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Berit Holmeslet

Ocular Vestibular Evoked Myogenic Potentials

Stimulus aspects of the oVEMP test

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Trondheim, October 2016

Department of Neuroscience

Norwegian University of Science and Technology

Trondheim, Norway

Department of Otolaryngology, Head and Neck surgery

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Trondheim, Norway

1 Norsk sammendrag

Balanseorganene ligger dypt inne i tinningbeinet på hver side og samarbeider med det øvrige balansesystemet, bl.a. øyemuskulatur. Hver gang vi forflytter oss oppstår det hodebevegelser og for vi skal se klart, er vi helt avhengig av at øynene beveger seg motsatt vei av hodebevegelsen. Refleksbuen mellom balanseorganet og øyemuskulatur sørger for dette. Ved skade eller sykdom i et av de to balanseorganene endres signaloverføringen til balansesystemet, resultatet er varierende grad av svimmelhet, synsforstyrrelser, falltendens og kvalme. Symptomer som kan være langvarige og invalidiserende.

De siste årene har økt interesse innen feltet ført til at det er utviklet nye og nyttige metoder som kan bidra til bedre diagnostikk av den svimle pasienten. Testene kartlegger funksjonen i de ulike delene – tre bueganger og to otolitter – til balanseorganet; vHIT undersøker buegangene, cVEMP sacculus og oVEMP utriculus. Beinledet lyd på hodeskallen aktiverer utriculus og refleksbuen, elektroder plassert under øynene måler øyemuskelresponsen. Testen er lovende, men har vist seg noe ustabil, da den er avhengig av både testutførelse, valg av stimulus og stimulussted. Vi har gjort tre studier som har hatt som mål å bidra til bedre standardisering av metoden.

I den første studien ble et kraftig stimulus benyttet på fire ulike steder: panne, ørebeinet på begge sider og i bakhodet, hos friske og syke. Vi fant at alle friske ører gav god respons, mens det syke ikke gjorde det. Valg av stimulus sted påvirker oVEMP responsen; mer spesifikt, ved stimulering på ørebeinet (samme side som stimulus blir gitt) og i bakhodet, blir en større del av balanseorganet

aktivert enn ved de øvrige stedene. For øvrig, gir stimulering i bakhodet dårlige responser og bør ikke brukes som stimuleringssted.

I det andre studiet undersøkte vi om et mindre kraftig stimulus satt midt på hodet, utløste responser hos friske og syke – og fant at en lav frekvens (125 Hz) gav god respons fra alle friske ører, og ingen fra den syke. Høyere frekvenser (250-500 Hz) gav dårlig respons og egnet seg ikke. Videre, utløses det bedre responser om slagretningen til stimulus (i startfasen) er vendt mot skallen. Stimulering midt på hodet med en lav frekvens og slagretning mot hodet, egner seg for screening,

I tredje studiet undersøkte vi på friske om de to stimulerings stedene; midt på hodet og panne, gir stabile responser når oVEMP testen gjentas. Vi fastslo at det er minst variasjon dersom pannen brukes som stimuleringssted. Pannen anbefales som stimuleringssted.

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2 Acknowledgements

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A great thank to everyone who have contributed to this work. The project was initiated and in its first phases supervised by Krister Brantberg. He had his main position at Karolinska Hospital in Stockholm, combined with his professor position at the NTNU and St. Olav Hospital. The studies were greatly helped out by Magnus Westin, the chef engineer in Stockholm. When Brantberg returned to be a full time work at his mother department, the project needed local supervision, and my medical colleague Trude Basso introduced me to her supervisor, Olav Foss, Orthopedic Research Center (ORC), St. Olav Hospital. He most kindly continued the supervision and phased out the project. I am very grateful for the warm welcoming Olav Foss and his collaborators at the Orthopedic Research Center have given me. In this context, a special great honor to Jomar Klaksvik, the chef engineer at the Orthopedic Research Center.

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Finally, I have to thank my husband, Marcus, for your mental and practical support and to my lovely daughters, Åsa and Ingvild, most of all for reminding me, that there is more to life than working on a PhD.

3 List of Papers

I. Ocular vestibular evoked myogenic Potential: Skull taps can cause a stimulus direction dependent double peak.

II. Ocular vestibular-evoked myogenic Potentials (oVEMPs) in response to bone-conducted vertex vibration.

III. The repeatability of oVEMP in response to low-frequency vibration against the vertex and forehead.

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4 Abbreviations

ACS = air conducted sound

aVOR = angular Vestibulo Ocular Reflex

BCV = Bone Conducted Vibration

cVEMP = cervical Vestibular Evoked Myogenic Potential

Fz = Forehead

oVEMP = ocular Vestibular Evoked Myogenic Potential

SVH = Subjective Visual Horizontal

SVV = Subjective Visual Vertical

tVOR = translational Vestibulo Ocular Reflex

vHIT = video Head Impulse test

VOR = Vestibulo Ocular Reflex

5 Introduction to the study

The complex vestibular system – in the inner ear and brain – is critical to our sense of hearing and equilibrium. Studies of inner ear disorders are demanding due to both the location within the temporal bone and the small size of the inner ear structures. Direct microscopy is not possible, while sampling requires advanced and often destructive surgery. The anatomy and function of the inner ear's fine structures cannot be adequately evaluated with the imaging techniques currently used.

Recent research has advanced our understanding of vestibular physiology and new noninvasive methods are developed that objectively measure the function in the five different parts of the vestibular labyrinth; the three semicircular canals (superior, lateral and posterior) and the otoliths (the utricle and the saccule). The methods are based on head accelerations, sound and vibration; stimuli that naturally activate the vestibular labyrinth. oVEMPs is a new method that measures the utricle function by recording evoked responses in the inferior oblique eye muscle (Weber et al., 2012). Development of oVEMP is a step forward in the understanding of vestibular function, but the test needs further validation and standardization before it can be included in the standard clinical test battery for inner ear disorders. This PhD thesis concerns standardization and clinical application of oVEMP methodology, and describes how various stimulation conditions impact the oVEMP test.

6 Anatomy and neurophysiology

6.1 Evolution of the vestibular system

Living organisms have adapted into a gravitational environment and share several similarities in the construction of hearing- and equilibrium organs, most likely due to a common phylogenetic origin (Carey and Amin, 2006). The otoliths – which literally means “hearing stones” (Straka et al., 2016) – developed to discover near-field sound, whole body-acceleration and gravity. Fish and frogs are examples of organisms that have preserved a common auditory- and vestibular function in the otolith organ (Lundberg et al., 2015). The otoliths later developed to also respond to far-field sound through the ear (Todd et al., 2009). Birds are examples of organisms that, in addition to the otolith, have developed a pouch– the lagena – which correlates to the cochlear duct in humans (Lundberg et al., 2015). Otolith organs in humans have preserved a sensitivity to loud low-frequency sound, that form the basis of vestibular-evoked myogenic potentials (Emami et al., 2012). To ensure balance, all organisms have two mechanisms in common: one to keep eyes on targets during quick angular head movements and one to maintain an upright position and gait in darkness (von Baumgarten and Thumler, 1979).

6.2 The human postural system

The human postural system (figure 1) consists of a peripheral and a central system. In the periphery; the eyes, the vestibular labyrinths and the proprioceptive system detect changes in head and body positions. Signals are transmitted via afferent nerve fibers (after this referred to as afferents) to the brain for integration in the brainstem and cerebellum. The brain modulates the information and produces compensatory motor responses in the ocular and skeletal muscles that

ensure upright position and forward moving – locomotion – and prevent us from falling. Despite the system's complexity – many neural connections between the peripheral and central nerve system – robustness is ensured by its duality.

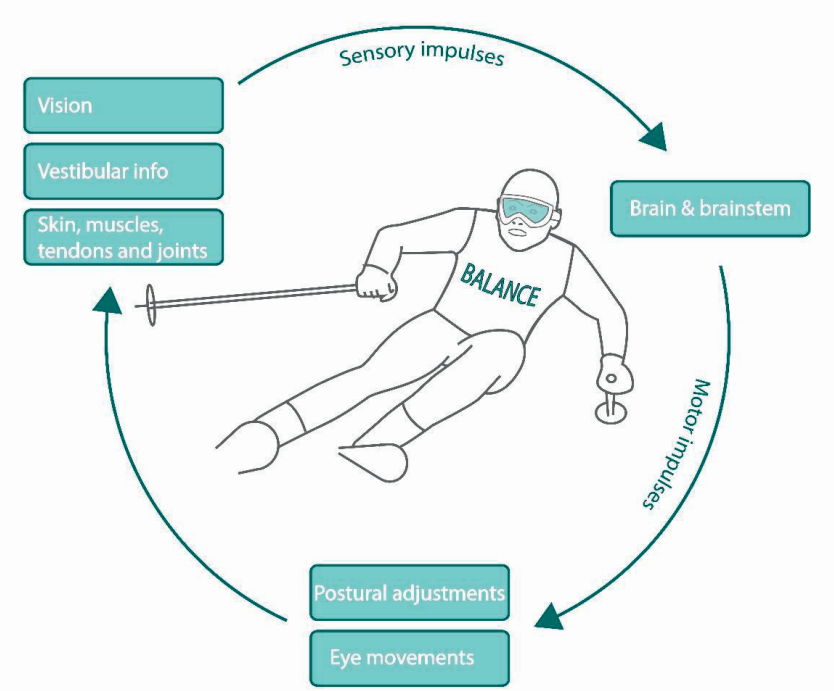

Figure 1. The vestibular labyrinth, vision and proprioceptive system (skin, muscles, tendons and joints) detect changes in head and body position and provide information to the brain via afferent nerve fibers ("afferents"). The brain produces compensatory motoric eye and body movements to maintain postural control and stable vision during upright posture and locomotion.

6.3 The vestibular labyrinth and neurophysiology

The membranous vestibular labyrinth (figure 2) is about 1.5 cm and filled with endolymph. It is located in the temporal bone, in a similar-shaped cavity filled with perilymph (Flock, 1964). The superior vestibular nerve innervates the anterior and lateral semicircular canals, the utricular macula and a small part of the saccular macula (the "hook"). The inferior vestibular nerve innervates the posterior canal and the main part of the saccular macula. The vestibular labyrinth constitute a part of the peripheral postural system, but is closely connected to the central vestibular system.

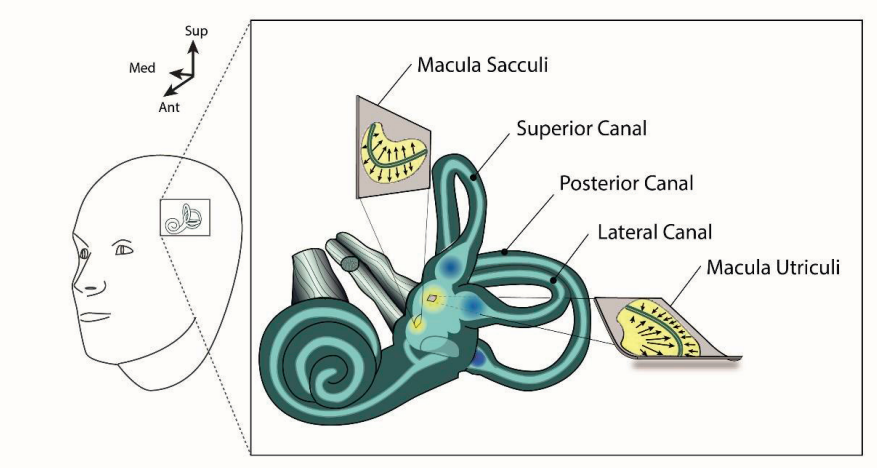

Figure 2. The left vestibular labyrinth and its approximate position and size within the temporal bone. There are three semicircular canals and two otoliths; the horizontal utricular macula and the vertical saccular is shown with directions of polarization vectors.

6.3.1 The semicircular canals

The semicircular canals (figure 3) are arranged ~ perpendicular to each other. The lateral canal is mainly horizontal, but the anterior part is tilted 30 degrees upwards. The superior and posterior canals are vertical and form a 90 degree angle to the lateral canal. From the sensitive crista – located at the base of the membranous ampulla – ~ 7600 hair cells (surface 1.0 mm^2) protrude into the cupula (Rosenhall, 1972a). The construction is similar in all three canals, but the cilia have different orientations to enhance the canals' sensitivity to angular acceleration within their plane (Lowenstein, 1959).

The canals respond to angular accelerations and work in pairs with the corresponding canals on the opposite side; turning the head to the right side activates the right lateral canal and inhibits the left lateral canal. The right superior canal works with the left posterior canal and the right posterior canal works with the left superior canal. Bending the head backwards activates the posterior canals and inhibits the anterior canals. This perpendicular, pairwise organization and orientation of the cilia, makes the canals extremely efficient in detecting angular accelerations in all directions (Bronstein, 2013).

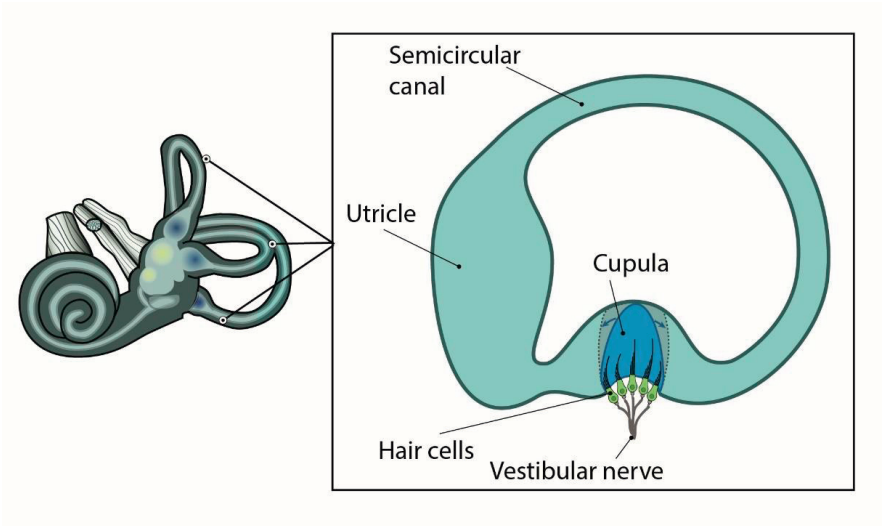

Figure 3. The canals are semicircular, and have an open end into the utricle. The ampulla is in the other end (expanded area). At the base of the ampulla, is the crista, from which hair cells protrude into the cupula. The cupula fills all the space of the ampulla and the endolymph cannot pass.

When an individual starts to rotate at a constant speed, the cupula is deflected in the opposite direction of the rotation due to inertial forces in the endolymph. The cupula is set in a neutral position when the rotation and the inertial forces achieve the same speed. When the rotation ceases, the cupula is deflected in the same direction as the initial rotation due to lag in the inertial forces of the endolymph. The endolymph and the cupula have the same density; therefore they do not react to linear accelerations (Bronstein, 2013).

6.3.2 The otoliths

The utricular and the saccular sac constitute the otolith organ. The utricle is horizontally oriented and all canals open into it. The saccule is inferior to the utricle, vertically orientated and detached to the temporal bone on its medial side. The utricle is twice as large as the saccule and only attached to the temporal bone

in its rostral area. The main part rests on a thin membrane over the endolymph (Curthoys et al., 2009). The macula is the sensitive area in the otoliths and is localized on the floor in the utricle and on the medial wall in the saccule. The macula has a three-layer construction that is similar in both otoliths (figure 4), except that the kinocilium is closest to the striola in the utricle and farthest out in the saccule. The polarization vector therefore differs (figure 2) in the two otoliths (Lowenstein, 1959, Flock, 1964).

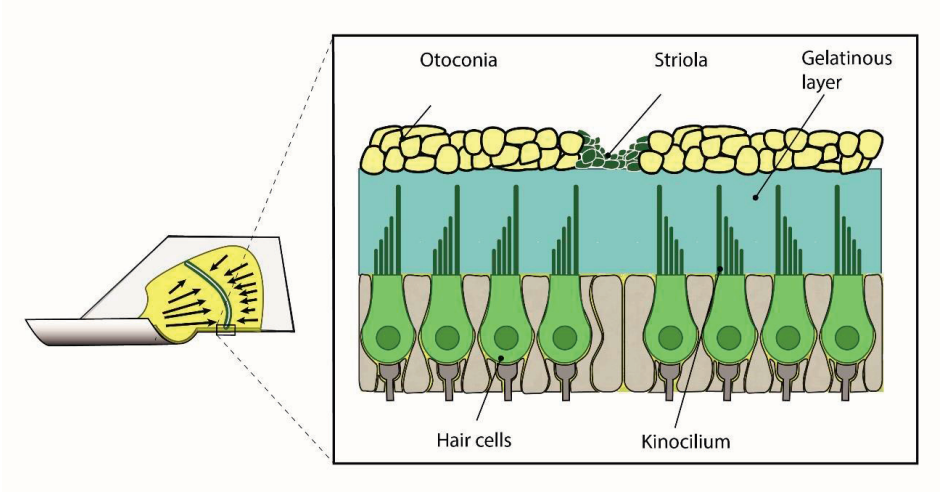

Figure 4. The three-layer macula; at the base is a layer of hair cells that protrudes into a gel, on top is a layer of otoconia. The striola is a "cleft" which divides the macula into a medial and a lateral part. The cilia on either side of the striola are organized differently in the utricle than in the saccule; the kinocilium (longest hair) is closest to the striola in the utricle and farthest out in the saccule.

The otoconia are carbonate crystals formed as hexagons (3-30 µm in diameter, specific weight 2.95 g/cm 3) (Bronstein, 2013). Presence of the otoconia adds more mass to the macula than to the cupula of the canals. The specific weight of the otoconia layer is greater than that of the surroundings, a feature that enable the otoliths to detect linear accelerations in the horizontal and vertical plane, e.g.

gravity. The fixed cilia at the bottom of each macula do not distinguish between linear accelerations and head tilts (Paige and Seidman, 1999, Goplen, 2009, Bronstein, 2013). Further, otoconia formation and reabsorption is an ongoing process, aided by supporting- and surrounding dark cells in the macula (Flock, 1964, Furman, 2003, Lundberg et al., 2015).

The utricular macula is kidney-shaped. It contains hair cells, dark cells and supporting cells (Rosenhall, 1972b). The striola divides the macula into a medial and a lateral part which vary in size between individuals, but the medial utricular macula is usually the largest (Goldberg and Fernandez, 1971, Rosenhall, 1972b). The total mean utricular macula surface of 4.3 mm^2 is however relative constant (Rosenhall, 1972b, Tribukait and Rosenhall, 2001).

The utricular macula is most active when a person holds the head in an upright position and the largest polarization vector is in the anteromedial direction – correlates to locomotion – and the smallest in the posterior direction (Tribukait and Rosenhall, 2001). Commissural inhibition enhances the linear sensitivity in the utricular macula (Uchino et al., 1999, Uchino and Kushiro, 2011), e.g. activates the right lateral macula and inhibits the left lateral macula. The underlying mechanism is that neurons in the vestibular nucleus receive afferents from both sides that activate (monosynaptic) afferents on the stimulated side and inhibit (disynaptic) afferents on the non-activated side (Goldberg et al., 1987, Uchino et al., 2001). In general, excitatory stimuli cause larger responses than inhibitory stimuli (Fernandez and Goldberg, 1976).

The shape of the saccular macula is usually oblong with an anterior bulge and a posterior tip (figure 2). The S-formed striola – actually a thickened area in the

saccule – divides the macula into a superior and inferior part which varies in size between individuals. The total mean saccular surface of ~2.35 mm^2 is however relative constant (Tribukait et al., 2005).

The macula is fixed to the temporal bone (Curthoys et al., 2009, Todd et al., 2009) and responds to head tips (gravitation); the otoconia are dislodged up or down, thereby deflecting hair cells in the underlying gel. Cross-striolar inhibition enhances the saccule's sensitivity to gravity; vestibular nucleus neurons are excited or inhibited by ipsilateral monosynaptic and disynaptic afferents (Uchino et al., 1999).

6.3.3 The hair cells

The hair cells (figure 5) are mechanoreceptors that transform mechanical energy – from a deflected cupula or dislodged otoconia – into electrical impulses in the vestibular nerve (Vollrath et al., 2007).

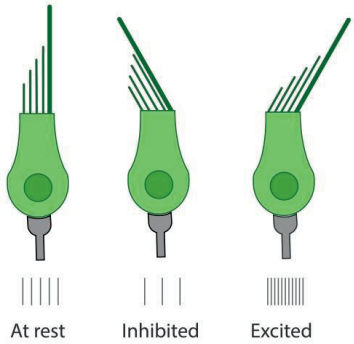

Figure 5. Hair cells at rest, inhibited (repolarized) and excited (depolarized). The state of hair cells determines the intensity of the firing rate in vestibular afferents.

On top of the cell surface are the kinocilium (longest and thickest) and stereocilia (smaller and thinner) in the range ~1:50-100 (Bronstein, 2013); ~7600 in each

crista (Rosenhall, 1972a); 33 100 in the utricle and 18 800 in the saccule (Rosenhall, 1972b). The cilia are connected by tip links (Vollrath et al., 2007) that enable the cilia to move synchronic with the dominant kinocilium. Deflection towards the kinocilium stretches the tip links and opens channels that allows for potassium flow down the electrical gradient. The hair cell depolarizes and releases neurotransmitters onto afferent vestibular nerve endings, figure 5). Deflection in the opposite direction, repolarizes the hair cells and fewer neurotransmitters are passed on to the vestibular nerve (Vollrath et al., 2007).

In the vestibular system, there are mainly two types of hair cells; type I are "bottle shaped" and surrounded by a large calyx with one afferent nerve ending connected to the cell basis. Type I cells are typically located in the summits of the crista and in central parts of the striola. Type II are cylinder shaped and surrounded by a large calyx with multiple afferent nerves at the cell basis, typically located around the crista and in the outer edges of the macula (Rosenhall, 1972a, b).

6.3.4 Vestibular afferents

The vestibular nerve contains more afferents than efferents (Bergstrom, 1973b). Each afferent contains 25000 neurons (Park et al., 2001) that enable them to respond quickly. The afferents consists of myelinated and unmyelinated fibers of various diameters (Lorento de No, 1932, Bergstrom, 1973b). Type I afferents have mainly thick (6-9 μ) irregular fibers, while type II afferents have mainly thin (1-2 μ) regular fibers. Both afferents are also endowed with medium-sized afferent fibers (Wersall, 1956). The resting potential differs from one afferent neuron to another, for example 90 spikes/s in the canals and 60 spikes/s in the

otoliths. In general a high resting potential enhances the firing rate in the afferents (Fernandez and Goldberg, 1976). The firing rate allows for both excitation and inhibition in an afferent (Goldberg and Fernandez, 1971). An excitatory response can be as high as 400 spikes/s; far more sensitive than the inhibitory response. Utricular afferents have mainly strong afferent projections to the external eye muscles (vestibulo-ocular reflex) and the saccule to the neck muscles (vestibulo-cervical reflex) (Uchino and Kushiro, 2011).

