and 10 Hz repetition rates produced the maximum response rate, and highest F_{sp} values The rate of 10 Hz requires a considerably shorter time for recording compared to 1 and 5 Hz rates, so there appears to be an optimal trade-off between recording time and response detection for the majority of subjects.

4.3 Experiment 3: Effects of plateau duration on cVEMPs in response to 500 Hz tone-burst

4.3.1 Introduction

The cVEMP is frequency-specific to lower frequencies such as 500 Hz tone-bursts (Marimuthu & Harun, 2016). Tuning investigations showed that the largest amplitude and lowest threshold of cVEMP response occurred at approximately 500 Hz tone-burst stimuli in AC mode (Akin et al., 2003; Park et al., 2010; Piker et al., 2013; Rauch et al., 2004). Therefore, 500 Hz tone-bursts are widely used for eliciting cVEMP responses. It has also been reported that the cVEMPS are highly affected by the duration of 500 Hz tone-burst stimulus (Singh et al., 2014b). An illustration of typical tone-burst stimulus components is shown in Figure 4.13 below. A few studies have investigated the effects of different plateau durations of 500 Hz tone-burst stimuli on the amplitude and latency of cVEMP response in AC mode (Cheng & Murofushi, 2001b; Marimuthu & Harun, 2016; Singh et al., 2014b). Singh et al. (2014 b) reported that a 2 ms rise/fall time and 1 ms plateau duration yielded the largest amplitude of cVEMP response among other combinations of rise/fall and plateau durations. However, Cheng & Murofushi. (2001b) recommended a plateau duration of 2 ms for clinical recording of cVEMP. The same authors (Cheng & Murofushi, 2001a) found that a 1 ms rise/fall time yielded the largest amplitude of cVEMP response. According to Marimuthu & Harun (2016), a plateau duration of 0 ms (rise/fall time was kept constant at 2 ms) produced the highest amplitude of cVEMP response amongst the four plateau durations (0, 2, 4, and 10 ms). In contrast, for eliciting oVEMP response, Kantner et al. (2014) concluded that a stimulus with adequate plateau duration was more effective, compared to a stimulus without plateau duration. However, in their study, there was no benefit of increasing the plateau duration above 2 ms (ramp time was kept constant at 2 ms) (Kantner et al., 2014). Hence, previous studies concluded that using shorter plateau durations produced a higher amplitude of cVEMP response, compared to longer plateau

durations. However, using longer plateau durations reduces spectral splatter and would be more frequency-specific to elicit cVEMP response. Nevertheless, none of the previous studies have used a plateau duration longer than 10 ms for evoking cVEMP or oVEMP. Therefore, in the present study, it was decided to use both short and long plateau durations to elicit the cVEMP response. Moreover, previous studies have focused on measuring the effect of changes in ramp and plateau durations on the amplitude and latency, but not on the quality of cVEMP responses. Furthermore, as mentioned at the beginning of the chapter, there is a gap in the knowledge about what generates the cVEMP response with different ramp or plateau durations. Exploring where the cVEMP occurs would be clinically important in identifying the ideal ramp and plateau durations of tone-burst stimuli for eliciting responses.

Therefore, the purpose of this experiment was to explore what triggers or generates cVEMP responses through finding the effect of changing stimulus plateau durations on the quality, amplitude and latency of cVEMP responses, while keeping the other parameters constant (rise/fall time of 2 ms with a linear ramp, repetition rate of 10 Hz and stimulus frequency of 500 Hz).

Rationale: there are two possible expectations for where the cVEMP originates:

- First, the cVEMP occurs at the 'integrating' point of the stimulus. The term 'integrating' means that the energy of the stimulus builds up over time to the point where the reflex is triggered. If this is true, it would be expected that latency would increase for a longer plateau (see the example in Figure 4.14). If the energy is integrated, the longer plateau would produce a larger cVEMP response. A possible advantage for longer stimuli is that they are more frequency specific than short stimuli, as spectral splatter reduces with increasing duration of stimuli.

- A second possibility is that the cVEMP is primarily an 'onset' response. For the current study, the onset of the stimulus is defined as the time for the stimulus to reach half or 2/3 the maximum value. If the onset duration (ramp) changes, it would be expected that there would be an increase in latency. If this is true, most of the cVEMP response with maximum amplitude occurs at the onset of the stimulus and the rest of the stimulus is not important. In the current study, the ramp time was kept constant, so if VEMP is an 'onset' response, latency would not change (see the example in Figure 4.15). For the

previous experiment on S-VEMP (Chapter 3), the S-VEMP stimulus effectively has a very long ramp with no sharp peak (see the example in Figure 4.16 (A)). This could reduce muscle synchrony but would be more frequency specific. Increasing the ramp time of a stimulus could increase the duration of the action potential of a muscle fibre within the muscle and cause asynchrony of firing of the muscle fibres, to generate simultaneous action potentials (Daube & Rubin, 2009, p.334). This could be the reason for the low response rate of S-VEMP in the majority of subjects in the previous experiment (1).

4.3.2 Aims and Hypothesis

The aims of this study were as follows:

- To find out how latency and amplitude are affected by plateau duration; and
- To identify the optimal plateau duration with maximum amplitude and quality for clinical recording of cVEMP evoked by 500 Hz tone burst in AC mode.

The following hypotheses were developed for this study:

- Hypothesis 1: VEMP is an 'onset' response.
- Hypothesis 2: VEMP is an 'integrating' response.

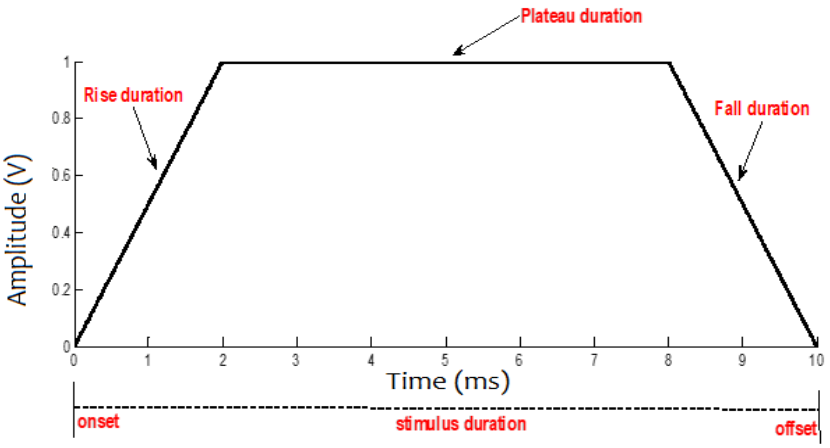

Figure 4.13: Schematic diagram of tone-burst stimulus components that represent stimulus duration, including linear ramp (rise/fall time), and plateau duration.

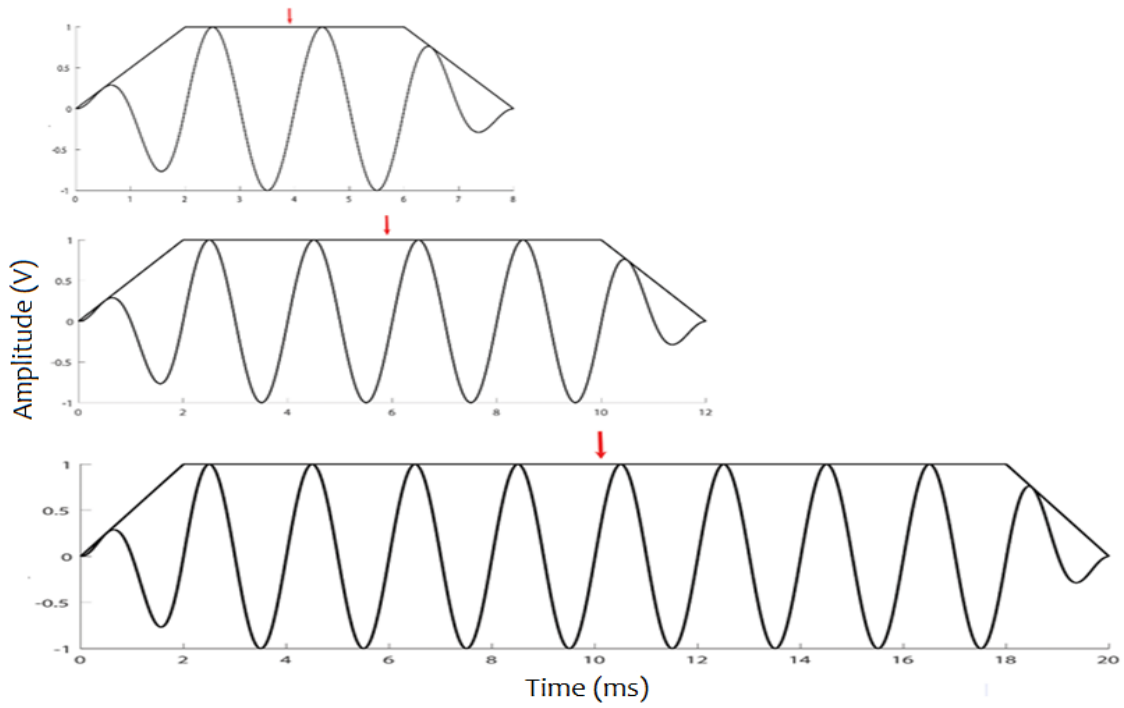

Figure 4.14: Examples of 500 Hz tone-burst stimulus with different plateau durations (4, 8, and 16 ms). The ramp time was kept constant at 2 ms. The red arrows display cVEMP response, which occurs at the 'integrating' point of stimulus in this figure. The term 'integrating' means that the energy of the stimulus builds up over time to the point where the reflex is triggered. In this case, VEMP latency would increase with plateau length.

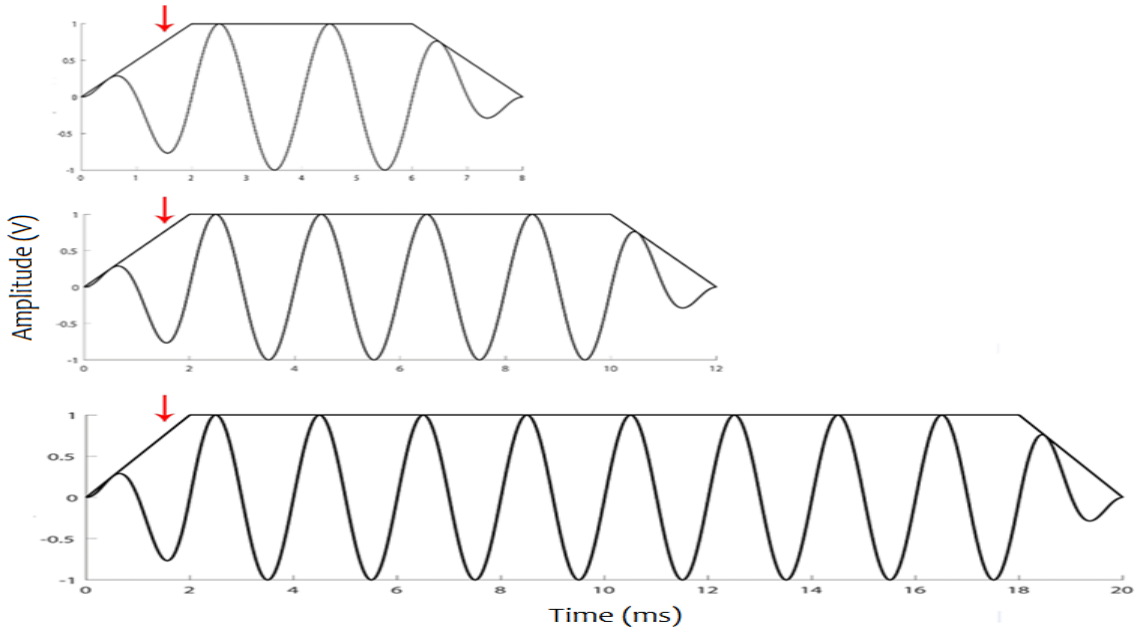

Figure 4.15: Examples of 500 Hz tone-burst stimulus with different plateau durations (4, 8, and 16 ms). The ramp time was kept constant at 2 ms. The red arrows display the cVEMP response, which would occur if the cVEMP was an onset response. In the current study, the onset of the stimulus is defined as the time for the stimulus to reach half or 2/3 the maximum value. In this case, VEMP latency would not change with plateau length.

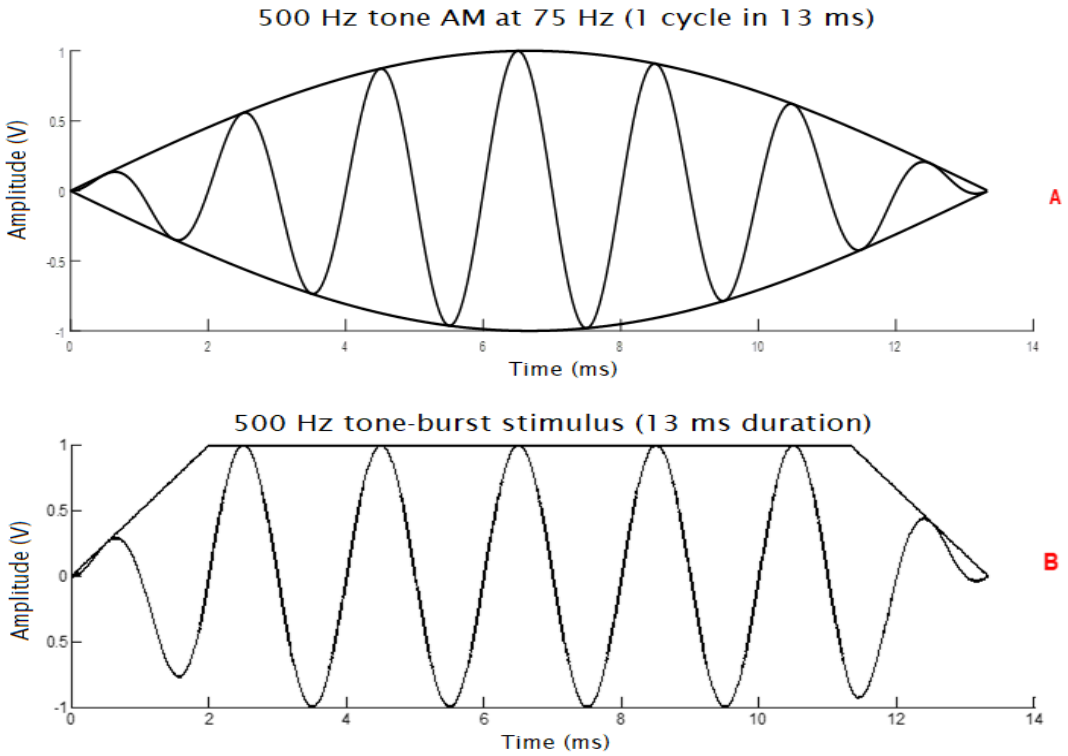

Figure 4.16: Examples of stimuli used for eliciting S-VEMP and standard cVEMP response (A and B respectively). The A waveform represents 500 Hz carrier frequency, 100 % amplitude modulated at 75 Hz, and the B waveform represents a 500 Hz tone-burst stimulus. The duration of the 75 Hz modulated tone used for eliciting S-VEMP response is roughly equal to 13 ms of 500 Hz tone-burst. The A stimulus has effectively a very long ramp, so it is not clear which part of the stimulus triggers the cVEMP response, whereas stimulus B has a linear ramp with sharp onset. The absence of a linear ramp in stimulus A could reduce muscle synchrony.

4.3.3 Research questions:

- What generates or triggers cVEMP?
- What is the effect of stimulus duration of ACS 500 Hz tone-bursts on the quality and amplitude of cVEMP response?
- Does varying the plateau duration (while keeping the ramp time fixed) have an effect on detection of cVEMP response?

4.3.4 Methods

The methods used in this experiment were largely identical to those used in experiment 2, apart from some changes to the number of participants, the nature of the stimuli, and the procedures. No other changes were made in these sections, so further details regarding the inclusion/exclusion criteria, how cVEMP was conducted, the equipment setup (apparatus), the bootstrap technique for objective detection of cVEMP and the procedures used with subjects to conduct this experiment are as described in section 4.2.4 above. The changes were as follows:

4.3.4.1 Participants

The current study included 23 healthy subjects in the age range of 23–49 years (mean age = 29.25 years). Based on a paired-sample t-test in IBM SPSS SamplePower 3, this study required approximately 23 adult (aged 18 years or older) subjects to obtain a power of ≥ 80 %. The required sample size (N = 23) was determined using data from a previous study on cVEMP (Cheng & Murofushi, 2001b), assuming that the mean difference in p13 latency for 52 subjects is 1.1 ms and SD of the difference is 1.75 ms, for two plateau durations (2 and 10 ms) of 500 Hz tone-bursts. For 23 subjects, the difference in latency of p13 between two plateau durations was predicted to be detected with a power of 81 %. Responses were recorded from 23 right ears from 23 subjects.

4.3.4.2 Stimuli

VEMP responses from SCM muscle were measured using 500 Hz tone-burst stimuli with different plateau durations, at a rate of 10 Hz. Six different plateau durations were used in this experiment, 0, 4, 8, 16, 32, and 64 ms, for a fixed stimulation level of 115 dB p.e. SPL (Table 4.2 shows the stimulus levels in dB LEQ (A) for all plateau durations). All the stimuli had 2 ms rise/fall time. The time window used for analysis of each stimulus was 40 ms (with a single point at 20 ms) and the responses to 150 stimuli were averaged for each run. The repetition rate (10 Hz) was constant, so total duration for 150 epochs was similar for all stimuli (recording time=15 s) (Table 4.2). Further details regarding the calibration and analysis of the stimuli can be found in section 4.2.4.2.

Table 4.2: Total recording time (in ms), dB LEQ (A) level and dB peak sound level for all stimuli used in the present experiment. All stimuli had a 2 ms rise/fall time (one cycle rise/fall). The duration of each cycle of 500 Hz tone-burst was 2 ms and the epoch length was 0.1 s (100 ms) for all stimuli, for a repetition rate of 10 Hz. As the repetition rate and the number of epochs were fixed, total duration for 150 epochs was similar for all stimuli (0.1 X 150=15 s).

Plateau durations (ms)	**0**	**4**	**8**	**16**	**32**	**64**
Total number of cycles (rise/fall and plateau)	2	4	6	10	18	34
Total recording time (in s)	15	15	15	15	15	15
A-weighted sound level (dB LEQ (A))	93.6	98.8	101.1	103.8	106.6	109.4
Sound level in dB p.e. SPL	115	115	115	115	115	115

4.3.4.3 Procedures

cVEMP stimulation was carried out on the participants using 500 Hz tone-bursts with varying plateau durations at a fixed stimulation level of 115 dB p.e. SPL. A total of six different recordings was carried out for each subject's right ear. The ipsilateral ART was compared for people with and without cVEMP responses, at long plateau durations (8 ms and longer) (8 and 7 subjects, respectively). In this section, all other procedures used with subjects in this experiment to conduct ART and cVEMP measurements were identical to those used in Experiment 2, as described in section 4.2.4.4.

4.3.4.4 Pilot Study

Piloting was performed in order to determine the existence of cVEMP response to 500 Hz tone-bursts across different plateau durations, while keeping the ramp time fixed at 2 ms (one cycle rise/fall with a linear ramp). cVEMP was conducted using a 500 Hz tone-burst at six different plateau durations, presented at 115 dB p.e. SPL. For five subjects, peak-to-peak amplitude and the F_{sp} values in response to unilateral stimulation were measured at all six plateau durations. Ipsilateral cVEMP responses

to 0, 4, 8, 16, 32, and 64 ms plateau durations were found in the five tested ears. However, three of the ears were devoid of response at 16, 32, and 64 ms.

4.3.5 Results

4.3.5.1 Assessing Normality

Testing of normality was carried out on the data using the one-sample Shapiro-Wilk test, because the sample size was 23 (n< 50). The test results revealed that normality was rejected (p < 0.05). Therefore, a non-parametric Friedman's test was used to analyse the data for the two parameters (F_{sp} values and amplitude data). However, it was noted from the results of p13 and n23 latencies and acoustic reflex measurement that normality was not rejected, and thus a parametric one-way repeated measures ANOVA and Independent-measures t-test respectively were used to analyse the data for these parameters.

4.3.5.2 Objective detection of cVEMP using a bootstrap technique

The presence of the cVEMP response for 23 healthy volunteers was objectively detected using a bootstrap analysis and the p-value was obtained for all plateau durations for the 23 subjects, using MATLAB software. Figure 4.17 below shows the number of subjects with a real or significant cVEMP response (p<0.05), as a function of plateau duration. The cVEMP was successfully recorded at all the 6 plateau durations for the study; however, the number of subjects showing the presence of cVEMP varied between different plateau durations. The response rate had a general trend of reduction when the plateau duration of the stimulus was increased beyond 4 ms. The response rate was 100 % only for the plateau duration of 4 ms (1 cycle rise/fall with a linear ramp), while the response rate decreased after that to 78, 43, 26 and 17 % of the tested ears at plateau durations of 8, 16, 32 and 64 ms respectively. The shortest plateau duration, of 0 ms, produced a response rate of 65 %, which was less than that of 4 and 8ms plateau durations. The grand averaged waveforms of cVEMP were recorded from all of the participants and the results are presented in Figure 4.18.

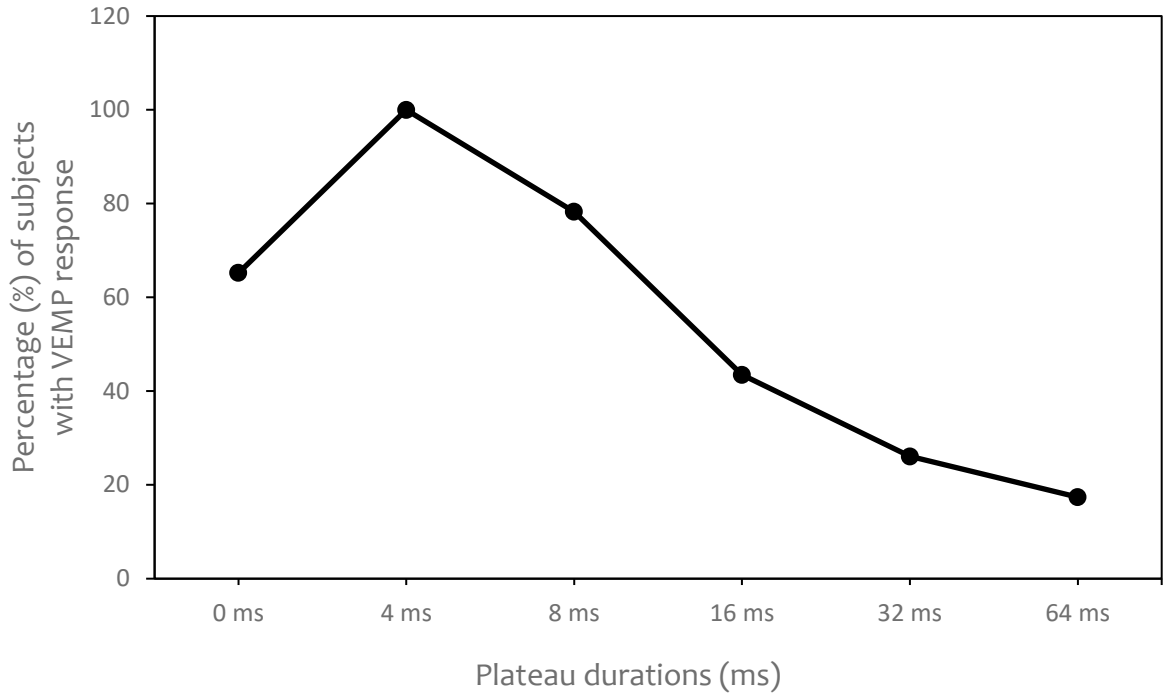

Figure 4.17: Percentage (%) of subjects with cVEMP response as a function of plateau duration.

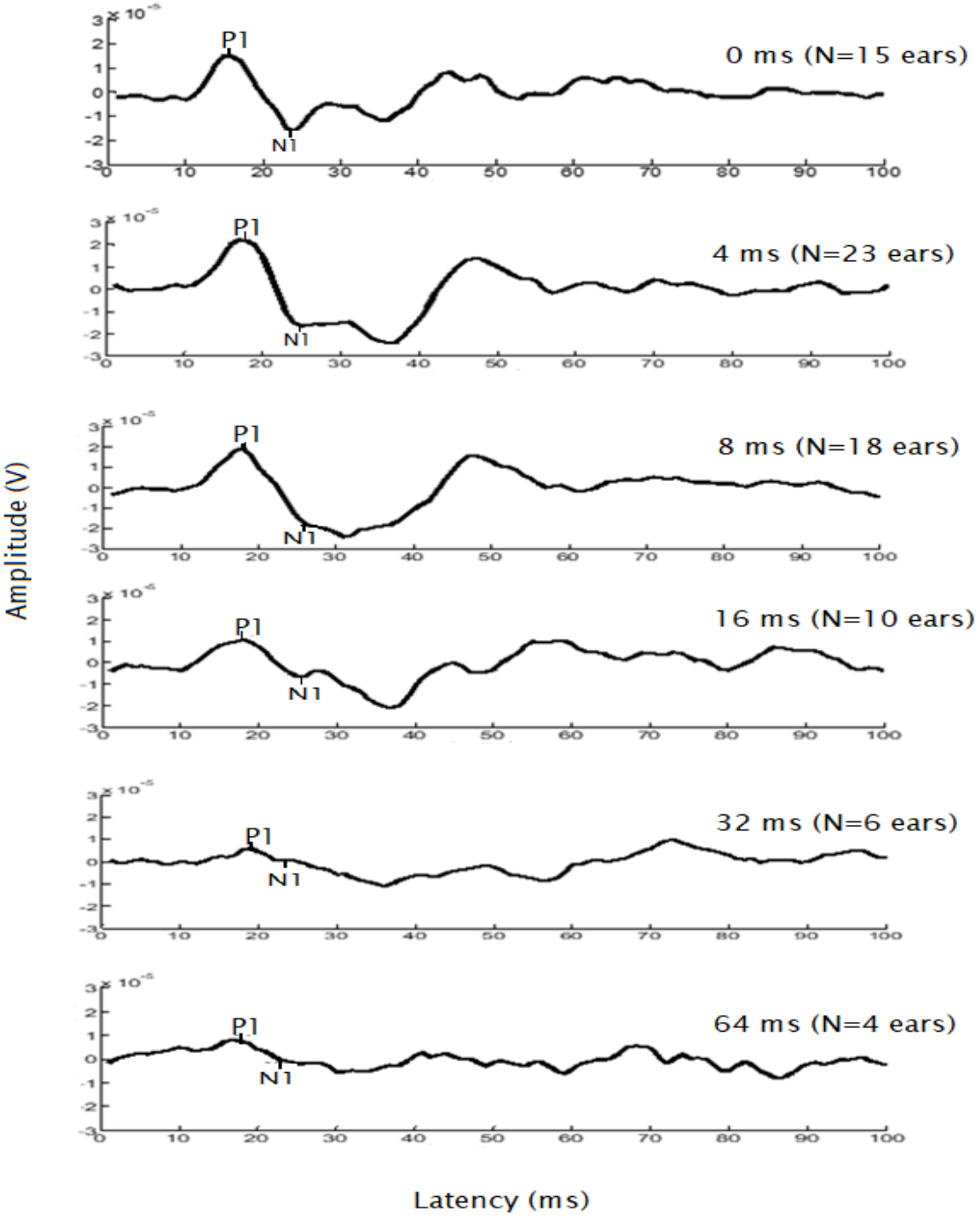

Figure 4.18: Grand averages of cVEMP waveforms from all participants, displaying changes in amplitude with increasing plateau durations. In these images, the upward positive peak (P1) and downward negative peak (N1) represent the actual cVEMP waveform response. N is the number of subjects with positive cVEMP responses at each plateau duration.

Using the p-values produced by the bootstrap analysis, it was found that the majority of subjects did not show cVEMP responses at 32 and 64 ms plateau duration. Therefore, these plateau durations were not included in the statistical analysis.

4.3.5.3 F_{sp} values of cVEMP response as a function of plateau duration

The effect of changing the plateau durations on the quality of cVEMP responses was measured using the F_{sp} values, which were obtained for all plateau durations, using MATLAB software. The F_{sp} values for cVEMP were measured at 115 dB p.e. SPL for 23 ears. F_{sp} values of the non-responsive ears were set to one in the comparative analysis of F_{sp} values (on average this is the F_{sp} obtained when no response is present in the data). From the results, it appeared that there was a significant effect of plateau duration on the F_{sp} value [$X^2(3) = 11.731$, $p < 0.05$]. Wilcoxon signed-rank-testing was conducted for multiple comparisons to identify which plateau durations were significantly different in F_{sp} value from one another. The results showed the F_{sp} value at 4 ms to be significantly higher than at the plateau duration of 16 ms ($p<0.05$). The remaining comparisons between different plateau durations showed a lack of significant difference. Figure 4.19 below shows the comparison of mean and ±1 SE of the mean F_{sp} value for different plateau durations.

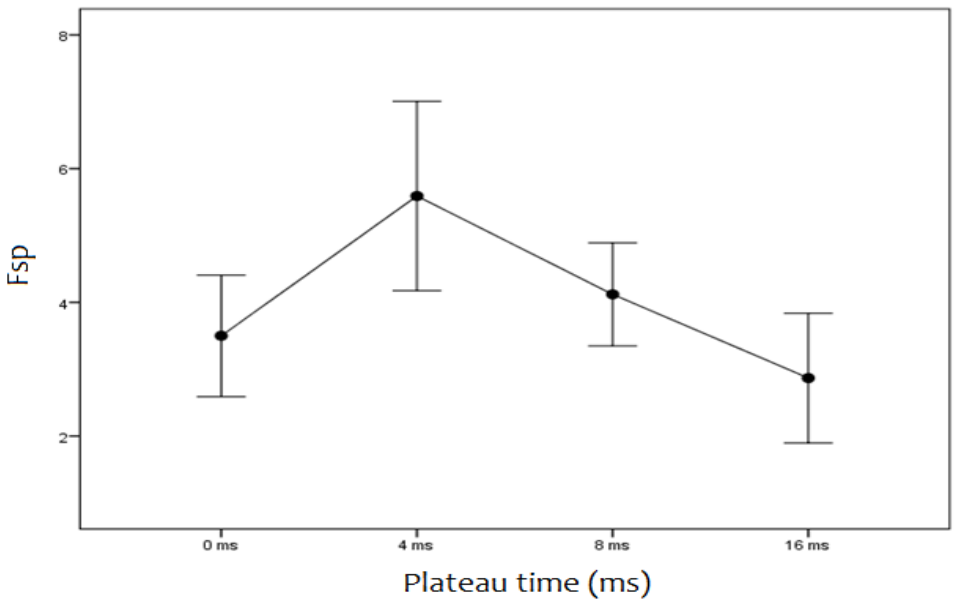

Figure 4.19: F_{sp} (dB) values of cVEMP as a function of plateau duration. Error bars represent ±1 SE of the mean. The F_{sp} values of recordings with no significant response on bootstrap analysis in the tested plateau durations were set to 1 for missing data.

4.3.5.4 Peak-to-peak amplitude of cVEMP at different plateau durations

The effect of variation in plateau durations on cVEMP amplitude was evaluated. The mean peak-to-peak amplitudes for p13 and n23 of cVEMP waveforms at 115 dB p.e. SPL were subjectively determined (by visual inspection) for a total of 23 ears using MATLAB software. The amplitude values of the non-responsive ears were set to zero in the comparative analysis of amplitude values (if no response indicated in the data, then there should be no measurable amplitude; hence it was set to zero). No significant effect of plateau duration on peak-to-peak amplitude [$X^2(3) = 6.835$, $p > 0.05$] was observed. However, the plateau duration of 4ms produced higher amplitude than all other plateau durations. Figure 4.20 below shows the comparison of mean and ±1 SE of the mean peak-to-peak amplitude for plateau durations.

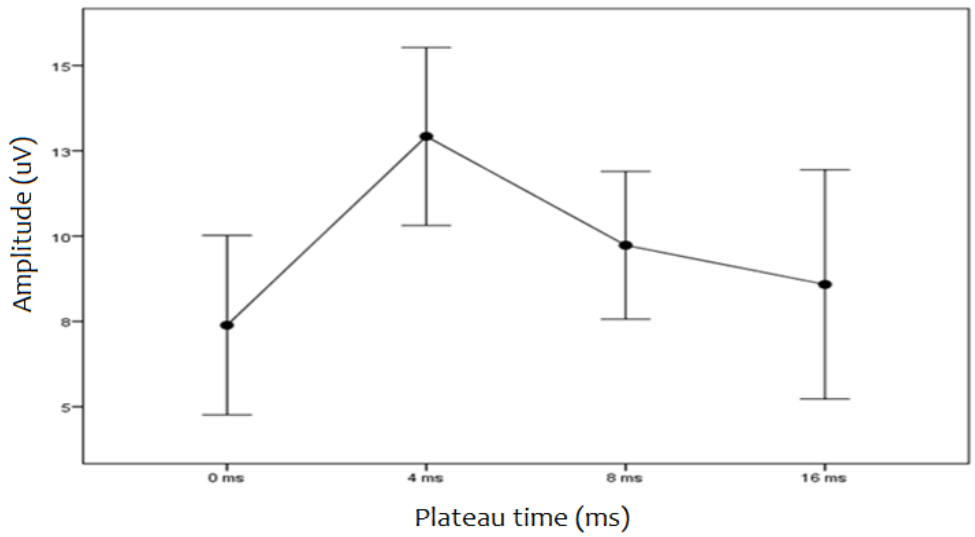

Figure 4.20: Peak-to-peak amplitude of cVEMP as a function of plateau duration. Error bars represent ±1 SE of the mean. The amplitude values of recordings with no significant response on bootstrap analysis in the tested plateau durations were set to zero for missing data.

4.3.5.5 Individual variations of peak-to-peak amplitude of cVEMP between subjects as a function of plateau duration

The individual amplitude of cVEMP responses for each participant was evaluated. Figure 4.21 below shows the peak-to-peak amplitude of cVEMP across different

plateau durations for each subject. For the majority of subjects, the amplitude of cVEMP response tended to drop with an increase in plateau duration beyond 4 ms. The plateau duration of 0 ms produced lower amplitude than 4, 8 and 16 ms plateau durations. The plateau duration of 4 ms produced higher amplitudes than all other plateau durations for the majority of subjects (Figure 4.21).

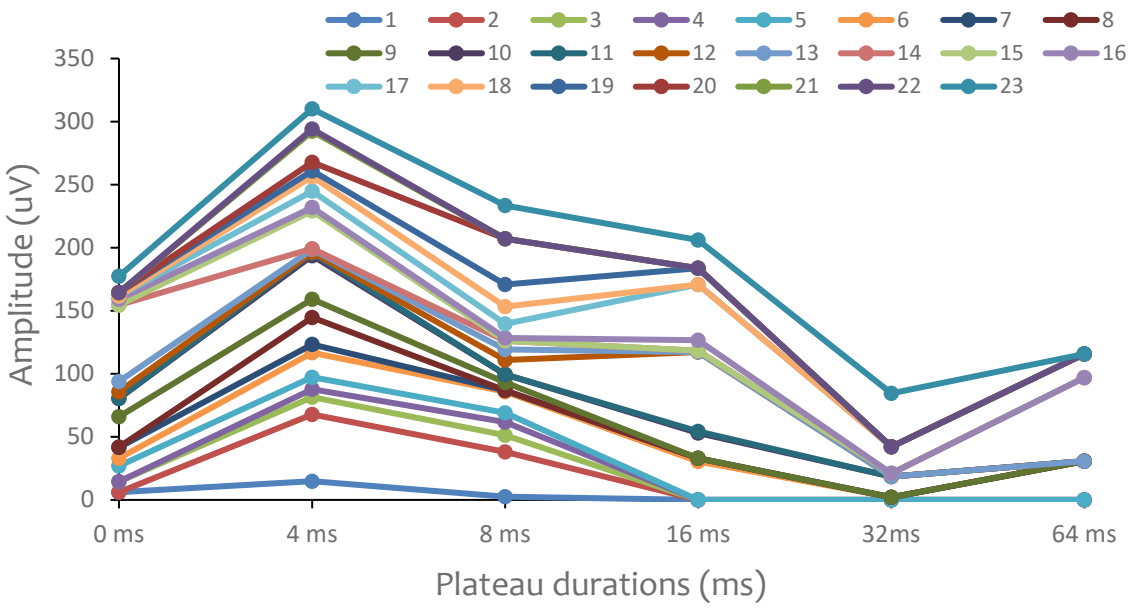

Figure 4.21: Peak-to-Peak amplitude of cVEMP as a function of plateau duration for all 23 subjects.

