MODULATION OF VESTIBULAR-EVOKED REFLEXES AND OCULOMOTOR FUNCTION WHEN STANDING UNDER HEIGHT-INDUCED STATES OF FEAR, ANXIETY AND AROUSAL

by

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Abstract

As an important source of information for postural control, the Vestibular System may contribute to anxiety-related effects on balance control during stance and gait, particularly through increases in the vestibulospinal reflex (VSR) gain. While vestibulo-ocular reflex (VOR) gain has been associated with chronic anxiety, it is unclear if VOR and VSR gains are also sensitive to acute threat-related changes in fear, anxiety and arousal. Vestibular Evoked Myogenic Potentials (VEMPs) and Head Impulse Tests (HIT) can be used to test the gain of VSR and VOR pathways. Having subjects stand at the edge of an elevated platform can be used to threaten standing balance and induce arousal, anxiety and fear related to falling; known as a height-induced postural threat. The first aim of this thesis was to investigate how postural threat-related changes in arousal, anxiety and fear influence VEMP and HIT outcomes. Since the VOR depends also on visual pathways receiving signals relating to visual field motion and eye movements, a second study was designed to examine the independent effect of postural threat on oculomotor function using eye saccades, smooth pursuit and optokinetic nystagmus. For the first time, VEMPs were simultaneously recorded while standing from different muscles representing the three distinct vestibular reflexes. Likewise, this thesis is the first to investigate functional VOR and oculomotor outcomes with changes in state anxiety.

The results from both studies provide robust evidence for increased VSR and VOR gain with acute negative emotional states. Furthermore, the observed increased gain of oculomotor function suggests that part of the VOR modulation occurs in neural centres not related to the vestibular system. These observations not only shed a light on how the VOR and gaze control are

affected by state anxiety, fear and arousal, confirming previous reports on the VSR, but have also shown how emotions could alter the outcomes of clinical tests commonly used for assessing the vestibular and oculomotor function.

Preface

The experimental procedures used in the studies included in this thesis were reviewed by The University of British Columbia Clinical Research Ethics Board (UBC CREB# H06-70316). All subjects provided written informed consent prior to participation in these experiments and every effort has been made to ensure that those subjects are not identified in this thesis.

I was the lead investigator of the two studies conducted, responsible for experimental design, data collection, data analyses and manuscript composition. The concept of the thesis was developed together with my program supervisor, Dr. Mark G. Carpenter, and Prof. John H.J. Allum. Along with Prof. J. Timothy Inglis, they both advised throughout the whole process, being all supervisory authors of the project. Mr. Taylor Cleworth developed the algorithms applied for analyzing the data from the three experiments, with the advice of Prof. Allum.

The studies included in this thesis have not been submitted for publication at the time of the document completion.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ACQEM:	Anti-compensatory quick eye movements
ACS:	Air conducted sound
ASSC:	Anterior semicircular canal
BGA:	Background activity
cVEMP:	Cervical vestibular evoked myogenic potential
EDA:	Electrodermal activity
EMG:	Electromyography
EOG:	Electro-Oculography
EVAR:	Earth vertical axis rotation
GVS:	Galvanic vestibular stimulation
HcVEMP:	High-cervical vestibular evoked myogenic potential
HIT:	Head impulse test
HSSC:	Horizontal semicircular canal
HVOR:	Horizontal vestibulo-ocular reflex
IO:	Inferior oblique
LARP:	Left anterior /right posterior
LcVEMP:	Low-cervical vestibular evoked myogenic potential
legVEMP:	Leg vestibular evoked myogenic potential
LVN:	Lateral vestibular nucleus
LVST:	Lateral vestibulospinal tract
MLF:	Medial longitudinal fasciculus

MVN:	Medial vestibular nucleus
MVST:	Medial vestibulospinal tract
OKN:	Optokinetic nystagmus
OMF:	Oculomotor function
OVAR:	Off-earth vertical axis rotation
PSSC:	Posterior semicircular canal
ptp:	Peak-to-peak
RALP:	Right Anterior /left posterior
RMS:	Root mean square
SCM:	Sternocleidomastoid
SD:	Standard Deviation
SOL:	Soleus
SPL:	Sound pressure level
SSC:	Semicircular canal(s)
STB:	Short tone bursts
SVS:	Stochastic vestibular stimulation
TRP:	Trapezius
VCR:	Vestibulocollic reflex
VEMP:	Vestibular Evoked myogenic potential
vHIT:	Video Head impulse test
VOR:	Vestibulo-ocular reflex
VSR:	Vestibulospinal reflex
VVOR:	Vertical vestibulo-ocular reflex

Acknowledgements

This document represents the story of how I decided to start again, to pursue a dream long time due. I arrived in Vancouver in the summer of 2012, full of excitement upon the new turn my life was about to take, but full of fear too. Here I found excellent persons that not only made my adaptation easy, but that guided me through all the necessary steps in order to achieve one of the most important accomplishments in my life. I would like to thank everyone that helped me during this process.

First of all, thanks to the members of my committee. Dr. Carpenter, you believed in me and made this enterprise possible: thanks for your generosity, your kindness and your guiding hand. Prof. Inglis, thanks for being there every time I needed your expertise, for the good vibrations you gave me since the first time we met, for your contagious enthusiasm. And Prof. Allum, thanks for your help and warmness, always accessible and willing to share your vast knowledge.

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Thanks to all my fellow students in the Neural Control of Posture and Movement Laboratory: Taylor (I am so, so grateful for your help and hard work during the last two years: you are a genius), Brian, Eveline, Shannon, Jordan, Chantelle, Adam. Thanks for all your help and for the

good times we shared together. Also to all the current and former graduate students in the School of Kinesiology of UBC that at some point shared the path with me.

Finally, thanks to my lovely family. To my partner in this adventure, Myriam: you supported this dream through your daily sacrifice and made it with the best of your smiles. I love you. To my precious kids, Eduardo and Jaime, the reason of everything. To my dear parents for their understanding and unconditional love.

Thanks so much!

'The fear thou art in prevents thee from seeing or hearing correctly, for one of the effects of fear is to derange the senses"

Don Quixote, Miguel de Cervantes, 1605

For Myriam, Eduardo and Jaime

Chapter 1: Introduction

Highly threatening situations evoke negative emotional changes, such as fear and anxiety, and augment vigilance, expressed by an increase in autonomic arousal (Adkin et al., 2000, Oken et al., 2006). These changes are known to affect postural control (Adkin et al., 2000; Carpenter et al., 2001; Davis et al., 2009) and could explain why vulnerable populations, such as the elderly, are more likely to fall when they feel anxious or fearful (Maki et al., 1991). Falls have a strong prevalence in elderly populations (Blake et al., 1988). Therefore, it is critical to achieve a better understanding of the mechanisms that underlie this phenomenon in order to design more effective prevention strategies.

The threat of falling from height, by placing a subject at the edge of an elevated surface, has proven effective for inducing fear and anxiety, and therefore, can be used to study how these factors affect changes in postural control (Adkin et al., 2000; Carpenter et al., 2001; Davis et al., 2009). This experimental scenario is commonly referred to as a height-induced postural threat. Subjects typically demonstrate a smaller amplitude and a higher frequency of center of pressure displacements as postural threat is increased from relatively low to high surface heights (Carpenter et al., 1999; Adkin et al., 2000). In these conditions a significant increase in perceived fear and anxiety are reported, as well as a decrease in confidence in maintaining balance and perceptions of stability. These emotional effects are often accompanied by an increase in autonomic arousal (Adkin et al., 2000; Davis et al., 2009, Horslen et al., 2013).

The height paradigm was applied in several studies to investigate the effects of postural threat on different sensory systems that are involved in postural threat responses. When the proprioceptive system was explored though this paradigm, a higher sensitivity in the muscle spindles during states of fear and anxiety was suggested through a significant increased gain in the tendon tap reflex, but not in the H Reflex (Horslen et al., 2013), whereas no change in afferent feedback to the cortex was observed (Davis et al., 2011). Regarding the visual system, a high prevalence of visual height intolerance has been described (Brandt and Huppert, 2014) when subjected to similar threatening scenarios. However, little is known about the role of the vestibular system, a main contributor to postural control, in threat-induced changes to postural control. While some studies have shed light on the effects of threat on vestibular-evoked postural responses (Osler et al., 2013; Horslen et al., 2014) and vestibulospinal reflexes (Naranjo et al., 2015), the extent to which other vestibular reflexes or pathways are affected by threat is still not clear.

1.1 Neuroanatomy and neurophysiology of the vestibular system: an overview

The vestibular system is one of the main sources of information used in balance control, and is part of a complex sensory-motor organization that involves communication between the vestibular receptors, eye muscles, postural muscles, the somatosensory and visual systems, brainstem, cerebellum and the cortex. We may distinguish between the peripheral and the central vestibular system. The peripheral vestibular apparatus is found in the bony labyrinth of the inner ear in the temporal bone. The labyrinth consists of the three semicircular canals (SSC) (anterior, posterior and horizontal), the vestibule and the cochlea (where the auditory component of the inner ear is located). Suspended within the bony labyrinth is a membranous labyrinth that

contains a fluid named the endolymph and the vestibular organs. The SSC are formed by the semicircular ducts, having each at its base a widened part called the ampulla. Each SSC is oriented in a different plane for detecting the angular accelerations that arise from the tridimensional head movements. The otolith organs (the utricule and the saccule) located in the vestibule detect linear accelerations of the head. The utricle is oriented in a nearly horizontal plane, while the saccule is oriented more vertically. Head movements are detected by a specialized epithelium within the membranous labyrinth that contains hair cells that are sensitive to lateral bending, and have slightly different features for both types of receptors (Purves et al., 2001).

The sensory epithelium within the ampulla of each SSC is called the crista. The hair cells are organized in bundles inside a gelatinous structure called the cupula. Inertial drag, from head movement in the appropriate plane, makes the endolymph lag and press against the cupula, causing it to bend and therefore displacing the hair cells. Bending of the hair cells cause the opening of ion channels to depolarize the cell and thereby activate the attached afferents. The information originating from both vestibular apparati is integrated centrally, where excitation of canals on one side is mirrored with decreased activation on the opposite side of the head, coding for angular velocities and accelerations of the head. In the otolith receptors, the sensory epithelium where the hair cells are located is called the macula, which is covered by a layer of calcium carbonate crystals known as otoconia, that is imbedded in a jello like membrane, the otolithic membrane. When the head moves linearly, the translation of the otolithic membrane due to inertial lag causes shear forces that mechanically activate the hair cells and increase firing. Again, the information from both apparati is integrated to code for linear velocities and

accelerations of the head. Due to its orientation, the saccule codes for linear accelerations in the vertical and antero-posterior directions and the utricule codes for linear accelerations in the antero-posterior and medio-lateral directions (Purves et al., 2001).

1.1.1 Primary vestibular afferents and central processing of vestibular reflexes

The information from the peripheral vestibular apparatus is projected to the central vestibular system through the vestibular afferents, through the vestibular nerve. This nerve is a branch of cranial nerve VIII or vestibulocochlear nerve, with the cell bodies located in the vestibular or Scarpa's ganglion, and has two different divisions: inferior and superior. The central vestibular system is located in the brainstem. It is formed by the vestibular nuclei, located within the pons and extending caudally into the medulla, and, although receiving bilateral inputs, integrates the information originating mainly from the ipsilateral peripheral vestibular apparatus. There are four vestibular nuclei on each side: lateral (or Deiters' nucleus) (LVN), medial (MVN), superior (SVN) and inferior vestibular nuclei (IVN). Each vestibular nucleus receives projections originating from all ipsilateral and contralateral vestibular receptors (Blumenfeld, 2002; Uchino and Kushiro, 2011). The vestibular nuclei neurons drive the different vestibular reflexes necessary to compensate for movements of the head. There are three vestibular reflexes processed at the central vestibular system: the vestibulospinal reflex (VSR), that generates automatic postural adjustments of the body to head movements, the vestibulocollic reflex (VCR), that activates neck muscles to maintain the head stabilized, and the vestibulo-ocular reflex (VOR), that counteracts head movements for gaze stabilization (Highstein and Holstein, 2006) (Figure 1).