6.3.5 The vestibular nerve

The vestibular nerve contains afferents from all five parts of the vestibular labyrinth. The cell bodies are located in the Scarpa's ganglion where an isthmus divides the nerve into a superior and an inferior branch. Afferents in the superior branch originate in the utricle, the superior and the lateral canals and the "hook" of the saccule. Afferents in the inferior branch originate in the posterior canals and the "hook" of the saccule (Lorento de No, 1932). Both branches proceed in the internal auditory canal via the cerebellopontine angle before they enter the brainstem and terminate in the vestibular nuclei. Ascending fibers proceed mainly to the cerebellum and descending fibers (Lorento de No, 1932, Bronstein, 2013) precede into white matter tracts that inter-connecting information from the spinal cord, brainstem and cerebellum. The medial longitudinal fasciculus tract provide information from the peripheral to the central vestibular system, it is particularly important to maintain stable gaze (Bronstein, 2013).

6.3.6 The vestibular nuclei complex

The vestibular nucleus complex is a set of four overlapping nuclei located in the brainstem; the medial vestibular nucleus; the lateral vestibular nucleus (Deiters'nucleus); the superior vestibular nucleus and inferior vestibular nucleus.

Each part of the vestibular labyrinth, terminates in defined areas in the vestibular nuclei (Lorento de No, 1932). Neurons in the nuclei, can be arranged in groups according to their sensitivity to head – and eye movements (Angelaki, 2004). Further, numerous projections run from the vestibular nuclei complex to the cerebellum, extraocular motor nuclei, reticular substance, cerebral cortex and spinal cord. The vestibular nuclei regulate between afferent and efferent impulses that are essential for maintaining symmetry (Furman, 2003). Efferents in the vestibular nerve respond to asymmetric activity in the vestibular nuclei, but the efferents cannot distinguish normal head movements from inner ear pathology, thus any asymmetry is always interpreted as head movements (Furman, 2003).

6.3.7 Vestibulo Ocular Reflex

VOR is a short three-neuron arch that runs from the vestibular labyrinth to the external eye muscles (figure 6). VOR generates compensatory eye muscle rotations at the same speed as quick head movements, an act that ensures still eyes and a clear vision during head movement (Angelaki, 2004).

The two distinct VOR systems– aVOR and tVOR – are short latency reflexes that generate quick eye rotations in response to angular – and linear accelerations. aVOR is mediated by the canals and is the best defined of the two (figure 6). Typical for aVOR is that one single eye movement is sufficient to stabilize the visual image on the retina during head rotations (Angelaki, 2004). tVOR is more complex and at present less understood. It depends more on the viewing distance, the response is delayed and the eyes have more smoot pursuits and saccadic eye movements than the aVOR (Angelaki et al., 2000). The main function of the tVOR is to equalize the differences between close and distant objects that arise on the retina during locomotion (Angelaki, 2004).

The eyes need to discriminate between a flow of visual impressions – sights in the depth and close – that move in different spatial directions during locomotion. tVOR stabilizes the visual field by minimizing visual image slips on the retina; close objects are moved peripherally and far-away objects kept central on the retina. People perceive that close objects moves faster than objects far away. The visual field is stabilized at one point by objects moving in only one direction. The eyes always look at an object at different angles and distances, thereby creating an image mismatch on the retina; tVOR adjusts this mismatch by equalizing the differences (Angelaki and Hess, 2001, Angelaki, 2004). To ensure a stable vision, both VORs depend strongly on connections to and in the central nerve system, as well as on structures in the brain. The neural basics and contributions from the central nervous system in the VORs are not fully understood (Angelaki, 2004).

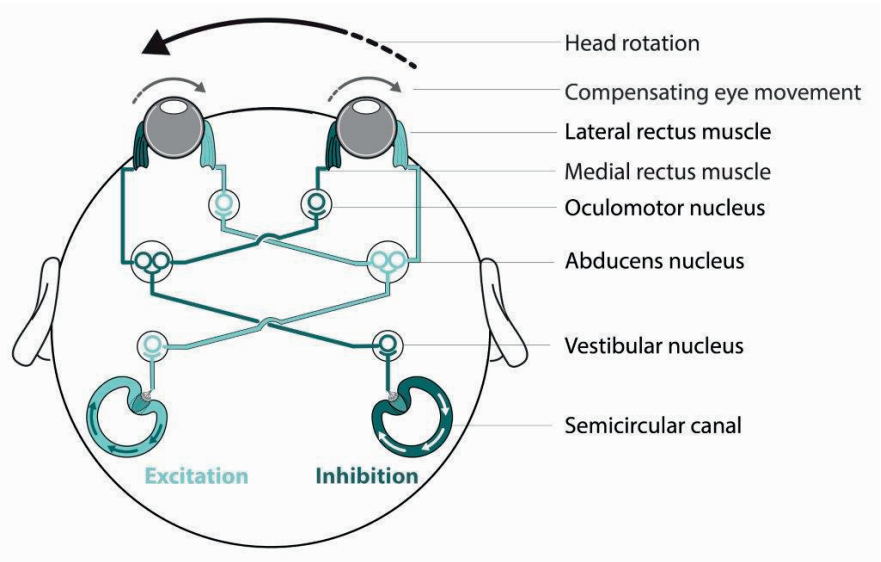

Figure 6. The principles of the Vestibulo Ocular Reflex (VOR). A head rotation to the left activates the left lateral canal and transmits signals in the vestibular nerve to the left vestibular nucleus. VOR then crosses in the brainstem before entering the right abducens nucleus, which contains motoneurons for the eye muscles. The right lateral rectus muscle and the left medial rectus are contracted to ensure eye rotation to the right. The right VOR transmits inhibitory signals. Interaction between the right and left VOR ensures stable vision during head movements.

7 Vestibular function testing

In 1914, Robert Barany received the Nobel Prize for discovering that water irrigation in the external ear canals set the endolymph in motion and the patient had "water nystagmus" (Wiest, 2015). The caloric test was established as the "gold standard" for vestibular function testing. Other tests were introduced, but without the same success. After a long period of "vestibular hibernation" new interest has been aroused and research instigated. Understanding and knowledge within the field therefore have increased. New tests have been introduced that ensure a more complete vestibular test battery; cVEMPs, oVEMPs and vHIT.

7.1 Nystagmus

In 1892, Ewald described that stimulation of one canal produces eye movements – nystagmus – in the same plane as the canal. This is known as Ewald's first law, and is a considered basic vestibular knowledge (Bronstein, 2013).

The definition of nystagmus is involuntary periodic eye movement that arises in the non-vestibular (congenital or lesions in the central nerve system) or vestibular system. Vestibular nystagmus (after this referred to as nystagmus) occurs due to unilateral vestibular pathology, which causes asymmetric impulses between the healthy and lesioned side, observed as nystagmus. Typically, the eyes move smoothly in one direction, cancelled by a quick movement in the other direction. Vestibular symptoms – vertigo, nausea, vomiting and a feeling of falling to one side – are often present. Nystagmus acts in accordance to the underlying pathology in the vestibular labyrinth. Vestibular neuritis, labyrinthitis and Meniere's disease are examples of diseases that affect the entire labyrinth typically on one side. In the acute stage, asymmetry between the healthy and

lesioned side is usually presented as horizontal nystagmus towards the healthy side (Strupp and Magnusson, 2015).

Benign Paroxysmal Positional Vertigo (BPPV) affects the canals, commonly the posterior canal. When the subject lies down on the affected side, otoconia in the posterior canal disturbs the free float of endolymph, and asymmetric impulses are observed as torsional nystagmus toward the affected side. Torsion occurs due to one vertical and one diagonal downward component. Pure up-beating nystagmus is occasionally observed in posterior canal tests (Ichijo, 2013, Fay, 2016), but central pathology is a more common reason and should be ruled out (Janssen et al., 1998, Kim et al., 2005, Ong et al., 2015). BPPV also affects the lateral canal and horizontal nystagmus, that is apogeotrop (beats upward from the ground) or geotropic (beats downward to the ground), is observed in positional tests (Brandt, 1990). Down-beating nystagmus is mainly caused by central disorders, but occasionally by BPPV (Bertholon et al., 2002). Isolated otolith nystagmus probably exists, but is rare, most likely because central compensatory mechanisms quickly rules out any present otolith nystagmus (Paige and Seidman, 1999).

Present nystagmus is a symptom of asymmetry between the two labyrinths. The brain immediately starts a process that adjusts the signal flow to the vestibular nuclei, resulting in diminished nystagmus commonly within days to a week. During recovery, the nystagmus can transiently change direction before it disappears. A late clinical sign of previous vestibular pathology is to shake the head, immediately afterwards a transitory nystagmus is often observed towards the non-affected side. Investigation of nystagmus is essential in vestibular assessments, but the interpretation can be challenging and complex. For all cases, use of specialized glasses that magnify the eyes and inhibit eye fixation (Frenzel

glasses, video nystagmus googles), ease the investigation (Goplen, 2009, Bronstein, 2013).

7.2 Caloric test

Caloric test measures the function of the lateral canal. Irrigation with warm (44^0) or cold water (30^0) – or hot and warm air – into the external ear canal causes a temperature difference between the body's temperature and the applied water. Warm water leads to increased endolymph in the irrigated ear, and thereby increased firing rates in vestibular afferents, resulting in horizontal nystagmus towards the irrigated ear. Cold water irrigation causes decreased endolymph and less firing in vestibular afferents, resulting in nystagmus away from the stimulated ear (Warm Same Cold Away). The lateral canals are considered to have normal function when the asymmetry is < 25 %, while asymmetry > 25 % represents unilateral pathology. If no nystagmus present, ice water is sometimes used (Bronstein, 2013).

7.3 Video head impulse test

The vHIT is based on quick eye and head movements that are not visible for the human eye. Saccadic eye movements can be observed by performing bedside head impulse test during acute vestibular attacks by turning the head quickly towards the side with pathology (Halmagyi and Curthoys, 1988), however compensatory mechanisms immediately adjust for the asymmetry by making the saccades even quicker. Within short time, saccades are no longer visible for the human eye. Studies that combined head impulse test and scleral search coil technique, led to the development of the video head impulse test (MacDougall et al., 2009). An infrared camera was mounted in the frame of googles; the camera capture compensated saccades from all six canals during head turns (Macdougall et al.,

2013). With perfected technique, the vHIT test is performed within 10 minutes. The vHIT and the caloric test, both investigate the SCCs. The vHIT performs the test at higher frequencies that are closer to our normal behavior and also all six canals are investigated, it also better tolerated by the subjects. The caloric test investigate only the horizontal SCC and at lower frequencies than vHIT. The test takes longer time and causes more discomfort for the patient. The two tests are complementary (Redondo-Martinez et al., 2016) and both are at present needed, but the vHIT will probably develop to be the screening test of choice of the SCCs, while caloric test is for those with a negative vHIT, but still suspect vestibular disease.

7.4 The subjective visual horizontal and subjective visual vertical

The SVH and SVV are valuable tests of otolith dysfunction (Clarke et al., 2001, Schonfeld et al., 2010), that indicate an individual's perception of the horizontal and vertical gravitation lines without referring landmarks (Bohmer and Mast, 1999). In an upright subject with otolith dysfunction, SVH/SVV is often deviated from the horizontal or vertical line. Dysfunctional otoliths recognize linear accelerations as head tilts, while the sensors – eyes and somatosensory systems – suggest that one is upright. The sensory conflict between the vision and the otoliths results in a short counter-rolling of the eyes that tilts the subject's vision; it is measured with the subject in a dark room outfitted with a lit bar. The subject must orient the lit bar horizontally or vertically and tilt the lit bar towards the dysfunctional side; at the point where the subject feels a normal upright position. The deviation varies in line with time from onset and the degree of dysfunction and compensation (Halmagyi and Curthoys, 1999).

7.5 Posturography

Posturography assesses static or dynamic postural control in the upright position, often located in vestibular test laboratories to evaluate a subject's posture and balance before rehabilitation. Posturography is valuable to evaluate the subject's progression, adjust exercises and the final effects of rehabilitation (Marioni et al., 2013).

7.6 Vestibular Evoked Myogenic Potential

VEMPs are short- latency myogenic responses that originate in the otoliths. Electrodes placed over neck- or eye muscles record the biphasic vestibular myogenic Potential evoked by ACS through the hearing chain or by BCV to the skull. VEMP was reported in 1964 by Bickford, who described short latency myogenic responses, measured at the occiput in response to ACS. The response was present in deaf as well as normal hearing subjects, suggesting a vestibular origin (Bickford et al., 1964). VEMP was again described in the beginning of the 1990ies, this time in a contracted m. Sternocleidomasteoideus after stimulating the ear with ACS (Colebatch and Halmagyi, 1992, Colebatch et al., 1994). Today, cVEMP is established as a test of saccular function (Rosengren and Kingma, 2013). Further investigation, discovered myogenic responses from nearby the eyes, today known as oVEMPs and considered as a test of utricular function (Rosengren et al., 2005, Todd et al., 2007).

7.7 Cervical VEMP

cVEMP test the function in sacculus and the inferior vestibular nerve (Colebatch et al., 1994), commonly evoked by ACS. BCV is an alternative in cases with middle ear pathology. One ear is stimulated at a time, but requires almost identical contraction on both sides for reliable results. It is essential that the

Sternocleidomasteoideus muscle is contracted, if not, responses are weak or absent (Colebatch et al., 1994). The vestibulo-cervical reflex provides the signal transmission from the saccule to the neck muscles. cVEMP is an inhibitory myogenic response (Colebatch and Rothwell, 2004) with a bi-phasic curve; initial downward (positive) peak at ~ 13 ms (p13), followed by an upward (negative) peak at ~ 23 ms (n23) (Welgampola and Colebatch, 2001). Asymmetric cVEMP (asymmetry ratio > 2.5) are often present in in Meniere's disease, superior canal dehiscence syndrome, vestibular neuritis, and some neurologic diseases, e.g. multiple sclerosis (Brantberg, 2009). With increasing age, there is a natural course in the general muscular atrophy as well as a loss of inner ear structures (Bergstrom, 1973c), therefore cVEMP is suggested to be most reliable in individuals below 65 years.

8 Ocular Vestibular Evoked Myogenic Potential (oVEMP)

8.1 Background

Increased interest for vestibular research discovered that ACS in the ears or BCV to the skull evoked myogenic responses underneath the eyes (Rosengren et al., 2005, Iwasaki et al., 2007, Todd et al., 2007). Also, in patients with total unilateral vestibular loss, responses were absent in the eye contralateral to lesioned ear but were evoked in deaf ears. These findings gave an idea of an apparently crossed response originating in the vestibular labyrinth and the test had the potential to be an additional useful vestibular test (Chihara et al., 2007, Iwasaki et al., 2007, Todd et al., 2007, Welgampola et al., 2008). Today, the test is known as oVEMP and it is a general understanding of oVEMP as an excitatory myogenic response originating predominantly in the utricle. And, that a potential saccular

contribution from the “hook” is insignificant (Todd et al., 2007, Welgampola et al., 2009). Impulses from the utricle are mediated via afferents in the crossed tVOR (superior vestibular nerve) to the contralateral eye muscle (Todd et al., 2007, Iwasaki et al., 2008b, Curthoys and Vulovic, 2011); the m. Obliquus inferior (Weber et al., 2012). The optimal test procedure is; elevated head, gazed eyes (Rosengren et al., 2005, Govender et al., 2009, Welgampola et al., 2009) and placement of bipolar electrodes under the eyes (Rosengren et al., 2005, Iwasaki et al., 2007, Todd et al., 2007, Welgampola et al., 2009) (figure 9). oVEMP is a relatively new and promising test of utricular and superior vestibular nerve function, complementary to cVEMP that has not been taken fully into the clinic. Studies that aim to improve standardization of the method are needed to validate and establish the test.

8.2 Waveform and nomenclature

oVEMP is an excitatory short latency biphasic response with an initial upward (negative) peak, followed by a downward (positive) peak (figure 7). Initial oVEMP studies described a peak at ~ 10 ms (n10), and a second peak at ~ 15 ms (p15) (Rosengren et al., 2005, Iwasaki et al., 2007), thereby the denomination. Other denominations have also been used – for example n1/ p1 – (Chihara et al., 2007, Welgampola et al., 2008). We have consistently used n10/p15 in our articles and in the thesis.

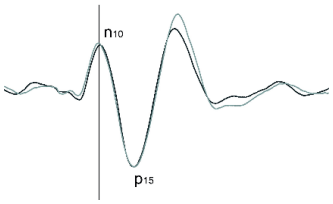

Figure 7. Typical oVEMP waveform with an upward negative peak (n10) and a downward positive peak (p15).

8.3 Asymmetry ratio

The basis for asymmetry ratio calculation is to use amplitudes from baseline to the n10 peak or the peak-to-peak amplitude. The asymmetry ratio is calculated by using the formula:

$$AR(\%) = \frac{\text{amplitude}_{large} - \text{amplitude}_{small}}{\text{amplitude}_{large} + \text{amplitude}_{small}} \times 100$$

According to the formula, AR will always be a positive (absolute) value between 0-100 %. Normal and pathological limits are at present not affirmed. Latency is discussed as a potential useful parameter for vestibular diagnostic (Taylor et al., 2014a), but is not used for oVEMP interpretation at present.

8.4 Bone Conducted Vibration and Air Conducted Sound

BCV applied to the skull at low thresholds or intense sound via the normal hearing chain, are sufficient stimuli to activate irregular afferents in the otoliths (Murofushi and Curthoys, 1997, Curthoys et al., 2006) and thereby evoke oVEMPs.

Bone-conducted stimuli reach the vestibular system through the auditory channel and start a cascade of events; involving the middle ear, ossicular chain, CSF pressure waves and inertial forces in the endolymph. BCV is complex, as it relies

on individual skull geometry, soft tissue (skin, hair), layered bone and brain tissue (Stenfelt et al., 2000) as well as on the type of bone-conducted device and its configuration (Iwasaki et al., 2007, Taylor et al., 2014a).

ACS also initiates a cascade of events that stimulates the inner ear; saccule and the cochlea. Sound waves – in the auditory canal – fluctuates the tympanic membrane, reinforced by the ossicular chain. The stapes footplate transmits vibrations onto the oval window, resulting in endolymph flow in the vestibule that stimulates sacculus and cochlea at sufficient sound intensity. ACS depends on a normal middle ear for sound transmission to the inner ear.

Irregular afferents in the otoliths are highly sensitive to BCV at low thresholds (Curthoys et al., 2006, Todd et al., 2008b, Chihara et al., 2009), but less so to sound. Higher thresholds are needed for ACS (Todd et al., 2007, Curthoys and Vulovic, 2011). The reason why both stimuli – BCV and ACS – activate the otoliths is most likely the evolutionary theory of a common origin for hearing and otoliths in vertebrates (Carey and Amin, 2006). In daily life, head displacements are the normal activator (Karlberg et al., 2003).

8.5 Bone-conducted devices

Different types of bone-conducted devices evoke oVEMP (Rosengren et al., 2010, Taylor et al., 2012b); a normal tendon hammer is the simplest and most available device. By tapping on the skull, oVEMPs are recordable. The taps are given manually; the varying strength of the taps results in different oVEMP morphology (Rosengren et al., 2009). The tendon hammer does therefore not improve standardization of the oVEMP method.

A custom made electro-dynamic exciter (figure 8) – developed for vestibular research – was introduced as an attempt to standardize hammer tapping (Brantberg et al., 2008). The electro-dynamic exciter is a solenoid mounted in a wooden box. On top of the box, is a scaled handle with a double spring system, in the other end is the box extended with a round bass stud with a diameter of 2.35 cm. The "Skull Tapper" delivers a strong impulse that causes both vibrations in the skull and head accelerations.

Figure 8. The Skull Tapper (photo by Magnus Westin).

Introduction of the Minishaker (Bruel and Kjaer Model 4810, Denmark) – a commercial made electro-dynamic with a solenoid and a moving coil – has improved standardization of the oVEMP method because it delivers constant linear accelerations (Rosengren et al., 2009).

8.6 Stimulus site

oVEMP can be evoked from sites located in the midline of the skull, running from the forehead to the back of the skull, or from the mastoid bones. All sites evoke oVEMP, but the stimulus site affects the oVEMP morphology.

The mastoids are efficient stimulus sites and good bone conductors, evoking large oVEMP amplitudes. They have close proximity to the utricle and the direction of the stimulus is in the plane of the utricle. The mastoid bone is relatively flat and stiff, which enhances their flexibility compared to midline sites (Stenfelt and Goode, 2005, Todd et al., 2008b, 2009, Tseng et al., 2011). Due to the normal constraints of the skull, a Minishaker applied at the mastoid bone, causes linear accelerations that initially affect the contralateral utricle, followed by backward linear accelerations that affect the ipsilateral utricle. oVEMPs are evoked and recorded from both eyes, but the response is strongest from the contralateral mastoid (Todd et al., 2008a, Govender et al., 2016). The test must therefore be taken on each mastoid – preferably under identical conditions – to achieve similar oVEMPs from each side.

Midline sites activate the vestibular labyrinths synchronously and oVEMPs are immediately recordable from under the eyes. Fz is located in the forehead, about 1/3 of the distance between the nasion and the inion (Agrawal 2013). Fz delivers consistent oVEMP and is the site most commonly used (Iwasaki et al., 2008b, a, Lin et al., 2010). The conduction properties are good, due to high bone density in the forehead (Lin et al., 2010). Occiput – in the back of the skull – is the midline site that has the shortest distance to the inner ear. oVEMPs are evoked, but the latency is often longer than in the forehead; explained by thinner bone and therefore less efficient conduction properties (Lin et al., 2010). We also disfavor

occiput as a stimulation site in our study due to small responses (Holmeslet et al., 2011).

Vertex is at the top of the skull and farthest away from the vestibular labyrinths (Stenfelt and Goode, 2005). The bone is relatively thin and the vertex lacks the feedback mechanisms found at the other sites. Stimulation at the forehead or the occiput causes feedback accelerations between each other and between the mastoids. Such feedback accelerations exist due to the normal constraints of the skull. The feedback theory does not apply to the vertex, because there is only soft tissue in the other end and the acceleration most likely just fades out into the soft tissue (Stenfelt and Goode, 2005). Vertex evokes oVEMPs and seems to share stimulation properties with the mastoids, but vertex has longer latencies and smaller amplitudes than the mastoids (Westin and Brantberg, 2014). Vertex show promising results in the investigation of superior canal dehiscence (Taylor et al., 2014a, Verrecchia et al., 2016).