4.3.5.6 The Latency of cVEMP at Different Plateau Durations

The effect of changing the plateau duration on latency was measured in the current study. The latencies of p13 and n23 were subjectively measured for all participants from plots of response waveforms. The latency values of the non-responsive ears were not included in the comparative analysis of latency values. The within-subject effect showed that the means of p13 and n23 latencies did not differ significantly between plateau durations ($p>0.05$), p13 [$X^2(5) = 8.243$, $p>0.05$] and n23 [$X^2(5) = 9.565$, $p>0.05$]. Figure 4.22, below, shows the comparison of mean and ±1 SE of the mean for p13 and n23 latencies of cVEMP for different plateau durations.

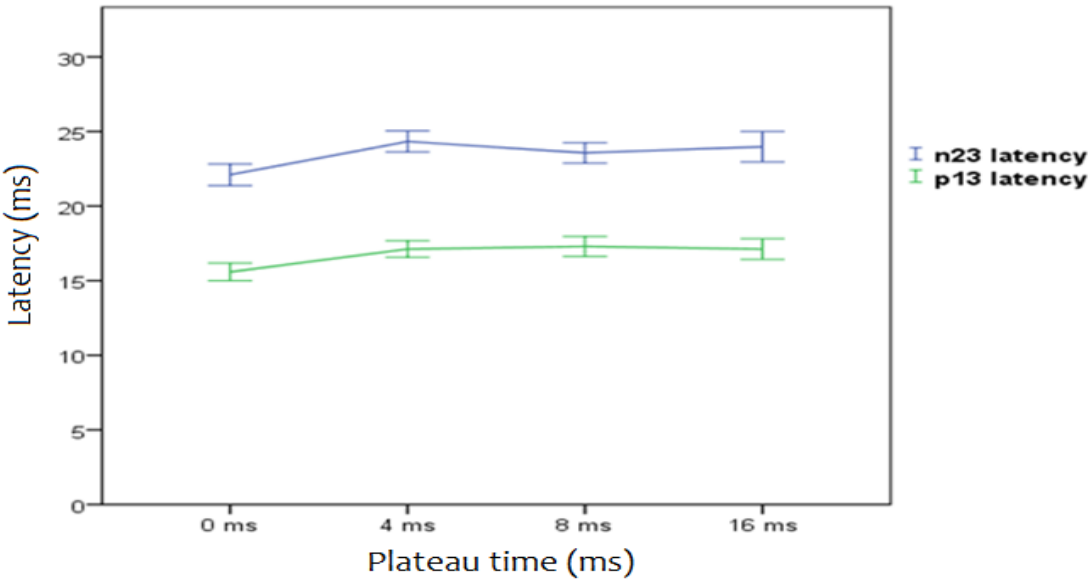

Figure 4.22: Mean p13 and n23 latencies of cVEMP at different plateau durations. Error bars represent ±1 SE of the mean. The latency values of recordings with no significant response on bootstrap analysis in the tested plateau durations were not included in the comparative analysis of latency values.

4.3.5.7 ART measurement for subjects with and without cVEMP responses at long stimulus plateau durations

In this work, it was expected that the small number of subjects who reported cVEMP responses at long stimulus plateau durations (8 ms and longer) may have abnormally elevated or absent ARTs compared with those without responses. Thus, the measurement of ipsilateral ART was compared for people with and without cVEMP responses at long plateau durations (8 ms and longer). A parametric independent-measures t-test showed that there was a significant difference in ART for subjects with (m=88.6, SD=4.3) and without (m=82, SD=5.7) cVEMP responses at long plateau durations of 500 Hz tone-burst: t (13) =2.419, p=0.03 (Figure 4.23). Subjects who reported cVEMP response at 8 ms plateau duration and longer had statistically significantly higher ART than subjects who reported no response.

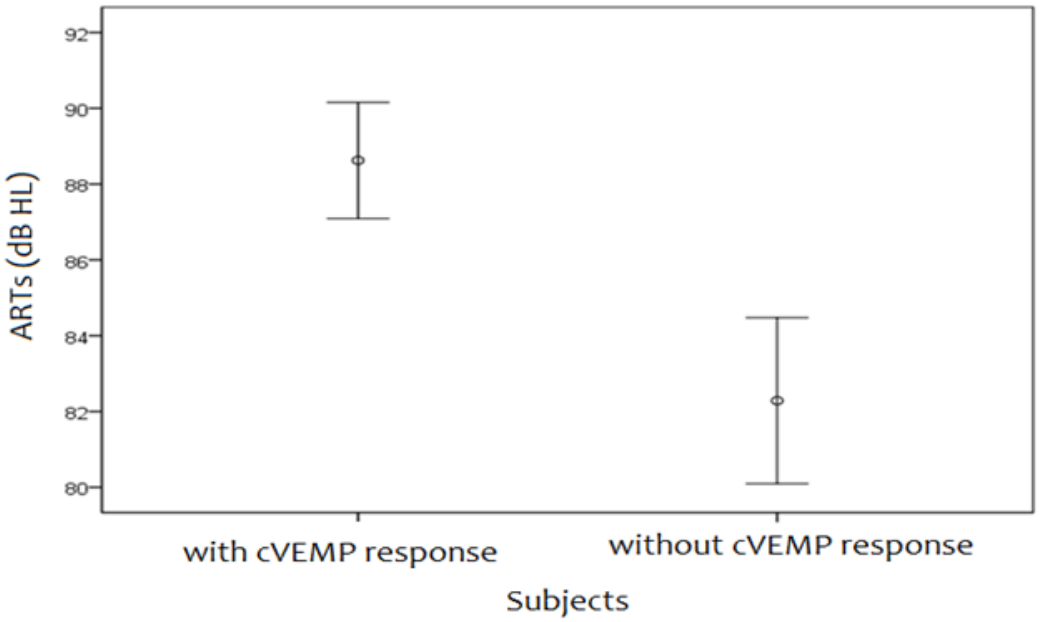

Figure 4.23: Ipsilateral acoustic reflex measurement (in dB HL) of 500 Hz pure-tone stimulus for 8 subjects with and 7 subjects without cVEMP response at long plateau durations (8 ms and longer). Error bars represent ±1 SE of the mean.

4.3.6 Discussion

The motivation behind this work was to improve frequency specificity of cVEMP responses by exploring how they adapt with long plateau times. Response rate was 100% for 4 ms plateau duration and decreased progressively for longer plateau durations. The shortest stimulus of 2 ms rise/fall time without a plateau produced a response rate less than that of 4 and 8 ms plateau durations. This short stimulus could have produced a response that is not representative of the characteristic frequency stimulated (see the illustration in Figure 4.24 below). As the tone-burst stimuli have a brief onset, spectral splatter of acoustic energy to other unwanted frequencies may occur (Katz et al., 2015). As a result, the frequency specificity of the VEMP evoked by a short stimulus will be decreased, as contributions could arise from frequencies other than the characteristics frequency being tested. Thus, a longer tone-burst stimulus will be more frequency-specific for eliciting cVEMP response. Figure 4.24 shows the spectrum of a 500 Hz tone-burst with different durations, as used in the current research. The spectral splatter has been defined by the -3 dB point; -3 dB is the point where the power has dropped to a value of 50 % of the maximum (taken from http://www.sengpielaudio.com/calculator-bandwidth.htm). As can be seen, as the stimulus duration is increased by increasing

the number of cycles, the 3-dB bandwidth of the stimulus is reduced, and, consequently, the stimulus become more representative of the characteristics frequency stimulated. As losses of energy to unwanted frequencies decrease, the bandwidth becomes sharper, since the energy is well focused on the centre frequency. The overall response becomes more representative of the characteristics frequency stimulated (500 Hz tone-burst). Thus, the longer the duration of the stimulus, the less spectral splatter occurs.

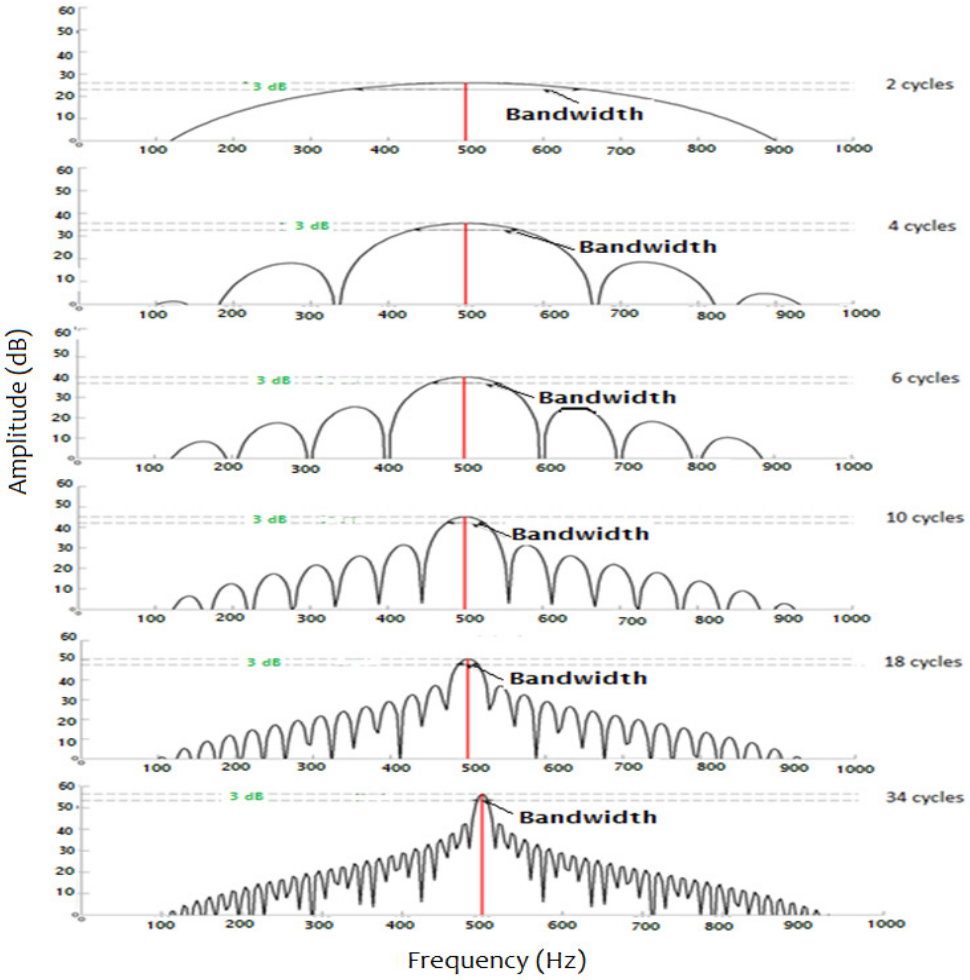

Figure 4.24: The spectrum of a 500 Hz tone-burst with different durations (2, 4, 6, 10, 18, and 34 cycles) as used in this work. The red line in the plots represents the centre frequency. In the top plot, the stimulus with a short duration occupies a broader spectral area since the acoustic energy spreads out to other unwanted frequencies. Conversely, the stimulus with a long duration (bottom plot) produces a discrete peak in the spectrum, so the spectral splatter is greatly reduced.

In the present study, it was expected that the small number of subjects who reported cVEMP responses at long stimulus plateau durations may have abnormally elevated or absent ARTs compared with those without responses. According to the results obtained, it appeared that the ART for subjects with cVEMP responses at long plateau durations was within the normal range of ART (usually occurring at around 75.5-90.5 dB HL). However, subjects with cVEMP response at long plateau durations had statistically significantly higher ARTs than subjects without responses. Thus, acoustic reflex could be one of the reasons for the reduced response rate for the majority of subjects with lower ARTs at long plateau durations.

4.3.6.1 Amplitude and F_{sp}

There was a trend of progressive decrease in amplitude and F_{sp} with an increase in the duration of the stimulus plateau duration beyond 4 ms. The plateau duration of 4 ms showed the highest amplitude and quality among all the plateau duration. None of the previous studies measured the effect of variation in plateau durations on the quality of cVEMP response. Although there was no significant difference with variation in plateau duration, the morphology of the cVEMP waveform was affected beyond the 8 ms plateau duration.

It was expected in this work that the amplitude would increase with increasing plateau durations. Although the peak level for all stimuli was constant in this study, at 115 dB p.e. SPL, the stimuli with longer durations were observed to be louder than stimuli with shorter durations. This is because the total energy of the stimulus becomes bigger with increasing plateau duration. Although the amplitude increased as the plateau duration increased from 0 ms to 4 ms, the amplitude decreased afterwards. The reduction in the amplitude and the response rate following increases in plateau duration could probably be attributed to SCM muscle fatigue (within the stimulus). Increasing the stimulus duration would cause multiple firing for muscle fibres, and consequently lead to muscle fatigue (Brahme, 2014. p.248). In addition, fatigue could be associated with recruitment of further muscle fibres in order to maintain the required force produced by the muscle (Paul & Wood, 2002). Using long stimulus plateau duration would require recruitment of further muscle fibres to maintain muscle contraction and, consequently, the rate of fatigue would be exacerbated. Others have attributed the decline in the amplitude with increased plateau duration to the impact of stapedial reflex (Cheng & Murofushi, 2001b). As

the stapedial reflex occurs at a lower threshold than VEMP, any VEMP recording will be influenced by the reflex response (Singh et al., 2014a). As the total energy of the stimulus gets bigger with increased plateau duration, the stimuli with longer duration would experience a greater effect of stapedial reflex (see Figure 4.25 below).

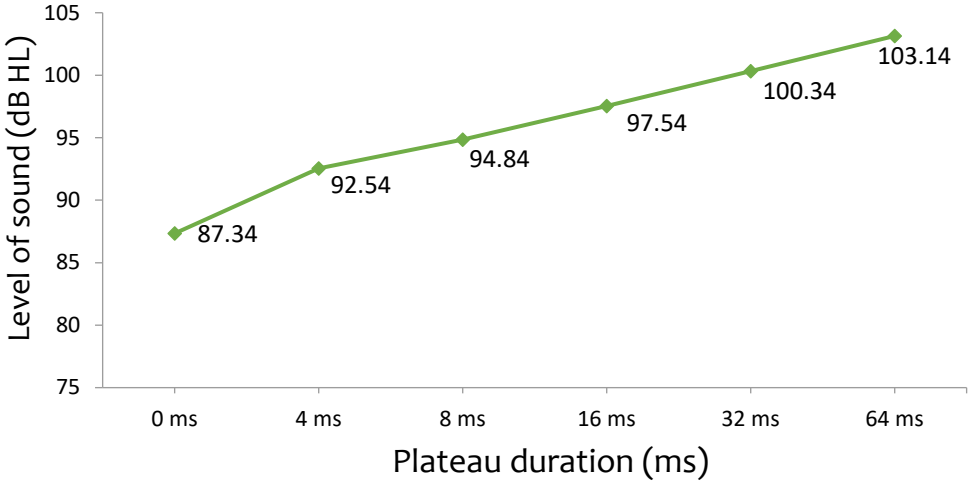

Figure 4.25: Levels of a stimulus sound (in dB HL) at each plateau duration used in the current study. The stimulus levels for all plateau durations used for eliciting the cVEMP response were above the normal range of ART, which usually occurs around 75.5-90.5 dB HL (equivalent to 85-100 dB SPL). Therefore, any cVEMP recording could be influenced by the reflex response, in the present study.

Marimuthu & Harun (2016) investigated the effect of the plateau duration on amplitude and found the 0 ms plateau duration produced the largest amplitude amongst the four plateau durations, which was not in accordance with the present study, where the best amplitude was obtained at 4 ms plateau duration. However, in their study, the ramp times used to elicit cVEMP were different between plateau durations. Cheng & Murofushi (2001b) investigated the effect of variation in plateau durations on amplitude and reported a progressive decrease in amplitude with plateau times, similar to that of the current study. These authors attributed the decline in amplitude following increases in plateau duration to the effect of stapedial reflex. In their study, although the 5 ms plateau showed the highest amplitude among the other plateau durations, it was not considered as an optimal rate for cVEMP recording. This is because the plateau duration of 5 ms could have elicited

the cVEMP response and induced the stapedial reflex at the same time (Cheng & Murofushi, 2001b). In an experiment on cats, the EMG activity of the middle ear muscle in response to a loud clicking sound was recorded using implanted electrodes (Salomon, 1966). The stapedius muscles showed an initial contraction, with latency in the range between 4.5 and 10 ms (Salomon, 1966). Thus, in their study, Cheng & Murofushi (2001b) recommended the stimulus duration of 2 ms (0 ms rise/fall and 2 ms plateau duration) as an optimal duration for evoking cVEMP, as it can elicit the cVEMP response prior to inducing stapedial reflex. In the present study, stimulus durations of 8, 12, and 20 ms could have elicited the cVEMP response and induced the stapedial reflex simultaneously, whereas stimulus duration of 4 ms could elicit a cVEMP response prior to the stapedial reflex. Thus, the stapedial reflex could have cancelled the intensified energy of long stimulus duration (Cheng & Murofushi, 2001b), and consequently the amplitude of cVEMP response decreased as the plateau duration increased. It would be assumed that the longer tones are loud enough to elicit a reflex, but the shorter tones are not. If this is the case, the shortest stimulus duration of 2 ms rise/fall and 0 ms plateau duration would produce higher cVEMP amplitude compared to the other plateau durations. However, this short stimulus without an adequate plateau duration produced the smallest amplitude among the four plateau durations. This might be as a result of spectral splatter of energy to unwanted frequencies, so this stimulus would seem to be not representative of the characteristics frequency being tested.

In both the current and previously mentioned studies, there was no increase in amplitude of cVEMP evoked by ACS when using plateau durations longer than 4 and 5 ms, despite the increased energy being supplied. In addition, the subject's exposure to sound was higher for longer plateau durations than for shorter plateau durations, as the energy builds up with an increase in the plateau duration. Hence, using shorter plateau durations, such as 4 ms, would lessen the subject's exposure level to sound and produce higher amplitude and better morphology of cVEMP response, compared to longer plateau durations. However, longer plateau durations reduce spectral splatter and would be more frequency specific to elicit cVEMP response. Nevertheless, none of the previous studies used longer plateau duration than that of 10 ms for evoking cVEMP. In this work, a plateau duration of 0 ms produced the smallest amplitude of cVEMP response among the other plateau durations, and thus was not considered as an effective frequency-specific stimulus

for evoking cVEMP response. Additionally, increasing the plateau duration beyond 4 ms did not produce any certain benefit concerning F_{sp} value and amplitude of cVEMP response.

4.3.6.2 Latency

Although there was a general trend of increase in the mean p13 and n23 latencies as the plateau durations increased from 0 ms to 16 ms, the differences in these increments were not statistically significant. This was not in agreement with the outcomes of the previous studies regarding the effect of plateau time on latency of cVEMP (Cheng & Murofushi, 2001b; Marimuthu & Harun, 2016; Singh et al., 2014b). Cheng & Murofushi. (2001b) reported a significant prolongation in latency as the plateau durations increased from 1 ms to 10 ms. However, there were no significant differences between some plateau times in their study. Marimuthu & Harun. (2016) also reported a significant prolongation in p13 and n23 latency, with increases in the plateau durations. However, in their study, the rise/fall time used to elicit cVEMP was different between plateau durations. Cheng & Murofushi (2001b) attributed the prolongation in the latencies of p13 and n23 to the changes in stimulus duration used to elicit cVEMP response, which related to changes in the plateau duration. The same authors (Cheng & Murofushi, 2001a) measured the latency of cVEMP at different ramp times with keeping the plateau duration constant and found a progressive prolongation of latencies as the ramp time increased. The increase in latency of cVEMP response consequent to changes in the ramp time might add to the evidence that cVEMP is primarily an onset response. In this case, most of the VEMP response with maximum amplitude occurs at the onset of the stimulus and the rest of stimulus is not important.

None of the previous studies explored where the VEMP occurs. For the current study, the ramp time and the peak level were kept constant for all plateau durations to find out what generates VEMP. The results of the present study support the theoretical hypothesis that cVEMP is primarily an onset response. The lack of significant shifting in latency across different plateau durations might exclude the theoretical prediction that the 'integrating' point of tone-burst stimulus is what triggers cVEMP response. However, it may be that some integration occurs in the plateau region, but very rapidly, so that beyond the first ms or so the length of the plateau does not have an

effect on the latency of the response observed. Additionally, it appeared that energy of the stimulus is not the main generator of cVEMP, as the amplitude decreased with increased plateau durations for both the present study and all of the previous studies. Therefore, it can be concluded that VEMP is primarily an onset response. Based on these results, the lack of a sharp-onset of AM tone in experiment 1 (reported in Chapter 3) might explain the low response rate of S-VEMP in that study.

4.3.7 Conclusions

This study established that responses to 500 Hz tone-bursts from the SCM muscle can be recorded at different plateau durations. However, the majority of subjects had responses only to short plateau duration, whereas only a small number of subjects had responses to long plateau durations. This might be attributed to either SCM muscle fatigue or the impact of stapedial reflex at long plateau durations, for the majority of people. The lack of significant shifting in latency with an increase in the plateau durations, keeping the peak level and the ramp duration constant for all recordings, suggests that it is the onset of the stimulus that generates the cVEMP response. There is no benefit of using tone-burst plateau durations longer than 4 ms. Using shorter plateau durations reduces the level of sound exposure during cVEMP recording although it reduces frequency-specificity.

In this chapter (in both experiments), the optimal stimulation parameters of cVEMP have been found. The main conclusions were: the optimal trade-off between recording time and response detection for the majority of subjects appears to be a rate of 10 Hz, and the onset of the stimulus is most likely what generates the cVEMP response, so there might be no benefit in increasing tone-burst durations for the VEMP response. The next chapter aims to optimize detection of the cVEMP response.

Chapter 5 : A comparison of objective and subjective detection of cVEMP responses

Acknowledgements:

I would like to express my deepest appreciation and thanks to Michael Chesnaye for his contributions towards this study, which include writing the code for the statistical analysis and constructing the figures. In addition, I would also like to thank him for his explanations regarding the technical details of the statistical detection methods.

5.1 Introduction

Although cVEMP has been widely used in clinical practice as an objective measure of the VCR, the interpretation of data still relies upon subjective (visual) interpretations, and so results are highly dependent upon clinical expertise. Different criteria have been used in the literature to visually judge the presence of a cVEMP response. Park et al. (2010) based response detection on the premise that the cVEMP waveform should be biphasic, with a first positive component (p1) at 13 ms followed by a negative (n1) component at 23 ms (see example Figure 2.3 in Chapter 2). Similarly, Isaradisaikul et al. (2012) stated that the p1 latency should be at 12.6-20.10 ms and n1 latency at 19.7-27.6 ms for the cVEMP response to be present. In 2012, the British Society of Audiology (BSA) suggested two criteria as a basis to determine the presence of a response. First, the cVEMP waveform should be biphasic with a positive (p1) component followed by a negative (n1) component, and second the amplitude (inter-amplitude p1-n1) of the response should be 20-150 μV. Previously, Brantberg and Fransson (2001), attempted to indicate the presence of the cVEMP response objectively, based on four criteria to determine the response presence: the myogenic potential should be biphasic with a first positive peak (p1) at 13.5 ms followed by a negative (n1) peak at 15-20 ms, the positive and negative peaks should be repeatable in two recordings of cVEMP, and the amplitudes of p1 and n1 of the myogenic response should exceed the variations in voltage for about three standard deviations

through the first 5 ms after stimulus presentation. However, their method for response detection is not a standard one for evoked response detection. It seems that the criteria used for visual judgment of the presence of a cVEMP response varies considerably across studies. Even when specified criteria are used for visual interpretation of the presence or absence of a cVEMP response, subjective judgment is typically required to decide if the criteria are met.

To reduce cVEMP measurement noise, a number of epochs are averaged (typically around 150). The visual evaluation of the averaged response is problematic when the SNR is poor, due to a small response relative to the physiological background noise (Don et al., 1984). When the SNR is poor, the waveform morphology of the response is affected by noise, and this causes difficulty in identifying the presence of the cVEMP response. Furthermore, replications of significant peak and trough components can be unreliable due to variations in the background noise between testing sessions (Don et al., 1984). For cVEMP tests, using a fixed number of epochs (averages) can never guarantee the repeatability of the waveforms, as SNR and muscle tension often vary between testing runs. More repetitions of the waveform should be conducted for low SNR responses than for high SNR responses, to obtain a clear response. However, performing a large number of recordings of the cVEMP may not be possible due to the effect of muscle fatigue (recording 150 epochs at 5 Hz stimulation rate takes 30s which is around the limit that a patient may be maintain good neck tension for). Hence, visual identification is subjective, and considerable variations may occur between experienced audiologists in reporting the presence of a cVEMP response for a given stimulus. In a clinical setting, caution should be exercised when visually identifying the presence and absence of the cVEMP response.

The problem of subjective identification of response thresholds has been recognised for other evoked responses. For example, Vidler & Parker (2004) compared the threshold estimates of 16 professionals on ABR data. The difference between threshold estimates was in some cases more than 40 dB. However, to date, this variability has not been well studied for cVEMP thresholds, and the statistical methods that have been used for detection of other evoked responses have not been well tested for the measurement of VEMP tuning curves. Several statistical approaches have been explored and used for automated detection of evoked potentials such as the ABR, Auditory Steady State Responses and the Auditory Late

Response. A review of approaches is given in Chesnaye et al. (2018). In the case of ABR, Elberling and Don (1984) established one of the first methods and proposed the F_{sp} (F at a single point) statistic as an objective estimate of response quality. This approach (and a variant termed the F for multiple points (F_{mp})) is now available in several commercially available clinical measurement systems. Threshold values for F_{sp} can be used to indicate when a response is significantly different from noise. It is possible to convert F_{sp} values to p values, indicating the significance of a response, but only if the degrees of freedom of the signals being measured are known. Elberling and Don. (1984) made such an estimate of degrees of freedom for ABR, but the degrees of freedom of VEMP data have not been well explored. Therefore, the F_{sp} value does not provide a statistical p value indicating when a VEMP response is significant. Lv et al. (2007) directly overcame the problem of unknown degrees of freedoms by evaluating and calculating the significant value of a parameter (i.e. F_{sp}) using a bootstrap analysis without needing to estimate the degrees of freedom. Bootstrapping is an objective resampling approach that has been widely used for automated detection of evoked potentials, such as ABR (Lv et al., 2007). This is an approach first presented by Efron. (1979), which is based on random resampling of the data to estimate the null distribution of a F_{sp}, which would be anticipated if there was no stimulation present. From this distribution, the p value for a given F_{sp} value can be established (Lv et al., 2007) (see Chapter 4, section 4.2.4.5 for more details).

Another objective method that has been well used for auditory evoked response detection is the Hotelling's T^2 test (HT^2) (Hotelling, 1931), which is a multivariate extension to the student's t-test and can be used to evaluate whether the variables' means are significantly different from the hypothesized values. A number of authors have applied the Hotelling's T^2 approach for automated response detection of slow Cortical Auditory Evoked Potentials (Carter et al., 2010; Chang et al., 2012; Golding et al., 2009; Van Dun et al., 2012; Van Dun et al., 2015). Recently, Chesnaye et al. (2018) investigated and compared the objective detection of ABR response using HT^2 statistics and several objective approaches, including F_{sp} and the magnitude squared coherence (amongst others) and showed that HT^2 was the most sensitive approach.

Based on the preceding review, both the F_{sp} evaluated by the bootstrap approach and the HT^2 test were selected as objective detection methods for detecting the cVEMP

response in this study. To the best of my knowledge, neither of these methods has yet been evaluated for automated detection of cVEMP responses.

The goal of this work is to explore if objective analysis methods can be used to improve the measurement of cVEMP thresholds and hence reduce reliance on subjective interpretation approaches. The objectives were 1) to compare the sensitivity (threshold estimates) and specificity (false positive rate-FPR- which is defined as the percentage of cases that were wrongly considered as having a response when the response was actually absent) of objective detection methods—the F_{sp} bootstrapping approach and HT^2 test—to results obtained through subjective inspection by experienced observers, and 2) to compare detection times for the two statistical objective measures when detecting cVEMP responses.

Research questions:

- Can cVEMP response be detected objectively using statistical approaches?
- How do subjective and objective estimates of cVEMP threshold compare?

5.2 Methods

The experimental protocol for this study was approved by the Human Experiment Safety and Ethics Committee (ERGO: 23879) of the University of Southampton before the research commenced.

5.2.1 Participants

13 subjects (7 females and 6 males) with normal hearing and balance function in the age range of 22-48 years participated in this study. The required sample size was determined using data from a previous study on objective detection of evoked potentials (Lv et al., 2007), assuming that the minimum Chi-Square value is 21.3 for 12 subjects to obtain significant results at alpha=0.05. The Chi-Square Power shows that for 86 % power, 13 subjects are needed to detect a significant result (alpha=0.05).

Details regarding the inclusion/exclusion criteria used to recruit healthy subjects in this experiment are identical to those described in the previous experiments; see

section 3.2.1 in Chapter 3. Responses were recorded from 13 ears from the 13 subjects in the present study.

5.2.2 Stimuli

For the current study, 500 Hz 1:2:1 (one cycle rise/fall and two cycles plateau) tone-burst stimuli were presented using insert earphones (Etymotic ER-3A). The stimulus level was decreased in 3 dB steps from 109 dB A (A-weighted sound level) until the level was significantly below that where a response could be seen (around 86 dB A typically). Each recording consisted of 150 repeats of an 8 ms short tone-burst. A repetition rate of 10 Hz was fixed for all measurements, so total duration for each recording was 15 s. The rate of 10 Hz was found to be the optimal trade-off between recording time and response detection for the majority of subjects, from the previous study (Experiment 2). Responses were recorded ipsilaterally from the left SCM muscle for normal subjects. The order of presentation of stimulus intensities was randomised among subjects. One recording was made at each stimulus intensity for each subject. Two recordings at each stimulus intensity to obtain repeatable waveforms were not performed, since a long recording period would exceed the maximum allowable amount for a typical exposure and could cause muscle fatigue. Thus, to minimise the measurement time and reduce the risk of muscle fatigue, it was decided to measure the response once at each stimulus intensity. In addition, to avoid muscular fatigue, a one-minute break was given to each subject after each recording.

5.2.3 Apparatus

The equipment setups used with subjects to conduct this experiment were identical to those used in Experiment 1, as described in section 3.2.3.

5.2.4 cVEMP recording

VEMPs from the SCM muscle were recorded ipsilaterally for all subjects. All procedures used with subjects in this experiment to conduct cVEMP measurements were identical to those used in Experiment 1, as described in section 3.2.4.

5.2.5 Detection methods

5.2.5.1 Visual inspection of the response

cVEMP thresholds were separately estimated by three experienced audiologists (A, B & C), who were blinded to the experimental conditions. In this work, cVEMP responses were defined as present if the cVEMP was a reproducible biphasic waveform with a positive (P1) peak followed by a negative (N1) peak; the inter-amplitude (P1-N1) of the response was between 20 and 150 μV, based on the criteria presented by the BSA (2012); the waveform should be larger than the rest of the response in the overall average and the acceptable latency range of p1 was 13.9-19.2 ms and n1 was 22.9-30.3 ms. These latency values were determined from a recent study (Blakley and Wong, 2015) of cVEMP evoked by 500 Hz tone-bursts in 48 adults (23-64 years) with no history of hearing and balance problems. If any of these criteria were not met, then the cVEMP response was judged to be absent. Examples of a clear (A), probable or possible (B), and absent (C) cVEMP response to ACS are shown in Figure 5.1 below. In example A, all the criteria for response presence have been met, so the response is judged to be present. In example B, there is a peak response, but not larger than the rest of noise in the overall average, so it is difficult to be sure that it is not noise. In example C, the response was judged to be absent as all the criteria for response presence were not met.

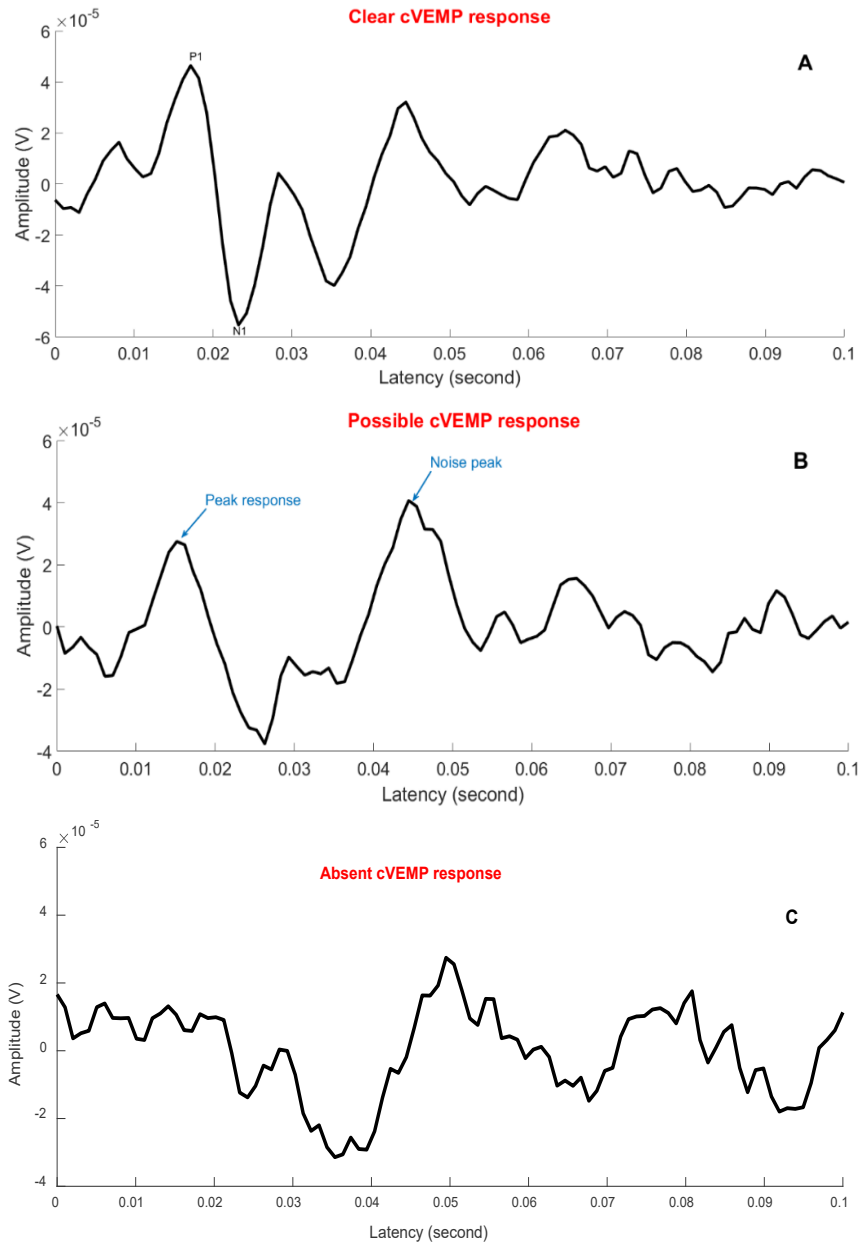

Figure 5.1: Example data for cVEMP responses at 500 Hz tone-burst stimulus. Plot A represents a clear cVEMP waveform (all criteria were met). Plot B represents a possible cVEMP response; this is because the cVEMP waveform is not larger than the rest of the response in the overall average (the noise peak is higher than the cVEMP peak). Plot C shows an absent cVEMP response.

