ADVANCEMENT IN CVEMP'S

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"A PESSIMIST SEES THE DIFFICULTY
IN EVERY OPPORTUNITY;
AN OPTIMIST SEES THE OPPORTUNITY
IN EVERY DIFFICULTY."

- Sir Winston Churchill

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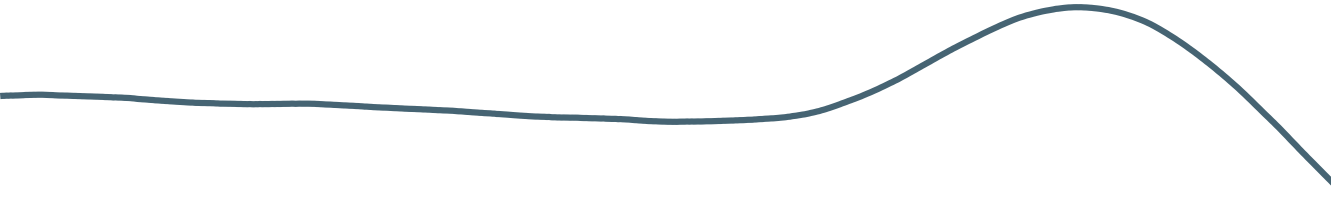

GENERAL INTRODUCTION

INTRODUCTION

Vestibular problems are very common. An average of 22 persons per 1000 between age 30 and 75 in the Netherlands were known by their primary care physician to have a vestibular diagnosis in 2013 (source: CBS). Although only a fraction of these patients will ever need to consult a specialist, balance disorders are a large portion of physical complaints in our society.

CLINICAL PROBLEM

When a dizzy patient consults a specialist, the typical evaluation consists of: a careful history, physical examination, an audiogram and perhaps an ENG. These tests grossly evaluate the middle ear, cochlea and horizontal semicircular canal. The balance organ, however, is much more than just the horizontal semicircular canal, and while much is known about the vestibular apparatus in humans, there are very few tests available to evaluate all parts of the balance organ. This problem is especially relevant in vestibulopathies that do not (always) involve the horizontal semicircular canal such as Meniere's disease and superior canal dehiscence (SCD). In superior canal dehiscence a bony defect of the superior semicircular canal creates a third window, which allows acoustic energy to flow toward the balance part of the labyrinth. This, among other complaints, causes patients to get dizzy in response to loud sounds. Patients diagnosed with Meniere's disease typically suffer from vertigo attacks lasting between 20 minutes and 12 hours with fluctuating hearing loss, aural fullness and tinnitus. The disease could eventually lead to a deaf ear and loss of balance on the affected side. In these patients there is a need for more elaborate evaluation besides the semicircular canals. A number of these tests are described in chapter 1. The cervical vestibular evoked myogenic potential (cVEMP) is such a test, although it has some serious shortcomings limiting its clinical use. This thesis aims to improve the clinical application of the cVEMP.

CVEMP

The cervical vestibular evoked myogenic potential (cVEMP) is a vestibular test that uses sound (or vibration) to elicit a vestibular response resulting in an inhibition in the ipsilateral, contracted sternocleidomastoid (SCM) muscle that can be recorded using EMG electrodes. In recent years the cVEMP has gained attention because it is believed to primarily evaluate the saccule, as

opposed to most other vestibular tests which evaluate mainly horizontal semicircular canal function. Testing the saccule might especially be relevant in patients suffering from Meniere's disease, in which endolymphatic hydrops of the saccule seems to be associated with the disease. There are over 1300 publications on cervical vestibular evoked myogenic potentials in current literature. Even though many studies discuss its clinical use, several have indicated high variability in cVEMP measures. Also, it is still unclear what outcome measure should be used. This doctoral thesis begins with a historic overview of cVEMP discovery and testing followed by the practical parameters involved in cVEMP testing. The second chapter gives an overview of current electrophysiological testing in Meniere's disease. After that a series of publications are presented that attempt to bring the cVEMP closer to reliable clinical use, mainly by correcting for muscle activation and increasing the stimulation rate.

HISTORY OF CVEMPS

Although the primary function of the saccule is to detect linear acceleration in the vertical plane and the position relative to gravity, the cVEMP uses acoustic stimuli to assess the saccule. The acoustic sensitivity of the saccule seems to be an evolutionary remnant since several non-mammalian species, that have no cochlea, use the saccule for sound detection. Humans do not use this function since the cochlea is much more sensitive for processing auditory information.

The acoustic sensitivity of the vestibular system was first investigated by Nobel Prize nominee dr. Pietro Tullio in 1929 (Tullio, P., Das Ohr und die Entstehung der Sprache und Schrift. Berlin: Urban and Schwarzenberg, 1929). He made detailed observations of sound evoked head movement, eye movement and postural changes in animals following surgical fenestration of the bony labyrinth. In 1935, von Bekesy reported vestibular responses to sound in healthy human subjects and provided evidence that the responses were not mediated by the cochlea. After that Huizinga and de Vries recorded the electrical response to sound from the vestibular system from a pigeon (1).

With the development of the digital averager in 1954, small amplitude click-evoked responses could be distinguished from larger background noise by averaging many single responses together. In 1962 Bickford et al. recorded an averaged response on the skull (inion) to loud air conducted tone bursts (2). They found that this

response was primarily myogenic in origin and that the amplitude of the response was related to the tension of the cervical muscles (3). Further investigation by Bickford and colleagues showed that in deaf patients, with intact vestibular functions, the response was still present which led them to the assumption that the response was of vestibular origin. A loud noise to the ear elicits a cochlear response in addition to a saccular one. Bickford showed that the cochlear response differed from the vestibular responses in both latency and amplitude indicating a different origin (3).

A study by Cazals et al. in 1983 showed evidence for the acoustic sensitivity of the saccule by showing acoustic responses in guinea pigs after significant ampullar and utricular destruction in addition to total cochlear destruction with preservation of the saccule(4). Acoustic responses from the vestibular system were demonstrated by McCue and Guinan in cats. Single afferent fibers were stimulated and a sizable fraction of the fibers with irregular activity were acoustically responsive. Using intracellular labeling, the origin of these fibers was found to be the saccule. They found that these acoustically responsive vestibular afferents had a shorter latency to sounds compared to cochlear afferents and a higher threshold (>90dB SPL). The irregular vestibular afferents also had a smaller frequency range to which they were responsive, between 0,1-3kHz.(5). Townsend later found that in patients with Meniere's disease (MD), which is associated with endolymphatic hydrops of the saccule, the VEMP response changed (6). Together with evidence of altered tuning in MD patients, the saccule was thought to be the end organ activated by sound.

In 1994 Colebatch et al. examined the phenomenon and described the same response using electrodes placed on the sternocleidomastoid muscle (SCM) instead of in the inion (7). The characteristics of the VEMP showed a largely linear relationship between sound intensity and tonic EMG level (8). The first positive and negative peaks had a latency of 11.7 ms (SD 0.89) and 20.5 (SD 1.89) respectively (figure 1).

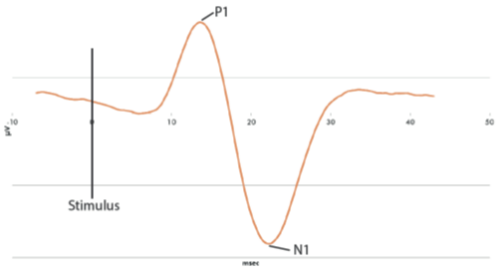

Figure 1. Typical cVEMP response with P1 at 11.7 ms and P2 at 20.5ms.

An early study by Bath et al. showed that if the SCM muscle was

not contracted there was no response, independent of the stimulus intensity (8). This means that the VEMP response depends on a sufficient level of muscle contraction. We now know that the amount of muscle contraction is more or less linearly related to the amplitude of the cVEMP response. Since the purpose of the test is to determine saccular function, elimination of the confounding effect of muscle "noise" is an important subject of research in the present day. There have been serval different methods described to control for muscle contraction variability during cVEMP testing. Examples of these methods are a blood pressure cuff that had to be kept at a certain pressure (9-11). By providing feedback of the amount of muscle contraction subjects could correct activation if needed. Other studies used an EMG graph that showed the subject whether the appropriate contraction strength was applied (12). All these measures were used to reduce the variability of the cVEMP due to muscle contraction with variable ways of success. An automatic correction for muscle activation would be preferable. This process is called normalization and is a major focus of this thesis.

ANATOMY OF HUMAN HEARING AND BALANCE

In humans the hearing and balance organ are located in the inner ear and are closely related. The cochlea and the vestibular labyrinth are in continuity with each other and each consists of a bony labyrinth with a membranous structure inside. The space between the two parts is filled with perilymph, which is flowing from the subarachnoid space. The membranous part is filled with endolymph. The vestibular labyrinth consists of two otolith organs (the utricle and the saccule) and three semicircular canals. In humans the utricle and saccule are sensitive to linear acceleration, with the utricle positioned mostly horizontal and the saccule mostly vertical. The three semicircular canals are placed in different planes, each one perpendicular to the two other canal planes and respond to circular movement in each specific plane.

CVEMP PHYSIOLOGY

Cervical VEMP's depend on the vestibulocollic reflex which arises from the acoustically responsive sensory cells and neurons in the saccule and utricule and is conducted centrally via the vestibular nerve (5). The afferents (nerves leading towards the brain) are susceptible to noise and are known as the otolith irregular afferents (13-15). These afferents project to the vestibular nuclei and cause an inhibition of the contracted ipsilateral SCM when activated by a loud acous-

tic stimulus via the accessory nerve. Single motor unit firing studies showed that the SCM is the dominant muscle for cVEMP response (16). A large body of evidence indicates that the SCM response is predominantly determined by saccular activation (and not utricular). Basta et al. obtained direct evidence concerning the laterality and peripheral origin of the VEMP in humans by recording EMG of the SCM in response to direct electrical stimulation of the vestibular nerve in seven patients during cerebellopontine angle surgery (17). EMG responses were present in the ipsilateral SCM in all patients following electrical stimulation of the inferior vestibular branch of the vestibular nerve (i.e. saccular) whereas they were absent in the contralateral SCM. Electrical stimulation of the superior nerve failed to produce an EMG response from the SCM muscle.

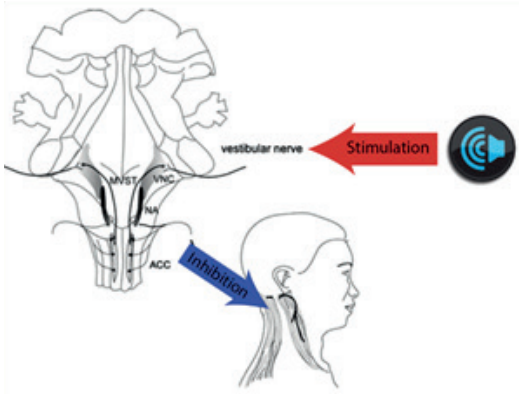

Figure 2. cVEMP physiology: an acoustic stimulus triggers the vestibulocollic reflex throught the saccule and via de vestibular nerve, vestibular nuclei through the accessory nerve to the sternocleidomastoid muscle.

If the inferior vestibular nerve function is lost (for example due to a tumor) cVEMP's (elicted by sound) are absent, but are preserved in patients with selective lesions of the superior part of the vestibular nerve (18). This, however, does not mean that utricular afferents are not activated by sound. The utricular afferents are activated by sound but do not have strong projections to the SCM muscle. The cervical VEMP (cVEMP) evokes the response in the SCM through saccular activation and therefore gives an indication of saccular function (15).

Many methods of evoking VEMP's have been described since (e.g. air conducted sound, bone conducted vibration and galvanic stimulation) and other VEMP pathways have been described (e.g. ocular VEMP's, in which loud sounds provoke a response in ocular muscles).

OCULAR VEMP (OVEMP)

Ocular VEMP's are similar to cervical VEMPs in that they are both elicited by acoustic stimulation of the vestibular organs and that the response is measured in muscle EMG (19). The oVEMP is recorded by

placing recording electrodes below the eyes and maintaining an upward gaze during recording, forcing the inferior oblique muscle closer to the recording electrode(20). However, there are some important differences between cVEMP and oVEMP, not all of which are clearly understood.

A major difference between cVEMP and oVEMP is that the oVEMP is a contralateral response whereas the cVEMP is ipsilateral. This is shown in a study by Chihara et al. using patients with single sided vestibulopathy where the oVEMP tested on the affected side showed no or decreased oVEMP's on the contralateral side, but when tested on the unaffected side normal responses were present on the contralateral side. In this study cVEMP's showed the opposite findings (i.e. no responses on ipsilateral side of vestibulopathy and normal responses on unaffected side) (21).

Secondly the cVEMP is an inhibitory response and the oVEMP is excitatory, as shown in a single motor unit recording study by Colebatch et al. (16). The origin of the responses are also different. The saccule has a strong connection with the SCM muscle and therefor the cVEMP mostly represents saccular function whereas the utricle has a strong connection with the eye muscles, making the oVEMP more likely to be of utricular origin (15, 22). Clinical studies in vestibular neuritis (VN) patients have been used to study the source of oVEMP versus cVEMP responses. The inferior vestibular nerve contains afferents from the saccule and posterior semicircular canal whereas the superior vestibular nerve carries mainly utricular, lateral and superior semicircular canal afferents. Several studies have shown that patients with superior vestibular neuritis have oVEMP responses that are reduced or absent, while the cVEMP is intact (23) (24). This evidence is not conclusive since a small part of the saccular afferents run through the superior vestibular nerve (Voit's nerve), which could provide an alternate mechanism for the observed responses. Others feel this is unlikely based on observations in an animal model; the saccular-ocular connection is shown to be weak in cats (25). This thesis focusses on cVEMP.

VEMP VS AUDITORY BRAINSTEM RESPONSE (ABR)

The equipment used for cVEMP testing is often not specifically designed for this. Mostly it is done using auditory brainstem response (ABR) equipment with little to no modification to recording protocols or signal processing. As a result, the difference between

the underlying mechanisms generating the ABR and VEMP have often been ignored. The differences, which are substantial, are discussed below.

In essence, the ABR is a cochlear test and the cVEMP is a vestibular test. Although both tests use surface electrodes to record a response evoked by sound, the origin of the VEMP response is fundamentally different as is the background noise. The ABR is an excitatory far-field electrical potential that reflects the summation of synchronized neural activity starting from the excitation of the auditory nerve and ascending up the central auditory pathway. Each peak in the response relates to activation of an additional "station" in this ascending activation pathway. The first two waves reflect activation of the cochlear nerve, the third wave is associated with activation of the superior olivary complex, the fourth wave is attributed to activation of the pons and lateral lemniscus and finally the fifth wave is associated with activity in the midbrain, lateral lemnicus and inferior colliculus. The most common outcome measures are amplitude, latency and threshold. Clinically the ABR is used to assess hearing sensitivity in young children or adults that are unable to perform other audiometric tests. It was also used to assess the presence of retrocochlear disease, most commonly an acoustic tumor. Although the MRI is now the investigation of choice for space-occupying lesions.

Even though the same equipment is used to record both ABR and cVEMP, it is imperative to understand the fundamental differences between the two tests. ABR is an excitatory test of the cochlea and central auditory pathways. This is in contrast to cVEMP which is a peripheral vestibular (saccular) response that inhibits ongoing EMG activity of the SCM. An ABR is performed at rest with as little movement from the subject as possible, whereas cVEMP requires the subject to actively contract the SCM muscle. Although detection of the ABR depends on a low level of background noise and sufficient signal averaging; the amplitude of the response does not vary with subject background noise level. In cVEMP the contraction strength of the muscle has a substantial influence on the amplitude of the response which is one of the main subjects of this thesis. Also since muscle contraction is essential to evoke a cVEMP it is not possible to average endlessly because of muscle fatigue, limiting the time-window the record a response.

The differences are summarized in table 1.

	ABR	cVEMP
Nature	Excitatory	Inhibitory
Origin	Cochlear and VIII nerve	Saccule and Inferior vestibular nerve
Averaging	Decrease background noise to detect response	Modulation of background noise to obtain response
Subject	Inactive	Active

Table 1. The differences between auditory brainstem responses and cervical vestibular evoked myogenic potentials.

SIGNAL PROCESSING IN CVEMP

Besides appropriate equipment, processing the recorded signals is also essential to get a reliable outcome. Since signal processing is not often done by physicians, a short outline concerning signal processing in cVEMP and its challenges is described in this section.

The VEMP waveform is obtained by simple waveform averaging. Waveform averaging is appropriate when each trace consists of the same "true" response embedded in a random noise that is independent of the signal. Many auditory-evoked potentials fit this model, for instance, the auditory brainstem response (see above). However, in the case of a VEMP measurement, the underlying physiology reveals that the "measurement noise" primarily originates in the random nature of the motor-unit activity, and the saccule's response is encoded by the modulation of this random process. VEMP noise and signal are therefore neither additive nor independent. The influence of the vestibular input on the EMG is more like the multiplication of the vestibular signal with the EMG noise, rather than the addition of the two. So the normal theory worked out for additive signal and noise (such as in the ABR) does not work for a VEMP. While averaging may capture some aspects of the signal involved, it ignores other information available in the individual traces.

To capture more of this information different techniques can be used and are currently investigated. This thesis focusses on reducing the muscle noise in order to get a more uniform measurement of saccular function.

CVEMP TESTING

There is great variability in the methods used to elicit cVEMP's. This section describes the different variables that need to be considered when testing cVEMP's. Significant variability also exists between subjects and even within sessions making it difficult to interpret the results. Since all other tests in the vestibular test battery rely mostly on horizontal semicircular canal function, there are no alternatives to assess the saccule. Improvement of the current testing method seems to be the only way available now to improve our ability to assess saccular and utricular function.

The kind of stimulus that is used to evoke the response will be discussed first followed by the frequencies at which cVEMP's are evoked. Thirdly, the analysis of cVEMP outcomes will be outlined and lastly a global overview of our method to elicit cVEMP's is demonstrated.

Stimulus

A transient (short) sound stimulus causes the saccule to activate a reflex arc terminating at the sternocleidomastoid muscle which inhibits the firing rate of motor units. This stimulus can be air-conducted sound (ACS), bone-conducted vibration (BCV) or galvanic stimulation (GS). Either tone bursts or clicks are used for ACS or BCS.

Air conducted sound

Welgampola (2001) compared cVEMP's evoked by tone bursts to those evoked by clicks. They found that the responses were similar but that tone bursts require a lower absolute intensity to evoke a response (14). Rauch confirmed this finding in healthy subjects. Rauch et al. assessed cVEMP's to evaluate the side of disease in unilateral MD patients and compared cVEMP with the conventional vestibular test battery (electronystagmography and sinusoidal vertical axis rotation testing) (26). They found that threshold testing using tone bursts at 250Hz was highly sensitive to side of disease (27). Clicks were poorer compared to all other tests used. Currently tone burst are the stimulus of choice when testing cVEMPs using ACS.

Bone conducted vibration

It is not always possible to use ACS to elicit VEMPs. When a subject has a conductive hearing loss it is difficult to interpret the results (15). Using bone conducted vibration (BCV) the middle ear conductive mechanism can be substantially reduced. Since BCS activates both labyrinths, a bilateral response in SCM muscles is usual-

ly produced. The response in the ear ipsilateral to the bone conduction transducer occurs earlier and is usually larger (28). Both ACS and BCV are suited to elicit cVEMPs, although they might not activate all the same neurons.

Galvanic stimulation

Galvanic stimulation uses an electric current to evoke a VEMP response. It bypasses the labyrinth and stimulates the vestibular nerve directly. If a response is present this is evidence for the presence of functioning primary vestibular afferents (15). Using this characteristic galvanic VEMP (gVEMP) in combination with cVEMP can be useful to distinguish labyrinthine (e.g. Meniere's disease) from retro-labyrinthine lesions (e.g. vestibular schwannoma) (29). Because the labyrinth is the side of disease, responses in Meniere's disease would involve elevated thresholds and decreased amplitudes or even no response in cVEMP, but an intact gVEMP response is expected because the vestibular nerve is intact. If the nerve itself is damaged (for instance by a tumor) then a gVEMP response is expected to be diminished or absent as well, however this is not yet clinically proven (30).

Frequency

Frequency is the number of occurrences of a repeating event per unit time. The unit for frequency is Hertz, in which one Hertz stands for 1 repetition every second. In sound, frequency is the primary determinant of pitch. The human ear can roughly detect sounds between 20 and 20.000Hz.
In 1997 McCue and Guinan Jr. showed in cats that primarily vestibular afferents responded to sound (31). The most optimal frequency to provoke this response was between 500 and 1000Hz. A later study by Welgampola found the same frequencies to be optimal in humans (14). In this study 6 subjects were tested using a broad spectrum of frequencies (range 100-1000Hz) and the peak of the tuning curve was found at 700Hz. Todd et al (2000) tested cVEMPs at a large range of frequencies (100, 200, 400, 800, 1600 and 3200Hz) and found that the most optimal frequency lays between 300 and 350Hz, however in this study 1ms rise-fall time was used which causes spread of energy across frequencies, particularly at low frequencies which might explain the lower optimal frequency in this study (32). Welgampola's study used a broader range of frequencies and the best tuning showed to be at 700Hz (14). All named studies were done in healthy subjects. When comparing cVEMPS in healthy subjects with pathological subjects between 250 and 1000 Hz, Rauch et al (2004) found that the most consistent difference

between normal subjects and MD patients was found at 500Hz (27). This difference was found both in threshold and in peak-to-peak amplitude. Furthermore, in healthy subjects the tuning curve centered at 500Hz whereas in Meniere's patients this tuning curve was altered (Figure 3).

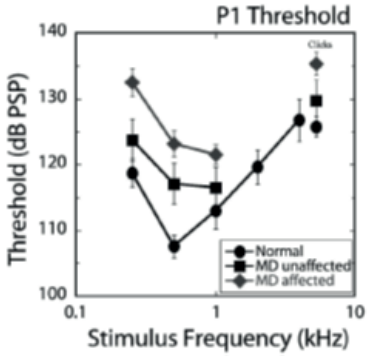

Figure 3. Altered tuning curve of cVEMP threshold in Meniere's patient compared to healthy controles.

ANALYZING THE CVEMP

Since variability is high in cVEMP it is difficult to decide which parameter is most informative as an outcome. In cVEMP, latency, peak to peak (PP) amplitude and threshold can be used as outcome parameter.

Latency

Latency refers to a short period of delay between the stimulus and the response. In cVEMP testing the time between the stimulus and the emerging of the positive and negative peak can be measured and used as an outcome. The latency can be altered in patients with nerve affecting pathologies or central pathology.

Peak to peak amplitude

Peak to peak amplitude (PP) measures the distance between the positive and negative peak. PP amplitude varies between frequencies and therefore it is preferable to assess multiple frequencies (27, 33). Reduced PP amplitude could indicate vestibular function loss. However PP amplitude also covaries (almost linearly) with muscle contraction intensity, i.e. a strong muscle contraction gives a larger PP amplitude and vice versa. This can be a significant confounding variable.

Interaural asymmetry ratio

Recent studies have described the use of the interaural asymmetry ratio (IAR) to compare the left with the right ear in order to aid in identifying the affected ear in Meniere's disease (34) where the peak to peak amplitude of both ears is compared by calculating the ratio between them, by using the following formula:

$$\frac{\text{PP amplitude AS - PP amplitude AD}}{\text{PP amplitude AS + PP amplitude AD}} * 100$$

In strictly unilateral diseases this could be a helpful outcome but would be problematic in bilateral disease.

Threshold

Threshold is the lowest intensity of the stimulus at which a cVEMP response is still detectable and can be measured at different frequencies (e.g. 93dB in figure 6). This parameter is very similar to audiometric evaluation in which thresholds at different frequencies are measured. Furthermore, using only a present/absent criterion, the degree of damage to the otoliths over time is not measurable, using threshold this is possible.

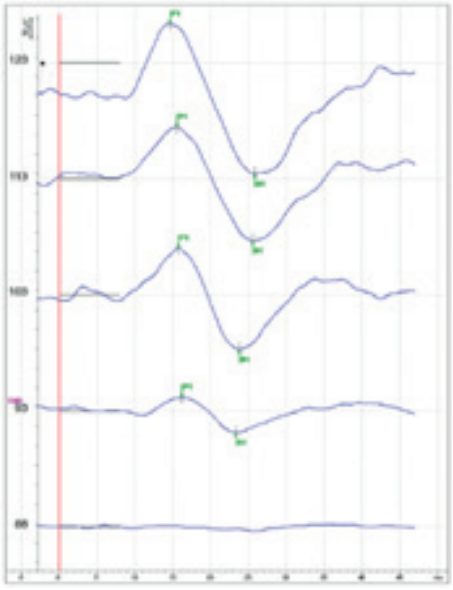

Figure 6. Threshold measurement in cVEMP, in this case threshold is found at 93dB

CVEMP TESTING AT THE MASSACHUSETTS EYE AND EAR

As described in the sections above, cVEMP testing is not plug and play. Meticulous preparation and execution are essential in order to obtain reliable results. In this section our cVEMP setup is shown. For a more detailed description see further chapters.