8.7 Frequency

The frequency of the given stimulus varies, this also affect the oVEMP response (Taylor et al., 2012a, Zhang et al., 2012). The frequency is defined as low (< 400 Hz) or high (> 400 Hz) (Stenfelt and Goode, 2005). Low frequencies have long wavelengths, i.e. the corresponding wavelength is longer than the dimension of the head. During stimulation the skull and associated low frequency, tend to move as one unit. Also, the frequency and the direction of the stimuli are in the same direction at low frequencies (Stenfelt and Goode, 2005). The resonance of the utricle is ~ 100 Hz (Todd et al., 2008b, 2009), most likely because the utricle is dominated by irregular afferents that are highly sensitive to low frequency BCV (Todd et al., 2008b). 100 Hz is reported as the most effective frequency for

utricular activation (Todd et al., 2008b, 2009, Zhang et al., 2012, Dennis et al., 2016).

High frequencies have short wavelengths that cause "travelling" waves that go in different directions within the skull. The waves activate different compartments (liquid, soft tissues) at different times, and phase out when they meet an anti-resonance wave (Henry and Letowski, 2007). This is often explained as the "ringing" effect of the skull or elastic wave propagations around the skull (Todd et al., 2008b, Jombik et al., 2011). At high frequencies, the stimulus direction is mainly in the interaural direction irrespective of the frequency (Stenfelt and Goode, 2005, Henry and Letowski, 2007, Zhang et al., 2012).

At present, no optimal oVEMP frequency has been identified, but 500 Hz is frequently used and evokes consistent and reliable oVEMP at the forehead (Iwasaki et al., 2008a, b, Lin et al., 2010). Further testing is needed to determine one frequency suitable for screening.

8.8 Initial stimulus direction and head acceleration

The initial stimulus direction – positive or negative – of the bone-conducted device determines the activation of the utricle. Positive initial stimulus motion is when the outbound signal leaving the bone conductor is directed towards the head (figure 9). Negative initial stimulus motion is when the outbound signal from the bone conductor is directed away from the head. The initial stimulus motion determines which part of the utricle – medial or lateral – that firstly is activated (Lin et al., 2012). In oVEMP the activation is reflected as a short (negative) or long (positive) latency time (Holmeslet et al., 2015).

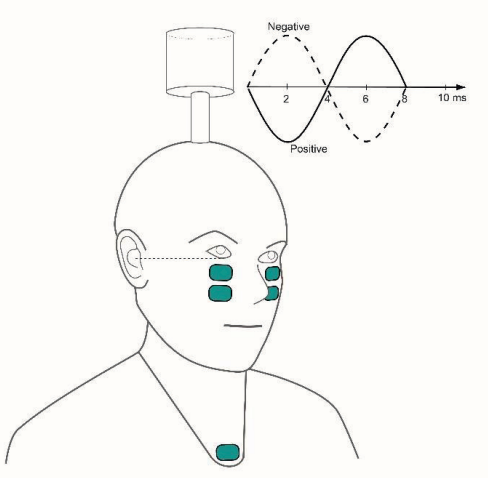

Figure 9. The figure illustrates the bipolar oVEMP electrode montage underneath each eye and the reference electrode. The Minishaker is placed at the vertex. The directions of the initial motions from the Minishaker are illustrated in the outlined box; initial positive motion is directed towards the head (black line), initial negative motion is directed away from the head (dotted line).

Initial positive stimulus motion – at the vertex and contra-lateral mastoid – causes outward linear accelerations and lateral utricular activation. Initial negative stimulus motion causes inward linear accelerations and medial utricular activation (Jombik et al., 2011, Westin and Brantberg, 2014). When Fz is used as the stimulation site, the initial stimulus motion causes opposite head accelerations; initial positive motion causes inward accelerations and initial negative motion outward accelerations (Iwasaki et al., 2008b, Cai et al., 2011, Jombik et al., 2011, Lin et al., 2012, Govender et al., 2016). The medial-lateral activation is most likely due to the different orientation in the cilia on each side of the striola (Cai et al., 2011, Jombik et al., 2011), or that the hair cells interpret the opposed acceleration directions to be different (Todd et al., 2008a). Initial positive stimulus motion is reported to cause more reliable responses and improved readability of

amplitudes (Cai et al., 2011). Initial stimulus motion has a more dominant influence on the evoked oVEMP for the low frequencies (Zhang et al., 2012). The lateral side in the macula is suggested to be the major activator of tVOR (Angelaki, 2004).

BCV causes linear head acceleration in a 3-dimensional plane (Hakansson et al., 1996) (figure 9); the y line (interaural direction); the x line (naso - occipital direction) and the z line (vertical direction) (Stenfelt and Goode, 2005).

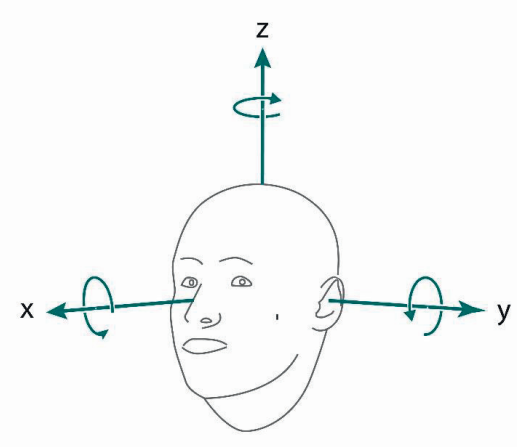

Figure 9. The figure illustrates the 3-dimensional linear head accelerations. The interaural line (y), the naso-occipital line (x) and the vertical line (z).

8.9 Test performance

Several technical issues and procedural factors may affect the oVEMP response. The subject sits on a chair (vertex) or lies on a bench with the chest elevated 30 degrees (forehead). The skin on the cheeks and sternum is rubbed to remove oil and dust before electrode mounting, modest skin preparation with 70% isopropyl alcohol swabs is reported as sufficient (Taylor et al., 2014b). A pair of electrodes – one passive and one active – is mounted underneath each centered pupil and at

the proximal part of the sternum (figure 9); accuracy in electrode placement is important for optimal responses. The impedance (resistance) should be kept < 10 kΩ to reduce stimulus artefacts, especially for the BCV oVEMP (Taylor et al., 2014b).

To set the vestibular labyrinths in a "neutral position", the head is brought into Reid's plane – an imaginary line from the lateral eyehook to the orifice of the ear – parallel with the floor (Blanks et al., 1975). The subject must center the pupils and focus on an upper point at the wall in front of the sitting place. Upward gaze bring the muscle-belly closer to the skin surface and ensures better contact with the electrodes, thus better recordings of oVEMPs (Rosengren et al., 2005, Govender et al., 2009, Welgampola et al., 2009, Kantner and Gurkov, 2014). A band pass filter is added to reduce background noise. The width of band passing can affect the evoked oVEMP and varies between studies; some keep it low (3-500 Hz) (Taylor et al., 2014a), other broader (2-2000 Hz) (Holmeslet et al., 2011). The given stimulus has a rise-duration-fall time and a short rise time is sufficient for evoking larger oVEMP (Burgess et al., 2013).

In general, VEMP testing is not advised in elderly (i.e. > 65 years), due to the normal aging process of the inner ear. Studies suggest a hair cell loss of 5-6 % per decade between 40-90 years ((Bergstrom, 1973a, c, Rosenhall, 1973, Welgampola and Colebatch, 2001).

9 VEMPs in vestibular diseases

9.1 Vestibular neuritis

Vestibular neuritis is the third most common cause for vertigo with an incidence of 3.5/100000 (Sekitani et al., 1993) t. Evidence suggests a viral etiology (Hirata et al., 1989, Hirata et al., 1995, Baloh, 2003). Vestibular neuritis commonly affects the superior vestibular nerve (Fetter and Dichgans, 1996) or both branches, while isolated inferior neuritis is rare (Halmagyi et al., 2002, Kim and Kim, 2012). The complementary VEMP methods are excellent to distinguish between neuritis in the superior (oVEMP) and the inferior branch (cVEMP) (Iwasaki et al., 2009, Manzari et al., 2010a, Curthoys et al., 2011).

9.2 Meniere's disease

Meniere's disease affects the inner ear, normally only one side. Malabsorption of the endolymph causes hydrops in the endolymphatic duct or sac and the subject suffers from attacks (of at least 20-minute duration) with aural pressure, vertigo, tinnitus and progressing hearing loss (Paparella and Djalilian, 2002). Recurrent attacks often occur during several weeks or months, followed by attack-free periods of variable length and restored vestibular function. Vestibular investigation is most relevant under or in relation to attacks; oVEMPs and cVEMPs are reported as abnormal in Meniere's disease. cVEMP is most relevant since sacculus is mainly affected (Taylor et al., 2011, Murofushi, 2016). oVEMP amplitude is reported increased in relation to a Meniere's attack, while it is normalized in a quiet phase (Manzari et al., 2010b).

9.3 Benign Paroxysmal Positional Vertigo (BPPV)

BPPV is the most common reason for vertigo and the estimated one-year incidence is 0.6% (von Brevern et al., 2007). The incidence increases sevenfold in subjects > 60 years in relation to subjects < 40 years. Women are affected twice as often as men. The underlying pathology is unknown, but BPPV is associated with osteoporosis and migraine; conditions that also affect women more than men (Brandt et al., 2006). Dislodged otoconia – mainly from the utricle – enters the canals (commonly the posterior canal) and fleet in one of the arms of the canal (canalolithiasis) or fastens in the cupula (cupulolithiasis) (Bronstein, 2013). The diagnosis is mainly based on characteristic history and diagnostic position tests where nystagmus – in the plane of the positioned canal –is typically observed. History, clinical investigation and repeated maneuvers are often sufficient to diagnose and treat BPPV. Studies report that oVEMPs are abnormal, more than cVEMPs, suggesting utricular involvement (Singh and Apeksha, 2015, Xu et al., 2016). Increased oVEMP amplitude is also suggested as an objective parameter to evaluate if reposition maneuvers are successful (Bremova et al., 2013). At present the role of the VEMP tests in BPPV is not clear.

9.4 Vestibular migraine

The diagnosis vestibular migraine (VM) was recently implemented in the International Classification Disease (ICD 2010) code register. VM can cause symptoms in the same manner as other vestibular diseases; the underlying pathophysiology is often the same as for periphery disorders like Meniere's disease. oVEMP and cVEMP tests do not necessarily contribute to discriminate between vestibular migraine and Meniere's disease, but frequency tuning in

cVEMP is reported to possibly increase accuracy of differential diagnostics (Taylor et al., 2012b, Zuniga et al., 2012, Murofushi, 2016).

9.5 Semicircular dehiscence

Semicircular dehiscence is a congenital disorder of the inner ear. The temporal bone overlying the superior canal is very thin at birth. The thinning process proceeds during life, and an open defect – "window" – in the temporal bone is seen on CT scans. Sound or pressure (Valsalva's maneuver) triggers vertigo attacks in the patient. cVEMP and oVEMP are both enlarged in this relatively rare condition (Brantberg et al., 2004, Brantberg and Verrecchia, 2012, Taylor et al., 2014a, Manzari et al., 2015, Verrecchia et al., 2016).

9.6 Other diseases

oVEMP and cVEMP have potential to aid investigation of other peripheral and central disorders. Introduction of cVEMP and oVEMP have given new insight in the vestibular labyrinth and coherent structures and pure otolith vertigo – that gives a sensation of lateral tilts in the roll or pitch plane – is recently suggested as a new diagnosis (Murofushi et al., 2012, Murofushi et al., 2013). VEMPs also contribute to map the size and location of acoustic neuroma (Brantberg, 2009, Lin et al., 2014). The VEMP tests can also predict the course of sudden deafness; in those with normal cVEMP and reduced oVEMP the hearing improved (You et al., 2014). VEMPs also contribute in the investigation of central disorders like multiple sclerosis (Shimizu et al., 2000, Murofushi et al., 2001), brainstem infarction (Itoh et al., 2001, Ahn et al., 2011, Oh et al., 2013, Weng and Young, 2014) and in the different stages of Parkinson disease (de Natale et al., 2015) (Potter-Nerger et al., 2015).

10 General aims

oVEMP is a new promising vestibular test, but better standardization of the method is needed before it can be taken fully into the clinic. A clinical useful test must be “safe, practical, robust and reproducible” (Halmagyi and Curthoys, 1999). The overall aim of this thesis was to define if some stimulation sites are more practical, robust and reliable in response to low frequency BCV than others.

Specific aims:

10.1 Paper one

The aim was to document possible underlying mechanisms after using a skull tapper to induce strong oVEMP impulses at different sites on the skull in healthy subjects and in patients with unilateral vestibular loss. The skull tapper activates the vestibular labyrinth via direction-independent vibration and direction-dependent head acceleration and the choice of stimulus site may have an impact on the evoked oVEMP.

10.2 Paper two

The primary aims were to investigate if low frequency BCV vertex stimulation evokes oVEMP in healthy subjects and in patients with unilateral vestibular loss, and possibly specify an AR cut-off value. The secondary aims were to explore what possible effects the initial stimulus motions and use of higher frequencies have on vertex-evoked oVEMP.

10.3 Paper three

The aim was to calculate an asymmetry ratio in healthy subjects after low frequency BCV in the forehead and at the vertex, and define which of the two

sites have the most consistent AR with respect to repeatability, magnitude and variance in a test-retest situation.

11 Materials and methods

11.1 Study design

The thesis consists of three separate studies. Study one and two are case- control studies, while the third study includes controls only. The controls for study one and two are recruited from the colleges and staff at the St. Olav Hospital, Norway and the Karolinska Hospital, Sweden. In study three, all subjects were recruited at the St. Olav Hospital. The "cases" were recruited from a well-known "patient pool" of severe unilateral vestibular pathology at the Yrselsentralen, Karolinska Hospital in Stockholm. The inclusion of controls started in May 2009 and was completed in January 2015; separate groups were enrolled over some weeks for each study. Patient inclusion started in May 2009 and was completed in 2010. The study was in accordance with the Helsinki Declaration and also approved by the Regional Committee for Medical Research.

11.2 Inclusion and exclusion

All participants were between 18 – 70 years old, not pregnant or suffering from any serious disease. For the participants in the control group (all three studies) no previous history of vestibular disorder was accepted. The participants in the patient group (study one and two), were selected from a patient pool with well-known vestibular loss at Karolinska Hospital. The patients were initially diagnosed with vestibular diseases – Meniere's disease, acoustic neuroma, sudden deafness and infections with vestibular symptoms – and treated with

intratympanic gentamicin, labyrinthectomy or other surgery. Postoperative tests – caloric test and cVEMP – had confirmed unilateral vestibular loss.

11.3 Recordings

11.3.1 Preparing the subject before testing

Before performing the oVEMP test, the skin underneath each eye, the cheek and proximal sternum were rubbed with fine sandpaper (Trace Prep, 3M Dot, Ontario, Canada). The redundant skin was then wiped carefully with cotton gauze. The subject centered the pupils and elevated the eyes by looking to an upper marked point placed on the wall in front of them. Adhesive skin electrodes (Neuroline 720, Ambu and Ballerup, Denmark) were fastened to the prepared skin areas in a line underneath centered pupils and elevated eyes. The upper recording electrode was placed just underneath the infraorbital margin and the lower recording electrode approximately 2 cm below. The ground electrode was in most cases fastened at the proximal sternum (figure 9), but in cases of hairy chest, the soft tissue above the sternum was used.

11.3.2 Software settings

For stimulus set-up and oVEMP recordings, we used the commercially available Medelec Synergy Viasys Healthcare system (Warwick, UK), developed for neurophysiologic testing. The impedance was measured before recording. We aimed to keep the impedance < 10 kΩ and achieve as similar as possible values from all four electrodes.

The Medelec system delivered a force level re 1N of 115 dB, and the amplifier (Power Amplifier Type 2718 Brüel and Kjaer) adds an extra 20 dB. The total given intensity was 135 dB peak-to-peak equivalent force level re 1N; meaning

that the peak sound pressure level (SPL) was 135 dB (equivalent to 1 μN for the Minishaker and 150 dB re -1 μN for the wooden box). The peak sound pressure, expressed as decibel, refers to 1 μPa (units of pressure [μPa]). The rise–duration–fall time was of 2–8–2 ms for the studies with the Minishaker. The band-pass filter was set at 4 or 20–2000 Hz. In the Medelec system, the initial stimulus motion was decided as positive or negative.

11.3.3 Bone conducted devices

In our first study we used the electro-dynamic exciter, the "skull tapper" (see 6.5.5), which was placed at the mastoids, the forehead and the occiput in alternating order. The handle was pressed against the skull – in practice when the armature reached the bass stud. The pressure was monitored by the scaled handle. The force of the skull tapper to the skull during stimulation was 30 N and the peak value of the impulse was 33 N (Brantberg et al., 2008). The stimulus rate was 0.5 Hz (eight skull taps lasting 16 s). The electro-dynamic exciter generates sound that possibly contributes to secondary inner ear activation and the test person therefore wore ear protections.

In the second and third study we used the Minishaker (see 6.5.5), fitted with a polyoxymethylene (Delrin) rod (5-mm long, 20-mm diameter) and slightly sloped in the contact area (175^2 mm). The frequency of the stimulus was set to 125-Hz single-cycle tone-burst and the repetition rate of the stimulus was set to 5 stimuli/s (one stimulus sequence lasted 12.8 s). The maximum force of the Minishaker is 10 N.

In the second study all healthy subjects were investigated with both positive and negative initial stimulus motion in alternating order, some of the healthy subjects

were stimulated at 250 and 500 Hz. In the third study, we only used 125-Hz and positive initial stimulus motion.

11.3.4 Test situation

The subject was seated; the head was slightly bowed forward and kept in this position during the stimulus sequence. For forehead stimulation in the third study, the subject lay down on a bench, with the chest elevated approximately 30^0, and the head slightly bowed forward. For all studies, the subject elevated the centered eyes (Manzari et al., 2010a) and was encouraged not to blink.

11.4 Statistics

All statistical calculations were performed using IBM SPSS statistics (version 17.0 (study one) and version 21 (study two and three). Sigma Plot software (version 12) calculated the ROC analyses in study two.

For each study, different statistical approaches were used. The statistical significant level was $p > 0.05$ in all analyses.

11.4.1 Study one

We used the analysis of variance (**ANOVA**) to test for statistical significant differences in the mean oVEMP response, latency and amplitude at the four different stimulation sites; forehead, occiput, right and left mastoid, in healthy subjects and in a group with severely unilateral vestibular pathology. ANOVA is a general statistical model that is useful to compare means in three or more groups, and to describe variations among and between groups. The ANOVA model assumes normal distribution of the data, which was the case for the latencies. The amplitudes were supposed to follow a normal distribution in a large

enough population. For post hoc analysis, we used Least Significant Difference test.

11.4.2 Study two

In study two, we investigated to what extent BCV at vertex discriminates between healthy and lesioned ears. We had four continuous outcome parameters –

n10 latency, p15 latency, n10 amplitude and p-p amplitude – in our analysis presented as means with standard deviations.

The study had repeated measurements in the same individual, and there was 7 % missing values in the dataset. We compared the responses in the two groups by using the **Linear Mixed Model.** The model is preferred with repeated measurements in individuals.

We used **Akaike's information criterion** to identify the better model. In all models, the **residuals** were normally distributed. We defined the AR cut-off value from **ROC (Receiver Operating Characteristics) curves** for the n10p15 and the n10 amplitudes, both with positive initial stimulus motion.

11.4.3 Study three

Three criteria were used to determine the site – vertex or forehead – with the most consistent AR. We first identified the site with the lowest repeatability coefficient, second we explored the site with the lowest median AR and thirdly we defined the site with the least variance. Q–Q plots were used for visual inspection of data normality. The AR was calculated after subtracting the response from the left side from that of the right side. By this, ARs could take both positive and negative values, a **signed AR.** This procedure is in contrast to

the one used in a normal clinical setting where the lowest response is subtracted from the highest, regardless of side. By this, the AR will always hold a positive value. The signed AR was normally distributed and these data were subsequently used in the analysis of variance. We wanted to compare the forehead with the vertex with respect to the AR value. We pooled the signed ARs and used **Levene's test** to gain statistical power and to compare the variance in the data between the vertex and the forehead. Levene's test assumes that the variances are equal in the populations.

Like study two, this study also contains repeated measurements in an individual. We used the Linear Mixed Model to analyze the differences of the signed AR at each site. The fixed effects were the stimulation site, the test sequence and the type of amplitude. The residuals were normally distributed.

The **absolute AR** value was calculated to present the results similar to the one used in the clinic. We presented the absolute values from the three tests and both stimulation sites in **Box-plots**. For descriptive statistics, we presented the data from the first test with 95% confidence interval.

For further analyzes we defined two test conditions. Condition A compares test 1 and 2, which are two following tests without change of electrodes. Condition B compares test 1 and 3 and mimics a clinical situation where the subject needs to return to the clinic for new tests. Therefore, the subject had a break between the two tests before new electrode mounting. The differences of the ARs between the two test conditions with the corresponding SD were calculated. The differences were normally distributed. Further, the **repeatability coefficient** was calculated from the SD of these differences and multiplied with 1.96. The repeatability

coefficient indicates where the result between two repeated tests will be found, with a probability of 95%.

Further, we want to clarify the difference between r**epeatability and reproducibility**. The repeatability describes the variation in a test under repeatability- or reproducibility conditions. Under repeatability conditions the test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment, within a short interval of time (ref ISO/TR 24498:2006(en)) – which was the case in study three. We did not find significant effects in the oVEMP response at any of the two locations for any of the three tests. Change of electrodes did not influence the oVEMP response. In contrast, under reproducibility conditions the test results are obtained by the same method on identical test items in different laboratories with different operators, using different equipment.

12 Results of the papers

12.1 Paper one

The aim was to document possible underlying mechanisms after using a skull tapper to induce strong oVEMP impulses at different sites on the skull in healthy subjects and in patients with unilateral vestibular loss.

We investigated the oVEMP response after stimulating the right versus left mastoid, the forehead and the occiput with a skull tapper. Typical oVEMPs were evoked at the forehead and the contra-lateral mastoid in all healthy ears, atypical oVEMPs with a double-peak was evoked at the ipsi-lateral mastoid and at the occiput. When a double peak was present, the p15 latency occurred later. There were no significant differences in latency or amplitude between the forehead,

right- and left mastoids. Occiput stimulation showed significant smaller amplitudes and significantly longer latencies than the response at the three other sites. However, from our results, we disfavor occiput as stimulation site due to generally small responses.

12.2 Paper two

The primary aims were to investigate if low frequency BCV vertex stimulation evokes oVEMP in healthy subjects and in patients with unilateral vestibular loss, and possibly specify an AR cut-off value. The secondary aims were to explore what possible effects the initial stimulus motions and use of higher frequencies have on vertex-evoked oVEMP.

oVEMPs were symmetrically evoked after 125 Hz BCV at the vertex in all healthy ears, but not in lesioned ears; vertex as stimulation site can be used to define vestibular hypofunction. The correlation between the n10 amplitude (baseline to peak) and the n10-p15 amplitude (peak-to-peak) is excellent – 0.934 with positive motion ($p < 0.01$) and 0.976 with negative motion ($p < 0.01$) – both can therefore be used for AR calculation. The AR cut-off value was about 20 % for both amplitudes and stimulus motions, while ROC estimates an AR cut-off value at 37 % for the n10-p15 amplitude, and 51 % for the n10 amplitude with positive initial stimulus motion (specificity 0.98 and sensitivity 0.70).