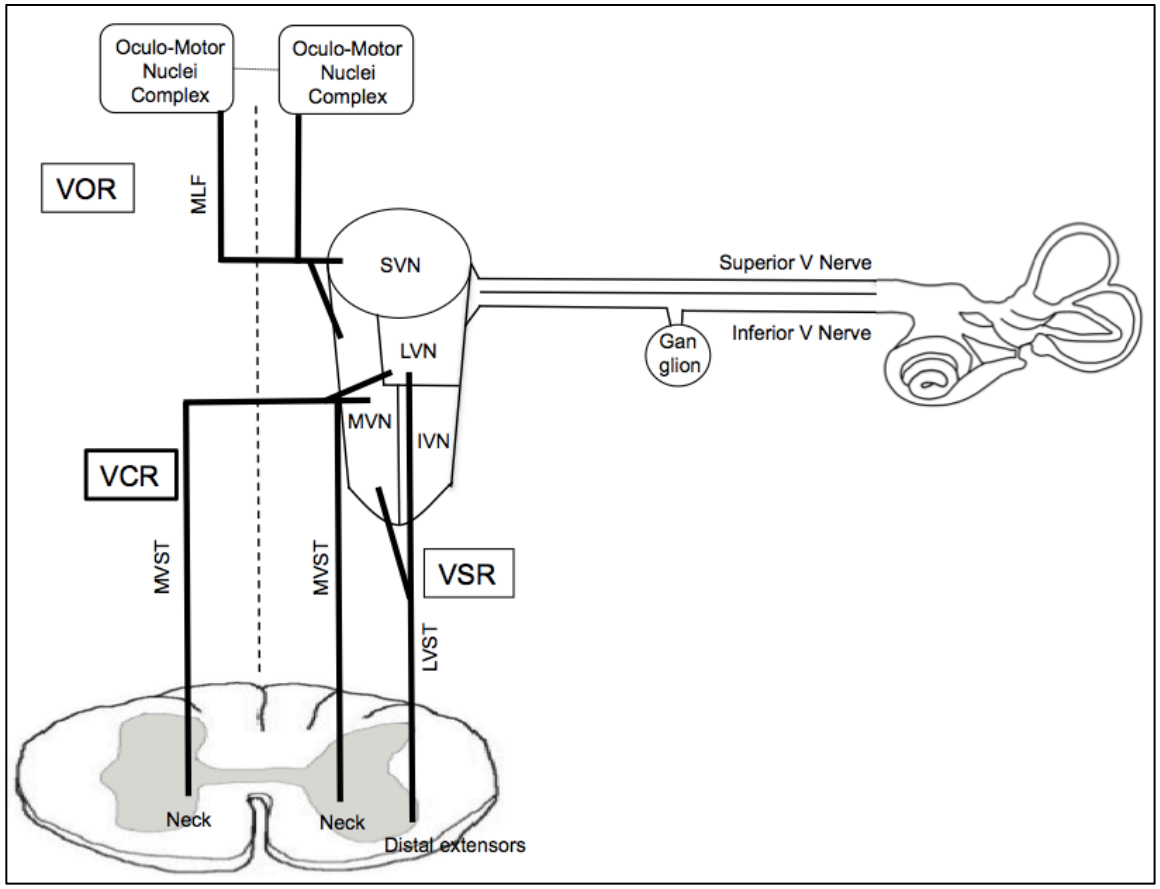

Figure 1. Pathways of the vestibular reflexes. Information from the peripheral vestibular apparatus is carried to the vestibular nuclei complex in the brainstem, with the superior vestibular nucleus (SVN), inferior vestibular nucleus (IVN), lateral vestibular nucleus (LVN) and medial vestibular nucleus (MVN). The vestibular reflexes are then processed in different vestibular nuclei and projected through different pathways: the vestibulo-ocular reflex (VOR) is projected via the medial longitudinal fasciculus (MLF) to the oculo-motor nuclei complex, the vestibulocollic reflex (VCR) is projected via the ipsilateral and contralateral medial vestibulospinal tracts (MVST) to the proximal motorneuron pools at the cervical segment of the spinal cord, and the vestibulospinal reflex (VSR) is projected via the ipsilateral lateral vestibulospinal tracts (LVST) to the distal motorneuron pools in the spinal cord with potential contributions from the reticulospinal tract.

The VSR and the VCR mainly originate in the LVN and the MVN. The VSR projects ipsilaterally from the vestibular nuclei via the lateral vestibulospinal tracts (LVST) to the

motorneuron pools of the distal limb muscles, and bilaterally to the motorneuron pools of the neck and trunk muscles via the medial vestibulospinal tract (MVST). There are also contributions from the reticulospinal vestibular pathways in this reflex. VSR are used to generate postural adjustments to compensate for head movements. The axons from the vestibulospinal tract excite the motorneurons of the extensor muscles, inhibiting the flexor muscles. As a result of this projection there is a strong tonic input to the extensor antigravity muscles. The VCR project bilaterally, decussating at the brainstem level, via the MVST to the ipsilateral and contralateral motorneuron pools in the upper cervical segment that control the neck muscles responsible for stabilization of the head (Purves et al., 2001; Blumenfeld, 2002). For the purpose of this thesis, and according to the descending projections to the spinal cord, the VCR will be considered as a type of VSR.

The VOR is a three-neuron arc with synapses at the vestibular nuclei (mainly medial and superior, depending on the vestibular receptor activated) and the oculomotor nuclei complex, which include 3 different brainstem nuclei (oculomotor, abducens and trochlear) responsible for controlling the muscles of the eye. Depending on the direction of the head movement, and therefore the receptors activated, different extra-ocular muscles will be activated or inhibited in order to maintain gaze fixation. In the event of a horizontal rotation, the MVN receives rotation signals originating from the horizontal SSC (HSSC). Neurons stemming from the MVN project to both the contralateral abducens nucleus and the ipsilateral oculomotor nucleus. In the abducens nucleus, motorneurons projecting directly to the lateral rectus muscle of the eye are excited and interneurons that inhibit the oculomotor nucleus (same side as the abducens) are activated, causing de-activation of the medial rectus muscle. The ipsilateral oculomotor nucleus

activates the medial rectus muscle on its side and uses inhibitory projections to the abducens nucleus to de-activate the lateral rectus (Blumenfeld, 2002). This way, a leftward rotation of the head in the yaw plane will excite the right lateral rectus and the left medial rectus, and will inhibit the left lateral rectus and the right medial rectus. Different pathways are involved in the vertical VOR resulting from head displacements in the pitch plane. When the head is rotated in the pitch plane, angular acceleration information is obtained from both the anterior (ASSC) and posterior (PSSC) semicircular canals. Furthermore, changes in angular position are registered by the otoliths due to a rotation in the gravity field. This information is projected to the vestibular nuclei, and from there to the oculomotor nuclei that activate the superior recti and inhibit the inferior recti in a downward rotation, or the opposite in an upward rotation, to maintain the gaze. The torsion of the eye is controlled in both directions by the superior oblique (receiving projections from the trochlear nucleus) and the inferior oblique (Uchino and Kushiro, 2011).

1.1.2 Other projections from the central vestibular system

Other outputs from the vestibular nuclei include projections to the cerebellum, the thalamus, the cortex and the medial pontomedullary reticular formation. The latter, known to participate in postural adjustments (Prentice and Drew, 2001), sends projections to the motorneurons of proximal muscles via the reticulospinal tracts and is considered to modulate the VCR and VSR, generating additional tonic activation or inhibition of the extensor muscles (Barnes, 1984).

1.1.3 Potential modulatory inputs to the vestibular system

The vestibular nuclei receive strong inputs from the cerebellum. Due to the interaction between the vestibular nuclei and cerebellum, via the vestibular-cerebellar tract, the cerebellum is considered the adaptive processor of the vestibular system, monitoring performance and readjusting the central vestibular processing (Hain and Helminski, 2007). These connections allow the central vestibular system to also integrate information originating from proprioceptive and visual inputs via the cerebellum (Krebs et al., 2012). The vestibular nuclei receive inputs directly from ascending somatosensory pathways originating mainly from skin receptors and type II muscle afferents that are not relayed through the cerebellum (Krebs et al., 2012).

Recent work has shown how multisensory visuo-vestibular cortical areas may exert an influence on the VOR when engaging subjects in visuo-spatial tasks, suggesting a role of cortical inputs in the modulation of vestibular reflexes (Bronstein et al., 2015).

It has been demonstrated that the peripheral vestibular receptors receive innervation from efferent cell groups located in the brainstem immediately lateral to the abducens nucleus (Rasmussen and Gacek, 1958). Although it was thought that this efferent system could modulate the resting firing discharge of hair cells during active head movements, studies on animal models showed a similar activity in the afferents when comparing active and passive head rotations (Cullen and Minor, 2002), leaving the role of these vestibular efferents still unknown.

Neuroanatomical evidence with animal models has also shown strong excitatory inputs to the vestibular nuclei from neural regions involved in processing emotional and affective responses. It has been postulated (Balaban and Thayer, 2001; Balaban, 2002; Staab et al., 2013) that the parabrachial nucleus, which processes convergent vestibular, somatic, and visceral information to mediate avoidance conditioning, anxiety and conditioned fear responses, projects to the medial, inferior and superior vestibular nuclei. The vestibular nuclei receive noradrenergic projections from the locus coeruleus, via the coeruleo-vestibular pathway, and serotonergic projections from the dorsal raphe nucleus and the nucleus raphe obscurus (Halberstadt and Balaban, 2003). These neural connections between centers responsible for processing emotional responses and the vestibular nuclei, could explain the influence that emotions have on postural control and how the dynamics of the vestibular reflexes could be affected during threatening conditions.

It is well known how histamine mediates fear, anxiety and arousal responses (Dere et al., 2010). Other projections from histaminergic neurons located mainly in the hypothalamus to the medial and inferior vestibular nuclei have been described (de Waele et al., 1992; Serafin et al., 1993; Yabe et al., 1993; Peng et al., 2013), causing excitation of those nuclei by activation of H1 and H2 receptors.

1.2 Vestibular contributions to negative emotional changes: body of evidence

Most of the studies that examined the influence of fear and anxiety on the vestibular system have focused on people with chronic disorders, such as chronic anxiety (Furman et al., 2006), which

are also known to have a clear predisposition to balance deficits. However, it is difficult to control for anxiety levels when sampling from these populations. Also, transient states of fear, anxiety and arousal are more likely to be present as risk factors that facilitate falls in otherwise healthy subjects (Maki et al., 1991). Other experiments induced mental stress and arousal through mental arithmetic (Yardley et al., 1995) or sleep deprivation that led to increased anxiety (Collins, 1988; Quarck et al., 2006), but again the levels of anxiety generated were not measured nor controlled. All these studies provided converging evidence of a positive relationship between chronic anxiety and/or arousal and vestibular gain, but restricted to VOR, which involve different nuclei and pathways than the VSR.

A recent study by Osler et al. (2013) used square-wave Galvanic Vestibular Stimulation (GVS), which consists of the application of an electrical current via electrodes placed on the mastoid processes, to examine how postural threat influences the vestibular system. When subjects stood at high heights, the initial kinematic response to GVS was unchanged, whereas the later response period after 800 ms was found to be significantly attenuated. Horslen et al. (2014) recently conducted experiments evoking ground reaction forces with Stochastic Vestibular Stimulation (SVS) in subjects standing at different heights. Their findings contrast with those of Osler et al. (2013) in that they suggest an increase in the gain of VSR during height-induced states of fear and anxiety, expressed by an increased gain and coherence between the galvanic input and the ground reaction force outputs measured through a force plate. Similar findings were observed by Lim (2014) with threat of perturbation.