All subjects underwent a DPOAE screening before and after cVEMP testing (EchoScreen Natus Inc.) to evaluate cochlear hair cell function. The setup for cVEMP testing is showed in figure 7. Every subject sat on a chair and electrodes were placed as shown. The active (positive) electrode was most commonly placed at the midpoint of the SCM muscle. A recent study showed that the placement of the electrodes in either the lower quarter, the midpoint or the top quarter of the muscle makes little difference in result with the exception that when placing in the lower quarter, the polarity of the response flips. The first positive wave became negative and the second negative wave became positive. This could be explained by the possibility that the muscle might have multiple innervation zones (35). When measuring both the left and right side it is important that both the electrodes are attached at the same point of the muscle, otherwise false positive left right differences could be found. For the reference electrode it is possible to use either two electrodes (one on each side) or one electrode placed centrally, above the ma-

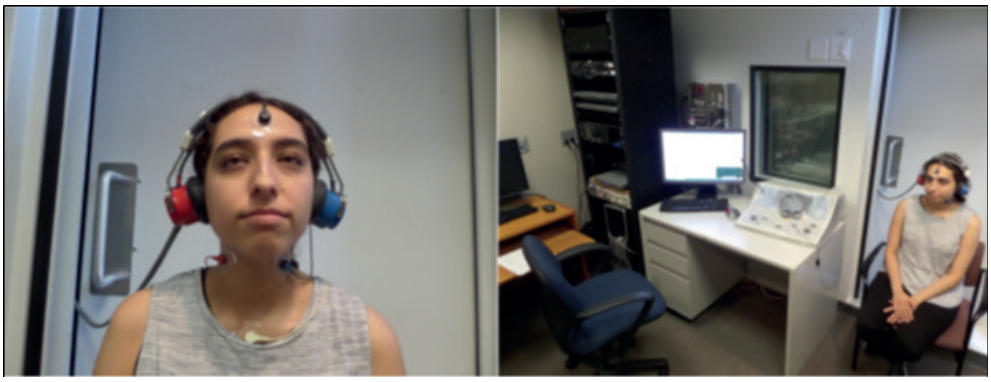

Figure 7. cVEMP test setup.

nubrium of the sternum. We found that in identical conditions, with the only difference being the use of either one or two electrodes, the VEMP response did not change (unpublished data). Each side was tested separately. The muscle was contracted by turning the head to the non-tested ear and tilting the chin slightly down (about 30 degrees). The strength of contraction is constantly displayed on the monitor in front of the observer. The software is programmed in a way that only contractions with an RMS amplitude above 65μV are included. No ceiling for contraction strength was applied except exclusion of signals at the limits of the amplifier capacity. Unpublished data shows that the highest contractions do not alter the outcome significantly. The observer verbally stimulated the subject to increase contraction when the strength seemed to decline. No other feedback method was used. For each cVEMP waveform between 200 and 300 responses were averaged, this includes only the responses above 65μV of contraction strength. All information regarding the described variables (and more) are shown in a single screen as shown in figure 8. CVEMPs were recorded at multiple frequencies and intensities which were randomly assessed. More details on cVEMP testing are discussed further along in this thesis.

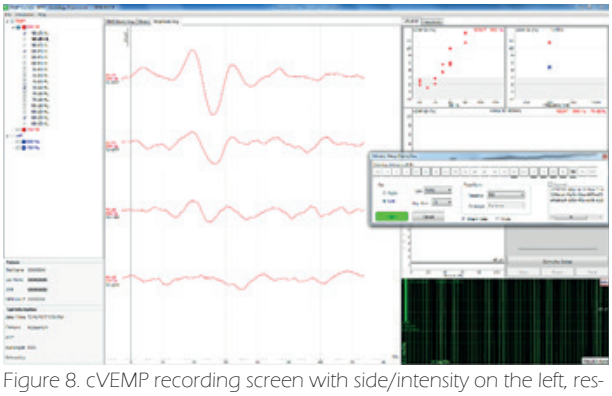

Figure 8. cVEMP recording screen with side/intensity on the left, response view in the middle, the window on the left shows the settings for a single measurement (frequency/intensity/side).

AIM OF THIS THESIS

Improving the cVEMP for better clinical use was the aim of this thesis. Both fundamental and clinical relevant aspects were assessed by asking the following questions:

What is the current status of electrophysiological testing for Meniere's disease?

A number of different electrophysiological tests have been described to diagnose Meniere's disease. Chapter 2 gives an overview of these different tests and evaluates their usefulness in the diagnosis of Meniere's disease. The cVEMP is a promising test, however there are some clear issues that need to be addressed before it can be used widely.

How can the large intersubject variability in cVEMPs be overcome?

The large variability in cVEMP amplitude appears to be generated by intersubject muscle contraction variability. Chapter 3 describes two studies that show that normalization significantly reduces intersubject variability and which normalization method is most effective.

Using normalized cVEMP's, can we show disease progression in Meniere's disease?

Meniere's disease is a progressive disease and in this chapter we investigated whether serial cVEMP testing can show this progression. Also, the diagnosis of Meniere's disease is sometimes difficult, especially to differentiate between Meniere's disease and Vestibular Migraine. In this study we evaluated different outcomes and how they change over time in these two pathologies.

Is there a method to make cVEMP testing more tolerable?

CVEMP testing can be time consuming and difficult for patients. We increased the stimulation rate to reduce test time and burden and evaluated whether this affected the outcome of the cVEMP.

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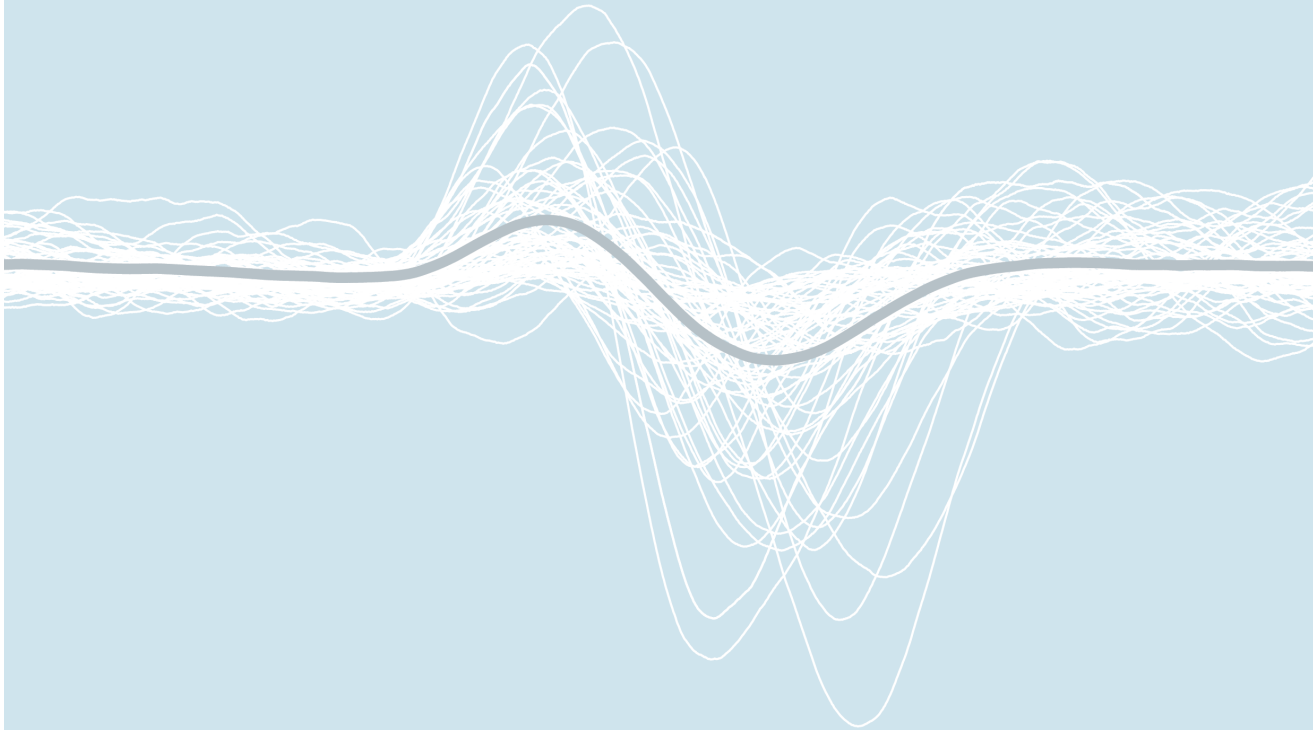

CHAPTER 2

Electrophysiologic diagnosis of Meniere's disease

ELECTROPHYSIOLOGIC DIAGNOSIS OF MENIERE'S DISEASE

ABSTRACT

Meniere's disease was first described in 1861 by Prosper Meniere, the exact pathophysiology is still unclear. The history taken by the physician in combination with audiometric testing are still the most important diagnostic tools, however multiple electrophysiological tests have been described to aid in the diagnosis of Meniere's disease. These tests are used because of their presumed sensitivity to structural and/or electrochemical changes brought about by hydrops, although it is still not clear what the exact relation of hydrops with Meniere's disease is. Here we discuss the electrophysiologic tests that are currently used in Meniere's disease, namely the vestibular evoked myogenic potential (VEMP), electrocochleography (ECoG) and the cochlear hydrops analysis masking procedure (CHAMP). Until the pathophysiology of the disease is figured out and more sensitive test are available we will have to focus on the history the patient tells and use electrophysiological tests to support the diagnosis, not the other way around.

INTRODUCTION

Diagnosis of Meniere's disease

Prosper Meniere was the first to describe the syndrome that consisted of continuous or intermittent head noises accompanied by diminution of hearing and intermittent attacks of vertigo, dizziness, uncertain gait, staggering and falling, accompanied by nausea, vomiting and syncope[1]. The syndrome that now carries his name still mostly follows this description: fluctuating hearing loss, aural fullness and episodic vertigo in which the vertigo attacks typically last between 20 minutes and 24 hours[2]. The diagnosis is a clinical one and based on the history taken by the physician and the exclusion of other causes.

While there is no definitive "Meniere test", a number of tests have been developed that can support the diagnosis of Meniere's disease. Here we discuss the most relevant electrophysiological tests currently used clinically. None of these tests can substitute for a careful history, physical exam, and audiogram as the essential tools for diagnosing Meniere's disease.

Meniere's disease affects both cochlear and vestibular endorgans. There are electrophysiologic tests of each that may be informative in the evaluation of Meniere patients; especially vestibular evoked myogenic potentials (VEMP) for vestibular testing, and electrocochleography (ECoG) and modified Auditory Brainstem Response (ABR) for cochlear testing. Since Hallpike and Cairns [3] and Yamakawa[4] first described endolymphatic hydrops as the signature histopathologic finding in Meniere's disease, there has been a presumption that endolymphatic hydrops is also the essential pathophysiologic abnormality, causing all the clinical symptoms of fluctuating and progressive sensorineural hearing loss, episodic vertigo, tinnitus, and aural fullness. This presumption is now considered to be highly suspect, if not downright incorrect. There are numerous studies confirming endolymphatic hydrops in Meniere's temporal bones post mortem (e.g. [5], [6], [7]). However, there are many cases with endolymphatic hydrops that lack clinical symptoms of Meniere's disease[6, 8] and rare cases of Meniere's disease that lack hydrops[9]. More recently magnetic resonant imaging (MRI) has been used to identify endolymphatic hydrops in living patients[10],[11]. Although this method is still in development, studies have shown that not all MD patients have endolymphatic hydrops that could be visualized using specific MRI protocols. Ambiguity about the precise relationship of endolymphatic hydrops to clinical Meniere's

disease notwithstanding, the electrophysiologic tests discussed below are used in evaluation of Meniere's disease because of their presumed sensitivity to structural and/or electrochemical changes brought about by hydrops.

VESTIBULAR EVOKED MYOGENIC POTENTIAL (VEMP)

Physiology

Acoustic sensitivity of the vestibular system was first investigated by Nobel Prize nominee dr. Pietro Tulio in 1929 [12]. He made detailed observations of sound-evoked head movement, eye movement and postural changes in animals following surgical fenestration of the bony labyrinth. In 1935 von Békésy was the first to report vestibular responses to sound in healthy human subjects and provided evidence that the responses were not mediated by the cochlea [13]. He confirmed the assumption that the response was of vestibular origin by showing that it was preserved in deaf patients with intact vestibular function.

In 1994 Colebatch et al. re-examined the phenomenon sound-evoked vestibular responses and showed that responses could be recorded from the ipsilateral sternocleidomastoid muscle (SCM) as well as from the inion as described earlier by others [14] [15]. The characteristics of this vestibular evoked myogenic potential (VEMP) showed a linear relationship between sound intensity and tonic EMG level[16]. Murofushi and Curthoys found more evidence for the saccular origin of the response by retrograde tracing of the click-sensitive afferents showing most neurons to originate in the saccular macula[17].

Cervical vestibular evoked myogenic potentials (cVEMP) depend on the vestibulocollic reflex which arises from the acoustically responsive sensory cells and neurons in the saccule and utricule with signals conducted centrally via the vestibular nerve[18]. The afferents which are susceptible to noise are known as the otolith irregular afferents [19] [20] [21]. These afferents project to the vestibular nuclei and cause an inhibition of the contracted ipsilateral SCM when activated by a loud acoustic stimulus [22]. Since saccular endolymphatic hydrops is the most consistent histopathologic change seen in Meniere temporal bones, it has been hypothesized that VEMP behavior, either threshold, amplitude, or frequency tuning will be altered in Meniere ears[23] [24] [25].

Air conducted sound (ACS) is the most commonly used stimulus for eliciting cVEMPs. Tone bursts are preferable above clicks because the latter have less reliable results and need

higher absolute intensities to evoke a response [20] [24]. Since high intensity sound is required to evoke the VEMP response, the response is easily obliterated by even a small degree of conductive hearing loss. In such cases bone conducted vibration (BCV) can be used as an alternative stimulus to bypass the middle ear problem and provide adequate stimulus to evoke the VEMP [21]. Usually a bilateral response in SCM muscles is produced, where the response in the ear ipsilateral to the bone conduction devise occurs earlier and is usually larger[26].

In patients with Meniere's disease cVEMP typically show lower peak-to-peak amplitude and an elevated threshold [27] [24]. Also, VEMP tuning is flattened in Meniere patients. Rauch et al. found that the tuning for cVEMP in different frequencies showed a "V" shape threshold curve, with the optimal response at 500Hz and higher thresholds at higher and lower stimulus frequencies. In Meniere patients the "V" flattens and the optimal response shifts to higher frequency [24]. In early stage Meniere's an augmented response may be found, attributed to saccular dilatation that abuts the stapes footplate leading to increased saccular sensitivity[28]. Murofushi (2001) showed an absent or delayed response in 51% of MD patients [27]. Young et al. (2003) found abnormal cVEMPs in 82% of Meniere's patients [28] and deWaele et al. (1999) found abolished cVEMP responses in 54% of MD cases[29]. This indicates that the binary assessment of present vs. absent VEMP is not very useful. Rauch et al. showed a statistically significant difference between the thresholds in healthy ears compared to Meniere ears at 500Hz, indicating that threshold might be a more sensitive way to assess cVEMP results[23]. Other methods to improve sensitivity of the cVEMP include calculation of interaural asymmetry and assessment of VEMP threshold or amplitude slope as a function of stimulus frequency [20] [23, 30].

Interaural asymmetry ratio

The interaural asymmetry comparison is usually expressed as a ratio, the interaural asymmetry ratio (IAR). This ratio compares the left and right ear within a subject [20]. Most often cVEMP amplitude is used for this calculation. Normative data suggest that normal subjects have an asymmetry ratio less than 47% (calculated by the mean + 2 standard deviations)[31] [32] [20]. Young et al. showed that the IAR differed significantly between the various stages of Meniere' disease[28]. It is important to note that Lin et al. showed that 27% of asymptomatic ears of unilateral Meniere patients manifested a reduced response VEMP (i.e. elevated threshold and/or flattened tuning curve)[33]. In the same study they examined pairs of temporal

bones from 17 cases of unilateral Meniere's disease and found that 35% of cases had saccular endolymphatic hydrops in the asymptomatic ear. These findings are highly suggestive that altered VEMP response in the asymptomatic ears of unilateral Meniere patients might be a sign of "preclinical" Meniere's disease, since approximately 25-35% of Meniere patients eventually develop bilateral disease[34] [35]. This also means that the calculated IAR in unilateral Meniere cases may be less sensitive to detection of an abnormal ear because the contralateral ear has reduced cVEMP as well, resulting in less interaural difference. IAR therefore must be interpreted with caution in cases of unilateral Meniere's disease.

VEMP testing has also been used to distinguish Meniere's disease from some other vestibulopathies. Most notably, superior semicircular canal dehiscence (SSCD) can have overlapping symptoms with Meniere's disease (e.g. Tulio phenomenon). VEMP is very useful to differentiate between the two because cVEMP amplitudes are markedly increased and thresholds are lowered in SSCD and other "third window" conditions, just the opposite of findings in Meniere's disease[36] [37].

Vestibular migraine is a relatively new diagnosis that can present with similar complaints as Meniere's disease [38, 39]. Baier et al. (2009) looked at 63 vestibular migraine patients and found significantly reduced cVEMP amplitudes compared to normal controls[40]. Zuniga et al. used cVEMP to attempt to differentiate between vestibular migraine and Meniere's disease [41]. They found decreased cVEMP amplitudes in both groups, with no significant difference, and concluded that VEMP was not useful to separate the two.

Correcting for muscle contraction

One major drawback of VEMP is the degree of test variability, especially between subjects[20] [42] [43]. In order to obtain a reliable cVEMP result it is important to reduce the variability of test as much as possible. The VEMP response is a measure of inhibitory modulation of SCM muscle activity, not a "positive" waveform hidden in stochastic noise, as seen in the auditory brainstem response. Therefore, simple signal averaging cannot reduce noise to enhance the signal-to-noise ratio. One method to control this muscle "noise" (i.e. the SCM EMG activity that is not modulated by the sound-evoked cervicollic reflex) is to attempt to standardize the magnitude of muscle contraction effort across test subjects by providing visual feedback of muscle effort to the test subject. Different forms of feedback mechanisms (e.g. a blood pressure cuff that had to be held at a certain pressure or visual feedback that showed the EMG level

of the contraction) have been shown to decrease test-retest variability[44] [45]. Another method to control for the difference in muscle contraction level between subjects is by using a form of normalization. Normalization is accomplished by dividing the cVEMP waveform by a constant that resembles the individual muscle activity. By correcting for the muscle activation, the cVEMP response is less variable between subjects, which should make it easier to distinguish between a healthy and a pathological response. Previous studies have shown that test-retest variability within subjects is so low that it is not significantly reduced by normalization[32] [42]. However, using normalization, the variability between subjects is significantly reduced showing that the normalized cVEMP offers a more accurate indication of saccular and inferior vestibular nerve function and the potential for improved sensitivity to detect abnormal ears[46].

Ocular VEMP (oVEMP)

Ocular VEMPs are similar to cervical VEMPs in that they are both elicited by acoustic stimulation of the vestibular organs and that the response is measured in muscle EMG [47]. The oVEMP is recorded by placing recording electrodes below the eyes and maintaining an upward gaze during recording, forcing the inferior oblique muscle closer to the recording electrode[48]. There are some important differences between cVEMP and oVEMP, which are not all clearly understood yet. A major difference is that the oVEMP is a contralateral response whereas the cVEMP is ipsilateral. This is shown in a study in which the oVEMP was absent on the contralateral side in patients with unilateral vestibular function but present on the ipsilateral side [47]. Secondly the cVEMP is an inhibitory response and the oVEMP is excitatory, as shown in a single motor unit recording study [49]. The more uncertain parts of the test relate to the the endorgan responsible for the response. It has been proposed that the oVEMP is mainly mediated by utricular stimulation while the cVEMP is a saccular response[50]. Clinical studies in vestibular neuritis (VN) patients have been used to study the source of oVEMP vs. cVEMP responses. The inferior vestibular nerve contains afferents from the saccule and posterior semicircular canal whereas the superior vestibular nerve carries utricular, lateral and superior semicircular canal afferents. Several studies have shown that patients with superior vestibular neuritis have oVEMP responses that are reduced or absent, while the cVEMP is intact [51] [52]. This evidence is not conclusive since a small part of the saccular afferents run through the superior vestibular nerve (Voit's nerve), which could provide an alternate mechanism for the observed responses. Others feel this is unlikely based on observations in an

animal model; this saccular-ocular connection is shown to be weak in cats [53]. Whether the oVEMP is exclusively a utricular response or a response mediated by both otolith organs (i.e. saccule and utricule) is still heavily debated (e.g. [54]) and further studies will be needed to resolve the issue.

Clinical use of oVEMP is even less standardized than is use of cVEMP. The choice of air- (ACS) vs. bone-conducted stimuli is controversial. Todd et al. (2007) showed that using ACS generally causes an upward eye movement and BCV a downward eye movement in humans, which indicates a different endorgan pattern [55]. Curthoys et al. (2010) showed in guinea pigs that ACS activates utricular receptors [50]. Iwasaki used recordings of ACS and BCV and different head positions in a guinea pig model to demonstrate that the two different stimuli are activating different populations of otolith afferents[56]. One possibility is that ACS and BCV both can activate irregular otolith neurons, but not all neurons activated by BCV can be activated by ACS. Thus ACS responses most likely are a subset of BCV responses[21].

In Meniere's disease, the oVEMP has been shown to have enhanced amplitudes in the early stages of the disease [51] and decreased amplitudes and increased thresholds in later stages [57] [58]. Others have studied the tuning of the response by looking at the 500Hz and 1000Hz stimulus amplitude ratio and found that this ratio was statistically lower in Meniere's patients[59]. This flattening of the tuning curve was previously described for cVEMP [24]. Huang et al. (2010) used a variety of vestibular tests to show the location of hydrops formation in 20 patients with Meniere's disease [57]. They used audiometry (cochlea), caloric responses (horizontal semicircular canal), oVEMP (utricle), cVEMP (saccule) as pointers to which part of the inner ear was affected by hydrops. They found the prevalence of abnormal function in decreasing order in the cochlea, saccule, utricule and semicircular canals that would represent the sequence of hydrops formation in Meniere's disease.

Conclusion

The vestibular evoked myogenic potential test depends upon integrity of the otolith organs and vestibular nerves. The cVEMP is primarily a test of saccular and inferior vestibular nerve integrity, while the oVEMP appears to be more dependent on utricular and superior vestibular nerve function. VEMP testing is an emerging and valuable addition to the vestibular function testing "toolbox" since it enables assessment of each otolith organ in a way not previously available. The details of the underlying physiology and the precise me-

thods of performing, analyzing, and interpreting VEMP responses are still evolving and not yet standardized. Much more research is needed to determine how best to utilize VEMP testing for diagnosis and monitoring of Meniere's disease and other peripheral vestibulopathies.

ELECTROCOCHLEOGRAPHY (ECOG)

ECoG is an electrophysiological test that records summating potential (SP) and the compound action potential (AP) of the cochlea and auditory nerve elicited by an acoustic stimulus[60]. The SP is a reflection of the out hair cell integrity whereas the AP reflects auditory nerve integrity[61]. The electrode used to measure the electrical potentials can be placed though the tympanic membrane (transtympanic) or against the tympanic membrane. The transtympanic membrane method gives more reliable results but is also more invasive, which is why in most clinics in the US the electrode is placed either against the tympanic membrane or in the external auditory canal[61].

The glycerol test, a modification of the ECoG, was proposed as a way of improving diagnostic accuracy of the audiogram and ECoG by challenging inner ear fluid homeostasis. However, studies show only about a 50% incidence of glycerol-induced threshold shift (a "positive" test) in Meniere patients[62] [63]. The combination of low sensitivity and significant patient discomfort in performance of the test have led to its abandonment at most centers.

SP/AP amplitude ratio

The ratio of the summating potential (SP) and the nerve action potential (AP) in response to auditory stimuli is used as an indicator of endolymphatic hydrops. The exact mechanism of the SP/AP amplitude ratio increase is still uncertain, but it is hypothesized that the excessive fluid volume caused by endolymphatic hydrops deforms the basilar membrane, thereby altering the amplitude and latency of the response[60] [64]. Thus an enhanced SP/AP ratio is considered by many to be a positive indicator for Meniere's disease. Early studies of the SP/AP ratio showed that the measurement was highly variable and deemed its clinical use limited[65] [66]. The sensitivity of the test lies around 60%, while specificity is reported around 90%[67] [68], meaning that the test has a strong positive predictive value but low negative predictive value. Only around half of Meniere patients have enhanced SP/AP ratio. Another limitation of ECoG is that its reliability depends on integrity of outer hair cells so it can only be tested in a patients with

hearing threshold ≤60dB[61]. Since Meniere's disease is associated with progressive hearing loss, ECoG is usually of no use in more advanced disease.

SP/AP area ratio

Approximately 20 years ago reports were made of an abnormal AP-N1 latency in ECoG when using condensation vs. rarefaction clicks in Meniere patients[69] [70]. The hypothetical explanation for this phenomenon was a change in the velocity of the traveling wave in an hydropic cochlea. The vibration of the cochlear partition may be abnormally restricted or enhanced under such conditions according to the direction of basilar membrane motion. As a result the traveling wave (on which the AP-N1 should be dependent) will differ accounting to whether the initial deflection of the partition is toward the scala vestibuli (as with rarefaction clicks) or the scala tympani (as with condensation clicks). In order to increase the sensitivity of ECoG Ferraro et al. tried to combine the findings of enlarged SP/AP ratio and the increased duration of the AP-N1 latency by calculating the area under the curve and named this the "area ratio". In a study to test this hypothesis, Al-momani et al. looked retrospectively at area ratio and SP/AP ratio and showed that both of the ECoG parameters were statistically significantly different between the Meniere and control group. The calculated sensitivity and specificity were 83.9% and 92% respectively. However, a subsequent study by Baba et al. could not replicate this finding[71].

It is possible that using both the SP/AP amplitude ratio and SP/AP area ratio in combination could improve sensitivity and clinical utility of ECoG for evaluation of patients in early stages of Meniere's disease, for example, when the diagnosis is unclear. However, though ECoG specificity is high, the test is sometimes positive in other diseases that can look like Meniere's disease, such as superior semicircular canal dehiscence[72]. Vestibular Migraine is also known to have symptoms similar and sometimes indistinguishable from Meniere's disease[38]. No studies have been conducted to investigate ECoG results in vestibular migraine patients and compare them to results in Meniere's disease.

Conclusion

In conclusion, ECoG is most useful in the early stages of Meniere's disease, however its diagnostic capabilities are limited by a relatively low sensitivity, the inability to distinguish between others diseases and the fact that it can only be used in patients with hearing thresholds above 60dB. Furthermore, studies are needed to assess the relation between ECoG and vestibular migraine, the most common confounding diagnosis.