Initial negative motion caused significantly shorter latencies ($p < 0.001$) and initial positive motion had larger amplitudes (not significant). The initial stimulus direction did not affect the n10-p15 interval.

All healthy subjects responded 100% to 125 Hz and positive stimulus motion and 95% with negative initial stimulus motion. In the group investigated at higher

frequencies, the response to 250 Hz was 50% (positive motion) and 34.6 % (negative motion). The response to 500 Hz was 0 % (positive motion) and 20 % (negative motion). Frequencies > 125 Hz were considered ineffective in evoking oVEMP at vertex.

12.3 Paper three

The aim was to calculate an asymmetry ratio in healthy subjects after low frequency BCV in the forehead and at the vertex, and define which of the two sites have the most consistent AR with respect to repeatability, magnitude and variance in a test-retest situation.

Each subject had three tests at the forehead and three tests at the vertex. We did not find significant differences in the AR between the tests. Change of electrodes did not affect the AR, neither in the forehead or the vertex. To identify the site with the more consistent AR of the two, we calculated the repeatability coefficient, which was lowest in the forehead. The median AR was significantly lower in the forehead ($p < 0.005$), and there was less variance in the forehead ($p \leq 0.001$). By using our three statistical tools, we unambiguously ascertain the forehead as the best site to obtain a consistent AR.

13 General discussion

13.1 Methodological considerations

13.1.1 Inclusion criteria

The healthy subjects were included among the colleagues and staff at St. Olav Hospital, Trondheim and at the Karolinska Hospital, Stockholm. Since all

included controls are from a working place, they are also all between 18-70 years. However, to include subject < 70 years old is in accordance with previous reports about weakened oVEMP after the age of 60-70 years (Nguyen et al., 2010, Tseng et al., 2010, Rosengren et al., 2011, Piker et al., 2013). Evoked oVEMP is reported in children from two –three years old (Huang et al., 2012, Wang et al., 2013a), but it was not an aim of our studies to include children under the age of 18. Any previous history of vestibular disorder (healthy subjects) was an exclusion criterion to avoid interference with the recording data. Vestibular diseases are often manifested later in life and the subjects in the patients group were in general older (~15 years) than the healthy subjects.

13.1.2 Software

oVEMP was diminished if the impedance was high or differed much between the electrodes. In cases of impedance > 10 kΩ it was impossible to reduce it despite careful skin preparation. For all cases, we strived to achieve ~ equal impedance from each electrode, as that seemed important for symmetrical oVEMP. Just recently, the impedance has been described to not affect the amplitude or latencies significantly, but high impedance (limits are not described) might diminish the oVEMP amplitude (Taylor et al., 2014b).

A bandpass filter adjusts for electrical background noise in the recorded muscle; lower limit is classified as a high-pass filter and upper limit as a low-pass filter. We had a relatively broad bandpass, 20-2000 Hz (study one and two) and 4-2000 Hz (study three) compared to other studies where 5-1000 Hz is more commonly used (Rosengren et al., 2010, Wang et al., 2013b). It is suggested that high-pass filter < 10 Hz and low-pass filter > 1000 Hz allows more low frequency background noise to pass through and diminish the oVEMP waveform (smaller

amplitudes and longer latencies) (Wang et al., 2013b). The evoked oVEMP in our studies might have been affected by the relatively wide bandpass filter we have used. The optimal bandpass filter is suggested to lie between 1-1000 Hz (Wang et al., 2013b).

The stimulus' repetition rate influences the evoked oVEMP response (Chang et al., 2010); the optimal repetition rate relies on the intensity of the used stimulus. In her review, Rosengren summarizes that 100-500 repetitions is quite common for BCV, whereas a tendon hammer only needs 20-50 repetitions (Rosengren et al., 2010). The Minishaker we used had a repetition rate at 5 Hz (192 repetitions) while the skull tapper had 0,5Hz (24 repetitions). The repetition rates reported in our studies should therefore be in accordance with what Rosengren summarizes in her review.

13.1.3 Test performance

During the test sequence, the subjects gaze upward and fixed the eyes on marked dots in front of the subject, the test performer judged if the eyes were in position and kept there throughout the test. Also, whether the head is placed in Reid's line is a subjective judgement. The importance of this position is also pointed out by Verrecchia (Verrecchia et al., 2016). The subject should also keep the eyes as much elevated as possible during the test. Maximal upward gaze results in the largest oVEMP amplitudes (Chihara et al., 2007, Iwasaki et al., 2008b, Govender et al., 2009, Smulders et al., 2009, Murnane et al., 2011).

13.2 Discussion of results

13.2.1 Study one

The skull tapper is a powerful stimulus and normally less power is needed to evoke oVEMP (Todd et al., 2008a). The vibration from the powerful skull tapper is suggested to cause two types of head accelerations in the skull; one that is not direction dependent and one that is direction dependent (Brantberg et al., 2008, Brantberg et al., 2009), thus double peaks. The first peak is most likely a direct result of the vibration and occurs regardless of stimulus site. The second peak relies on the site and the direction of the head accelerations and occurs only at those sites where the stimulus direction activates additional vestibular afferents. A similar double peak is also described in cVEMP with the same skull tapper (Brantberg et al., 2008, Brantberg et al., 2009). In presence of a double peak, the n10p15 interval was also prolonged, which to some extent also indicates more vestibular activation. It is suggested that other parts of the vestibular labyrinth contribute to the oVEMP because afferent nerve fibers also run between the canals to the Oblique muscle (Uchino et al., 1982), however they are less responsive to BCV than the irregular afferent nerve fibers from the otoliths (Curthoys et al., 2006). Further, the scanty connection between the saccule to the obliquus muscle (Uchino and Kushiro, 2011) is weak and its contribution to the oVEMP response is most likely insignificant (Curthoys, 2010). We therefore argue that the direction-dependent n10b peak is a muscular response due to additional vestibular activation, predominantly from the utricle.

13.2.2 Study two

Vertex evokes typical oVEMP in healthy ears, but the n10 latency is somewhat longer than other midline sites (Lin et al., 2010). The longer latency in our study

is in accordance with other studies using low frequency BCV at vertex (Lin et al., 2010, Taylor et al., 2014a, Verrecchia et al., 2016). Westin et al. have recently described that stimulation at the mastoids and vertex cause similar head accelerations within the skull, but the responses are smaller at vertex (Westin and Brantberg, 2014).

The AR cut-off value varies between studies (table 1), possibly because the studies have few participants and that clinics use different oVEMP approaches. The AR defines a limit of normality and is the clinical parameter that is most important for oVEMP interpretation. Govender et al. show how the AR cut-off value depends on stimulus conditions. They investigated 22 patients with vestibularis neuritis and 22 healthy subjects. Three different stimuli were used to evoke oVEMP; 500 Hz ACS, 500 Hz BCV at the forehead, and BCV to the mastoids. The AR cut-off value was 57% after ACS and decreased with BCV (Govender et al., 2015). Iwasaki et al. calculated the AR in 11 patients with unilateral vestibular loss (vestibular neurectomy due to vestibular schwannoma). They used different BCV stimuli; skull taps with a tendon hammer, mini shaker (positive - and negative polarity) and a mini tone burst at 500 Hz BCV, all at the forehead. They calculated similar ARs for all stimuli approaches and averaged the AR cut-off value at ~ 40% for all test conditions (Iwasaki et al., 2008a, b). Manzari et al. investigated 133 subjects with unilateral vestibularis neuritis, the AR cut-off value at 46.5 % was determined by ROC (diagnostic accuracy at 94 %) (Manzari et al., 2010a). Taylor compared 35 normal subjects with 77 patients with unilateral Meniere's disease, and used ACS and BCV at the forehead to evoke oVEMP – the AR cut-off value was suggested ~ 40% regardless of stimulus condition (Taylor et al., 2011). The AR cut-off value in our second study was 37

% calculated from the n10p15 amplitude and 51 % from the n10 amplitude (Holmeslet et al., 2015). From these studies, it seems to be a tendency to set the AR cut-off limit at ~ 40 %, regardless of stimulus approach. The affected ear is not always totally damaged, therefore the AR is not 100% for all cases.

Stimuli	Site	Amplitude	Disease	AR in healthy subjects	AR in lesioned subjects
G:500 Hz ACS	Fz	p-p	Vestibular neuritis	57%	100%
G:500 Hz BCV	Fz	p-p	Vestibular neuritis	36 %	100%
G:500 Hz BCV	Mast	n10	Vestibular neuritis	46%	100%
I: BCV	Fz	n10	Vestibular Schwannoma	40%	92%
M: 500 Hz BCV	Fz	n10	Vestibular neuritis	46.5%	86,1 %
H: 125 Hz BCV	Vertex	p-p n10	Unilateral Vestibular Loss	37 % 51 %	100%
T:ACS and BCV	Fz	n10	Meniere's disease	~ 40% (39-42%)	

G=Govender. I=Iwasaki. M=Manzari. H=Holmeslet. T=Taylor.

Table 1: Studies discussing the AR cut-off value at various stimulus conditions.

We introduce a new method to present grand mean curves; by aligning the first peak of the individual curves along the time axis, we were able to present curves with a more realistic shape, and also define more reliable values of the AR with narrower 95 % confidence intervals (only test 1 in study 3). In our opinion, this is a better way to achieve realistic values, instead of the more commonly used grand means curves which tend to smear out the characteristic oVEMP shape.

We report that positive initial motion causes longer latencies than negative motion. The longer latency is explained by activation of the smaller lateral utricular macula, while the shorter latency possibly correlates to activation of the

larger medial macula (Zhang et al., 2012). The evoked oVEMP is influenced by the initial stimulus motion for lower frequencies (< 400 Hz), while this is of less importance for the higher frequencies (Cai et al., 2011, Zhang et al., 2012). The n10-p15 interval is not affected by the initial stimulus motion. One patient responded weakly to positive initial stimulus motion, but not to negative. We know that this was just one patient, but theoretically the shift of stimulus motion could be used to map the macular response.

oVEMPs were successfully evoked at 125 Hz BCV, while 250 and 500 Hz were ineffective. This finding is in contrast to other studies, because 500 Hz is the most commonly used frequency for evoking oVEMPs. However, 250 Hz BCV is reported to evoke oVEMP just as efficient as 500 Hz at the forehead (Chihara et al., 2009) and 100 Hz BCV at the mastoids also evokes oVEMPs efficiently (Todd et al., 2008b, 2009, Zhang et al., 2012). Most studies evoke oVEMPs sufficiently at 500 Hz regardless of stimulus site, so why did we not achieve that? The conduction properties at the vertex are suggested less appropriate, because of the thinner bone and more soft tissue. Higher frequencies cause ringing effects, activating additional afferents, also the feedback mechanism is weak. We also had a broad bandpass filter; these are conditions that possibly contribute to diminished oVEMP responses. 125 Hz is a low frequency that makes the head move as an unit – ringing effects are avoided – and the frequency is also in line with the resonance of the utricle (Todd et al., 2009), conditions that possibly favors utricular activation and better recordable responses.

13.2.3 Study three

We wanted to minimize systematic errors like oVEMP montage, upward gaze and random errors like different test personnel and performed our tests under repeatability conditions. Test personnel affect the oVEMP greatly (Ertl et al., 2015), thereby allowing for greater variance between results. Further, more reproducibility studies would most likely determine the precision of AR oVEMPs under conditions found more often in daily clinical practice. In general, oVEMPs are reported repeatable (Nguyen et al., 2010); the time interval between our tests was small, but re-tests with longer time intervals (weeks-months) – or change of electrodes – do not have significant effects on the oVEMPs (Iwasaki et al., 2008b).

AR cut-off limits are described on a group level, but applied on individuals. We have investigated the repeatability coefficient, the magnitude and the variance of the AR, at two different sites in a test-retest situation. By using the three different statistical tools, we have investigated aspects related to AR and site that have not previously been described, aspects that are of great individual importance. Further, we ascertain the forehead as the preferred stimulus site.

The forehead is previously reported to be consistent and repeatable in response to BCV (Nguyen et al., 2010). Most other oVEMP test-retest studies (Nguyen et al., 2010, Piker et al., 2011, Kim and Ban, 2012) – ACS or BCV – use Inter Class Correlation (ICC) as statistical tool to describe how strongly groups of measures resemble each other. Often, values smaller than 0.4 indicate poor repeatability, values in the range 0.4–0.74 indicate fair to good repeatability and values larger than 0.75 indicate excellent repeatability. We regard ICC analyses to be scaled too roughly and study results are too often presented without confidence intervals.

13.3 Strengths and limitations

13.3.1 Strengths

We have investigated how to evoke oVEMP during clinical relevant conditions. The site used for stimulation must be consistent, reliable and not at least clinical useful. We have systemically, thoroughly and in a stepwise manner looked into how stimulus conditions – frequency and stimulus motions – affect the oVEMP morphology at the different sites. The clinically valuable parameter of oVEMP is the AR, which we also have estimated and validated. Based on the data in our three studies, we ascertain the most clinically relevant and consistent site in healthy subjects and patients.

13.3.2 Limitations

In study two, vertex vibration (at 250 Hz and 500 Hz) did not evoke oVEMP sufficiently; we could therefore have investigated if this was the same when we stimulated the forehead. In addition, we could have tested the response at different band passing filters. We have not measured accelerations within the skull with our test set up.

13.4 Future perspectives

We should perform tests with narrower bandpass filter to see if we can evoke sufficient oVEMP at frequencies > 125 Hz. More oVEMP studies for age, gender and specific vestibular diseases are warranted, and from such studies, AR cut-off limits should be defined. Different stimulus conditions – initial stimulus motion, rise-duration-fall time, band pass filter, frequencies and site – may contribute in the mapping and understanding of various vestibular symptoms and thereby improve vestibular diagnostic, treatment and rehabilitation approaches.

14 Conclusion

Paper 1:

The present data support a theory that skull tapping causes a double peak when the stimulus is applied at specific sites; the first double peak is independent of stimulus site and occurs due to the vibration from the skull tapper, whereas the second peak is direction dependent – it depends on the stimulus site and the direction of the stimulus. The double peak most likely represents activation of different utricular components.

Paper 2:

oVEMPs in response to 125 Hz vertex vibration is three times larger in healthy controls compared to patients with unilateral vestibular function loss. The initial stimulus affects the oVEMP latency and the amplitude.

Paper 3:

The forehead should be used as stimulation site for low-frequency bone-conducted vibration in the clinic because the site produced significant lower median AR, lower repeatability coefficients and less variance than the vertex.

15 References

Ahn BH, Kim HA, Yi HA, Oh SY, Lee H (2011) Abnormal cervical vestibular-evoked myogenic potential in anterior inferior cerebellar artery territory infarction: frequency, pattern, and a determinant. J Neurol Sci 307:114-119.

Angelaki DE (2004) Eyes on target: what neurons must do for the vestibuloocular reflex during linear motion. J Neurophysiol 92:20-35.

Angelaki DE, Hess BJ (2001) Direction of heading and vestibular control of binocular eye movements. Vision Res 41:3215-3228.

Angelaki DE, McHenry MQ, Hess BJ (2000) Primate translational vestibuloocular reflexes. I. High-frequency dynamics and three-dimensional properties during lateral motion. J Neurophysiol 83:1637-1647.

Baloh RW (2003) Clinical practice. Vestibular neuritis. N Engl J Med 348:1027-1032.

Bergstrom B (1973a) Morphology of the vestibular nerve. 3. Analysis of the calibers of the myelinated vestibular nerve fibers in man at various ages. Acta Otolaryngol 76:331-338.

Bergstrom B (1973b) Morphology of the vestibular nerve. I. Anatomical studies of the vestibular nerve in man. Acta Otolaryngol 76:162-172.

Bergstrom B (1973c) Morphology of the vestibular nerve. II. The number of myelinated vestibular nerve fibers in man at various ages. Acta Otolaryngol 76:173-179.

Bertholon P, Bronstein AM, Davies RA, Rudge P, Thilo KV (2002) Positional down beating nystagmus in 50 patients: cerebellar disorders and possible anterior semicircular canalithiasis. J Neurol Neurosurg Psychiatry 72:366-372.

Bickford RG, Jacobson JL, Cody DT (1964) NATURE OF AVERAGE EVOKED POTENTIALS TO SOUND AND OTHER STIMULI IN MAN. Ann N Y Acad Sci 112:204-223.

Blanks RH, Curthoys IS, Markham CH (1975) Planar relationships of the semicircular canals in man. Acta Otolaryngol 80:185-196.

Bohmer A, Mast F (1999) Assessing otolith function by the subjective visual vertical. Ann N Y Acad Sci 871:221-231.

Brandt T (1990) Positional and positioning vertigo and nystagmus. J Neurol Sci 95:3-28.

Brandt T, Huppert D, Hecht J, Karch C, Strupp M (2006) Benign paroxysmal positioning vertigo: a long-term follow-up (6-17 years) of 125 patients. Acta Otolaryngol 126:160-163.

Brantberg K (2009) Vestibular evoked myogenic potentials (VEMPs): usefulness in clinical neurotology. Semin Neurol 29:541-547.

Brantberg K, Lofqvist L, Fransson PA (2004) Large vestibular evoked myogenic potentials in response to bone-conducted sounds in patients with superior canal dehiscence syndrome. Audiol Neurootol 9:173-182.

Brantberg K, Lofqvist L, Westin M, Tribukait A (2008) Skull tap induced vestibular evoked myogenic potentials: an ipsilateral vibration

response and a bilateral head acceleration response? Clin Neurophysiol 119:2363-2369.
Brantberg K, Verrecchia L (2012) Effectiveness of different click stimuli in diagnosing superior canal dehiscence using cervical vestibular evoked myogenic potentials. Acta Otolaryngol 132:1077-1083.
Brantberg K, Westin M, Lofqvist L, Verrecchia L, Tribukait A (2009) Vestibular evoked myogenic potentials in response to lateral skull taps are dependent on two different mechanisms. Clin Neurophysiol 120:974-979.
Bremova T, Bayer O, Agrawal Y, Kremmyda O, Brandt T, Teufel J, Strupp M (2013) Ocular VEMPs indicate repositioning of otoconia to the utricle after successful liberatory maneuvers in benign paroxysmal positioning vertigo. Acta Otolaryngol 133:1297-1303.
Bronstein AM (2013) Oxford Textbook of Vertigo and Imbalance. London, United Kingdom: Oxford University Press.
Burgess AM, Mezey LE, Manzari L, MacDougall HG, McGarvie LA, Curthoys IS (2013) Effect of stimulus rise-time on the ocular vestibular-evoked myogenic potential to bone-conducted vibration. Ear Hear 34:799-805.
Cai KY, Rosengren SM, Colebatch JG (2011) Cervical and ocular vestibular evoked myogenic potentials are sensitive to stimulus phase. Audiol Neurootol 16:277-288.
Carey J, Amin N (2006) Evolutionary changes in the cochlea and labyrinth: Solving the problem of sound transmission to the balance organs of the inner ear. Anat Rec A Discov Mol Cell Evol Biol 288:482-489.
Chang CM, Cheng PW, Wang SJ, Young YH (2010) Effects of repetition rate of bone-conducted vibration on ocular and cervical vestibular-evoked myogenic potentials. Clin Neurophysiol 121:2121-2127.
Chihara Y, Iwasaki S, Fujimoto C, Ushio M, Yamasoba T, Murofushi T (2009) Frequency tuning properties of ocular vestibular evoked myogenic potentials. Neuroreport 20:1491-1495.
Chihara Y, Iwasaki S, Ushio M, Murofushi T (2007) Vestibular-evoked extraocular potentials by air-conducted sound: another clinical test for vestibular function. Clin Neurophysiol 118:2745-2751.
Clarke AH, Schonfeld U, Hamann C, Scherer H (2001) Measuring unilateral otolith function via the otolith-ocular response and the subjective visual vertical. Acta Otolaryngol Suppl 545:84-87.
Colebatch JG, Halmagyi GM (1992) Vestibular evoked potentials in human neck muscles before and after unilateral vestibular deafferentation. Neurology 42:1635-1636.
Colebatch JG, Halmagyi GM, Skuse NF (1994) Myogenic potentials generated by a click-evoked vestibulocollic reflex. J Neurol Neurosurg Psychiatry 57:190-197.
Colebatch JG, Rothwell JC (2004) Motor unit excitability changes mediating vestibulocollic reflexes in the sternocleidomastoid muscle. Clin Neurophysiol 115:2567-2573.
Curthoys IS (2010) A balanced view of the evidence leads to sound conclusions. A reply to J.G. Colebatch "Sound conclusions?". Clin Neurophysiol 121:977-978.