5.2.5.2 Description of the methods

The two statistical methods (HT^2 and F_{sp} bootstrapping, described below) were applied to the responses recorded from otologically normal subjects and cVEMP thresholds were then identified for each statistical method. In this work, the cVEMP threshold was defined as the lowest stimulus level (in dB A) at which a significant response ($p \leq 0.05$) is attained, with a significant response for all higher stimulus levels (see example in Table 5.1). Each statistical parameter was calculated for the analysis time window 10-30 ms following stimulus onset. These thresholds were compared to those subjectively estimated by three observers who independently estimated the cVEMP threshold.

Table 5.1 shows the p-values at all intensity levels for the two objective methods used for measuring the cVEMP threshold for one participant. In this example, the minimum stimulus intensity for detection of the response was 103 and 100 for the bootstrap approach and HT^2 test, respectively. For all higher levels, the p-values showed a significant response ($p \leq 0.05$).

Table 5.1: An example of p-values for the two objective methods at different stimulus levels for one participant. The marked p-value shows the cVEMP threshold for one subject.

Stimulus level (in dB A)	F_{sp} bootstrapping	HT^2
97	0.85	0.68
100	0.21	**0.03**
103	**0.01**	0.001
106	0.001	0.001
109	0.001	0.002

A. Bootstrap analysis of F_{sp} values

For each cVEMP recording, the F_{sp} of the coherent average was first calculated (as explained in Chapter 3, section 3.2.5). A bootstrap approach was then applied to determine whether the value of F_{sp} for each recording indicated a significant response (for more details of the bootstrap method, see section 4.2.4.5 in Chapter 4).

B. Hotelling's T^2 test (HT^2)

The one-sample Hotelling's T^2 test is the multivariate extension to the student's t-test, and can be used to test whether the means of N features (in this work N time-voltage means) are significantly different from N hypothesized values. In the present work it is assumed that the expected values of the features (the N hypothesized values) are zero. The statistic itself is a weighted sum of the N feature means where the weights are determined by the variances and covariances of the features. These weights are furthermore optimal in the sense that they maximize the resulting T^2 value, which maximizes the sensitivity of the test when using the N features in question (Simaika, 1941). The weights have the additional property of normalizing the N means, which allows features with different scales and units to be combined appropriately. For more details of the methods see Chesnaye et al. (2018).

Before applying the HT^2 approach, each recorded epoch (n=150) with a duration of 20 ms was reduced to a number of average voltages, with each average having been taken within a time window covering a particular latency range. For example, with 5 windows used, the 5 time windows covered the range from 10 to 30 ms, with each feature being 4 ms wide. The effect of different numbers of time windows from 2 to 20 on threshold estimates was explored. A repeated-measures ANOVA with Greenhouse-Geisser corrected values showed a statistically significant effect of the number of features of the HT^2 test on the cVEMP threshold (F (2.144, 25.731) =9.548, $P < 0.05$) (see Figure 5.2). However, on paired comparison, only the choice of 2 features raised thresholds compared to the other approaches. 5 features appeared to be the best trade-off between sensitivity and analysis complexity and this number was used in the results section below.

Response detection was based on the cut-off p-value obtained from a one-sample HT^2 test on the time window averaged data using MATLAB software. The one-sample HT^2 test is a multivariate extension of the ordinary one-sample t-test; it tests a null

hypothesis, which has been defined in this work as that the expected averaged value in every time window is zero. A cut-off of $p<0.05$ results in a FPR of 5 %.

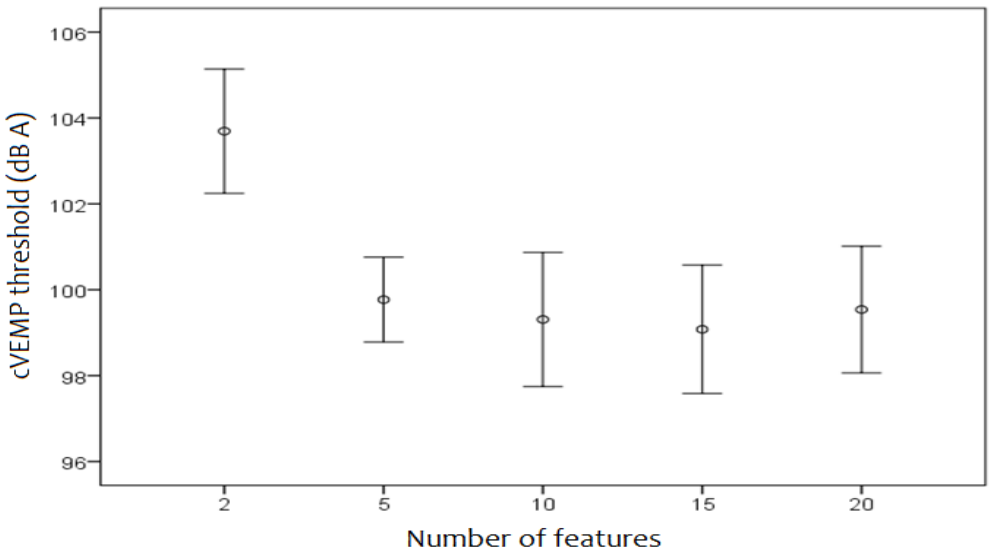

Figure 5.2: cVEMP thresholds for 13 subjects as a function of the number of features of the HT^2 test. The error bars represent ±1 SE of the mean.

Specificity evaluation using simulations

In statistics, a FPR (also identified as 1-specificity) refers to the probability of incorrectly rejecting the null hypothesis for a specific test. The FPR is defined as the percentage of the total number of recordings conducted for the no stimulus condition that were wrongly considered as positive test results when the response was absent. To determine if the estimated FPR of 5 % was achieved when no stimulation was present, 8 recordings of 150 no-stimulus epochs were collected from 17 subjects (this included all subjects who had been tested before in the stimulus condition and 4 additional subjects, to obtain extra no-stimulus recordings). The 136 recordings (17 subjects × 8 sets) were structured into 136 recordings, where each recording contained 150 epochs, and each epoch was 100 ms (corresponding to a repetition rate of 10 Hz). The epochs were then split into 5 segments (0-20; 20-40; 40-60; 60-80 and 80-100 ms) to generate more data; however, these segments may be correlated with each other, so independence between statistical tests cannot be guaranteed. There were then 680 recordings of epochs, where each recording still contained 150 epochs, but the duration was 20 ms.

As there was no stimulus response, for a p-value of 0.05, the FPR was expected to occur in 5 % of cases and was tested using the HT^2 test and bootstrap approach. The HT^2 test was applied to each recording of the epochs. The HT^2 test was then further applied to features extracted from the epochs. Hence, the 20 ms segments listed above were split into 5 segments (so that each segment had a 4 ms duration). The mean across each segment was taken, so each recording contained 150 × 5 features. For the bootstrap, the F_{sp} value was evaluated by generating 500 additional 20 ms epochs for each of the 680 recordings of no-stimulus data. The 20 ms bootstrapped segments were selected randomly from within the 100 ms windows from the original recording.

Detection times for statistical methods

The required time to detect a cVEMP response was measured by finding the number of stimuli (expressed in seconds) required for the p-value to drop and remain below the 0.05 threshold for the remainder of the test. The significance of a statistic was evaluated by taking the first 10 epochs and obtaining the p-value for the HT^2 test and the F_{sp} bootstrapping method, then taking the first 20 epochs and generating the p-value for both objective methods, and so on.

5.2.6 Pilot study

Piloting was performed to determine if cVEMP responses can be detected objectively using statistical methods. The cVEMP was conducted using a 500 Hz 1:2:1 (cycles) tone-burst for four otologically normal subjects. The cVEMP thresholds for these subjects were determined at 500 Hz using 3 dB steps from 109 to 97 dB A. A p-value produced by the HT^2 test and bootstrap approach was used to determine the presence of a response. A p-value of less than 0.05 suggests a cVEMP response is likely to be present, whereas a p-value equal to or greater than 0.05 suggests that the response is absent. From piloting, it was found that the cVEMP response can be detected using objective statistical methods.

5.3 Results

5.3.1 Subjective inspections of cVEMP threshold

Figure 5.3 shows the cVEMP thresholds estimated by the three experienced audiologists (A, B & C) for 13 subjects. The inter-observer reliability for detecting a cVEMP threshold was assessed using a Fleiss' kappa test. The degree of agreement given by Fleiss' kappa between the three observers was 0.5314. For the reliability of judges to be regarded as high, Fleiss' Kappa values should be ≥ 0.90, according to Arnold (1985). Thus, the reliability between observers was not high and this indicates disagreement in the identification of cVEMP thresholds, which is consistent with effects seen for ABR threshold estimation (Vidler & Parker, 2004).

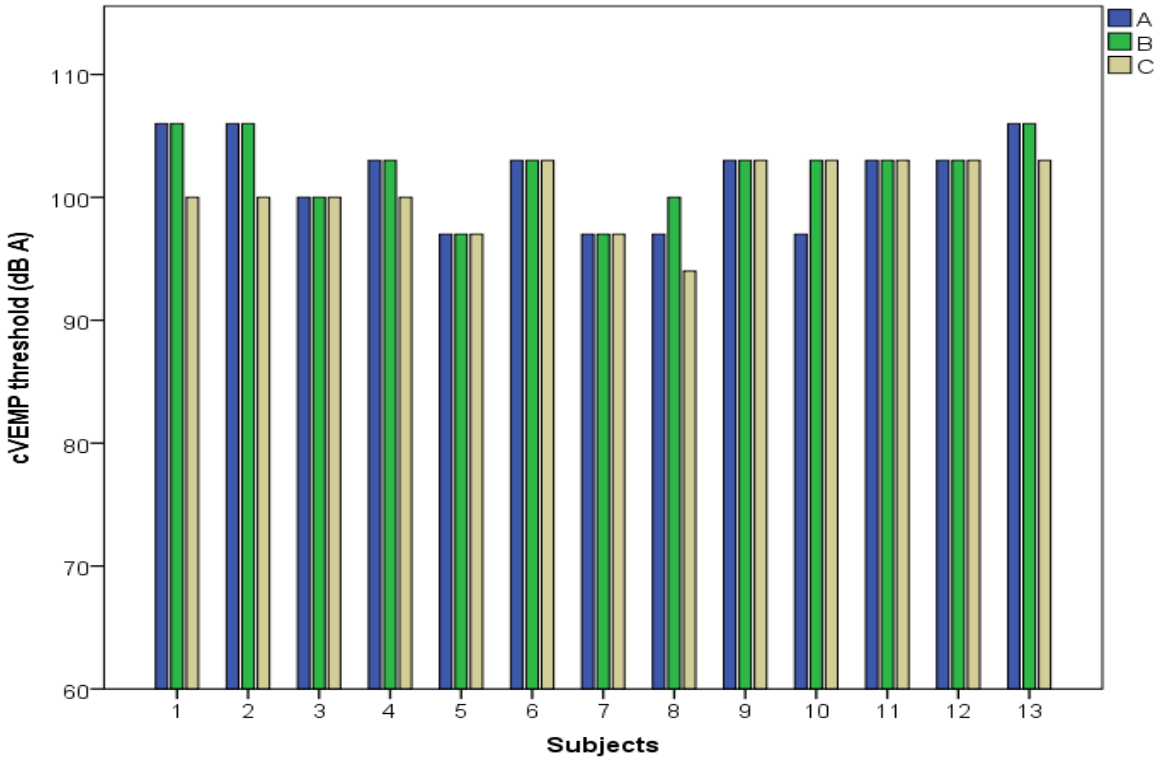

Figure 5.3: cVEMP thresholds for 13 subjects with normal hearing and balance function, as determined through subjective detection by three experienced audiologists (A, B, & C). For each participant, the three bars show the cVEMP threshold estimated by observers A, B, and C respectively.

5.3.2 Comparison of subjective and objective estimates of threshold

Figure 5.4 shows the mean cVEMP threshold for 13 subjects estimated subjectively by three observers (A, B, & C) and by using the objective methods (HT^2 and bootstrap for a significance level of α =5 %) for the same number of averages, n=150. A non-parametric Friedman test showed a highly significant difference between the estimates from the methods [χ^2 (4) = 15.118, p <0.001]. Wilcoxon signed-rank testing was conducted to further explore where these differences lay and showed that the HT^2 test was sensitive in finding cVEMP thresholds at significantly lower stimulus levels than found by observers A & B (p<0.05). The bootstrap method also detected the cVEMP response at significantly lower thresholds compared to one of the observers (B). Observer C detected the cVEMP threshold at a significantly lower stimulus level compared to observer B. HT^2 produced the numerically lowest value of threshold, with a significantly lower threshold than two of the three raters.

Figure 5.4: The mean cVEMP threshold for 13 subjects estimated subjectively by three observers (A, B & C) and objective methods (HT^2 and F_{sp} bootstrapping) for a significance level of 5 %. Subjective and objective thresholds were determined for 150 sweeps. The error bars represent ±1 SE of the mean.

5.3.3 FPR using simulated data

For the no-stimulus data, the FPRs were 2.79 % for the HT^2 test and 3.52 % for the bootstrapped approach. The lower and upper boundaries for significant (p<0.05) deviations from an expected FPR of 0.05 can be found with the theoretical binomial distribution by setting the number of Bernoulli trials to the number of tests performed (680), and the probability of observing a successful trial to the expected FPR (5 %). The lower and upper 95% confidence intervals for an expected 5 % FPR are then given by 24/680 = 3.53 % and 47/680 = 6.91 %, respectively. Figure 5.5 shows the probability distribution of the number of false positives when the probability of observing a false positive is 5 %, and when 680 tests are performed. The expected FPR is 0.05, so 680*0.05 = 34 of the tests are expected to be false positives. Thus, in theory, the binomial distribution is distributed around 34. Using the recorded

signals, the number of false positives were 18 (2.79 % * 680) for the HT^2 test and 24 (3.52 % * 680) for the bootstrapped approach. The HT^2 FPR was therefore significantly ($p<0.05$) conservative. The FPR for F_{sp} bootstrapping, on the other hand, was very close to the expected FPR limit of 5 %. The FPR for HT^2 was not within the boundaries of the expected FPR, which could be possibly due to independence violation between epochs. The consequence of this is that the test will be more conservative, i.e. less likely to detect a response. This could mean that detection times are longer than they should be. Adjusting the HT^2 criterion for detection by increasing the value of alpha from 0.05 until the FPR reached 5 percent was explored. However, this did not improve the detection times of HT^2 test. An alpha of 0.05 was used in the final analysis of data in this work.

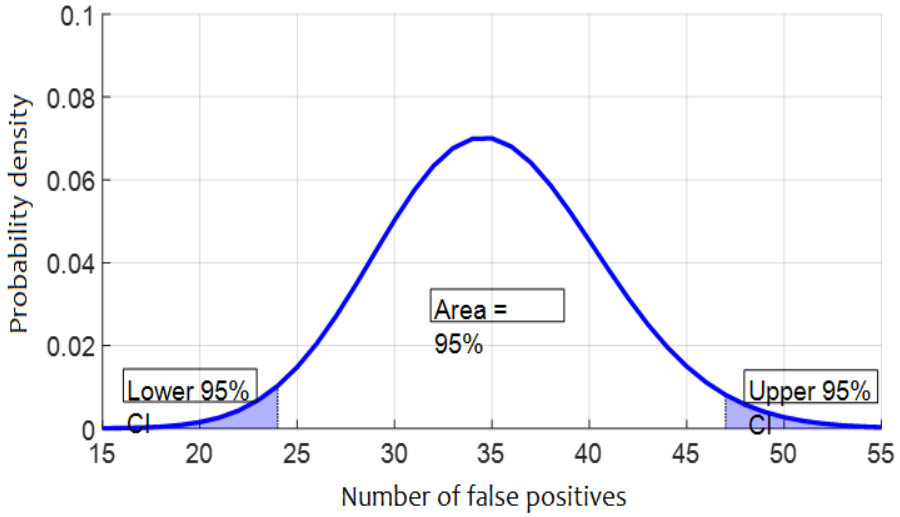

Figure 5.5: The theoretical binomial distribution for 680 Bernoulli trials, using a 5 % probability for a successful trial. In other words, there were 680 'Bernoulli trials' for 680 tests. In each trial, there was a 5 % chance for a false positive, so the probability of a 'successful trial' (observing a false positive) is 5 % per trial, or 5 % per test. Probability density on the y-axis represents the probability of observing some false positives given by the area under the curve. 5 % of 680 trials is 34 and hence the distribution is centred on that. The 95 % confidence intervals for the expected 34 false positives had an upper and lower FPR boundary of 47/ 680 = 6.91 % and 24 / 680 = 3.53 %, respectively.

5.3.1 Comparison of detection time

Figures 5.6 and 5.7 illustrate the effect of the number of stimuli (epochs) recorded at each stimulus level on the ability to detect the cVEMP response for the HT^2 test and F_{sp} bootstrapping method, respectively. It was found that the detection rate of the cVEMP response increased with the increasing number of stimuli recorded and with the stimulus level. At 109 and 106 dB A, 50 and 110 stimuli, respectively, were enough to receive 100 % detection using the HT^2 test. In contrast, the bootstrap method required 120 stimuli at 109 dB A to detect responses in all thirteen subjects, whereas more than 150 epochs were required to achieve 100 % detection at 106 dB A.

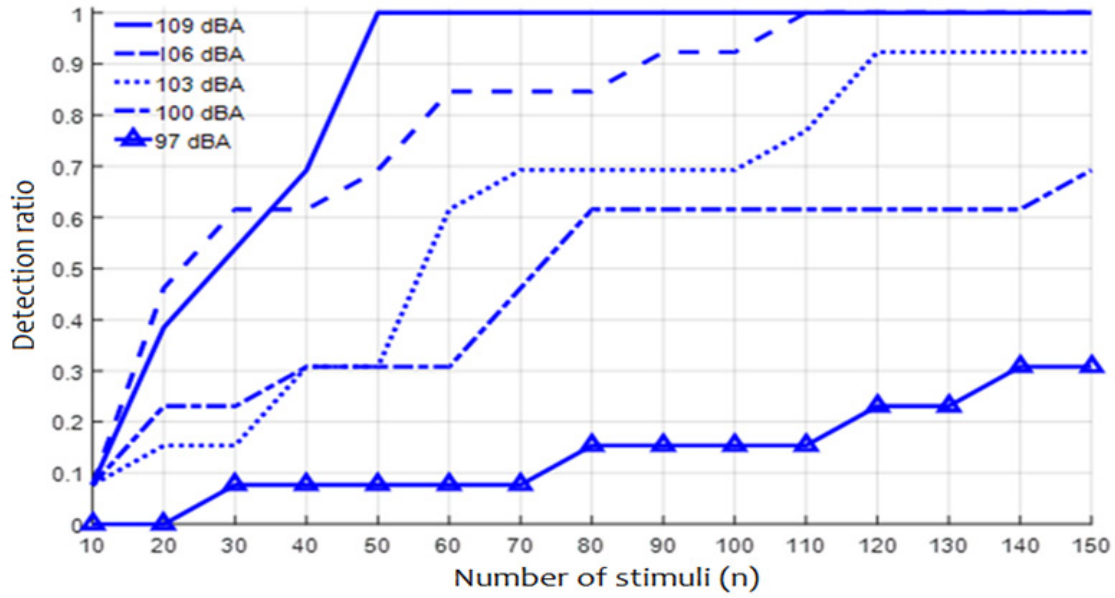

Figure 5.6: The detection rate of cVEMP response as a function of the number of stimuli recorded and the stimulus intensity, using the HT^2 test.

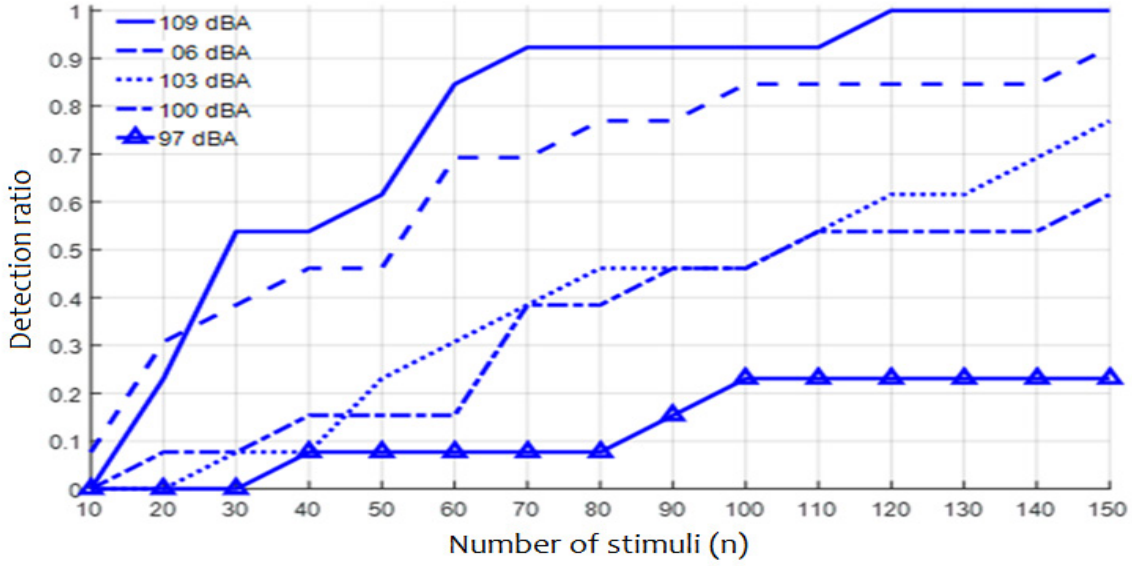

Figure 5.7: The detection rate of cVEMP response as a function of the number of stimuli recorded and the stimulus intensity, using the F_{sp} bootstrapping method.

Figure 5.8 shows the fraction of subjects in which the cVEMP response was detected as a function of the stimulus levels for the HT^2 test and the bootstrap method, using 150 epochs recorded. As expected, the detection rate of the cVEMP response increases with increasing stimulus levels. At 106 dB A, the cVEMP responses were detected in all of the 13 subjects with the HT^2 test (with 150 sweeps), while a stimulus

intensity of 109 dB A was required to obtain 100 % detection with the bootstrap method (with 150 sweeps). Consequently, using the HT^2 test at a stimulus level of 106 dB A with 150 sweeps for detecting cVEMP responses can slightly reduce the sound exposure for human ears, by 3 dB.

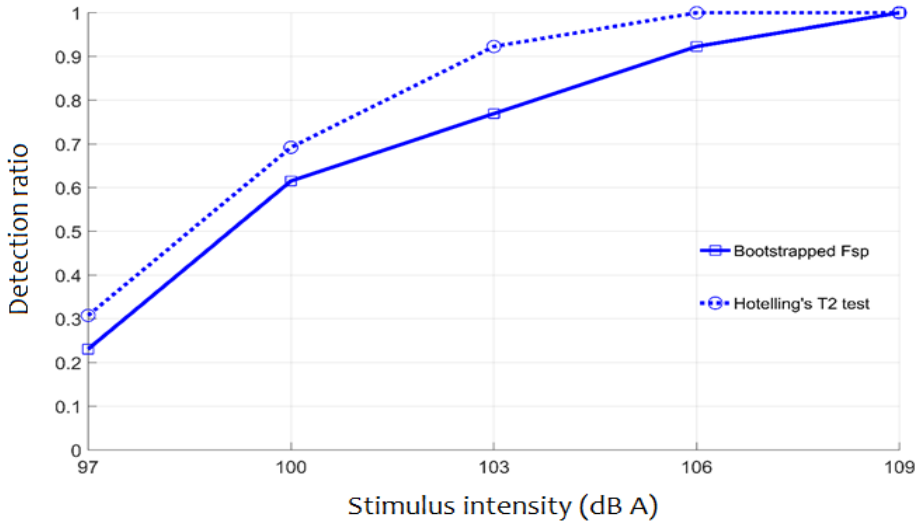

Figure 5.8: The proportion of cases in which the cVEMP responses were detected, as a function of stimulus level (in dB A) for HT^2 and the bootstrap method.

Figure 5.9 illustrates the average detection time (in seconds) for both HT^2 test and the bootstrap method at different stimulus intensities. A non-parametric Wilcoxon test revealed that the detection time was significantly shorter for the HT^2 test compared to the F_{sp} bootstrapping method at 109 and 106 dB A. No significant difference was found in the detection time between the two methods for the remaining stimulus intensities. The average detection time (in seconds) was shorter for the HT^2 test compared to the F_{sp} bootstrapping method. On average, it appears that the HT^2 test can detect cVEMP responses faster than the bootstrap approach, taking less than five seconds at 109 and 106 dB A. Hence, the measurement time of the cVEMP response can be considerably reduced with the HT^2 test which will consequently lessen fatigue and neck tension.

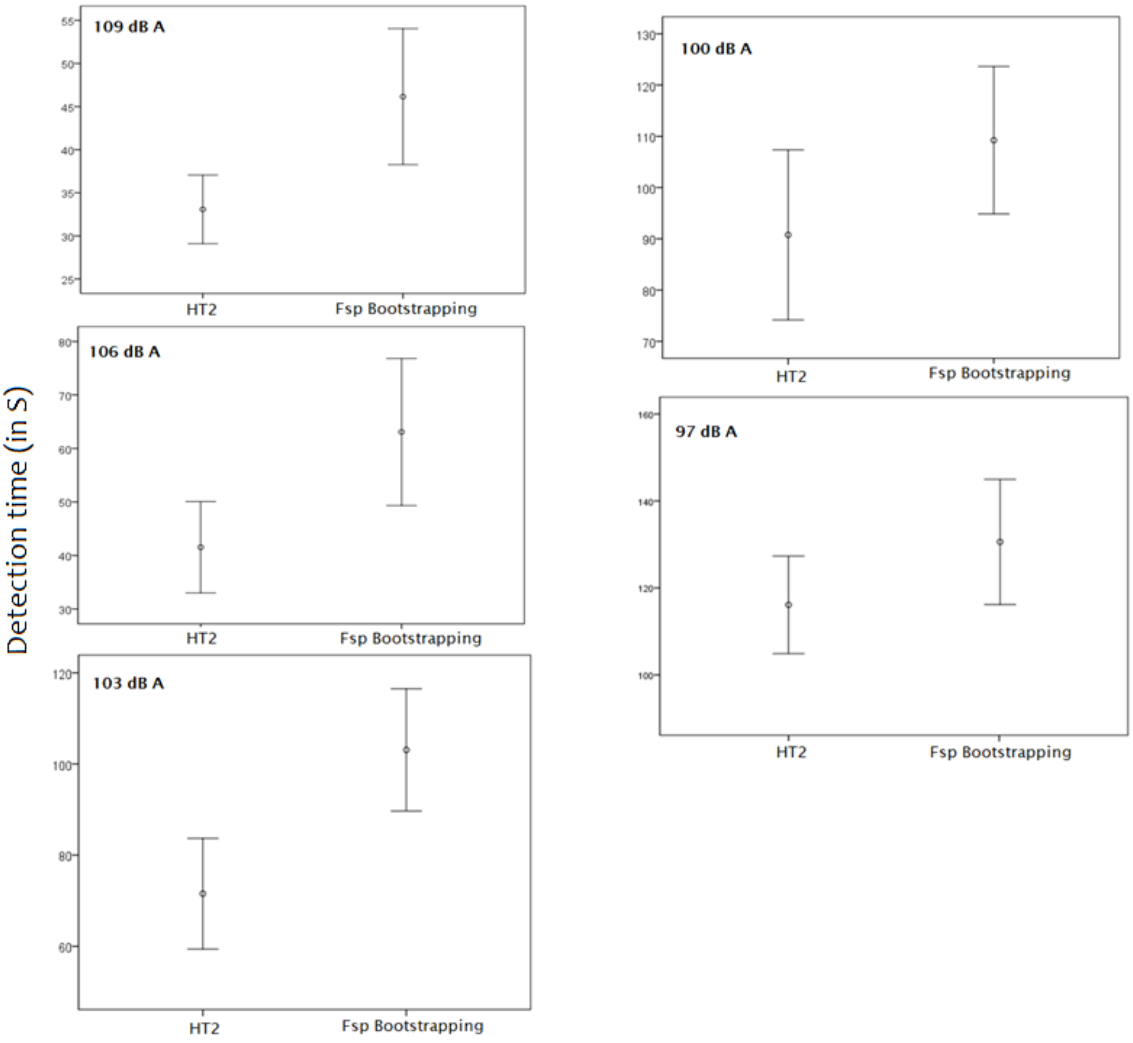

Figure 5.9: The average detection time (in seconds) for both HT^2 test and F_{sp} bootstrapping approach at different stimulus levels. Error bars represent ±1 SE of the mean. The detection time values for missing data were set as 16 seconds (for 160 sweeps with 0.1-second time window).

5.4 Discussion

Although the cVEMP is considered as an objective measurement of saccular function, its objectivity is diminished by conventional visual analysis of the response. Subjective analysis of response presence remains the most common approach for cVEMP threshold estimates. The motivation behind this work was to detect cVEMP responses objectively in order to eliminate the high variability of visual judgements and to increase the sensitivity of response detection, which is a novel contribution of the present study.

This work showed that cVEMP responses can be estimated objectively using statistical approaches. The objective estimate of the presence of the response with the HT^2 test was the most sensitive method, identifying cVEMP responses at lower stimulus levels than either visual detection or the bootstrap F_{sp} method. There was a considerable level of disagreement between the three experienced audiologists (A, B and C) in their decisions regarding identification of response thresholds of cVEMP, as reflected by a moderate reliability kappa value. This is consistent with the subjective variability that has been found between different experienced professionals in subjective threshold determination of ABR data (Vidler and Parker, 2004). The advantage of using objective methods over subjective identification of cVEMP responses is that they are not affected by the subjectivity of visual judgement. However, for clinical use, some subjective inspection of waveforms is still helpful to check the morphology of the response, which could be used for diagnosing particular disorders. This could be done for only the highest stimulation levels, where the VEMP response should be clear. For example, prolongation of p1-n1 peak latencies and reduced amplitudes of cVEMP responses are common in patients with central vestibular disorders (e.g. multiple sclerosis, brain stem tumour, acoustic neuroma and vestibular migraine), owing to the slowing of nerve-impulse conduction by demyelination along the vestibulospinal pathway (Rosengren et al., 2010). Several studies showed that cVEMP latency was prolonged in patients with multiple sclerosis (Alpini et al., 2004; Oh et al., 2016; Shimizu et al., 2000; Versino et al., 2002), whereas cVEMP amplitudes were found to be reduced in patients with vestibular migraines (Baiser et al., 2009; Baiser & Dieterich, 2009; Oh et al., 2016; Zuniga et al., 2012). A study by Angunsri et al. (2010) showed that reduced amplitude and

prolonged latency of cVEMP responses were found in patients with acoustic neuroma. Hence, looking at cVEMP parameters, such as latency and amplitude, would help in distinguishing these pathologies from other aetiologies affecting the brain stem (e.g. brain stem tumour).

In the results for specificity, a lower-than-expected FPR was observed for the HT^2 test: significantly ($p<0.05$) conservative. The FPR for F_{sp} bootstrapping, however, was very close to the expected FPR limit of 5 %. The lower-than-expected FPRs might be attributed to independence violations, i.e. when one response is not completely independent from the next. It is probable that low-frequency noise was present, which introduced some correlation across epochs. Chesnaye et al. (2018) concluded that the stimulus rate and filter settings interact in terms of the amount of violation of independence. Probably further work is needed to explore this in more depth for VEMP and to find the best combinations of rate and filter settings for clinical work. For now, this method can be used, but with the caveat that it does have reduced sensitivity.

On average, the HT^2 test can detect cVEMP response quickly, in less than 5 seconds at 109 and 106 dB A (when the cVEMP is clear). This can considerably reduce the duration of the test and consequently reduce the fatigue of the subject, by reducing the time needed to maintain neck tension. The bootstrap approach performs significantly worse than HT^2 in terms of test duration, but it is still more sensitive than visual inspection of response presence. Therefore, for people with normal vestibular function, using the HT^2 test at these stimulus intensities (109 and 106 dB A) can considerably reduce the duration of the measurement. This requires fewer sweeps and can thus lessen the subject's fatigue from having to maintain neck tension. The results of the present study are consistent with a previous study on ABR by Chesnaye et al. (2018) who found an advantage in terms of sensitivity and detection time for the HT^2 test when detecting ABRs. In clinical settings, rapidly identifying the cVEMP threshold with the HT^2 test can maximise time effectiveness, especially for measuring the cVEMP frequency tuning curve, which may help in the diagnosis of patients with MD.

With respect to how the number of features of the HT^2 test affects the cVEMP thresholds, it was found in the current work that using the HT^2 test with five features appeared to offer a good trade-off between sensitivity and analytic complexity.

In summary, significant variability was seen between subjective estimates of cVEMP thresholds. Objective analysis with the HT^2 test was more sensitive than 2 of 3 experts in detecting responses. Moreover, the measurement time of cVEMP was considerably reduced with the HT^2 test.

5.5 Conclusions

There is significant variability between subjective estimates of VEMP threshold by experienced raters. Objective analysis methods are more sensitive than subjective analysis, can detect responses rapidly and have the potential to reduce variability in threshold estimates, hence they present promising approaches towards automated threshold estimation of VEMP tuning curves.

The next Chapter explores the use of the objective statistical response detection to estimate the frequency-tuning curve of the saccule in Ménière's patients.

Chapter 6 : Comparing objective cVEMP-tuning curves with ECochG for the diagnosis of Ménière's disease

6.1 Introduction

MD is a disorder that affects the inner ear and is associated with episodic cochlear symptoms that include low to medium frequency SNHL, fluctuating aural symptoms (hearing, tinnitus and the sensation of ear pressure or fullness in the ear) and vestibular symptoms including recurrent spells of vertigo (Furman et al., 2010). The mechanism underlying these episodic symptoms is poorly understood although previous studies (de Waele et al., 1999; Furman et al., 2010; Paparella & Kimberley, 1990) have suggested that the presence of endolymphatic hydrops can be considered a pathological condition underlying the development of MD. Endolymphatic hydrops is believed to be caused by an abnormal accumulation of endolymph fluid that fills the hearing and balance structures in the inner ear (Gürkov et al., 2016). This disorder results in an enlarged endolymphatic space, referred to as endolymphatic hydrops (Gürkov et al., 2016). Researchers long believed that endolymphatic hydrops was necessarily associated with the symptoms experienced by patients suffering from MD and could be a histological substrate for the disease (de Waele et al, 1999; Furman et al., 2010; Paparella & Kimberley, 1990). However, evidence from human temporal bones studies revealed that although all patients with MD symptoms in life had evidence of endolymphatic hydrops in at least one ear post-mortem, there were also patients with endolymphatic hydrops without signs or symptoms of MD (Merchant et al., 2005; Rauch et al., 1989). This suggests that pre-existing hydrops is not directly responsible for the symptoms of MD, but it can merely be an epiphenomenon of the pathophysiological mechanism of the disease (Merchant et al., 2005). If hydrops is causative, then not only every patient with a history of MD symptoms during life would have evidence of hydrops but also every case of hydrops would have symptoms of MD.

The cause of the development of hydrops is still unknown, but many theories have been proposed and the majority of them are based on the abnormal development or reabsorption of endolymph (Pirodda et al., 2010; Schuknecht, 1982). The most widely accepted hypothesis, proposed by Schuknecht (1982), suggests that hydrops may rupture the membranous labyrinth in the inner ear leading to potassium intoxication in the vestibular hair cells. Mixing high potassium endolymph with low potassium perilymph can alter the neural discharge rate of the vestibular nerves. Thus, both the cochlear Reissner's membrane and the vestibular saccular membrane, delicate membranes that separate the endolymph from perilymph space, can be affected by hydrops, causing vertigo attacks and hearing loss.

To date, there is no internationally approved clinical test for the diagnosis of MD. In 1995, the AAO-HNS proposed a set of criteria to help in the diagnosis of MD, which are now widely used. Based on these criteria, patients are clinically classified as having definite, probable and possible MD. The diagnosis of MD can be difficult, especially if vestibular symptoms occur in isolation, so objective tests are essential in addition to historical information for identification of the disease.