CHAMP (COCHLEAR HYDROPS ANALYSIS MASKING PROCEDURE)

The Cochlear Hydrops Analysis Masking Procedure (CHAMP) uses wave V from the auditory brainstem response (ABR), evoked by clicks, to assess a change in the traveling wave velocity (TWV) by measuring these traveling wave velocities in different portions of the cochlea. This method assumes that cochlear hydrops is the cause of Meniere's disease, and that this could alter the basilar membrane which in turn could affect the electrical potentials [73]. It is hypothesized that the increased endolymphatic volume increases the stiffness of the basilar membrane which would influence the TWV. Previous studies have calculated increased TWV in patients diagnosed with Meniere's disease[74] [75]. The hypothesis that the stiffening of the basilar membrane increases the TWV forms the basis of CHAMP.

The CHAMP methodology was described by Don et al. The cochlea is masked with different frequencies in order to get ABR threshold responses from different areas along the cochlea partition (i.e. basal vs. more apical). In a healthy response, the latency is expected to increase when the masking noise frequency is higher (i.e. more basal), since the sound has to travel farther up the cochlea to evoke a response in an unmasked frequency domain. If the TWV is pathologically increased, there is less latency difference between the different portions of the cochlea [73].

In their first CHAMP study Don et al. tested 38 normal hearing subjects and 23 definite Meniere patients, comparing click-evoked wave V latency with and without a 500Hz masking noise. They found a 100% sensitivity and 100% specificity for differentiating active Meniere patients from controls. This result supports the authors' hypothesis that the TWV is increased in definite MD patients causing the latencies to be similar in different parts of the cochlea. The Meniere subjects in this study represent a subset of the most severe and active cases. Other studies have shown less sensitivity and specificity. Ordonez-Ordonez et al. performed a prospective validation study to evaluate the usefulness of CHAMP [76]. Their study consisted of three groups, definite Meniere's disease (n=32), other vestibulopathic and neuropathic patients (n=35), and normal (n=32). Results showed a 100% specificity for definite Meniere patients but only 31% sensitivity. Kingma and de Wit analyzed CHAMP results from 22 unilateral definite Meniere patients, defined as sen-

sorineural hearing loss of more than 60dB, tinnitus and periodic vertigo attacks (at least 2, lasting more than 20 minutes), and found sensitivity of only 32%[77]. DeValck et al. looked retrospectively at 45 patients with oto-vestibular complaints [78]. They wanted to assess the usefulness of the test in a more diverse group, by including not just definite Meniere's patients (n=14) but also probable (n=5), possible (n=13) and non-Meniere (n=25) patients. Disappointingly, 49% of performed tests were not interpretable, and in those with an interpretable result, sensitivity was 31% and specificity was 28%. The high proportion of uninterpretable tests might be due to the more severe hearing loss in the Meniere group. In the definite Meniere group, average threshold were 47.8dB, SD 18.8. Overall, they found that subjects with a normal CHAMP had an average threshold of 18.4dB (SD 12.5), those whose CHAMP was deemed "indicative for ELH group" had average threshold of 37dB (SD 15.2), and the "uninterpretable" group had average threshold of 39.7dB (SD 22.5). When the uninterpretable data were excluded, the sensitivity was 53% and specificity 70%. If only definite Meniere patients were included, 100% sensitivity and 80% sensitivity was obtained.

Conclusion

The CHAMP test exhibits very consistent abnormalities in definite Meniere patients compared to normals but has a low sensitivity and specificity in patients with uncertain diagnosis. Therefore it offers little by way of diagnostic power, serving at best as a means of confirming what is already known. Perhaps future research will determine if altering the parameters of the test will help making it a more accurate test but presently it lacks clinical utility.

CONCLUSION

Diagnosing Meniere's disease is based on criteria set by the AAO-HNS and besides an audiogram no other tests are needed for diagnosis. Still there are some tests available that can aid in the physicians confidence to support the diagnosis of Meniere's disease. The VEMP has the ability to test the otoliths which was not possible before, this makes it a valuable addition to current tests and has shown to be useful in Meniere's disease. However there is much more research needed to determine how best to utilize this test. The ECoG seems to have some value in identifying MD in its early stages, however the negative predicting value is low (i.e. low sensitivity). The recently described CHAMP test has shown little diagnostic power, perhaps future research will determine if altering the parameters of the test will help making it a more accurate test but presently it lacks clinical utility.

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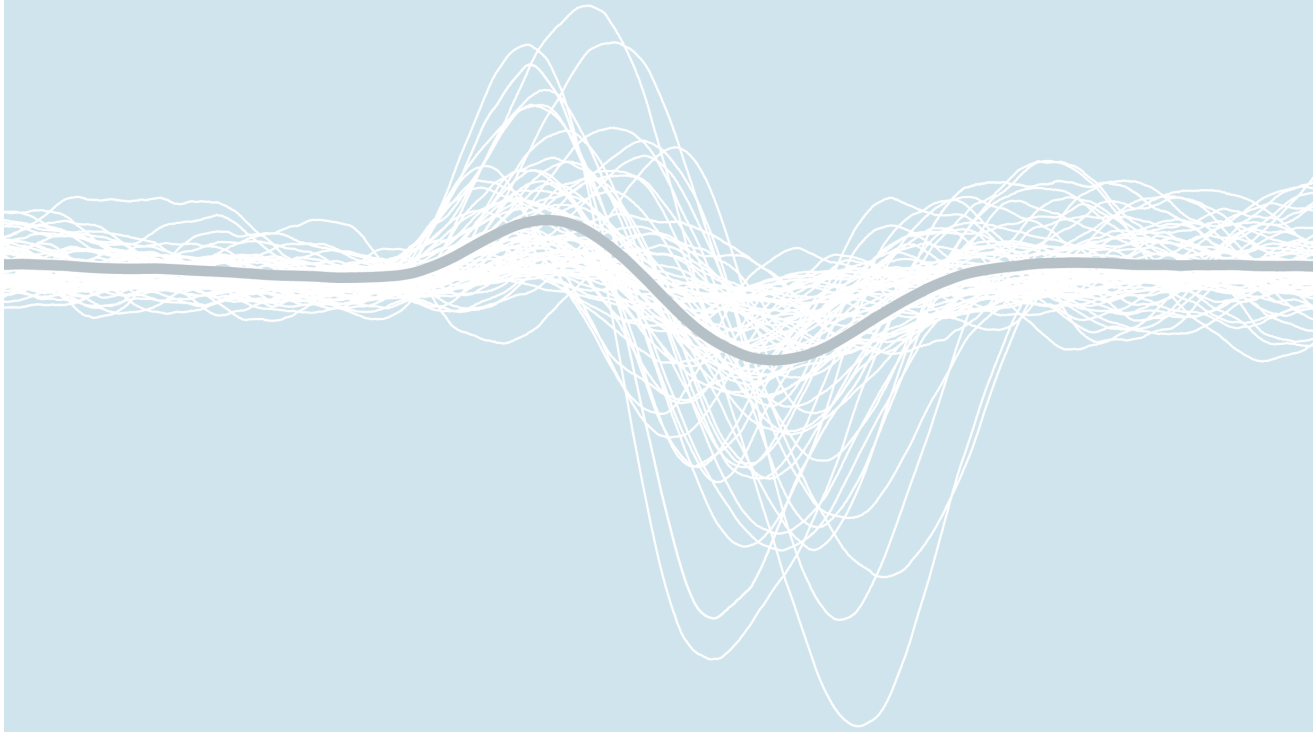

CHAPTER 3.1

Normalization reduces intersubject variability in Cervical Vestibular Evoked Myogenic Potentials (cVEMPs)

NORMALIZATION REDUCES INTERSUBJECT VARIABILITY IN CERVICAL VESTIBULAR EVOKED MYOGENIC POTENTIALS (CVEMPS)

ABSTRACT

Objective: Cervical vestibular evoked myogenic potentials are used to assess saccular and inferior vestibular nerve function. Normalization of the VEMP waveform has been purposed to reduce the variability in vestibular evoked myogenic potentials by correcting for muscle activation. In this study, we test the hypothesis that normalization of the raw cervical VEMP waveform causes a significant decrease in the intersubject variability.

Study Design: Prospective cohort study.

Setting: Large specialty hospital, department of otolaryngology.

Subjects: Twenty healthy subjects were used in this study.

Intervention: All subjects underwent cervical vestibular evoked myogenic potential testing using short tone bursts at 250, 500, 750 and 1000 Hz. Both inter- and intrasubject variability was assessed.

Main Outcome Measures: Variability between raw and normalized peak-to-peak amplitudes was compared using the coefficient of variation. Intrasubject variability was assessed using the intraclass correlation coefficient and interaural asymmetry ratio.

Results: cVEMPs were present in most ears. Highest peak-to-peak amplitudes were recorded at 750Hz. Normalization did not alter cVEMP tuning characteristics. Normalization of the cVEMP response caused a significant reduction in intersubject variability of the peak-to-peak amplitude. No significant change was seen in the intrasubject variability.

Conclusion: Normalization significantly reduces cVEMP intersubject variability in normal subjects without altering cVEMP characteristics. By reducing cVEMP amplitude variation due to non-saccular, muscle-related factors, cVEMP normalization is expected to improve the ability to distinguish between healthy and pathological responses in the clinical application of cVEMP testing.

Words: cervical vestibular evoked myogenic potential, normalization, intersubject variability.

INTRODUCTION

Vestibular evoked myogenic potentials (VEMPs) are electromyogenic (EMG) potentials elicited by high-intensity transient acoustic stimuli and recorded from surface electrodes over tonically contracted muscles. Different types of VEMPs are recorded from neck muscles or ocular muscles (for overview see Curthoys1) and both have been incorporated as part of the vestibular testing battery in many clinics worldwide. This paper focuses on cervical VEMPs (cVEMPs) which are primarily ipsilateral inhibitory responses measured by EMG electrodes placed on the skin above the ipsilateral sternocleidomastoid (SCM) muscle. Air conducted sound stimuli have been shown to stimulate not only the saccule but also the utricle, and the anterior and horizontal canals2. Despite this the cVEMP is mostly of saccular origin since the SCM response is predominantly determined by saccular activation3,4 and thus reflects the status of the saccule and inferior vestibular nerve.

A typical cVEMP response consists of an initial positive peak (P1) occurring approximately 13 ms after the stimulus, followed by a negative peak (N1) at a latency around 23 ms. The most common cVEMP measure is the amplitude difference between these two peaks , i.e. the peak-to-peak (PP) amplitude . This measure has been reported to show high variability, especially between subjects 5,6. Aside from actual differences in saccular response to acoustic stimuli, the high intersubject variability in cVEMPs from normal subjects can originate from differences in muscle contraction effort, muscle mass, age, and/or electrode position7-9.

The normal variability of the cVEMP amplitude confounds the ability of this metric to reveal changes due to altered saccular or inferior vestibular nerve function. Reduction of VEMP variability is thus an important goal in the clinical application of cVEMPs. As early as 1994 when Colebatch et al.10 found a linear relationship between the cVEMP amplitude and tonic EMG activity, it was proposed that normalization of the cVEMP response by the ongoing EMG activity might control for differences in muscle activation across subjects and thereby reduce variability. Since then normalization has been used in research settings, but only limited studies have been conducted to evaluate its usefulness.

The few published studies on normalization have focused on intrasubject variability (interaural and test-retest variability) not the intersubject variability from subject-to-subject. For example, McCaslin et al.8 found no significant difference in the variation of the interaural asymmetry ratio when comparing raw and normalized cVEMP PP amplitudes. Ochi et al.6 studied cVEMP

test-retest variability and also found no statistically significant difference between raw and normalized cVEMP PP amplitudes. No study has systematically compared the effect of normalization on intersubject variability. Because the variability in cVEMPs is largest among, rather than within, normal subjects, we hypothesize that normalization will have its most significant effect on intersubject variability. Here we test this hypothesis using data from 20 normal subjects.

MATERIALS AND METHODS

Subjects

20 healthy subjects (9 male, 11 female) were used (mean age 29, range 20-48). On each, an audiogram was obtained prior to cVEMP testing to verify that the subject did not have an air-bone gap at any test frequency. Otoacoustic emissions (OAE's) were measured before and directly after cVEMP testing to determine if cochlear status was affected by the testing. Subjects were excluded if they had a history of neck injuries or balance problems. Informed consent was signed. This study was approved by the Human Studies Committee of the Massachusetts Eye and Ear Infirmary.

Stimuli

Tone bursts were generated by custom-programmed evoked potential software (National Instruments 16-bit digital I/O board) using a Blackman gating function with the rise and fall each two cycles and no plateau for the frequencies 250, 500, 750 and 1,000 Hz. The resulting rise time for each frequency was 8.0 ms. at 250Hz, 4.0 ms. at 500Hz, 2.5 ms. at 750Hz and 2.0 ms. at 1000Hz. Stimuli were presented at a rate of 13/s to circumaural headphones (Telephonics TDH-49) at a level of 123 dB peak-equivalent sound pressure level (peSPL) for each frequency10. During averaging, the phase of each stimulus was alternated.

cVEMP recordings

Vestibular evoked myogenic potentials were recorded using a custom-programmed evoked potential system. Each ear was stimulated separately and cVEMPs were recorded from the SCM ipsilateral to the stimulus. Subjects sat upright with their head turned toward the non-test ear 11; this contracted the SCM on the test side. EMG activity was recorded from surface electrodes. A positive electrode was placed on the middle of the belly of each SCM, reference electrodes were placed on the SCM tendons just above the clavicle and a ground electrode was placed on the forehead. SCM muscle EMG activity was amplified, bandpass-filtered and sampled for 30ms after stimulus onset at a sampling rate of 50kHz using a 16-bit analog to

digital converter (National Instruments). For each waveform between 200 and 300 responses were averaged. Separate averages were stored for stimuli with rarefaction or condensation initial phases. These averages were combined into one average for alternating phase stimuli. To ensure adequate muscle contractions during the measurement, EMG was continuously monitored, and only contractions that yielded rms EMGs above 65μV were included.

Each subject was tested in 2 sessions with at least 1 week between sessions. In each session, three cVEMP responses were recorded to the highest stimulus level (123 dB peSPL) for each of four tone-burst frequencies: 250, 500, 750 and 1000 Hz. The order of each frequency and test side was randomized for each test session. A cVEMP was visually judged to be present when the response was greater than 1.5 times the residual noise in the overall average. For each subject and session, the measured values from the three replications at each frequency were averaged to obtain overall values at that frequency.

Raw and normalized cVEMP responses were recorded simultaneously. Trace-by trace normalization was used to obtain the normalized waveforms. In other words, the raw EMG trace was digitized after each stimulus and was divided by the overall RMS amplitude of that individual trace. The normalized traces were then averaged to obtain the normalized cVEMP average response.

Analysis

At each frequency, means and standard deviations for each side and session were calculated from raw and normalized cVEMP PP amplitudes, and for P1 and N1 latencies. Student's t-tests, applied across subjects, were used to compare cVEMP responses from each side, and from test-retest sessions. The raw results are calculated in microvolts and the normalized results are dimensionless. In order to compare the two we used the coefficient of variation (CV) to compare the variability between raw PP amplitude and normalized PP amplitude. This statistic is calculated by dividing the standard deviation by the mean. Since the data were non-parametrically distributed a non-parametric Levene's test was used to compare the coefficients of variation using a p-value of <0.05 for statistical significance. Test-retest variability was assessed using the intraclass correlation coefficient (ICC). As in previous studies, we classified an ICC value of 1.00 as perfect reliability, 0.75 to 1 as excellent reliability, 0.40 to 0.75 as fair-to-good reliability and less than 0.40 as poor reliability. Since we do not know of any test to statistically

compare ICCs we used the CV on this metric as well. Interaural asymmetry ratios (IAR) were calculated by the formula: ((PP amplitude left – PP amplitude right)/(PP amplitude left + PP amplitude right)*100). The results from both sessions were used separately in the asymmetry calculations yielding 40 comparisons across subject and sessions comparisons. Besides assessment of variability using the CV, repeated measures analyses of variance (ANOVA) was used to detect differences in IARs between raw and normalized PP amplitude.

Results

Cervical vestibular evoked myogenic potentials were present for 123 dB peSPL stimuli in all of the 20 subjects but not always at all frequencies (65% at 250Hz, in 95% at 500Hz, 100% at 750Hz and 100% at 1000Hz). Sample cVEMP waveforms are shown in Figure 1: raw waveforms on the left and normalized waveforms on the right. No significant differences were found in raw VEMP PP amplitudes or latencies between the first and second session or between the right and left sides. All subjects passed a test for the presence of otoacoustic emissions before and after each session.

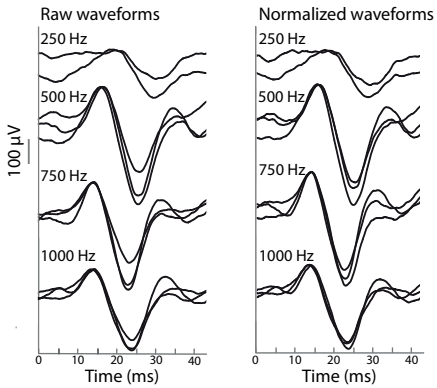

Figure 1. cVEMP responses from one subject during a session at 123 dB peSPL (90 dB HL) for each of the toneburst frequencies. The left panel shows the raw waveforms while the right panel shows the corresponding normalized waveforms. Toneburst frequency is designated on the left.

While waveforms fitting the expected cVEMP morphology and latency range could be identified from each subject, there was significant variability in the voltage of the cVEMP waveform across subjects. The variability in cVEMP responses at 500Hz for all subjects and all sessions is illustrated in figure 2. The bold line indicates the average of all responses and is consistent with expected cVEMP morphology. However, variability in both peak-to-peak amplitude and latency between subjects is obvious.

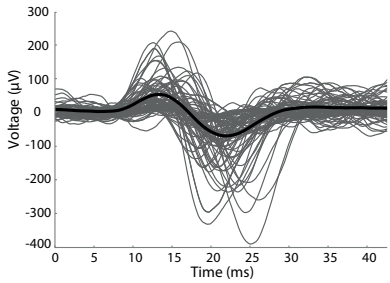

Figure 2. cVEMP responses for a 500 Hz toneburst from all subjects and all sessions. The light gray lines indicate the individual cVEMP responses while the black line indicates the grand average across all the individual responses.

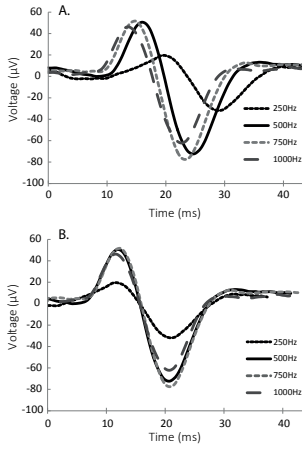

Figure 3. A: the mean cVEMP response across subjects and sessions for each test toneburst frequency. B: the mean cVEMP response for each toneburst frequency after the rise time of the toneburst was subtracted of each sample point. The result of subtracting the toneburst rise time is that the peaks at the different frequencies now align.

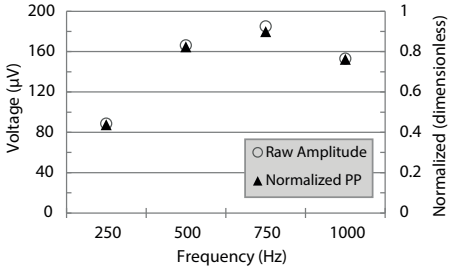

Figure 4. Mean raw PP cVEMP amplitudes and normalized PP cVEMP amplitudes for each toneburst frequency. Tuning characteristics of cVEMP amplitude is unaffected by normalization.

The average cVEMP response across all subjects and ears is plotted in figure 3a for each test frequency. The latency of the response was inversely related to the frequency of the stimulus. This latency dependence was also noted by Rauch et al. 10 and attributed to the rise-time of the toneburst. Interestingly, the cVEMP peaks align when the sum of the toneburst rise and fall times, is subtracted from the latency at each frequency (Figure 3b).

For each frequency, the means of the raw PP and normalized PP amplitudes are shown in Figure 4. The expected amplitude distribution is present with the largest mean response at 750 Hz and the smallest at 250 Hz. cVEMP tuning was similar for raw and normalized cVEMP PP amplitudes indicating that normalization did not alter cVEMP tuning. The patterns across frequencies of raw and normalized cVEMP PP amplitudes were similar. Detailed data are given in table 1.

Since no significant difference was found between the two sessions and sides, data from both sessions were used in assessing variability between raw and normalized results and interaural asymmetry ratios. For the ICC both

Parameter	250 Hz	500 Hz	750 Hz	1,000 Hz
Raw PP amplitude in µV (SD)	88.60 (79.03)	166.18 (150.56)	184.98 (167.60)	153.01 (135.95)
95% CI	65.01–102.35	132.46–199.91	147.68–222.28	122.76–183.27
Normalized PP amp. in µV (SD)	0.44 (0.24)	0.81 (0.44)	0.90 (0.41)	0.76 (0.34)
95% CI	038–0.50	0.73–0.92	0.81–0.99	0.68–0.84
ICC (95%CI) raw PP	0.91 (0.80–0.96)	0.95 (0.91–0.98)	0.95 (0.91–0.96)	0.92 (0.84–0.96)
ICC (95%CI) normalized PP	0.83 (0.65–0.92)	0.96 (0.91–0.98)	0.96 (0.92–0.98)	0.96 (0.92–0.98)
IAR (SD) raw PP	20.8 (14.1)	23.7 (19.3)	22.5 (15.0)	20.0 (15.4)
IAR (SD) normalized PP	17.0 (12.4)	17.6 (13.7)	15.1 (11.9)	15.9 (14.2)
P1 Latency in ms (SD)	13.2 (2.7)	14.4 (2.3)	14.5 (2.1)	14.4 (2.2)
N1 Latency in ms (SD)	21.2 (3.1)	21.8 (2.4)	22.0 (2.2)	21.6 (2.3)

Table 1. Details of tested parameters for both raw and normalized data

sides were used to calculate the test-retest variability. The variability of raw and normalized cVEMP metrics, compared using coefficients of variation (CVs) is shown in Figure 5. Normalized cVEMP PP amplitudes had lower CVs (i.e. lower variability) than raw cVEMP PP amplitudes at all frequencies (Fig. 5A). The variability of the normalized PP amplitude was significantly lower (Levene's test, $p < 0.001$ at all frequencies) than the variation of raw PP amplitudes with the CV cut in half at 750Hz (0.91 to 0.45). In contrast to the large effect of normalization on the variation of VEMP PP measures, normalization did not reduce variability much when applied to within-subject measures. Test-retest reliability, as calculated by the intraclass correlation coefficient, was excellent (ICC>0.70) for both raw and normalized cVEMP PP amplitudes, so that the ICC variability was low and not changed much by normalizing (Fig. 5B). The effect of normalization on the variability of the interaural asymmetry ratio was also limited (Fig. 5C). IARs variability was high and no significant difference was found between the raw and normalized IARs ($p > .05$) at any frequency (Fig. 5B). The coefficient of variation ratios are listed in table 2. In summary, Figure 5 illustrates that CVs for intrasubject parameters (ICC and IAR) show little or no improvement with normalization whereas intersubject variability is significantly decreased with normalization. Finally, cVEMP latency did not significantly differ between raw and normalized cVEMPs.

CV Parameter	250 Hz	500 Hz	750 Hz	1,000 Hz
ICC raw PP	0.15	0.08	0.07	0.14
ICC normalized PP	0.30	0.07	0.07	0.07
IAR raw PP	0.81	0.73	0.70	0.76
IAR normalized PP	0.89	0.68	0.75	0.78
Raw PP	0.89	0.91	0.91	0.89
Normalized PP	0.56	0.54	0.45	0.45

Table 2 Coefficients of variation for both inter- and intrasubject parameters

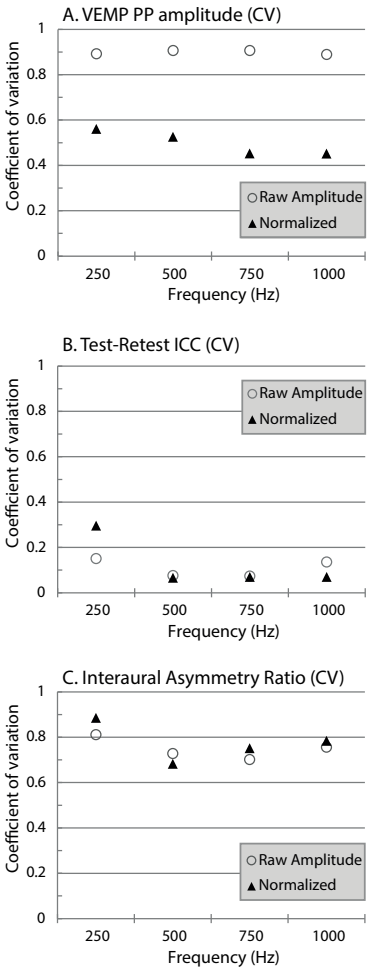

Figure 5. A. Coefficient of variation (CV) of raw PP and normalized PP, with a significant reduction in variability in the normalized cVEMP responses. B. CV of interaural asymmetry ratio (IAR) between raw PP and normalized PP cVEMP results. C. CV of intraclass correlation coefficient (ICC) between raw PP and normalized PP cVEMP results.

Discussion

The purpose of this study was to show the effect of normalization on the variability of cVEMP PP amplitudes. In particular we were interested in the effect of normalization on intersubject variability since we hypothesized that this would be the situation most influenced by normalization. Our data show that cVEMP intersubject variability is significantly reduced by trace-by-trace normalization.