Curthoys IS, Iwasaki S, Chihara Y, Ushio M, McGarvie LA, Burgess AM (2011) The ocular vestibular-evoked myogenic potential to air-conducted sound; probable superior vestibular nerve origin. Clin Neurophysiol 122:611-616.
Curthoys IS, Kim J, McPhedran SK, Camp AJ (2006) Bone conducted vibration selectively activates irregular primary otolithic vestibular neurons in the guinea pig. Exp Brain Res 175:256-267.
Curthoys IS, Uzun-Coruhlu H, Wong CC, Jones AS, Bradshaw AP (2009) The configuration and attachment of the utricular and saccular maculae to the temporal bone. New evidence from microtomography-CT studies of the membranous labyrinth. Ann N Y Acad Sci 1164:13-18.
Curthoys IS, Vulovic V (2011) Vestibular primary afferent responses to sound and vibration in the guinea pig. Exp Brain Res 210:347-352.
de Natale ER, Ginatempo F, Paulus KS, Manca A, Mercante B, Pes GM, Agnetti V, Tolu E, Deriu F (2015) Paired neurophysiological and clinical study of the brainstem at different stages of Parkinson's Disease. Clin Neurophysiol 126:1871-1878.
Dennis DL, Govender S, Colebatch JG (2016) Properties of cervical and ocular vestibular evoked myogenic potentials (cVEMPs and oVEMPs) evoked by 500Hz and 100Hz bone vibration at the mastoid. Clin Neurophysiol 127:848-857.
Emami SF, Pourbakht A, Sheykholeslami K, Kamali M, Behnoud F, Daneshi A (2012) Vestibular hearing and speech processing. ISRN otolaryngology 2012:850629.
Ertl M, Boegle R, Kirsch V, Dieterich M (2015) On the impact of examiners on latencies and amplitudes in cervical and ocular vestibular-evoked myogenic potentials evaluated over a large sample (N = 1,038). Eur Arch Otorhinolaryngol.
Fay JL (2016) Benign Paroxysmal Positional Vertigo in 2 Children: A Case Series. Pediatr Phys Ther [Epub ahead of print].
Fernandez C, Goldberg JM (1976) Physiology of peripheral neurons innervating otolith organs of the squirrel monkey. I. Response to static tilts and to long-duration centrifugal force. J Neurophysiol 39:970-984.
Fetter M, Dichgans J (1996) Vestibular neuritis spares the inferior division of the vestibular nerve. Brain 119 (Pt 3):755-763.
Flock A (1964) STRUCTURE OF THE MACULA UTRICULI WITH SPECIAL REFERENCE TO DIRECTIONAL INTERPLAY OF SENSORY RESPONSES AS REVEALED BY MORPHOLOGICAL POLARIZATION. J Cell Biol 22:413-431.
Furman JM, Cass, S. P. (2003) Vestibular disorders, a case-study approach. New York, USA: Oxford University Press, Inc.
Goldberg JM, Fernandez C (1971) Physiology of peripheral neurons innervating semicircular canals of the squirrel monkey. I. Resting discharge and response to constant angular accelerations. J Neurophysiol 34:635-660.
Goldberg JM, Highstein SM, Moschovakis AK, Fernandez C (1987) Inputs from regularly and irregularly discharging vestibular nerve afferents to

secondary neurons in the vestibular nuclei of the squirrel monkey. I. An electrophysiological analysis. J Neurophysiol 58:700-718.
Goplen FK (2009) Svimmelhet. Diagnostikk og behandling. Kompetansesenter for vestibulære sykdommer, Haukeland universitetssykehus, Bergen, Norway: Kompetansesenter for vestibulære sykdommer.
Govender S, Dennis DL, Colebatch JG (2015) Vestibular evoked myogenic potentials (VEMPs) evoked by air- and bone-conducted stimuli in vestibular neuritis. Clin Neurophysiol 126:2004-2013.
Govender S, Rosengren SM, Colebatch JG (2009) The effect of gaze direction on the ocular vestibular evoked myogenic potential produced by air-conducted sound. Clin Neurophysiol 120:1386-1391.
Govender S, Rosengren SM, Dennis DL, Lim LJ, Colebatch JG (2016) Contrasting phase effects on vestibular evoked myogenic potentials (VEMPs) produced by air- and bone-conducted stimuli. Exp Brain Res 234:141-149.
Hakansson B, Carlsson P, Brandt A, Stenfelt S (1996) Linearity of sound transmission through the human skull in vivo. J Acoust Soc Am 99:2239-2243.
Halmagyi GM, Aw ST, Karlberg M, Curthoys IS, Todd MJ (2002) Inferior vestibular neuritis. Ann N Y Acad Sci 956:306-313.
Halmagyi GM, Curthoys IS (1988) A clinical sign of canal paresis. Arch Neurol 45:737-739.
Halmagyi GM, Curthoys IS (1999) Clinical testing of otolith function. Ann N Y Acad Sci 871:195-204.
Henry P, Letowski TR (2007) Bone conduction: Anatomy, Physiology and Communication. In: US Army Research Laboratory, vol. ARL-TR-4138, pp 30-52, 92-108.
Hirata T, Sekitani T, Okinaka Y, Matsuda Y (1989) Serovirological study of vestibular neuronitis. Acta Otolaryngol Suppl 468:371-373.
Hirata Y, Gyo K, Yanagihara N (1995) Herpetic vestibular neuritis: an experimental study. Acta Otolaryngol Suppl 519:93-96.
Holmeslet B, Foss OA, Bugten V, Brantberg K (2015) Ocular vestibular-evoked myogenic potentials (oVEMPs) in response to bone-conducted vertex vibration. Clin Neurophysiol 126:608-613.
Holmeslet B, Westin M, Brantberg K (2011) Ocular vestibular evoked myogenic potentials: skull taps can cause a stimulus direction dependent double-peak. Clin Neurophysiol 122:391-397.
Huang YC, Yang TL, Young YH (2012) Feasibility of ocular vestibular-evoked myogenic potentials (oVEMPs) recorded with eyes closed. Clin Neurophysiol 123:376-381.
Ichijo H (2013) Cupulolithiasis of the posterior semicircular canal. Am J Otolaryngol 34:458-463.
Itoh A, Kim YS, Yoshioka K, Kanaya M, Enomoto H, Hiraiwa F, Mizuno M (2001) Clinical study of vestibular-evoked myogenic potentials and auditory brainstem responses in patients with brainstem lesions. Acta Otolaryngol Suppl 545:116-119.
Iwasaki S, Chihara Y, Smulders YE, Burgess AM, Halmagyi GM, Curthoys IS, Murofushi T (2009) The role of the superior vestibular nerve in

generating ocular vestibular-evoked myogenic potentials to bone conducted vibration at Fz. Clin Neurophysiol 120:588-593.
Iwasaki S, McGarvie LA, Halmagyi GM, Burgess AM, Kim J, Colebatch JG, Curthoys IS (2007) Head taps evoke a crossed vestibulo-ocular reflex. Neurology 68:1227-1229.
Iwasaki S, Smulders YE, Burgess AM, McGarvie LA, Macdougall HG, Halmagyi GM, Curthoys IS (2008a) Ocular vestibular evoked myogenic potentials in response to bone-conducted vibration of the midline forehead at Fz. A new indicator of unilateral otolithic loss. Audiol Neurootol 13:396-404.
Iwasaki S, Smulders YE, Burgess AM, McGarvie LA, Macdougall HG, Halmagyi GM, Curthoys IS (2008b) Ocular vestibular evoked myogenic potentials to bone conducted vibration of the midline forehead at Fz in healthy subjects. Clin Neurophysiol 119:2135-2147.
Janssen JC, Larner AJ, Morris H, Bronstein AM, Farmer SF (1998) Upbeat nystagmus: clinicoanatomical correlation. J Neurol Neurosurg Psychiatry 65:380-381.
Jombik P, Spodniak P, Bahyl V (2011) Direction-dependent excitatory and inhibitory ocular vestibular-evoked myogenic potentials (oVEMPs) produced by oppositely directed accelerations along the midsagittal axis of the head. Exp Brain Res 211:251-263.
Kantner C, Gurkov R (2014) The effects of commonly used upward gaze angles on ocular vestibular evoked myogenic potentials. Otol Neurotol 35:289-293.
Karlberg M, Aw ST, Black RA, Todd MJ, MacDougall HG, Halmagyi GM (2003) Vibration-induced ocular torsion and nystagmus after unilateral vestibular deafferentation. Brain 126:956-964.
Kim JS, Choi KD, Oh SY, Park SH, Han MK, Yoon BW, Roh JK (2005) Medial medullary infarction: abnormal ocular motor findings. Neurology 65:1294-1298.
Kim JS, Kim HJ (2012) Inferior vestibular neuritis. J Neurol 259:1553-1560.
Kim MB, Ban JH (2012) The efficiency of simultaneous binaural ocular vestibular evoked myogenic potentials: a comparative study with monaural acoustic stimulation in healthy subjects. Clin Exp Otorhinolaryngol 5:188-193.
Lin CM, Wang SJ, Young YH (2010) Ocular vestibular evoked myogenic potentials via bone-conducted vibrations applied to various midsagittal cranial sites. Otol Neurotol 31:157-161.
Lin KL, Chen CM, Wang SJ, Young YH (2014) Correlating vestibular schwannoma size with vestibular-evoked myogenic potential results. Ear Hear 35:571-576.
Lin KY, Wang SJ, Young YH (2012) Influence of head acceleration on ocular vestibular-evoked myogenic potentials via skull vibration at Fz versus Fpz sites. Int J Audiol 51:551-556.
Lorento de No R (1932) Anatomy of the eighth nerve. . The Laryngoscope 43:1-38.
Lowenstein O (1959) A functional interpretation of teh electron-microscopic structure of the sensory hairs in the crista of the elasmobranch Raja clavata in terms of directional sensitivity. Nature 184:1807-1808.

Lundberg YW, Xu Y, Thiessen KD, Kramer KL (2015) Mechanisms of otoconia and otolith development. Dev Dyn 244:239-253.
Macdougall HG, McGarvie LA, Halmagyi GM, Curthoys IS, Weber KP (2013) The video Head Impulse Test (vHIT) detects vertical semicircular canal dysfunction. PLoS One 8:e61488.
MacDougall HG, Weber KP, McGarvie LA, Halmagyi GM, Curthoys IS (2009) The video head impulse test: diagnostic accuracy in peripheral vestibulopathy. Neurology 73:1134-1141.
Manzari L, Burgess AM, MacDougall HG, Curthoys IS (2015) Superior canal dehiscence reveals concomitant unilateral utricular loss (UUL). Acta Otolaryngol 135:557-564.
Manzari L, Tedesco A, Burgess AM, Curthoys IS (2010a) Ocular vestibular-evoked myogenic potentials to bone-conducted vibration in superior vestibular neuritis show utricular function. Otolaryngol Head Neck Surg 143:274-280.
Manzari L, Tedesco AR, Burgess AM, Curthoys IS (2010b) Ocular and cervical vestibular-evoked myogenic potentials to bone conducted vibration in Meniere's disease during quiescence vs during acute attacks. Clin Neurophysiol 121:1092-1101.
Marioni G, Fermo S, Zanon D, Broi N, Staffieri A (2013) Early rehabilitation for unilateral peripheral vestibular disorders: a prospective, randomized investigation using computerized posturography. Eur Arch Otorhinolaryngol 270:425-435.
Murnane OD, Akin FW, Kelly KJ, Byrd S (2011) Effects of stimulus and recording parameters on the air conduction ocular vestibular evoked myogenic potential. J Am Acad Audiol 22:469-480.
Murofushi T (2016) Clinical application of vestibular evoked myogenic potential (VEMP). Auris Nasus Larynx 43:367-376.
Murofushi T, Curthoys IS (1997) Physiological and anatomical study of click-sensitive primary vestibular afferents in the guinea pig. Acta Otolaryngol 117:66-72.
Murofushi T, Komiyama S, Yoshimura E (2013) Do patients who experience episodic tilting or translational sensations in the pitch plane have abnormal sacculo-collic reflexes? Neurosci Lett 553:95-98.
Murofushi T, Nakahara H, Yoshimura E (2012) Assessment of the otolith-ocular reflex using ocular vestibular evoked myogenic potentials in patients with episodic lateral tilt sensation. Neurosci Lett 515:103-106.
Murofushi T, Shimizu K, Takegoshi H, Cheng PW (2001) Diagnostic value of prolonged latencies in the vestibular evoked myogenic potential. Arch Otolaryngol Head Neck Surg 127:1069-1072.
Nguyen KD, Welgampola MS, Carey JP (2010) Test-retest reliability and age-related characteristics of the ocular and cervical vestibular evoked myogenic potential tests. Otol Neurotol 31:793-802.
Oh SY, Kim JS, Lee JM, Shin BS, Hwang SB, Kwak KC, Kim C, Jeong SK, Kim TW (2013) Ocular vestibular evoked myogenic potentials induced by air-conducted sound in patients with acute brainstem lesions. Clin Neurophysiol 124:770-778.

Ong C, Patel K, Musiek E, Van Stavern G (2015) Mystery case: a young woman with isolated upbeating nystagmus. Neurology 84:e17-19.
Paige GD, Seidman SH (1999) Characteristics of the VOR in response to linear acceleration. Ann N Y Acad Sci 871:123-135.
Paparella MM, Djalilian HR (2002) Etiology, pathophysiology of symptoms, and pathogenesis of Meniere's disease. Otolaryngol Clin North Am 35:529-545, vi.
Park JJ, Tang Y, Lopez I, Ishiyama A (2001) Age-related change in the number of neurons in the human vestibular ganglion. J Comp Neurol 431:437-443.
Piker EG, Jacobson GP, Burkard RF, McCaslin DL, Hood LJ (2013) Effects of age on the tuning of the cVEMP and oVEMP. Ear Hear 34:e65-73.
Piker EG, Jacobson GP, McCaslin DL, Hood LJ (2011) Normal characteristics of the ocular vestibular evoked myogenic potential. J Am Acad Audiol 22:222-230.
Potter-Nerger M, Govender S, Deuschl G, Volkmann J, Colebatch JG (2015) Selective changes of ocular vestibular myogenic potentials in Parkinson's disease. Mov Disord 30:584-589.
Redondo-Martinez J, Becares-Martinez C, Orts-Alborch M, Garcia-Callejo FJ, Perez-Carbonell T, Marco-Algarra J (2016) Relationship between video head impulse test (vHIT) and caloric test in patients with vestibular neuritis. Acta Otorrinolaringol Esp 67:156-161.
Rosengren SM, Govender S, Colebatch JG (2011) Ocular and cervical vestibular evoked myogenic potentials produced by air- and bone-conducted stimuli: comparative properties and effects of age. Clin Neurophysiol 122:2282-2289.
Rosengren SM, Kingma H (2013) New perspectives on vestibular evoked myogenic potentials. Curr Opin Neurol 26:74-80.
Rosengren SM, McAngus Todd NP, Colebatch JG (2005) Vestibular-evoked extraocular potentials produced by stimulation with bone-conducted sound. Clin Neurophysiol 116:1938-1948.
Rosengren SM, Todd NP, Colebatch JG (2009) Vestibular evoked myogenic potentials evoked by brief interaural head acceleration: properties and possible origin. J Appl Physiol 107:841-852.
Rosengren SM, Welgampola MS, Colebatch JG (2010) Vestibular evoked myogenic potentials: past, present and future. Clin Neurophysiol 121:636-651.
Rosenhall U (1972a) Mapping of the cristae ampullares in man. Ann Otol Rhinol Laryngol 81:882-889.
Rosenhall U (1972b) Vestibular macular mapping in man. Ann Otol Rhinol Laryngol 81:339-351.
Rosenhall U (1973) Degenerative patterns in the aging human vestibular neuro-epithelia. Acta Otolaryngol 76:208-220.
Schonfeld U, Helling K, Clarke AH (2010) Evidence of unilateral isolated utricular hypofunction. Acta Otolaryngol 130:702-707.
Sekitani T, Imate Y, Noguchi T, Inokuma T (1993) Vestibular neuronitis: epidemiological survey by questionnaire in Japan. Acta Otolaryngol Suppl 503:9-12.

Shimizu K, Murofushi T, Sakurai M, Halmagyi M (2000) Vestibular evoked myogenic potentials in multiple sclerosis. J Neurol Neurosurg Psychiatry 69:276-277.
Singh NK, Apeksha K (2015) Efficacy of cervical and ocular vestibular-evoked myogenic potentials in evaluation of benign paroxysmal positional vertigo of posterior semicircular canal. Eur Arch Otorhinolaryngol.
Smulders YE, Welgampola MS, Burgess AM, McGarvie LA, Halmagyi GM, Curthoys IS (2009) The n10 component of the ocular vestibular-evoked myogenic potential (oVEMP) is distinct from the R1 component of the blink reflex. Clin Neurophysiol 120:1567-1576.
Stenfelt S, Goode RL (2005) Transmission properties of bone conducted sound: measurements in cadaver heads. J Acoust Soc Am 118:2373-2391.
Stenfelt S, Hakansson B, Tjellstrom A (2000) Vibration characteristics of bone conducted sound in vitro. J Acoust Soc Am 107:422-431.
Straka H, Zwergal A, Cullen KE (2016) Vestibular animal models: contributions to understanding physiology and disease. J Neurol 263 Suppl 1:10-23.
Strupp M, Magnusson M (2015) Acute Unilateral Vestibulopathy. Neurol Clin 33:669-685, x.
Taylor RL, Blaivie C, Bom AP, Holmeslet B, Pansell T, Brantberg K, Welgampola MS (2014a) Ocular vestibular-evoked myogenic potentials (oVEMP) to skull taps in normal and dehiscent ears: mechanisms and markers of superior canal dehiscence. Exp Brain Res 232:1073-1084.
Taylor RL, Bradshaw AP, Halmagyi GM, Welgampola MS (2012a) Tuning characteristics of ocular and cervical vestibular evoked myogenic potentials in intact and dehiscent ears. Audiol Neurootol 17:207-218.
Taylor RL, Schulin M, Goonetilleke S, Welgampola MS (2014b) Does electrode impedance affect the recording of ocular vestibular-evoked myogenic potentials? J Am Acad Audiol 25:969-974.
Taylor RL, Wijewardene AA, Gibson WP, Black DA, Halmagyi GM, Welgampola MS (2011) The vestibular evoked-potential profile of Meniere's disease. Clin Neurophysiol 122:1256-1263.
Taylor RL, Zagami AS, Gibson WP, Black DA, Watson SR, Halmagyi MG, Welgampola MS (2012b) Vestibular evoked myogenic potentials to sound and vibration: characteristics in vestibular migraine that enable separation from Meniere's disease. Cephalalgia 32:213-225.
Todd NP, Rosengren SM, Aw ST, Colebatch JG (2007) Ocular vestibular evoked myogenic potentials (OVEMPs) produced by air- and bone-conducted sound. Clin Neurophysiol 118:381-390.
Todd NP, Rosengren SM, Colebatch JG (2008a) Ocular vestibular evoked myogenic potentials (OVEMPs) produced by impulsive transmastoid accelerations. Clin Neurophysiol 119:1638-1651.
Todd NP, Rosengren SM, Colebatch JG (2008b) Tuning and sensitivity of the human vestibular system to low-frequency vibration. Neurosci Lett 444:36-41.

Todd NP, Rosengren SM, Colebatch JG (2009) A utricular origin of frequency tuning to low-frequency vibration in the human vestibular system? Neurosci Lett 451:175-180.
Tribukait A, Rosenhall U (2001) Directional sensitivity of the human macula utriculi based on morphological characteristics. Audiol Neurootol 6:98-107.
Tribukait A, Rosenhall U, Osterdahl B (2005) Morphological characteristics of the human macula sacculi. Audiol Neurootol 10:90-96.
Tseng CC, Wang SJ, Young YH (2011) Comparison of Bone-Conducted Vibration for Eliciting Ocular Vestibular-Evoked Myogenic Potentials: Forehead versus Mastoid Tapping. Otolaryngol Head Neck Surg 146:289-294.
Tseng CL, Chou CH, Young YH (2010) Aging effect on the ocular vestibular-evoked myogenic potentials. Otol Neurotol 31:959-963.
Uchino Y, Hirai N, Suzuki S (1982) Branching pattern and properties of vertical- and horizontal-related excitatory vestibuloocular neurons in the cat. J Neurophysiol 48:891-903.
Uchino Y, Kushiro K (2011) Differences between otolith- and semicircular canal-activated neural circuitry in the vestibular system. Neurosci Res 71:315-327.
Uchino Y, Sato H, Kushiro K, Zakir M, Imagawa M, Ogawa Y, Katsuta M, Isu N (1999) Cross-striolar and commissural inhibition in the otolith system. Ann N Y Acad Sci 871:162-172.
Uchino Y, Sato H, Zakir M, Kushiro K, Imagawa M, Ogawa Y, Ono S, Meng H, Zhang X, Katsuta M, Isu N, Wilson VJ (2001) Commissural effects in the otolith system. Exp Brain Res 136:421-430.
Verrecchia L, Westin M, Duan M, Brantberg K (2016) Ocular vestibular evoked myogenic potentials to vertex low frequency vibration as a diagnostic test for superior canal dehiscence. Clin Neurophysiol 127:2134-2139.
Vollrath MA, Kwan KY, Corey DP (2007) The micromachinery of mechanotransduction in hair cells. Annu Rev Neurosci 30:339-365.
von Baumgarten RJ, Thumler R (1979) A model for vestibular function in altered gravitational states. Life Sci Space Res 17:161-170.
von Brevern M, Radtke A, Lezius F, Feldmann M, Ziese T, Lempert T, Neuhauser H (2007) Epidemiology of benign paroxysmal positional vertigo: a population based study. J Neurol Neurosurg Psychiatry 78:710-715.
Wang SJ, Hsieh WS, Young YH (2013a) Development of ocular vestibular-evoked myogenic potentials in small children. Laryngoscope 123:512-517.
Wang SJ, Jaw FS, Young YH (2013b) Optimizing the bandpass filter for acoustic stimuli in recording ocular vestibular-evoked myogenic potentials. Neurosci Lett 542:12-16.
Weber KP, Rosengren SM, Michels R, Sturm V, Straumann D, Landau K (2012) Single motor unit activity in human extraocular muscles during the vestibulo-ocular reflex. J Physiol 590:3091-3101.
Welgampola MS, Colebatch JG (2001) Vestibulocollic reflexes: normal values and the effect of age. Clin Neurophysiol 112:1971-1979.

Welgampola MS, Migliaccio AA, Myrie OA, Minor LB, Carey JP (2009) The human sound-evoked vestibulo-ocular reflex and its electromyographic correlate. Clin Neurophysiol 120:158-166.
Welgampola MS, Myrie OA, Minor LB, Carey JP (2008) Vestibular-evoked myogenic potential thresholds normalize on plugging superior canal dehiscence. Neurology 70:464-472.
Weng YC, Young YH (2014) Mapping affected territory of anterior/posterior inferior cerebellar artery infarction using a vestibular test battery. Acta Otolaryngol 134:268-274.
Wersall J (1956) Studies on the structure and innervation of the sensory epithelium of the cristae ampulares in the guinea pig; a light and electron microscopic investigation. Acta Otolaryngol Suppl 126:1-85.
Westin M, Brantberg K (2014) Mastoid and vertex low-frequency vibration-induced oVEMP in relation to medially directed acceleration of the labyrinth. Clin Neurophysiol 125:615-620.
Wiest G (2015) The origins of vestibular science. Ann N Y Acad Sci 1343:1-9.
Xu H, Liang FY, Chen L, Song XC, Tong MC, Thong JF, Zhang QQ, Sun Y (2016) Evaluation of the utricular and saccular function using oVEMPs and cVEMPs in BPPV patients. J Otolaryngol Head Neck Surg 45:12.
You TZ, Wang SJ, Young YH (2014) Registering grades of sudden deafness to predict the hearing outcome via an inner-ear test battery. Int J Audiol 53:153-158.
Zhang AS, Govender S, Colebatch JG (2012) Tuning of the ocular vestibular evoked myogenic potential to bone-conducted sound stimulation. J Appl Physiol 112:1279-1290.
Zuniga MG, Janky KL, Schubert MC, Carey JP (2012) Can vestibular-evoked myogenic potentials help differentiate Meniere disease from vestibular migraine? Otolaryngol Head Neck Surg 146:788-796.

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Ocular vestibular evoked myogenic potentials: Skull taps can cause a stimulus direction dependent double-peak

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ABSTRACT

Objective: To explore the mechanisms for skull tap induced ocular vestibular evoked myogenic potentials (oVEMP).
Methods: An electro-mechanical "skull tapper" was used to test oVEMP in response to four different stimulus sites (forehead, occiput and above each ear) in healthy subjects (n = 20) and in patients with unilateral loss of vestibular function (n = 10).
Results: In normals, the oVEMP in response to forehead taps and the contra-lateral oVEMP to taps above the ears were similar. These responses had typical oVEMP features, i.e. a short-latency negative peak (n10) followed by a positive peak (p15). In contrast, the ipsi-lateral oVEMP to the laterally directed skull taps, as well as the oVEMP to occiput taps, had an initial double negative peak (n10 + n10b). In patients with unilateral loss of vestibular function, the crossed responses from the functioning labyrinth were very similar to the corresponding oVEMP in normals.
Conclusions: The present data support a theory that skull tapping may cause both a response that is more stimulus direction dependent and one that is less so.
Significance: Whereas the stimulus direction dependent occurrence of the negative double-peak might reveal the functional status of one part of the labyrinth, the rather stimulus direction-independent response might reveal the functional status of other parts.