Naranjo et al. (2015) extended this work by using Vestibular Evoked Myogenic Potentials (VEMPs) as the method for probing changes in the gain of vestibulospinal reflexes. Results demonstrated a significant increase in the peak amplitudes of VEMPs recorded from the sternocleidomastoid (SCM) and of VEMPs recorded from the soleus (SOL), which were associated with increases in fear and anxiety when standing at an elevated surface height, as shown through the significant correlations found between increases in VSR reflex amplitude of the SCM and SOL and increases in psycho-social measures of emotional changes. This was interpreted as an increase in the gain of VSR, but only in those muscles that were posturally engaged in the quiet standing task.

One of the limitations of these previous projects is that they were focused on VSR. Therefore the results may not be generalized to changes in VOR that involve different neural structures and pathways. Furthermore, they may be susceptible to changes in muscle background activity caused by leaning at height. Thus, there is a need to determine if there is neurophysiological or functional evidence for changes in VOR pathways due to fear of falling, state anxiety and increased arousal, that parallels previously reported changes in VSR gain.

1.3 Clinical assessment of the vestibular function

1.3.1 Neurophysiological assessment of the otolithic function: Vestibular Evoked Myogenic Potentials

As aforementioned, recent studies conducted in our laboratory (Horslen et al., 2014; Naranjo et al., 2015) used 2 common methods for evoking and physiologically measuring the vestibular reflexes: GVS and VEMPs. Although GVS has been proven to elicit vestibular reflexes (Welgampola and Colebatch, 2005; Rosengren et al., 2010), the stimulus affects the whole vestibular afferent, not specific end-organs, and influences postural sway (Pavlik et al., 1999) in a way that could alter the measure of those evoked myogenic potentials. By recording VEMPs from different muscles, a direct and simultaneous measure of the 2 vestibular reflex pathways (VOR and VSR) may be obtained.

VEMPs constitute a reliable diagnostic technique and a powerful research tool for testing the vestibular reflexes from the end-organs via the vestibular nerve, the vestibular nuclei and descending projections to the motorneuron pools. A VEMP is usually defined as a short latency potential recorded from tonically contracted muscles in response to auditory or bone-conducted stimuli (Welgampola and Colebatch, 2005; Rosengren et al., 2010). For standard clinical screening, air-conducted sound (ACS), such as clicks or short tone bursts (STB), is used to evoke responses from the SCM. These responses are recorded with surface electromyography (EMG). The evoked potential elicited from the ipsilateral SCM is known as cervical VEMP (cVEMP), driven by the saccule and projected mainly via the MVST (Welgampola and Colebatch, 2005).

CVEMPs are therefore interpreted as a test for saccular function. A characteristic waveform is obtained when averaging unrectified EMG responses from several pulses. The first component of this waveform is a positive-negative complex, labeled p13-n23 because of the mean peak latency times. As evident by recording directly from the SCM motorneuron pool, Colebatch and Rothwell (2004) showed that the VEMP initial positivity (p13) corresponds to inhibition of the underlying motor unit firing, the subsequent negativity (n23) corresponding to underlying excitation. VEMPs may be elicited from neck extensor muscles, such as the ipsilateral Trapezius (TRP), which was the first neck and head extensor muscle where published auditory myogenic evoked potentials were observed (Bickford et al., 1964). VEMPs from the ipsilateral TRP would also represent a saccular response of the VCR, but projected mainly from the LVN via the ipsilateral LVST to the muscle's motorneuron pool (Uchino and Kushiro, 2011). The averaged waveform obtained is similar to VEMPs obtained from upper limb muscles (Naranjo et al., 2015) with peaks that could be labeled as n1-p1. For the purpose of this thesis, we will label cVEMPs from the SCM as high-cervical VEMPs (HcVEMP) and cVEMPs recorded from TRP as low-cervical VEMPs (LcVEMPs) due to the spinal level (Figure 2).

Ocular VEMPs (oVEMPs) may be elicited also from the extraocular muscles contralateral to the stimulated ear, normally from the inferior oblique (IO), and are often used as a standard clinical test of the VOR pathways (Figure 2). Although it remains unclear whether it is the utricle or the saccule which is responsible for ACS oVEMP responses, it is accepted that oVEMPs are mediated by the superior division of the vestibular nerve (Rosengren and Kingma, 2013), as observed in clinical cases of patients with superior vestibular neuritis that showed abnormal oVEMPs and normal cVEMPs (Shin et al., 2012) and others with inferior vestibular neuritis that

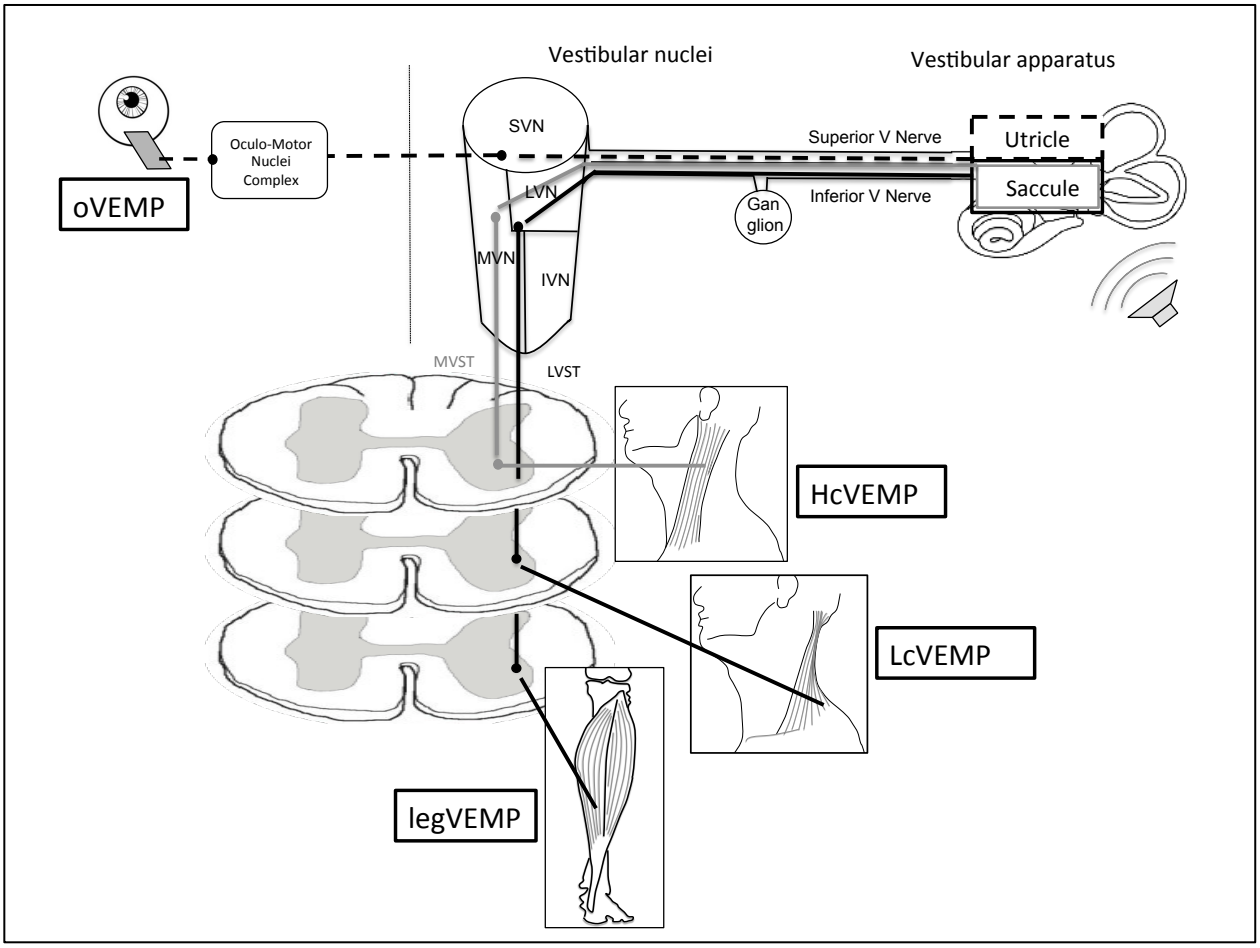

Figure 2. Vestibular Evoked Myogenic Potential (VEMP) pathways. Air-conducted sounds stimulate the otolith receptors, the utricle and the saccule, that project to the vestibular nuclei at the brainstem. The ocular vestibular evoked myogenic potential (oVEMP) is recorded from the contralateral Inferior Oblique via the oculomotor nuclei complex. The high-cervical vestibular evoked myogenic potential (HcVEMP) is recorded from the ipsilateral Sternocleidomastoid, whereas the low-cervical vestibular evoked myogenic potential (LcVEMP) is recorded from the ipsilateral Trapezius. The leg vestibular evoked myogenic potential (legVEMP) is recorded from the ipsilateral Soleus.

showed the opposite results (Manzari et al., 2010). The elicited and recorded VOR response through oVEMPs is therefore processed mainly in the superior vestibular nucleus (Rosengren

and Kingma, 2013). A similar averaged waveform to the cVEMP is obtained when using unrectified EMG data from the IO muscle contralateral to the stimulated ear, resulting in a typical averaged waveform. OVEMP peaks are normally labeled as n10-p15 and show slightly earlier peak latencies than cVEMPs (10 ms and 15 ms, respectively) (Rosengren and Kingman, 2013).

VEMPs can also be elicited and recorded from limb muscles (Watson and Colebatch, 1998; Naranjo et al., 2015), representing a saccular response of the VSR projected via the LVST (Uchino and Kushiro, 2011). VEMPs evoke a consistent negative peak in the ipsilateral soleus (SOL), demonstrated by rectifying the EMG data, labeled p1 and argued to be of vestibular origin because the responses are similar to those evoked with GVS (Watson and Colebatch, 1998). In a recent study, this response in the SOL was labelled a legVEMP (Naranjo et al., 2015; Figure 2). Examples of VEMPs recorded from the different muscles are represented in Figure 3.

Previous work (Osler et al., 2013; Horslen et al., 2014; Naranjo et al., 2015) has only examined the effects of induced states of fear, anxiety and arousal on the VSR, but not on the VOR. This thesis was the first to investigate if the observed physiological vestibulospinal changes apply to VOR, measured through oVEMPs.