We also confirmed that the cVEMP has excellent intrasubject test-retest variability for both raw and normalized cVEMP PP amplitudes. Both have high (>0.70) intraclass correlation coefficients (ICCs), at all frequencies. Isaradisaikul et al. 12 found similar results (ICC 0.86, sd 0.75-0.92), as did Meas et al. 13 (ICC 0.90) and Vanspauwen et al. 14 (ICC 0.89). The high test-retest reliability of cVEMP PP amplitudes, and the resulting limited effect of normalization on this reliability, are not surprising considering muscle activation in an individual subject is not expected to differ greatly between sessions. Ochi et al. 6 also found the effect of normalization on test-retest variability to be limited.

The interaural asymmetry ratio, a measure of the difference between the right and left cVEMP PP amplitudes, is sometimes used as a way to have one ear of a subject serve as a control for testing the other ear. McCaslin et al. 8 showed that cVEMP normalization did not significantly improve the variability of the interaural asymmetry ratio, we found similar results in our study. Since most normal subjects have similar muscle activation on both sides, the influence of normalization is expected to be minimal.

In contrast to the small effects of cVEMP normalization found within individual subjects, our results show that normalization has a significant effect on intersubject variability. It reduces the CV as much as 50% (Table 1 and Figure 5).

The high intersubject variability of raw cVEMP PP amplitudes could be due to variation in normal saccular sensitivity or to muscle-related variation. We hypothesized that raw cVEMP variability from differences in normal saccular function are dwarfed by muscle-related variation, including variation in contraction effort. This hypothesis is strongly supported by the demonstration that normalization (which accounts for differences in muscle factors) significantly reduces intersubject variability without changing cVEMP characteristics such as tuning or latency (Figures 3 and 4).

Thus, normalization aids in reducing the interference of non-vestibular influences like muscle activation on the measurement of cVEMP amplitudes.

The key to normalization is to obtain from the EMG a measure that represents the contraction strength and to divide the cVEMP waveform by this measure. There are different ways to obtain a normalization measure. We used the rms EMG whereas other groups have used the rectified EMG (8,15). Normalization can be done trace-by-trace (e.g. each trace is divided by the rms EMG of that trace) or on the averaged EMG (e.g. the averaged cVEMP waveform is divided by the rms from the averaged waveform). Another variable is the time-frame over which the normalization constant was obtained: either the pre-stimulus part of the waveform or the entire waveform (including the response) can be used. It remains to be determined if any particular normalization method is best.

In conclusion, we have shown that normalization reduces cVEMP intersubject variability in normal subjects significantly. By reducing cVEMP amplitude variation due to non-saccular, muscle-related factors, cVEMP normalization is expected to improve the ability to distinguish between healthy and pathological responses in the clinical application of cVEMP testing.

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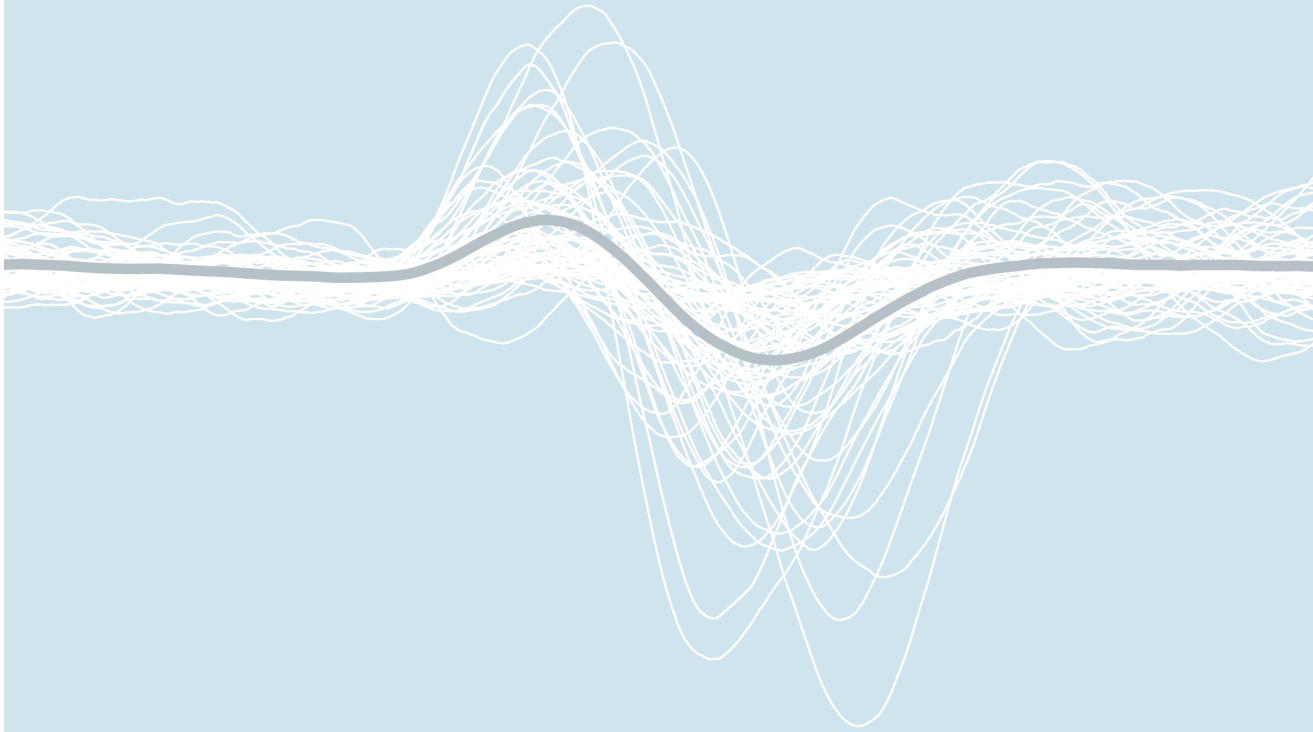

CHAPTER 3.2

Normalizing cVEMPs:
Which method is the most effective?

NORMALIZING CVEMPS: WHICH METHOD IS THE MOST EFFECTIVE?

ABSTRACT

Objectives: *To determine the most effective method for normalizing Vestibular Evoked Myogenic Potentials (VEMPs).*

Design: *VEMP data from 20 normal subjects were normalized using 16 different methods. All methods used the peak-to-peak value of an averaged VEMP waveform (VEMPpp) and obtained a normalized VEMP (VEMPn) by dividing VEMPpp by a measure of the electromyogram (EMG) amplitude. EMG metrics were obtained from the EMG within short- and long-duration time windows. EMG amplitude was quantified by its root-mean-square (RMS) or average full-wave-rectified (RECT) value. The EMG amplitude was used by (1) dividing each individual trace by the EMG of this specific trace, (2) dividing VEMPpp by the average RMS or RECT of the individual trace EMG, (3) dividing the VEMPpp by an EMG metric obtained from the average VEMP waveform, or (4) dividing the VEMPpp by an EMG metric obtained from an average VEMP "noise" waveform. Normalization methods were compared by the VEMPn coefficient of variation (CV) across subjects and by the area under the curve (AUC) from a receiver-operating-characteristic (ROC) analysis. A separate analysis of the effect of EMG-window duration was done.*

Results: *There were large disparities in the results from different normalization methods. The best methods used EMG metrics from individual-trace EMG measurements, not from part of the average VEMP waveform. EMG quantification by RMS or RECT produced similar results. For most EMG quantifications, longer window durations were better in producing receiver operating characteristics with high areas under the curve. However, even short window durations worked well when the EMG metric was calculated from the average RMS or RECT of the individual-trace EMGs. Calculating the EMG from a long-duration window of a VEMP "noise" average waveform was almost as good as the individual-trace-EMG methods.*

Conclusions: *VEMP normalization using EMG quantification from individual-trace EMGs substantially reduces VEMP variability across subjects and also yields a VEMPn value that accurately detects the presence of a VEMP response.*

INTRODUCTION

Cervical vestibular evoked myogenic potentials (cVEMP) are used clinically to evaluate saccular and inferior vestibular nerve function, as well as in assessing the presence of a superior semicircular canal dehiscence (SCD). A major drawback of cVEMP measurements is that the results can vary greatly among healthy subjects which limits their clinical value (Welgampola and Colebatch 2001). A considerable portion of the variability is due to differences in muscle activation. Several methods have been proposed to control muscle activation, e.g. using feedback mechanisms where subjects have to maintain their contraction in a certain range, or the use of a blood pressure cuff that has to be kept at a certain pressure (Vanspauwen, Wuyts et al. 2006, Isaradisaikul, Strong et al. 2008). The downside of these methods is that they depend on the subject to control their muscle contraction within a narrow range. It would be preferable to have this done automatically.

One way to correct for variations in muscle contraction amplitude is to computationally normalize the raw cVEMP measurement by a measure of the muscle electromyographic (EMG) amplitude (Colebatch, Halmagyi et al. 1994). This works because there is a nearly linear relation between muscle contraction amplitude and the EMG amplitude during cVEMP measurements (Ochi, Ohashi et al. 2001). However there are many ways in which muscle EMG amplitude can be measured and VEMP measurements can be normalized. For instance, Rosengren et al. divided the peak-to-peak value of the average VEMP waveform (VEMPpp) by the average of the rectified EMG response measured in a pre-stimulus time period (Rosengren, Welgampola et al. 2010). In contrast, van Tilburg et al. used a trace-by-trace normalization in which each trace was normalized by the root-mean-square (RMS) EMG of the trace and the resulting normalized traces were averaged(van Tilburg, Herrmann et al. 2014). This yielded a normalized VEMP waveform from which the VEMPpp was calculated. Van Tilburg et al. found that, in healthy subjects, this type of normalization produced a significant reduction in intersubject variability of the normalized VEMP (VEMPn) compared to VEMPpp, which demonstrated that this particular method of normalization greatly reduces VEMP variability. There has, however, been no study that assesses the relative merits of the various methods of normalization.

Here we describe a variety of methods for using the EMG amplitudes obtained during cVEMP measurements

to calculate a VEMPn. We ask the question: "What is the best way to normalize cVEMP, and how big are the differences between different methods of normalizing cVEMP?" There are at least 2 definitions of "best": 1. The method that makes the VEMPn amplitudes be most consistent across subjects, and 2. The method that produces the VEMPn that is best at distinguishing when a VEMP waveform is present or absent as assessed by ROC curves. We will consider outcome measures from both.

	Normalization Method Described		EMG Metric		Application of EMG				EMG Metric Time Window			
Article Reviewed (Total = 14)	Yes	Partial	RMS	RECT	Trace by Trace (Mode A)	EMG From Trace, Averaged (Mode B)	Waveform Ave (Mode C)	Other	10 msec	20 msec	100 msec	Entire Window
Welgampola and Colebatch (2001)	X	–	–	X	–	X	–	–	–	X	–	–
Ochi et al. (2001)	X	–	–	X	–	X	–	–	–	X	–	–
Isaacson et al. (2006)	–	X	–	–	–	–	–	X	–	–	–	X
Ito et al. (2007)	X	–	–	X	–	X	–	–	–	X	–	X
Osei-Lah et al. (2008)	–	X	–	X	–	–	–	–	–	–	–	–
Lee et al. (2008)	X	–	–	X	–	–	X	–	–	X	–	–
Wang et al. (2008)	X	–	–	X	–	X	–	–	–	–	–	X
Murofushi (2009)	X	–	–	X	–	X	–	–	–	X	–	–
Nguyen et al. (2010)	X	–	–	X	–	X	–	–	X	–	–	–
Lim (2013)	X	–	–	X	–	X	–	–	–	–	–	–
Bogle et al. (2013)	X	–	–	X	–	X	–	–	–	X	–	–
McCaslin et al. (2013, 2014)	X	–	X	X	–	X	–	–	–	–	X	–
van Tilburg et al. (2014)	X	–	X	–	X	–	–	–	–	–	–	X
Total	11	2	2	11	1	9	1	1	1	6	1	4
Column (%) of total	85	15	15	85	8	69	8	8	8	46	8	31
Heading (%) of total	100		100		92				92			

Table 1. Summary of different normalization methods as reported in the literature. Capital "X" indicates when a normalization variable was included in the description of cVEMP normalization method (Ochi, Ohashi et al. 2001, Welgampola and Colebatch 2001, Isaacson, Murphy et al. 2006, Ito, Karino et al. 2007, Lee, Cha et al. 2008, Osei-Lah, Ceranic et al. 2008, Wang, Yeh et al. 2008, Murofushi 2009, Nguyen, Welgampola et al. 2010, Bogle, Zapala et al. 2013, Lim, Dennis et al. 2013, McCaslin, Fowler et al. 2014, van Tilburg, Herrmann et al. 2014).

METHODS

Normalization Methods

All methods obtained the VEMP amplitude from the difference between the positive and negative peaks in an averaged VEMP waveform (VEMPpp). cVEMP normalization methods vary in how they measure muscle activation from the recorded EMG and in how the EMG measurement is applied to the VEMP data. There are three main elements in each normalizing method:

Time Window: *A time period (a "window") over which to measure the EMG. Most published methods used a pre-stimulus window. In our main across-normalization comparison, we used a pre-response window and a window that contains the whole period between each stimulus onset. We also compare different window lengths.*

Quantification of EMG metric: *The method to quantify the EMG in the window. Two methods have been used to quantify the EMG: (A) calculating the RMS value, and (B) calculating the average full-wave-rectified (RECT) value.*

Application of EMG metric: *The calculation used or mode of applying the EMG metric to the recorded responses to obtain a normalized cVEMP.*

We considered 4 modes:

Mode A: *The recorded EMG response following each stimulus (called a "trace") was divided by the EMG metric obtained from that trace. The resulting normalized traces were averaged to yield a normalized VEMP waveform. VEMPn was obtained from the peak-to-peak value of this waveform.*

Mode B: *All of the traces were averaged to obtain the un-normalized VEMP waveform from which VEMPpp was obtained. From the windowed part of each trace, a trace-EMG-metric was obtained. The overall-EMG-metric was the average of all the trace-EMG-metrics. VEMPn was obtained by dividing VEMPpp by the overall-EMG-metric.*

Mode C: *As in B, all of the traces were averaged to obtain the un-normalized VEMP waveform from which VEMPpp was obtained. The overall-EMG-metric was obtained from this un-normalized VEMP waveform by calculating the RMS or RECT value in the EMG window. As above, VEMPn was obtained by dividing VEMPpp by the overall-EMG-metric.*

Mode D: *As in B and C, all of the traces were averaged to obtain the un-normalized VEMP waveform from which VEMPpp was obtained.*
The overall-EMG-metric was obtained from an average
EMG "noise-waveform"

in which the VEMP response part was cancelled. To cancel the VEMP response, alternate post-stimulus EMG traces were reversed in sign and averaged (an even number only). This yielded a measure of the EMG noise (plus some added variation due to the less-than-perfect cancellations of sound-evoked EMG response components). An example VEMP and the corresponding noise waveform are shown in Figure 1. The overall-EMG-metric was obtained from this noise-waveform by calculating the RMS or RECT value in the EMG window. As above, VEMPn was obtained by dividing VEMPpp by the overall-EMG-metric.

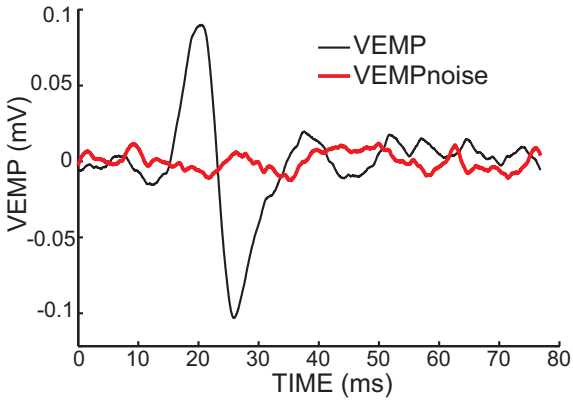

Figure 1. A VEMP waveform (thin line) and the corresponding "noise" waveform (thick line) calculated by averaging the same traces as in the VEMP waveform, but with every-other trace reversed in sign. This noise waveform was used to calculate the EMG metric for the Mode D method of normalization.

Modes C and D are similar in that both calculated the EMG-metric from an averaged EMG waveform, but in Mode-D the alternation of polarity canceled out the sound-evoked VEMP response. The cancellation of the VEMP response is relevant when the EMG-metric window includes all of the EMG-response trace, which would include the VEMP response, if present. In contrast, for modes A and B, reversing the sign of alternate traces has no effect because both modes use individual traces for the EMG-metric calculations and RMS and RECT are insensitive to the sign of the trace. There are two time windows (pre-stimulus and entire trace), two EMG quantifications (RMS and Rectified) and four ways of applying the EMG quantification (see above Modes), forming 16 normalization combinations. We compared these 16 combinations with respect to reduction of cVEMP variance and the accuracy of cVEMP detection.

Subjects and Stimuli

We measured VEMPpp and applied the 16 normalization methods to the data from the same 20 healthy, young subjects (mean age, 29 y; range: 20-48 y) that were used by van Tilburg et al. (2014). Each subject was tested in two sessions with sound stimuli at 250, 500, 750 and 1000 Hz. We did not use the 250 Hz data because they did not appear to provide good, consistent VEMPs.
Stimuli were tone bursts with 2-cycle rise and fall times

and no plateau. In our system, a trace started 3 ms before the sound onset. In each session, at least two measurements were done at 123 dB peSPL (123 dB peSPL is equivalent to 90 dB nHL) at each frequency, and at 500 Hz a level series was done in 5 dB steps from 93 to 123 dB peSPL. The stimulus presentation rate was 13/s which allowed us to do all the required runs in a single session. Although a 13/s presentation rate gives slightly lower VEMP amplitudes than 5/s, the resulting VEMPs have similar reliability (van Tilburg, Herrmann et al. 2016). One consequence of using 13/s is that the pre-response time and the total time available between stimuli are much less than with 5/s. For the pre-response measurement, our system provided 3 ms that was pre-stimulus and to this we added the following 5 ms which is earlier than the beginning of VEMP responses. This yielded a 8 ms "pre-response" time.

Electrodes were placed on the sternocleidomastoid muscle as described previously (van Tilburg, Herrmann et al. 2014). EMGs were sampled at 50 kHz. For each recording, 200 to 300 single EMG responses were obtained and stored. Data analysis considered only response traces with EMGs above 65 μV RMS.

Deciding whether a VEMP response was present, or not.

For 500, 750 and 1000 Hz stimuli at the highest sound level, VEMP responses were almost always clearly identifiable in the average EMG waveform. The latency of the high-level VEMP response served as a guide for deciding whether responses were present at lower levels. We kept in mind that the latency tends to be a fixed value after the peak of the sound stimulus, and is therefore delayed by two cycles of the stimulus frequency relative to sound onset (Rauch, Silveira et al. 2004, van Tilburg, Herrmann et al. 2014).

To ascertain whether a given EMG average waveform contained a VEMP response, all of the average waveforms from a given subject and session were displayed on a single screen from high levels to low levels with successive traces displaced slightly vertically so that they did not overlap. This allowed the viewer to see if a putative VEMP response in a waveform aligned with the VEMPs at other sound levels. The waveforms were viewed and scored as VEMP "present" or "absent" by 2 of the authors. In 89% of cases, they agreed in their scoring. For the purpose of this paper, we considered a VEMP waveform to be present when both viewers scored the waveshape as "VEMP present", otherwise it was considered to be absent. The results and conclusions are little affected by

using the scoring of either one of the viewers alone (data not shown).

The VEMP response window and measuring VEMP peak-to-peak amplitudes

VEMP amplitudes were measured as the peak-to-peak values of the waveforms, using separate time windows for discerning the positive and negative peaks. To decide the time range in the EMG response in which to look for VEMP peaks, we used an iterative procedure. We started with wide time windows, and for each waveform containing a VEMP response we found the peak positive value and the peak negative value, and their latencies. We then adjusted the windows with the goal of choosing windows that included as many as possible of the peaks in the EMG waveforms that were judged to be VEMP response peaks, while excluding nearby peaks judged NOT to be part of VEMP waveforms. In individual subjects VEMP's at each sound frequency align if the time axis is adjusted to be relative to the peak of the sound stimulus (Rauch, Silveira et al. 2004, van Tilburg, Herrmann et al. 2014). To account for this, the windows were adjusted by adding the stimulus rise time (two cycles of the stimulus frequency). Based on these procedures we chose an acceptance window of 9 to 20 ms for the positive VEMP peak and up to 28 ms for the following negative VEMP peak. Although there were a few cases where a VEMP waveform was judged to be present and the peak was outside of the acceptance window, in all of these cases the peak was very close to the window edge and the most positive (or negative) point within the window (which was then used as the peak) was only slightly smaller than the actual peak.

The VEMP peak-to-peak amplitude (VEMPpp) was measured by an automated algorithm using the VEMP response windows described above. The VEMP positive peak was taken to be the largest positive value of the average EMG within the time window from 9 to 20 ms after the stimulus peak. The VEMP negative peak was taken to be the largest negative value of the average EMG within a time window that started after the VEMP positive peak and ended 28 ms after the stimulus peak. When there was a clear VEMP response, this algorithm always picked out the correct VEMP peaks. When there was not a clear VEMP response, the algorithm usually picked the peaks chosen by a human observer, but when it didn't, there was usually little difference in the peak-to-peak values obtained by the algorithm and by the human observer. Despite the difficulty when VEMP response amplitudes were low or absent, it is necessary to quantify the peak-to-peak amplitude for all VEMP waveforms to be able to determine how well the normalizations tracked the visual judgment of VEMP present/absent.
Receiver Operating Characteristic

(ROC) curves and bootstrapped error bars

An alternate way to evaluate the normalization methods is to determine how well the resulting VEMPn values agree with the visual determination of which EMG waveforms showed a VEMP response and which did not. An analytical method that does this is the receiver operating characteristic (ROC). In a ROC analysis, a tentative-threshold criterion is varied in small steps from zero to the highest VEMPn value obtained. At each criterion value, if the VEMPn from a particular subject is greater than the criterion, the VEMPn is considered to indicate that a VEMP response had occurred. A VEMPn greater than the criterion from a waveform that did have a VEMP response was scored as a "true positive". A VEMPn greater than the criterion from a waveform that did not have a visually-determined VEMP response was scored as a "false positive". When the criterion is very low many cases will be false positives and as the criterion rises there will be fewer false positives and more true positives. The area under the ROC curve (AUC) is a single-number metric that characterizes how well the VEMPn matches the visual evaluation of VEMP present/absent.

To get an estimate of the standard deviations of the VEMPn AUC values, we used a bootstrap method. For each set of VEMPn's (and for the un-normalized VEMPpp's), there were 1142 VEMPn waveforms, of which 786 were judged to have a VEMP present. For each VEMPn set, a new set of 1142 VEMP waveforms was formed by randomly choosing a waveform from the original set of 1142 waveforms (without removing this waveform from the original set, i.e. it could be chosen again), with the VEMP present/absent preserved, and the AUC was calculated from this new VEMPn set. For each VEMPn set, this was done 10,000 times and the SD of the distribution of the resulting AUCs was taken as the SD of AUC of the original VEMPn data. In each of the 10,000 randomizations, the same randomization was used for all VEMPn sets.

RESULTS

Coefficients of Variation

One goal for VEMP normalization is to make the VEMPn values across subjects as uniform as possible for VEMP's obtained at the same sound level and frequency. Variation in VEMPn amplitudes across subjects can be quantified by the coefficient of variation (CV), which is the standard deviation of the data divided by the

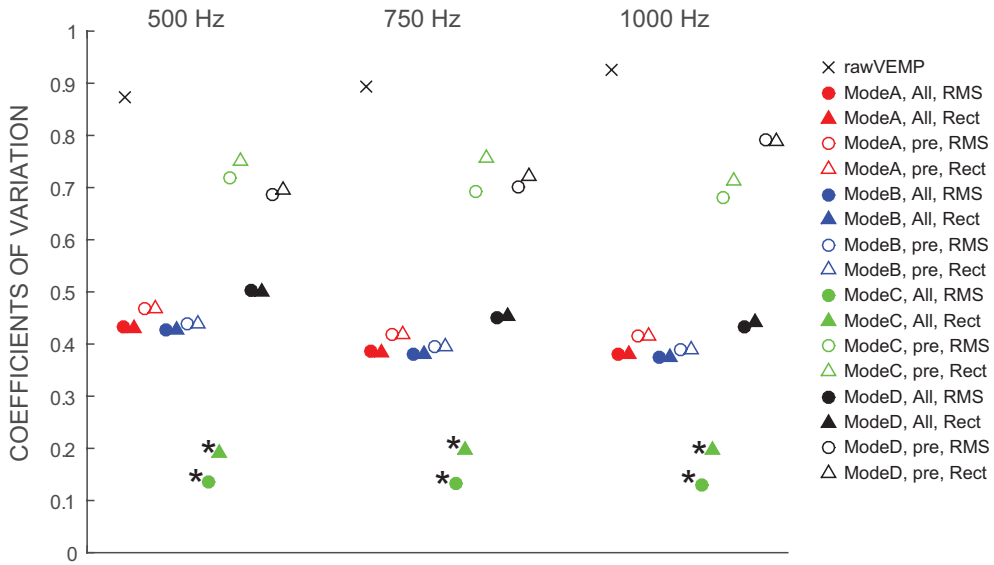

Figure 2. The coefficients of variation (CVs) from normalized VEMPs (VEMPn's) and for the un-normalized (raw) cVEMP, as indicated by the key. Each point is the CV for the 123 dB peSPL runs of the 40 ears stimulated at the frequency indicated at top. Mode = how the EMG metric was applied (see text). All = the EMG widow included the whole 77 ms trace. Pre = the EMG window included 8 ms before the VEMP response. RMS = root mean square EMG. Rect= average rectified EMG. *indicates Mode C points normalized by the all-trace EMG window. They represent a partial self-normalization that produces an artificially low CV without providing more consistent VEMP information. These points were included for completeness.