1. Introduction

Since the early studies by Colebatch and co-workers (Colebatch and Halmagyi, 1992; Colebatch et al., 1994), testing vestibular evoked myogenic potentials (VEMP) has gained increased attention (for review: Welgampola and Colebatch, 2005). Most studies have dealt with cervical muscular activity within the sternocleidomastoid muscle (cVEMP) in response to air conducted sounds, and this set-up has established itself as a clinical test for saccular function. However, it has also been demonstrated that skull tap-induced cVEMP, in contrast to sound-induced, depend on two different stimulus mechanisms; one that is more, and one that is less stimulus direction dependent (Brantberg et al., 2008, 2009).

VEMP can also be recorded from under the eyes the with a bipolar electrode montage. These responses are called ocular VEMP (oVEMP) and represent synchronous activity of the extra-ocular muscles (Rosengren et al., 2005; Todd et al., 2007; Iwasaki et al., 2007). Both air conducted sounds and bone conducted stimulation were used in the initial studies. It was suggested that the oVEMP are mainly due to a crossed reflex and that they mainly reflect activity in the inferior oblique muscle.

In a recent study on healthy subjects, it was demonstrated that the character of oVEMP in response to impulsive transmastoid accelerations was determined mainly by the direction of the imposed acceleration (Todd et al., 2008). The authors speculated that, given the nature of the stimuli, utricular afferents were likely to be powerfully activated. Hence, testing skull tap-induced oVEMP might, potentially, constitute a means of evaluating utricular function. This possibility is significant, considering that, at present, there is no available non-perceptual and easily applicable clinical test for utricular function.

The aim of the present study was to explore further how skull tap oVEMP depend on the direction of head acceleration. More specifically, by tapping the skull on different sites, both in healthy subjects and in patients with only one functioning labyrinth, our aim was to test whether skull tap oVEMP depend on more than one stimulus mechanism (which seems to be the case for skull tap-induced cVEMP). Such a finding could be of great significance, because separation of different oVEMP components might reveal the status of different labyrinthine functions.

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2. Methods

2.1. The subjects

The study group consisted of twenty healthy subjects, 19–58 years old (mean age 38.2 years, 12 women and 8 men). According to their own statements, none had any history of vestibular or neurologic disorders, or any chronic ear disease. Data were also obtained from 10 patients, 32–67 years old (mean age 52.8 years, 4 women, 6 men), with severe unilateral loss of vestibular function.

The ten patients were selected on the basis of their complete unilateral vestibular loss, either after surgical labyrinthectomy (one patient with Ménière's disease), after acoustic neuroma surgery (two patients with small tumors) or after intratympanic gentamicin instillations because of recurrent attacks of vertigo (in four patients related to Ménière's disease, in one related to unilateral deafness after an acoustic trauma and in two related to unilateral deafness because of parotitis). In all of the seven patients treated with intratympanic gentamicin, test of caloric response and of sound-induced cervical VEMP had suggested complete loss of vestibular function in the lesioned ear.

The study was approved by the local ethic committee and all included subjects gave their informed consent to participation.

2.2. The oVEMP stimuli

The electro-mechanical skull tapper has previously been described in detail (Brantberg et al., 2008). In brief, a pull type linear solenoid is mounted in a wooden box at the bottom of which there is a round brass stud (diameter = 2.35 cm). The peak value of the impulse from the skull tapper, measured on an artificial mastoid (B&K 4930), is 33 N (=150 dB re 1 μN). The skull tapper also generated a loud sound and the subjects/patients, and also the test personnel, wore noise protection during the stimulations.

The subjects/patients were in the sitting position and during the skull tap stimulation the head was held so that the imaginary line between the opening of the outer ear canal and the lower margin of the orbit (Reid's plane) was close to horizontal. The skull taps were applied in the midline of the upper forehead and at the occiput. Skull taps were also applied approximately 2–3 cm above the opening of the outer ear canal on the right and the left side. These skull taps will be referred to as "lateral taps". Further, only one stimulation direction, i.e. positive acceleration, was used. The direction of these stimulations/accelerations will be given by specifying the site of the tapping, e.g. right side taps caused an acceleration of the right ear medially and the left ear laterally. The force of the skull tapper on the skull during stimulation was 30 N, and the skull tapper was held approximately horizontal. Each stimulus sequence consisted of three repetitions of 8 skull taps during a 16 s period (i.e. stimulus rate 0.5 Hz). There was a short break after each round of 8 taps. The same procedure was used for all four stimulus locations: right side, left side, forehead and occiput. Moreover, the stimulus order was systematically varied.

2.3. The oVEMP recordings

A Medelec Synergy signal averager was used to measure the oVEMP. Two-channel recordings of surface electromyographic activity were obtained using small disc electrodes (Neuroline 720, Ambu, Ballerup, Denmark). For recording, a pair of self-adhesive electrodes was attached beneath each eye, directly below the pupil. The active (+) self-adhesive recording electrode was placed close to the infra-orbital ridge, approximately one cm below the lower eyelid and the reference (−) electrode was placed about two cm below the first electrode (but, in illustrations, negative potentials were depicted as upward deflections). The self-adhesive electrodes were cut to allow the two electrodes to come closer together. The ground electrode was placed on the uppermost part of the sternum. Care was taken to minimize skin resistance by gentle rubbing (Trace Prep, 3M Dot, Ontario, Canada). During recording, the subject/patient focused on an imaginary point straight ahead and as high up as possible (while keeping his/her head in the same position). The subject/patient was instructed not to blink during the recording.

2.4. Analysis of oVEMP

For analysis of the oVEMP, the unrectified response was amplified and analogue filtered (passband 20–2000 Hz). The oVEMP latency and amplitude were then measured from each individual recording. Baseline-to-peak and peak-to-following amplitude were measured for the first negative peak. This peak has been called n10 and the following first positive has been called p15 (Rosengren et al., 2005), a nomenclature that will also be used in this presentation. However, as it turned out, there was an extra negative peak shortly after n10 in response to some of the skull tap stimulations. This peak will be referred to as n10b. In addition, we computed grand-mean curves and the amplitude and the latency were also computed from these curves. Further, grand-mean curves to opposite tapping sites were subtracted (for mid-sagittal taps it was the forehead from occiput response and for lateral taps it was the contra-lateral from ipsi-lateral response). Our reasoning here was that any direction-independent response would be common to both of these responses and therefore removed by subtraction. Hence, the resulting difference curve should be useful for the demonstration of direction-dependent responses (Brantberg et al., 2008).

2.5. Statistics

SPSS (version 17.0, IBM, Chicago, Illinois) was used for statistical analysis. The oVEMP recorded from under the left and the right eye turned out to be very similar in response to forehead and occiput

Table 1
oVEMP latency and amplitude in response to skull taps for normals (n = 20). For the laterally directed taps (applied above the right and the left ear), the mean values from recordings below the two ipsi-lateral respectively the two contra-lateral eyes are given. For frontal and occiput taps, the mean values from recordings below the two eyes are given. Mean ± SD and the number of observations ("eyes" = 40) for each value are given. In addition, the corresponding latency and amplitude from the grand-mean curves are presented within parenthesis.

	n10 latency (ms)	n10b latency (ms)	p15 latency (ms)	n10 amplitude (μV)	n10b amplitude (μV)	n10-p15 amplitude (μV)	n10b-p15 amplitude (μV)
Lateral tap ipsi-lateral response	8.3 ± 0.7	9.7 ± 0.5	14.2 ± 1.0	8.2 ± 5.7	8.2 ± 4.5		20.4 ± 10.2
	39/40 (7.9)	36/40 (9.4)	38/40 (14.1)	39/40 (5.3)	36/40 (4.6)		36/40 (15.1)
Lateral tap contra-lateral response	8.4 ± 1.2		13.1 ± 1.8	7.6 ± 5.3		16.7 ± 10.6	
	39/40 (7.8)		39/40 (11.8)	39/40 (4.6)		39/40 (11.1)	
Frontal tap	8.2 ± 1.0		12.6 ± 1.7	6.8 ± 5.7		14.6 ± 10.9 38/40	
	38/40 (7.8)		38/40 (12.5)	38/40 (4.3)		(10.6)	
Occiput tap	9.0 ± 1.2	10.9 ± 1.1	15.1 ± 1.7	6.3 ± 3.6	6.2 ± 3.7		13.0 ± 9.0
	40/40 (8.1)	36/40 (10.4)	40/40 (15.3)	40/40 (2.6)	36/40 (3.7)		36/40 (9.6)

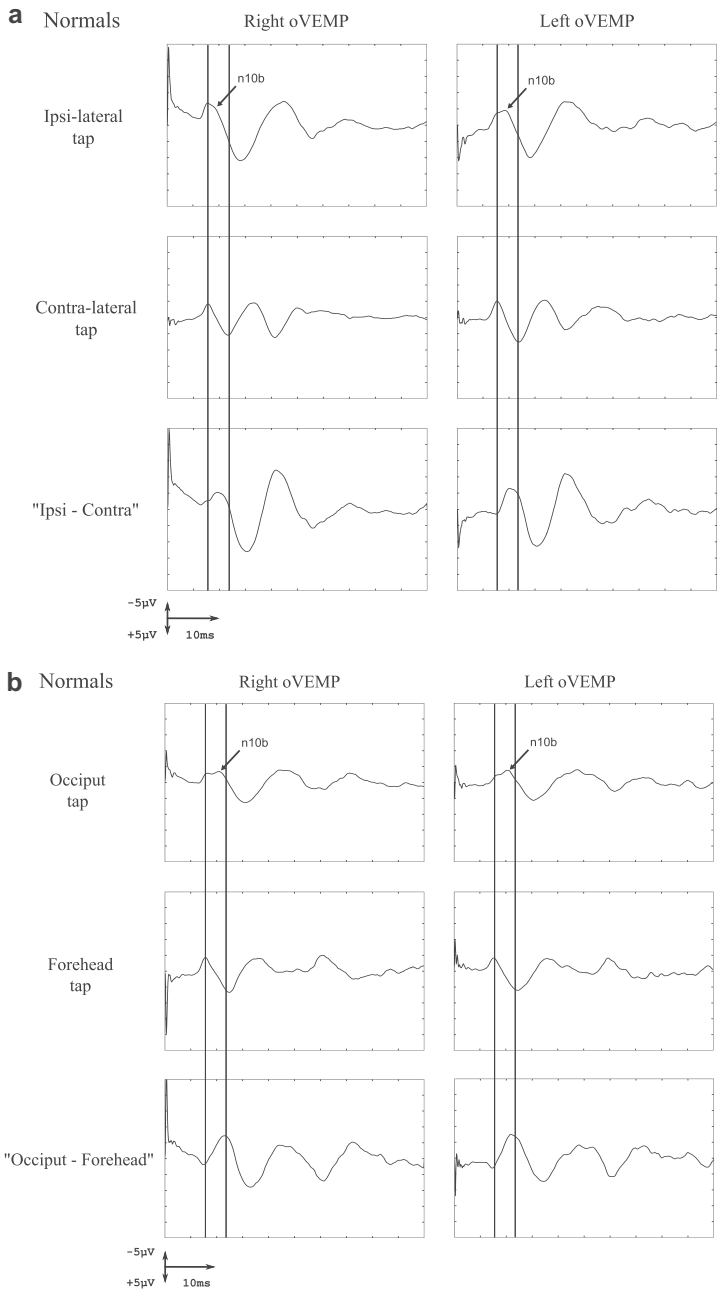

Fig. 1. The grand mean oVEMP of the 20 normal subjects in response to lateral (Fig. 1a) and mid-sagittal taps (Fig. 1b). The responses from under the right eye and from under the left eye are presented separately. Negative values are depicted upwards. In order to make the latency differences clearly discernible, the two vertical lines show the latency for the first negative (n10 = 7.7 ms) and the first positive peak (p15 = 11.9 ms) of the left oVEMP in response to a contra-lateral tap. In addition, the third rows demonstrate the effect of subtraction. In Fig. 1a, oVEMP curves from the eye contra-lateral to the tapping have been subtracted from the ipsi-lateral oVEMP ("Ipsi-Contra"). In Fig. 1b, oVEMP curves in response to forehead taps were subtracted from the response to occiput taps ("Occiput-Forehead"). Thus, for each eye, curves without n10b peaks are subtracted from curves with n10b peaks. These subtraction curves clearly demonstrate a negative peak occurring at approximately 10 ms for lateral taps and at approximately 12 ms for mid-sagittal taps.

taps. These mean responses from the two eyes were therefore used for further statistical evaluation. In response to lateral taps, both the two contra-lateral oVEMP and the two ipsi-lateral oVEMP were similar. Hence, the mean responses from the two contra-lateral

eyes as well as the two ipsi-lateral eyes were likewise used for the statistical evaluation. Differences in oVEMP amplitude and latency between the four different stimuli sites were assessed using an analysis of variance model (ANOVA). The Least Significant Difference test was used for post hoc analysis. A probability of $p < 0.05$ was considered significant.

3. Results

3.1. Normals

Whereas the oVEMP amplitudes differed between subjects, there were only small inter-subject latency differences (Table 1). Thus it seems reasonable to construct grand-mean curves for visualization of the responses (Fig. 1). In response to forehead taps, the oVEMP recorded from under each of the two eyes was very similar. There was a short-latency negative peak (n10) followed by a positive peak (p15). In response to laterally directed skull taps (presented above the right/left ear) there was, on the side contra-lateral to the tapping, a very similar oVEMP to that in response to forehead taps, i.e. a short-latency negative peak (n10) followed by a positive peak (p15). In contrast, the first negative peak on the side ipsi-lateral to the tapping was followed by a second negative peak (n10b). This second negative peak occurred approximately 1–2 ms after the first negative peak (and before the first positive peak (p15)). In response to occiput taps, a similar double-peak (n10 + n10b) was recorded from under both eyes. Although the separation is not obvious in all grand-mean curves, these two components of the double-peaks were clearly separated in most individual recordings (Fig. 2 and Table 1).

In addition to the stimulus site dependent occurrence of n10b, statistical analysis revealed a significant stimulus site effect on oVEMP amplitudes ($p < 0.001$). Post hoc analysis showed the n10 amplitude to be significantly smaller for occiput taps compared with the ipsi-lateral response to lateral taps ($p < 0.05$). There was also a statistically significant stimulus site effect on oVEMP latencies ($p < 0.001$). Post hoc analysis suggested that the n10 latency to occiput taps was longer compared with the other stimulus sites ($p < 0.001$ compared to frontal taps; $p < 0.05$ compared to the contra-lateral response to lateral taps; $p < 0.01$ compared to the ipsi-lateral response to lateral taps). Hence, the post hoc analysis suggested that most of these stimulus site effects were related to a different response to occiput taps. Consequently, it is suggested that the normals' oVEMP to frontal and right/left taps are very similar (except for the absence/presence of the n10b peak). Nevertheless, the positive peak (p15) did occur later whenever it was proceeded by a double negative peak compared to when it followed a single peak ($p < 0.001$ for occiput taps compared with both frontal taps and the contra-lateral response to lateral taps; $p < 0.01$ for the ipsi-lateral response to lateral taps compared with frontal taps; $p < 0.05$ for the ipsi-lateral response compared with the contra-lateral response to lateral taps).

3.2. Patients

In patients, there were no obvious oVEMP from the eye on the side opposite to the lesioned labyrinth, i.e. recorded from under the eye on the healthy side, in response to taps on any of the four sites (Fig. 3). However, the oVEMP on the side opposite to the functioning labyrinth had features that were very similar to the response among the normals (Table 2 and Fig. 3). oVEMP recordings from this side consisted of a single negative early peak (n10) followed by a positive (p15), both in response to forehead taps and lateral taps above the healthy ear (which is an analogous finding to those for the normals). In response to lateral taps over the lesioned ear there was a double-peak (n10 + n10b) ipsi-laterally, which is also analogous to the findings for the normals. Likewise, the patients "crossed" response to occiput taps also suggested a double-peak.

4. Discussion

In the present study it was found that skull tap oVEMP are to some extent dependent on the stimulus site, both in the mid-sagittal and the lateral plane. There was a double negative early peak (n10 + n10b) from the ipsi-lateral eye to lateral taps and from both eyes to occiput taps. There was a single negative early peak (n10) from the contra-lateral eye to lateral taps and from both eyes to forehead taps. Consequently, a stimulus direction dependent mechanism is suggested as the cause for the negative "extra peak" (n10b). The same stimulus site dependency was seen in patients with only one functioning labyrinth (in recordings from the eye opposite to the functioning labyrinth). Further, the latency of the

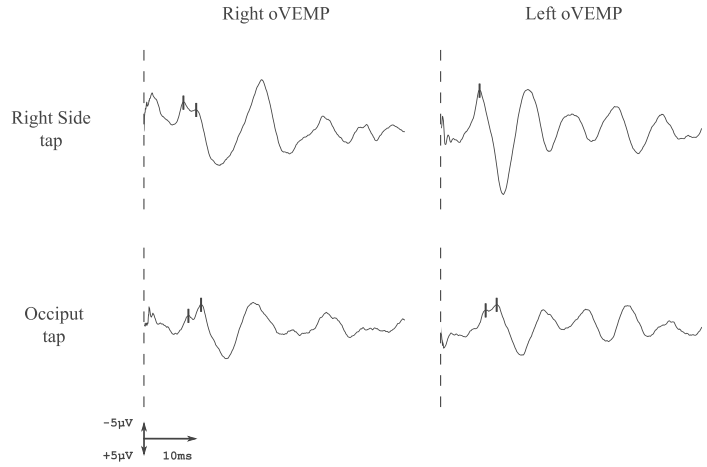

Fig. 2. Individual oVEMP recordings in one of the normal subjects. Note the double-peaks (n10 + n10b) from the ipsi-lateral eye to lateral taps and from both eyes to occiput taps. Note also a single peak (n10) from the contra-lateral eye to lateral taps. Stimulus onset is indicated by the dashed lines.

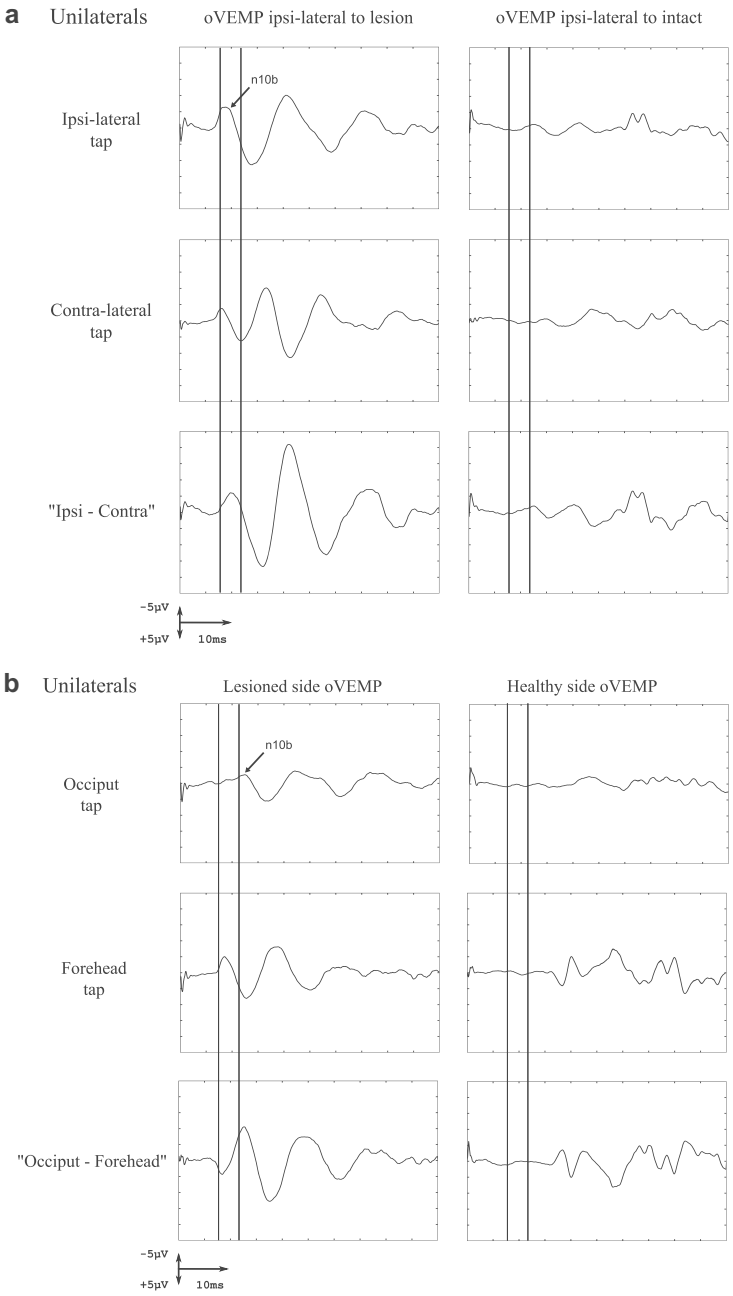

Fig. 3. The grand mean oVEMP for the ten patients with severe unilateral loss of vestibular function. Data are presented as in Fig. 1 (and the two vertical lines depict the same latencies, 7.7 and 11.9 ms, respectively, as in Fig. 1).

first positive peak (p15) was not just related to the n10 peak. The p15 occurred later whenever there was a double negative peak as opposed to when there was a single peak. This finding suggest that n10b truly represents an electromyographic signal, and not just electroencephalographic or electro-oculographic activity.

4.1. The mechanism for n10

In addition to the stimulus site dependent occurrence of the n10b, there also seem to be oVEMP responses to skull taps that are not so stimulus site dependent. The latency and amplitude of

Table 2
oVEMP latency and amplitude in response to skull taps in patients with unilateral loss of vestibular function (n = 10). Data are presented as in Table 1, however, in patients the data represent only oVEMP from recordings under the opposite eye ("eyes" = 10) to the functioning labyrinth (i.e., from under the eye on the lesioned side).

	n10 latency (ms)	n10b latency (ms)	p15 latency (ms)	n10 amplitude (μV)	n10b amplitude (μV)	n10-p15 amplitude (μV)	n10b-p15 amplitude (μV)
Lesioned side tap	8.4 ± 0.3	9.6 ± 0.6	13.8 ± 0.4	8.8 ± 3.0	8.0 ± 3.8		19.6 ± 8.4
	9/10 (8.2)	9/10 (9.7)	9/10 (14.0)	9/10 (5.0)	9/10 (5.8)		9/10 (17.1)
Healthy side tap	8.9 ± 2.8		12.3 ± 3.4	8.0 ± 3.8		14.3 ± 6.6	
	9/10 (8.2)		9/10 (11.9)	9/10 (3.8)		9/10 (10.0)	
Frontal tap	8.8 ± 0.6		12.5 ± 0.6	9.2 ± 3.7		19.5 ± 8.1	
	7/10 (8.5)		7/10 (12.7)	7/10 (5.0)		7/10 (13.0)	
Occiput tap	10.4 ± 1.4	11.7 ± 1.7	14.9 ± 1.8	5.7 ± 2.2	5.8 ± 4.1		15.9 ± 10.3
	10/10 (8.9)	8/10 (12.5)	9/10 (17.0)	10/10 (1.1)	8/10 (2.7)		7/10 (8.3)

the n10, in both normals and patients, was rather similar to both frontal and right/left side lateral taps. This suggests a less stimulus direction dependent mechanism, possibly bone vibration, to be of importance for the n10 peak.