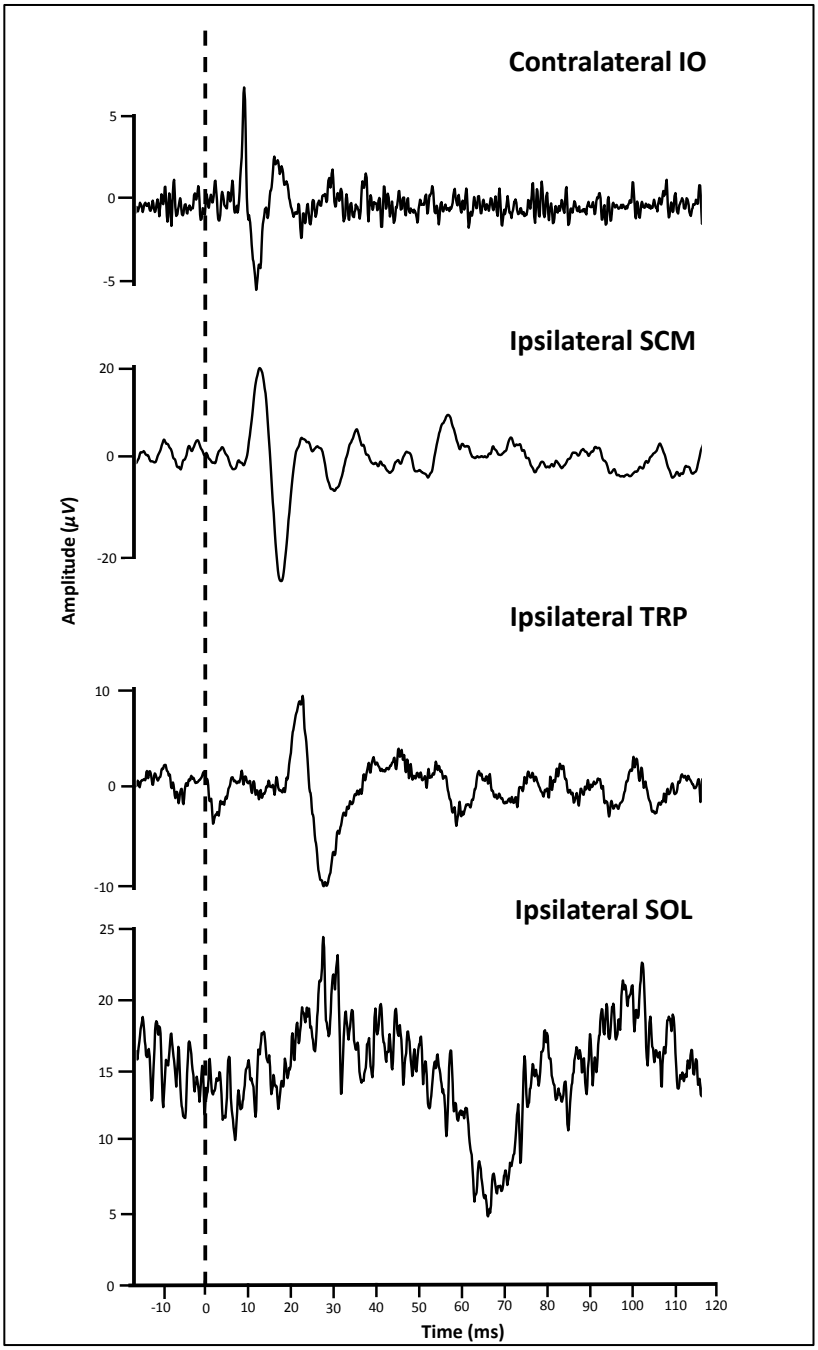

Figure 3. Examples of Vestibular Evoked Myogenic Potentials (VEMPs) averaged from 256 short-tone bursts, elicited and recorded from: Inferior oblique (IO), sternocleidomastoid (SCM), trapezius (TRP) and soleus (SOL). The dashed line represents the onset of the pulse.

Therefore, the *first research question* of this thesis was: **do threat-induced changes in fear, anxiety and arousal cause increases in the gain of VOR and VSR?** The first *objective* of Study 1 was to address this question by comparing changes in oVEMPs, cVEMPs and legVEMPs from a low height condition to a high height condition, in order to investigate the physiological behavior of the VOR and VSR respectively, within the same subjects.

1.3.2 Functional assessment of the vestibulo-ocular function: the Head Impulse Test

Increases in the amplitude of oVEMPs do not necessarily mean there will be a significant functional change in the VOR. VEMPs are elicited as the result of an unnatural stimulation of the otoliths, and could only provide a representation of a small component of the vestibular system that would normally be integrated into the whole, including other receptors and pathways. In a clinical environment, 3 methods are commonly used for functionally assessing the VOR: the caloric test, the rotating chair and the head impulse test (HIT). In caloric tests the VOR is evoked by irrigating the ear with hot and cold water, which causes the endolymph to move due to the convection currents generated by differences in temperature (Purves et al., 2001). The need of a skilled professional to perform the test, in addition to the unpleasant experience that could affect the desired height effect and the subject needing to lie down, make this method difficult to apply on a height paradigm. The rotating chair evokes nystagmus responses that can be used for interpreting the VOR gain. These responses can originate from the HSSCs by using an earth vertical axis rotation (EVAR) -the most commonly used (Furman et al., 2006; Allum and Honegger, 2013), or combined otolith and SSC stimulation by using off-earth vertical axis

rotations (OVAR) (Hain, 1986; Furman et al., 2006). The EVAR rotating chair selectively stimulates the horizontal SSC, and head and eye movements are principally in the yaw plane. However, using and controlling a rotating chair system on an elevated surface seems difficult, and subjects could not stand at the edge of an elevated platform while seated in a chair. The HIT constitutes then the most feasible method for functionally assessing the VOR at height.

Initially described as a bedside test for rapid assessment of the vestibular function (Halmagyi and Curthois, 1988), the HIT involves monitoring eye movements as the subject fixates on a stationary target while the head is moved briskly to either side. When the clinician moves the head of the patient with short and fast movements, called thrusts (normally not larger than 30°, at velocities ranged from 80°/s to 250°/s), the eyes will continue gazing at the target because of the compensatory smooth eye movement opposite to the head provided by the VOR. As previously described, the VOR arc measured through a horizontal HIT, with head movements conducted on a strict yaw plane, involves the HSSC, the MVN and the oculomotor nuclei complex (abducens and oculomotor nuclei). At the same time, a vertical VOR response can be elicited by moving the subject's head in a pitch plane, therefore stimulating the saccule and anterior/posterior SSCs by adding vertical accelerations, and involving the trochlear nucleus in the resultant compensatory eye movement (Halmagyi and Curthois, 1988).

The VOR gain, a ratio that has a normal value close to 1 in healthy subjects, is quantified by dividing the eye velocity by the head velocity. Specific details relating to different methods are described in the methods section of this thesis. The video HIT (vHIT) is considered a valid and reliable method for assessing the VOR (Weber et al., 2009). With this system, the eye and the

head movements are registered through a built-in camera and gyroscopes installed in the goggle system, allowing a more accurate measure of the VOR gain and the evaluation of deficits not detected by simple observation (Weber et al., 2009). The modern vHIT systems will normally capture the different velocities of the head and the eye movements and will automatically apply an algorithm for rejecting incorrect trials and calculating the gain. When comparing VOR results from both vHIT and rotating chair, it has been shown that the VOR responses from the vHIT, in response to higher velocities of approximately 150 °/s (compared to the slower range, around 30 °/s, conducted by a rotating chair) better approximate head velocities occurring during gait-related balance tasks (Allum and Honegger, 2013).

Previous work that investigated the effects of emotions on functional VOR was conducted on subjects with increased trait anxiety rather than state anxiety (Furman et al., 2006), using a rotating chair as method to evoke the VOR, and with the limitation of not controlling for levels of anxiety experienced during the experiments.

Therefore, the *second research question* of this thesis was: **do threat-induced changes in oVEMPs accompany functional changes in the gain of the VOR?** The second *objective* of Study 1 was to address this question by comparing the gain of the VOR measured through vHIT between conditions, and correlating changes in functional VOR measures with changes in psychosocial measures of fear, anxiety and arousal.

1.4 Oculomotor function and anxiety

Increases in the VOR gain observed in chronic anxiety patients and induced states of arousal were argued previously (Yardley et al., 1995; Furman et al., 2006) to be generated in the vestibular nuclei via the described neurophysiological and neurochemical links from neural centres that mediate responses to fear and anxiety (Balaban and Thayer, 2001; Balaban, 2002; Staab et al., 2013). However, there are other potential neural machanisms for anxiety to influence VOR gain that do not involve the vestibular nuclei, and that may have been overlooked. These include serotonergic projections from the rostral dorsal raphe nucleus to the caudal part of the oculomotor nucleus and trochlear nucleus (Peyron et al., 1998) and serotonin receptors labelled in the abducens nucleus (Fay et al., 2000), together with histaminergic influences on the areas responsible of visual input processing (Harrington, 1997), with the potential contribution of the histaminergic pathways (de Waele et al., 1992; Serafin et al., 1993; Yabe et al., 1993; Peng et al., 2013). The influence of acute and transient states of anxiety, fear or arousal on the oculomotor function (OMF) has never been investigated (Staab, 2014).

A clinical battery composed by 3 different tests is normally conducted in order to assess the OMF: Eye saccades, smooth pursuit and optokinetic nystagmus (OKN).

The *eye saccade* test measures the rapid eye movements performed towards a determine stimulus. The abducens and oculomotor nuclei that contain the motorneurons that control eye movements receive projections from 2 critical centres for initiation and accuracy of the eye saccades: the superior colliculus in the midbrain and the frontal eye field of the cortex.

The *smooth pursuit* test measures the smooth voluntary tracking eye movements that allow close following of a moving target; pursuit is the most voluntary eye movement of the three discussed here. The pathway supporting eye saccades involves some of the same areas that are in the smooth pursuit pathway, including frontal eye fields, supplementary eye fields and the cerebellum. It also includes parietal eye fields, the superior colliculus, the basal ganglia, and the vergence centres that receive projections from the occipital visual cortex and modulate the divergent and convergent movements of the eye (O'Driscoll et al., 2000).

The *OKN*, a test to measure the optokinetic reflex, combines saccadic and pursuit eye movements. The OKN occurs in the eyes in response to a rotational, ongoing-large scale, movement of the visual scene. The OKN involves a slow phase where the eye follows a moving target similar to a smooth pursuit, and a fast phase where a saccade returns the eye to a central fixation point (Baloh and Honrubia, 1979). Despite the basic model of the neural pathways serving the OKN, it also involves the occipital and frontal visual cortices, and the oculomotor nuclei (Dix and Hood, 1971). Animal models suggest an involvement of the vestibular nuclei in the slow phase of the OKN, with suppression of the OKN after destruction of the vestibular nuclei, and activation of LVN neurons during normal OKN (Azzena et al., 1974). The same vestibular nuclei neurons respond to both passive head rotations and full field visual motion (Boyle et al., 1985). As such, there are similar dysfunctions in OKN and pathological spontaneous nystagmus, normally present in clinical central vestibular disorders. The oculomotor nuclei complex receive projections from the vestibulo-cerebellum via the vestibular nuclei (through the vestibular-cerebellar pathways) (Takemori and Cohen, 1974). The OKN is therefore

accepted as a method for testing vestibulo-cerebellar function, as it is usually diminished in cerebellar subjects. The OKN is not however entirely dependent on the cerebellum, as OKN responses persist in the absence of this neural structure. This preservation has been attributed to the projections from the pretectum in the midbrain (which receives projections from the retina) to the vestibular nuclei (Precht and Strata, 1980). Moreover, interactions between visual inputs and vestibular function have been thoroughly described in studies that showed how the neurons at the vestibular nuclei are activated by visual OKN stimuli in animal models in a velocity-dependent fashion (Allum et al., 1976; Waespe and Henn, 1979), and how the OKN stimulus depresses the slow phase velocities of the nystagmus evoked by true passive rotations of the head (Koenig et al., 1978).

Therefore, the eye saccades and smooth pursuit probe different parameters of OMF and are not dependent on vestibular involvement. In contrast, the OKN combines both movements, but also has a clear contribution from the vestibular system.

No significant changes in the eye saccades parameters were observed when studying attention deficits in subjects with chronic anxiety compared to controls (Derakshan et al., 2009), and peak velocities of saccadic eye movements were lower in subjects with panic disorders than in those with depressive syndromes (Derakshan et al., 2009). Furthermore, saccadic eye movements were found to be significantly more inaccurate in patients suffering from panic disorder compared to healthy controls (Jergelova and Jagla, 2010). When smooth pursuit tests were conducted on 3 groups of subjects diagnosed with trait anxiety, schizophrenia endophenotype and depression, the anxiety group showed a better smooth pursuit predictive performance in absence of vision

compared to those who showed depressive symptoms, but no changes in smooth pursuit gain was observed (Kattoulas et al., 2011), or no comparisons with healthy controls were made. In addition, subjects with high trait anxiety have a poorer gaze stability and accuracy that affects the ocular motor control compared to subjects with low trait anxiety (Laretzaki et al., 2011). Subjects with vestibular-cerebellar dysfunction and predisposition to panic disorders demonstrate higher gain of the OKN than healthy controls (Levinson, 1989).