ECochG has been widely used as an objective clinical tool for the diagnosis of cochlear hydrops. However, its clinical utility remains a subject of debate among researchers because 25 % to 54 % of patients who were considered to have MD based on the AAO-HNS guidelines showed normal ECochG results (Kim et al., 2005). The major debated point in the literature is the establishment of the SP/AP ratio cut-off (Mammarella et al., 2017) to indicate MD. Gibson et al. (1983) proposed a value of 0.29. Other authors proposed somewhat higher ratios as indicative of hydrops, between 0.40 and 0.45 (e.g. Al-Momani et al., 2009; Margolis et al., 1995; Wuyts et al., 1997). It has been speculated that the false negative rates for ECochG result from the transient nature of endolymphatic hydrops (Kim et al., 2005). However, Ohashi and others found that an elevated SP/AP ratio of ECochG is maintained over an extended period of time and despite medical and surgical treatment (Ohashi et al., 1991). Thus, it appears that the EcochG SP/AP ratio only indicates endolymphatic hydrops and it is not necessarily correlated with MD symptoms (Gibson, 2017). This is consistent with the findings of temporal bone studies that found the presence of hydrops does not necessarily correlate with MD symptoms (Merchant et al., 2005; Rauch et al., 1989). Overall, a number of factors may contribute to the reported low sensitivity of EcochG for MD, including fluctuation of Ménière's symptoms, lack of

standardization regarding EcochG stimulus parameters, recording techniques and interpretation, and distortion of ECochG components due to deterioration of cochlear hair cells in more advanced stages of the disease (Ferraro & Durrant, 2006). This has limited the clinical utility of ECochG in the diagnosis of MD (Lamounier et al., 2014).

Other measures than the SP/AP ratio have been proposed in the literature to increase the diagnostic sensitivity of EcochG in detecting cases of MD Combination of the area under the SP/AP curve and SP/AP amplitude ratios significantly improved the sensitivity to 92 % of extra-tympanic (ET) EcochG in the diagnosis of MD, while maintaining specificity as high as 84 % (Al-Momani et al., 2009). Ferraro & Tibbils (1999) found that the inclusion of the SP/AP area curve measurement resulted in improving the sensitivity of ET ECochG to 90 % in the diagnosis of definite cases of MD. Devaiah et al. (2003) conducted a retrospective study on 138 patients with possible MD undergoing trans-tympanic (TT) ECochG (an invasive technique which includes inserting a needle electrode through the tympanic membrane), and compared the sensitivity of SP/AP amplitude ratio and SP/AP area curve ratio in the diagnosis of endolymphatic hydrops. They found sensitivities of 50 % and 87.5 % for amplitude ratio and area ratio, respectively. In contrast, a retrospective study on 198 patients with MD showed that the inclusion of the SP/AP area ratio measurement in addition to the conventional SP/AP amplitude ratios, as well as using SP/AP area alone, did not increase the diagnostic sensitivity of TT ECochG in identifying MD cases (Baba et al., 2009). The sensitivities for SP/AP amplitude ratio and SP/AP area ratio in patients with definite MD were 57.1 % and 43.9 %, respectively (Baba et al., 2009). These authors attributed the discrepancy in results between their study and previous studies on sensitivity of the SP/AP area ratio (e.g. Al-Momani et al., 2009; Ferraro & Tibbils, 1999) to various methods of measurements, e.g. TT versus ET ECochG. Further research is necessary to assess the sensitivity of the inclusion area measurements with ET ECochG in the diagnosis of endolymphatic hydrops.

Measurement of the area under the SP/AP curve is not easily performed, and it requires specialist software, which is not yet commercially available (Ferraro, 2010; Mammarella et al., 2017). In addition, there is still no consensus among researchers about the most practical method to establish the SP/AP area measurement (Grasel et al., 2017).

Conlon & Gibson (2000) proposed measuring 1 kHz tone-burst (1 ms rise/fall and 14 ms plateau) evoked SP amplitudes and found that the diagnostic sensitivity of TT ECochG increased to 85 %, compared with the conventional SP/AP click (100 μs) amplitude ratios. A tone burst will test a more specific region of the cochlear than a click and it is possible that the 1 kHz frequency is more specific to MD than the range of frequencies tested with a click. Sass et al. (1998) found that the sensitivity of TT EcochG obtained by using measurements of SP/AP amplitude ratio and SP amplitudes at 1 KHz tone-burst increased from 62% to 82%, without changing specificity. As yet few other studies have explored the sensitivity of SP/AP measurements to MD using tone-burst stimuli. In the present study, the tone-burst elicited SP in MD with ET ECochG could not be performed, since this would exceed the maximum allowable amount for a typical noise exposure. Therefore, the only parameter considered in this study is the SP to AP (SP/AP) click amplitude ratio.

cVEMP responses to AC stimulation are primarily considered to be saccular in origin and it has been proposed that cVEMP response frequency will be altered in patients with MD, hence it may be sensitive to endolymphatic hydrops in the saccule. Previous studies on healthy subjects found that cVEMP exhibits a frequency-tuning curve with the best response (frequency tuning) at 500 (Akin et al., 2003; Park et al., 2010; Piker et al., 2013; Rauch et al., 2004; Timmer et al., 2006). Rauch et al. reported that subjects with MD exhibit a different cVEMP tuning pattern from healthy subjects and propose that dilatation of the saccule due to hydrops could raise its resonant frequency, and make it more sensitive to higher frequencies thus alters VEMP tuning curves. In their study, subjects with unilateral MD exhibited a higher threshold at all frequencies, with greater response at 1000 Hz than 500 Hz on the affected side, while the healthy subjects showed best cVEMP response at around 500 Hz. The unaffected ear of MD subjects also showed alterations in cVEMP tuning and a threshold shift compared to normal subjects; however, the reason was not identified. They suggested that an altered cVEMP tuning pattern could be a marker of saccular endolymphatic hydrops and hence it is believed that altered tuning in the unaffected ear may indicate early signs of the development of bilateral MD.

A common issue with cVEMP threshold measurements is that the waveforms are analysed subjectively. In the previous study described in Chapter 5, there was significant variability between experienced raters in their subjective identification of cVEMP response thresholds, which is consistent with effects seen for ABR threshold

estimation (Vidler & Parker, 2004). Hence, in a previous work (Experiment 4 described in Chapter 5), an objective analytical approach using the HT^2 test was introduced for the automated response detection of VEMPs. The results showed that it was faster and more sensitive than subjective analysis, so it presents a promising approach for the automated threshold estimation of VEMP tuning curves. No previous study has used objective methods to measure the saccular frequency tuning curves.

This work was motivated by the need for an objective estimation of the frequency tuning curve of the saccule. This study aimed to explore the applications of the statistical method (i.e., the HT^2 test) in measuring the saccular tuning curve in patients with MD. This study also aimed to evaluate the sensitivity and specificity of cVEMP and ECochG in the diagnosis of MD compared to a clinical diagnosis based on the 1995 AAO-HNS criteria, which is here considered a gold standard for the purpose of the calculation of sensitivity and specificity. In addition, the degree of agreement between the two objective tests was assessed.

Research questions:

- How do objective VEMP frequency-tuning curves (threshold, amplitude, and latency as a function of frequency) of the saccule compare between normal hearing subjects and patients with MD?

- What is the diagnostic power of ECochG and cVEMP in the diagnosis of MD, compared to the diagnosis based on the AAO-HNS criteria (hypothetical "gold standard")? What is the degree of agreement between the two diagnostic tests?

6.2 Methods

Ethics Committees of Jordan-Amman Hearing and Balance Centre and the University of Southampton (ERGO: 26674 for healthy subjects and ERGO 31024 for patients with MD) approved the experimental protocol for this study. The present study was performed using human subjects with normal hearing and vestibular function, and

patients with MD. All subjects signed an informed consent form for participation in the present study.

Note: The first part of this study (normative data on cVEMP) was carried out on normal subjects in the United Kingdom. However, recruiting patients was found to be challenging within the time scale of the PhD. Therefore, the second part of this work was done on patients with MD at the Middle East Hearing and Balance Centre in Jordan-Amman. Although the cVEMPs were carried out in different places, the equipment used with both control and study groups for cVEMP recording was the same. For ECochG recording, normative data were established from normal adults in Jordan in order to use same equipment on patients with MD.

6.2.1 Study population

Control group (normal subjects)

To obtain normative data of cVEMP recording, responses were recorded from 20 otologically normal subjects (20 ears), with a mean age of 30 years (range 20-45), who were fellow students and staff from the University of Southampton, and also their friends. Eleven men and nine women participated as normal subjects. In the current experiment, responses were recorded from the left SCM muscles on all normal subjects.

Normative data of ECochG were established in 20 normal healthy adults (40 ears), with a mean age of 35 years (range 18-40), of whom 9 were male and 11 were females, who were staff members of the Middle East Hearing and Balance Centre in Jordan-Amman, and their associates.

Details regarding the inclusion/exclusion criteria used to recruit healthy subjects in this experiment are identical to those described in the previous experiments, see section 3.2.1 in Chapter 3.

Study group (Ménière's patients)

The clinical test group with MD consisted of 15 patients (nine women and six men), with a mean age of 40 years (range 18-60), who had been diagnosed with definite MD according to the1995 AAO-HNS. Both ears were tested (30 ears). Based on these guidelines, both ears (n=30) of the 15 patients were symptomatic, but,

unexpectedly, left ears were more affected by the disease than the right ears. Testing was conducted at the Middle East Hearing and Balance Centre in Jordan-Amman. Patients had stronger subjective symptoms (tinnitus and ear fullness) and more severe hearing deterioration in the left ears, while they had either tinnitus or aural fullness and less severe hearing loss in the right ears. In line with the AAO-HNS guidelines, AC pure tone averages at 0.5, 1, 2, and 3 KHz frequencies were used to classify the stage of the hearing level. The worst audiometric results during the 6-month period prior to treatment were used for stage classification. Based on these guidelines, left ears were classified as stage 3, while right ears were classified as stages 1 and 2. As the progression of the disease in each ear was different, left and right MD ears were sub-classified into 'most' and 'least' affected ears respectively, according to the severity of the subjective symptoms (ear fullness, tinnitus) and the stage of hearing level. Some of the patients had been counselled to adopt a low sodium diet and to avoid caffeine, and some started diuretic drugs, to reduce natural deterioration of hearing function, as recommended by a study for Santos et al. (1993). Some of the patients were also undergoing laser treatment (Tinnitool-ear laser tinnitus treatment). Patients who were determined to have middle or external ear pathology, neck/back stiffness or pain, or allergy to alcohol swabs were excluded from the study. In addition, patients who had undergone surgical treatment were also excluded.

Table 6.1: Diagnostic criteria for definite MD (AAO-HNS, 1995).

1. Two or more definitive spontaneous episodes of vertigo ≥ 20 min
2. Audiometrically documented hearing loss on at least one occasion
3. Tinnitus or aural fullness in the treated ear
4. Other causes excluded

Table 6.2: Stages of hearing level proposed by AAO-HNS measured by pure tone average at frequencies of 0.5, 1, 2, and 3 KHz.

Stage of hearing level	Four-tone average
1	<26 dB
2	26-40 dB
3	41-70 dB
4	>70 dB

Sample size

Based on Piface (Lenth, 2009) power and sample size software, a One-way ANOVA indicated that this study would require a minimum of 18 normal subjects and 18 MD patients to obtain a power above 80 %. The required sample size (N = 18) was determined using data from a previous study on measuring the saccular tuning curve for normal subjects and patients with MD (Rauch et al., 2004), assuming that the mean difference in cVEMP thresholds of tone-burst stimuli between normal and Ménière's subjects at 500 tone-burst is around 12 dB p.e. SPL and the SD at 500 Hz tone-burst is about 5 dB p.e. SPL. The One-way ANOVA indicated that for 86 % power, a minimum of 18 subjects in both groups are needed to detect a significant result (alpha=0.01).

6.2.2 Stimuli

cVEMP recording

Tone-burst (250, 375, 500, 750, and 1000 Hz) stimuli with a one-cycle rise and fall and a two-cycle plateau were presented using insert earphones (Etymotic ER-3A). The number of cycles in the stimulus was kept constant, so that the spectral spread as a function of centre frequency was constant, and stimulus duration changed with frequency from 16 ms at 250 Hz to 4 ms at 1000 Hz. Each recording consisted of 150 epochs. The rate of stimulus presentation of 10 Hz was fixed for all measurements, so total duration for each recording was 15 s. The rate of 10 Hz was found to be the optimal trade-off between recording time and response detection for the majority of subjects in the previous study (Chapter 4). cVEMP thresholds were determined at five frequencies using 3 dB steps from 106 to 85 dB LAS (A-weighted

sound level with a slow time constant) (125 to 105 LLpK (peak): true peak level of the input signal in dB)). Levels in dB LEQ (A) (A-weighted equivalent continuous sound level) gave almost identical results. Table 6.3 shows the stimulus levels used in the present study in different units, for all frequencies. The order of presentation of stimulus intensities was randomised among subjects. To avoid muscular fatigue, a one-minute break was given to each subject after each recording. The equipment setup used with subjects to conduct cVEMP recording was identical to that used in Experiment 1, as described in section 3.2.3.

Table 6.3: The stimulus levels used in this study, in dB LAS, dB LAeq, and LLpK (peak), for all frequencies (250, 375, 500, 750, and 1000 Hz).

LAS (SPL)	LAeq (SPL)	LLpK (peak)
106	106	125
103	103	122
100	100	119
97	97	116
94	94	113
91	91	110
88	88	107
85	85	104

ET ECochG recording

The stimulus was a 100 μs broadband click generated using the Vivosonic Integrity™ V500, and delivered at intensity of 120 dB p.e. SPL (90 dB nHL) at a rate of 11.3 times per second. 1000 responses were collected in each run with an alternating polarity (500 rarefaction and 500 condensation), so total duration for each recording was 2 minutes (1000 repeats/11.3 Hz). Signals were filtered with 3000 Hz low-pass and 5 Hz high-pass filters. A sampling rate of 34 KHz was used and 50 K amplification. All subjects had bilateral recordings, and this was repeated in each ear. Of the repeats, the test run with the largest AP amplitude was chosen for analysis. Recordings were conducted in a sound-attenuated room.

6.2.3 Procedure

6.2.3.1 cVEMP recording

All procedures used with participants in this experiment to conduct cVEMP measurements were identical to those used in Experiment 1, as described in section 3.2.4.

6.2.3.2 ET ECochG recording

ECochG was recorded using an ET electrode. After otoscopic examination, the tested ear canal was irrigated with 0.9% saline solution and dried. The ET-Wick electrode (Sanibel) was inserted in the external auditory meatus and placed under otomicroscopy on the tympanic membrane. The foam rubber tip of the ER-3A insert phone was placed and helped to secure the ET-Wick electrode in place. Recording of ECochG was performed using the ET-Wick electrode as reference. An active electrode was placed on the earlobe of the non-test ear, and the ground (common) electrode was placed on the lower forehead. The impedance of the electrodes was maintained below 5 KΩ.

6.2.4 Response analysis

6.2.4.1 cVEMP

The presence of the responses was objectively detected using a cut-off p-value of 0.05 (FPR of 5 %) obtained from a one-sample HT^2 test on the array of cVEMP data. For more detailed of the method see section 5.2.5 in Chapter 5.

6.2.4.2 ET ECochG

A 10 ms analysis time window was used. Two runs were performed for each ear, so the test run with the largest AP amplitude in each ear is chosen for data analysis. As shown in Figure 6.1, the stimulus baseline, SP, and AP peaks were visually determined, and the SP/AP amplitude ratio was analysed and reported as a percentage. The amplitudes of SP and AP were measured with reference to the stimulus baseline. The SP amplitude was calculated from the stimulus onset (defined as baseline start) to the first peak, while AP amplitude was measured from the onset of the stimulus to its first peak, in the way described by Ferraro (2000). The AP

latency measured from stimulus onset to the AP peak should be identical to the latency of wave 1 in ABR, with normal range between 1.3 and 1.7 ms (Ferraro, 2000). Unfortunately, the SP/AP area under the curve ratio measurement was not available on the equipment used for the study. Hence SP/AP amplitude ratio was the primary ECochG parameter used in the analysis.

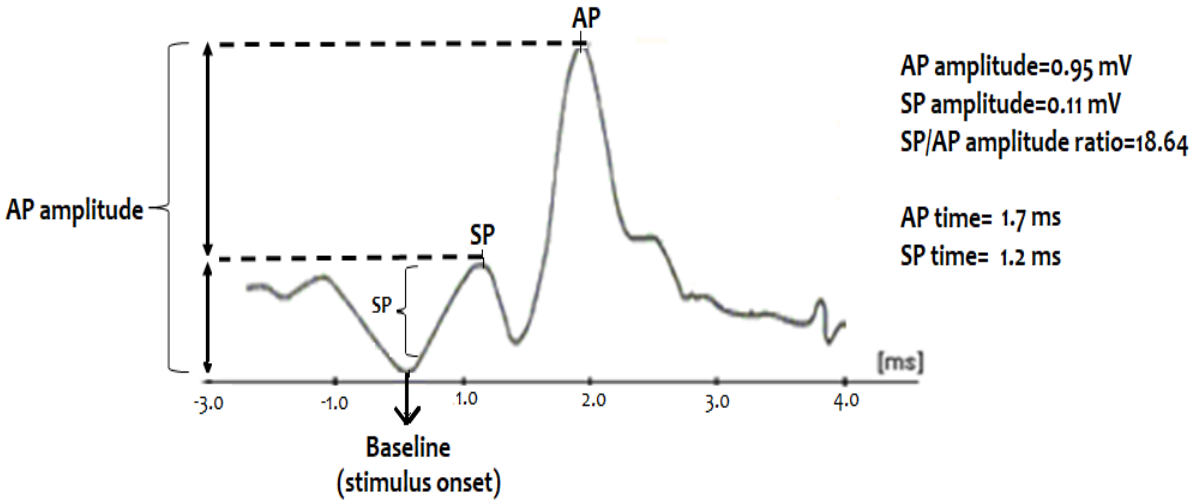

Figure 6.1: An example of normal ET ECochG tracing at 90 dB nHL using click sound. The ECochG is analysed by comparing the amplitude of the SP to the AP. The baseline is used as a reference point for SP and AP amplitude measurements.

6.2.5 Pilot study

Piloting was performed to determine if cVEMP responses could be obtained for different tone-bursts, using the maximum allowable stimulus level in this study (106 dB LAS). At the beginning, the cVEMP was conducted using 250, 375, 500, 750, 1000, 2000, and 4000 Hz 1:2:1 (cycles) tone-bursts for four otologically normal subjects. A p-value produced by the HT^2 test was used to determine the presence of a response. From piloting, it was found that the cVEMP responses were present for all tone-bursts except 2 and 4 KHz, in all four normal subjects. It seems likely that cVEMP thresholds at 2 and 4 KHz tone-bursts are higher than the maximum allowable stimulus level used in this study (106 dB LAS). Thus, recording cVEMP at levels higher than 106 dB LAS to obtain responses at higher frequencies such as 2 and 4 KHz could not be performed. Therefore, these two frequencies were not

included in the final study. Piloting was also performed to determine the applicability of recording ECochG with the ET-Wick electrode. ECochG recording was performed on two healthy subjects, and a sharp AP and SP peak and a smooth baseline were observed in the tracing.

6.3 Results

6.3.1 Objective estimate of cVEMP tuning curve

6.3.1.1 Threshold

Figure 6.2 shows the cVEMP thresholds (in dB LAS) for normal subjects' ears (n=20), and the 'most' (n=15) and 'least' (n=15) affected ears of bilaterally affected patients with MD, which were objectively detected by the HT^2 test for a significance level of α =5 % (150 sweeps) as a function of stimulus frequency. In the control group, it was found that cVEMPs were present for all tone-burst stimuli at different stimulus levels except 250 Hz. Ten otologically normal subjects did not have cVEMP responses to 250 Hz tone-bursts. Thus, the threshold values of recordings that did not show a significant response on the HT^2 test were set to 109 (3 dB step above the maximum level used in this study) for the analysis of threshold values. In the study group, 15 patients with bilateral MD were identified, so a total of 15 right MD ears and 15 left MD ears were affected by the disease. Out of 30 ears, 20, 13, 8, 10, and 6 ears showed absence of cVEMP waves at 250, 375, 500, 750, and 1000 Hz frequencies, respectively. The threshold values of recordings that did not show a significant response on the HT^2 test were set to 109 dB LAS for the analysis of threshold values (If the stimulus intensity had been increased, then it may have been possible to evoke responses. However, this was not done in the current study due to concerns over acceptable noise exposure for subjects).

Across normal subjects (control group), there was some variation in individual thresholds and the pattern of the saccular tuning curve varied a little, but in general it was U shaped with the best frequency response between 375 and 500 Hz, on average. The Friedman test showed a statistically significant difference in cVEMP thresholds between frequencies ($P < 0.001$). The Wilcoxon signed-rank testing

revealed that the frequency of 500 Hz produced a significantly lower cVEMP threshold than all of the other frequencies ($p < 0.05$), except 375 Hz.

Compared to normal ears (n=20), the Mann-Whitney test showed that the 'most' affected MD ears (n=15) had a significant threshold increase for all tone-bursts (250-1000 Hz). The saccular tuning curve for the 'most' affected ears with MD no longer clearly indicated its best frequency response at 500 Hz, showing that the 500 Hz frequency tuning had either been lost or shifted. The Mann-Whitney test also showed that the MD 'least' affected ears had a significant threshold increase for tone-bursts of 375-1000 Hz, compared to normal subjects. For MD 'most' and 'least' affected ears, Wilcoxon signed-rank testing showed that MD 'most' affected ears produced significantly higher cVEMP threshold at 500 and 750 Hz tone-bursts compared to MD 'least' affected ears. The MD 'least' affected ears had less shift in the cVEMP threshold than the MD 'most' affected ears. The frequency tuning in the MD 'least' affected ears was not changed, suggesting that the 500 Hz frequency tuning had not been either lost or shifted. The largest difference between the groups was seen at 500 Hz (see p-values of multiple comparison between ears in Table 6.4). It appears that 500 Hz is the best frequency to differentiate normal ears from MD affected ears, based on threshold alone.

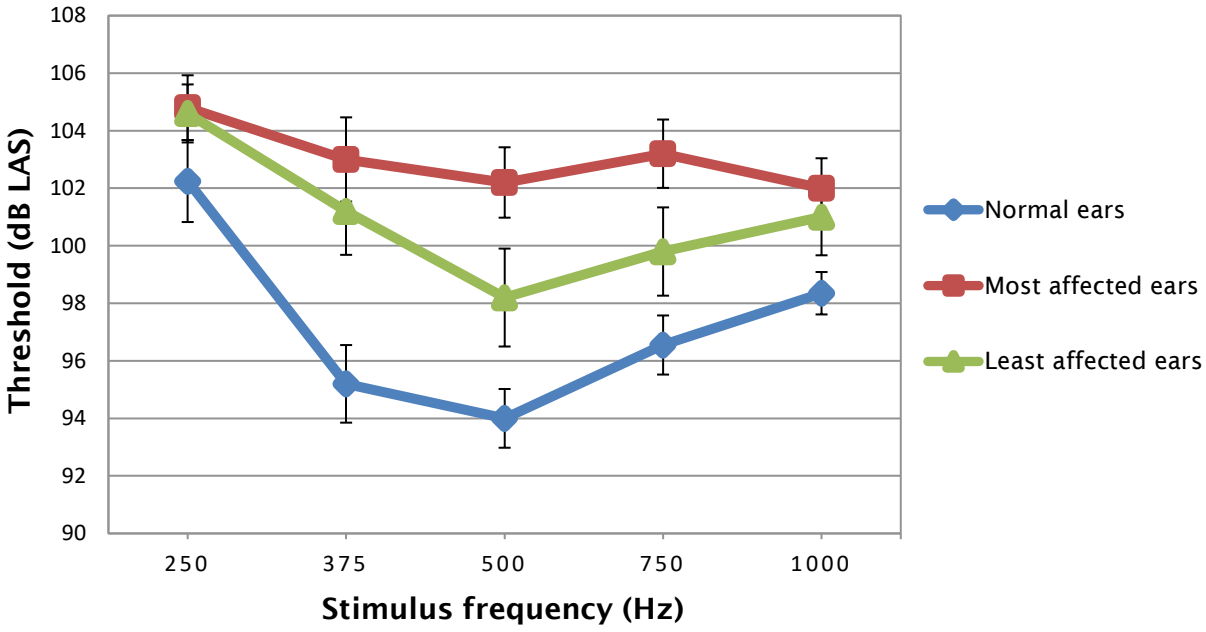

Figure 6.2: Mean cVEMP thresholds at different tone-burst stimuli for 'most' and 'least' affected ears of bilaterally affected patients with MD (n=30), and for normal subjects' ears (n=20). The error bars indicate ±1 SE of the mean. For the analysis, the threshold value of recordings that did not show a significant response on the HT^2 test was set to 109 (3 dB step above the maximum stimulation level used in this study).

Table 6.4: P-values of multiple comparisons (using the Wilcoxon signed-rank test) of cVEMP threshold at different frequencies between normal ears and MD 'most' and 'least' affected ears, and between 'most' and 'least' affected ears. The highlighted p-values indicate significant results.

Comparisons	250 Hz	375 Hz	500 Hz	750 Hz	1000 Hz
Normal / MD 'most' affected ears	0.364	**0.001**	**0.000**	**0.001**	**0.006**
Normal / MD 'least' affected ears	0.441	**0.010**	**0.043**	0.096	0.068
MD 'least' / 'most' affected ears	0.493	0.199	**0.007**	**0.012**	0.298

6.3.1.2 Peak-to-peak amplitudes

Figure 6.3 shows the peak-to-peak amplitudes (p13-n23) of cVEMP at 103 dB LAS as a function of tone-burst stimuli for normal subjects' ears (n=20), MD 'most' affected (n=15) ears and MD 'least' affected (n=15) ears. The amplitude values of the cVEMP recordings that showed a significant p-value on the HT^2 test at 103 dB LAS were subjectively measured from plots of response waveforms, using MATLAB software.

Across normal subjects (control group), a Friedman test showed that there was a significant effect of frequency on the peak-to-peak amplitude [χ^2 (4) = 29.519, p <0.000]. Wilcoxon signed-rank testing was conducted for multiple comparisons and revealed that the frequency of 250 Hz produced significantly lower cVEMP amplitude than all of the other frequencies.

Compared with normal subjects, the Mann-Whitney test revealed that the MD 'most' and 'least' affected ears showed significantly lower amplitudes for tone-bursts (375-1000 Hz), with more differences in the MD 'most' affected ears than in the 'least' affected ears (see p-values of multiple comparison between ears in Table 6.5 below). For MD 'most' and least affected ears, the Wilcoxon signed-rank test showed that the frequency of 250 Hz produced significantly lower cVEMP amplitude than the 500, 750, and 1000 Hz frequencies. On average, normal ears showed the largest cVEMP amplitude at 500 Hz tone-burst; however, there was insufficient power to detect the statistical significance of the difference between 500 Hz and the other remaining frequencies. The MD 'most' affected ears showed loss or shift of frequency tuning in comparison with normal subjects.

Both amplitude and threshold measures showed cVEMP frequency tuning, with the strongest response at 500 Hz. Similarly, both measures showed impairment of both ears for bilaterally MD patients, with more alteration in the most-affected ear. However, cVEMP amplitudes showed more variance than threshold measurements, and thus threshold appears to be the best measure to use to discriminate the groups.

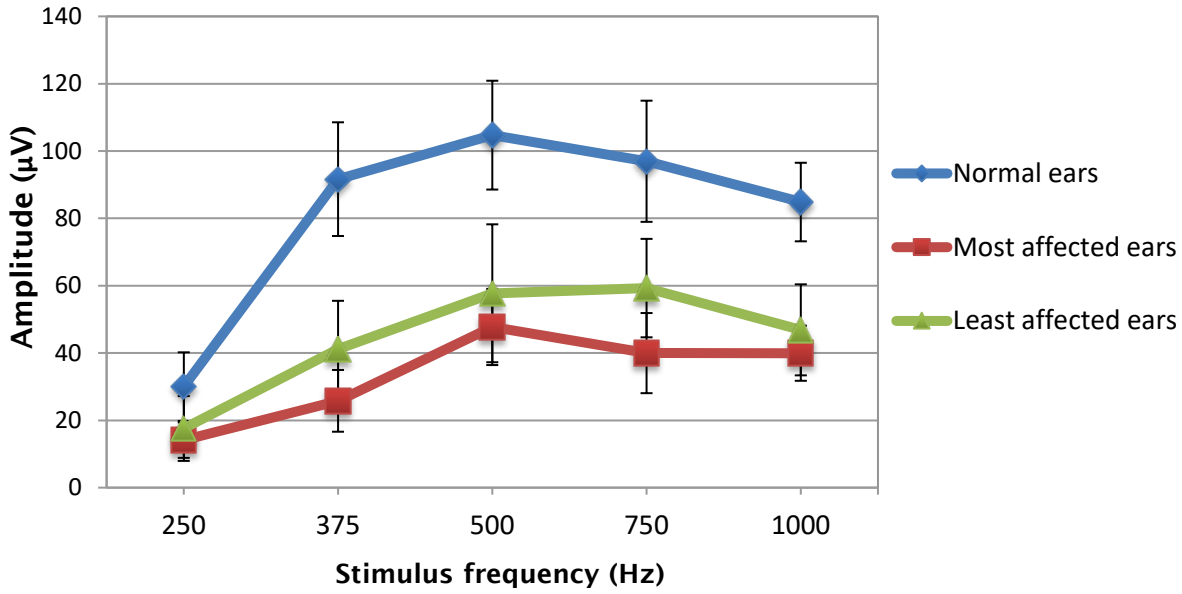

Figure 6.3: Mean peak-to-peak amplitude of cVEMP as a function of tone-burst frequencies for normal subjects (n=20), and for 'most' and 'least' affected ears of bilaterally affected patients with MD (n=30) at 103 dB LAS (122 LLpK (peak)). Error bars represent ±1 SE of the mean. The amplitude values of recordings that did not show a significant response on the HT^2 test at 103 dB LAS were set to zero for the analysis of amplitude values (if there is no response indicated in the data then there should be no measurable amplitude; hence it was set to zero).

Table 6.5: P-values of multiple comparisons (using the Wilcoxon signed-rank test) of cVEMP amplitude at different frequencies between normal ears and MD 'most' and 'least' affected ears, and between 'most' and 'least' affected ears. The highlighted p-values indicate significant results.

Comparisons	250 Hz	375 Hz	500 Hz	750 Hz	1000 Hz
Normal / MD 'most' affected ears	0.323	**0.000**	**0.007**	**0.012**	**0.008**
Normal / MD 'least' affected ears	0.274	**0.007**	**0.020**	0.148	**0.006**
MD 'least' / 'most' affected ears	0.753	0.333	0.508	0.114	0.875

6.3.1.3 Latency

The mean cVEMP latencies of p13 and n23 for normal ears (n=20), MD 'most' affected (n=15) and MD 'least' affected (n=15) ears as a function of stimulus frequency are plotted in Figure 6.4. Generally, the latencies of p13 and n23 of cVEMP decreased with increasing stimulus frequency for all groups, and this could be attributed to shortening of the rise time of tone-burst stimuli. There was no significant difference in latencies for any tone-burst stimulus in any of the three groups (normal ears, MD 'most' affected, and MD 'least' affected ears) as shown by the Wilcoxon signed-rank test ($p>0.05$).

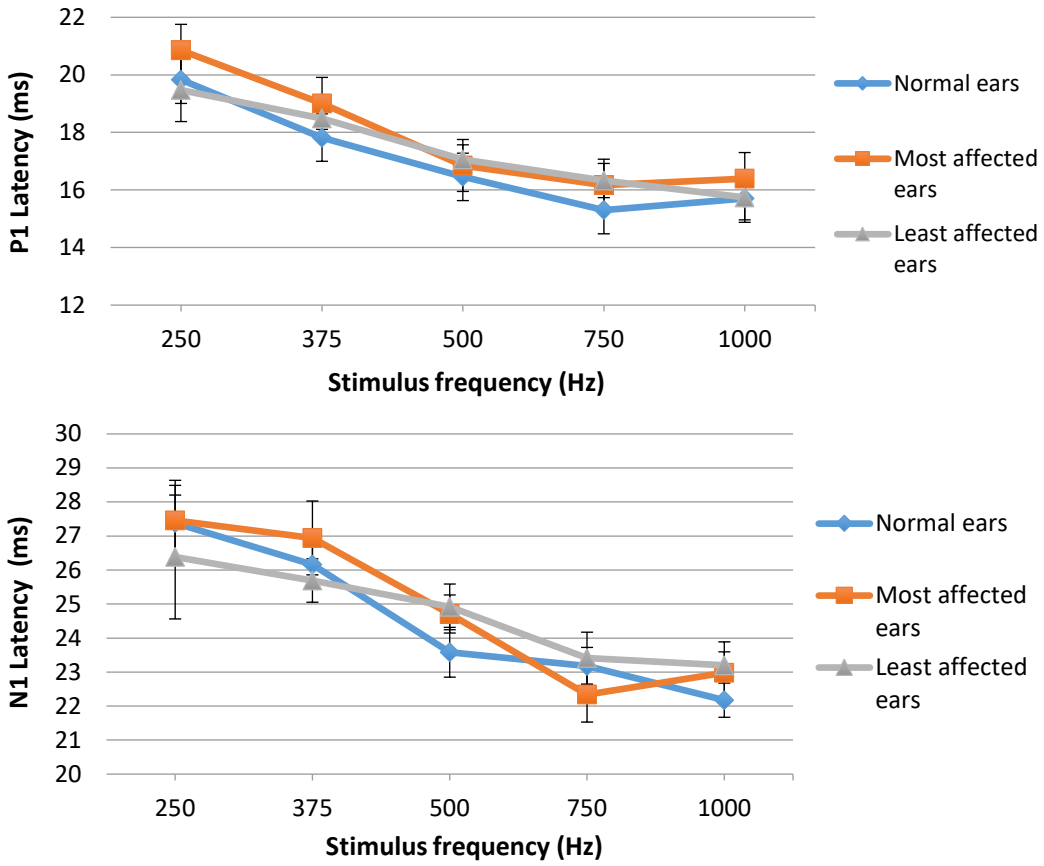

Figure 6.4: Mean of cVEMP latencies of p13 and n23 as a function of tone-burst stimuli for normal subjects (n=20), and for 'most' and 'least' affected ears of bilaterally affected patients with MD (n=15) at 103 dB LAS (122 LLpK (peak)). Error bars indicate ±1 SE of the mean. The latency values of recordings that did not show a significant response on the HT^2 test at 103 dB LAS were not included in the analysis of the latency values.

Table 6.6 below shows the response rate and means, with 95 % confidence intervals of the mean (mean ± 1.96×SD) of p1-n1 of threshold (dB LAS), amplitude (μV), and latencies p1-n1 (ms) of cVEMP at different tone-burst frequencies in the control group. Test results exceeding the 95 % confidence intervals of the mean for cervical VEMP parameters of the normative data (threshold, amplitude, and peak latencies) were considered abnormal in the study group.

Table 6.6: Normative cVEMP data: detection rate, and the means (95 % confidence intervals of the mean: mean ± 1.96×SD) of P1-N1 Amplitude (μV), latencies P1-N1, and threshold (dB LAS) of cVEMP at different tone-burst frequencies in healthy subjects.

cVEMP parameters	250 Hz	375 Hz	500 Hz	750 Hz	1000 Hz
Detection rate	50 % (10/20)	100 % (20/20)	100 % (20/20)	100 % (20/20)	100 % (20/20)
p1-n1 amplitude (μV) at 103 dB LAS	30 (-59.2 to 119.2)	91.6 (-56.4 to 239.7)	104.7 (-36.9 to246.4)	96.9 (-60.7 to 254.7)	84.8 (-17.4 to 187.1)
P1 latency (ms) at 103 dB LAS	19.8 (13.6 to 26)	17.8 (13.5 to 22)	16.4 (13 to 19.8)	15.3 (12 to 18.5)	15.6 (10.7 to 20.6)
N1 latency (ms) at 103 dB LAS	27.3 (20.1 to 34.5)	26.1 (21 to 31.2)	23.5 (17.1 to 29.9)	23.1 (18.5 to 27.8)	22.1 (17.7 to 26.5)
Threshold (dB LAS)	102.2 (89.7 to114.7)	95.2 (83 to 107)	94 (85 to 102.9)	96.5 (87.5 to 105.5)	98.3 (91.8 to 105.8)

6.3.2 ECochG in normal subjects and study group

This section firstly presents the normative data for SP/AP amplitude ratio that were established in 20 normal hearing adults (40 ears) who did not have symptoms of MD based on the 1995 AAO-HNS criteria. The SP/AP amplitude ratio between normal ears and MD affected ears is then compared.