The n10 peak did, however, differ in one respect. It was smaller and it occurred later in response to occiput taps. Similar findings to mid-sagittal taps have recently been reported by Lin and co-workers (Lin et al., 2010). They found occiput (inion) taps to cause n10 peaks with longer latencies compared to forehead taps. There was speculation as to whether this difference was due to differences in conduction velocity, because the latency alteration was unrelated to the distance between the stimulus sites and the vestibule. The same authors also found n10 amplitudes to be smaller for occiput taps, at least in comparison to taps at the nasion. A study on cVEMP also showed the response to occiput taps to have longer latencies compared to forehead taps (Brantberg et al., 2008). In view of the fact that n10 both occurs later and has a smaller amplitude, it is suggested that occiput taps might not be the most favorable stimulation site for testing the n10 of the oVEMP. Whether other midline taps or lateral taps are the better for clinical testing is probably, at present, not known. On the one hand, midline taps are likely to stimulate both labyrinths equal; on the other, with lateral accelerations it is possible to cause different directionally specific responses from each of the two labyrinths.

There is, at present, an ongoing discussion as to the origin for the n10 peak of oVEMP (Colebatch, 2010; Curthoys, 2010). Recent studies have demonstrated that the n10 peak of the oVEMP is distinguished from the R1 of the blink reflex (Chihara et al., 2009; Smulders et al., 2009). The n10 peak not only has an earlier onset, it also differs with regard to laterality, modulation by gaze position and patient groups. Further, animal experience has shown that irregular otolithic primary neurons are selectively activated by bone-conducted vibration (Curthoys et al., 2006). Many of these neurons originate from the utricular macula and they also pass though the superior division of the vestibular nerve. In addition, electrical stimulation of the nerve from the utricular macula in the cat has been shown to activate the contra-lateral inferior oblique muscle (Suzuki et al., 1969).

Human studies do also support a utricular origin for n10 in response to certain bone conducted stimuli (Todd et al., 2008; Iwasaki et al., 2009; Halmagyi and Carey, 2010). However, this oVEMP peak is not just evoked by bone vibration, but can also be evoked by air conducted sounds, i.e., suggesting a less stimulus direction dependent mechanism for n10. Based on known weak saccular-ocular projections and clinical studies demonstrating absence of n10 in most patients with superior vestibular neuritis, some authors suggest n10 to be predominately a utricular response (Curthoys, 2010). Others suggest that oVEMP to sounds could be due to saccular activation (Colebatch, 2010), i.e., different oVEMP mechanism for sound compare to some bone-conducted stimulations. Recent studies give support for such a possibility. Sound and bone vibration evoke different ocular movements and frequency tuning to sound and bone vibration is also different for oVEMP (Todd et al., 2007, 2009). Further, the interpretation that n10 to sounds could be of saccular origin must not be in conflict with findings of absent oVEMP in patients with superior vestibular neuritis. This could be explained by convergence between saccular and utricular pathways underlying the projection to the inferior oblique muscle. Removal of tonic facilitation from the utricular input might then leave the remaining saccular input sub-threshold for producing oVEMP (Colebatch, 2010).

4.2. The mechanism for n10b

Stimulus direction dependent oVEMP in response to impulsive transmastoid accelerations have recently been demonstrated (Todd et al., 2008). When using inferior electrode positions and up-gaze, the response to the same principal head acceleration as in the present study caused an initial negativity from the contralateral eye and a delayed negativity from the ipsi-lateral eye. Those authors also noticed a double negative peak in the ipsi-lateral oVEMP in some subjects, i.e. a similar double-peak with the same polarity as in the present study. However, it seems that the first negativity was larger in our study compared to theirs. Theoretically, this could be due to differences in the nature of the stimulus used. The peak value of the impulse from our skull tapper was 33 N, whereas the output from their "Minishaker" was 3.6 N. Although "our" skull tapper has been useful for exploring the two mechanisms for VEMP (Brantberg et al., 2008, 2009), the recent report from Todd et al. suggests that such a strong stimulus might not be necessary for testing VEMP (Todd et al., 2008). Consequently, the impulsive transmastoid accelerations we used should probably not be recommended for clinical routine testing. Further, in the study by Todd et al., care was taken to minimize any angular accelerations to the laterally directed linear impulse stimuli. In the present study, we used the same stimulus sites that were previously used to demonstrate that cVEMP depend on two different stimulus mechanisms (Brantberg et al., 2008, 2009), i.e. stimuli that were also likely to cause some angular head accelerations.

Then what might the origin for the n10b be? A blink reflex origin for n10b seems less likely for similar reasons as for the n10. In addition, n10b peaks were absent for forehead taps but present for occiput taps. The occiput is outside the distribution of the trigeminal nerve and such stimulations do not cause blinks to tactile stimulation (and influence from acoustic blink reflexes can be ruled out due to their much longer latency (Aramideh and Ongerboer de Visser, 2002)). Further, oVEMP are known to be critically dependent on gaze direction (Rosengren et al., 2005). The present normals/patients were instructed to look up and straight ahead during testing. An involuntary change of gaze, for example related to whether skull taps were applied to the right or left side, could theoretically have influenced the oVEMP. However, in view of the

fact that the first peak (n10) changed very little between right and left skull taps, it can be concluded that a change of gaze direction was not the cause for the n10b. It should also be pointed out that an analogue filter with a passband of 20–2000 Hz was used in the present study. A filter with a lower cut-off frequency could have obscured the double-peak by merging the two peaks.

In agreement with previous studies, the oVEMP seems to be caused almost entirely by contra-lateral labyrinthine activation. In the present patients with unilateral vestibular lesions, both n10 and n10b were absent on the side contra-lateral to the lesion. Hence, a labyrinth origin (also) for n10b is suggested. Further, as regards which part of the labyrinth, a semicircular canal contribution can probably not be ruled out for the n10b response. The applied stimuli probably caused angular head accelerations and thus excited the semicircular canals. Animal experiments also suggest that anterior semicircular canals have excitatory pathways to the contra-lateral inferior oblique muscle (Uchino et al., 1982). However, in the study by Todd et al., care was taken to minimize any angular accelerations to the laterally directed linear impulse stimuli. Nevertheless, they also noted, as mentioned above, a similar stimulus direction dependent oVEMP as the one we refer to as n10b. This may suggest an otolithic rather than a semicircular canal origin for n10b. Further, whereas the saccule is known to have only sparse connections to extra-ocular muscles, the utricle is known to have connections to the contra-lateral inferior oblique muscle (Suzuki et al., 1969). This might suggest a utricular origin for n10b. The utricule also has a structure which is consistent with different responses to the two directions, i.e. one response representing excitation of the medial part and one response representing excitation of the lateral part.

4.3. Summing up

It is suggested that not only cVEMP, but also oVEMP, are a summed response of two different stimulus mechanisms, one more and one less stimulus direction dependent. For cVEMP, the less direction dependent mechanism seems to depend on saccular function and it causes an ipsi-lateral inhibition. The more direction dependent cVEMP seem to be related to utricular function and to cause both an ipsi-lateral inhibition and a contra-lateral excitation (Brantberg and Tribukait, 2002; Brantberg et al., 2008, 2009; Rosengren et al., 2009). For oVEMP, a direction dependent mechanism seems to cause the delayed contra-lateral excitation (n10b). The exact labyrinthine origin for this is, at present, probably not known but it might also be utricular. Finally, the origin for the less stimulus direction dependent first contra-lateral excitation (n10 peak) is under debate.

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References

Aramideh M, Ongerboer de Visser BW. Brainstem reflexes: electrodiagnostic techniques, physiology, normative data, and clinical applications. Muscle Nerve 2002;26:14–30.
Brantberg K, Tribukait A. Vestibular evoked myogenic potentials in response to laterally directed skull taps. J Vestib Res 2002;12:35–45.
Brantberg K, Löfqvist L, Westin M, Tribukait A. Skull tap induced vestibular evoked myogenic potentials: an ipsilateral vibration response and a bilateral head acceleration response? Clin Neurophysiol 2008;119:2363–9.
Brantberg K, Westin M, Löfqvist L, Verrecchia L, Tribukait A. Vestibular evoked myogenic potentials in response to lateral skull taps are dependent on two different mechanisms. Clin Neurophysiol 2009;120:974–9.
Chihara Y, Iwasaki S, Ushio M, Fujimoto C, Kashio A, Kondo K, Ito K, Asakage T, Yamasoba T, Kaga K, Murofushi T. Ocular vestibular-evoked myogenic potentials (oVEMPs) require extraocular muscles but not facial or cochlear nerve activity. Clin Neurophysiol 2009;120:581–7.
Colebatch JG, Halmagyi GM. Vestibular evoked potentials in human neck muscles before and after unilateral vestibular deafferentation. Neurology 1992;45:1635–6.
Colebatch JG, Halmagyi GM, Skuse NF. Myogenic potentials generated by a click-evoked vestibulocollic reflex. J Neurol Neurosurg Psychiatry 1994;57:190–7.
Colebatch JG. Sound conclusions? Clin Neurophysiol 2010;121:124–6.
Curthoys IS, Kim J, McPhedran SK, Camp AJ. Bone conducted vibration selectively activates irregular primary otolithic vestibular neurons in the guinea pig. Exp Brain Res 2006;175:256–67.
Curthoys IS. A balanced view of the evidence leads to sound conclusions. A reply to J.G. Colebatch "Sound conclusions?" Clin Neurophysiol 2010 [Epub ahead of print].
Halmagyi GM, Carey JP. Vestibular evoked myogenic potentials – we live in interesting times. Clin Neurophysiol 2010;121:631–3.
Iwasaki S, McGarvie LA, Halmagyi GM, Burgess AM, Kim J, Colebatch JG, Curthoys IS. Head taps evoke a crossed vestibulo-ocular reflex. Neurology 2007;68:1227–9.
Iwasaki S, Chihara Y, Smulders YE, Burgess AM, Halmagyi GM, Curthoys IS, Murofushi T. The role of the superior vestibular nerve in generating ocular vestibular-evoked myogenic potentials to bone conducted vibration at Fz. Clin Neurophysiol 2009;120:588–93.
Lin CM, Wang SJ, Young YH. Ocular vestibular evoked myogenic potentials via bone-conducted vibrations applied to various midsagittal cranial sites. Otol Neurotol 2010;31:157–61.
Rosengren SM, McAngus Todd NP, Colebatch JG. Vestibular-evoked extraocular potentials produced by stimulation with bone-conducted sound. Clin Neurophysiol 2005;116:1938–48.
Rosengren SM, Todd NP, Colebatch JG. Vestibular evoked myogenic potentials evoked by brief interaural head acceleration: properties and possible origin. J Appl Physiol 2009;107:841–52.
Smulders YE, Welgampola MS, Burgess AM, McGarvie LA, Halmagyi GM, Curthoys IS. The n10 component of the ocular vestibular-evoked myogenic potential (oVEMP) is distinct from the R1 component of the blink reflex. Clin Neurophysiol 2009;120:1567–76.
Suzuki JI, Tokumasu K, Goto K. Eye movements from single utricular nerve stimulation in the cat. Acta Otolaryngol 1969;68:350–62.
Todd NP, Rosengren SM, Aw ST, Colebatch JG. Ocular vestibular evoked myogenic potentials (OVEMPs) produced by air- and bone-conducted sound. Clin Neurophysiol 2007;118:381–90.
Todd NP, Rosengren SM, Colebatch JG. Ocular vestibular evoked myogenic potentials (OVEMPs) produced by impulsive transmastoid accelerations. Clin Neurophysiol 2008;119:1638–51.
Todd NP, Rosengren SM, Colebatch JG. A utricular origin of frequency tuning to low-frequency vibration in the human vestibular system? Neurosci Lett 2009;451:175–80.
Uchino Y, Hirai N, Suzuki S. Branching pattern and properties of vertical- and horizontal-related excitatory vestibuloocular neurons in the cat. J Neurophysiol 1982;48:891–903.
Welgampola MS, Colebatch JG. Characteristics and clinical application of vestibular-evoked myogenic potentials. Neurology 2005;64:1682–8.

Paper II

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Ocular vestibular-evoked myogenic potentials (oVEMPs) in response to bone-conducted vertex vibration

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HIGHLIGHTS

- oVEMPs in response to low-frequency bone-conducted vibration are evoked symmetrically at vertex.
- oVEMPs depends on the initial stimulus motion, and a positive initial stimulus motion generated larger amplitudes and longer latencies compared to the negative motion.
- The method can be used to discriminate between healthy and lesioned ears.

ABSTRACT

Objective: To investigate low-frequency vertex bone-conducted (BC) vibration for evoking ocular vestibular myogenic potentials (oVEMPs) and its ability to discriminate between lesioned and healthy ears.
Methods: oVEMPs were analysed in response to 125-Hz single cycle vertex BC vibration in healthy subjects ($n = 50$) and in patients with severe unilateral vestibular loss ($n = 10$). Both positive and negative initial stimulus motions were used.
Results: In most healthy subjects, vertex BC vibration oVEMPs was successfully and symmetrically evoked from both ears. The response was dependent on the direction of the stimulus motion. The latency was shorter with negative initial stimulus motion; however, a positive initial stimulus motion generated somewhat larger amplitudes. Furthermore, there was no significant response from lesioned ears, whereas oVEMPs from the patients' healthy ears were similar to the responses in healthy subjects.
Conclusion: The oVEMP low-frequency BC response was dependent on the direction of the initial stimulus motion. Testing oVEMPs in response to low-frequency vertex vibration can discriminate patients with unilateral vestibular function loss from healthy controls.
Significance: Low-frequency vertex BC vibration oVEMPs should be considered a possible clinical screening test to evaluate vestibular function.

1. Introduction

Efficient and informative clinical tests to investigate vestibular function are useful for diagnosing vestibular disease. The measurement of vestibular evoked myogenic potentials (VEMPs) has made it possible to evaluate parts of the balance organs that were previously not easily accessible. Cervical VEMPs (cVEMPs) are used to evaluate the functions of the sacculus and inferior vestibular nerve (non-crossed vestibulo-collic reflex) (Rosengren et al., 2005). Recently, ocular VEMPs (oVEMPs) have been reported (Iwasaki et al., 2007; Rosengren et al., 2005; Todd et al., 2007). An oVEMP is a short-latency, biphasic, negative–positive reflex (crossed vestibulo-ocular reflex) that mainly represents vestibular functions of the utricle and superior vestibular nerve (Todd et al., 2007). oVEMP testing is therefore suggested as a complementary method to evaluate vestibular function.

There is no established consensus on an optimal procedure for testing oVEMPs and previous studies described varied stimulus conditions. oVEMPs can be evoked by air-conducted (AC) sounds or BC vibrations, but the latter are considerably more powerful (Curthoys et al., 2011; Rosengren et al., 2005). Most previous

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oVEMP studies were performed with 500-Hz BC vibrations (Chihara et al., 2009; Iwasaki et al., 2008a,b; Lin et al., 2010, 2012; Manzari et al., 2010; Rosengren et al., 2009). However, frequencies below 200 Hz are capable of evoking oVEMPs (Chihara et al., 2009; Todd et al., 2008; Zhang et al., 2012), and frequencies as low as 100 Hz are reported to be most effective for evoking oVEMPs in response to mastoid stimulation (Todd et al., 2008, 2009b; Zhang et al., 2012). It has also been shown that the direction of the initial stimulus motion affects oVEMP latency and amplitude (Cai et al., 2011; Jombik et al., 2011). The most common stimulation sites are the mastoids and the forehead midline; other midline sites (vertex, occiput) are less common.

As an anatomical stimulus site, the vertex has several clinical advantages. The test is performed with the subject in a sitting position, which makes the standard BC stimulation easy with regard to head position and stimulus direction. However, little is known about the advantage of this site for vestibular testing. Consequently, the aim of this study was to investigate the vertex as a site for clinical vestibular function testing. We investigated the abilities of different frequencies to evoke oVEMPs and the ability to discriminate between patients with unilateral vestibular function loss and healthy controls.

2. Methods

2.1. Subjects

Two groups of subjects were assessed.

The control group included 50 healthy individuals (mean age 46.8 years, range 23–63, 25 women and 25 men). According to their own statements, none of them had any history of chronic ear disease or vestibular or neurologic disorders.

The patient group comprised 10 patients with severe unilateral vestibular loss (mean age 61.6 years, range 52–70, 3 women and 7 men). One patient had undergone small acoustic neuroma extirpation. The other nine patients had received treatment for severe recurrent vertigo attacks. Seven of them were locally treated with gentamicin. Of these, five had vertigo attacks because of Ménière's disease, one had vertigo attacks related to an intralabyrinthine acoustic neuroma and one had vertigo attacks related to sudden idiopathic hearing loss. The two remaining patients with recurrent vertigo attacks suffered from Ménière's disease and had undergone labyrinthectomy. All patients submitted to post-treatment caloric and cVEMP testing, and the results suggested severe loss of vestibular function in the lesioned ear and normal vestibular function in the other ear. The patient group in this study is the same patient group reported by Westin and Brantberg (2014). The study was approved by the local ethical committees in Trondheim and Stockholm. All included individuals provided informed consent to participate in accordance with the Helsinki Declaration.

2.2. oVEMP stimulation

A commercially available handheld BC vibrator (Minishaker 4810, Brüel and Kjaer, Naerum, Denmark) was used for stimulation. It was fitted with a 55-mm long, 20-mm diameter rod made of polyoxymethylene (Delrin). The end of the rod was slightly sloped, and the surface of the flat end had a contact area of 175 mm^2. BC stimuli signals were generated by a commercially available system for evoked responses (Medelec Synergy Viasys Healthcare, Warwick, UK). The stimulus intensity was 135 dB peak-to-peak equivalent force level re 1 μN. The force was achieved from programming the Medelec to 115 dB and adding 20 dB with an amplifier (Power Amplifier Type 2718 Brüel and Kjaer). The repetition rate was 5 stimuli/s, and one stimulus sequence lasted 12.8 s. The sequence was repeated three times with a short break in between, for a total of 192 stimuli to each participant for each stimulus mode. oVEMPs were recorded in response to a 125-Hz single-cycle tone-burst (duration 8 ms) and in response to 250- and 500-Hz tone bursts with a rise–duration–fall time of 2–8–2 ms.

All subjects were tested with 125-Hz (n = 50). In addition, some of the controls were tested with 250- (n = 26) and 500-Hz (n = 5). The stimulus order was systematically alternated to avoid bias.

Positive and negative initial stimuli motions were assessed in all participants. The positive and negative initial stimuli motions were directed towards and away from the head, respectively (Fig. 1, box).

During stimulation and recording, the participant's head was orientated so that Reid's line (an imaginary line from underneath the eye to the orifice of the ear) was close to the horizontal. The subject gazed forward and upward during each test sequence. Participants were encouraged not to blink. The device was placed at the vertex and held there with no additional force applied (static force = 10 N) (Fig. 1).

2.3. oVEMP recordings

Small self-adhesive skin electrodes (Neuroline 720, Ambu and Ballerup, Denmark) were used to record responses. Two pairs of electrodes were placed underneath each eye along an imaginary vertical line underneath the pupils. The active electrode was placed in the area of the infra-orbital ridge, approximately 1 cm below the lower eyelid, and the reference electrode was placed approximately 2 cm below the active electrode. The ground electrode was placed superiorly on the sternum (Fig. 1). To minimize resistance, the skin

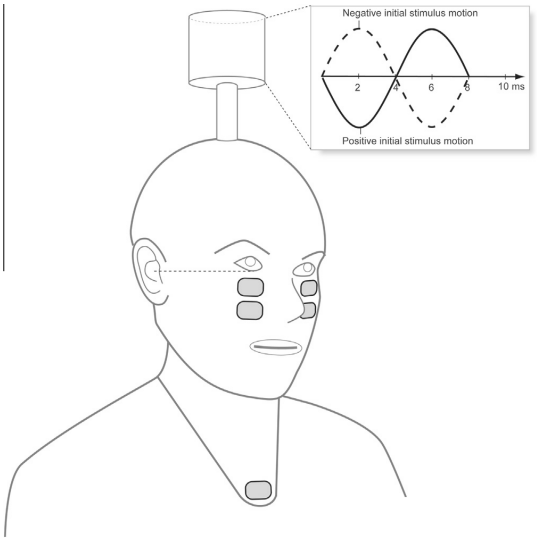

Fig. 1. The oVEMP experimental setup. Two pairs of electrodes were vertically placed under the subject's pupils, and a ground electrode was placed proximally at the sternum. During the stimulation sequence, the subject was asked to keep their eyes elevated. The head is held in a position so that Reid's line (an imaginary line from underneath the eye to the orifice of the ear [horizontal hatched line]) is close to the true horizontal. The Minishaker was placed at vertex and kept there with no additional force except the sideway support from the test personnel's hand. In the highlighted box, a single 125-Hz cycle for the positive (black line) and negative (dashed line) initial stimulus motions are shown.

was prepared by gentle rubbing with very fine sandpaper (Trace Prep, 3M Dot, Ontario, Canada) and the impedance was kept below 8 kΩ in most cases. The recorded oVEMPs were processed with a Medelec Synergy signal averager.

2.4. oVEMP analysis

For oVEMP analysis, the unrectified response was amplified and analog filtered (Bandpass 20–2000 Hz). The oVEMP latency and amplitude were measured from each individual recording. According to Rosengren's nomenclature (Rosengren et al., 2005), the first negative peak was referred to as n10, and the following positive peak was p15. The n10 and p15 latencies and the n10p15 (peak-to-peak) and n10 (baseline-to-peak) amplitudes were noted. The recordings were exported to, and analyzed by, a custom-made analysis program (Labview 8.6, National Instruments, Austin, TX, USA). Moreover, for each subject, an asymmetry ratio (AR) for oVEMP amplitude was calculated as the difference between the larger and smaller amplitudes, regardless of side, divided by the sum: AR (%) = ([amplitude$_{larger}$ – amplitude$_{smaller}$]/[amplitude$_{larger}$ + amplitude$_{smaller}$] × 100).

Receiver operating characteristic (ROC) analyses were employed to determine the AR's ability to discriminate between patients and controls.