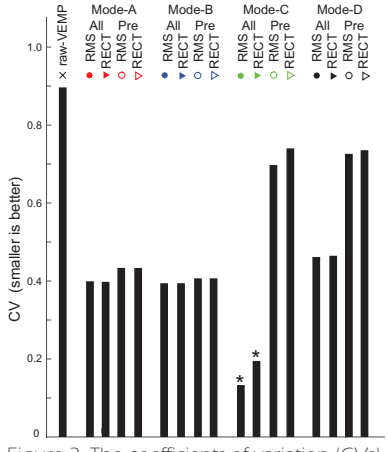

Figure 3. The coefficients of variation (CVs) from the data of Figure 2 averaged across frequency. *indicates Mode C points normalized by the all-trace EMG window. They represent a partial self-normalization that produces an artificially low CV without providing more consistent VEMP information.

mean. The 16 normalization methods that we tested produced a wide range of CVs. We concentrate here on VEMPn CVs for the responses obtained at the highest sound level. A comparison of VEMPs from low sound levels is less useful because VEMP threshold varied across subjects causing the low-level data to be less comparable. The CVs from each frequency are shown in Figure 2 and the CVs averaged across all frequencies are shown in Figure 3. Also included in these figures are the CVs for the un-normalized VEMPpp. All of the VEMPn's had CVs that were less than the CV of VEMPpp (note that a lower CV is better).

The VEMPn values fell into several clusters (Figs. 2 and 3). The biggest cluster was formed by the Mode-A and Mode-B CVs. For these, and for all of the VEMPn's, there was very little difference between CVs that used RMS versus RECT to quantify the EMG amplitude. The Mode-A and Mode-B VEMPn's that used the all-trace window had very similar CVs and were slightly better than the CVs from VEMPn's that used the shorter, pre-response window. For VEMPn's that used the pre-response window, Mode-B CVs were slightly lower than Mode-A CVs.

The Mode-C VEMPn's produced the lowest CVs and almost the highest CVs, depending on the timing of the EMG window. When the pre-response window was used, Mode-C VEMPn's produced high CV values that were lower than those from un-normalized VEMPs, but they provided less than half of the reduction in CVs that was produced by the best Mode-A and Mode-B VEMPn's (Figs. 2 and 3). On the other hand, when the whole-trace window was used, Mode-C VEMPn's produced extremely low CV values. The whole-trace window included the VEMP response, so that bigger VEMP responses resulted in bigger overall-EMG-metrics. Thus these whole-trace-window VEMPn's are a self-normalization of the VEMP average waveform. As would be expected this self-normalization certainly produced the lowest CVs, however the ROC analysis described in the next section indicates that it significantly decreases cVEMP detection.

The Mode-D VEMPn's obtained their overall-EMG-metric from noise waveforms in which the VEMP-response wave was cancelled out. This removed the self-normalization that made the Mode-C CVs very low. As a result, Mode-D CVs did not have very low CVs. Mode-D CVs that used the full-trace window had values that were only slightly higher than the Mode-A and Mode-B CVs. On the other hand, the Mode-D CVs that used the pre-response window were much higher and similar to the Mode-C CVs that used the pre-response window. This similarity makes sense because both of the Mode-C and Mode-D EMG metrics used a time window that did not contain the VEMP response and reversing every-other trace before averaging is not expected to make much difference in these overall-EMG-metrics.

ROC curves

ROC curves that include the VEMPn data from all three frequencies and all levels are shown in Figure 4. Data from all levels are included because ROC curves need to have cases from below to above the VEMP threshold. ROC curves that are

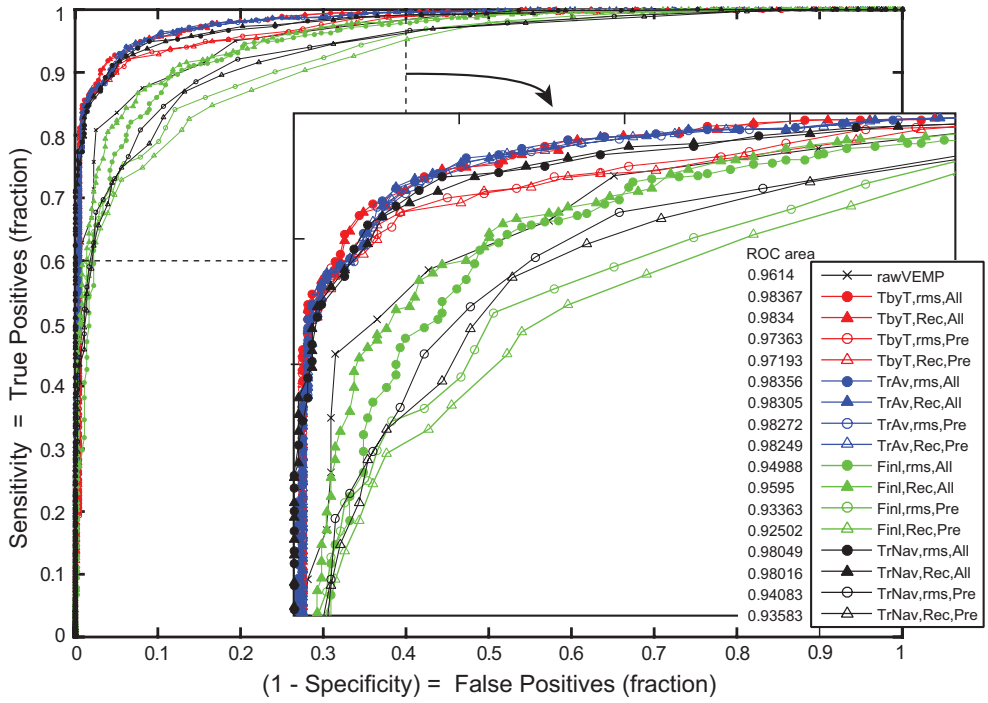

Figure 4. Receiver-Operating-Characteristic (ROC) curves from the normalized cVEMP's (VEMPn's) and for the un-normalized (raw) cVEMP, as indicated by the key. ROC curves show the fraction of true positives (the "sensitivity" of the test) versus the fraction of false positives (unity minus the "specificity" of the test). All of the data at all frequencies are included.

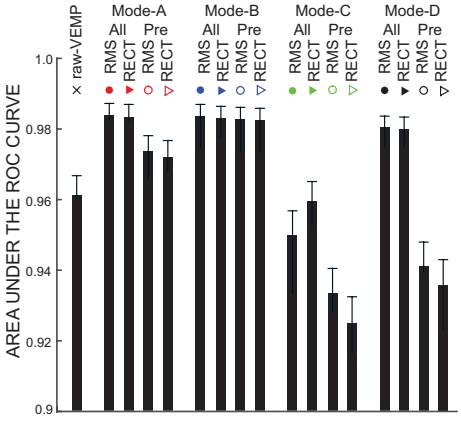

Figure 5. The Area Under the Curve from the ROC analysis of each normalization method and for the raw VEMPpp. All data were included. The error bars are estimated standard deviations.

closer to the upper-left corner are better because they achieve a higher true-positive fraction (also called the "sensitivity" of the test) for any given false-negative fraction (which is unity minus the "specificity" of the test). A single-number metric that characterizes how well the VEMPn matches the visual evaluation of VEMP present/absent is the area under the curve (AUC). AUC values are shown as bar graphs in Figure 5, along with bootstrapped error bars (see Methods) that indicate the estimated standard deviation for each AUC.

The ROC curves and their AUCs paint a different picture than the CVs for which VEMPn methods are best. In particular, the Mode-C VEMPn's that used the all-trace window, which were the best using the CV metric, had lower AUCs than no normalization at all. Even worse were AUCs for Mode-C and Mode-D VEMPn's that used the pre-response window. All of these had lower AUCs than no normalization at all. The only one of the VEMPn's calculated from an averaged waveform that was better than no normalization was the Mode-D VEMPn's that used the all-trace window. The best AUCs were from Mode-A and Mode-B VEMPn's that used the all-trace window. Very slightly lower were the Mode-B VEMPn's that used the pre-response window and a bit lower were Mode-A VEMPn's that used the pre-response window.

To determine whether the differences between the various VEMPn ROC results are statistically significant, paired t-tests were done on the bootstrapped distributions of various combinations of VEMPn ROC results. This analysis showed that even when there were extremely small difference between a pair of ROC results, this difference is highly statistically significant. However, the usefulness of this test is questionable. These distributions started with two lists VEMPn values and randomized them in exactly the same way so that the small original difference persisted and determined the outcome of the paired t-test. A better estimate of whether two ROC-AUC bars in Figure 5 are statistically significantly different is shown by whether their error bars overlapped. From the error bars it appears that there are negligible differences between the AUCs from Mode-A and Mode-B VEMPn's that used the all-trace window and between them and the Mode-B VEMPn's that used the pre-response window. The Mode-A VEMPn's that used the pre-response window appear to be significantly worse than the set of best VEMPn's. Perhaps most surprising, the Mode-D VEMPn's that used the all-trace window had quite high AUCs, almost as high as the best Mode-A and Mode-B AUCs.

The effect of window duration

The above data show that the all-trace window always did better than the pre-trace window. It seems likely that this is because capturing a longer sample of the EMG provides a better estimate of the EMG amplitude. To determine how long the EMG window should be, we did normalizations and calculated the resulting ROC-AUCs using a wide range of window durations. To enable the window to be longer than the 77 ms duration of our stimulus period, we extended each trace

(starting with the third response trace) earlier and later in time by concatenating it with the two earlier and two later traces to produce a series of 385 ms long (overlapping) records of the EMG. This provided long pre-response and post-stimulus periods that can be used for Mode-A and Mode-B normalizations. For Modes C and D, there was no useful way to extend these averages past their 77 ms duration (if we averaged including the prior and post 77 ms we would get 3 almost identical concatenated averages and no new information). For Modes A and B, we started at the sound onset (3 ms after the trace start in our system) and separately extended the window earlier or later in duration. For Modes C and D, we could not get an earlier duration that was longer than 3 ms, so instead the window started at the end of the averaged period and extended earlier in time, and in separate calculations the window started at the beginning of the waveform and extended later in time.

The results from changes in EMG window duration are shown in Figure 6. For Modes A, C and D, short window lengths produced low AUCs, which is what was expected. However, for Mode-B, we found very little dependence of AUC on window length, which was unexpected (see Discussion). As expected, Mode-C showed big divergences when the window included, or partly included, the VEMP response, which occurred at different durations for the windows extending forward versus backwards in time. On the other hand, for Modes A, B and D there was very little difference between windows going forward or backwards in time (so only the backwards plots are shown in Fig. 6). In particular, there was only a small deviation between forward and backward Mode-A and Mode-B data when their windows in-

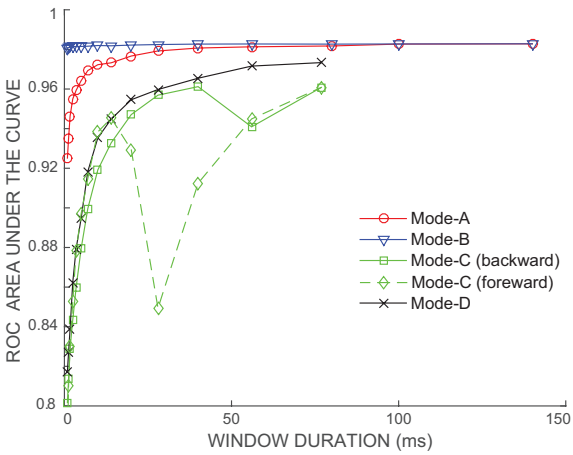

Figure 6. The ROC AUCs as functions of the duration of the EMG window. All VEMP's are included. Mode-A used a trace-by-trace normalization. Mode-B obtained the EMG metric from an average of the individual trace EMGs. Mode-C obtained the EMG metric from the averaged VEMP waveform. Mode-D obtained the EMG metric from the averaged "noise" waveform. See text for further details. All ROCs used RMS. Mode A, B & D window durations extended backward in time. Forward in time plots from Modes A, B & D are very similar. Forward and backward data are shown for Mode C because these windows include the VEMP response at different times and including the VEMP response produces a dip.

cluded the time of VEMP responses. Presumably, this is because in an individual trace, the EMG response is much bigger than the deviation caused by the VEMP response so that the VEMP response has little effect on the EMG metric. Finally, the results for RMS versus RECT quantification of the EMGs showed almost no difference in the overall shape of the AUCs as functions of window duration, so only RMS values are shown in Figure 6.

DISCUSSION

Our evaluation of VEMP normalizing methods using a variability measure (CVs) and a measure of signal detection (ROC AUCs) yielded results that were similar in some aspects and different in others. All VEMPn's had CVs that were lower (better) than the un-normalized VEMP CV, but 6 of the 16 VEMPn's had AUCs that were worse than the un-normalized-VEMP AUC. The most dramatic difference between the CV and AUC results was for the Mode-C VEMPn's that used the all-trace window. These had the lowest (i.e. best) CVs, but poorer-than-average AUCs. The Mode-C VEMPn's that used the all-trace window are different because their overall-EMG-metric included the VEMP itself and provided a kind of self-normalization. As shown by the AUCs, this type of normalization is worse than most of the other VEMPn's in signaling the presence/absence of a VEMP response in an average EMG waveform. To our knowledge, a normalization of this kind has never been used and we will not consider it further. When the Mode-C VEMPn's from the all-trace window are dropped from consideration, the rest of the VEMPn CVs and AUCs showed the same pattern of which normalization method was better or worse (Figs. 2-5).

Another consistent pattern across the data is that RMS versus RECT methods of quantifying the EMG produced little difference in the resulting VEMPn's. RECT yielded slightly better CVs than RMS but RMS yielded better AUCs more often than RECT. These metrics appear to be equal in value.

Mode-A and Mode-B are both commonly used in current VEMP clinical testing. Both used EMG metrics that were derived from the EMGs of individual traces, whereas Mode-C and Mode-D used EMG metrics derived from the VEMP waveform after it was averaged. The individual-trace methods are definitely superior and preferred. Rosengren et al. used a Mode-B method and averaged rectified EMG traces using a separate signal-recording channel (Rosengren, Welgampola et al. 2010). However, only a

single channel is needed and the trace EMG metrics can be obtained in software, which is what we did. The only normalization method that used averaged waveforms for the EMG metric and that was almost as good as the trace-by-trace methods was the Mode-D-all-trace method. However, this method got its EMG-metric from calculated "noise" waveforms and these still require signal processing that is more complex than straight averaging.

Our analysis of the effect of EMG-window duration showed that longer windows are better, except when the window extends into an average-waveform region that contains the VEMP response. However, the effect of extending the window duration saturates and not much benefit accrues from making the window longer than 50 ms. It is not surprising that longer windows are better because they gather more data and give a better estimate of the overall EMG activity. It was surprising, however, how little difference the window duration made for Mode-B VEMPn's. Even when the Mode-B EMG window was only 1 ms long, it did a good job of normalizing the data. In comparison, a 1 ms Mode-A EMG window was much worse. With a 1 ms window, the EMG values from the individual traces vary greatly from one trace to the next and in Mode-A this will make the individual normalized-traces vary greatly from one to the next. In contrast, with Mode-B, the individual EMG values are never used alone, they are only used as an average across all of the traces. The good normalization derived from a 1 ms Mode-B normalization tells us that a 1 ms sample of the waveform taken every 77 ms gives a good overall measure of the EMG amplitude from that run.

Comparison with previous papers that used VEMP normalization

To show how they affect the results, we used a wide variety of normalization methods, but only a small number of these have been used in published papers. Table 1 summarizes a review of 14 cVEMP papers {Isaacson, 2006}{Ito, 2007}{Lee, 2008}{Osei-Lah, 2008}{Wang, 2008}{Murofushi, 2009}{Nguyen, 2010}{Bogle, 2013}{Lim, 2013}{McCaslin, 2014}. Many papers provided only limited data on the way the normalization was performed. However, when described, most papers used the Mode B normalization (averaged waveform, RECT EMG (85%). and pre stimulus time EMG window (62%)).

CONCLUSIONS

Normalization is a widely accepted method of reducing variability. In the case of cVEMP testing, the objective of normalization is to "cancel out" intersubject variations in muscle effort noise so differences in cVEMP measurement more accurately reflect actual differences in the saccular response

to acoustic stimuli. In the clinical setting, some form of normalization is mandatory if cVEMP is to be truly useful in detecting abnormalities, intersubject differences, and intrasubject test-retest differences. There can be large differences in the results from different normalization methods, consequently it is essential to clearly describe how normalization has been applied in every paper. The methods that reduce cVEMP variance while retaining good cVEMP detection obtain the EMG metric from EMG measurements on individual traces (Modes A & B), not from the averaged VEMP waveform (Modes C & D). RMS and RECT methods for quantifying the EMG produce similar results. In most cases it is better to use a longer window duration for the EMG quantification, but the window duration matters very little when the EMG metric is calculated from the average of the individual-trace EMGs. Calculating the EMG from a long duration window of a VEMP "noise" averaged waveform is almost as good as the individual-trace methods, but it still requires more signal processing than a simple VEMP average. For EMG metrics obtained from individual EMG traces, including the time of the VEMP response does not prevent the result from providing a good normalization.

ACKNOWLEDGEMENTS

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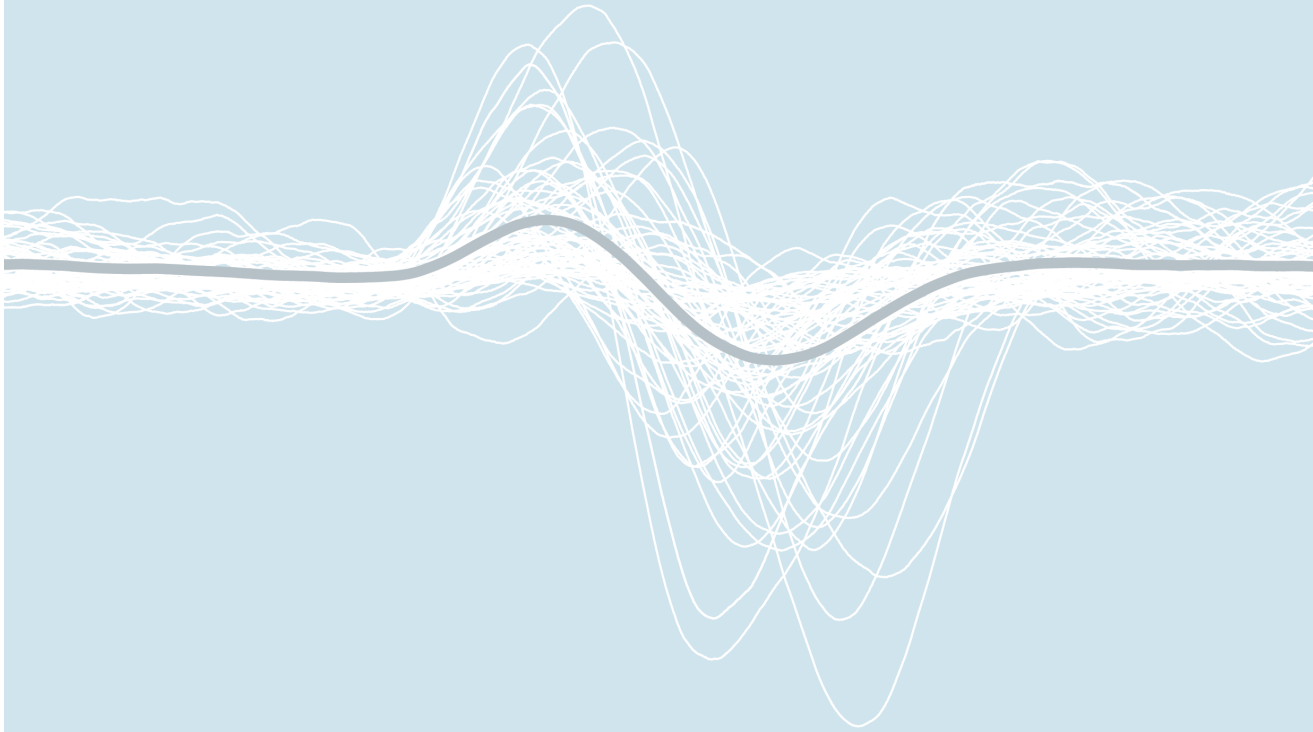

CHAPTER 4

Serial cVEMP testing is sensitive to disease progression in Meniere patients

SERIAL CVEMP TESTING IS SENSITIVE TO DISEASE PROGRESSION IN MENIERE PATIENTS

ABSTRACT

Objective: To assess the cVEMPs ability to track disease progression in Meniere's disease patients over time and identify the most sensitive outcome measurement.
Study design: Retrospective
Setting: Large specialty hospital, department of otolaryngology.
Subjects: Twenty nine Meniere's patients and seven Migraine Associated Vertigo patients
Intervention: All patients underwent two cervical vestibular evoked myogenic potential tests at 250, 500, 750 and 1000Hz with a minimum test interval of three months.
Main Outcome Measures: Threshold, peak-to-peak amplitude, interaural asymmetry ratio and effect size.
Results: In affected Meniere's ears all outcome measures were worse during the second test, for threshold this difference was statistically significant at 750 and 1000Hz compared to the first test. Compared to healthy ears the threshold was significantly lower at all frequencies. Peak-to-peak amplitude was significantly decreased at the second test at 750Hz compared to the first test. In Migraine Associated Vertigo no significant difference between tests was found at any frequency in peak-to-peak amplitude or threshold. In Meniere's ears, threshold showed a higher effect size at 500, 750 and 1000Hz compared to peak-to-peak amplitude.
Conclusion: cVEMP is able to track progression in Meniere's disease over time. Thresholds were the most effective outcome measure to both track progression and to distinguish between Migraine Associated Vertigo and Meniere's patients.
Words: Cervical vestibular evoked myogenic potential, Meniere's disease, Migraine Associated Vertigo

INTRODUCTION

The cervical vestibular evoked myogenic potential (cVEMP) is a test used to aid in the diagnosis of Meniere's disease. Classic Meniere's disease cVEMP responses are characterized by elevated thresholds, decreased peak-to-peak amplitudes and increased asymmetry ratios (1, 2). The latency is usually unchanged in Meniere's patients. Peak-to-peak (PP) amplitude and the interaural asymmetry ratio, calculated from the PP amplitude, are widely used outcome measures. PP amplitude varies with muscle contraction intensity, which can be corrected by applying a form of normalization (3). The interaural asymmetry ratio (IAR), that compares the left ear with the right ear, has the disadvantage that 30% of Meniere patients show "Meniere-like" changes on the unaffected ear, which would reduce IAR and thereby underestimate pathology (4). Threshold (i.e. the lowest intensity at which a response is still detectable) is used less often, but has shown to be a highly reliable metric (5). Which outcome is the most useful in the diagnosis of Meniere's disease is unclear.

cVEMP responses in patients with Meniere's disease have been described in several studies. For example, in patients with more severe disease (i.e. drop attacks) cVEMP thresholds are even higher compared to "regular" Meniere's patients (4). Young et al. (2003) showed that VEMPs (both cervical and ocular) could help stage Meniere's disease (6). It is not known if the cVEMP is able to track disease progression over time. The purpose of this study was to assess whether cVEMPs are sensitive to progression of Meniere's disease over time and thus show worsening of the outcome as the disease progresses. As comparison groups we selected normal healthy subjects and patients with Migraine Associated Vertigo (MAV). MAV patients can exhibit cVEMP abnormalities similar to Meniere's disease (7) but are not predicted to show progression comparable to Meniere's cases. An additional goal was to determine if a particular cVEMP measure, threshold, amplitude or amplitude ratio, changed over time with disease progression.

To test our hypothesis we retrospectively compared cVEMP results from Meniere and MAV patients who had two cVEMP clinical evaluations during their disease and analyzed which parameter of the test was best able to track progression of the disease.

METHODS

Subjects

All patients diagnosed at the Massachusetts Eye and Ear Infirmary (MEEI) with unilateral Meniere's disease between 2006-2013 and who had two cVEMP tests were included in this retrospective study. Their charts were analyzed and Meniere's disease was verified to be the final diagnosis. Patients with both migraine and Meniere's disease were excluded. The flowchart for the patient inclusion is shown in figure 1. Twenty-nine patients (15 female) with unilateral Meniere's disease met the inclusion criteria (mean age 59, SD 12 years). The average time between tests was 28.3 months (SD 23.4 months).

Patients with migraine associated vertigo (MAV) were included using the same protocol. Seven patients met the inclusion criteria (4 females, mean age at first test 50 (SD 6.8)) with an average time between tests of 24.9 months (SD 13.8).

As controls, we used twenty healthy subjects (described previously in (3)). In these controls, PP measurements were made at 123 dB pSPL for all four stimulus frequencies (250, 500, 750 and 1000Hz) and cVEMP thresholds were determined at 500Hz. The controls provide cVEMP statistics from normal, young ears (mean age 29, range 20-48). They were not age-matched to the Meniere's and MAV patients.

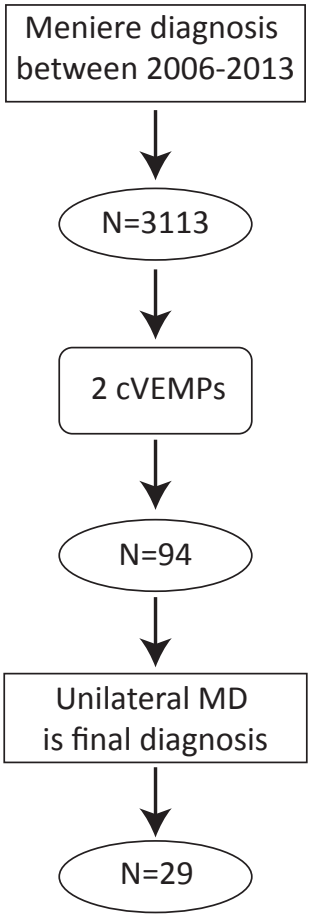

Figure 1. Meniere's patient inclusion flow chart.

cVEMP recording

cVEMPs were recorded to tonebursts (Blackman gating function, 2 cycle rise/fall, no plateau) at four frequencies: 250, 500, 750 and 1000Hz (see (1)for details), as part of a clinical cVEMP evaluation. Stimulus intensity was varied at each frequency for determination of cVEMP threshold. All cVEMP waveforms were stored and available for retrospective analysis. Peak-to-peak P1-N1 amplitude was measured retrospectively on the normalized cVEMP waveform at the 123 dB pSPL or 127 pSPL intensity level (90 or 95 dB HL except at

250 Hz where they are 80 or 85 dB HL). A trace-by-trace RMS normalization was used (3). If no response was present at 123 or 127 dB pSPL, the residual noise of the waveform was measured at two points in the cVEMP time window (0-15 and 15-30ms). The residual noise was defined as the average of the two largest peak-to-peak amplitudes in the waveform where no response was present. This value was then used as the peak-to-peak amplitude for the absent response. In addition, cVEMP threshold was visually determined from the intensity series. If the cVEMP response was absent at the highest stimulus intensity (i.e. at the equipment limits), cVEMP threshold was defined as 10 dB higher than the equipment stimulus level limit. In other words, 10dB was added to the higher stimulus level and set as threshold. All measurements were made by one person, blinded for the side of disease, using the normalized waveform.