For the control group (n=20), all 40 ears showed AP and SP. There was no significant difference in the SP/AP amplitude ratio between right and left ear, based on the repeated measures t-test ($p>0.05$), so data from both ears were combined. Mean SP/AP amplitude ratio was 0.205±0.074 (mean ± SD) for 40 ears. In this study, the variability in the SP/AP amplitude ratio was low, and thus the 95 % cut-off value (mean +1.96×SD) was 0.35 (see Figure 6.5). In the clinical test populations, ECochG results exceeding the 95 % range of SP/AP amplitude ratio were considered abnormal. For patients with MD (n=30 ears), the SP/AP amplitude ratio for MD affected ears (n=30) was 0.43 (SD=0.166): for 'most' affected ears (n=15) it was 0.51 (SD=0.164) and for the 'least' affected ears (n=15) was 0.34 (SD=0.121) (Figure 6.5). An increased value of SP/AP amplitude ratio is consistent with endolymphatic hydrops (MD). An example of normal SP/AP amplitude ratio and enlarged SP/AP amplitude ratio in an endolymphatic ear from a patient with left MD is shown in Figure 6.6.

As normality was not rejected, a t-test was conducted to find out if there was a significant difference in the SP/AP amplitude ratio between normal and MD affected ears. Independent Samples Tests revealed that normal ears showed significantly lower SP/AP amplitude ratios than MD 'most' affected ears $t(33) = -7.246$, $p<0.001$, and MD 'least' affected ears $t(33) = -4.061$, $p<0.001$. A Paired Samples Test within the MD group showed that the MD 'most' affected ears had significantly higher SP/AP amplitude ratios in comparison to MD 'least' affected ears $t(14) = 3.495$, $p < 0.001$.

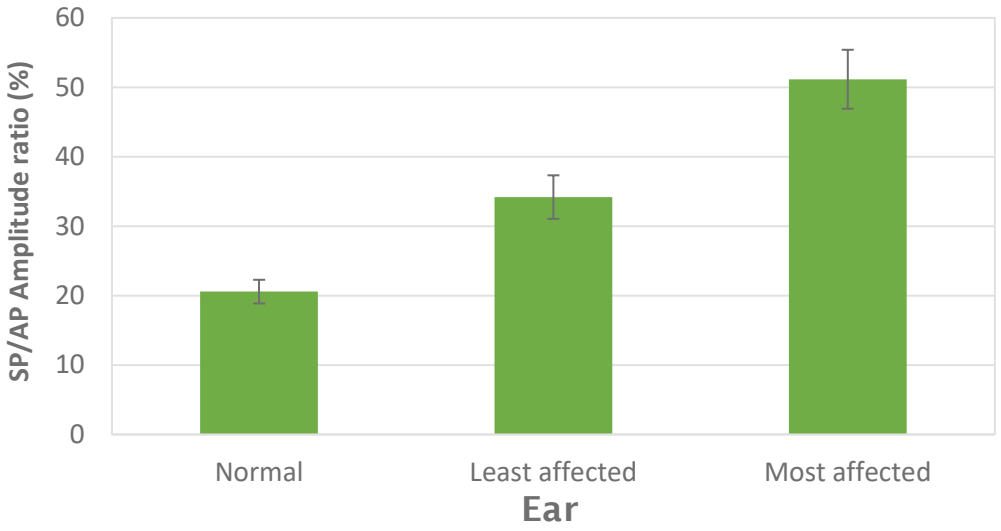

Figure 6.5: ECochG mean percentage SP/AP amplitude ratios for normal ears (n=40), and for 'least' (n=15) and 'most' (n=15) affected ears of patients with MD. Error bars represent ±1 SE of the mean.

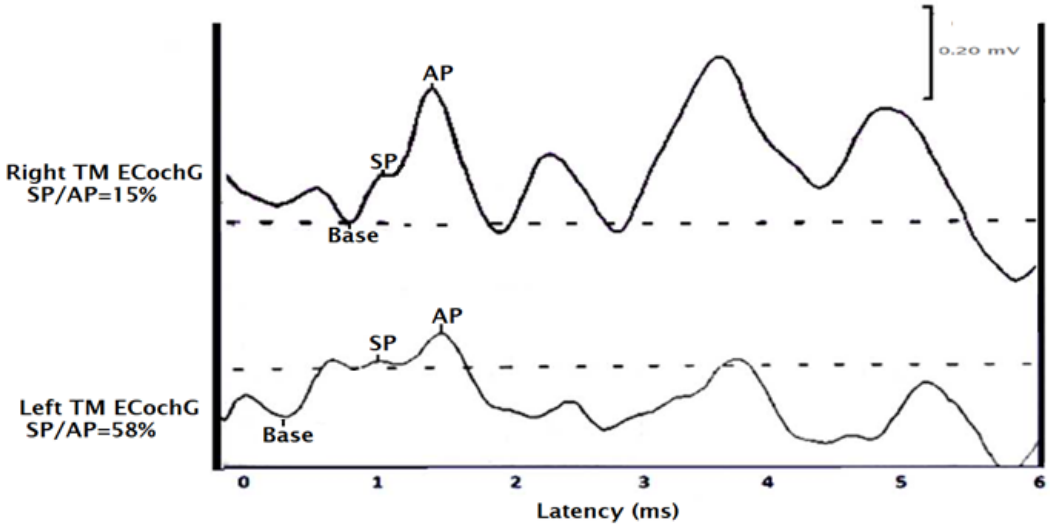

Figure 6.6: TM click ECochG recording from a patient with left MD (note the higher SP). The amplitudes of summating potential (SP) and action potential (AP) were measured with reference to the baseline value. Top trace represents normal right ear with normal SP/AP amplitude ratio and bottom trace displays endolymphatic left ear with enlarged SP/AP amplitude ratio beyond the normal cut-off criterion 35 % (0.35) in this study. Note: this figure has been scanned from a paper copy, so the resolution has reduced a little.

6.3.3 Sensitivity and specificity of ECochG and cVEMP

The diagnostic power of the two objective tests to detect the presence or absence of MD was calculated by comparing results from the MD group to the 95 % normative ranges of cVEMP threshold to 500 Hz tone-bursts (see Table 6.6) and the ECochG SP/AP amplitude ratio defined from normative data.

Based on the ECochG test, patients with any SP/AP amplitude ratio for clicks exceeding the normative data values (or the 95 % range of the SP/AP amplitude ratio), were classified as "positive", while, those with SP/AP amplitude ratio within the 95 % normative range were classified as "negative".

For cVEMP tests, patients were similarly classified as "positive" or "negative" based on threshold: those with cVEMP thresholds for 500 Hz tone-burst greater than the 95 % normative range (mean + 1.96×SD, or 102.9 dB LAS) and also those with no response at the highest stimulus level used were also considered as "positive". While, patients with cVEMP thresholds for 500 Hz tone-burst within the 95% normative range were classified as "negative". This was chosen because threshold appears to be the best measure to use to discriminate the groups, as previously reported in section 6.3.1.

Measures taken by the three diagnostic tools were compared: AAO-HNS criteria, ECochG, and cVEMP, with the AAO-HNS criteria considered as the "gold standard" for the purpose of sensitivity and specificity analysis. The diagnostic power of the two objective diagnostic tests were calculated using the formula below:

Hypothetical "gold standard" AAO-HNS criteria			
	Positive		Negative
Diagnostic test (ECochG or cVEMP)	Positive	True positive (A)	False negative (C)
	Negative	False positive (B)	True negative (D)

Sensitivity =A/ (A + C) **Specificity=D/ (D + B)**

Figure 6.7 shows the sensitivity and specificity for the presence and absence of MD in the 'most' and 'least' affected ears for ECochG (SP/AP amplitude ratio) and cVEMP threshold at 500 Hz. For both tests and both ears, the results indicated that the ability of the two objective tests to identify healthy cases was high, with specificity of 83.3 % for cVEMP and 100 % for EcochG. However, the ability of the two diagnostic tests in both ears to identify MD cases varied from low to moderate, with sensitivity of 22.2 % and 71.4 %, respectively.

The agreement between the two diagnostic tests (regarding whether the MD 'most' and 'least' affected ears were normal or abnormal) was measured by the kappa coefficient. In the Cohen's kappa (k) test, the agreement of both tests was equal to 0.69 in the MD 'most' and 0.58 in the 'least' affected ears, indicating a low and moderate agreement between the two tests' outcomes, based on the guidelines proposed by Landis & Koch (1977).

To see if the diagnostic power of cVEMP can be affected by the amplitude parameter, sensitivity and specificity were also measured based on the 95 % normative data of peak-to-peak amplitude at 500 Hz tone-burst. For MD 'most' and 'least' affected ears, the measurements indicated poor sensitivity (33.3 % and 22.2 %, respectively), and poor specificity (33 % and 16 %, respectively). Therefore, it appears that the specificity of the cVEMP is reduced using amplitude parameters, compared to threshold measures, while sensitivity is still low.

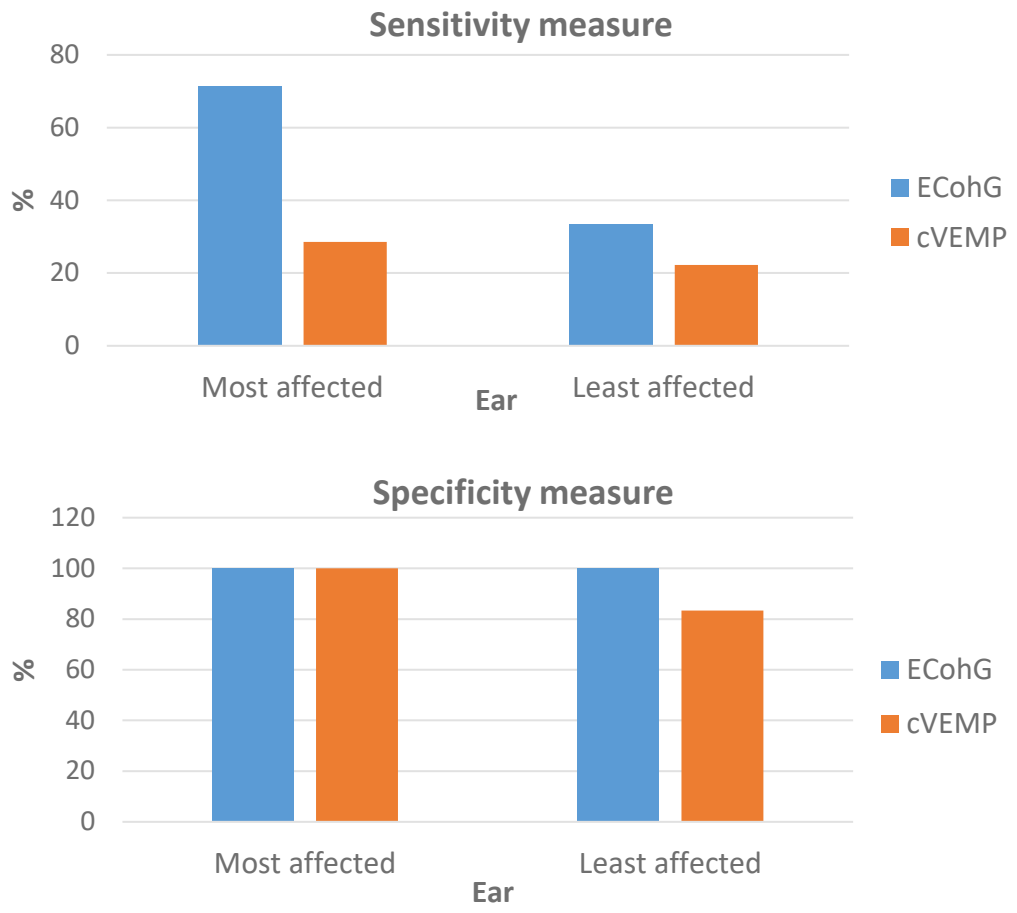

Figure 6.7: cVEMP (based on 95 % normative ranges of cVEMP threshold at 500 Hz tone-burst) and ECochG (based on 95 % range for normative SP/AP ratio) sensitivity and specificity (%) in MD 'most' and 'least' affected ears.

6.3.4 Sensitivity analysis for patients with acute symptoms on the day of testing

The symptomatic status of MD patients was examined and compared to the ECochG and cVEMP test results on the day of recording (see Tables 6.7 and 6.8). Symptoms included vertigo, tinnitus, hearing loss, or ear pressure, and all combinations of these four. At the time of testing, the 9 'most' affected and 4 'least' affected out of 30 Ménière's ears were symptomatic; symptoms included combinations of ear fullness, hearing loss, and tinnitus. Of the 9 MD 'most' affected ears, 8 ears showed elevated SP/AP amplitude ratio and 3 ears showed absent cVEMP (at 500 Hz), giving sensitivity values of 89 % and 33 % for ECochG and cVEMP, respectively. Of the 4

'least' MD affected ears, one ear showed abnormal ECochG and cVEMP, giving a sensitivity of 25 %. However, the sample of least affected ears is small, so the result for least affected ears should be treated with caution.

Table 6.7: Clinical symptoms, ECochG and cVEMP test results on the day of recording for the most affected ears of the 15 patients.

Patient no	ECochG	cVEMP	Symptoms during testing
1	abnormal	normal	Yes
2	normal	normal	No
3	normal	normal	No
4	abnormal	normal	Yes
5	abnormal	normal	No
6	normal	normal	Yes
7	abnormal	normal	Yes
8	abnormal	normal	No
9	abnormal	abnormal	Yes
10	abnormal	abnormal	Yes
11	abnormal	normal	Yes
12	abnormal	abnormal	Yes
13	normal	normal	No
14	normal	normal	No
15	abnormal	normal	Yes

Table 6.8: Clinical symptoms, ECochG and cVEMP test results on the day of recording for the least affected ears of the 15 patients.

Patient no	ECochG	cVEMP	Symptoms during testing
1	normal	normal	No
2	normal	abnormal	No
3	normal	normal	No
4	abnormal	abnormal	No
5	normal	normal	No
6	normal	normal	Yes
7	normal	normal	Yes
8	abnormal	normal	Yes
9	normal	normal	No
10	normal	normal	No
11	normal	normal	No
12	normal	abnormal	Yes
13	normal	normal	No
14	normal	normal	No
15	normal	normal	No

6.4 Discussion

This work has established that cVEMP tuning curves can be estimated objectively using statistical approaches, which is a novel contribution of the present study. This work also aimed to measure the diagnostic power of two objective tests (cVEMP and ECochG) in the diagnosis of MD and compare it to a diagnosis based on the AAO-HNS criteria.

Surprisingly, in the present study, the left ears in all 15 patients were more affected than the right ears were. It is unlikely that the probability of detecting patients with MD in all 15 left ears is a random result: if MD were equally prevalent in the left and right ears, there would be only a 1 in 16,384 chance that the threshold would be

higher in the left ear of all subjects. At present, there is no evidence for lesion preferences in MD in one ear or the other. It is not certain that this feature was unusual in the test population recruited in the study. However, a retrospective study conducted by Devaiah et al. (2003) on eight patients with possible MD as defined by the AAO-HNS criteria found that the left ears were symptomatic in seven of the eight patients, which suggests a higher prevalence on the left side.

6.4.1 Objective estimate of VEMP tuning curves

Objective estimate of the saccular tuning curve showed cVEMP frequency tuning with lowest thresholds at 500 Hz, on average, which is broadly consistent with the subjective estimate of response tuning found in previous studies (Akin et al., 2003; Park et al., 2010; Piker et al., 2013; Rauch et al., 2004). It appears that objective detection methods can be used to measure saccular tuning curves (which has not been previously reported in the literature). cVEMP tuning curves that were affected by the presence of MD showed flatter tuning than those in the control group. While other studies reported that ears with Ménière's exhibited a shift upward from 500 to 1000 Hz with a rise in the thresholds of all frequencies (e.g. Rauch et al., 2004; Timmer et al., 2006). In previous tuning studies, tuning curves were measured using quite high-level sounds, especially when testing frequencies away from the minimum of the saccular tuning curves. A concern of using such high stimulus levels is noise exposure, especially in patients with tinnitus. In the current study, out of 30 ears, 20, 13, 8, 10, and 6 ears showed absence of cVEMP waves at 250, 375, 500, 750, and 1000 Hz frequencies, respectively. If I the stimulus intensity had been increased then it may have been possible to measure more responses at high frequencies. However, this was not done in the current study due to concerns over acceptable noise exposure for subjects.

The focus of this work was on measurement of the cVEMP threshold. Such threshold measurements have been used in several previous studies as a diagnostic indicator: for example, Rauch et al. (2004) reported that patients with unilateral Meniere's disease had significantly increased cVEMP thresholds in the affected ears compared to unaffected ears or normal ears. Streubel et al. (2001) found that for patients with superior canal dehiscence syndrome, the cVEMP threshold from the affected side was significantly lower than that for unaffected ears. However, an alternative

diagnostic approach is to measure amplitude asymmetries between the right and left sides (e.g. Kingma and Wit, 2011).

6.4.2 ET ECochG

The ECochG testing used in this study was ET, which, based on the literature (Lamounier et al., 2014), is an effective and non-invasive measure used to identify cochlear hydrops. In this research, the only parameter considered was the SP/AP amplitude ratio, because of the simplicity of applying it to the types of equipment that were available. Normative data for the SP/AP amplitude ratio were established in 20 normal hearing subjects (40 ears) who had no hearing or balance problems. The variability in the SP/AP amplitude ratio was low among the healthy subjects; thus, the 95 % cut-off value of the ET electrode was 0.35. This finding was consistent with the data reported in Grasel et al. (2017) and Pou et al. (1996). Other previous studies found somewhat higher ratios, between 0.40 and 0.45 (Al-Momani et al., 2009; Margolis et al., 1995). This difference in SP/AP ratio cut-off values between studies probably results from differences in the method to determine baseline, as well as the AP and SP peaks in ECochG data. Roland and Roth (1997) compared the SP/AP amplitude ratios calculated from the same ECochG tracings by 10 audiologists. The inter-interpreter difference between SP/AP amplitude ratios was found to be significant. Thus, ECochG is vulnerable to subjective bias, which cannot be completely avoided, although an area for future research may be to apply objective (statistical) measures to ECochG.

Patients with MD showed a significant increase in the SP/AP amplitude ratio compared with the control subjects, and the increases were highest in the MD most affected ears. It is widely accepted that the elevation in SP amplitude relative to AP amplitude is a positive indicator of endolymphatic hydrops in patients with suspected MD, consistent with the notion that SP is enlarged in ears with hydrops due to the distention of the BM toward the scala tympani (Ferraro & Tibbils, 1999).

6.4.3 Sensitivity and specificity of ECochG and cVEMP

In the identified MD cases, both cVEMP and ECochG showed high specificity of 83.3 % and 100 %, respectively, and low to moderate sensitivity at 22.2 % and 71.4 %,

respectively. This result is consistent with previous findings in the literature, indicating a good ability for cVEMP and ECochG to correctly rule out the disease (Lamounier et al., 2017). The sensitivity of cVEMP in the most and least affected ears was 29 % and 22.2 %, respectively. This was lower than that found in previous studies, which ranged between 40 % and 63.6 % (de Waele et al., 1999; Lamounier et al., 2017; Young et al., 2003), although they did not use objective methods to define thresholds. The sensitivity of ECochG was 71.4 % in the most affected ears and 33 % in the least affected ears. Previous findings ranged between 57 % and 71 % (Lamounier et al., 2017), so the results of the present study were in the upper range that has been reported previously. The threshold of SP/AP amplitude ratio used to define abnormality varies in the literature, which leads to variation in the sensitivity and specificity of the ECochG test in the diagnosis of MD. When the cut-off value of the abnormal SP/AP ratio is increased, the specificity of ECochG in identifying endolymphatic hydrops is improved at the cost of sensitivity. When the SP/AP ratios are decreased, the reverse occurs (Kim et al., 2005). In this work, the cut-off limit for a defined MD was a little lower than in other studies. In this study, 4 of 15 Ménière's ears (the 'most' affected) in patients who were considered to have definite MD based on the AAO-HNS criteria showed normal SP/AP amplitude ratios in the ECochG test (28.6 % false negative rate), resulting in a sensitivity of 71.4 %.

In previous work, different ECochG and cVEMP sensitivities have been reported based on the stage of the disease. For ECochG, an elevated SP/AP ratio has been found to be maintained for a long time and even in the asymptomatic period (i.e. the time between attacks) and despite successful treatment (Kim et al., 2005; Ohashi et al., 1991). For cVEMP, the measurements vary based on the stage of MD. Although responses may disappear or remain altered during the 24 h of a Ménière's attack, they may return to normal after 48 h or with medical intervention, if the hair cells of the saccule remain undamaged (Kuo et al., 2005). In the present study, none of the patients was tested during the first 24 h of a Ménière's attack, but some patients were in the symptomatic period, experiencing vertigo, tinnitus, ear pressure, hearing loss, or combinations of these four symptoms. This finding could explain the low sensitivity of cVEMP in the diagnosis of MD, even in the most affected ears, in contrast to ECochG, which had the higher sensitivity of 71 % in the most affected ears. In general, this finding is consistent with the concept that cVEMP testing has increased sensitivity to MD in the acute phase of the disease. Compared to the least

affected ears, the sensitivity of ECochG in the most affected ears was higher, consistent with a longer duration of the disease. This is also consistent with Kim et al. (2005), who found that ears with longer disease duration and/or more severe symptoms showed a more elevated SP/AP ratio.

Although cVEMP has lower sensitivity than ECochG for MD, especially in the most affected ears, it has the significant advantage that the saccule is not affected by cochlear dysfunction, and hence can be performed in patients with significant hearing loss. For ECochG, a hearing loss greater than 40–50 dB HL reduces the amplitude of SP and AP due to cochlear hair cell damage and/or fewer cochlear hair cells and nerve fibres, resulting in distortion of the SP/AP amplitude ratio (Ferraro, 2010). Thus, ECochG is best performed in the primary stage of the disease before the hearing loss has progressed. In the present study, no patients had severe deterioration in hearing function. Although the ears that were the most affected by MD were classified as stage 3 (41-70 dB HL), there was no distortion in SP and AP components. Thus, the amplitude ratio could still be measured precisely. The potential disadvantages of VEMP testing include high sound levels in patients with tinnitus (the stimulus levels used in this study were limited compared to other studies due to safety concerns) and a long test duration (see next section 6.4.4 for further details).

The relationship between cVEMP and ECochG test results and the symptomatic status of patients with MD at the time of testing were examined. The sensitivity of ECochG was as high as 89 % in the most affected ears during a symptomatic period, which was higher than the percentage shown in the cVEMP test (33 %). Thus, when patients are symptomatic, ECochG is more sensitive to MD. However, it could be difficult to schedule patients for ECochG testing during the symptomatic period, as patients typically feel sick, fatigued and have poor concentration during this period. From the data of the present study, the clinical utility of cVEMP for the diagnosis of MD appears limited. The sensitivity of ECochG was increased in patients who had symptoms of MD on the day of testing, including a combination of ear fullness, hearing loss, and tinnitus. This finding was consistent with that of Ferraro et al. (1985), who found abnormal ECochG results in over 90 % of patients who were suffering ear pressure and hearing loss on the day of recording. These authors

considered the presence of the clinical symptoms of ear pressure and hearing loss as the strongest predictor of positive ECochG outcomes (Ferraro et al., 1985).

The agreement between the two diagnostic tests, which was measured by Cohen's kappa, was moderate for the 'most' affected ear and low for the 'least' affected ear, based on the guidelines proposed by Landis and Koch (1977). Although due to the low number of least affected ears in the MD group, this result should be treated with some caution. The agreement between the two tests was not expected to be perfect, because they evaluated different vestibular structures in the inner ear, which was suggested by Lamounier et al. (2017).

6.4.4 Measurement time of ECochG and cVEMP

In this study, the duration of the ECochG testing of each patient was between 60 and 90 minutes. In contrast, the duration of the cVEMP testing of each patient was between 90 and 150 minutes. The estimated time required for each test included taking the patient's history, preparing the patient for testing, and taking breaks between recordings. Thus, the testing time for ECochG was shorter than the cVEMP testing of all patients in this study. This discrepancy was because the cVEMP thresholds at five frequencies of both ears in each patient were measured. The patients were dissatisfied with the total duration of the testing because they became exhausted. To reduce the duration of testing, the cVEMP threshold could be measured only at 500 Hz (recording 150 epochs at a stimulation rate of 10 Hz requires 15 s), because the largest difference in threshold between the normal ears and the MD-affected ears was observed at this frequency. The testing duration could also be reduced by decreasing the intensity of the steps between recordings. In the present study, the use of 3 dB in the steps increased the test recording time. However, high sound levels and testing patients with tinnitus would still pose limitations in cVEMP testing.

6.4.5 Limitations

The present study has some limitations. First, the generalisability of the results would be improved if a greater number of patients with the same duration of the disease were recruited. Second, SP/AP area ratio could not be measured because it was not available in the equipment used for the ET ECochG recording. Thus, further

research should be conducted to assess the sensitivity of the inclusion area ratio of SP/AP and the tone-burst elicited SP in MD with ET ECochG and to compare them with the objective VEMP tuning curve over a prolonged period in patients with definite MD.

6.5 Conclusions

The cVEMP-tuning curves provided by objective detection were broadly similar to those provided by visual estimation in previous studies. In patients with MD, the tuning curve was flatter. The ears that were the most affected by MD showed higher thresholds than the ears that were the least affected by MD, which showed higher thresholds than those of the control group. Objective analytical methods appear well suited to measuring cVEMP tuning curves. However, the measurement of VEMP tuning curves using objective analytic measures to define threshold is not very sensitive to MD in patients with long-term disease. VEMP sensitivity may increase in the acute phase, but even then does not appear high. Furthermore, noise exposure and test duration are concerns in VEMP testing. The ECochG SP/AP amplitude ratio measure gives high, but not perfect, sensitivity for the diagnosis of MD. Both ECochG and VEMP testing show fair specificity for MD.

Chapter 7 : Clinical evaluation of vestibular function in unilateral cochlear implant candidates

7.1 Introduction

Because of the close anatomical connection between the cochlea and the vestibulum, it is possible that patients with severe to profound SNHL may exhibit abnormalities in balance function. A review conducted by Santos et al. (2015) showed that almost 70 % of children with SNHL have vestibular loss, and 20-40 % of them have severe bilateral vestibular dysfunction. They report that many aetiologies of SNHL are associated with balance impairment, such as Usher syndrome, meningitis, and cytomegalovirus infection. Fujimoto et al. (2015) found that 40 % of patients with idiopathic sudden SNHL also complained of vestibular loss.

CIs are widely used to initiate or restore hearing for patients who do not get benefit from conventional hearing aids (Santos et al., 2015). The effect of severe to profound hearing impairment on quality of life is well established (e.g. Tatovic et al., 2011) and the benefits of CI to improve hearing, speech perception, language production and quality of life are well documented (for example see systematic reviews on the clinical effectiveness of CI in severely-profoundly deaf children and adults such as Forli et al., 2011 and Berrettini et al., 2011). Although fitting a CI is considered a safe procedure, opening a hole in the cochlea when inserting the electrodes has potential to damage the neighbouring vestibular organs through trauma, infection, haemorrhage, vascular changes due to inserting of the electrodes, or pathologic disruption of the endolymphatic system (Santos et al., 2015). Examples of this have been confirmed by histopathological studies (Handzel et al., 2006; Tien and Linthicum, 2002) and several studies that used clinical tests pre and post-surgery, such as the vHIT (Batuecas-Caletrio et al., 2015), and VEMPs (Basta et al., 2008; Jin et al., 2008; Licameli et al., 2009; Psillas et al., 2014; Robard et al., 2015).

Recently, the risk of vestibular damage following CI surgery has received more attention because of the possibility of bilateral implantation (Wagner et al., 2010). Several studies have evaluated the incidence of vestibular damage after CI surgery (Basta et al., 2008; Jacot et al., 2009; Jin et al., 2008). In these studies, the reported incidence of vestibular function loss (change in vestibular end-organs's function as measured objectively by clinical tests) following implantation varies widely, with estimates, ranging between 23 and 100 % of patients (Robard et al., 2015). This vestibular loss seems to be not related to the aetiology of hearing loss and could come from surgical trauma or from electrical stimulation of the CI electrodes (Filipo et al., 2006). Although vestibular loss following implantation has been proven in histopathological studies (Handzel et al., 2006; Tien and Linthicum, 2002), the mechanisms to explain this damage after operation are still poorly understood (Abouzayd et al., 2016). An additional complication is that there is no correlation between the patient's vestibular symptoms measured by the dizziness handicap inventory (DHI) questionnaire and the outcome of the objective balance tests (Abouzayd et al., 2016): None of these objective tests, cVEMP, caloric or vHIT has been found to have a good correlation with a patient's balance symptoms. A possible explanation for the poor correlation between the outcome of these objective tests and a patient's subjective symptoms may be that objective testing measures end organ function, whereas dizziness will depend on whether or not central compensation for vestibular dysfunction has occurred and this may not be measured directly by objective tests (Abouzayd et al., 2016).

Another reason for poor correlation may arise from testing only part of the vestibular end organs, so other untested organs could be responsible for symptoms that are not detected by only one or a few objective balance tests (Abouzayd et al., 2016). Unfortunately, it is unlikely that the five vestibular organs can be measured with a single balance test. Consequently, it has been suggested that measuring balance function following CI surgery should involve a comprehensive assessment for all vestibular end organs (Abouzayd et al., 2016) and should include, for example, cVEMP (saccule), oVEMP (utricle), and vHIT (all three SCCs) (Maheu et al., 2017), although there is still some debate as to whether it is the type of measurement (e.g. cVEMP or oVEMP) or the stimulation method (sound or vibration) that selects whether the sacculus or utricle is being tested. Curthoys (2010) proposed that oVEMP reflects the contralateral utricular function and cVEMP reflects the ipsilateral saccular

function. Thus, oVEMP and cVEMP are independent measures that can be used to differentiate the functions of the saccular and utricular macula, not by measuring the stimulus selectivity in either of the two maculae but by measuring the responses, which are determined by the differential neural projections of the utricular and the saccular neural information conveyed to various muscle groups (Curthoys et al., 2018). In other words, the specificity of the vestibular system to acoustical and vibratory stimulation is motor-related rather than sensory-related (Curthoys, 2010). However, Todd et al. suggest that oVEMPs produced by head translations in the horizontal plane from a mini-shaker are likely to arise predominantly from the utricle (Todd et al., 2008). Thus, the origin of oVEMPs is still being debated in the literature (see Todd, 2014, for a review).

VEMP is widely used in clinical practice as an objective vestibular technique for measuring the function of otolith organs. Sound evoked cVEMP is thought to predominately represent the saccular pathway function (Rosengren et al, 2010). The saccular macula is the closest vestibular organ to the cochlea, and, consequently, it is the vestibular organ most frequently impaired by CI surgery, and also the sensor most likely to be damaged following implantation, according to histological studies (Handzel et al., 2006; Tien and Linthicum, 2002). As mentioned earlier, sacculus damage from using postoperative cVEMP testing has been reported in between 21–100 % of implanted patients (Basta et al., 2008 ; Jin et al., 2008; Krause et al., 2010; Licameli et al., 2009; Melvin et al., 2009; Robard et al., 2015; Todt et al., 2008; Xu et al., 2014). More recently, in a large systematic review and meta-analysis of 16 studies, Abouzayd et al. (2016) reported a sensitivity of 32 % for cVEMP testing to detect saccular dysfunction in symptomatic CI users. However, the 16 studies included in their review had many methodological differences, for instance, in terms of stimulus type, level of stimulation, and criteria for analysing the presence of the cVEMP response, which varied between studies.

The evaluation of the effect of CI on utricular function has received little attention in the literature. To the best of my knowledge, only two studies have measured utricular function before and after implantation, using oVEMP testing. Xu et al. (2014) reported that 82 % (18 out of 22 young patients) of children with clear AC oVEMP responses preoperatively showed utricular dysfunction in the implanted ear following surgery. Maheu et al. (2017) found that 75 % (3 out of 4) of patients showed

a complete loss of ocular VEMP responses in the implanted ear postoperatively. This postoperative loss of oVEMP responses in the implanted ears reflects the possible damage to the utricle by implantation (Xu et al., 2014).

One possible explanation for changes in the response of some objective measures of vestibular end-organ function (i.e., VEMP) could be that these are due to a change in sound transmission in the implanted ear rather than a saccular disorder (Basta et al., 2008). An association between dizziness and VEMP function might indicate that the change is not simply due to changes in the pathway of sound transmission. However, it is still debatable whether oVEMP to acoustical stimulation is a saccular or utricular response (more details are provided in Appendix C). Todd et al. (2008) argue that the vibration elicited oVEMP is primarily a measure of utricular function, so the oVEMP to vibration at 100 Hz may be a more selective test of utricular function than the oVEMP to sound stimulation. Therefore in the present study, oVEMP to vibratory stimulation was used as a test of utricular function. As the technique of oVEMP in response to mini-shaker vibration is new, normative values to define the presence or absence of a response are still sparse in literature. Thus, one component of the present study was to obtain normative data for oVEMP to vibration (mini-shaker) on a small sample of healthy human subjects, which could then be compared to clinical measurements pre and post implantation.

To date, relatively few studies have evaluated the function of both vertical and horizontal SCCs before and after implantation, using vHIT or 3D HIT. For example, Migliaccio et al. (2005), Maheu et al. (2017), and Melvin et al. (2009) tested each of the three canals. More studies have tested only specific types of canal: e.g. Basta et al. (2008) tested only the vertical canals, and others tested only the horizontal canals (Batuecas-Caletrio et al., 2015; Jutila et al. 2013; Robard et al. 2015; Thierry et al., 2015; Todt et al., 2008). Migliaccio et al. (2005) found one significant decrease in VOR gain for all three canals, using vHIT on the side of the implant for one symptomatic patient (9 % of patients in their study) following CI surgery. Melvin et al. (2009) found one patient (3.6 % of patients) with a significant drop in vestibular function in all three SCCs (from normal, to severe, to profound vestibular dysfunction on the 3D HIT). However, Maheu et al. (2017) did not report any loss of function in any patient on the vHIT and suggested that the function of SCCs is not affected by CI surgery. Basta et al. (2008) failed to find any patient with a loss of vertical canal function on the vHIT following implantation. Thus, there are conflicting findings in

the literature and more studies are required to evaluate the function of vertical canals before and after CI surgery in order to confirm the slight reduction in vertical canal function following implantation, as reported in the previous two studies (Melvin et al., 2009; Migliaccio et al., 2005). Batuecas-Caletrio et al. (2015) found a change in the horizontal SCCs in 10 out of 30 patients (30 %) on the vHIT. In the literature, the reported deterioration in the function of horizontal SCCs after CI surgery varies from 19 % (Todt et al., 2008) to 72.4 % (Robard et al., 2015). The variability could be attributed to differences in the test techniques used for recording vHIT and/or the criteria (low cut-off value) used to differentiate between normal and abnormal responses.

Up to now, to the best of my knowledge, only two recent studies have measured each of the five sensory vestibular organs separately using vHIT, and VEMPs and none used vibration elicited VEMP. Maheu et al. (2017) measured the impact of unilateral implantation on balance function using vHIT, cVEMP and oVEMP, and showed that 75 % (3 out of 4) of patients showed a complete loss of cervical and ocular VEMP responses in the implanted ear following surgery. None of the 4 patients reported a change in vHIT, which suggests that the function of SCCs was not affected by CI surgery in patients in their study. However, this study has some limitations, which could affect its validity to determine the effect of implantation on balance function. Only 4 patients were recruited in the study, and no reason for this small sample size is given in the paper. In addition, the authors did not mention any of the stimulus parameters, e.g. stimulus type, intensity, and duration, that were used to elicit cVEMP and oVEMP in their study, and the mean delay between CI surgery and the vestibular tests was not stated.