Therefore, the *third research question* of this thesis was: **do fear, state anxiety and/or arousal influence OMF?** The *objective* of Study 2 was to address this question by investigating the effects of fear, anxiety and arousal on the 3 oculomotor tests, in order to determine if the hypothesized increase in VOR gain could be also modulated at the level of the oculomotor nuclei complex.

1.5 Summary of the introduction

Induced states of fear of falling, anxiety and arousal could increase the gain of vestibular reflexes. This thesis was designed to investigate the modulation of the three different vestibular reflexes during height-induced emotional changes. A significant focus of this thesis is on changes to the VOR, explored with oVEMPs and, the more functional VOR measure, the vHIT. Furthermore, changes in the gain of vestibular reflexes and changes in emotional measures were correlated to explore inter-relationships. Finally, in a separate study, OMF was tested in the presence of a height-induced postural threat to determine the involvement of the oculomotor nuclei complex in the changes to VOR responses at height.

1.6 Hypotheses

Consistent with previous research the following was hypothesized:

Study 1:

- *Hypothesis 1*: The gain of VOR and VSR will increase with threat, as evident by a significant effect of height on the normalized amplitudes of oVEMP, HcVEMP, LcVEMPs and legVEMPs, and a significant positive correlation between increases of induced fear and anxiety and increases in VEMP amplitudes.

- *Hypothesis 2*: The VOR gain measured through the vHIT will significantly increase in the high condition compared to the low condition, finding significant correlations between changes in the VOR gain and changes in fear, anxiety and arousal.

Study 2:

- *Hypothesis 3*: There will be no significant changes in the gain of OMF measured through eye saccades and smooth pursuit between the low and the high height conditions, whereas the gain of the OKN will be significantly larger in the high condition due to its modulation at the vestibular nuclei level. Significant correlations will be found between changes in psycho-social and autonomic emotional outcomes and changes in the OKN gains.

Chapter 2: Methods

2.1 Common methods for Study 1 and Study 2

Postural threat was manipulated by having subjects stand on a hydraulic lift (M419-207B10H01D, Pentalift, Guelph, Ontario, Canada) that was positioned at two different height conditions. In the Low Condition subjects stood away from the edge of the platform at a height of 0.8 m; in the High Condition subjects stood at the edge of the platform at a height of 3.2 m. The order of presentation of heights was fixed to always begin at the low height condition, to account for known order effects of height (Adkin et al., 2000). Subjects were secured with a harness attached to the ceiling and were accompanied by an experimenter at all times.

Autonomic arousal was measured by electro-dermal activity (EDA), recorded by electrodes placed on the hypothenar and thenar eminences in the palm of the non-dominant hand (Skin Conductance Module 2502, Cambridge Electronic Design, Cambridge, UK). EDA was sampled at 100Hz (Spike 2 and Power 1401, Cambridge Electronic Design, Cambridge, UK) over the course of each condition. Psycho-social aspects regarding perceived confidence, stability, fear and anxiety were measured through questionnaires (Appendix) (Adkin et al., 2000; Carpenter et al., 2001; Davis et al., 2009; Horslen et al., 2013; Horslen et al., 2014). Confidence was assessed immediately prior to each condition, while anxiety, fear and stability were assessed immediately following the completion of each condition.

2.2 Study 1

Twenty-five young healthy subjects (10 males; age = 27.76 ± 5.95; weight = 68.4 ± 9.22 kg; height = 168.44 ± 9.37 cm), undergraduate and graduate students from the University of British Columbia, volunteered to participate in the study. Subjects were free from any neurological or non-neurological cause of balance, hearing or cognitive impairment, extreme fear of heights, frequent or severe headaches, pregnancy and/or history of low blood pressure or fainting, history of chronic neck pain or/and whiplash syndrome, or severe neck movement restrictions, as verified by self-report. All subjects provided informed consent prior to participation. All experimental procedures were approved by the UBC Clinical Research Ethics (UBC CREB# H06-70316).

This study was conducted in 2 different experiments. All 25 subjects performed Experiment 1 (VEMP eliciting); while a sub-set of nineteen (19) of the participants from Experiment 1 (8 males; age = 27.73 ± 6.24; weight = 69 ± 9.40 kg; height = 168.52 ± 9.61 cm) also performed Experiment 2 (vHIT). Experiments were performed by subjects on separate days, and the order of presentation of the experiments was counter-balanced across subjects.

2.2.1 Experiment 1: VEMPs

2.2.1.1 Procedures

Acoustic stimulation of the right ear was used to elicit VEMPs from the left IO, right SCM, right TRP and right SOL. Subjects stood with feet shoulder width apart and body oriented 90° to the edge of the platform (left shoulder toward edge). Both heels were raised with wedges (30°) to increase tonic activation of SOL. A forceplate (Bertec, Columbus, Ohio, USA) was used by the experimenter to monitor antero-posterior and medio-lateral moments online, and provide verbal feedback, if necessary, to assist the subjects in maintaining a constant body position within and between conditions.

The head was rotated 60° in the yaw plane (toward platform edge), and 30° downward to increase tonic activation of SCM and TRP. A laser pointer attached on top of the head was used to provide visual feedback for the subject to help maintain constant head position between conditions. During the experiment subjects were asked to look upwards as much as possible along the vertical target line in order in increase tonic activation of IO muscles, and to try and avoid excessive blinking.

VEMPs were elicited by STB of 4 ms in duration (alternating polarity tone burst, rise/fall time of 1 ms and a plateau time of 2 ms) at a 500 Hz frequency, delivered at an intensity of 125 dB sound pressure level (SPL). STBs were delivered at a rate of 5 Hz (i.e. 1 tone burst every 200 ms), addressing one of the limitations of the study conducted by Naranjo et al. (2015), in which

the stimuli were delivered at a slower rate due to a biomechanical effect observed through forceplate data. The slower rate (0.5-0.8 Hz) had the disadvantage of extending the duration of the trials and thus increasing the potential for a habituation effect at the high condition. The intensity of the STB was below safety limits determined by the Canadian Center for Occupational Health and Safety. Following clinical protocols (Welgampola and Colebatch, 2005; Rosengren et al., 2010), 256 pulses were presented on each condition in order to observe a consistent waveform by averaging the responses to all tone bursts. STB were delivered monaurally in the right ear through headphones (model 296D 100-1, Telephonics, USA) via a stereo amplifier (model SX-650, Pioneer, Kawasaki, Kanagawa, Japan). The STB's were generated with a custom written computer program (Spike 2, Cambridge Electronic Design, Cambridge, UK) and calibrated before every test using a sound pressure meter (model CR250, Cirrus, Hunmanby, North Yorkshire, UK). The experimental protocol for Experiment 1 is represented in Figure 4.

2.2.1.2 Data acquisition and analyses

For obtaining VEMPs, EMG data was recorded from all muscles using surface electrodes. Data was pre-amplified 500x, sampled at 3000 Hz and band-pass filtered 10Hz – 1000Hz (Telemyo 2400R, Noraxon, Scottsdale, Arizona, USA), then analog-digital sampled at 5000 Hz (Spike 2 and Power 1401, Cambridge Electronic Design, Cambridge, UK) and analyzed offline using a custom written program (Spike 2, Electronic Design, Cambridge, UK). Pairs of surface electrodes were placed approximately 2 cm apart on each muscle belly. For TRP, electrodes were placed over the intersection between the upper and middle fibers, and with the ground electrode

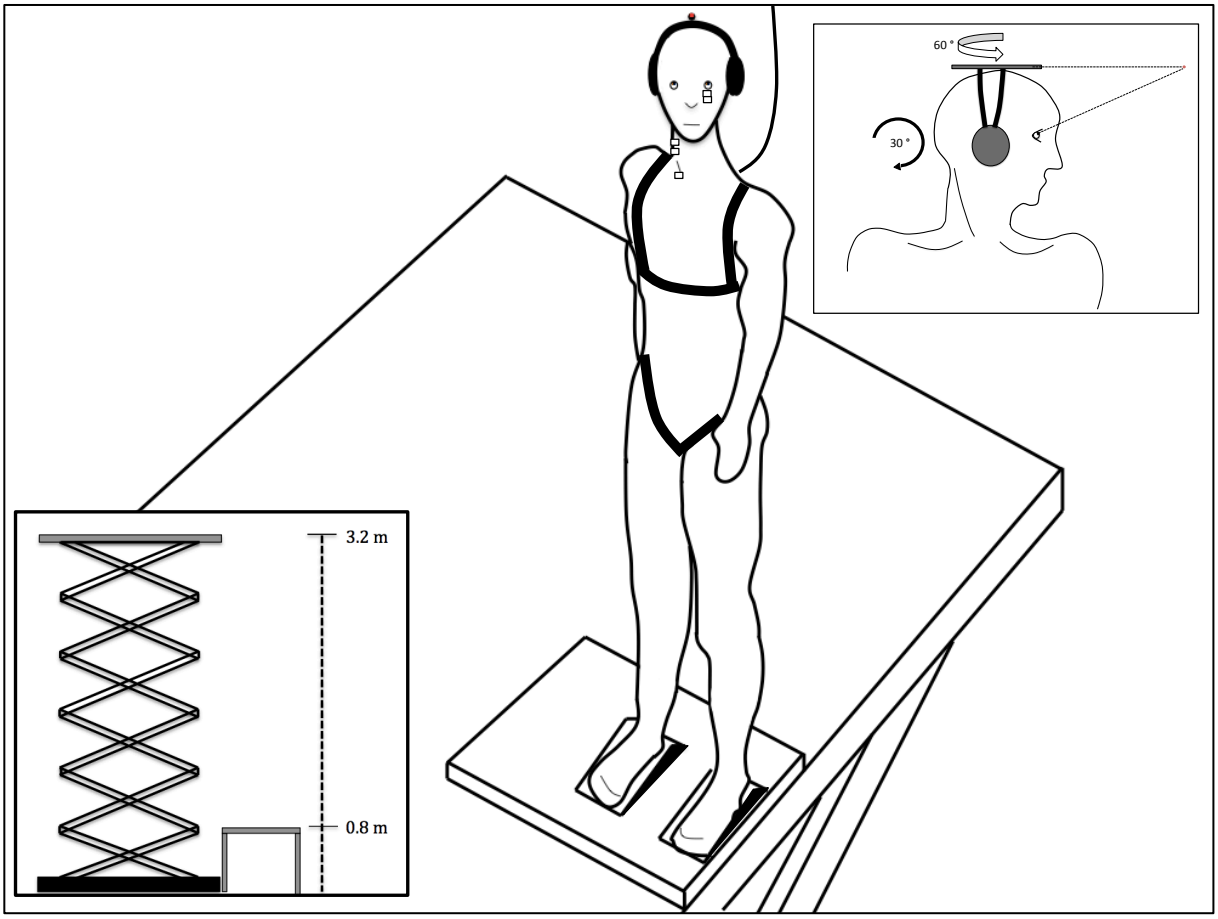

Figure 4. Experimental set-up for Experiment 1, Study 1 (VEMP testing). Subjects were placed sideways at the edge of a hydraulic lift, on a force plate, and on wedges to activate the soleus. Bottom left corner shows the 2 height conditions, with a table placed at the low condition for removing subjects from the edge. Top right corner shows the subject's head and neck position for activating the target muscles: leaving the trunk in a neutral position, the neck and head will be turned 60° and flexed 30°; a laser pointer attached to the headphone set and aligned with the horizontal line projected a laser beam to the wall, forcing the subject to look upward.

applied over on the medial aspect of the right clavicle. For the IO, electrodes were placed on the face immediately below the left eye, following the midline of the eyeball. This method of electrode placement has been shown effective in order to record clear VEMP responses from the

IO with less variability in the results (Sandhu et al., 2013). For the SOL, the electrodes were placed slightly lateral to the midline of the calf immediately distal to the junction of the Achilles Tendon and the belly of the Lateral Gastrocnemius. EMG data was rectified only for the SOL (Watson and Colebatch, 1998; Naranjo et at., 2015).