Analysis

The ears of the Meniere's subjects were categorized as affected or unaffected by the disease. Average threshold, peak-to-peak amplitudes and interaural asymmetry ratios (IAR) were calculated for each group and the two groups were compared using t-tests. The IAR was calculated using the formula: ((AS-AD) / (AS+AD))*100, but substituting "AS" for "unaffected ear" and "AD" for "affected ear", so that a positive outcome meant the amplitude on the affected side was smaller.

Normal and MAV patients showed no evidence of asymmetry so, within subjects, their ears were grouped for calculation of average threshold and peak-to-peak amplitude. The IAR was calculated by the following formula: ((AS-AD) / (AS+AD))*100. Time between tests was calculated in months.

To assess whether PP amplitudes or thresholds were best able to detect progression of Meniere's disease the effect size was calculated. The effect size is the difference between two means (the two tests) divided by the standard deviation. The higher the effect size, the bigger the difference between the two tests (i.e. a high effect size suggests a bigger progression of the disease).

RESULTS

cVEMP Threshold

cVEMP Thresholds in Meniere's patients

29 patients (15 female) with unilateral Meniere's disease met the inclusion criteria (mean age at first test 59 years, SD 12 years). The average time between tests was 28.3 months (SD 23.4 months). The biggest change between tests was in the affected ear (Fig. 2). For the affected ear, thresholds from the second test were higher than thresholds from the first test at all frequencies, and were significantly higher at 750 and 1000 Hz (P<0.02 and <0.01 respectively). In contrast, the unaffected ear showed little difference in thresholds between the first and the second test at any frequency. Comparing the cVEMP thresholds in the affected vs. unaffected ears (the interaural threshold difference), there was little difference at the first test but at the second test there were large, statistically-significant differences at all four frequencies (P=0.03 at 250 Hz, P<0.01 at 500 Hz, P=0.02 at 750 Hz and P<0.01 at 1000 Hz). The thresholds at 500Hz of the Meniere's patients were significantly higher than the thresholds of the control subjects (P<.05) (Fig. 2).

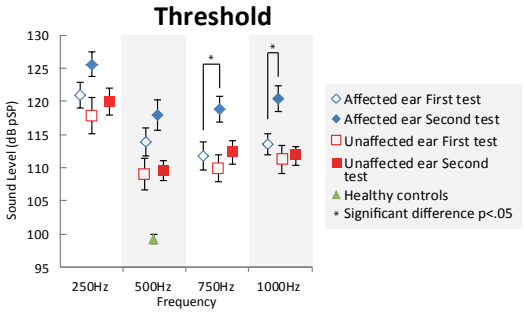

Figure 2, Mean cVEMP thresholds measured at 4 frequencies showing the results for the affected and unaffected ears (diamonds and squares) of the Meniere's patients during the first and second test (filled and unfilled symbols). Error bars indicate standard error of the mean. Significant differences are marked with an asterisks and are present between the first and second test of the affected ear at 750 and 1000 Hz.

cVEMP Thresholds in Migraine Associated Vertigo patients

Seven MAV patients were included (4 females, mean age at first test 50 years, SD 6.8 years). The average time between tests was 24.9 months (SD 13.8 months). Both ears were grouped together since no patient had hearing loss or other symptoms suggesting unilateral disease. No significant difference in MAV thresholds were found between the first and second test at any frequency. Comparing the thresholds of the MAV patients to the second-test thresholds of the affected ear of the Meniere's patients, the thresholds were significantly lower for the MAV patients at all frequencies (P=0.01 at 250 Hz, P=0.02 at 500 Hz, P=0.01 at 750 Hz and P=0.01 at 1000 Hz) (Fig. 3A). No significant difference was found between the thresholds of

the MAV patients and the first-test thresholds of the unaffected ears of Meniere's patients (Fig. 3B). Thresholds at 500Hz of the MAV patients were all significantly higher than the thresholds of the control subjects (P<0.01) (Fig. 3A, B).

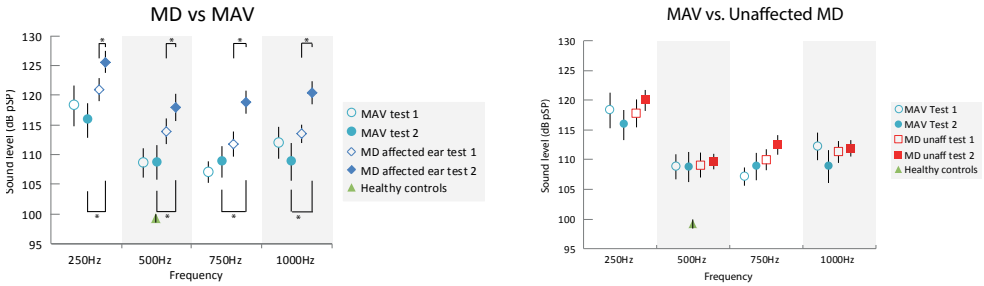

Figure 3a (top). Mean cVEMP thresholds measured at 4 frequencies showing the results for the MAV patients (circles) and the affected Meniere's ears (diamonds) during the first (unfilled) and second (filled) tests. B (bottom) cVEMP threshold measured at 4 frequencies showing the results for the MAV patients (circles) and the unaffected Meniere's ears (squares) during the first (unfilled) and second (filled) tests. Error bars indicate standard error of the mean. No significant differences were present between tests in the MAV patients.

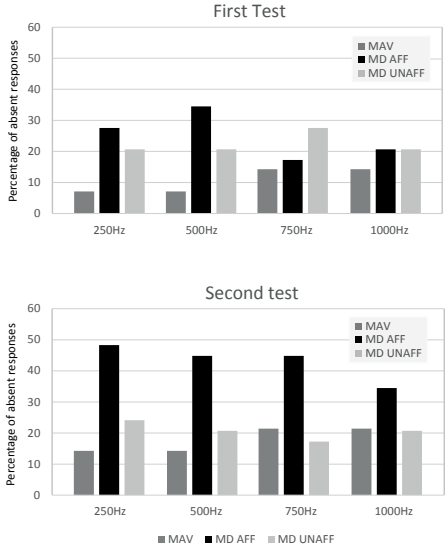

Figure 4A (left). Prevalance of absent cVEMP responses in the affected ear during the first (light grey) and second (black) test. 4B (right). Prevalance of absent cVEMP responses in the unaffected ear during the first (light grey) and second (black) test.

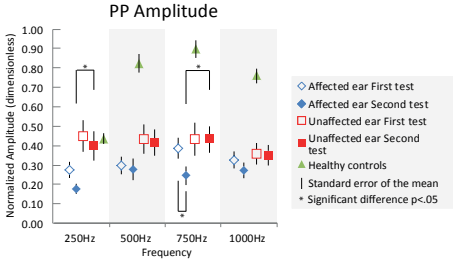

Figure 5. Mean peak-to-peak amplitudes at 4 frequencies measured in the controls (triangles), affected (diamonds) and unaffected (squares) Meniere's ear during the first (unfilled) and second (filled) test. Error bars indicate standard error of the mean. A significant difference was found only for 750 Hz between the first and second test of the affected ear in Meniere's patients.

Peak-to-peak (PP) amplitude

Regardless of metric or frequency, the incidence of absent cVEMP responses was substantially more prevalent in the second test of the Meniere's affected ears (figure 4a, b). For calculating PP averages, these absent responses were given PP values equal to their noise floors (see Methods). The PP amplitudes of the Meniere's affected ears were smaller in the second test than in the first test, at all frequencies (Fig. 5) and the difference was significant at 750Hz ($P<0.05$). The PP amplitudes from the second test of Meniere's patients were smaller in the affected ear than in the unaffected ear, at all frequencies, with the difference statistically significant at 250Hz and 750Hz ($P=0.01$ and $P=0.03$, respectively).

For the MAV patients, there were no significant differences between the average PP amplitudes of the first and second tests (Fig. 6). In addition, no significant differences in average PP amplitudes were found between the MAV ears and the unaffected Meniere's ears. Comparing the MAV ears and the affected Meniere's ears, the Meniere's ears had lower average PP amplitudes at all frequencies, with the difference significant at 250Hz and 750Hz ($P=0.02$ and $P=0.03$, respectively) (Fig. 6). All of the average PP amplitudes at 500, 750 and 1000Hz from MAV patients and from Meniere's patients were lower than the PP amplitudes of the control subjects and the differences were statistically significant ($P<.05$) (Figs. 5, 6).

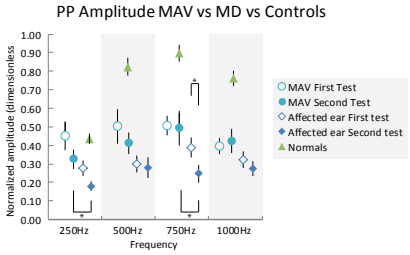

Figure 6. Mean peak-to-peak amplitudes at 4 frequencies measured in the controls (triangle), MAV patients (circles) and the affected (diamonds) Meniere's ear during the first (unfilled) and second (filled) test. Error bars indicate standard error of the mean. No significant differences were found between the first and second tests.

Interaural Asymmetry Ratio (IAR)

For the affected Meniere's ear, the average IAR from the second test was larger than from the first test at all frequencies, and the difference was significant at 750Hz ($P=0.03$) (Figure 7). At the second test the IAR from Meniere ears differed significantly from controls at 250, 500 and 750Hz ($p<0.04$, $p<0.05$ and $p<0.03$ respectively). For the MAV group there was a significant difference with the MD group for both tests at 250Hz

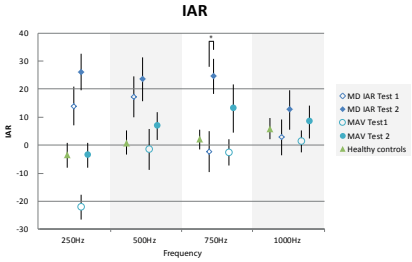

Figure 7. Mean interaural asymmetry ratio calculated at 4 frequencies for the healthy controls (triangle), Meniere's patients (diamonds) and MAV patients (circles). Error bars indicate standard error of the mean. The only significant difference between the first and second was at 750 Hz for the Meniere's patients.

(P.01 for test 1 and P.04 for test 2) (figure 7).

Effect size

The effect size was used to assess which test parameter (PP amplitude or threshold) was best able to show a change between the first and second test. At 500, 750 and 1000Hz the threshold showed higher effect sizes, at 250Hz the PP amplitude was slightly better able to show a difference between the first and second test (fig 8).

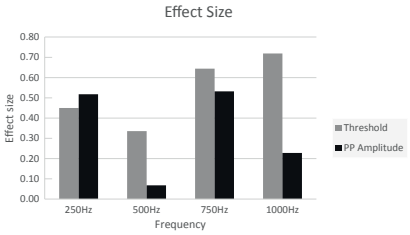

Figure 8. The effect size calculated at 4 frequencies for mean cVEMP thresholds (black) and mean cVEMP PP amplitudes (gray).

DISCUSSION

The purpose of this study was to evaluate whether progression of Meniere's disease could be tracked using the cVEMP. We also assessed whether peak-to-peak amplitude or threshold was more sensitive to disease progression and, therefore, better suited for monitoring Meniere's disease progression. Our data show that cVEMP threshold is the metric most sensitive to progression of Meniere's disease, showing significant threshold elevation from first to second test. Peak-to-peak amplitudes were a less sensitive measure to track disease progression.

Threshold

The average time between the two cVEMP tests was around two years for both pathological groups. During this time the thresholds of the affected ear in Meniere's disease worsened significantly for the higher frequencies, indicating progression of Meniere's disease. Various hypotheses have been put forward for the underlying cause of this change. Todd et al. hypothesized that the resonance in the saccule might be changing as Meniere's disease progresses (8). This could explain the change in sensitivity to different frequencies that we found (i.e., a significant increase in threshold at 750 and 1000 Hz).

Age is also known to have an effect on cVEMP peak-to-peak outcomes, namely a reduction of PP amplitude across frequencies (9). When we compare the results from our pathological subjects to our earlier obtained control data (who have a substantial lower mean age) we, too, find worse responses in the older group. However, age does not explain the significant worsening in the higher frequencies seen only in the affected ears in Meniere patients. Also, the threshold difference between

the affected and unaffected ear increased significantly at all frequencies, making it more likely to be an effect of pathology than of age.

Our patients with MAV showed no significant increase in threshold over time but their baseline cVEMP thresholds were elevated and PP amplitudes reduced compared to normal controls. The precise mechanism by which MAV might cause cochleovestibular (endorgan) damage is unknown. The work by Vass and colleagues suggests that trigeminovascular modulation of bloodflow to the inner ear, a phenomenon that is active in migraine, is a plausible explanation (10). Multiple studies have shown that the cVEMP peak-to-peak amplitude is reduced in MAV patients compared to healthy subjects and does not distinguish between MAV and Meniere's disease (7, 11). Our data are consistent with these reports. However, our data suggest that the poorer cVEMP threshold in MAV patients is stable over a time period during which Meniere's patients developed a significant threshold worsening in the affected ear. We propose that it could be useful to obtain a baseline cVEMP test and repeat this later if the pathological cause of the complaints (i.e. MAV or Meniere's) is still unclear.

Quantitative and statistical treatment of absent responses

Analysis and interpretation of absent responses at the intensity limits of the equipment is an issue that has plagued studies using audiometric outcome measures. (12). Likewise this is an issue for VEMP studies, many of which encounter absent responses in pathologic ears. In the present study we managed absent threshold responses by adopting the method often used in studies using pure tone audiometric outcomes: We added 10dB to the uppermost stimulus intensity and called this the threshold. Addressing the issue of absent PP amplitude response is somewhat more difficult as there is not a standardized approach elsewhere in our literature. In this study we used the peak-to-peak amplitude of the residual noise of the waveforms where no cVEMP response was present to assure that a value for each data point was present. By so doing, we could always compare PP amplitudes quantitatively and statistically even if no cVEMP response was present.

Threshold vs. peak-to-peak amplitude

Results indicate that cVEMP threshold is a more sensitive outcome metric for tracking progression of Meniere's disease. The use of effect size made it possible to compare the two outcome parameters (i.e. threshold and PP amplitude) and showed that threshold is more sensitive to changes between the two tests. Especially at 500 and 1000Hz threshold was more sensitive than PP amplitude.

Interaural Asymmetry Ratio (IAR)

As in the PP amplitude measure, the IAR also showed a significant difference at 750Hz between serial measures in the affected ears of Meniere patients. No significant IAR difference was found between the MAV group and the MD patients in first or second test, challenging the usefulness of the IAR in separating these groups.

Also the IAR needs to be interpreted with caution since approximately 30% of Meniere patients have bilateral disease or Meniere-like cVEMP responses in their "unaffected" ear (5). This leads to a reduced IAR and underestimation of the disease. Since a high IAR indicates a large difference between ears, the predictive value of an increased IAR is high. However, if the IAR is low it could be that both ears are healthy or that both ears have reduced responses. Thus the predictive value of a low IAR is low and no conclusions can be drawn from such an outcome.

Shortcomings

The lack of age matched controls in this study makes it difficult to interpret the comparison between healthy and pathological subjects. However it does not influence the results we found in the pathological group. Since current literature lacks consensus on the exact influence of age on the cVEMP response we could not correct our data for this variable. In future studies an age-matched control group should be used.

Our retrospective study could not control the time between the two tests, which averaged 28.3 months for the Meniere's group (SD 23 months). With this considerable spread in a relatively small group (N=29), we cannot determine the temporal characteristics of progressive cVEMP changes. We do not know if they were gradual, sudden, or stepwise. However the effect of time on the cVEMP has been clearly demonstrated. Future studies are needed to provide more details about the relationship between progressive Meniere's disease and the cVEMP.

Also our selected patient population was limited to those with an unambiguous single diagnosis of Meniere's disease or MAV; uncertain cases or those with both conditions were excluded. In the general Meniere's and MAV population there is more heterogeneity and the diagnosis is not always as

clear. In a prospective study a more representative population should be included.

In conclusion, the present study shows that the cVEMP is able to track progression in Meniere's disease over time. Thresholds were the most effective outcome measure to both track progression and to distinguish between MAV and Meniere patients.

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CHAPTER 5

Increasing the stimulation rate reduces cVEMP testing time by more than half with little change in threshold

INCREASING THE STIMULATION RATE REDUCES CVEMP TESTING TIME BY MORE THAN HALF WITH LITTLE CHANGE IN THRESHOLD

ABSTRACT

Objective: *Assessing the effect of a higher stimulation rate in cVEMP outcome measurements*

Study design: *Prospective cohort study*

Setting: *Large specialty hospital, department of otolaryngology.*

Subjects: *Eleven healthy subjects were used in this study*

Intervention: *All subjects underwent a cervical vestibular evoked myogenic potential test at 500, 750 and 1000Hz.*

Main Outcome Measures: *Threshold, peak-to-peak amplitude and interaural asymmetry ratio.*

Results: *Peak-to-peak cVEMP amplitudes were larger at 5/s than at 13/s. The 5/s to 13/s differences were statistically significant at 500 and 750Hz (p <.02). The variation in PP amplitudes across subjects, was not significantly different at any frequency for 5/s vs. 13/s stimuli. No significant difference was found in the interaural asymmetry ratio at any frequency. The cVEMP thresholds were similar between stimulation rates.*

Conclusion: *No significant differences in sensitivity, accuracy or precision outcomes were found between 5/sec and 13/sec stimulation rates, though intrasubject PP amplitude was significantly lower at the faster stimulation rate. To keep patient discomfort and test time to a minimum, the faster stimulation rate is recommended.*

Words: *Cervical vestibular evoked myogenic potential, Stimulation rate, threshold, peak-to-peak amplitude*

INTRODUCTION

Cervical vestibular evoked myogenic potentials (cVEMPs) are used worldwide to assess saccular and inferior vestibular nerve function (1). They are evoked by high intensity acoustic stimuli and recorded from the contracted ipsilateral sternocleidomastoid muscle (SCM). The contraction of the muscle is essential since the response causes an inhibition of motor unit action potentials that cannot be measured without muscle contraction (2). The longer the recording epoch, the more difficult and uncomfortable it is for the patient to maintain consistent SCM contraction. To get a predetermined number of cVEMP responses, the duration of contraction depends on the speed at which the acoustic tone burst stimuli are presented (i.e. the stimulation rate). It is important to select a stimulation rate that shows both clear cVEMP responses and is fast enough to keep the contraction time as short as possible for the patient.

Wu et al. reported that the cVEMP response to click stimuli was present in 100% of subjects using 1, 5 or 10 clicks per second stimulation rates (3). Since the highest VEMP peak-to-peak (PP) amplitudes were found at 1 and 5/sec stimulation rates, they concluded that 5/sec was the best choice since it took a shorter time to collect the data compared to 1/sec stimulation rate.

Currently most clinics use a 5/sec stimulation rate for cVEMPs. A typical high quality cVEMP recording requires averaging the responses to approximately 200 stimulus repetitions (4). Using a 5/sec stimulus repetition rate requires at least 40 seconds per measurement (i.e., 40 seconds of SCM contraction). In our clinic we use a 13/sec stimulus repetition rate, which requires only 15 seconds of muscle contraction to get 200 sweeps. This study compares 5/sec and 13/sec stimulation rates to assess their impact on cVEMP test outcomes.

METHODS

Eleven subjects (8 male, mean age 28.4, range 24-39) participated in the study. All subjects had normal hearing threshold sensitivity and no air-bone gaps as indicated by an audiogram done prior to cVEMP testing. Distortion product otoacoustic emissions (DPOAEs) were also measured before and directly after cVEMP testing. DPOAEs were present in all subjects and were similar before and after cVEMP testing. Subjects were excluded if they had a history of neck injuries or balance problems. All subjects were asked to subjectively describe the test burden of

both stimulation rates. Informed consent was signed. This study was approved by the Human Studies Committee of the Massachusetts Eye and Ear Infirmary.

cVEMP recordings

Cervical vestibular evoked myogenic potentials were recorded using a custom-programmed evoked potential system. Each ear was stimulated separately and cVEMPs were recorded from the SCM ipsilateral to the stimulus. Subjects sat upright with their head turned toward the non-test ear to contract the SCM on the test side. EMG activity was recorded from surface electrodes on the SCM. A positive electrode was placed on the belly of each SCM muscle . The reference electrode was placed between the SCM tendon attachments at the clavicle. A ground electrode was placed on the forehead. SCM muscle EMG activity was amplified, bandpass-filtered and sampled for 30ms after stimulus onset at a sampling rate of 50 kHz using a 16-bit analog to digital converter (National Instruments). Two hundred EMG traces were averaged for each cVEMP response. To correct for differences in muscle contraction a RMS trace-by-trace normalization was used and all measurements were made using the normalized waveforms.

Stimuli

Tone bursts were generated by custom-programmed evoked potential software (National Instruments 16-bit digital I/O board) using a Blackman gating function with a two-cycle rise/fall and no plateau. Toneburst frequencies were 500, 750 and 1,000 Hz. Stimuli were presented at a rate of either 5/s or 13/s to circumaural headphones (Telephonics TDH-49) at a level of 123 dB peak-equivalent sound pressure level (peSPL) for each frequency. The 5/s and 13/s stimulation rates were recorded in a single session in randomized order (5/sec or 13/sec first). During the session the electrodes stayed in place and a break was taken between the different stimulation rate sessions to avoid muscle fatigue. At each frequency two waveforms were recorded at 123 dB peSPL (90 dB HL) and threshold was determined at 750Hz using 5dB steps from 123 to 98 dB peSPL. The order of stimulus presentation was also randomized within each rate session (5.sec or 13/sec).

Analysis

The two VEMP peak-to-peak (PP) amplitudes at the 123 peSPL level were averaged for each frequency to have a single outcome. The interaural asymmetry ratio (IAR) was calculated from the PP amplitudes using the formula: IAR = ((AL-AR) / (AL+AR))*100, where AL and AR are the VEMP PP amplitudes in the left and right ears, respectively. Thresholds, PP amplitudes and IARs were averaged across subjects and compared using t-tests.

The coefficient of variation (standard deviation/ mean) was used to compare the

variation across subjects between the two stimulation rates using Levene's test.

RESULTS

cVEMP responses were present in all 22 ears of the 11 subjects, at all frequencies and at both 5/s and 13/s repetition rates. Peak-to-peak cVEMP amplitudes were larger at 5/s than at 13/s, and the percent PP difference between rates gradually decreased with increasing stimulus frequency (Fig. 1). At, the 5/s stimulation rate VEMPs had bigger PP amplitudes than at 13/s, by 27% at 500 Hz, by 25% at 750Hz, and by 13.3% at 1000Hz. The 5/s to 13/s differences were statistically significant at 500 and 750Hz ($p < .02$).

Despite the cVEMP amplitudes being smaller at 13/s than at 5/s, other cVEMP metrics were little changed. The variation in PP cVEMPs across subjects, calculated using the coefficient of variation, was not significantly different at any frequency for 5/s vs. 13/s stimuli ($p=0.41$ at 500 Hz, $p=0.08$ at 750 Hz, and $p=0.49$ at 1000 Hz, by Levene's test) (Fig. 2). No significant difference was found in the interaural asymmetry ratio at any frequency ($p=0.10$ at 500 Hz, $p=0.11$ at 750 Hz, $p=0.10$ at 1000 Hz) (Fig. 3). Thresholds at 750Hz were similar on both sides and for both stimulation rates. For 13/second the average thresholds were 108.5 dB pSP for left-ear stimulation and 108.0 dB pSP for right-ear stimulation. At 5/second the thresholds were 107.4 dB pSP for left-ear stimulation and 106.1 pSP for right-ear stimulation (Fig. 4). The cVEMP thresholds were the same despite the cVEMP PP amplitudes being different for 123 dB peSPL.

DISCUSSION

The purpose of the study was to assess whether different tone burst stimulation rates yielded different cVEMP measurements and whether such differences had an impact on clinically relevant outcomes. Our results show that there was a significant reduction of intrasubject PP amplitude at the faster simulation rate but the faster stimulation rate did not cause a significant change in threshold, interaural asymmetry ratio, or intersubject variability of PP amplitudes. However, our subjects were young and healthy whereas patients undergoing a cVEMP exam usually are older and in less good condition. The major advantage of using a faster stimulation rate is that the muscle contraction time required to obtain a 200-sweep cVEMP measurement is reduced from 40 seconds to 15 seconds. Our data indicate that the faster stimulation rate gives this advantage with little or no sacrifice in the usefulness of cVEMP in

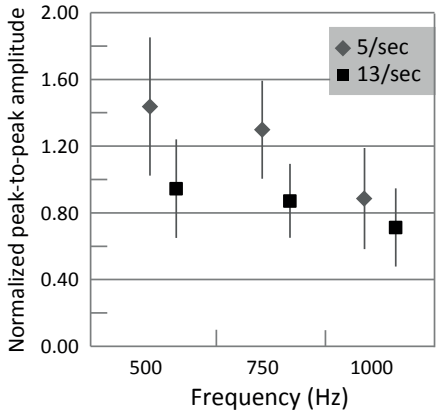

Figure 1. Average normalized peak-to-peak (PP) amplitudes for 500, 750 and 1000Hz at 5/sec and 13/sec stimulation rates. As frequency increases the differences in PP amplitude decreases. Error bars indicate SD.