Another study by Janky and Givens (2015) measured the vestibular system postoperatively for children with CI compared to healthy children. They found that the rate of vestibular loss in the SCCs' and otolith organ's function was higher in children with CI compared with healthy children. Their result is consistent with a strong association between vestibular dysfunction and congenital deafness. In their study, vestibular function was not tested prior to implantation; therefore, the effect of CI surgery on balance function could not be separated from any pre-existing vestibular disorder. Deafness is associated with vestibular dysfunction in several aetiologies, such as Usher syndrome and malformations in the inner ear (Thierry et

al., 2015) which may cause pre-implantation vestibular disorders. Previous studies have reported that the proportion of deaf children with vestibular dysfunction ranged from 30 % to 70 % (Ito, 1998; Jacot et al., 2009; Thierry et al., 2015; Tribukait et al., 2004). Thus, in order to assess if there is an effect of CI surgery on balance function, balance testing should be performed before and after the CI surgery.

The goal of the present study was to examine the effects of CI surgery on each sensory organ of the balance system separately, in adult patients with bilateral severe to profound SNHL undergoing unilateral implantation. Specifically, the function of saccules, utricles, and the three SCCs was assessed using ACS cVEMP, BCV oVEMP with a mini-shaker, and vHIT, respectively in each ear, both pre- and post-operatively. The use of these testing methods to measure the effect of CIs on different components of the vestibular system separately (the otolith organs and the SCCs) is rare in the literature. Indeed, to the best of my knowledge, (when the current study is started), none of the previous studies had assessed the function of the vestibular system completely before and after implantation using VEMP and vHIT. As the technique of oVEMP in response to mini-shaker vibration is new, normative values to define the presence or absence of a response are still sparse in literature, so this study also aimed to explore normative data for oVEMP to vibration (mini-shaker) on a small sample of healthy human subjects.

The **aims** of this project were as follows:

- To explore normative data for oVEMP in response to vibration with a mini-shaker on normal human subjects.
- To obtain information about the balance function of patients before implantation.
- To determine if any vestibular organs (SCCs, utricle or saccule) are affected by the CI surgery.
- To compare vestibular function pre and post unilateral implantation.
- To find out if a CI induces or aggravates balance disorders.
- To compare the prevalence of balance dysfunction in the implanted ear and the non-implanted ear within the same CI patient.

The present study specifically aimed to answer the following **research question**:

Does unilateral cochlear implantation affect vestibular function in adults?

7.2 Methods

The methods used in this study with hearing impaired subjects were approved by both the Human Experiment Safety and Ethics Committee of the University of Southampton's Institute of Sound and Vibration Research (ISVR) and the NHS Ethics Integrated Research Application System (REC reference: 14/WA/1015 and IRAS project ID: 156658) before the research commenced. Ethics approval for the normative study of oVEMP in response to mini-shaker vibration was also obtained from the University of Southampton Ethics Committee. The current study was independent of the clinical management of patients, so although the results of the testing were passed to the clinical team, the results were not used to inform the clinical management of the patients.

7.2.1 Study design

The aim of the present study was to measure if there is a significant effect of unilateral CI on balance function for deaf people. The study was thus designed as a cross-sectional comparison of vestibular function pre and post CI among a group of adults with severe to profound SNHL. The aim was to measure the function of the otolith organs and three SCCs before the CI surgery and two to three months after surgery by using cVEMP and oVEMP with two types of stimulation (ACS and BCV) and the vHIT. The second aim was to acquire normative data for oVEMP to vibration (mini-shaker) on healthy subjects.

Estimating the sample size is considered an important part in the early stages of conducting clinical research (Faber & Fonseca, 2014).The required sample size (n=12) was estimated using the data from a previous study in which the saccular function was measured before and after the CI surgery (Basta et al., 2008), where it was found that the mean difference in amplitude of cVEMP for 18 patients was 46.9 mV, and SD of the difference (using an effect size of 0.5) was 39.0 mV. This difference in the amplitude of cVEMP between pre-and postoperative groups can be

detected with a power of 81 %, using 12 subjects. The power of a study is defined as the probability of the test correctly rejecting the null hypothesis (H_0) (Faber & Fonseca, 2014). The null hypothesis is defined in this study as the proposition that there is no significant effect of CI on balance function. However, as it was challenging to recruit patients within the planned time for this study, only seven patients were recruited. Difficulty in recruiting patients could be due to the following reasons:

- These patients went through stressful assessments to determine their eligibility for CI surgery before my study was carried out.
- Children were not included in this research, although approximately 25 % of patients at the AIS centre were under 18 years of age (at the time of the study).
- Adults over 70 were not included; however, many of patients in the AIS centre were over 70 years of age.
- Patients who were invited to take part in this research may have decided to not participate as they already had a large number of appointments and did not wish to attend additional appointments.
- Patients electing not to participate in research in general.
- The geographical area AIS covers is very large, so patients may not have wished to travel.

With this underpowered study, it is difficult to generalise the statistical analysis of this research to the whole population. Because of the small sample size, a case-by-case approach was adopted for CI recipients instead of statistically analysing the data. This approach presents the patient's history, as described by the CI recipients in the questionnaire, and the results of the objective balance tests that were conducted before and after the CI surgery.

7.2.2 Study population

Normative study of oVEMP to vibration with a mini-shaker

10 subjects (6 male and 4 female) aged between 18 and 45 years, with a mean age of 26 years, were recruited in the study, which included fellow students and staff

from the University of Southampton, and also their friends. Screening questionnaires were used to rule out balance problems, and significant problems in eye vision that could not be reversed with eyeglasses or contact lenses. It was also ensured that subjects had normal hearing using PTA, and all subjects had pure-tone thresholds of around or better than 20 dB HL (hearing level). oVEMP responses to vibration (mini-shaker) were recorded from 10 ears from the 10 subjects.

Study group (CI patients)

This study was conducted on seven deaf CI recipients aged between 18 to 70 years (four women, three men) who were met the criteria of the present study and were operated on at the University of Southampton Auditory Implant Service (USAIS) between 2015 and 2017. All CI recipients included in the study were implanted using a similar surgical approach, known as the extended round window approach (O'Connell et al., 2016).

The adult patients were selected for implantation based on NHS criteria (NICE TAG 166 Guidelines). The main criterion for implantation was having severe to profound SNHL with no benefit from conventional hearing aids. All adult patients (18-70 years) who expected to receive a CI and agreed to take part in this study, were included in the study. Patients who had back/ neck pain, visual problems, outer and middle ear pathology (e.g. excessive wax, infection, ear discharge or bleeding), and allergy to alcohol swab were excluded from the study. The majority of the patients (n=5) included in the study had acquired SNHL from unknown causes. Two patients had congenital deafness due to maternal rubella and bacterial meningitis, respectively. The complete patients' information is shown in Table 7.1. All patients gave their written informed consent. The medical records for each patient were obtained from USAIS in order to document any history of balance problems or any relevant balance testing results, to compare them with the results of this study. The patient's evaluation of the vestibular function was collected from each patient before and after CI surgery, using a modified version of the Vestibular Rehabilitation Benefit Questionnaire (VRBQ) (http://www.isvr.soton.ac.uk/audiology/vrbq.htm, see Appendix F).

Table 7.1: Demographic data for seven implanted patients who took part in this study. F denotes female, M denotes male, and CI denotes cochlear implant. A, B, and C represent the surgeons who carried out the surgery.

Patient number	Sex	Age at onset of hearing loss	Aetiology of hearing loss	CI device	Surgeon symbol
1	F	52 years	Unknown	Cochlear CI512	B
2	M	54 years	Unknown	AB Mid Scala	A
3	M	54 years	Unknown	Cochlear CI512	B
4	F	Congenital	Maternal Rubella	Cochlear CI512	C
5	M	6 years	Unknown	Cochlear CI512	B
6	F	Early childhood	Bacterial meningitis	AB HiRes Ultra CI	A
7	F	39 years	Unknown	Cochlear CI512	C

7.2.3 Stimuli and Apparatus

Evaluation of otolith organs and the three SCCs was carried out before the CI surgery and two to three months after implantation in all patients. The measurements were performed for the implanted and non-implanted ear for each patient included in this study when the CI device was off. The VOR and VCR functions were measured using cVEMP and oVEMP with two types of stimulation (ACS and BCV) and the vHIT. All procedures were carried out in the same session.

7.2.3.1 vHIT testing

vHIT was measured by the ICS Impulse System manufactured by GN Otometrics (2011). A laptop running OTOsuite software was connected to a pair of light-weight goggles that were firmly fixed to the subject's head. The goggles contained both a high-speed video oculography camera that measured the subject's eye movement and a gyroscope that measured the subject's head velocity, as described in MacDougall et al. (2009). The eye position camera analysed the eye movement with a sampling rate of up to 250 Hz (MacDougall et al., 2009). The system measured the VOR of each SCC individually at high frequencies. The gain of the VOR is defined as the ratio of compensatory eye velocity to head impulse velocity, which is usually around one in subjects with normal vestibular function (Alhabib & Saliba, 2017). VOR

gains which are significantly less than one indicate reduced SCC function (Curthoys & Manzari, 2017).

7.2.3.2 VEMP testing

Cervical and ocular VEMPs were measured using the Bio-Logic Auditory Evoked Potentials system (AEP version 6.2.0) monaurally through headphones (TDH-39P) and a mini-shaker vibrotactile perception meter (VPM). For AC cervical VEMP, stimuli were 500 Hz tone-burst stimuli with 1-cycle rise/fall, and 2-cycle plateau at sound intensity level of 90 dB nHL. 10-1500 Hz band-pass filters were applied to the data. Each recording required 150 repeats of a 0.008 s tone pip, (4 cycles with a cycle duration of 0.002 s), making the total stimulus duration for one recording 30 s. The repetition rate (5 Hz) was constant, so the total recording time for 150 epochs was 30s (time window of 0.2 s × 150 epochs). The output from the Bio-Logic AEP system (version 6.2.0) was routed monaurally through headphones (TDH-39P). Four recordings (2 recordings for each ear) were repeated to give a total exposure time of 120 s.

Vibration ocular VEMPs (via a mini-shaker) were measured using 0.4 s tone bursts at 100 Hz with an rms (root mean square) level of 1ms^{-2} (metre per second squared) placed on the mastoid bone directly behind the auricle. For each vibration, 150 epochs of 0.4 s were generated (60 s exposure). Four recordings were repeated to give a total exposure time of 240 s. The output from the Bio-Logic AEP system (version 6.2.0) was routed into the VPM and the acceleration signal from the mini-shaker was measured on an oscilloscope. The oscilloscope was calibrated to target a peak-to-peak value of 5.2 volts when the measurement was made on the patient, in order to obtain the target acceleration of 10 ms^{-2} rms (5.2 V peak to peak = 14 ms^{-2} or 10 ms^{-2} rms). The mini-shaker was applied to the head with a force of 10 N (Newtons), as indicated on a calibrated force meter.

Table 7.2 below shows the stimulus parameters of AC S and BCV used for eliciting cVEMP and oVEMP, respectively.

Table 7.2: The stimulus parameters for ACS and vibration used to elicit cVEMP and oVEMP, respectively. AC refers to air conduction; TB refers to tone burst; Hz refers to Hertz, dB nHL refers to decibels normal hearing level; VPM refers to Vibrotactile Perception Meter; ms^{-2} refers to metres per second squared; and rms refers to root mean square.

Stimulus	Frequency	Polarity	Filter (Hz)	Transducer	Stimulus rate	Stimulus level
AC	500 Hz TB (1 cycle rise/fall and 2-cycle plateau)	Alternating	10-1500 Hz	Head Phone TDH-39P	5 Hz	90 dB nHL
Vibration	100 Hz TB	Condensing	10-1500 Hz	Mini-shaker VPM 1.0	5 Hz	Acceleration target 10 ms^{-2} rms

7.2.4 Procedures

For the study group (CI patients), balance function was evaluated before and two to three months after CI surgery for the implanted patients, using vHIT, AC cVEMP, and vibration oVEMP. For normative data on oVEMP, normal human subjects underwent oVEMP in response to mini-shaker vibration. The following procedures were used to perform these objective balance tests.

The participants attended the clinic room at the ISVR. They were given an information sheet and consent form and were then asked to complete a self-report questionnaire, in order to ensure that the patients did not have back/ neck pain, visual problems, outer and middle ear pathology (e.g. excessive wax, infection, ear discharge or bleeding), and allergy to alcohol swab. The vestibular function was then subjectively evaluated by each patient before and after CI surgery, using a questionnaire. After that, vestibular function was objectively evaluated by the examiner in the implanted and non-implanted ear for each patient through two balance tests.

7.2.4.1 vHIT

First, each participant underwent vHIT; the measurements were performed based on the protocol used by GN Otometrics (2011), which took a maximum of 30 minutes and involved wearing a pair of lightweight, securely-fitting goggles whilst the tester held the participant's head firmly but comfortably and made small rapid unpredictable head movements in several directions. The head impulses were unpredictable, to reduce the risk of compensation by the patient (predictive saccade). The goggles were comfortably positioned on the bridge of the nose and around the eye sockets to minimise the slippage of the camera relative to the head, which can cause artefacts. Eye position was recorded with an infrared camera in the system. It was decided to start with the lateral head impulses before the vertical ones. Vertical head impulses were more difficult for participants than lateral ones, and therefore lateral impulses were recorded first to increase the participant's familiarity with the test.

Patients were instructed to stare at a fixation dot located at a distance of about 1.20 m, measured with a tape measure (see green dot on the wall in Figure 7.2). This distance was used in previous studies of the vHIT system, such as Bell et al. (2015), to avoid activation of the convergence system (which occurs when the subject is too close to the visual target), which could raise the VOR gain above unity. Based on information from GN Otometrics (2011), the subject should be at least 1.00 m away from the target. Testing followed the protocol recommended by GN Otometrics (2011), which is shown in Table 7.3 below. About 20 head impulses in the planes of the horizontal and vertical canals were manually delivered by the experimenter with unpredictable timing and direction. During this test, the head turns were passive (i.e., conducted by the examiner) rather than active (i.e., voluntary head turns executed by the subject). This is supposed to improve the sensitivity of the vHIT testing, through reducing the possibility of predictive saccades (a non-VOR fast eye movement) occurring during head turns to correct for a known VOR deficit, as found by Black et al. (2005). These voluntary saccades generated by the subject during head rotations are hard to detect and can produce false negative results in patients with peripheral vestibular loss (Bell et al., 2015). Throughout this test, the head has to be moved with high acceleration, which should be sufficient to test the VOR of

each SCC in effective isolation from the same canal on the other side (Alhabib & Saliba, 2017).

Table 7.3: Stimulus parameters used with vHIT in the lateral and vertical planes (GN Otometrics, 2011); deg refers to degrees; deg/s refers to degrees per second, deg/s ^{2} refers to degrees per second squared.

Stimulus	**Lateral head impulse**	**Vertical head impulse (RALP/LARP)**
Angular displacement	10-20 deg	10-20 deg
Peak Head Velocity	100-250 deg/s	50-250 deg/s
Peak Head Acceleration	1000-2500 deg/s ^{2}	750-5000 deg/s ^{2}

To test the lateral SCCs, the tester rotated the patient's head horizontally in an abrupt, brief and unpredictable manner at a small angle and in a random direction (right or left). Peak head velocity of the horizontal impulses ranged from 100 to 250°/s (acceleration 1000–2500°/s^2, amplitude 10–20°) (GN Otometrics, 2011).

To test the anterior and posterior SCCs, the head rotations were delivered in the planes of the vertical canals – left anterior-right posterior (LARP) and right anterior-left posterior (RALP). These canals lie in planes, which are approximately 45° to the sagittal plane of the head. The person's head was positioned about 30–40° turned to the left or right with respect to his/her body, so that the targeted vertical canal plane was approximately aligned with the body's sagittal plane; diagonal head movements were then delivered in the plane of the vertical canals while gaze was directed at the fixed target. The tester placed one hand (dominant) on the top of the patient's head and the other beneath the chin, in order to control the direction of rotation. The patient's head was rotated vertically via the dominant hand in an abrupt, brief and unpredictable manner with a small angle (about 10-20 degrees), while the patient attempted to maintain fixation on the fixed target. Peak head velocity of the vertical

impulses ranged from 50 to 250°/s (acceleration 750–5000°/s², amplitude 10–20°) (GN Otometrics, 2011). The direction of the head movement determines which canal of the pair is activated: so for example, downward head impulses stimulate the left/right anterior canal and the upward ones stimulate the right/left posterior canal (GN Otometrics, 2011).

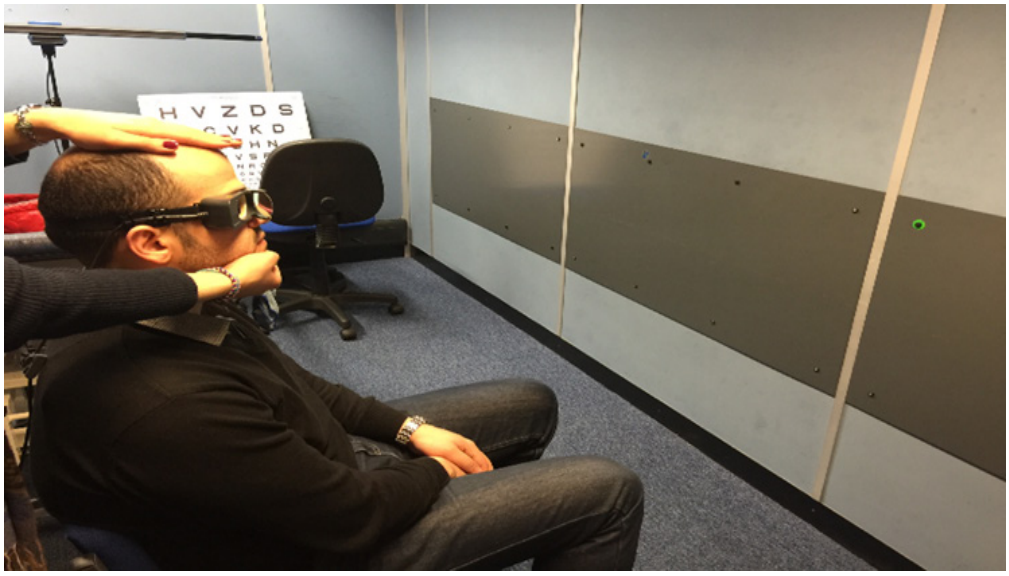

Figure 7.1: Experimental environment of vHIT. The green fixation dot is located around 1.20 m away on the surface in front of the participant.

7.2.4.2 VEMP recording

VEMPs from the SCM muscle were recorded ipsilaterally while subjects were seated upright on a chair with their chin turned over the contralateral shoulder to tense the SCM muscle. All procedures used with patients in this study to conduct cVEMP measurements were identical to those used in Experiment 1, as described in section 3.2.4.

7.2.4.3 oVEMP recording

VEMPs from the ocular muscles were recorded contralaterally to the stimulated ear, in response to vibration. The responses were recorded by placing five electrodes on the face. The two active pairs of electrodes were placed on the orbital margin below the centre of the eye and referred to two reference pairs of electrodes approximately 15-30 mm below, on the cheek, and the ground electrode low on the forehead (see

Figure 7.1 below). The impedance of the electrodes was maintained below 10 KΩ. Patients sat on a chair and were asked to look up at a target located 2 m away, with an elevation of 25 degrees, whilst vibrations were applied to patient's tested ear via the mini-shaker at the mastoid process (behind the pinna of the ear). The patients were tested with the same angle of elevation and with rest breaks in order to allow the patients to blink.

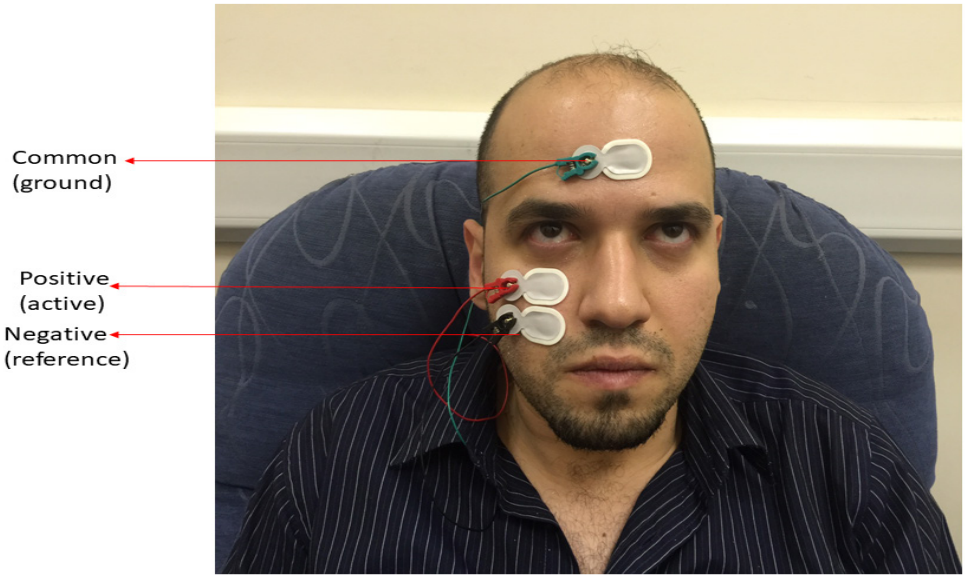

Figure 7.2: Positions of the electrodes in oVEMP recording.

7.2.5 Response analysis

Balance function in both the implanted and non-implanted ears was evaluated when the CI device was in the off condition.

7.2.5.1 cVEMP

The presence of a cVEMP response was visually evaluated based on specific criteria identical to those used in experiment four, as described in Chapter 5, section 5.2.5. If any of these criteria were not met, then the cVEMP responses were judged to be absent. The objective analysis with the HT^2 test for VEMP responses was not applied in the present study with CI patients, as there was not an appropriate programming environment in the Bio-Logic AEP system for implementing the methods; this can be considered as a limitation for the method. There was also a timing issue, in that

when the CI data collection began, the objective HT[2] method had not been developed and thus was not implemented at that point.

7.2.5.2 oVEMP

The normative range for oVEMP parameters to vibration with a mini-shaker at intensity of 90 dB nHL was explored in normal hearing subjects in the present study. Table 7.4 shows 95 % confidence intervals for the mean (mean ± 1.96×SD) of amplitude (uV), and latencies P1-N1 (ms) of oVEMP at 90 dB nHL in 10 normal human subjects. Test results exceeding the 95 % confidence intervals of the mean for oVEMP parameters of the normative data (amplitude, and peak latencies) were considered abnormal in the study group (CI patients). Thus, oVEMP responses were accepted as present if the oVEMP waveform was reproducible, biphasic with a positive (P1) peak followed by a negative (N1) peak, peak-to-peak amplitude and peak latencies should be within the 95 % range shown in Table 7.4. An example of normal ACS cVEMP and BCV oVEMP responses obtained from a healthy subject and abnormal responses recorded from a patient with bilateral otolithic dysfunction using the Bio-Logic system is shown in Figure 7.3 below.

Normative vibration oVEMP data

Table 7.4: Normative oVEMP data: 95% confidence intervals of the mean (mean ± 1.96×SD) of P1-N1 amplitude (uV), and latencies P1-N1, of oVEMP in 10 healthy subjects.

oVEMP parameters	Lower 95 % range	Upper 95 % range
P1-N1 amplitude (uV) at 90 dB nHL	1.02	3.89
P1 latency (ms) at 90 dB nHL	15.52	26.38
N1 latency (ms) at 90 dB nHL	19.92	32.48

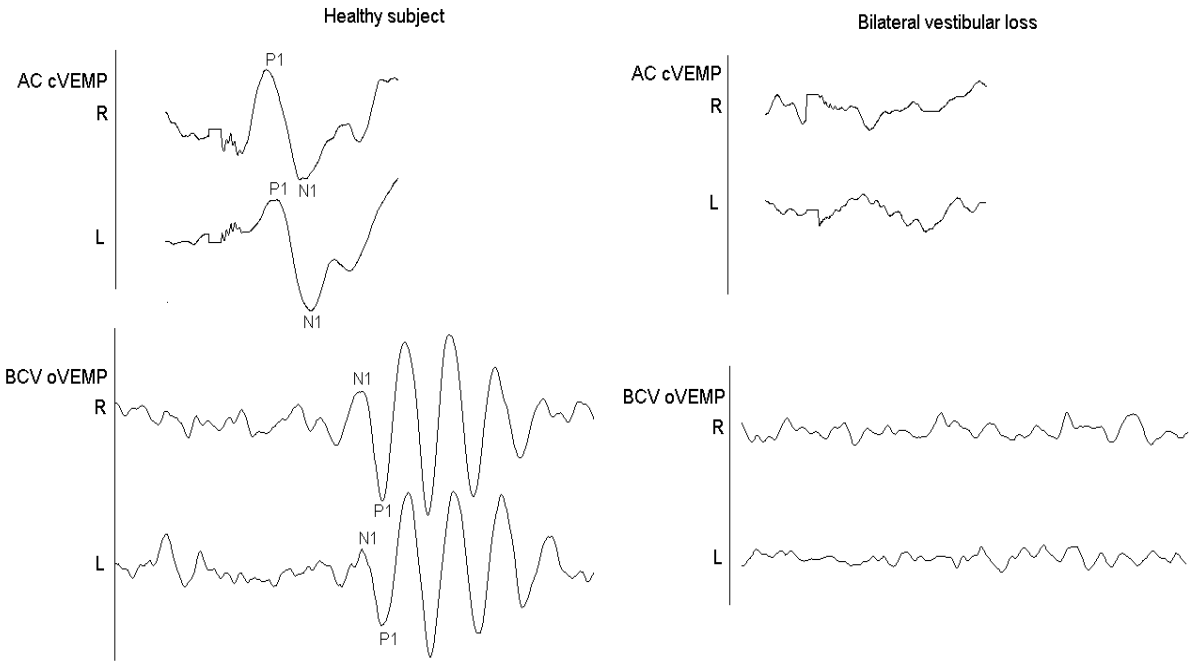

Figure 7.3: ACS cVEMP and BCV oVEMP responses obtained from a healthy subject (left panel), and a patient with bilateral vestibular loss (right panel) using the Bio-Logic Auditory Evoked Potentials system (version 6.2.0). The healthy subject displays normal P1-N1 from both SCM muscles and N1 responses beneath both eyes. The patient with bilateral vestibular loss has no P1-N1 from both SCM muscles and N1 responses beneath both eyes, which is consistent with bilateral loss of otolithic function.

7.2.5.3 vHIT

VOR gain (eye velocity/head velocity) was calculated automatically by a software package which divides the area under the curve of eye velocity by the area under the curve of head velocity (Janky and Givens, 2015). The VOR gain was considered normal if it was >0.85 for the horizontal canal, and >0.65 for the vertical canals, based on the manufacturer's suggestion of a low cut-off VOR gain (GN Otometrics, 2011), although it is not clear which peer reviewed research journals these normative VOR values are based on (Bell et al., 2015).

7.3 Case studies

Note: Both cVEMP and oVEMP responses are categorized as either present or absent based on specific criteria for cVEMP and normative data for oVEMP (see section 7.2.5), whereas vHIT is categorized as either normal or abnormal based on the pathological cut-off VOR gain (>0.85 for the horizontal canal, and >0.65 for the vertical canals) according to the manufacturer's suggestion of a low cut-off VOR gain (GN Otometrics. 2011).

Patient 1

History A 66-year-old woman had had bilateral severe to profound SNHL for 14 years. The cause of hearing loss was unknown. She reported that her balance was generally good, and she had not previously suffered from balance problems. She underwent CI surgery on 18th of July 2017, in the right ear. She reported that her balance was generally good, and she did not notice any balance problems after the surgery, except one accident, which happened within 5 weeks after the surgery. While she was riding her bicycle, she lost her balance and fell off her bicycle. Her arm was broken, and she had a pain in her back due to that accident. Apart from this accident, she reported that her balance was usually fine. Although she did not notice a change in balance function following surgery, this accident could be consistent with a short-term effect on balance function and it highlights the impact that balance dysfunction can have. Her balance was objectively assessed one month before and three months after the CI surgery, using vHIT, AC cVEMP and vibration oVEMP. Vestibular investigations before and after the surgery revealed the following:

Balance status before the CI surgery

vHIT: the VOR gain in both ears was within the normal range for all canals (horizontal and vertical).

ACS cVEMP: Present in both ears.

BCV oVEMP: Present in both ears.

Balance status after the CI surgery

vHIT: The VOR gain in both ears was within the normal range for all canals (horizontal and vertical).

ACS cVEMP: Absent in the right ear (implanted ear) and present in the left ear.

BCV oVEMP: Present in both ears.

Summary of results

This patient was manifesting normal balance function before the implant. After the implant, the saccular function in the right implanted ear for this patient was affected (as indicated by the loss of cVEMP in the right ear, see Figure 7.4 below), but the left

saccular function (non-implanted ear), SCCs (see VOR gains in Table 7.5 below) and utricle were unaffected.

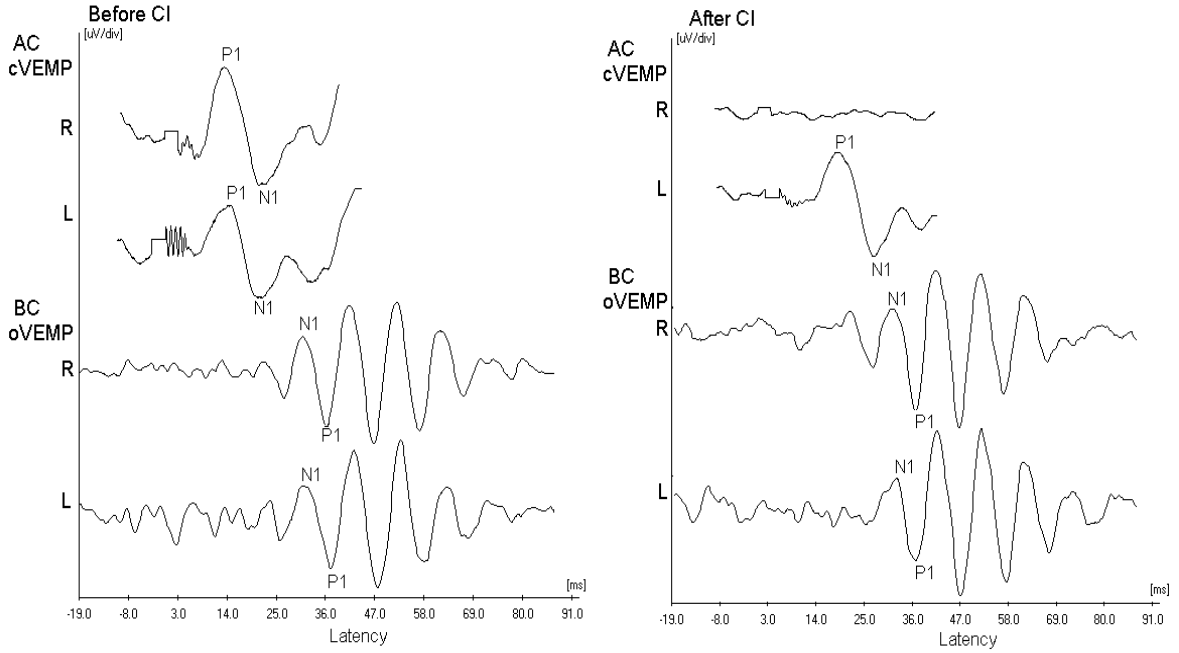

Figure 7.4: AC cVEMP and BCV oVEMP responses for right (implanted) ear (R) and left ear (L) obtained before and after implantation. The saccular function in the implanted ear appears to have been affected following implantation, as indicated by the loss of cVEMP responses (top right). However, the utricular function seems to have been unaffected by CI surgery. Note: the first peak (n1-p1) of BCV oVEMP response has been delayed by around 10 ms.

Table 7.5: VOR gain values for all SCCs in both ears pre- and post-CI. A green area indicates a normal result. The SCCs' function was not affected by CI surgery. RL: Right Lateral, LL: Left Lateral, LA: Left Anterior, RP: Right Posterior, RA: Right Anterior, LP: Left Posterior.

	RL	LL	LA	RP	RA	LP
Before CI	1.06	1.03	0.90	0.99	1.00	0.99
After CI	1.10	1.05	0.92	0.89	1.14	1.05

Patient 2

History A 67-year-old man had progressive bilateral hearing loss, which had progressed to severe to profound SNHL since 2005. He had bilateral hearing aids, but he reported that he got a little more information from the right hearing aid than the left. He also reported that he had a minor balance problem before the implantation, as he suffered from unsteadiness when listening to loud sounds and this lasted for a few minutes and sometimes he felt dizzy when standing up after lying down. He had been implanted in the left ear on 10th of January 2017. He did not notice any difference in his balance after being implanted. In addition, his balance was objectively assessed one day before and three months after the CI surgery, using vHIT, AC cVEMP and vibration oVEMP. Vestibular investigations before and after the surgery revealed the following:

Balance status before the CI surgery

vHIT: the VOR gain in both ears was within the normal range for all canals.

ACS cVEMP: Present in both ears. Some aspects of the patient's history seemed indicative of superior semi-circular canal dehiscence (SCC dehiscence), so the cVEMP threshold was measured in both ears on the same day as the testing session. The cVEMP threshold went down to 85 dB nHL in both ears, so the results did not suggest a diagnosis of SCC dehiscence based on this test.

BCV oVEMP: Present in both ears.

Balance status after the CI surgery

vHIT: The VOR gain in both ears was within the normal range for all canals (horizontal and vertical).

ACS cVEMP: Absent in the left ear (implanted ear) and present in the right ear.

BCV oVEMP: Present in both ears.

Summary of results

This patient was manifesting normal balance function before the implant**.** Following implantation, the saccular function in the left implanted ear for this patient was affected (as indicated by the loss of cVEMP in the left ear, see Figure 7.5 below), but

the right saccular function (non-implanted ear), SCCs (see VOR gains in Table 7.6 below) and utricle were unaffected. This patient reported in the self-reported questionnaire that he suffered from unsteadiness after exposure to loud sound and sometimes felt dizzy on standing up after lying down. This could be an indication of SCC dehiscence. However, results of cVEMP testing were not consistent with vestibular loss due to SCC dehiscence.

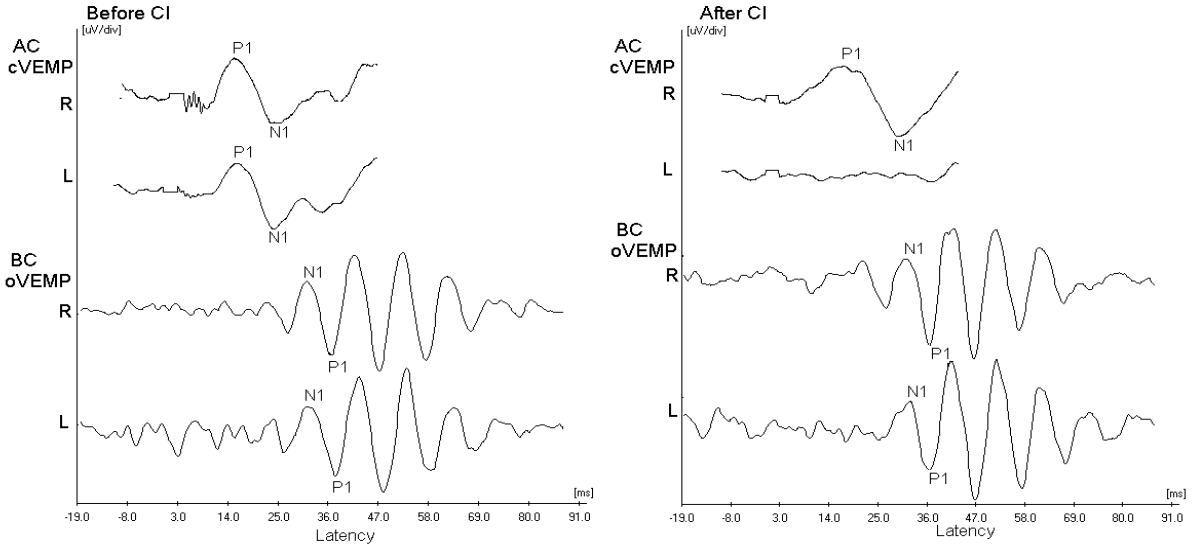

Figure 7.5: AC cVEMP and vibration oVEMP responses for right ear (R) and left (implanted) ear (L) obtained before and after implantation. The saccular function in the implanted ear appears to have been affected following implantation, as indicated by the loss of cVEMP responses. However, the utriclular function seems to have been unaffected by CI surgery. Note: the first peak (n1-p1) of BCV oVEMP response has been delayed by around 10 ms.