All 256 trials for each stimulus were spike trigger averaged to the tone bursts using Spike 2 software. The evoked EMG responses were analyzed separately for amplitude and latency for each participant. BGA of each muscle was calculated using the RMS amplitude of 20 ms pre-stimulus unrectified EMG (i.e. for the IO, SCM and TRP) and the mean amplitude for the rectified EMG (i.e. for the SOL). A 2 Standard Deviation (2SD) bandwidth based on that pre-stimulus RMS or mean amplitude was established for setting the threshold of significance of the VEMP peaks.

2.2.1.3 Controlling for Muscle Background Activity (BGA)

Muscle BGA is known to have a strong positive linear relationship with VEMP amplitude (Rosengren et al., 2010). However, it has been shown that with small levels of tonic activation, as the ones sustained by the subjects in this protocol, that linear correlation is not significant (Bogle et al., 2013). Therefore, following methods previously used in quiet standing VEMP recording (Naranjo et al., 2015), two different levels of control were set before normalizing the VEMP amplitude to muscle background activity. First, the position of the subjects were monitored to be similar between conditions: using the above mentioned laser pointer in the head and neck, through instructing to look as up as possible for the eye and monitoring online the

leaning attitude by observing the moment of force in the medio-lateral axis with the forceplate. Second, a screening protocol was used to identify any muscle with a mean BGA that could affect the response standardizing the tonic activation levels across conditions. This method consisted of calculating the mean BGA obtained through the average RMS amplitude (for unrectified EMG data) or mean amplitude (for rectified EMG data) from 20 ms prior to each stimulus for the Low condition. A conservative threshold of ± 1SD was set for each mean BGA, and those muscles with an average BGA that exceeded this threshold were subsequently removed from further analyses. Finally, as a standard method, the VEMP amplitude was normalized by the mean rectified EMG amplitude of 20 ms pre-stimulus for all muscles (Welgampola and Colebatch, 2001; Rosengren et al., 2010). Across all the participants, some muscles did not show a significant VEMP response, and therefore those muscles were not included in the analyses: 4 for the IO, 1 for the SCM, 3 for the TRP and 2 for the SOL. In addition, screening for changes in muscle background activity beyond the ± 1 SD threshold from Low to High, prior to normalization of VEMP amplitudes, resulted in the removal of 1 IO, 10 SCMs, 10 TRPs and 2 SOLs. Therefore, the final sample sizes included in the analysis were: 21 IO, 14 SCM, 12 TRP and 20 SOL.

Blinks can affect the calculations of muscle background activity in the IO, and therefore affect the values of VEMP amplitude. Subjects were instructed to try to control for blinking while sustaining upward gaze. Blinks that occurred were identified offline by plotting the RMS amplitude of 20 ms previous to each stimulus across time. Those pulses corresponding to data points that suggested a blink were removed before creating the VEMP averaged waveform.

The *dependent variables* included in this first experiment of Study 1 were the corrected peak-to-peak (ptp) amplitude for SCM (p13-n23), IO (n1-p1) and TRP (n1-p1), and the corrected peak amplitude for SOL (n1), expressed in microvolts (μV).

2.2.2 Experiment 2: vHIT

2.2.2.1 Procedures

A vHIT system (ICS Impulse, GN Otometrics, Taastrup, Denmark) was used for measuring the head and eye velocities for each condition. Subjects wore a goggles system that incorporated a video eye recorder (on the right eye) and 3 gyroscopes for registering head movements in the HSCC, ASCC and PSCC planes on the left and right side. The experimenter stood behind the subject to perform the head impulses. Head impulses were conducted in the yaw plane, to the left and to the right, and in the pitch plane, upward (chin up) and downward (chin down). The order of presentation for direction of head thrusts at each height condition was randomized beforehand. Head impulses were conducted until 20 correct trials in each direction (left and right for the horizontal and upward and downward for the vertical) were identified by the system's software.

Subjects stood facing forward at the edge of the lift. After calibrating the system, they were instructed to maintain the gaze at a central fixation point placed on the wall 3.10 m from the edge of the platform, at the level of their eyes. The experimenter stood on a 40 cm high platform, placed behind and to the left of the subject. For performing the head thrusts, and preventing the slippage of the goggles, the experimenter placed their hands on both sides of the subject's head

(slightly above the temporomandibular joints) for the lateral impulses, and the left hand on the mandible and the right hand on the apex for the vertical impulses. The experimenter then conducted head thrusts within 30° of movement to each direction, and target velocity of 100-150 °/s, while randomizing the directions within a plane to avoid any anticipatory responses from the subjects. Both the participants and the experimenter were secured with a harness and a safety line, and two hand rails were placed at both sides of the participant at a reachable distance as an extra safety measure (Figure 5).

2.2.2.2 VHIT data analyses

The vHIT system used in this experiment (ICS Impulse, GN Otometrics, Taastrup, Denmark) is designed to calculate VOR gains in a strict yaw plane and in the plane of the right ASCC/left PSCC (RALP) and left ASCC/right PSCC (LARP). The main challenge of this experiment was to calculate vertical VOR gains in the strict pitch plane using data from both oblique gyroscopes. All the raw data was extracted from the vHIT system and analyzed offline using a custom-made algorithm (Matlab 2007, Math Works Inc., Natick, MA, USA).

First, a dual pass Butterworth filter was applied with a corner frequency set at 0.2 of the sampling rate (or 200 Hz) for both head and eye velocities across time. Head accelerations were calculated for the vertical vHIT by the square root of the sum of LARP and RALP velocities squares $\sqrt{(RALP^2 + LARP^2)}$. The lateral head velocities were taken directly from the vHIT system data. Individual HIT events were identified with velocities greater than 60°/s. Eye velocity gains were calculated with respect to head velocity over the interval starting 100 ms

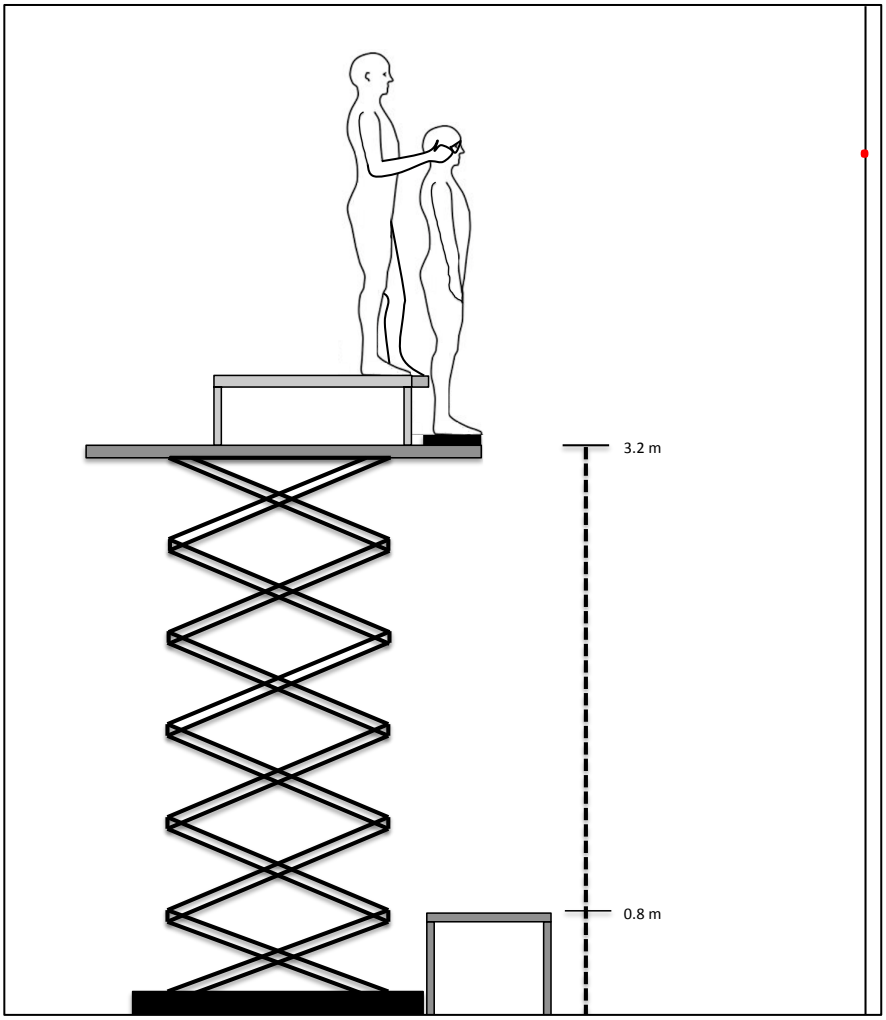

Figure 5. Experimental set-up for Experiment 2: video Head Impulse Test (vHIT). Subjects were placed facing forward at the edge of the hydraulic lift and wore the vHIT system while fixating the gaze in a central point on a wall 3m from them. The experimenter stood on another platform beside them and moved the head randomly to different directions in each plane.

before peak head velocity until head velocity first crossed zero velocity after its peak. Covert saccades, anti-compensatory quick eye movements (ACQEM) (Heuberger et al., 2014) and other artifacts (like slippage of the goggles) were identified and removed by applying a proprietary

technique based on previous work (Allum and Honegger, 2013). The remaining plots were examined and confirmed by a blinded external evaluator. After screening all the data, head and eye velocities were compared using the VOR gains, which were calculated as the ratio obtained when dividing the sum of the eye velocities by the sum of the head velocities within the previously described time frame (Allum and Honegger, 2013).

Although the VOR gains were separated in 2 different directions for each plane, left and right or upward and downward, the directions within a plane were combined for calculating the Horizontal VOR gain (HVORgain) and Vertical VOR gain (VVORgain), for the purpose of statistical analysis.

2.2.3 Statistical analysis

Paired samples *t*-tests were used to compare differences between height conditions for all dependent measures in both Experiment 1 and 2. Correlations between changes in psycho-social and autonomic measures, and changes in VEMP amplitudes (Experiment 1), and horizontal and vertical VOR gains (Experiment 2), were calculated using Pearson's Correlation Coefficient for those variables with a normal distribution and Spearman's Rho for those with a non-normal distribution. Normality was tested using the Shapiro-Wilk's test. All levels of statistical significance were set at $p = 0.05$.

2.3 Study 2

Twenty young healthy participants (12 males; age = 27.45 ± 5.68; weight = 67.9 ± 10.16 kg; height = 168.45 ± 10.55 cm) were recruited from the graduate and undergraduate student community of UBC. The exclusion criteria was the same as that for Study 1: no neurological or non-neurological cause of balance, hearing or cognitive impairment, extreme fear of heights, frequent or severe headaches, pregnancy and/or history of low blood pressure or fainting, history of chronic neck pain or/and whiplash syndrome, or severe neck movement restrictions, as verified by self-report. All subjects provided informed consent before participating. All experimental procedures were approved by the Clinical Research Ethics of the University of British Columbia (UBC CREB# H06-70316).