2.5. Statistics

The four outcome variables (dependent variables) were continuous. The dataset had 7% missing data with no consecutive replacement of missing values. Q–Q plots were used to examine data distributions. The latency data were found to be normally distributed and are presented as means and standard deviations (SDs). The amplitude data and AR (%) were nearly normally distributed, and they are also presented as means with SDs.

Linear mixed models were used to compare responses within individuals in the control group and between the control and patient groups. Akaike's information criterion was used in model comparisons to identify the most parsimonious models (Chang et al., 2010). The following significant covariates were ultimately included when comparing the left and right responses in the control and patient groups: (a) for the n10 latency time, covariates none; (b) for the p15 latency time, covariate n10 latency time; (c) for the n10p15 amplitude, covariates n10 latency time and n10 amplitude; and (d) for the n10 amplitude, covariate n10 latency time. The residuals were normally distributed in all models. Two ROC curves were calculated, one for the n10p15 amplitude and one for the n10 amplitude, both with positive initial stimulus motion. Differences were considered statistically significant at $p < 0.05$. IBM SPSS statistics software (version 21) was used for the statistical analyses, except for the ROC analyses, which were performed with SigmaPlot software (version 12).

3. Results

3.1. The effect of frequency

In the control group, the response rates for positive initial stimulus motion were 50% (26/52 ears) and 0% (0/10 ears) for 250- and 500-Hz BC vibration, respectively. The values for negative initial stimulus motion were 34.6% (18/52 ears) and 20% (2/10) for 250- and 500-Hz BC vibration, respectively. Consequently, these frequencies were considered ineffective in evoking oVEMPs. The following oVEMPs results were recorded in response to 125-Hz single-cycle tone-bursts.

3.2. Control group

There was a 100% response rate for positive initial stimulus motion from both ears. For negative initial stimulus motion, the response rate was 95%; two subjects did not respond from either ear, and one subject only responded to right ear stimulation (Table 1). There were no significant differences in the control subjects with regard to latency or amplitude between the two sides (Table 2). Therefore, mean values from the right and left ears were used when comparing control and patient data. The mean n10 latencies were significantly shorter for negative initial stimulus motion than for positive initial stimulus motion ($p < 0.001$). The mean p15 latency was also shorter for negative initial stimulus motion, but the difference was not significant. The n10 to p15 time interval was not significantly affected by the direction of the initial stimulus motion. However, the amplitudes were somewhat larger for the positive initial stimulus motion (Fig. 2, Table 2).

The mean AR (%) was slightly lower for the positive initial stimulus motion compared to the negative initial stimulus motion (Table 1).

The Pearson correlation coefficients for the n10p15/n10 amplitudes in response to positive and negative initial stimulus motion were 0.934 ($p < 0.01$) and 0.976 ($p < 0.01$), respectively.

3.3. Patient group

The response rate from the patients' healthy ears was 100% for both stimulus motions. The negative initial stimulus motion caused shorter latencies, and there was a tendency toward larger amplitudes for the positive initial stimulus motion (Fig. 2, Table 3).

There were no responses in 9/10 lesioned ears (recorded from the eye contralateral to the lesioned ear). One patient weakly responded to positive but not negative initial stimulus motion.

3.4. Patients versus controls

There were no significant differences in oVEMPs between controls and the healthy ears in the patient group (Table 4).

3.5. ROC curve analysis

The areas under the ROC curves for positive initial stimulus motion were 0.92 and 0.91 for the n10p15 and n10 amplitudes, respectively. There was no significant difference between the areas ($p = 0.67$). The best threshold value was 37% for the n10p15 amplitude, resulting in a specificity of 0.88 and a sensitivity of 0.80. For the n10 amplitude, the best threshold value was 51%, yielding a specificity of 0.98 and a sensitivity of 0.70 (Fig. 3).

4. Discussion

Our results demonstrate that low-frequency vertex BC vibration successfully evokes oVEMPs. The negative initial stimulus motion shortened n10 latency compared to the positive initial stimulus motion. However, all subjects responded to the positive motion, and the amplitude showed a stronger response compared to the negative motion. We also found that the AR was able to discriminate between healthy and lesioned ears. Either the n10p15 or n10 amplitude can be used to categorize a subject's oVEMP amplitude as within or outside the normal symmetry range considering the high correlation between the two amplitude measurements. The controls had a mean AR + 2 SD of approximately 50%; therefore, when the oVEMP amplitude is at least three times larger in one ear compared to the other, then it should be considered to be outside the normal symmetry range and thus asymmetric.

Table 1
Descriptive data of the oVEMP latencies, amplitudes, and amplitude asymmetry ratios (ARs) in response to 125-Hz BC vibration at the vertex with positive and negative initial stimulus motion in the controls (n = 50). The values are the means (averages of the recordings from both ears) and the standard deviations (SDs).

Initial stimulus motion (125 Hz)	n	n10 latency (ms)	p15 latency (ms)	n10p15 amplitude m(μV)	n10 amplitude (μV)	AR (%) n10p15 amplitude	AR (%) n10 amplitude
Positive (+)	50/50	14.3 (2.4)	18.7 (2.5)	10.0 (6.2)	4.2 (2.9)	20.9 (13.9)	21.3 (15.2)
Negative (−)	47.5[a]/50	12.6 (2.1)	17.0 (2.4)	9.4 (6.9)	3.8 (3.1)	19.6 (18.6)	22.5 (21.3)

[a] There were 48 oVEMP responses from the right ear and 47 from the left ear, yielding a mean value of 47.5.

Table 2
The oVEMP results from the left and right sides and the two different directions of the initial stimulus motion in the control group. These data were analyzed in a linear mixed model (LMM) to evaluate differences between the two sides (p side) and the effect difference in stimulus direction (p direction). *The direction of the initial stimulus motion affects the latency of the first peak (n10), and therefore the latency of the second peak (p15). This p value indicates that the n10 to p15 time interval is not affected by stimulus motion.

	n10 latency (ms)	p side	p direction	p15 latency (ms)	p side	p direction	n10p15 amplitude (μV)	p side	p direction	n10 amplitude (μV)	p side	p direction
Left +	14.2			18.6			4.4			10.3		
Right +	14.4			18.8			3.9			9.7		
		0.795	<0.001		0.222	0.809*		0.465	0.949		0.179	0.922
Left −	12.7			17.0			3.9			9.5		
Right −	12.7			17.2			3.6			9.4		

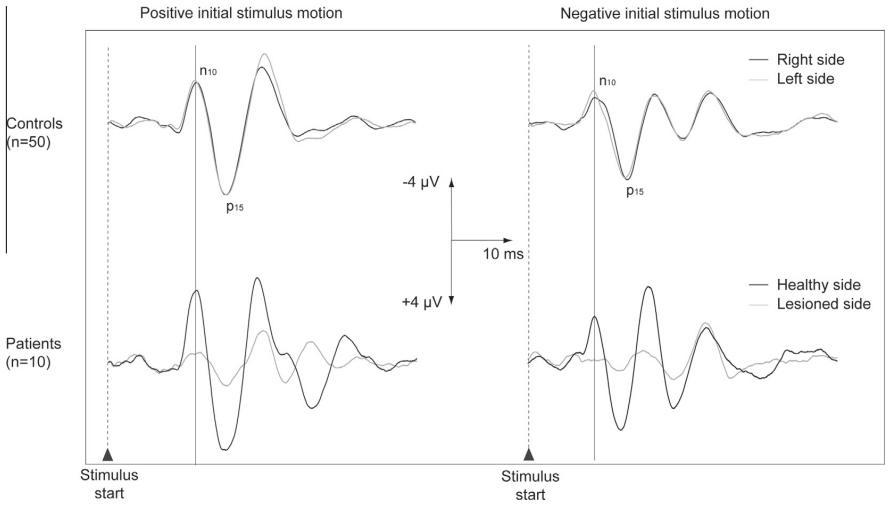

Fig. 2. Grand mean oVEMP for the 50 controls and 10 patients with univestibular loss after positive and negative initial stimulus motion, respectively. For the control subjects, the black and gray lines are the responses recorded from the right and left eyes, respectively. For the patients, the black and gray lines indicate the crossed responses from the healthy and lesioned ears, respectively (i.e. recorded from the eye opposite the healthy or lesioned ear).

Table 3
Descriptive data of the oVEMP latencies and amplitudes from the patients' healthy ears (n = 10) in response to 125-Hz BC vibration. The values are the means (SDs).

Initial stimulus motion (125 Hz)	Side	n	n10 latency (ms)	p15 latency (ms)	n10p15 amplitude (μV)	n10 amplitude (μV)
Positive (+)	Healthy side	10/10	14.7 (1.2)	19.3 (1.8)	13.2 (8.5)	5.5 (3.7)
Negative (−)	Healthy side	10/10	11.3 (1.2)	15.4 (1.8)	8.4 (6.1)	3.3 (2.1)

ROC analysis of the oVEMP amplitudes may also support a threshold value of approximately 50%.

4.1. Why vertex as stimulus site?

Clinical oVEMP testing in response to BC vibration is a new and fast evolving field. At present there is no consensus regarding the optimal stimulus site. Most prior reports concern oVEMP in response to mastoid stimulation or at different midline sites, especially Fz. Compared with other midline sites, vertex have both cons and pros. Vertex has been reported to cause oVEMP with lower amplitude compared with Fz (Lin et al., 2010). However, BC vibration at vertex is easy to standardize with regard to both stimulus direction and head position.

Table 4
The mean oVEMP results from the left and right sides in the control group with the healthy side in the patient group. Data are analyzed and presented as in Table 2.

	n10 latency (ms)	p case/ control	p direction	p15 latency (ms)	p case/ control	p direction	n10p15 amplitude (μV)	p case/ control	p direction	n10 amplitude (μV)	p case/ control	p direction
Control +	14.3			18.7			4.2			10.0		
Case +	14.7			19.3			5.5			13.2		
		0.431	0.001		0.944	0.700*		0.977	0.881		0.979	0.783
Control −	12.6			17.0			3.8			9.4		
Case −	11.3			15.4			3.3			8.4		

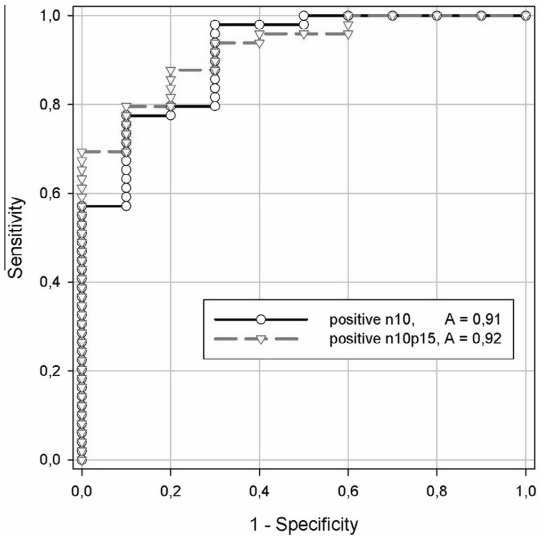

Fig. 3. The receiver operating characteristic (ROC) curves for the n10p15 and n10 amplitudes for positive initial stimulus motion in 50 controls and 10 patients.

In a seated patient with the head in a neutral position, it is simple to apply a standardized vertex BC stimulus. The Minishaker is easily placed at the stimulus site, and, although vertex is a curved elevation, the Minishaker can be held in position with just sideway support. The static BC stimulus force will thus merely depend on the Minishaker mass. The applied BC stimulus direction will also be in approximately the same direction as the gravity force vector. In addition, data are available from mastoid accelerometer recordings which have suggested that this low-frequency vertex stimulation cause labyrinthine acceleration in the same principal directions as if stimulus was applied at the contralateral mastoid (Westin and Brantberg, 2014).

Further, a well standardized head position during oVEMP testing seems justified, considering that the optimal head position during oVEMP testing is neither known in detail, nor has been unambiguously settled. Different head position causes not only variation in the spatial orientation of the utricular macula, but there is also modulation of intracranial pressure. Several recent studies have also pointed out alterations in oVEMP related to head position/intracranial pressure, a research area that is getting a lot of attention currently (Gurkov and Kantner, 2013; Iwasaki et al., 2012; Jerin and Gürkov, 2014; Taylor et al., 2014).

4.2. Vertex compared to other BC stimulus sites

oVEMPs due to vertex vibration exhibit longer latencies and smaller amplitudes compared to stimulation at other midline stimulation sites (Lin et al., 2010). This is most likely explained by anatomical properties: the vertex is the midline site that is usually furthest away from the labyrinth (Lin et al., 2010), and soft tissue on top of the skull and hair can distort the stimulation (Stenfelt and Goode, 2005). The occiput is the midline site closest to the labyrinth; however, a closer proximity does not necessarily lead to a shorter oVEMP latency because forehead vibration causes an earlier oVEMP than occiput stimulation (Lin et al., 2010). This could be related to its higher bone density and better conduction properties (Lin et al., 2010). Stimulation at the mastoids evokes oVEMPs with large amplitudes (Tseng et al., 2011). This is likely due to its optimal stimulus direction relative to the horizontally oriented utricle (Todd et al., 2008, 2009b), but some saccular contribution is not ruled out (Colebatch, 2010). A disadvantage of mastoid stimulation is that the test has to be performed on each side. Conversely, one stimulation sequence applied to the midline might be sufficient to evaluate both balance organs (Lin et al., 2010; Stenfelt and Goode, 2005).

4.3. Stimulus direction dependency

The oVEMP is sensitive to the initial stimulus motion direction, particularly with respect to latency. The present findings suggest that vertex vibration with a positive initial stimulus motion results in a longer latency than a negative stimulus motion. Furthermore, the latency was consistent with that reported by other studies in which an initially BC positive stimulus was applied at the vertex (Lin et al., 2010; Taylor et al., 2014; Westin and Brantberg, 2014). The overall response was also somewhat larger and more consistent with positive initial stimulus motion than with negative. A positive initial stimulus motion has also previously been suggested to be favorable for evoking oVEMPs, irrespective of the stimulation site (Cai et al., 2011).

4.4. AR

The AR is a continuous variable. The threshold value set to discriminate between patients and controls (binary classification) affects the number of false-positive and -negative results; thus, threshold value is a tradeoff, and clinical evaluation of the consequences of classification errors must be performed. ROC analysis is well suited to quantify errors expected at different threshold values. Manzari et al. reported that an AR threshold value of 46.5% was best to discriminate between normal and abnormal individuals when performing an ROC curve analysis of the n10 amplitude following 500-Hz BC vibration applied to the forehead (Manzari et al., 2010). In the presented study, ARs of 37% from the n10p15 amplitude and 51% from the n10 amplitude were the best threshold values; these are in line with the results of Manzari et al. However, our threshold values should be regarded as estimates because the data were collected from a relatively small study population.

4.5. Accelerations

The direction of head acceleration follows the direction of BC stimulus for frequencies <400 Hz (Jombik et al., 2011; Stenfelt

and Goode, 2005). It was also recently reported that the direction of measured labyrinthine accelerations were the same for BC stimulus applied at the vertex and at the contra-lateral mastoid (Westin and Brantberg, 2014). However, the amplitude of the accelerations was smaller for vertex compared with mastoid stimulation.

The head accelerations from the initial motion of the BC stimulus seem to determine the activation of macular haircells in the utricle (Lin et al., 2012). A positive initial stimulus motion applied to the vertex or contra-lateral mastoid causes initial lateral accelerations of the utricle, whereas a negative initial stimulus motion causes initial medial accelerations (Jombik et al., 2011; Westin and Brantberg, 2014). Further, the oVEMP latency for initial lateral acceleration is longer compared with initial medial acceleration. In contrast, the reverse occurs following forehead stimulation, that is, positive and negative initial stimulus causes shorter or longer oVEMP latency, respectively (Cai et al., 2011; Jombik et al., 2011; Lin et al., 2012). This phenomenon is most likely due to activation of oppositely oriented utricular hair cells (Cai et al., 2011; Jombik et al., 2011).

4.6. Frequency specificity

This study suggests that a 125-Hz vertex stimulus frequency could be useful to evoke oVEMPs in a clinical setting. The 250- and 500-Hz vertex stimulus frequencies were ineffective in consistently generating oVEMPs. The reason for this frequency dependency might be at least partly related to anatomical features. The utricle is only attached to the temporal bone in its rostral area; the majority of it rests on a thin membrane over the endolymph (Curthoys et al., 2009). The construction of the utricle is such that it responds well to a low frequency (Todd et al., 2009a), and its resonance has been described to be around 100 Hz (Todd et al., 2008, 2009a). Several studies have reported 100 Hz as the most effective stimulation frequency for utricular activation (Todd et al., 2008, 2009a; Zhang et al., 2012).

4.7. Patient group

In the patient group, there were seven participants who were diagnosed with Ménière's disease and treated with gentamicin, and none of them exhibited residual function in the lesioned ear after caloric stimulation or cVEMP testing. However, one of those patients had oVEMPs from both ears after positive initial stimulation. This suggests that there was still some residual function in the lesioned ear, but this only became apparent after positive initial stimulus motion.

4.8. Future

Additional studies with larger patient populations and various inner ear diagnoses are warranted to provide more graded information about different AR threshold values. Further, more basic knowledge about vertex BC vibration is needed, and future studies should evaluate test repeatability and to what extent oVEMPs are affected by small displacements of the stimulus rod.

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References

Cai KY, Rosengren SM, Colebatch JG. Cervical and ocular vestibular evoked myogenic potentials are sensitive to stimulus phase. Audiol Neurootol 2011;16:277–88.
Chang CM, Cheng PW, Wang SJ, Young YH. Effects of repetition rate of bone-conducted vibration on ocular and cervical vestibular-evoked myogenic potentials. Clin Neurophysiol 2010;121:2121–7.
Chihara Y, Iwasaki S, Fujimoto C, Ushio M, Yamasoba T, Murofushi T. Frequency tuning properties of ocular vestibular evoked myogenic potentials. Neuroreport 2009;20:1491–5.
Colebatch JG. Sound conclusions? Clin Neurophysiol 2010;121:124–6.
Curthoys IS, Uzun-Coruhlu H, Wong CC, Jones AS, Bradshaw AP. The configuration and attachment of the utricular and saccular maculae to the temporal bone. New evidence from microtomography-CT studies of the membranous labyrinth. Ann N Y Acad Sci 2009;1164:13–8.
Curthoys IS, Vulovic V, Burgess AM, Cornell ED, Mezey LE, Macdougall HG, et al. The basis for using bone-conducted vibration or air-conducted sound to test otolithic function. Ann N Y Acad Sci 2011;1233:231–41.
Gurkov R, Kantner C. Modulation of oVEMP amplitudes by lateral head tilts. Clin Neurophysiol 2013;124:1911–2.
Iwasaki S, Chihara Y, Egami N, Fujimoto C, Murofushi T, Yamasoba T. Different effects of head tilt on ocular vestibular-evoked myogenic potentials in response to bone-conducted vibration and air-conducted sound. Exp Brain Res 2012;223:389–96.
Iwasaki S, McGarvie LA, Halmagyi GM, Burgess AM, Kim J, Colebatch JG, et al. Head taps evoke a crossed vestibulo-ocular reflex. Neurology 2007;68:1227–9.
Iwasaki S, Smulders YE, Burgess AM, McGarvie LA, Macdougall HG, Halmagyi GM, et al. Ocular vestibular evoked myogenic potentials in response to bone-conducted vibration of the midline forehead at Fz. A new indicator of unilateral otolithic loss. Audiol Neurootol 2008a;13:396–404.
Iwasaki S, Smulders YE, Burgess AM, McGarvie LA, Macdougall HG, Halmagyi GM, et al. Ocular vestibular evoked myogenic potentials to bone conducted vibration of the midline forehead at Fz in healthy subjects. Clin Neurophysiol 2008b;119:2135–47.
Jerin C, Gürkov R. Posture-induced changes of ocular vestibular evoked myogenic potentials suggest a modulation by intracranial pressure. Exp Brain Res 2014;232:2273–9.
Jombik P, Spodniak P, Bahyl V. Direction-dependent excitatory and inhibitory ocular vestibular-evoked myogenic potentials (oVEMPs) produced by oppositely directed accelerations along the midsagittal axis of the head. Exp Brain Res 2011;211:251–63.
Lin CM, Wang SJ, Young YH. Ocular vestibular evoked myogenic potentials via bone-conducted vibrations applied to various midsagittal cranial sites. Otol Neurotol 2010;31:157–61.
Lin KY, Wang SJ, Young YH. Influence of head acceleration on ocular vestibular-evoked myogenic potentials via skull vibration at Fz versus Fpz sites. Int J Audiol 2012;51:551–6.
Manzari L, Tedesco A, Burgess AM, Curthoys IS. Ocular vestibular-evoked myogenic potentials to bone-conducted vibration in superior vestibular neuritis show utricular function. Otolaryngol Head Neck Surg 2010;143:274–80.
Rosengren SM, McAngus Todd NP, Colebatch JG. Vestibular-evoked extraocular potentials produced by stimulation with bone-conducted sound. Clin Neurophysiol 2005;116:1938–48.
Rosengren SM, Todd NP, Colebatch JG. Vestibular evoked myogenic potentials evoked by brief interaural head acceleration: properties and possible origin. J Appl Physiol 2009;107:841–52.
Stenfelt S, Goode RL. Transmission properties of bone conducted sound: measurements in cadaver heads. J Acoust Soc Am 2005;118:2373–91.
Taylor RL, Blaivie C, Bom AP, Holmeslet B, Pansell T, Brantberg K, et al. Ocular vestibular-evoked myogenic potentials (oVEMP) to skull taps in normal and dehiscent ears: mechanisms and markers of superior canal dehiscence. Exp Brain Res 2014;232:1073–84.
Todd NP, Rosengren SM, Aw ST, Colebatch JG. Ocular vestibular evoked myogenic potentials (OVEMPs) produced by air- and bone-conducted sound. Clin Neurophysiol 2007;118:381–90.
Todd NP, Rosengren SM, Colebatch JG. Tuning and sensitivity of the human vestibular system to low-frequency vibration. Neurosci Lett 2008;444:36–41.
Todd NP, Rosengren SM, Colebatch JG. A utricular origin of frequency tuning to low-frequency vibration in the human vestibular system? Neurosci Lett 2009a;451:175–80.
Todd NP, Rosengren SM, Govender S, Colebatch JG. Low-frequency tuning in the human vestibular-ocular projection is determined by both peripheral and central mechanisms. Neurosci Lett 2009b;458:43–7.
Tseng CC, Wang SJ, Young YH. Comparison of bone-conducted vibration for eliciting ocular vestibular-evoked myogenic potentials: forehead versus mastoid tapping. Otolaryngol Head Neck Surg 2011;146:289–94.
Westin M, Brantberg K. Mastoid and vertex low-frequency vibration-induced oVEMP in relation to medially directed acceleration of the labyrinth. Clin Neurophysiol 2014;125:615–20.
Zhang AS, Govender S, Colebatch JG. Tuning of the ocular vestibular evoked myogenic potential to bone-conducted sound stimulation. J Appl Physiol 2012;112:1279–90.

Paper III

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