Table 7.6: VOR gain values for all SCCs in both ears pre- and post-CI. A green area indicates a normal result. The SCCs' function was not affected by CI. RL: Right Lateral, LL: Left Lateral, LA: Left Anterior, RP: Right Posterior, RA: Right Anterior, LP: Left Posterior.

	RL	LL	LA	RP	RA	LP
Before CI	1.00	0.99	1.00	1.02	1.01	1.04
After CI	0.84	0.88	1.02	1.02	1.22	1.16

Patient 3

History A 66-year-old man had had bilateral profound SNHL for over 12 years. He had been fitted with bilateral hearing aids. The cause of hearing loss was unknown but could be due to significant noise exposure during his working life. He currently had tinnitus, worse in the left ear, which could be very annoying and affected his sleeping. He reported that he had normal balance and he did not suffer from problems in his balance system. He was implanted on 11th of August 2017, in the left ear. He reported that the intensity of the tinnitus was not suppressed or reduced with the CI, and still affected his quality of life. He did not notice any difference in his balance after being implanted. In addition, his balance function was objectively assessed one month before and two months after the CI surgery using vHIT, AC cVEMP and vibration oVEMP. Vestibular investigations before and after the surgery revealed the following:

Balance status before the CI surgery

vHIT: The VOR gain in both ears was within the normal range for all canals (horizontal and vertical).

ACS cVEMP: Missing data. This could be due to difficulty in identifying the SCM muscle, and this was because of thick neck tissue for this patient.

BCV oVEMP: Present in both ears.

Balance status after the CI surgery

vHIT: the VOR gain in both ears was within the normal range for all canals.

ACS cVEMP: Missing data.

BCV oVEMP: Present in both ears.

Summary of results

This patient showed bilateral normal function of both utricle and SCCs, and saccular dysfunction in both sides before the implant. Although the cVEMP results were consistent pre-post CI surgery (see Figure 7.6 below), the absence of cVEMP responses could be due to difficulty in identifying the SCM muscle, which was due to the thick neck tissue for this patient. After the implant, there was no effect of CI

surgery on the other vestibular organs (utricle and three SCCs (see VOR gains in Table 7.7 below)) for this patient.

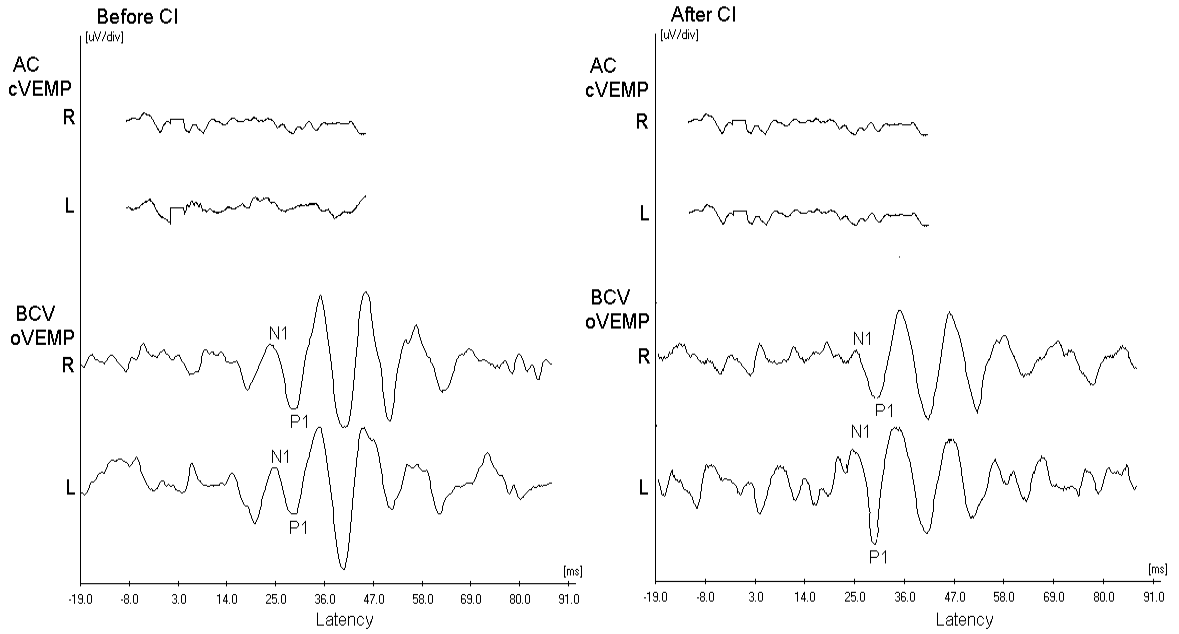

Figure 7.6: AC cVEMP and vibration oVEMP responses for right ear (R) and left (implanted) ear (L) obtained before and after implantation. The absence of cVEMP responses could be due to difficulty in identifying the SCM muscle, which was due to neck thickness. The utriclular function seems to have been unaffected by CI surgery. Note: the first peak (n1-p1) of BCV oVEMP response has been delayed by around 10 ms.

Table 7.7: VOR gain values for all SCCs in both ears pre- and post-CI. A green area indicates a normal result. The SCCs' function was not affected by CI. RL: Right Lateral, LL: Left Lateral, LA: Left Anterior, RP: Right Posterior, RA: Right Anterior, LP: Left Posterior.

	RL	LL	LA	RP	RA	LP
Before CI	**1.01**	**0.95**	**0.81**	**0.94**	**1.06**	**0.82**
After CI	**0.84**	**0.88**	**0.98**	**0.98**	**0.91**	**0.92**

Patient 4

History A 49-year-old woman had bilateral congenital severe to profound SNHL, which was attributed to maternal rubella. She was fitted with hearing aids to both ears at around two and a half years and had worn these consistently. Her hearing had deteriorated significantly over the past four years. She reported that her balance was generally good, and she had not suffered from any balance problems previously. She was implanted on 10th of August 2017 in the right ear. She had no significant balance problems immediately after surgery but had an episode of imbalance two weeks later, which could be consistent with short-term dizziness. Her balance was objectively assessed two weeks before and three months after the CI surgery, using vHIT, AC cVEMP and vibration oVEMP. Vestibular investigations before and after the surgery revealed the following:

Balance status before the CI surgery

vHIT: The VOR gain in both ears was within the normal range for all canals (horizontal and vertical).

ACS cVEMP: Present in both ears.

BCV oVEMP: Absent in both ears.

Balance status after the CI surgery

vHIT: the VOR gain in both ears was within the normal range for all canals (horizontal and vertical).

ACS cVEMP: Absent in the right ear (implanted ear) and present in the left ear.

BCV oVEMP: Absent in both ears.

Summary of results

This patient was manifesting abnormal utricular function in both sides, and normal function of both saccule and SCCs in both ears. After the implant, the saccular function in the right implanted ear for this patient was affected (as indicated by the loss of cVEMP in the right ear, see Figure 7.7 below), but the left saccular function (non-implanted ear), and SCCs (see VOR gains in Table 7.8 below) were unaffected.

This change in cVEMP result post implantation might be linked to the short-term dizziness reported by this patient.

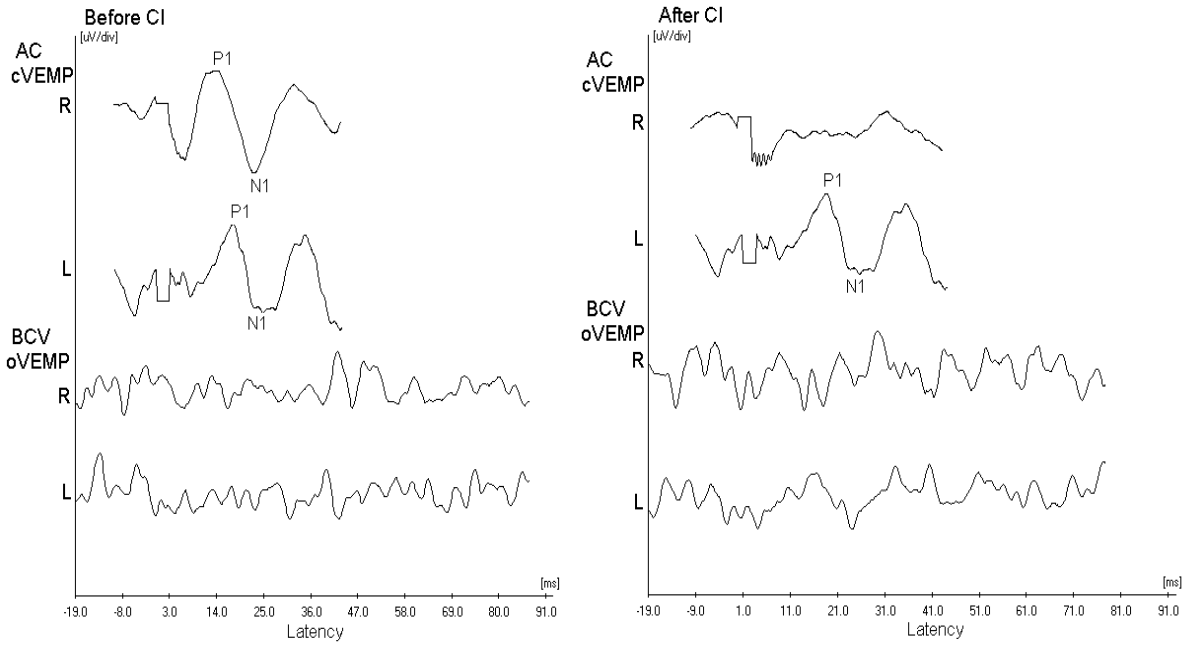

Figure 7.7: AC cVEMP and vibration oVEMP responses for right (implanted) ear (R) and left ear (L) obtained before and after implantation. The saccular function in the implanted ear appears to have been affected following implantation as indicated by the loss of cVEMP response (top right). However, the oVEMP results seems to have been consistent pre- and post-CI surgery.

Table 7.8: VOR gain values for all SCCs in both ears pre- and post-CI. A green area indicates a normal result. The SCCs' function was not affected by CI. RL: Right Lateral, LL: Left Lateral, LA: Left Anterior, RP: Right Posterior, RA: Right Anterior, LP: Left Posterior.

	RL	LL	LA	RP	RA	LP
Before CI	**0.88**	**0.85**	**0.99**	**0.75**	**1.21**	**0.85**
After CI	**0.79**	**0.84**	**0.77**	**0.88**	**0.24**	**1.00**

Patient 5

History A 43-year-old man had bilateral profound SNHL. He was first prescribed hearing aids when his hearing loss was identified at the age of 6. He was implanted on 4th of May 2016 in the left ear. He reported that he had started to suffer from balance problems in the last 10-12 years. He reported that he leant to the right side during walking most of the time, and that he did not notice any difference in his balance after being implanted. In addition, his balance was objectively assessed six weeks before and three months after the CI surgery using vHIT, AC cVEMP and vibration oVEMP. Vestibular investigations before and after the surgery revealed the following:

Balance status before the CI surgery

vHIT: The VOR gain in both ears was abnormal for all canals (horizontal and vertical). This could be due to a double vision problem (as the patient had reported).

ACS cVEMP: Present in right ear, but absent in left ear.

BCV oVEMP: Missing data. This could be due to his double vision problem.

Balance status after the CI surgery

vHIT: The VOR gain in both ears was abnormal in all canals.

ACS cVEMP: Present in right ear, but absent in left ear (implanted ear).

BCV oVEMP: Missing data.

Summary of results

This patient was manifesting abnormal function of utricles, bilateral SCCs, and left side saccule, and normal right side saccule before the implant (see Figure 7.8 below). So, he had only a small vestibular function before the implantation, which highlights the importance of performing the CI surgery on the side with poorer balance function. The patient had double vision, which may affect the performance of vHIT (see VOR gains in Table 7.9 below), and vibration evoked oVEMP, as both tests require the patient to stare at a target during testing. For example, the patient struggled to focus on the target due to his double vision, so he saw two targets on

the wall instead of one. After the implant, there was no effect of CI surgery on the saccular function in the non-implanted ear (right) for this patient.

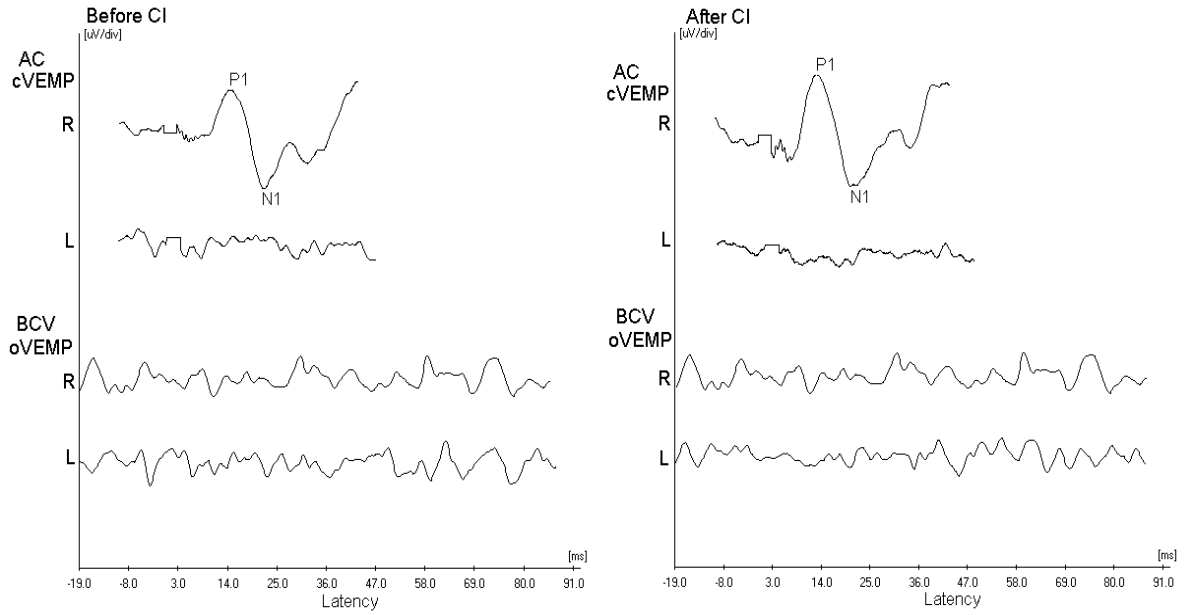

Figure 7.8: AC cVEMP and vibration oVEMP responses for right ear (R) and left (implanted) ear (L) obtained before and after implantation. The saccular function in the non-implanted ear (right) appears to have been unaffected by implantation. The oVEMP results seems to have been consistent pre- and post-CI surgery.

Table 7.9: VOR gain values for all SCCs in both ears pre- and post-CI. A red area indicates an abnormal result. The vHIT results were consistent pre- and post-CI surgery. RL: Right Lateral, LL: Left Lateral, LA: Left Anterior, RP: Right Posterior, RA: Right Anterior, LP: Left Posterior.

	RL	LL	LA	RP	RA	LP
Before CI	0.45	0.44	0.59	0.30	0.50	0.44
After CI	0.59	0.43	0.57	0.25	0.46	0.39

Patient 6

History A 55-year-old woman had had longstanding bilateral severe to profound hearing loss since early childhood, caused by bacterial meningitis which completely destroyed the balance and hearing parts of the ear. She had high-powered bilateral hearing aids but relied heavily on lip-reading to communicate and she reported that she struggled to hear accents. She reported regularly suffering from inflamed and red ear canals. She also reported that she suffered from severe balance problems, and that the symptoms were undoubtedly worse in the dark. She had a balance assessment on the 31 March 2017, conducted by Mr Ruddock at the Queen Alexandra Hospital, where she was diagnosed with bilateral peripheral vestibular hypofunction (as reported by the AIS centre). She underwent CI surgery on 26th of August 2017 in the right ear. Her balance was objectively assessed two days before and two months and one week after the CI surgery, using vHIT, cVEMP elicited by air conduction sound and oVEMP evoked by vibration. Vestibular investigations before and after the surgery revealed the following:

Balance status before the CI surgery

vHIT: The VOR gain in both ears was abnormal for all canals (horizontal and vertical).

ACS cVEMP: Absent in both ears.

BCV oVEMP: Absent in both ears.

Balance status after the CI surgery

vHIT: The VOR gain in both ears was abnormal for all canals (horizontal and vertical).

ACS cVEMP: Absent in both ears.

BCV oVEMP: Absent in both ears to 100 Hz tone-burst stimulus.

Conclusion

This patient was manifesting abnormal bilateral function of SCCs, utricle and saccule prior to implantation. Nothing had been changed in her balance results after the implant (see VEMP results in Figure 7.9 and VOR gains in Table 7.10 below). This

woman was very fit and active, which was slightly surprising given her vestibular hypofunction.

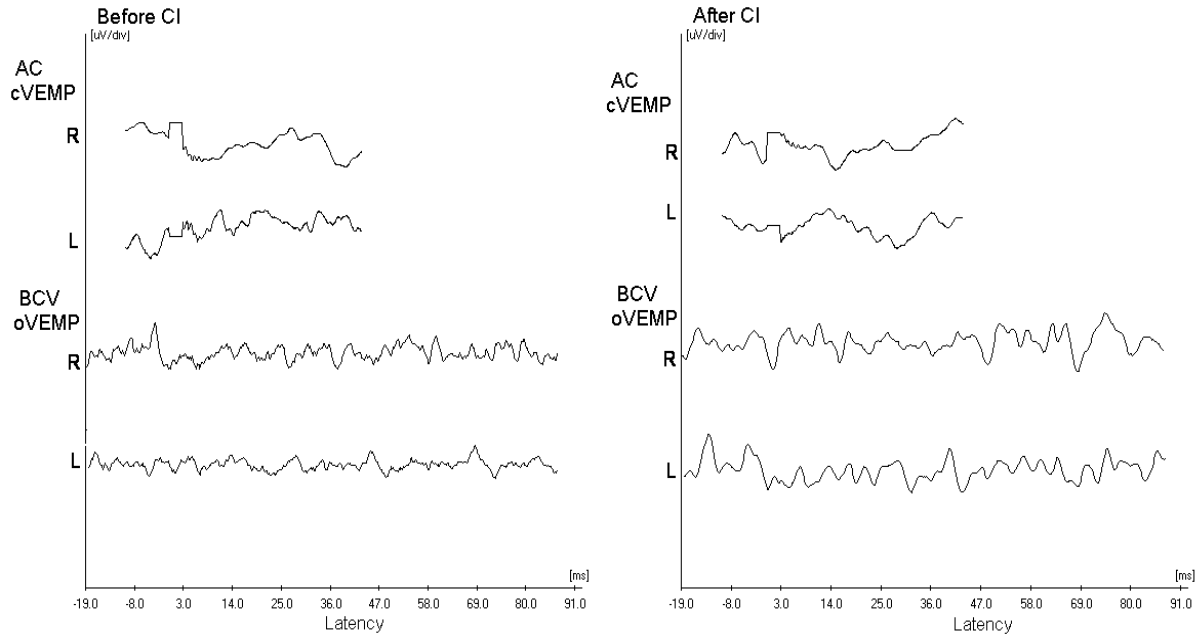

Figure 7.9: AC cVEMP and vibration oVEMP responses for right (implanted) ear (R) and left ear (L) obtained before and after implantation. The oVEMP and cVEMP results appears to have been consistent pre- and post-CI surgery.

Table 7.10: VOR gain values for all SCCs in both ears pre- and post-CI. A red area indicates an abnormal result. The vHIT results were consistent pre- and post-CI surgery. RL: Right Lateral, LL: Left Lateral, LA: Left Anterior, RP: Right Posterior, RA: Right Anterior, LP: Left Posterior.

	RL	LL	LA	RP	RA	LP
Before CI	0.35	0.31	0.29	0.32	0.49	0.40
After CI	0.42	0.41	0.54	0.36	0.61	0.49

Patient 7

History A 54-year-old woman had noticed a steady decline in her hearing approximately 15 years ago and was fitted with one hearing aid at that time. As her hearing loss progressed, she was fitted with a second hearing aid in 2005 and now had bilateral profound SNHL. The cause of her hearing loss was unknown. She reported that her balance was generally good, and she had not previously suffered from balance problems. She underwent CI surgery on 15th of November 2017, in the left ear. She was off balance for a couple of days following surgery (short-term vestibular disorders) but otherwise she had made an uneventful recovery. Her balance was objectively assessed four months before and two months after the CI surgery using vHIT, AC cVEMP sound and vibration oVEMP. Vestibular investigations before and after the surgery revealed the following:

Balance status before the CI surgery

vHIT: The VOR gain in both ears was within the normal range for all canals (horizontal and vertical).

ACS cVEMP: Present in both ears.

BCV oVEMP: present in left ear and absent from right ear.

Balance status after the CI surgery

vHIT: The VOR gain in both ears was within the normal range for all canals (horizontal and vertical).

ACS cVEMP: Present in both ears.

BCV oVEMP: absent in both ears.

Summary of results

This patient was manifesting normal function of bilateral SCCs, saccules, left side utricle, and abnormal right side utricle before the implant. After the implant, the utricular function in the left implanted ear for this patient was affected (as indicated by the loss of oVEMP in the left ear, see Figure 7.10 below), which might be consistent with the short-term feeling of being off-balance following surgery.

However, the saccular and SCCs function in both ears were unaffected after CI surgery.

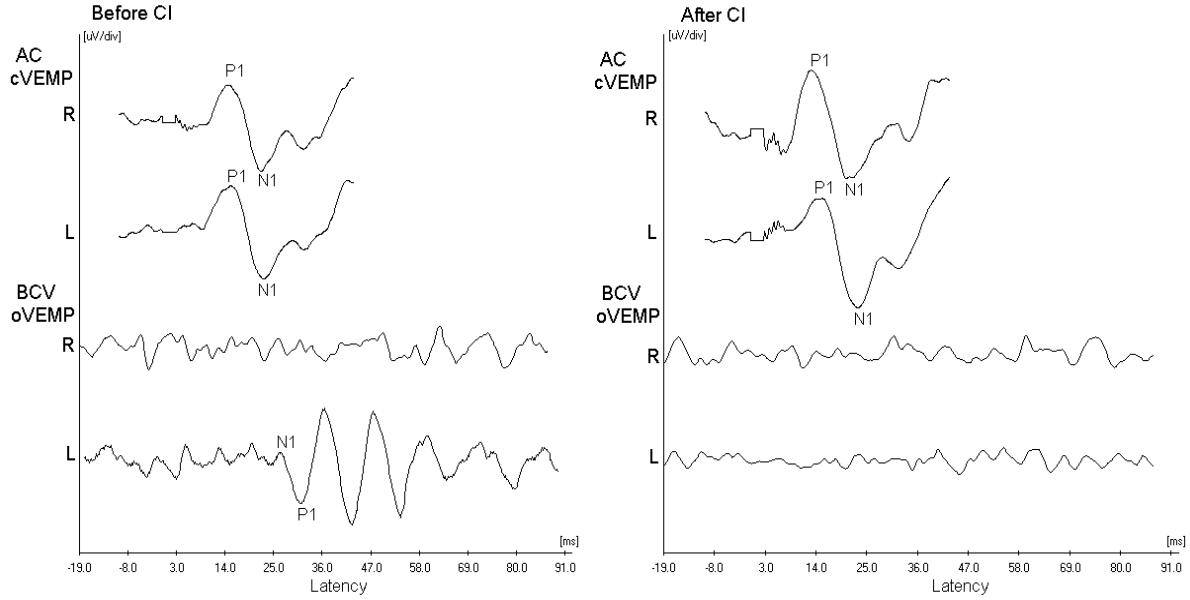

Figure 7.10: AC cVEMP and vibration oVEMP responses for right ear (R) and left (implanted) ear (L) obtained before and after implantation. The utricular function in the implanted ear appears to have been affected following implantation as indicated by the loss of oVEMP response. However, the saccular function in both ears appears to have been unaffected by CI surgery. Note: the first peak (n1-p1) of BCV oVEMP response has been delayed by around 10 ms.

Table 7.11: VOR gain values for all SCCs in both ears pre- and post-CI. A green area indicates a normal result. The SCCs' function was not affected by CI. RL: Right Lateral, LL: Left Lateral, LA: Left Anterior, RP: Right Posterior, RA: Right Anterior, LP: Left Posterior.

	RL	LL	LA	RP	RA	LP
Before CI	**1.06**	**1.13**	**0.92**	**0.83**	**0.92**	**0.99**
After CI	**1.21**	**1.04**	**0.90**	**0.86**	**1.00**	**0.94**

Table 7.12 below shows a summary of the changes in balance function for the seven deaf patients from pre to post-operatively in the implanted and non-implanted ears.

Table 7.12: Results of balance tests performed before and after the CI surgery for all seven patients, for the implanted and non-implanted ears. N (green area) refers to normal; A (red area) refers to abnormal; NA (yellow area) refers to not available (missing data), and a highlighted box with a thick black line represents a change in vestibular function pre- and post-CI surgery on the implanted side. Normal VEMP means a response was present. Normal vHIT means VOR gain >0.85 for horizontal canal and >0.65 for vertical canals.

Patient number	Age (years)	Implanted ear	Balance function preoperatively						Balance function postoperatively					
			Right ear			Left ear			Right ear			Left ear		
			vHIT	cVEMP	oVEMP	vHIT	cVEMP	oVEMP	vHIT	cVEMP	oVEMP	vHIT	cVEMP	oVEMP
1	66	Right	N	N	N	N	N	N	N	A	N	N	N	N
2	67	Left	N	N	N	N	N	N	N	N	N	N	A	N
3	66	Left	N	NA	N	N	NA	N	N	NA	N	N	NA	N
4	49	Right	N	N	A	N	N	A	N	A	A	N	N	A
5	43	Left	NA	N	NA	NA	A	NA	NA	N	NA	NA	A	NA
6	55	Right	A	A	A	A	A	A	A	A	A	A	A	A
7	54	Left	N	N	A	N	N	N	N	N	A	N	N	A

7.4 Discussion

The aim of the present study was to measure the effect of unilateral CI surgery on each vestibular organ separately in relation to balance function before implantation. It should first be mentioned that this study had some missing data for two of the patients. Patient 3 had thick neck tissue, which caused difficulty in identifying the SCM muscle for cVEMP testing. Patient 5 had double vision, which affected the performance of vHIT, and vibration evoked oVEMP, as both tests required the patient to stare at a target during testing.

Table 7.12 summarises of the results of all balance tests performed before and after the CI surgery for all seven patients. For patients 1, 2 and 4, the cVEMP response was altered in the implanted ear, resulting in unilateral saccular dysfunction. Patient 7 only had a change in utricular function in the implanted ear as indicated by the vibration oVEMP test. Unfortunately, this patient received her implant in the L ear with normal utricular function. Preimplant she had abnormal utricular function on the R, so ended up with bilateral utricular abnormality. It should be noted that the testing carried out was for research only and was not used to inform clinical decisions such as side of implantation. The other patients (3, 5, and 6) all had some vestibular dysfunction preoperatively, but showed no change in their balance function postoperatively. This could be related to their balance status prior to implantation: patient 6 had congenital deafness with poor balance function bilaterally before implantation, patient 3 had missing cVEMP data due to thick neck tissue, and patient 5 had missing oVEMP and vHIT data due to double vision; thus, the effect of CI surgery was not evident in these deaf patients.

The results of testing for patient 6 appear to demonstrate the sensitivity of the applied testing methods: the patient was known to have congenital bilateral hypofunction and reported a history consistent with this. All test results (SCCs with vHIT, saccule with sound evoked cVEMP and utricule with vibration evoked oVEMP) were bilaterally abnormal for this subject.

Variability in the results of objective balance tests before and after the surgery can be attributed to several factors, such as cause of deafness; type of CI device, and the occurrence of trauma to the inner ear in operations by different surgeons (see Table 7.1). Although statistical analysis of the results was not performed due to the small sample size, the results of the current study suggest that cVEMP and

oVEMP testing could be very informative if conducted prior to implantation. 50 % of deaf patients (i.e. three out of six, excluding patient 3 with missing cVEMP data), and 16 % of deaf patients (1 out of six, excluding patient 5 with missing oVEMP data) showed loss in saccular and ocular responses, respectively, following implantation. The cVEMP response was the most affected by surgery, followed by the oVEMP response, whereas the vHIT response yielded no change following CI surgery. Those results were consistent with those of Robard et al. (2015) and Maheu et al. (2017), who revealed a significant effect of CI surgery on cervical and ocular VEMP responses. Those results were also in agreement with those of histopathological studies, which showed that the saccule is the most affected vestibular organ following implantation, followed by the utricle, whereas the SCCs are the least affected by CI surgery (Handzel et al., 2006; Tien and Linthicum, 2002). As can be seen in Table 7.12, none of the deaf patients had any change in the vHIT outcome, showing that the SCCs' function was not affected by implantation in the patients in the present study. A further study with a larger sample of patients is needed to confirm these findings.

One patient (7) in the current study had received the implant on the ear with better balance function, ending up with bilateral utricular dysfunction. This result highlights the importance of performing a comprehensive balance assessment before unilateral implantation. Evidence of abnormal vestibular function on one side could influence the decision regarding which ear to implant. This will need to be weighed against other factors in the decision such as surgical considerations, audiological differences and patient preference (UK Cochlear Implant Study Group, 2004), but pre-implant assessment could help to avoid implanting an ear with good vestibular function over one with poor function. Generally, only caloric testing is used as a pre-implant assessment, so saccular and utricular function is not considered.

In the present study, there appeared to be an association between short-term dizziness and postoperative changes in VEMP responses in CI recipients, although the sample size is low. However, not all the implanted patients who showed loss of saccular and ocular function also suffered from dizziness postoperatively. The incidence of dizziness in the deaf patients was low and short-lived following implantation. Only three of the deaf patients (1, 4, and 7) reported some dizziness for a couple of days or weeks following surgery. In all these patients, otolith function was affected by implantation. This appears to be consistent with short-term dizziness resulting from a change in otolith function, which is then

compensated for. The short-term dizziness associated with a change in VEMP responses also suggests that the VEMP response indicates underlying otolith function and is not simply altered due to a change in sound transmission from implantation.

Three patients (2, 5, and 6) had abnormal vestibular function prior to implantation, but reported no change in their balance problems after implantation. For CI candidates with pre-operative balance problems, the importance of preserving residual function should be considered. In addition, knowing where vestibular function has been altered by CI may help to target vestibular rehabilitation for patients who may be at risk of post-surgical dizziness. In general the patients in this study showed a good compensation for their balance deficit following implantation, indicating that CI surgery did not affect the quality of life of these patients in the long term. These results were consistent with those of Abouzayd et al. (2016), who found a poor correlation between the outcome of the objective tests and patients' reported balance symptoms and attributed this to the central compensation for vestibular dysfunction. It would be useful to test balance function over a long postoperative period in order to evaluate the stability of the vestibular compensation for any deficit.

Improved vestibular test methods now allow differential assessment of individual vestibular organ function and this may lead to a better understanding of how CI affects the vestibular system. As many CI candidates have some vestibular function, pre-implant vestibular assessment may help to inform which side of implantation may best preserve that function, where other audiology and surgical considerations are equal. Post-implant assessment with VEMP may help to predict short-term dizziness. More work with a larger sample will be needed to make the case for routine clinical assessment.

7.5 Conclusions

In this study the sound evoked cVEMP, presumed reflecting saccular function, was most affected by implantation and was associated with short-term dizziness in some patients, although long term vestibular abnormality was not seen. Several patients had abnormal vestibular function pre-implant, which suggests that pre-implant multimodal vestibular assessment function may help to inform side of implantation and preserve residual vestibular function post implant. Post-implant

assessment including VEMP may help to predict short-term dizziness and target vestibular rehabilitation. This study highlights the potential benefits of conducting multimodal vestibular function pre and post cochlear implantation.

Chapter 8 : Conclusions, limitations and further research

This chapter summarises the main findings of the research studies reported in this thesis and draws conclusions based on these findings (section 8.1). It then discusses limitations of the experimental research studies and suggests potential further research (sections 8.2 and 8.3).

8.1 Summary and conclusions

The main goals of this research project were to improve cVEMP measurements by developing new stimulation and analysis approaches and to assess the clinical potential of these methods.

Measurement of cVEMP responses to AM tones (S-VEMP) was investigated in Experiment 1. The aim was to provide a frequency-specific stimulus that is more specific than the tone bursts that are commonly used in standard VEMP stimulation. Another aim was to reduce the testing time, because more than one frequency can be tested in both ears simultaneously. It appears that responses to 500 Hz AM tones could be recorded from the SCM muscle, but not in many subjects. Although they showed standard VEMP responses, most subjects did not respond to S-VEMP. Therefore, it can be concluded that the response to a short tone burst is not predictive of the response to an AM tone of long duration. It was unexpected that so few subjects would have S-VEMP responses. However, the characteristics of the stimulus that trigger the cVEMP response are not yet well understood, so it was decided to explore the adaptation of cVEMP to the repetition rate and stimulus duration.

From the results of Experiment 2, on the stimulus rate, it was established that responses could be recorded from the SCM muscle to 500 Hz tone bursts at high rates, but only in a few subjects. The variations in the percentages of fast- and slow-twitch fibres across the subjects might explain the finding, in the current study that only a few subjects reported responses at high repetition rates, while the majority did not. Regarding the trade-off between recording time and

response quality, the rate of 10 Hz appeared to be optimal in most subjects, based on the results of this study.

The results of Experiment 3, on the stimulus duration, suggested that it was the onset of the stimulus that generated the cVEMP response. This was supported by the lack of significant shifting in latency with increased plateau durations, in which the peak level and the ramp duration were constant in all recordings. Thus, these findings suggest there is no benefit in using plateau durations longer than 4 ms for AC cVEMP. Using shorter plateau durations reduced the level of sound exposure during cVEMP recording, in addition to frequency specificity. Thus, the low response rate of S-VEMP found in the first study could have been due to the absence of a sharp onset of the AM tone stimuli used to evoke S-VEMP. In addition, the thresholds of S-VEMP were higher than those for cVEMP. Therefore, noise exposure is a limitation of the S-VEMP. Such noise exposure is problematic for routine clinical work, which may suggest that S-VEMP is not promising, based on the current study.

Experiments 1 to 3 explored whether changes in the stimulation paradigms can improve cVEMP measurement. Experiment 4 turned to the question of whether cVEMP responses can be better detected using objective (statistical) analysis than by conventional subjective analysis. It was established that objective analysis methods are more sensitive than subjective analysis, can detect responses rapidly and can reduce the variability of subjective estimation of cVEMP thresholds. Because of their sensitivity and efficiency, objective analysis approaches appear to be a promising alternative to the subjective estimate of the presence of the response in threshold studies.

Experiments 1 to 4 have succeeded in improving the measurement paradigms for cVEMP. Experiment 5 turned to clinical applications of cVEMP in patients with MD. It was found that cVEMP-tuning curves provided by objective analysis methods were broadly similar to those provided by subjective analysis in previous studies. Hence, objective detection approaches can be used to measure saccular frequency-tuning curves, which has not been previously reported in the literature. Both the amplitude and threshold measures showed that the best frequency response in cVEMP tuning was 500 Hz. The impairment of both ears in bilateral MD patients can be detected using both these measures. However, the threshold measures demonstrated less variance compared with the amplitude measures, which indicated that the former was the superior clinical tool.