2.3.1 Procedure

Subjects were placed facing forward on the hydraulic lift, standing away from the edge in the Low condition and at the edge in the High. A 50-inch LCD screen (50PB560B, LG, Seoul, Korea) was placed at 1 m of distance, at the level of the eyes, suspended from the ceiling through a cable and pulley system. An electro-oculography (EOG) system (SC2000/2SP, UFI, Morro Bay, California, USA) was used for recording eye movements. Surface electrodes were placed at the lateral border of each eye. The ground electrode was placed centered on the forehead. EOG data was analog-to-digital sampled at 1000 Hz (Spike 2 and Power 1401, Cambridge Electronic Design, Cambridge, UK) and analyzed offline using a custom written program (Spike 2, Cambridge Electronic Design, Cambridge, UK). Subjects wore an accelerometer on the head that

combined linear accelerations to calculate angular accelerations in the yaw, pitch and roll planes during all procedures in this study. Head movements were monitored online to ensure subjects were maintaining a constant head position within a condition (Figure 6).

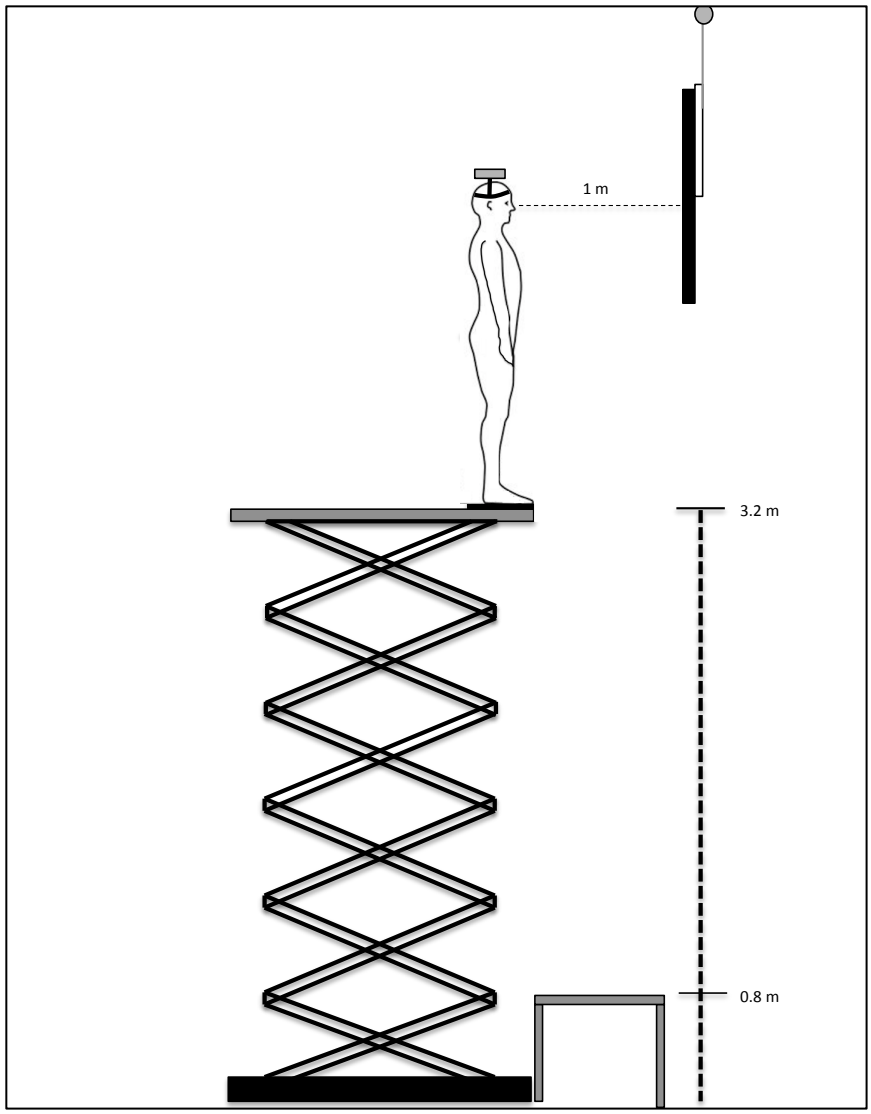

Figure 6. Experimental set-up for Study 2: oculomotor function (OMF) tests. Subjects were placed facing forward at the edge of the hydraulic lift and wore an accelerometer on the head. The visual stimuli were presented on a LCD screen 1m in front of them.

Participants were subjected to 3 OMF tests on each height: eye saccades, smooth pursuit and OKN. The order of presentation of the 3 oculomotor tests was randomized beforehand, being consistent between heights. Subjects were tested in the Low condition (0.8m) first and in the high (3.2 m) condition afterwards. Subjects performed the OMF tests in a slightly darkened room to ensure fixation on the visual stimuli.

2.3.2 Stimuli

The stimuli for the OMF tests were custom built using Vizard software (Worldviz, Santa Barbara, California, USA). For the eye saccades and smooth pursuit a black dot moved on a virtual white curved background. For the OKN, a simulated environment was created to resemble a rotating drum with a 1-meter radius. The stimuli were presented on the screen in a randomized order to every subject. The distance from the subject's eyes to the screen was 1 meter. When the platform was raised to the High condition, the screen was raised also to keep it at eye level. Before starting the first trial at the Low condition, a calibration test was performed, by having the subjects stare at a central fixation point that changed its position 27° alternatively to the left and to the right several times.

For the *eye saccades*, targets were presented randomly at different angles in the horizontal plane, with amplitudes that ranged from 8° to 45°. After having the subjects stare at a central fixation point, the target would change positions to either the right or left with a variable interstimulus interval (between 1 and 3s). Participants completed 52 saccades in total in each condition (Derakshan et al., 2009).

For the *smooth pursuit*, the subject was instructed to follow a target moving horizontally at a constant speed to either side. The target moved from the central fixation point to the right with a determined constant speed until it reached 20°, where it then changed direction without pause, as describing by a sawtooth waveform. The target moved at a given speed for approximately 40 s before stopping again in the central fixation point. Three target speeds, 15, 20, and 30°/s, were randomly presented with the target moving horizontally with an amplitude of 40° (20° from the central position).

The *OKN* was evoked by moving vertical black lines horizontally on a white background. The subject was asked to stare at a red central fixation point that disappeared after 3s and to keep the gaze at that same point. They were then instructed to focus on the line that was passing straight ahead of them and shift the gaze to the centre, approximately 1 or 2 lines back, when that line started exiting the center portion of the screen. The rotating drum was rotated at 3 different velocities of 15, 30 and 60°s for 20 seconds in each direction.

Eye position was calibrated using the EOG data from the eye saccade trials at the Low condition. The mean amplitude of 500 ms pre-stimulus position change from each trial was used for calculating the position change. The equation of the best fitting line between stimulus amplitude (in degrees) and the eye position change (in volts) for every trial was then used for calibrating the amplitudes. For those trials with repeated amplitudes, the average of all was calculated as amplitude for that specific target.

2.3.3 Eye saccade analysis

The methods used for analyzing the eye saccade data are described in previous work (Allum et al., 1998). Due to the existing noise in the EOG data, the eye saccades data was filtered using a 10Hz dual pass 2nd order Bessel filter. EOG signal was differentiated with an 8 point Remez exchange algorithm for calculating the eye velocity.

In healthy subjects, eye saccades consist of an *initial saccade* that reaches a maximum movement velocity, but typically undershoots the target, followed by a second corrective saccade (*post-saccade*) that moves the eye closer to the target position (used to measure the saccadic accuracy) (Baloh and Honrubia, 1979). The maximum peak in the velocity trace after the saccade onset was considered the saccade peak velocity. The pre-saccadic baseline position of the eye was calculated with the mean of a 50 ms time window 100 ms before the eye peak velocity (Schmitt et al., 2013). The amplitude of the initial eye saccade (saccadic amplitude) was calculated with the mean amplitude of 50 ms after the eye velocity returned to baseline velocity levels. This value was compared to the target amplitude for calculating the saccade gain. The post-saccadic amplitude, i.e. the amplitude of the corrective saccade, was calculated using the mean value of 50 ms of the eye position 200 ms after the initial saccade (an average time after which all subjects corrected the eye position on the target). This last value was used for calculating the post-saccadic gain, a measure of saccadic accuracy as described above (Figure 7).

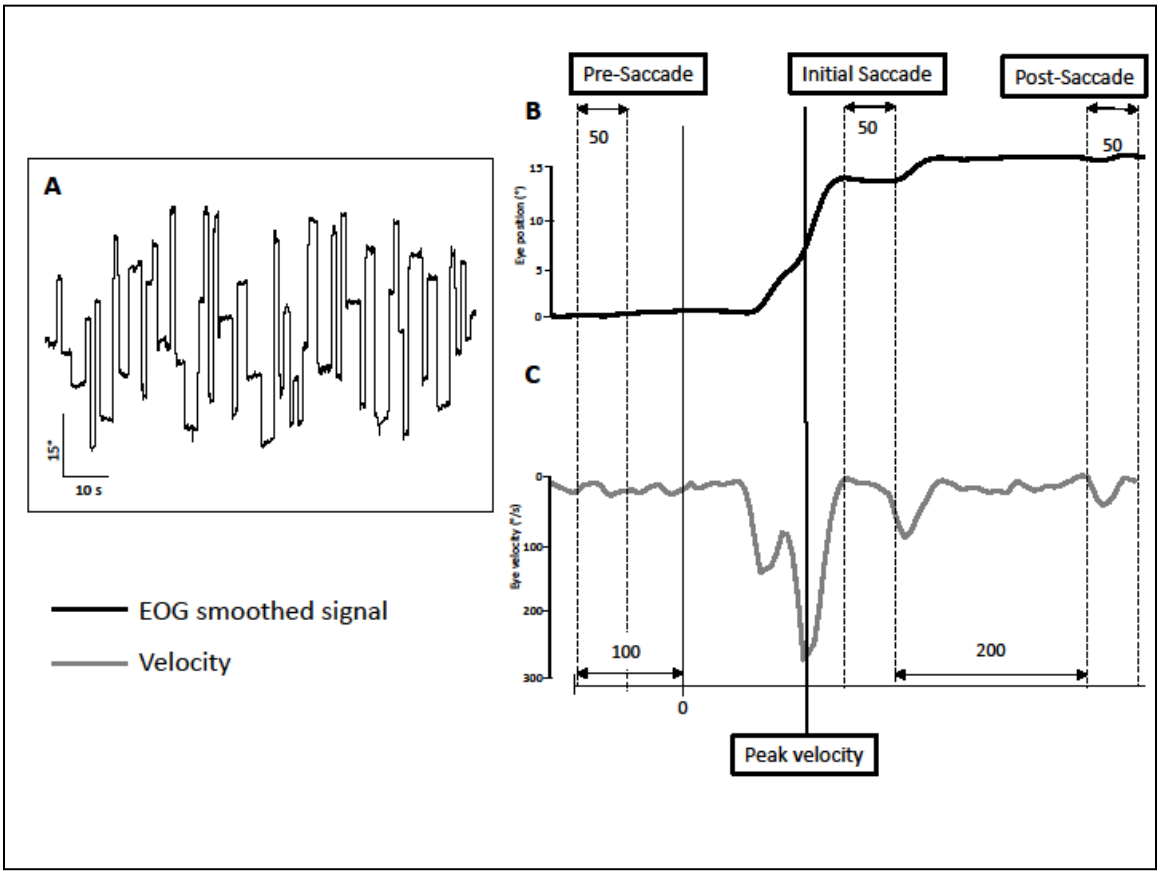

Figure 7. Eye Saccade analysis. A) Filtered electro-oculography (EOG) signal from an individual Saccade test. B) Filtered EOG signal from one Saccade trial with the eye moving to catch a determine target position changed. C) Eye velocity trace, with the peak velocity measure for that specific Saccade. Pre-saccadic eye position was calculated as the mean amplitude of the first 50 ms within 100 ms prior to the peak velocity. The initial saccade eye position was calculated with the mean amplitude of 50 ms after velocity returned to baseline. Post-saccadic eye position was calculated with the mean amplitude of 50 ms, 200 ms after the initial saccade. 0 marks the onset of the target displacement.