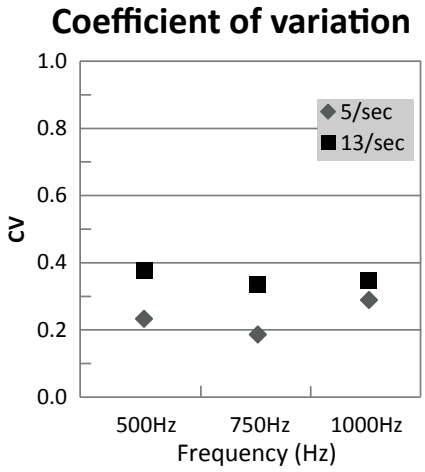

Figure 2. Coefficient of variation (SD/Mean) of the PP amplitude. No significant difference was found at any frequency between the two stimulation rates.

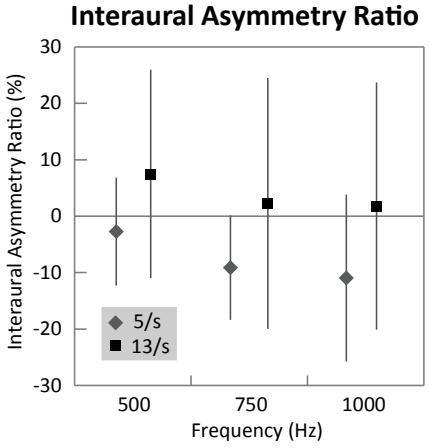

Figure 3. Interaural asymmetry ratios for the tested frequencies for both stimulation rates. Error bars represent standard deviation.

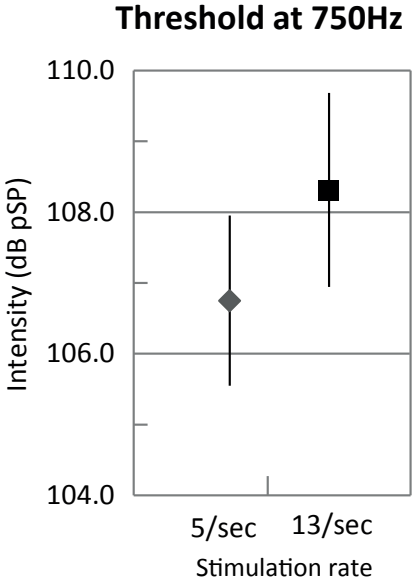

Figure 4. Thresholds for both stimulation rates at 750Hz. The combined average of both ears was used. Error bars represent SEM.

healthy subjects.

Wu and Murofushi (1999) reported that cVEMP responses were found in 100% of cases for repetition rates of 1/s, 5/s and 10/s, and in 63% of cases at 20/s. We also found cVEMPs in 100% of cases at both 5/s and 13/s. To our knowledge no other study has assessed the effect of sound stimulation rate on cVEMP outcomes. Similar to Wu and Murofushi we found that the cVEMP PP amplitude was smaller as stimulation rate increased with an amplitude decrease a little less than one-third (3). However, this decrease in amplitude did not result in significantly different cVEMP thresholds, IARs or increased cVEMP PP amplitude variability. Clinically it does not matter whether the peak-to-peak amplitude is large or small if test outcome is unaffected. cVEMP threshold depends upon the presence or absence of the response rather than the exact amplitude. The absence of a significant difference in cVEMP threshold between the two rates indicates that the decrease in amplitude noted at the higher level is likely not present close to threshold or is not great enough to significantly change the signal-to-noise ratio at threshold. The interaural asymmetry ratio assesses the left-right difference and since those factors that cause the decrease in cVEMP amplitudes with increasing stimulation rates can be expected to be the same in the right and left ears of a given subject, little change in interaural asymmetry ratio was expected nor found. However as seen in figure 3 the error bars for the IAR are much greater for the 13/sec data. This support our statement that the IAR is not a suitable outcome for cVEMPs, it is more variable compared to thresholds and in Meniere's patients there is a great risk of underestimating the IAR since the unaffected ear is affected in 30% of the cases. In addition, no significant difference in variability of cVEMP amplitudes across subjects was found between rates (Levene's test P>.05), indicating that the variability at the faster stimulation rate was not significantly greater than the variability at the slower stimulation rate.

It seems likely that the cVEMP amplitude declines as stimulus rate is increased because of adaptation at both the peripheral hair-cell-to-nerve-fiber synapses and at central synapses. That there is adaptation at peripheral synapses is shown by the decrease in saccular nerve responses with time during tone burst stimulation for sounds that are well above threshold (5). Adaptation at central synapses is shown by the lower amplitude cVEMPs at higher rates for galvanic-evoked cVEMP responses (6). A major factor in synaptic adaptation is the depletion of synaptic vesicles. Vesicle depletion increases with stimulation strength or rate, but is

very little near threshold where few vesicles are used. Different degrees of vesicle depletion may explain why cVEMP amplitude is considerably decreased by high stimulation rates for strong stimuli, but not for near-threshold stimuli.

All subjects were asked about the burden of the test vis-à-vis effort and discomfort. Faster stimulation rates are louder and could therefore be experienced as more uncomfortable. All subjects reported that the 5/sec test was substantially more difficult to complete while the 13/sec test was well tolerated, indicating that test time probably adds to the discomfort much less than a faster stimulation rate. It is possible that the faster stimulation rate could cause more stress because there is less time between the tone bursts to recover. However since the amount of stimuli (i.e. 200) was the same for both conditions (13/sec and 5/sec) the total sound exposure is not much different. Furthermore, in our study we found that threshold is the outcome of choice. In a protocol measuring thresholds one could use lower intensities to measure cVEMPs compared to levels normally used for cVEMP amplitude measurements lowering total sound exposure.

The difference in the difficulty of the test is likely due to the duration of muscle contraction needed between the two rates for each response and for the session as a whole. The duration of muscle contraction is nearly three times as long for the 5/sec than the 13/sec method (15.38 vs. 40 seconds) for each response. All participants in this study were young adults in good health; this test burden issue is likely of even greater importance with advancing age or poorer health. Keeping the test time to a minimum reduces the burden to the patient, increasing the probability of good results. In our clinic we assess cVEMP threshold at 4 frequencies in each ear. In a typical cVEMP test, 3-5 repetitions are made per frequency. Thus, 4 (frequencies) * 4 (average number of repetitions) * 2 (both ears) * 15.38 sec (testing time for one waveform) = 8 minutes of actually testing time, excluding preparations and explanations. At a stimulation rate of 5/sec this would increase to an untenable 21 minutes of testing time. This substantial increase in test time is both burdensome for the patient and costly for the clinic.

In our experience, cVEMP thresholds obtained at multiple frequencies is a more informative outcome measure than PP amplitude (7, van Tilburg et al. (in progress)). Since obtaining this outcome takes more time and effort it is essential to keep the recording time to a minimum. Using a higher stimulation rate does not significantly alter the threshold outcome but does reduce the testing time substantially.

In conclusion, no significant diffe-

rences in sensitivity, accuracy or precision outcomes were found between 5/sec and 13/sec stimulation rates, though intrasubject PP amplitude was significantly lower at the faster stimulation rate. To keep patient discomfort and test time to a minimum, the faster stimulation rate is recommended.

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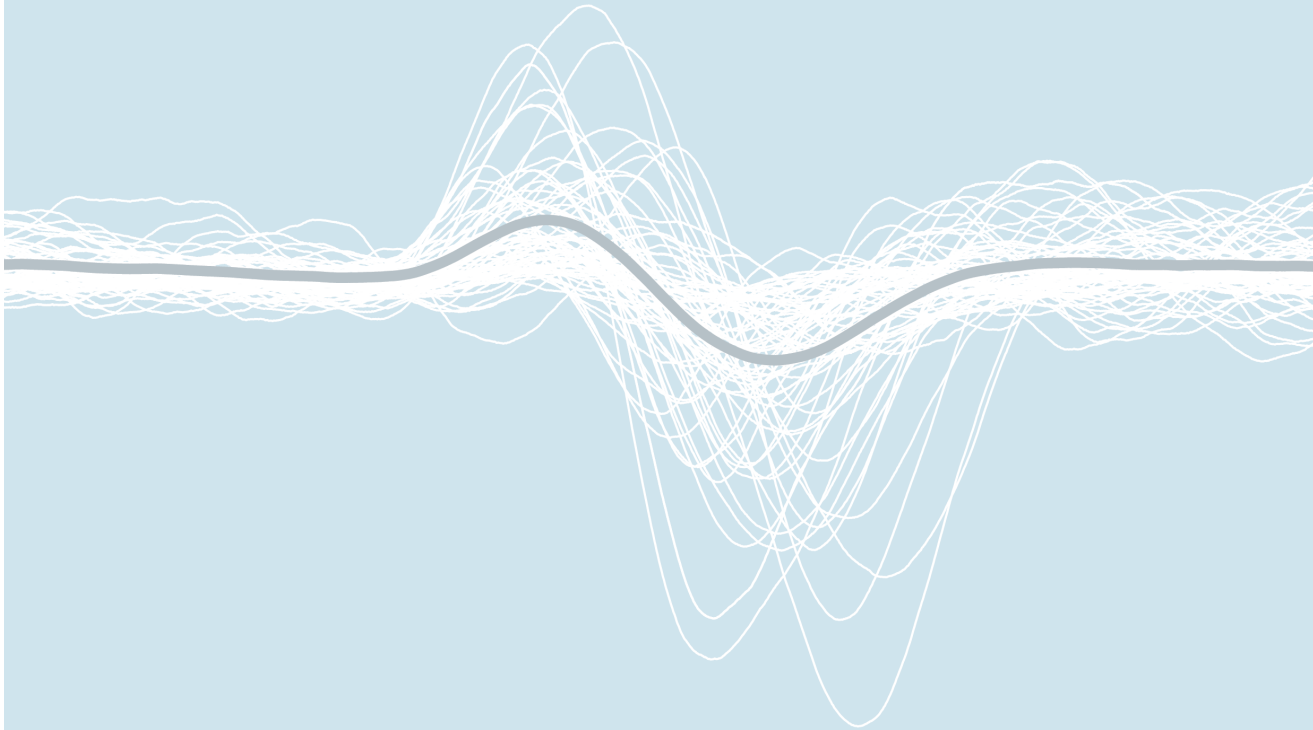


CHAPTER 6

Final discussion and valorisation

FINAL DISCUSSION AND VALORISATION

Since the renewed description of cVEMP's in 1994 the effect of muscle activation on test outcome has been described. The papers in this thesis are the first to systematically evaluate the effect of normalization (i.e. correcting for muscle activation) and to assess which method is most effective. Also, by reducing test time, demonstrating the ability to differentiate between pathologies and evaluating which outcome measure is most useful the clinical implementation of cVEMP has improved.

Current state of the art physiological tests available in diagnosing Meniere's disease show that there is no test that can prove Meniere's disease, especially in its early stages (chapter 2). Also, since the exact pathophysiology of Meniere's disease is still unclear, it is possible that patients with similar complaints have different underlying pathologies that can be distinguished more accurately as diagnostic abilities improve. For instance, we now know that clinical symptoms of vestibular migraine greatly overlap with Meniere's disease. Given the fact that vestibular migraine is much more prevalent compared to Meniere's disease, it is likely that patients with vestibular migraine have been (mis)diagnosed with Meniere's disease in the past. The distinction between the two entities is much better defined nowadays, although it can still be a challenge to distinguish the two in practice. Since diagnostic criteria have become better described, it is important to have well evaluated tests that aid in the diagnosis of different pathologies. This thesis describes a number of studies aimed to improve cVEMP testing and its clinical use, such as aiding in differentiating between vestibular migraine and Meniere's disease.

In cVEMP literature correcting for muscle activation has already been described (e.g. Welgampola 2001). By systematically evaluating the effect of normalization we showed that by correcting the EMG for muscle activation the variability of the cVEMP can be reduced significantly in normal subjects (chapter 3.1). Since the cVEMP response is largely linearly related to muscle EMG, correcting for differences in muscle strength makes sense. This means that when testing a person with a small SCM muscle contraction or a person with a strong SCM muscle

contraction, the influence of muscle strength will be reduced and thereby reflect saccular function more adequately. By reducing the variability, and thereby obtaining a more uniform

cVEMP outcome, it could also improve the ability to distinguish between a healthy and a pathological outcome.

The next step was to investigate different methods of normalization. In current literature it is often unclear if and how normalization of the muscle EMG was applied and thereby it is difficult to compare study outcomes. We found that there are important differences between the methods of normalization. For instance: normalization is more effective if every individual trace is normalized instead of normalizing the averaged waveform (chapter 3.2). Using a trace by trace normalization it is also possible to use a longer period of time to collect the EMG information. In our study a trace-by-trace normalization of either RMS or rectified EMG over an as long as possible time window yielded the optimal results. If there are limited signal processing capacities available using the average pre-stimulus EMG is the normalization method of choice.

After proving the effect of normalization and evaluating the optimal method we further explored the use of cVEMP in patient groups. Using normalized cVEMP we explored the effect of cVEMP outcomes in Meniere's patients over time and compared this with outcomes in Vestibular Migraine patients. Over an average of 2 years the affected side of unilateral Meniere's patients had significantly worse cVEMP outcomes (both PP amplitude and threshold), while the unaffected side was stable (chapter 4). When we compared these outcomes to Vestibular Migraine patients, we found that the cVEMP outcome in this group was stable over a 2 year period in all patients. An important limitation of study was the retrospective design and the small number of patients. However each individual patient in the Meniere's group showed worsening of cVEMP threshold whereas each vestibular migraine patient showed stable thresholds. Given the study results, it would be useful to further investigate this prospectively. Since vestibular migraine is a relatively new diagnosis there are many patients in which the diagnosis (Meniere's vs. Vestibular migraine) is unclear. Since these uncertainties may take years to assess, it would be helpful to incorporate cVEMP's routinely in the evaluation of these patients and track them over time.

One of the critiques of cVEMP's is that they are difficult to complete, especially in older patients. By increasing the stimulation rate (i.e. the amount of tone bursts per second) we were able to reduce the test time by almost 40% without significantly altering the thres-

hold in healthy subjects (chapter 5). Studies showed that the peak-to-peak (PP) amplitude is affected by the stimulation rate: a higher rate yields lower PP amplitudes. Even though this is true, in our study the lower PP amplitudes did not result in a significant change in threshold, which was the main outcome measurement. This study was done in healthy, young subjects and it could be argued that in more senior subjects the increase of stimulation rate could result in altered outcome. In our clinic a stimulation rate of 13Hz is standard practice since many years and outcomes previously published were no different from other studies (although not many studies used threshold as an outcome measure).

The cVEMP is a relatively new test that might be a valuable addition to the vestibular testing battery, however it also poses challenges for the professionals that work with it. For audiologists a new test is emerging which requires skills to perform and interpret. For engineers it possess challenges in signal processing and response detection as is shown by the development of VEMP inhibition depth (VEMPid, see below). For the physician, in the field of ENT or neurology, the vestibular test battery grows. It is important to learn about these tests and their possibilities in order to implement and apply them correctly. What the clinical impact is for cVEMP is yet to be determined but many clinics already use the test as an aid in diagnosing Meniere's disease and superior canal dehiscence (SCD).

We believe there is a role for cVEMP testing in patients with Meniere's disease, vestibular migraine and superior canal dehiscence. In patients where it is unclear if they suffer from Meniere's disease or vestibular migraine, cVEMP seems to be able to aid in diagnosis if multiple test are performed over time. In our study we have shown that over time, the cVEMP in Meniere's patients worsen and in vestibular migraine patients this does not seem to happen, although this needs to be confirmed in a prospective study. For superior canal dehiscence, studies have shown that high frequency (2000Hz) cVEMPs are evoked with low thresholds, caused by the third window effect leaking acoustic energy to the vestibulum. In healthy subjects significantly higher thresholds are needed to elicit a response.

The studies presented in this thesis include mostly healthy young subjects because, to improve cVEMP testing, it is necessary to make these improvements using subjects in which there is little risk of influencing the outcome by subject characteristics or pathologies.

In conclusion this thesis describes a number of studies that have the common goal to make the

cVEMP more reliable, by applying normalization, and more feasible clinically by increasing stimulation rate. Also we describe the potential of the test to differentiate between clinically similar vestibulopathies (Meniere Disease and vestibular migraine).

Despite the improvements in cVEMP testing and analyzing there is still a lot of work to be done. Some of these challenges are discussed below.

The cVEMP mechanism

Evaluating the mechanism of cVEMP's was outside the focus of this thesis but is a part of VEMP's that is poorly understood. Current literature is focusing on this topic and recently a review by Curthoys was published in which a suggested physiology was described how the vestibular organ can be sensitive for a wide arrange of frequencies. Curthoys describes the classic accelerometer mode of operation in which the otoconia move relative to the macula (sensitive for slow movement) and a new "seismometer" mode in which the otoconia remain at rest which the macula is moving (for high frequency movements). This could be an explanation to how the balance organ is sensitive to high speed movements.

Development of new analyzing methods

Normalization is a way to reduce the effect of muscle contraction in cVEMP testing. The next step is to make the testing process more automatized as well. A new outcome measure for cVEMP has been described as VEMPid, the inhibition depth of the VEMP. This should more accurately produce the saccular function by estimating the percentage of saccular inhibition. This method uses a template correlation value, which is a number that describes how well a (cVEMP) waveform resembles the template. In order to calculate the VEMPid a template is needed to which the measured cVEMP can be compared, this can be a template recorded from the subject or a generic template (Noij et al. 2018). Using a generic template is especially important in testing patients in whom it is often difficult or impossible to record a proper cVEMP waveform (e.g. in bilateral Meniere's patients). Studies have shown VEMPid to be able to assess cVEMP threshold in healthy subjects and future studies must show if VEMPid can be used in vestibulopathies as well (Prakash et al).

What outcome to use?

In current literature there are different parameters described as outcome of the cVEMP. Early studies described a present/absent outcome at one frequency. This seems to be a relative crude outcome since previous studies have shown that the saccule's response to sound is frequency dependent. Therefore, similar to audiometry, it is important to test multiple frequencies. In Meniere's patients a tuning curve has been described in which the cVEMP threshold sensitivity changes over frequencies. Also, the cVEMP is not a present/absent response, it seems likely that when the saccule degrades the cVEMP response becomes more difficult to elicit (i.e. more stimulation is needed in order to evoke a response). Like an audiogram, the cVEMP seems to track a sliding scale and does not appear to be an "on/off" response. To adequately measure this scale, peak-to-peak amplitude or threshold would be a better outcome measurement. In chapter 4 we describe that threshold is sensitive to progressive disease in all tested frequencies (i.e. all frequencies worsen over time) whereas peak-to-peak amplitude is significantly different in half of the frequencies. The results of this study support our hypothesis that threshold seems to be the most informative outcome for clinical use. At Mass Eye and Ear cVEMP threshold, measured at multiple frequencies, is the main test outcome since many years. An example of a cVEMP outcome for a unilateral Meniere's patient is shown below.

VEMP Threshold (dB HL)

Freq. (Hz)	Right	Left
500	95	80
750	95	85
1000	95	85

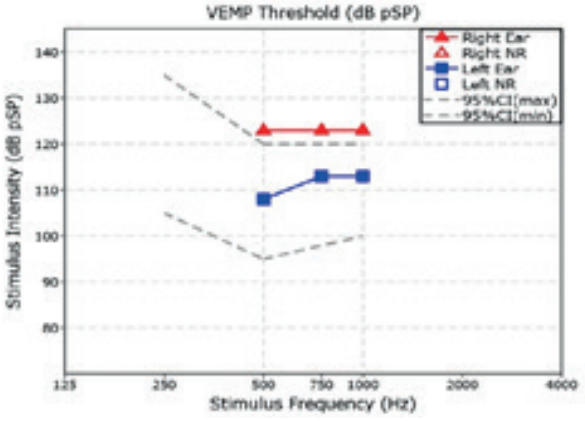


Figure 1. Example of the outcome figure of a cVEMP, a "VEMPogram".

Furthermore, in current literature the interaural asymmetry ratio (IAR) is an often used outcome measure. This outcome compares the PP amplitude from the healthy and the pathological side within a patient and gives a ratio as outcome. In a strictly unilateral disease this is a valua-

ble outcome, however in Meniere disease it is estimated that a third of the patients have (subclinical) bilateral disease. This means that the saccule can be affected on both sides but does not necessarily cause bilateral complaints. Separate studies have shown that about 30% of Meniere's patients' temporal bones show bilateral involvement. This number was also found in clinical studies and in our own cVEMP study we found that about of a third of the Meniere's patients diagnosed with unilateral Meniere's disease show increased cVEMP thresholds on the "unaffected" side. We do not yet know whether these patients will also develop bilateral disease, this is an important focus for future research. The contralateral affected cVEMP makes the IAR prone to under detection of Meniere's, therefore we recommend not to use this outcome in Meniere's patients. Future research will have to reveal which outcome is
most informative clinically.

Meniere's versus vestibular migraine

Vestibular migraine is a relatively new diagnosis. It is very likely that patients that have been diagnosed with Meniere's disease actually suffer from vestibular migraine. Especially in older literature it is important to keep this in mind and interpret results with caution. This is also true for VEMP testing, since our study suggests a different progression in time between these two pathologies. Even today it is sometimes difficult to differentiate clinically between the two. Since these uncertainties may take years to assess, it would be helpful to incorporate cVEMP's routinely in the evaluation of these patients and track them over time.

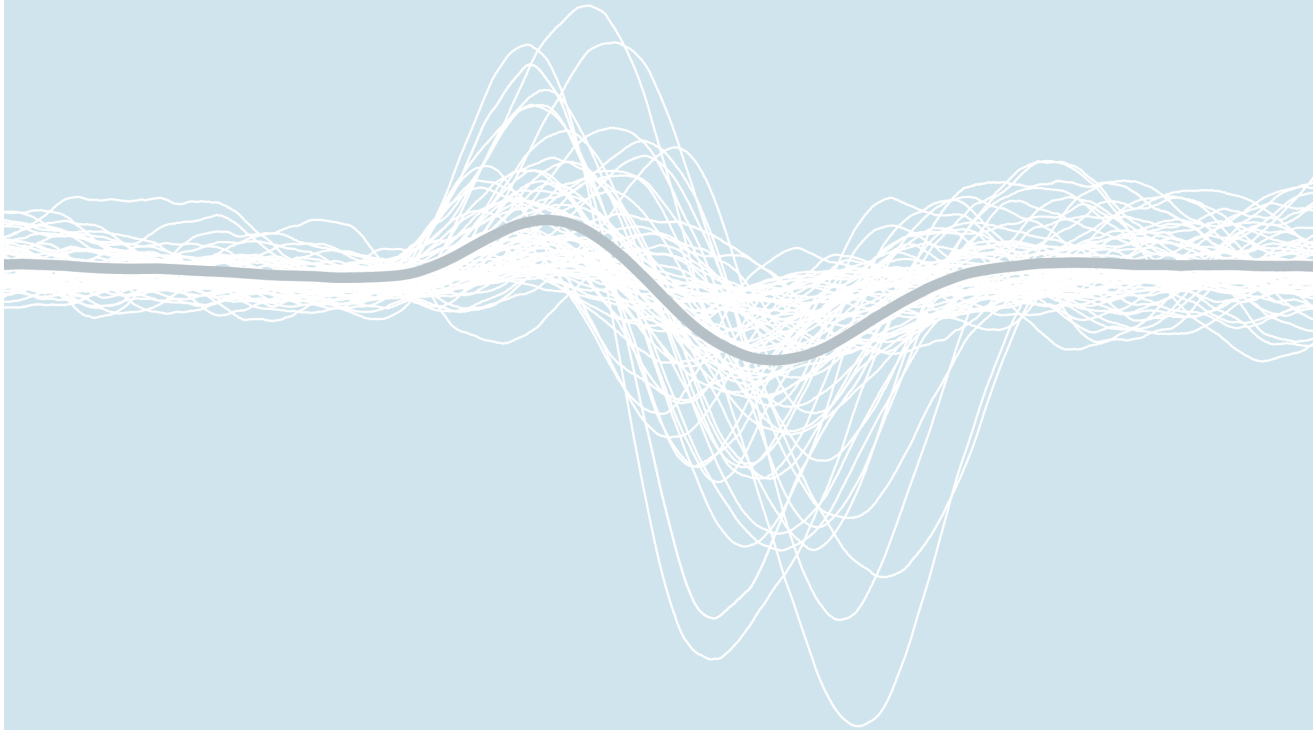
Effect of age on cVEMP

As described above and in current literature, the cVEMP is harder to elicit in older patients. In part this can be attributed to the difficulty of completing a cVEMP test, which takes a substantial amount of time and effort. Reducing test time and effort by increasing the stimulation rate and applying normalization could improve the ability to perform cVEMP in older patients. However the decline in vestibular function might also play an important role in this and could cause a higher amount of absent cVEMP responses in certain age groups, making the cVEMP less useful in these groups. However Meniere's disease, vestibular migraine and SCD usually have an age of onset at which the cVEMP is easy to elicit.

Standardizing

Since cVEMP testing is not standardized, it is necessary to specifically describe which variables are used for normalization. Applying adequate normalization methods has immediate clinical and research relevance. Our studies show the effect of normalization in a healthy, young population. Before standardizing cVEMP testing techniques it is important to assess how all outcome measures behave in pathological groups and across ages. This is already being done in current literature, however not always using optimal testing methods (i.e. the optimal normalization method). In order to make the cVEMP part of the regular "vestibular testing battery" it is important to investigate what is the optimal way of recording the response.