Experiment 5 was also conducted to compare the sensitivity and specificity of objective saccular tuning curves with other objective approaches to detect MD, such as ECochG. The results of both objective tests showed low to moderate sensitivity and high specificity in identifying MD cases. However, the sensitivity of ECochG to the disease was increased when the patients were symptomatic during test recording. Hence, the results indicated that the ECochG was superior to cVEMP tuning in clinical use. In addition, cVEMP tuning curves require a long duration and high sound levels, which are limitations of this test, particularly in patients with tinnitus.

Experiment 6 was conducted to examine the effects of unilateral CI surgery on each sensory organ in the balance system in deaf adults pre-and post-operatively, which are rarely considered in the existing literature. Although a statistical analysis was not performed because of the small sample size, the results of the preliminary study suggested the importance of evaluating the function of the otolith organs prior to implantation. The otoliths were more affected by the implantation than the SCCs were. It appeared from these results that VEMP is affected by implantation, which is correlated with short-term dizziness in some patients. Thus, post-implant assessment with VEMP may help to predict short-term dizziness. Several patients had vestibular function before the implantation. Hence, the vestibular function should be preserved because it may be important. However, clinical studies based on larger samples are necessary to confirm these findings.

8.2 Limitations of experimental research

Although the research aims were fulfilled, there were some limitations. The first study (Experiment 1) aimed to record VEMP responses to AM tone. However, modulated VEMP could not be recorded in the majority of potential volunteers, so the power of the study was low. It seems likely that it is hard to record S-VEMP while staying within ISVR noise exposure limits, although this is not conclusive; this is because S-VEMP thresholds are higher than those for standard cVEMP. Hence, avoiding breaching the ISVR noise exposure limits was a restriction in this study.

Experiment 4 aimed to explore objective methods for the detection of cVEMP response. Although it was found that objective analysis with an HT^2 test was

more sensitive than subjective analysis in detecting cVEMP responses, there was a lower-than-expected FPR for the HT^2 test: indicating it is significantly conservative. Probably further work is needed to explore this in more depth.

Experiment 5 aimed to compare the diagnostic power of the objective cVEMP-tuning curve and ECochG in the diagnosis of patients with definite MD. However, the conclusions of this study are limited, as ECochG testing was not used in as optimal a way as cVEMP measurement, which was optimized in this study through improved stimulation and analysis of responses. The sensitivity of ET ECochG testing would be improved if other parameters were included in the data analysis, such as the SP/AP area ratios, which could not be measured in this study because an outdated version of the equipment was used. Another limitation of this study is that it was not possible to evaluate the sensitivity of cVEMP and ECochG tests in identifying chronic and acute patients with MD over a lengthy period, because of the limited amount of time available for the PhD study. Furthermore, as mentioned previously (see Chapter 6), ECochG is vulnerable to subjective bias, which cannot be completely avoided. Comparing the SP/AP amplitude ratios determined from the same tracings by other audiologists was not possible, due to the limited amount of time available for conducting this study in Jordan.

Finally, Experiment 6 aimed to find out if there is an effect of CI surgery on balance function. However, the number of patients recruited within the limited amount of time available for the PhD limited my ability to conduct a statistical analysis of the data, and thus a case-by-case approach was presented for CI recipients. The generalisability of the results would be improved if a greater number of patients were recruited. Another limitation of this study is that objective analysis for VEMP responses could not be applied in the CI study, as there was not an appropriate programming environment in the Bio-Logic AEP system used with the CI study for implementing the methods. There was also a time problem, in that when this study began on patients, the objective method was not developed and thus was not implemented at that time.

8.3 Suggestions for further research

A number of areas for possible further research are proposed.

In Experiment 4, it was found that cVEMP can be automatically detected using statistical methods such as the HT^2 test, which outperformed the subjective estimations of the test results. Therefore, the HT^2 test should be studied in a larger population because of its sensitive and efficient detection technique. It was also found that there was a lower-than-expected FPR for the HT^2 test: it was significantly ($p<0.05$) conservative. The issue of low specificity (FPR) may be due to violation of independence. This needs more investigation, as does optimizing the analysis parameters for VEMP (there is probably an interaction between filter settings, stimulus rate, and degree of independence). A study by Chesnaye et al. (2018) explored this interaction in relation to ABR and it could be optimized for VEMP.

Due to time limitations, it was not possible in Experiment 5 to evaluate the sensitivity of ECochG and cVEMP tests in identifying chronic and acute MD patients over a long period of time. Further larger clinical studies are necessary to confirm the limited clinical utility of these objective methods, in particular cVEMP, for identifying MD cases. Furthermore, the ECochG SP/AP area ratio analysis approach could not be used for measurements in the present study because an outdated version of the equipment was used for the ECochG recording. Thus, further research should be conducted to assess which ET ECochG parameter or combination of parameters is the most sensitive and specific to diagnose MD, and to compare them with the objective VEMP tuning curve in patients with definite MD.

The effects of unilateral CI surgery on each sensory organ of balance were examined in this research (Experiment 6). However, this study was underpowered, making it difficult to generalise the statistical analysis of this research project to the whole population. Because of the small sample size, a case-by-case approach was presented for CI recipients instead of statistically analysing the data. The current study was only conducted on seven deaf CI recipients. As pointed out previously, it was not possible to recruit the intended number of subjects. Replicating the study with a larger number of patients would be necessary to draw solid conclusions about the separate effects of CI surgery on each vestibular end organ and to confirm the findings of Experiment 6.

A correlation was found between short-term dizziness and postoperative changes in VEMP responses in some, but not all, of the CI recipients in Experiment 6. Among these patients, otolithic function was affected by CI, and it appeared to coincide with short-term dizziness, which the body compensates for over time. Further work with a larger population is needed to measure postoperative function of vestibular end-organs and evaluate the stability of vestibular compensation.

Appendix A.

Anatomy and physiology of the vestibular system

The vestibular system in most mammals, is the sensory apparatus that is responsible for providing conscious realization of spatial orientation in relation to gravity, maintaining postural equilibrium and coordinating head position and eye movement throughout motion (Jacobson & Shepard, 2008). This system allows the body to change orientation and maintain sense of balance whilst making complex movements. The vestibular end organs send neural signals regarding head movement and gravitational forces primarily to the centres that control eye movements and to the muscles that keep the position upright.

In mammals, auditory and vestibular sensory receptors reside in the inner ear within the membranous labyrinth, which are in the bony labyrinth of the petrous part of the temporal bone. The bony and membranous labyrinth are separated by perilymph fluid, which has high sodium ion (Na+) concentration, and low potassium ion (K+) concentration and is formed by blood plasma (Jacobson & Shepard, 2008). The membranous labyrinth is filled with endolymph fluid, which is rich in potassium ions and deficient in sodium ions and is thought to be produced by the stria vascularis, which is located along the lateral wall of the cochlear duct, and absorbed by the endolymphatic sac (Jacobson & Shepard, 2008). It is thought that the stria vascularis helps in maintaining the high proportion of potassium ions to sodium ions in the endolymph fluid (Colman, 1987). It is also thought that the dark cells of the vestibular organs can maintain the ionic composition of inner ear fluids (Colman, 1987). However, the process for producing the endolymph and maintaining the ionic composition of the inner ear fluids are still poorly understood. Excess production or malabsorption of endolymph that fills both cochlear and vestibular structures may cause distention of the endolymphatic spaces, which is referred as endolymphatic hydrops, as seen in MD.

The vestibulocochlear nerve is the eighth paired cranial nerve (CN VIII), which is divided into two branches, vestibular and cochlear fibres, which are responsible for sense of hearing (cochlear nerve), and balance (vestibular nerve). The cranial

vestibular nerve is comprised of two divisions, superior and inferior fibres; the superior vestibular nerve carries vestibular afferent information to the brainstem from the utricular macula and the ampullary cristae of the anterior and horizontal SCCs, while the inferior vestibular nerve carries vestibular afferent information from the saccular macula and the ampullary cristae of the posterior SCCs (Fife, 2010). In normal vestibular function, the vestibular nerve carries the sensory information carried by the vestibular hair cells of the two otolith organs and the three SCCs through the vestibular ganglion that contains the cell bodies of the afferent vestibular neurons.

The sensory inputs from the vestibular end organs' receptors in the inner ear are processed through three reflex pathways which are involved in maintaining head and eye coordination, keeping upright posture and equilibrium, and providing awareness of spatial orientation in relation to gravity: the VOR and the VCR and the vestibulospinal reflex (VSR) (Jacobson & Shepard, 2008). The VOR, which is initiated when the SCCs detect head rotation, can maintain the stability of the images on the fovea of the retina through head movement by generating rapid compensatory eye movement. The eyes normally move in the opposite direction of head movement with the same velocity and this is expressed as the gain of VOR. It is defined as the ratio of eye velocity to head impulse velocity (Alhabib and Saliba, 2017). The amplitudes of the eye and corresponding head impulse should be equal with the opposite phases (gain is usually close to -1). The VCR stabilizes head position and the direction of gaze in space by generating a compensatory response by the neck muscle (Wilson and Schor, 1999). Movement of the head in space due to either rotation of the entire body or rotation of the head on the neck is detected by vestibular receptors (otolith organs and SCCs), leading to activation of the VCR reflex (Wilson and Schor, 1999). The VSR maintains upright posture and head stabilization. When vestibular sensory neurons detect body movements, the vestibulospinal tract send motor signals to specific muscles to respond to these movements and to re-stabilize the body and keep the upright posture (Jacobson & Shepard, 2008).

Appendix B.

Caloric Testing (Evaluation of horizontal canal function)

Caloric testing is the most frequently used objective test carried out in clinics to identify and localise peripheral vestibular disorder (Bell et al., 2015; Perez & Rama-Lopez, 2003). Nevertheless, a caloric test is limited, in that it stimulates and assesses the function of the right and left lateral (horizontal) SCCs only at very low frequencies (about 0.002-0.004 Hz) (Perez & Rama-Lopez, 2003). As a result, the sensitivity of the caloric test is not 100 %, as it is only able to detect vestibular loss in the low frequency range. Although 100 % canal paresis does not indicate complete vestibular loss, this may be due to a critical absence of low frequencies and the presence of high frequencies (Beynon et al., 1998). The poor sensitivity of the caloric test in detecting vestibular dysfunction in vertical canals and in the high-frequency range means that there is a need for other reliable and objective tests to reveal dysfunction in the peripheral vestibular system. The function of the VOR can be evaluated by using the caloric reflex test (Jacobson & Shepard, 2008). Vestibular responses are typically obtained by using bithermal caloric irrigation (cool water at 30° C and warm water at 44° C) or air irrigation (Perez & Rama-Lopez, 2003). The difference in temperature between the body and the injected water produces a current in the endolymph of the horizontal SCCs that can eventually cause cupular deflections similar to those produced by head rotation (Jacobson & Shepard, 2008). Irrigating the external ear with a medium that is warmer or cooler than the body temperature produces a temperature gradient in the irrigated ear (Jacobson & Shepard, 2008). The temperature gradient may change the density of the endolymph on the side of the horizontal SCCs (Jacobson & Shepard, 2008). Consequently, warm water causes the endolymph in the horizontal SCCs to become lighter and rise, causing deflection of the cupula (excitation), and therefore increasing the firing rate of the vestibular afferent nerve of the irrigated ear (Jacobson & Shepard, 2008). Such a deflection causes an excitation response in the lateral SCCs and produces horizontal nystagmus (compensatory eye movement without head movement) toward the ipsilateral ear. Conversely, cold water causes the endolymph in the ipsilateral horizontal SCCs to become denser and fall and thus decreases the

firing rate of the vestibular afferents (Jacobson & Shepard, 2008). Such a deflection causes an inhibitory response in the lateral SCCs and generates horizontal nystagmus toward the contralateral ear. Thus, for individuals with normal vestibular function, warm and cold water generate currents in opposite directions and consequently a horizontal nystagmus (fast phase) in opposite directions. A unilateral vestibular lesion is identified when the asymmetry in the maximum slow-phase velocity of nystagmus responses between the right and left ear exceeds 20 %. However, caloric stimuli are not always tolerated and are unpleasant for the patient. Thus, there is also a need for a reliable, fast, convenient, well-tolerated and objective method.

Appendix C.

Frequency Tuning of cVEMP and oVEMP in response to acoustical and vibratory stimulation

VEMPs can be evoked using both ACS and BCV, although the two methods of stimulation may differ with regard to the activation pattern of the end organs. Whether the end organ originally responsible for producing cVEMP and oVEMP in response to ACS and vibration is the utricle, saccule or both remains debateable (Brantberg et al., 2004; Curthoys, 2010; Curthoys et al., 2006; Curthoys et al., 2011; Curthoys, 2012; Curthoys & Vulovic, 2011; Govender et al., 2011; Park et al., 2010; Piker, 2013; Rosengren et al., 2010; Todd et al., 2009; Todd et al., 2000; Todd et al., 2007; Todd et al., 2008; Young et al., 1977; Zhang et al., 2011).

cVEMP and oVEMP evoked by ACS

Tuning investigations that measure AC-evoked cVEMP in healthy subjects have indicated peak response amplitudes (largest amplitude and lowest threshold) occurring at approximately 500 Hz for tone-burst stimuli (Akin et al., 2003; Murofushi et al., 1999; Park et al., 2010; Piker et al.,2013; Rauch et al., 2004). Welgampola and Colebatch (2001) found that the optimal tuning frequencies with the largest amplitude for VCR were evoked by ACS between 500 Hz and 1 KHz (around 700 Hz). Node et al. (2005) reported that the peak amplitude of cVEMP was between 500 Hz and 700 Hz. These findings are higher than those of by Todd et al. (2000), who observed a maximum response frequency between 300 and 350 Hz. The differences in the tuning frequencies of VEMP response obtained by different studies can be related to differences in the tone-burst stimuli that probably have different frequency specificity, particularly differences in the duration of tone bursts (rise/fall and plateau duration) and number of cycles used for VEMP stimulation. Node et al. (2005) evoked VEMP by tone-burst stimuli at different frequencies (250, 500, 700, 1000, 2000 and 4000 Hz) with a 6 ms duration (1 ms rise/fall time, 4 ms plateau). Todd et al. (2000) evoked cVEMP by 10 ms tone-burst stimuli at different frequencies (100, 200, 400, 800, 1600 and 3200 Hz) with a 1ms rise/fall time. However, the rise/fall duration of tone-burst stimuli might not be frequency specific for frequencies below 1 KHz, as the cycle

duration is 10 ms for 100 Hz, 5 ms for 200 Hz, 2 ms for 500 Hz, 4 ms for 250 Hz and 1.4 ms for 700 Hz. In other words, a 1ms rise/fall could produce a half cycle for 500 Hz and a quarter cycle for 250 Hz, which could affect the frequency specificity of the stimuli. Further research is required to measure the frequency specificity of different durations of tone-burst stimuli.

Park et al. (2010), Chihara et al. (2009) and Piker et al. (2013) reported maximum tuning of AC-evoked oVEMP amplitudes at 500 Hz, whereas Todd et al. (2009) reported the greatest amplitude response in the range 400-800 Hz. Results of tuning studies on AC-evoked oVEMP were similar to studies on AC-evoked cVEMP (Akin et al., 2003; Murofushi et al., 1999; Node et al., 2005; Park et al., 2010; Piker et al.,2013; Rauch et al., 2004; Todd et al., 2009; Welgampola and Colebatch, 2001). Todd et al. (2009) and Park et al. (2010) observed similar tuning for both reflexes in response to acoustical stimulation, with the largest amplitude response for stimuli at 400-800 Hz (Todd et al., 2009) and 500 Hz (Park et al., 2010). Thus, these findings support the suggestion that the same afferents contribute to both VCR and VOR reflexes, as the tuning of AC-evoked cVEMP is similar to that of AC-evoked oVEMP. One study by Zhang et al. (2011) reported an additional tuning peak of AC-evoked oVEMP at low frequency, 100 Hz. These authors described two separate peaks for AC-evoked oVEMP; the larger tuning peak occurred at around 600 Hz and the smaller tuning peak at approximately 100 Hz.

cVEMP and oVEMP evoked by BCV

Previous studies assessing vibration-evoked oVEMP and cVEMP have reported a low bass tuning, with the largest response at 100 Hz for both oVEMP and cVEMP (Todd et al., 2009). The largest response for vibration-evoked VEMP was obtained at 100 Hz for both projections, suggesting that this effect was not related to the end organ's response (Todd et al., 2009). Todd et al. (2008) reported that oVEMP showed a highly tuned response, with a maximal amplitude response at 100 Hz in response to whole head vibration (transmastoid plane). Sheykholeslami et al. (2001) reported that cVEMP showed a definite frequency-sensitive response, with a maximum amplitude at frequencies between 200 Hz and 400 Hz in response to bone-transmitted vibration using a bone conductor with short tone-bursts (100, 200, 400, 800, 1600 and 3200 Hz). Welgampola et al. (2003) reported that a frequency of 250 Hz delivered over the mastoid using BC tone-bursts at 250-2000 Hz frequencies produced the maximum amplitude response for cVEMP. This tuning frequency of 250 Hz was within the tuning range (200-400 Hz) suggested by Sheykholeslami et al. (2001). Again, the differences in the best frequency of VEMP

obtained by different studies can be attributable to differences in the tone-burst stimuli that probably have different frequency specificity, particularly differences in the duration of tone-bursts (rise/fall and plateau duration) and number of cycles used for VEMP stimulation. Sheykholeslami et al. (2001) used short tone-burst stimuli (100, 200, 400, 800, 1600 and 3200 Hz) with 10 ms duration (1 ms rise/fall and 8 ms plateau).Todd et al., (2009) used 10 ms tone-bursts with 5 ms rise/fall time (1:0:1 cycles). Welgampola et al. (2003) used tone-bursts at 250-2000 Hz frequencies with1 ms rise/fall time and a 7 ms plateau time. In a study by Todd et al., (2008), the length of tone-burst stimuli was 5 cycles (1cycle rise, 2 cycles plateau and 2 cycles fall). Further research is needed to measure the frequency specificity of different durations of tone-burst stimuli. From the tuning findings, the differences in the tuning frequencies between ACS and BCV stimuli for both reflexes are likely to originate from differences in the otolith organs, which may be preferentially stimulated by each stimulus. However, more research is required to investigate the tuning of cVEMP and oVEMP when evoked by BCV.

The origin of frequency tuning to ACS and BCV

It is essential to discuss the anatomical evidence of otolith organs before turning to the specificity of the response of utricular and saccular macula to acoustical and vibratory stimulation. Anatomical evidence regarding the otolith organs indicates that afferents from the utricular macula and the ampullary cristae of the anterior and horizontal SCCs travel in the superior vestibular nerve, together with a small bundle of the fibres from the hook region of the saccular macula, while the bulk of nerve fibres from the saccular macula together with afferents from the ampullary cristae of the posterior SCCs run in the inferior vestibular nerve (Uzun et al., 2007; Uzun-Coruhlu et al., 2007).

It has been reported that the ACS activates neural responses similar to those activated by the BCV. Evidence from animal studies on guinea pigs and squirrel monkeys suggests that both ACS and BCV can activate a high proportion of irregular vestibular neurons from both otolith organs (Curthoys & Vulovic, 2011; Curthoys et al., 2012; Young et al., 1977). Young et al. (1977) found that stimulation of both ACS and BCV activate the saccular afferents of squirrel monkeys; similarly, Curthoys and Vulovic (2011) observed that the utricular afferents of guinea pigs may be stimulated by both types of stimulation. Hence, the afferent specificity of one end organ for ACS and BCV was not supported in these studies, as most, but not all, of the vestibular afferent neurons that can be

evoked by ACS can also be activated by BCV (Curthoys, 2012; Curthoys, 2010). To sum up, evidence has indicated that both ACS and BCV can activate the utricular macula (Curthoys & Vulovic, 2011) and both ACS and BCV can activate the saccular macula (Young et al., 1977). However, these studies used 500 Hz frequency for BCV, which has been suggested as the frequency tuning for the saccule in several studies (Akin et al., 2003; Park et al., 2010; Piker et al., 2013; Rauch et al., 2004).

In order to understand how activation of the vestibular receptor by ACS and BCV can evoke behavioural responses that can be measured in individuals by cVEMP or oVEMP, evidence about the neural projections of VOR and VCR should be discussed. Uchino's research on cats to elucidate otolith-ocular and otolith-spinal neural projections has shown that the utricular and saccular maculae have different neural projections (Uchino et al., 2005). Uchino's group used localised electrical stimulation of 1228 vestibular nerve branches in anaesthetized cats to identify the VCR and VOR neural projections. These authors reported that only approximately 30 % of the extraocular muscle motoneurons responded to saccular nerve activation, while 100 % of the neck muscle motoneurons responded. Saccular neurons have a strong projection to the neck muscles and a weak projection to the extraocular muscles (Uchino et al., 2005). Accordingly, the neural connections of sacculo-collic and utriculo-ocular systems are relatively strong compared to the neural connections of the sacculo-ocular system (Uchino & Kushiro, 2011).

Based on the evidence of the differential strengths of the neural projections of the utricular and saccular maculae, and because of the stimulation of the otolithic receptor by both ACS and BCV, it is thought that these measured responses of the neck muscle to either ACS or BCV originate primarily from the saccular function, while the responses of the oculomotor muscle to either ACS or BCV are thought to originate primarily from the utricular function (Curthoys, 2012). Hence, the responses to ACS or BCV may represent either the saccular or the utricular contribution.

Clinical evidence from patients with acute vestibular neuritis (AVN) supports the hypothesis that cVEMP and oVEMP test the function of utriclular and saccular maculae independently. Manzari et al. (2010) and Iwasaki et al. (2009) measured the responses of oVEMP n10 and cVEMP for patients with unilateral superior AVN but preserved inferior vestibular nerve. All of these patients showed cVEMP P13-N23 to either ACS or BCV on the affected side, while oVEMP n10 to ACS or BCV was reduced or absent on the contralateral side (opposite to the affected ear). This outcome has been taken to suggest that utricular and saccular afferents are

activated by both stimulation of ACS and BCV, so oVEMP and cVEMP are independent measures that can be used to differentiate the function of saccular and utricular maculae (Curthoys, 2010).

However, previous studies on the effect of ACS on vestibular receptors have suggested that acoustical stimulation is probably appropriate for saccular afferent neurons based on both clinical (Brantberg et al., 2004; Murofushi et al., 1996; Tsutsumi et al., 2000; Welgampola & Colebatch, 2001) and animal studies (Murofushi & Curthoys, 1997). AC cVEMP was absent in patients with inferior vestibular nerve dysfunction, but present in patients with intact superior vestibular nerve (Colebatch et al., 1998; Murofushi et al., 1996; Tsutsumi et al., 2000). Tsutsumi et al. (2000) found a complete absence of AC cVEMP in patients with unilateral vestibular schwannomas in which the origin of the tumours was the inferior vestibular nerve. In addition, Murofushi and Curthoys. (1997) found that most saccular afferents in guinea pigs were sensitive to click sounds, but there was some labelling for utricular afferents. This was confirmed by tracing the origin of the sensitive afferents to click sounds by using extracellular biocytin injections. These findings suggest the saccular sensitivity of ACS and that is potentially due to the proximity of the saccule to the oval window, the stapes footplate and the cochlear duct (Rosengren et al., 2010; Todd et al., 2009). However, the mechanism that stimulates the stapes footplate by ACS, which leads to deflection in the vestibular hair cells, is still unknown (Curthoys, 2010).

Manzari et al. (2012) measured cVEMP and oVEMP in response to BCV at the mid forehead for 59 patients with unilateral inferior VN. All patients showed a reduced or absent cVEMP P13-N23 component on the affected side, while their oVEMP n10 component was normal. Based on this evidence, it appears that the inferior vestibular neuritis affects cVEMP, while superior VN affects oVEMP. This outcome has been taken to suggest that utricular and saccular afferents are activated by both stimulation of ACS and BCV, so oVEMP and cVEMP are independent measures that can be used to differentiate the function of saccular and utricular maculae (Curthoys, 2010). The reason could be related to the brainstem weighting of saccular function to the ipsilateral SCM and utricular function to the contralateral inferior oblique muscle (Curthoys, 2010; Piker, 2012). As a result, the specificity of the vestibular system to acoustical and vibratory stimulation is mistakenly interpreted in the literature as motor-related rather than sensory (Curthoys, 2010). Vibratory and acoustical stimulation produced similar patterns with few differences

in the tuning for the projection of both cVEMP and oVEMP (Todd et al., 2009). Vibration showed a low bass tuning with the biggest response at 100 Hz, which may be specific for utricular activation (Todd et al., 2008; Todd et al., 2009), whereas ACS produced the greatest amplitude of response in the 400-800 Hz range (Park et al., 2010; Todd et al., 2009), which may be specific for saccular activation.

Nevertheless, recent studies have reported the possible contribution of the utricular macula to acoustical stimulation, predominantly for oVEMP (Curthoys, 2010; Kinoshita et al., 2013; Shin et al., 2012). In patients with VN, selective involvement of the superior or inferior vestibular nerve suggests that the cVEMP and oVEMP test different vestibular nerve afferents. This has been confirmed by the dissociations in the abnormalities of cVEMP and oVEMP (Shin et al., 2012). In Shin et al.'s study, AC-evoked oVEMP was often abnormal and AC-evoked cVEMP was mostly unaffected in patients with unilateral superior AVN, suggesting that the oVEMP to ACS is predominantly mediated through the supervisor vestibular nerve, probably due to utricular receptors (Curthoys, 2010; Govender et al., 2011; Kinoshita et al., 2013; Shin et al., 2012). oVEMP is mediated by fibres in the superior part of the vestibular nerve, whereas cVEMP is mediated by fibres in the inferior part of the vestibular nerve. Piker (2012) and Curthoys (2010) have suggested that the saccule is the end organ responsible for generating cVEMP, whereas the utricle is the end organ responsible for generating oVEMP in response to ACS. These findings support the effect of ACS on the utricle, predominantly for frequencies of around 100 Hz, whereas frequencies from 400–800 Hz have a strong effect on the saccule (Zhang et al., 2011).

Both animal (Curthoys et al., 2006) and clinical studies (Govender et al., 2011) found that vibratory stimulation preferentially activated the utricular afferents. Curthoys et al. (2006) found that most of the irregular otolithic afferents (82.8 %) in guinea pigs which showed an increased response in the firing rate to BCV were probably from the utricular afferents, as they were in the superior division of the vestibular nerve. This was confirmed through the juxtacellular injection of neurobiotin into the activated neurons of guinea pigs to trace the site of their origin to the utricle macula. Govender et al. (2011) showed a similar reduction in the amplitudes of the response in the affected ear for both reflexes in response to lateral BCV impulses in patients with VN. This suggests that lateral impulses are expected for utricular receptors, in comparison with forehead taps and the lower rates of abnormalities for both cVEMP and oVEMP (Govender et al., 2011).

Variation in the tuning results of ACS maybe due to incorrect electrode placement, different stimuli for evoking VEMP, insufficient SCM neck muscle in cVEMP and low gaze in oVEMP. However, there is also some variation in BCV tuning, which may be due to differences in using different head acceleration directions. The direction of applied impulsive transmastoid acceleration is considered the main determinant of the vestibular response (Todd et al., 2008). From these findings, it appears that the sensitivity of the end organ afferent neurons to the direction and orientation of the linear acceleration of the bone vibrator at the mastoid may affect the tuning of otolith organs to bone vibratory stimulation. The two-otolith organs can be specifically stimulated by linear acceleration; the utricle receptors are most sensitive in the horizontal plane, whereas the saccule receptors respond best in the sagittal plane (Goldberg & Fernandez, 1984; Todd et al., 2008). This is because the utricle is located in the same plane as the lateral SCC and the saccule is located vertically when the head is upright. However, changing the orientation of the bone vibrator could stimulate different regions of the end organs. More research is needed to measure the directional selectivity of bone vibrator.

To sum up, it is still not known whether the specificity of the vestibular system to acoustical and vibratory stimulation is motor-related or sensory (Curthoys, 2010). In other words, either the VEMP projections (motor) or stimuli (sensory) can differentiate the utricular and saccular function. Some studies argue that ACS for both projections selectively activates the saccule and that BCV selectively activates the utricle, whereas others claim that utricular and saccular afferents are activated by stimulation of both ACS and BCV. According to this argument, cVEMP tests the saccular function, whereas oVEMP tests the utricular function. AC cVEMP may therefore be responsible for the saccule, whereas BCV oVEMP may be responsible for the utricle when the specificity of both the sensory and motor afferents by one sense organ is considered.

Appendix D.

Vestibular Migraine (VM) and MD

Several studies have reported that symptoms of VM have a significant overlap with those of MD (Baier & Dieterich, 2009; Battista, 2004; Ghavami et al., 2015; Shepard, 2006; Thompson & Amedee, 2009). Although both disorders may present similar clinical signs and symptoms, they have different pathophysiological mechanisms (Battista, 2004). Migraine is a dysfunction in the neural and vascular central nervous system as a result of successive contraction and dilation of intracranial vessels (Baloh, 1997; Thompson & Amedee, 2009). MD, in contrast, is a peripheral labyrinthine dysfunction and the presence of endolymphatic hydrops is considered the histological marker for developing the disease (Battista, 2004). Patients with migraine usually experience periodic headache attacks, but they also often experience other associated symptoms including sensitivity to daily stimuli such as sound (phonophobia) or light (photophobia) and head movements. They also suffer nausea (Shepard, 2015; Thompson & Amedee, 2009). It has been shown that migraine can also be associated with dizziness, referred to as migraine-associated dizziness (MAD) or VM. This has been defined as 'recurrent vertigo as a symptom of migraine' (Lempert & Neuhauser, 2009, p 334). Vertigo attacks (illusion of rotation) can occur simultaneously with headache attacks, during the attacks or after the headache attacks, but they most commonly present during the headache, as an aura, persisting from seconds to hours (Baier & Dieterich, 2009; Baloh, 1997). Dizziness has been reported in the majority of patients with VM in a study in which 38 % patients with migraine had vertigo attacks, as defined by the criteria of the International Headache Society (IHS) (Neuhauser et al., 2001; Thompson & Amedee, 2009).

MD is considered one of several vertigo syndromes that are epidemiologically associated with migraine. In one study, Ghavami et al. (2015) reported that the majority of patients with MD (51 %) had migraine headache, as defined by HIS criteria. Additionally, patients with VM may have all the otologic symptoms of definite MD, including low frequency fluctuating SNHL, spontaneous spells of vertigo (Baloh, 1997; Battista, 2004; Shepard, 2006; Thompson & Amedee, 2009), tinnitus and/or aural fullness (Battista, 2004; Shepard, 2006); these symptoms can

be unilateral or bilateral. For definite MD syndrome, episodic vertigo attacks and hearing loss should be present on the affected side (Surgery et al., 1995). Because of the similarities in symptoms between these two disorders, some patients with VM maybe misdiagnosed as having MD. There is a significant overlap in the symptoms of MD and VM, suggesting that differentiation between the two disorders maybe difficult (Baier & Dieterich, 2009). However, it is important to differentiate between the disorders, as the management options are very different for the two diseases (Battista, 2004). Hence, auditory symptoms may be used as an adjunct for differentiation between MD and VM (Battista, 2004).

Several studies have observed that auditory symptoms are generally unusual or less common in patients with VM (Baloh, 1997; Battista, 2004). Battista (2004) reported that PTA average and low-frequency PTA were significantly worse in patients with MD compared with VM patients, suggesting that audiometric results are often normal in patients with VM. However, 3 patients (3 %) with VM had abnormal PTA, but their hearing loss was mild to moderate low-frequency SNHL and relatively stable. The feature that differentiates definite MD from VM is that hearing loss in MD is progressive, and not stable as in VM. These results are considered a contradistinction for patients with MD, suggesting that fluctuating or permanent low-frequency SNHL is the rule (Battista, 2004). However, the audiometric findings become less useful when the patient is in the early stages of MD, where hearing loss may not yet be an issue or where both disorders are present in the same patient (Shepard, 2015). Consequently, segregating patients with MD from those with VM is still controversial. To sum up, these is no clinical test that can unequivocally differentiate between MD and VM. Currently, therefore, differentiation between the two disorders is based on the diagnostic criteria defined by the AAO-HNS (1995) for MD and Neuhauser et al. (2001) for VM (see Table below).

Diagnostic Criteria for MD developed by AAO-HNS in 1995 and VM Proposed by Neuhauser et al. (2001).

Diagnostic scale	Ménière's disease	Vestibular Migraine
Certain	Definite MD with the patient's history	
Definite	• At least two definite episodic spontaneous incidents of vertigo for at least 20 minutes. • Hearing loss in the affected ear. • Tinnitus or ear pressure in the affected ear.	• At least two episodic incidents of vertigo associated with one of the migrainous symptoms including migrainous headache, photophobia, phonophobia, visual or other aura • Current or previous history of migraine (HIS criteria). • Episodic vestibular symptoms (rotational or sensation of motion) with moderate severity.
Probable	• One definite episodic vertigo. Hearing loss. • Tinnitus or ear pressure in the affected ear.	• Episodic vestibular symptoms (rotational vertigo or sensation of motion) with at least moderate severity. • One of the followings: • Migraine (HIS criteria), migrainous symptoms during vestibular symptoms, or migraine precipitants of vertigo (foods, sleep disorders, response to medication (antimigraine drugs), or hormonal changes).
Possible	• Episodic vertigo without hearing loss or SNHL (fixed or fluctuating) with disequilibrium.	

Appendix E.

ECochG Recoding Techniques

The trans-tympanic (TT) and extra-tympanic (ET) are two general approaches for recording ECochG (Ferraro & Durrant, 2006). TT ECochG is an invasive technique which includes inserting a needle electrode through the tympanic membrane (TM), whereas ET ECochG is a non-invasive technique which involves resting electrodes on the TM (called the TM wick electrode) or against the skin of the external ear canal walls (the canal electrodes) (Ferraro & Durrant, 2006). The TT method produces a more robust and larger magnitude ECochG with less signal averaging time, resulting in a more reliable response than ET recording. Thus, the TT ECochG method is preferred to the ET method because of the higher SNR, which may be related to the close proximity of the recording to the cochlea (Filipo et al., 1997). Ruth and Lambert (1989) compared TT and ET ECochG methods in a study of 26 patients with MD. They found that both methods produced clear responses, but that the SP and AP amplitude were higher when using the TT method. However, the TT method has a major limitation related to its invasiveness: penetrating the TM is uncomfortable for patients even under local anaesthetic (Ferraro & Durrant, 2006). In comparison, the ET method can be performed without medical setting (sedation and physician), and with minimal discomfort for patients (Ferraro & Durrant, 2006). Nevertheless, the ECochG response to ET recording needs increased signal averaging time so that it produces a smaller magnitude ECochG response than the TT method (Ferraro & Durrant, 2006). Based on a meta-analysis of different studies, the normal SP/AP ratio for click sound should be greater than 35 % using TT ECochG and 42 % using ET ECochG (Wuyts et al., 1997).

Appendix F.

Cochlear implant questionnaire (A modified version of the VRBQ http://www.isvr.soton.ac.uk/audiology/vrbq.htm)

PRIVATE AND CONFIDENTIAL

The following questions ask about any balance problems or dizziness that you may have experienced. Please try answering the questions as accurately as possible. If you are unsure about how to answer a question, give the best answer you can and make any comments in the space available at the end of the questionnaire.

Name

Date questionnaire completed

Name of Centre Date of implant

Type of implants used (Please approximately specify the quantities of each type)

SECTIONS 1 –BEFORE COCHLEAR IMPLANTATION

Did you suffer from balance problems before you had a cochlear implant? (Yes/No)

If yes, please describe the balance problems that you experienced before cochlear implantation e.g. What were your symptoms? How long did it last? Is there a known cause for the balance problems? Did you receive any treatment for it? (Give a general overview, and if possible note down any specific cases.)

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Appendix F

SECTIONS 2 –In the months following the implant operation

Did you suffer from balance problems in the months following your implant operation?

(Yes/No)

If yes, please describe the balance problems that you experienced in the month following cochlear implantation e.g. what were your symptoms? How long did it last? Is there a known cause for the balance problems? Did you receive any treatment for it? (Give a general overview, and if possible note down any specific cases.)

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