This thesis shows the effect of normalization in cVEMP and which method is most effective. Outcomes of the test will more adequately reflect the saccular function. Further research into developing more automated response recognition is already underway. Also the burden of the test could be reduced by increasing the stimulation rate. Future research will have to show the role of cVEMP in different vestibulopathies, but first we need to focus on developing a standardized method of testing.



CHAPTER 7
Summary

SUMMARY

When a dizzy patient consults a specialist the typical evaluation consists of: a careful history, physical examination, an audiogram and perhaps an ENG or imaging. These tests grossly evaluate the middle ear, cochlea and horizontal semicircular canal. The balance organ, however, is much more than just the horizontal semicircular canal, and while much is known about the vestibular apparatus in humans, there are very few tests available to evaluate all parts of the balance organ. This problem is especially relevant in vestibulopathies that do not (always) involve the horizontal semicircular canal such as Meniere's disease and superior canal dehiscence (SCD). The cervical vestibular evoked myogenic potential (cVEMP) is a test that evaluates one of the different parts of the balance organ, namely the saccule.

The cervical vestibular evoked myogenic potential (cVEMP) is a vestibular test that uses sound to elicit a vestibular response resulting in an inhibition in the ipsilateral, contracted sternocleidomastoid (SCM) muscle that can be recorded using EMG electrodes. This inhibition is measured after averaging multiple acoustic stimuli (usually around 200-300 tone bursts) and in healthy people shows the typical waveform. In recent literature the test is gaining attention because it evaluates a part of the balance organ that was previously not possible.

The aim of this thesis was to increase the clinical applicability of the cVEMP and has led to the following questions. In the text below a summarized answer is given to these questions:

- What is the current status of electrophysiological testing for Meniere's disease?
- How can the large intersubject variability in cVEMPs be overcome?
- Using normalized cVEMP's, can we show disease progression in Meniere's disease?
- Is there a method to make cVEMP testing more tolerable?

In chapter two the evaluation of current electrophysiological tests available for Meniere's disease were assessed since this is a vestibulopathy in which cVEMP is mostly used as a diagnostic. Besides VEMP's the electrocochleografy (ECoG) and CHAMP test were evaluated. Electrocochleografy can be useful in the early stages of Meniere's disease, however given the low negative predicting value this test is scarcely used clinically.

The fact that it is not possible to perform the test in hearing loss above 60dB further limits clinical use. Cochlear Hydrops Analysis Masking procedure (CHAMPS) uses the auditory brainstem response (ABR) to assess latency changes that could imply Meniere's disease. Increasing latency would suggest cochlear hydrops which in turn could suggest Meniere's disease. In advanced and active Meniere's patients sensitivity and specificity are described to be 100%. However if the test is performed in a more diverse Meniere population the sensitivity drops to 31% and specificity to 28%. Furthermore it is not a vestibular test since it measures cochlear function. The strength of the test is to confirm the Meniere diagnosis in active and severe Meniere's, however as a diagnostic tool its value seems limited. For cVEMP's was found that it is not a plug and play test. Meticulous preparation and execution are essential in order to obtain reliable results. In current literature there are limited studies that describe these methods in detail. Furthermore the underlying physiology and optimal test parameters have not been standardized. More research is needed to determine how to best utilize the cVEMP in diagnosing and monitoring Meniere's disease and possibly other vestibulopaties.

One of the major shortcomings of cVEMP is its dependency on muscle tension, causing a great variability in test outcomes. The most important aim of this thesis was to reduce the influence of muscle contraction on the cVEMP outcome. By correcting for muscle contraction (i.e. normalizing) the variability of the test would be reduced and give a more reliable outcome. In chapter 3 the principle of normalization is applied to cVEMP's and a further study investigates which normalization method is most optimal. These studies are performed in a prospective cohort study in 20 healthy young test subjects. By correcting the output signal for the amount of muscle contraction (=normalization) a significant reduction in variability was achieved in healthy subjects (chapter 3.1). Simply put, normalization uses a coefficient that represents muscle contraction effort. This number is then divided by the outcome of the cVEMP test. By applying normalization the outcome of the cVEMP will more closely represent the saccular function instead of muscle strength.

The process of normalization can be applied in different methods. In other words we described different parameters that play a role in obtaining the normalization coefficient that represent the muscle contraction. In chapter 3.2 we described 3 parameters that influence the coefficient. These are:
the timing of the normalization (trace-by-trace of after
averaging all

traces), the type of EMG (RMS or rectified) and the time in which the coefficient is measured (pre-stimulus of during the entire measurement). By using each individual trace during the entire measurement the variability between subjects was substantially reduced. If a testing facility does not have the ability to use extensive signal processing we advise to use the pre-stimulus part of the averaged EMG to obtain a coefficient for normalization.

After demonstrating the effect of normalization the clinical application of cVEMP's was further investigated. In two patient groups consisting of Meniere's patients and vestibular migraine patients the effect of time on cVEMP output was evaluated. The hypothesis was that in the Meniere's group there would be a progressive worsening of the outcome whereas the vestibular migraine groups was anticipated to be more stable over time. Over an average of 2 years the affected side in Meniere's disease showed a significant worsening of both peak to peak amplitude and threshold measurements. The unaffected side remained stable over this time period. In the vestibular migraine group all patients showed stable cVEMP's over time. An important limitation of this study was its retrospective design and the small amount of patients. However each individual patient from both groups showed the same outcome (i.e. significant worsening in Meniere's patients and stable results in vestibular migraine patients). Vestibular migraine was only described recently and it is possible that in the past patients diagnosed with Meniere's disease actually suffered from vestibular migraine. Given this we advise to incorporate cVEMP as a regular test in the vestibular testing battery, so the progression of the test over time can be evaluated.

The last study described in this thesis investigated the improvement of clinical application of the cVEMP in which the stimulation rate was increased in order to complete the test in a shorter time span, making it more tolerable. In current literature the majority of studies use a stimulation rate of 5Hz. This means that five acoustic stimuli were presented each second in order to elicit a cVEMP response. In our study we increased the stimulation rate to 13Hz, 13 acoustic stimuli per second. By doing this, the test time was reduced by 40%. We did find that the peak to peak amplitude was reduced, however these did not alter the threshold measured in healthy subjects. Since the substantially less time and thereby effort needed to complete the test we advise to use 13Hz as stimulation rate.

The cVEMP is a relatively new test that has the ability to evaluate one of the parts of the balance organ that was previously not possible. This thesis demonstrates the effect of

normalization in cVEMP's and which method is most effective. Outcomes of the test will reflect saccular function more adequately and increase its clinical use. Further studies to the development of automated response recognition are already underway. Increasing stimulus rate can aid in reducing test burden without compromising test outcome. In monitoring Meniere's disease the cVEMP can play a role, for instance in evaluating before deciding whether or not to start more invasive treatment (intratympanic gentamicine or labyrinthectomy). Also in differentiating between Meniere's disease and vestibular migraine, the cVEMP can play a role. Further studies will have to show the role of cVEMP in diagnosing different vestibulopathies. But first standardization of the test should be the goal and with this thesis that goal has come closer.

CHAPTER 8

Dutch summary (Nederlandse samenvatting)

DUTCH SUMMARY (NEDERLANDS SAMENVATTING)

Wanneer een patiënt met duizeligheidklachten een KNO-arts bezoekt omvat een typische evaluatie veelal: een zorgvuldige anamnese, lichamelijk onderzoek, audiometrisch onderzoek en soms een ENG of beeldvormende diagnostiek. Hiermee worden het middenoor, de cochlea en het horizontale semicirculaire kanaal geëvalueerd. Het evenwicht systeem bestaat echter uit meer dan alleen het horizontale semicirculaire kanaal, maar het aantal testen wat dat onderzoekt is beperkt. Dit is in het bijzonder een probleem bij de diagnose van vestibulaire aandoeningen die zich buiten het horizontale semicirculaire kanaal bevinden, zoals bijvoorbeeld bij de ziekte van Ménière en bij superieure kanaal dehiscentie (SCD). De cervicale vestibular evoked myogenic potential (cVEMP) is een test die een uitspraak kan doen over een ander deel van het evenwicht systeem, namelijk de sacculus.

CVEMP's beschrijven de akoestische gevoeligheid van cellen in de sacculus. Deze cellen worden gestimuleerd door een luide akoestische stimulus aan te bieden in het oor aan de te testen zijde. De uitkomst van de test kan onder andere worden gemeten via een aangespannen musculus sternocleidomastoideus (SCM) aan ipsilaterale zijde die met elektromyografie gemonitord wordt. Door middel van inhiberende signalen die de sacculus naar de spier zendt wordt, na middelen van meerdere stimuli (meestal rond de 200-300 akoestische stimuli), de karakteristieke cVEMP vorm gezien op het electromyogram (EMG). De test krijgt in de huidige literatuur vrij veel aandacht omdat het een voorheen niet te testen onderdeel van het evenwichtsorgaan onderzoekt.

Het doel van deze thesis was om de klinische toepasbaarheid van de cervicale cVEMP te verhogen en dit heeft geleid tot de volgende vraagstellingen waarvan in onderstaand hoofdstuk een beknopt antwoord gegeven zal worden:

- Wat is de huidige state of the art in electrofysiologische testen voor de diagnose van de ziekte van Ménière?
- Hoe kan de intersubject variabiliteit van cVEMP's gereduceerd worden?
- Kunnen genormaliseerde cVEMP's progressie van de ziekte van Ménière over tijd aantonen?
- Is het mogelijk de cVEMP test praktisch beter uitvoerbaar te maken?

In hoofdstuk 2 werd een verkennend literatuur onderzoek gedaan naar de verschillende vestibulaire testen die de ziekte van Ménière evalueren omdat dit één van de meest voorkomende vestibulopathieën is waarbij de cVEMP gebruikt wordt. Naast de cVEMP, werd gekeken naar electrocochleo-

grafie (ECoG) en de Cochlear Hydrops Analysis Masking Procedure (CHAMP). Voor ECoG werd gevonden dat het een bruikbare test zou kunnen zijn in de vroege stadia van de ziekte. ECoG heeft echter een lage negatief voorspellende waarde waardoor deze test maar in weinig centra wordt uitgevoerd. Het feit dat de test niet meer uit te voeren is wanneer de patiënt gehoordrempels van boven de 60dB heeft, is daarnaast een praktisch probleem wat de diagnostische mogelijkheden verder beperkt. De CHAMP gebruikt een onderdeel van de auditory brainstem reponse (ABR) om de snelheid (= de latentie tijd) van het signaal door de cochlea te meten waarbij de hypothese is dat de latentietijd toeneemt indien de ziekte verergert. Deze test meet de functie van de cochlea, waarbij bij patiënten met vergevorderde en actieve ziekte een sensitiviteit en specificiteit van 100% werd beschreven. Echter wanneer deze test werd verricht onder een meer diverse Ménière populatie werd een sensitiviteit van slechts 31% gevonden en een specificiteit van 28%. Verder is het geen vestibulaire test omdat het de functie van de cochlea meet. De kracht van deze test is om bij actieve en ernstige patienten de diagnose van Ménière te ondersteunen, echter als diagnosticum lijkt de test van beperkte waarde. Voor de cVEMP werd gevonden dat het gebruik van deze test geen plug en play is. Nauwkeurige voorbereiding en uitvoering zijn nodig om betrouwbare test uitslagen te verkrijgen, waarbij deze in de huidige literatuur maar zeer beperkt beschreven zijn. Daarbij is de onderliggende fysiologie en de optimale uitvoering en interpretatie van de resultaten nog niet gestandaardiseerd. Meer onderzoek is nodig om te bepalen hoe de cVEMP het best kan worden ingezet ter ondersteuning en monitoring van de ziekte van Ménière en andere perifere vestibulopathieen.

Een van de belangrijkste tekortkomingen van de cVEMP is de afhankelijkheid van spierspanning waardoor de variabiliteit van de test groot is. Het belangrijkste doel van deze thesis was het verminderen van de invloed van spierspanning op de uitslag van de cVEMP. Door te corrigeren voor de spierspanning (i.e. normaliseren) zou de variabiliteit van de test verminderden en een meer betrouwbare uitkomst geven. Hiervoor wordt in hoofdstuk 3 het principe van normaliseren toegepast op cVEMP's en wordt onderzocht met welke methode dit zo optimaal mogelijk verricht kan worden. In een prospectieve cohort studie met 20 jonge, gezonde proefpersonen werd het effect van normaliseren op de variabiliteit in uitkomsten tussen de proefpersonen onderzocht. Door te corrigeren voor de hoeveelheid spierspanning (= normaliseren) is het gelukt om, in gezonde proefpersonen, een signifi-

cant lagere variabiliteit te verkrijgen wat de betrouwbaarheid van de test verhoogd. Simpel gezegd houdt normalisatie in dat voor elke gemeten respons er een coëfficiënt gemeten wordt die maat staat voor de geleverde spierspanning waardoor de respons wordt gedeeld. Hierdoor is de invloed van spierspanning op de uitslag van de cVEMP verminderd, een persoon met een krachtige halsspier zal daardoor vergelijkbare uitslagen hebben met iemand die een minder krachtige halsspier heeft indien de sacculus functie gelijk is.

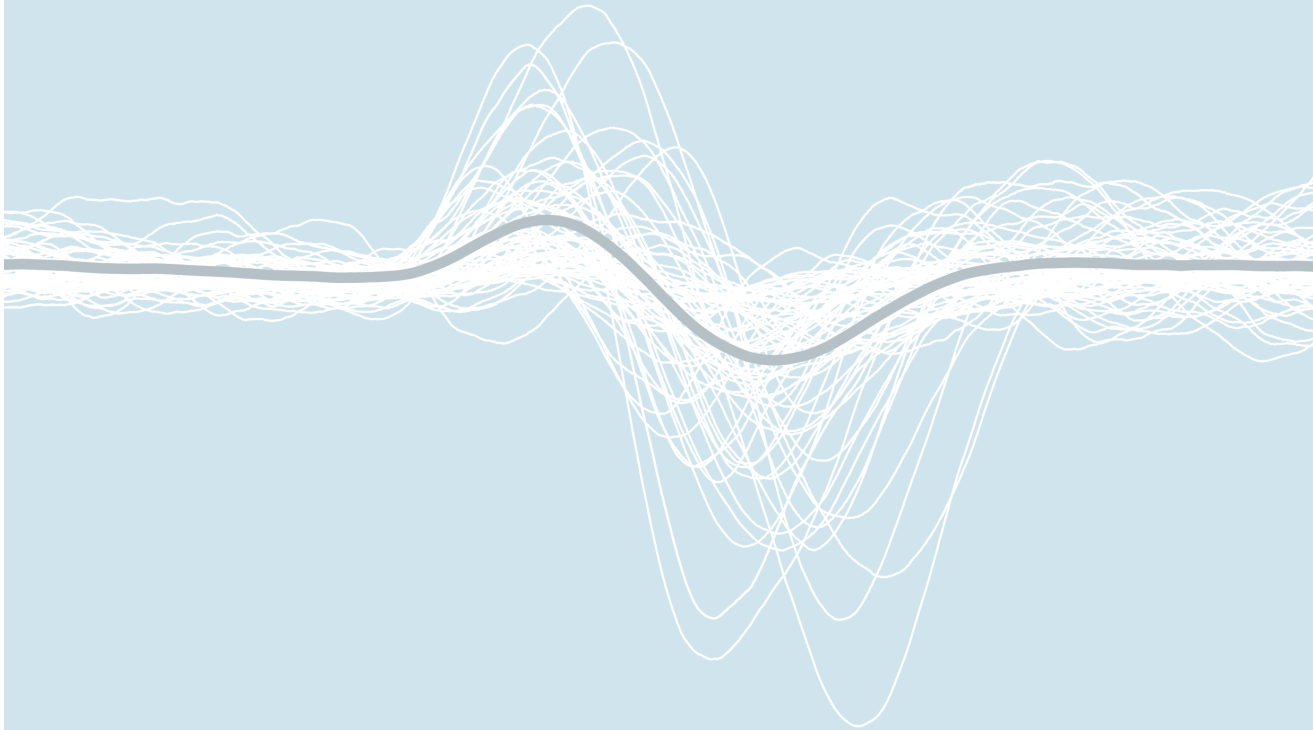
Het proces van normaliseren kan op verschillende manieren ingevuld en toegepast worden. Dat wil zeggen, er zijn verschillende parameters beschreven om de coëfficiënt te verkrijgen die maat staat voor de spierspanning. In hoofdstuk 3.2 hebben wij drie parameters benoemd die van invloed zijn om de coëfficient te bepalen. Dit zijn: de timing van de normalisatie (trace-by-trace of na het middelen van alle traces), het type EMG (root mean square of gerectificeerd) en de tijdsduur van het verkrijgen van de coëfficiënt (pre stimulus of gedurende de gehele meting). Door elke individueel gemeten trace gedurende de hele meting te gebruiken werd de variabiliteit van de cVEMP tussen personen substantieel gereduceerd. Indien een centrum geen mogelijkheid heeft om uitgebreide signaal processing toe te passen werd geadviseerd het pre-stimulus deel van het de gemiddelde EMG te gebruiken om de coëfficiënt voor normalisatie te verkrijgen.

Nadat het effect van normaliseren aangetoond was en de meest efficiënte methode beschreven was werd in hoofdstuk vier het klinisch gebruik van cVEMP verder onderzocht. Er werd voor 2 patiënten groepen, patiënten met de ziekte van Ménière en patiënten met vestibulaire migraine, onderzocht of het verloop van tijd invloed had op de uitkomsten van de cVEMP. Hierbij was de hypothese dat in de Ménière groep een progressieve verslechtering van de cVEMP te zien zou zijn omdat deze ziekte ook een progressief karakter heeft. Voor de vestibulaire migraine groep werd dit minder waarschijnlijk geacht. Over een gemiddelde tijd van 2 jaar toonde de aangedane zijde van de Ménière groep in elke patiënt dat er een significante verslechtering was opgetreden in zowel de peak to peak amplitude als in de drempel meting terwijl de onaangedane zijde stabiel bleef. Voor de vestibulaire migraine groep werd gevonden dat de cVEMP stabiel bleef in alle patiënten over de gemiddelde follow up van 2 jaar. Een tekortkoming van deze studie is het retrospectieve karakter en de kleine patiënten aantallen. Echter in elke individuele patiënt uit beide groepen werd dezelfde ontwikkeling gezien (achteruitgang bij Ménière en stabiel bij vestibulaire migraine). Omdat er waarschijnlijk in het verleden patiënten met Ménière gediagnostiseerd zijn terwijl ze vestibulaire

migraine hadden (een diagnose die pas veel recenter beschreven is) lijkt het ons nuttig om een uitgangs-cVEMP te verrichten bij patiënten met de verdenking op een van beide ziektebeelden zodat deze eventueel in de toekomst herhaald kan worden.

In de laatste studie (hoofdstuk 5) beschreven in dit proefschrift is gekeken naar de verbetering van de klinische uitvoerbaarheid van de cVEMP, in het bijzonder naar de verhoging van de stimulus snelheid zodat de test in kortere tijd te voltooien is en daarmee praktisch beter uitvoerbaar is. In de huidige literatuur worden in het overgrote merendeel van de studies stimulatie snelheden van 5Hz beschreven, dit houdt in dat er vijf akoestische stimuli per seconde werden aangeboden aan de te onderzoeken zijde. In onze studie hebben we deze stimulatie snelheid vergeleken met een snelheid van 13Hz, 13 stimuli per seconde. Hierdoor zou de test tijd, indien drempelwaardes op meerdere frequenties onderzocht worden, met 40% verkort worden. We vonden dat de peak to peak amplitudes lager werden bij een hogere stimulatie snelheid maar dat dit geen effect had op de gemeten drempels. Het advies is dan ook om de cVEMP uit te voeren met een stimulatiesnelheid van 13Hz.

De cVEMP is een vrij nieuwe test die de mogelijkheid biedt om eerder niet te onderzoeken delen van het labyrint te evalueren. Deze thesis demonstreert het effect van normalisatie in cVEMP's en welke methode het meest effectief is. Uitkomsten van de test zullen op deze manier meer adequaat de sacculus functie reflecteren en de klinische bruikbaarheid verhogen. Verder onderzoek naar de ontwikkeling van geautomatiseerde response herkenning is al onderweg. Daarnaast heeft het verhogen van de stimulatie rate ervoor gezorgd dat de last van de test verminderd kan worden zonder de uitslag negatief te beïnvloeden. Ook in het volgen van patiënten met de ziekte van Ménière lijkt de cVEMP een bijdrage te kunnen leveren, waarbij het ook een rol zou kunnen spelen in het besluit om meer invasieve therapie te verrichten (zoals intratympanale gentamicine of een operatieve labyrinthectomie). Eveneens kan er een bijdrage van de cVEMP in het onderscheid maken tussen Ménière en vestibulaire migraine. Verder onderzoek zal de rol van cVEMP in de diagnostiek van verschillende vestibulopathieën aan moeten tonen, maar eerst zal een gestandaardiseerde test methode moeten worden beschreven. Daar is met deze thesis een belangrijke stap in gezet.



CHAPTER 9

List of publications

LIST OF PUBLICATIONS

A Persistend Foramen of Huscke: a Small Road to Misery in Necrotizing External Otitis.
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Toward optimizing VEMP: Calculating VEMP Inhibition Depth (VEMPid) with a Generic Template.

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Mark J. van Tilburg, Barbara S. Herrmann, John J. Guinan Jr., Steven D. Rauch. Tijdens Association for Research in Otolaryngology (ARO), San Diego, Californie (USA), 2017

CHAPTER 10

Acknowledgements (Dankwoord)

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This is it, my thesis is done. Due to family expansions it took a little longer, but I am very happy with the result (both of my family and of this thesis). Since there was no chance for me to create this thesis alone there are many people that I want to thank.

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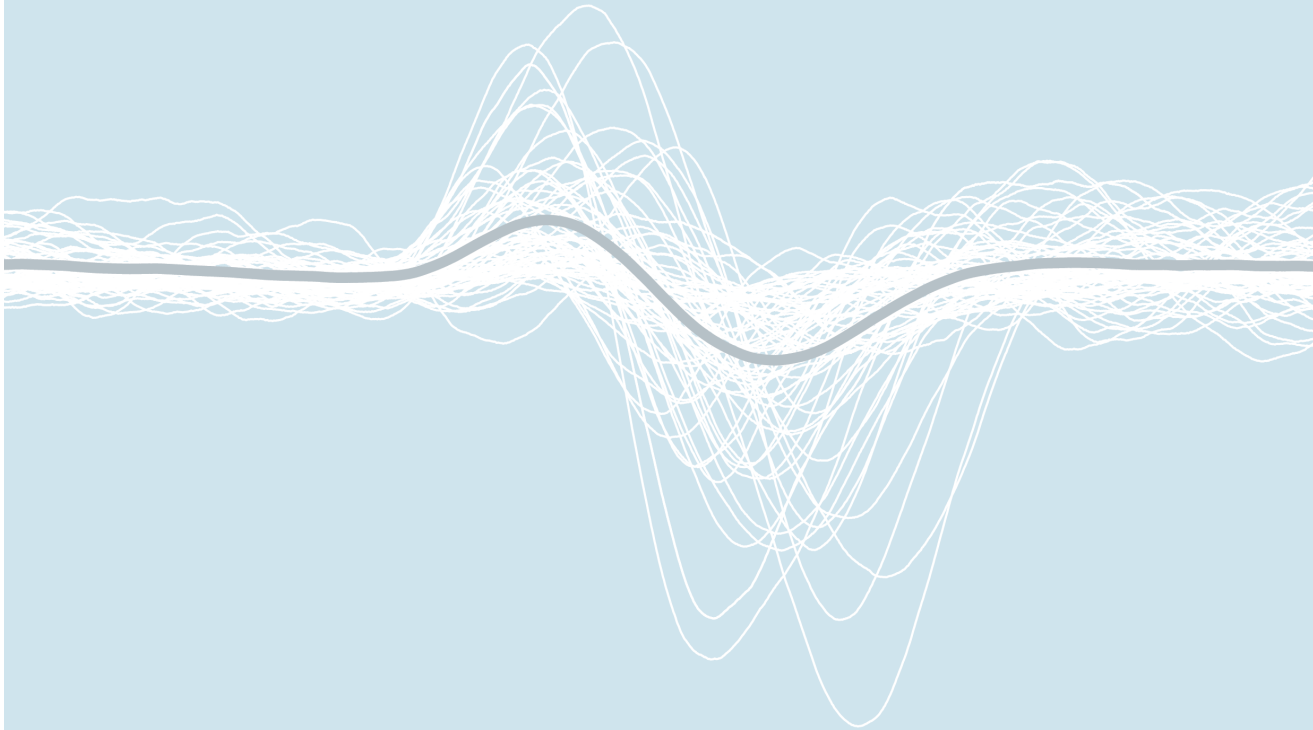

CHAPTER 11

Curriculum Vitae

CURRICULUM VITAE

Mark Johan van Tilburg was born in Venlo on August 9th, 1984. He grew up in Den Bosch with his parents and a younger sister and brother. In 2002 he graduated from high school in Den Bosch and after 2 years of studying psychology he started medical school at the Radboud University in Nijmegen. During these years he was a fanatic volleyball player. In his final year he completed a clinical internship at the department of Otorhinolaryngology and Head and Neck surgery of the Radboud University Medical Center. Before graduating he completed a scientific internship at Massachusetts Eye and Ear and Harvard Medical School (Boston, USA) under the supervision of prof. dr. Steve Rauch. After graduating from medical school in 2012 he returned to Boston as a research fellow, continuing the work he had started during his internship. In dr. Rauch's vestibular lab Mark worked on fundamental and clinical improvement of the cervical vestibular evoked myogenic potential. After two and a half year in Boston he started his medical residency in otorhinolaryngology at Maastricht University Medical Center in 2015 under supervision of prof. dr. B. Kremer and dr. J. Hof.