Trials were averaged within each direction, and combined across directions for the purpose of statistical analysis.

The *dependent variables* for the eye saccade tests were the following:

- *Saccadic gain*: target amplitude divided by initial saccade amplitude, expressed as a ratio and interpreted as a measure of the initial saccadic accuracy.
- *Post-saccadic gain*: the percentage of accuracy resulting from dividing the target amplitude by the post-saccadic amplitude.
- *Saccade peak velocity*: the average peak velocity from all saccade trials, expressed in °/s.

Due to the difficulty in calculating the exact delay between the target movement and the recording of eye movement, the onset latencies and durations of the saccades where not included in this study.

2.3.4 Smooth pursuit analysis

The methods used for analyzing the smooth pursuit data are also described in previous work (Allum et al., 1998). EOG data was smoothed by applying a 15Hz dual pass 2nd order Bessel filter. The smoothed EOG signal was differentiated with an 8 point Remez exchange algorithm for calculating the eye velocity in the left and right directions, (labelled negative and positive respectively). Mean velocities were combined across directions for each target velocity (15, 20, and 30°/s) and divided by the corresponding stimulus velocity to obtain the gain. Therefore, the

dependent variables for the smooth pursuit trials were the *smooth pursuit gains* for each target speed (Figure 8).

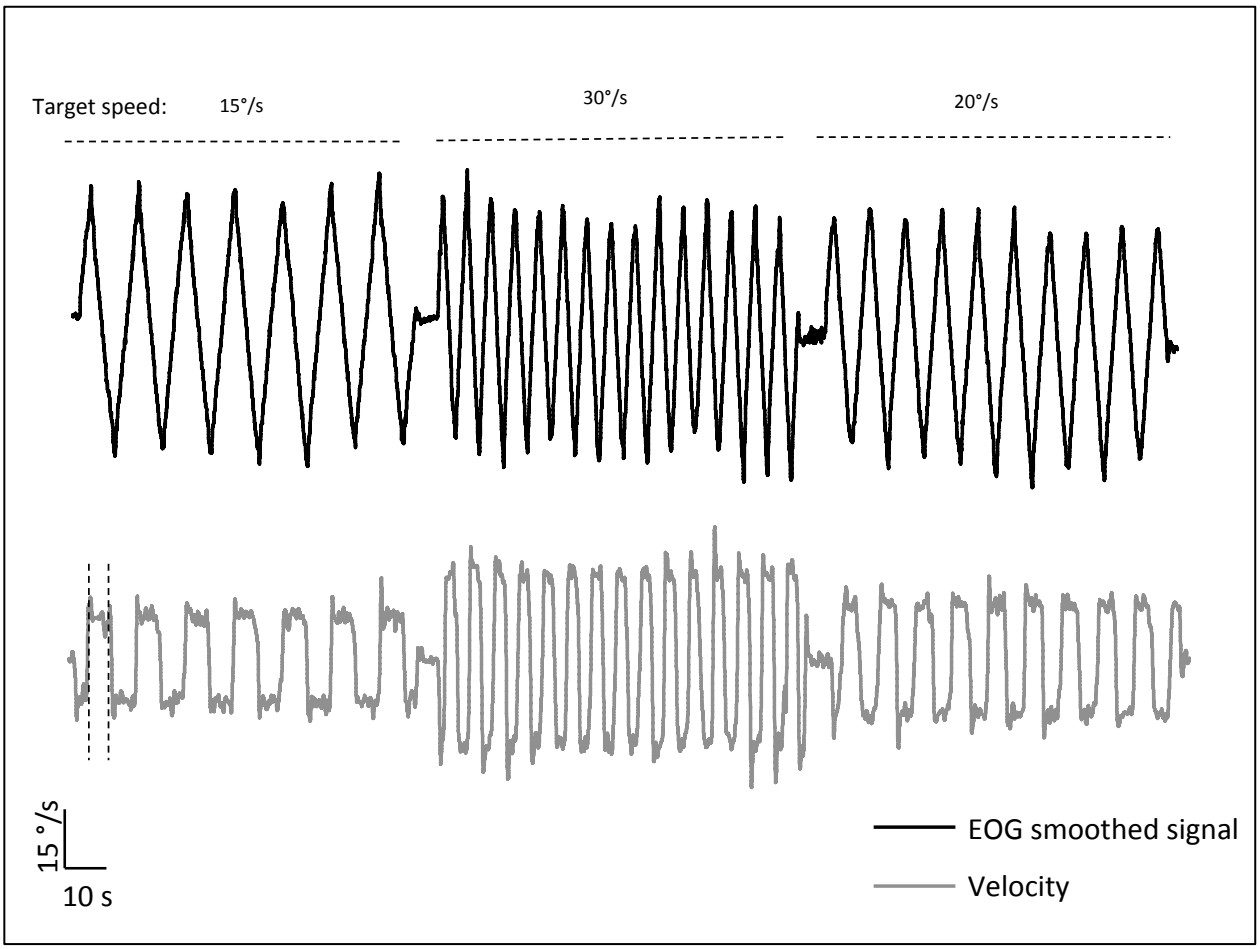

Figure 8. Smooth pursuit analysis. Traces in black represent the filtered electro-oculography (EOG) signal from an individual smooth pursuit test in response to the three different target velocities. Traces in grey represent the eye velocities for the same test. Means were obtained from all plateaus (dashed lines) to calculate an average eye velocity for each target speed trial and obtain a smooth pursuit gain.

2.3.5 Optokinetic nystagmus (OKN) analysis

Previously described methods (Allum et al. 1998) were used for analyzing the OKN responses. The EOG signal was smoothed using a 15Hz dual pass 2nd order Bessel filter before differentiating the signal by applying the previously described Remez exchange algorithm. The second process involved separating the slow phase velocity (SPV) from the fast phase velocity by applying a fast phase exclusion criteria through another algorithm. Once identified, the SPV signal was smoothed by a running average with exponentially weighted means. The mean SPV was calculated using 15 s of each direction and stimulus velocity. The combined mean SPV for each stimulus velocity (15, 30 and 60°/s) was divided by the true stimulus velocity to obtain 3 *OKN gains* (one for each speed trial) that were included as *dependent variables* for the OKN experiment (Figure 9).

2.3.6 Statistical analysis

Paired samples *t*-tests were used to compare differences between height conditions for all dependent measures in Study 2. Correlations between changes in psycho-social and autonomic measures and changes in saccade peak velocity and the different gains included as variables (saccade, post-saccade, smooth pursuit and OKN) were calculated using Pearson's Coefficient for those variables with a normal distribution and Spearman's Rho for those with a non-normal distribution. Normality was tested using the Shapiro-Wilk's test. All levels of statistical significance were set at $p = 0.05$.

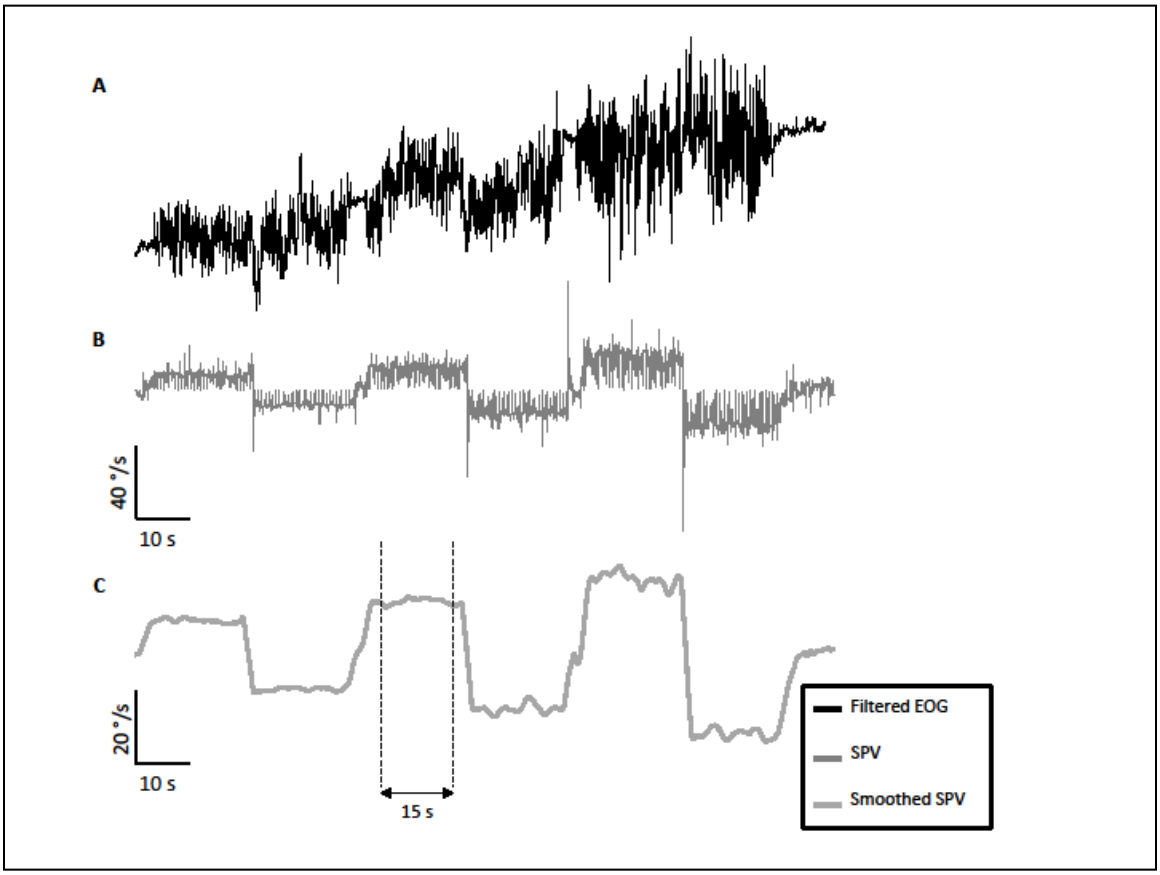

Figure 9. Optokinetic nystagmus (OKN) analysis. Traces in black represent the filtered electro-oculography (EOG) signal from an individual OKN test in response to the three different target velocities. Traces in dark grey represent the eye velocities for the same test. Traces in light grey represent the slow phase velocity (SPV) after applying the algorithm. Means were obtained from all plateaus (dashed lines) to calculate an average eye velocity for each stimulus speed trial and direction, to obtain a combined OKN Gain for each speed.

Chapter 3: Results

3.1 Study 1, experiment 1: VEMPs

Standing on the High compared to Low condition significantly increased EDA ($p = 0.007$), anxiety ($p < 0.001$) and fear ($p < 0.001$), and significantly decreased balance confidence ($p < 0.001$) and perceived stability ($p < 0.001$).

The average levels of tonic activation of each muscle in the Low condition (Table 1) were similar to those in previous work (Naranjo et al., 2015) and sufficient to elicit clear VEMP responses (Figure 3). Although BGA decreased in all muscles from Low to High (SCM = -4.3%; TRP = -2.7%; IO = -2.5%; SOL = -2.9%), the decrease in BGA was only statistically significant for SOL ($t(19) = 2.10$, $p = 0.049$).

3.1.1 VEMP latencies

Peak latencies for VEMPs in the Low condition (Table 1) are consistent with previously reported values for each muscle (Welgampola and Colebatch, 2005; Watson and Colebatch, 1998; Rusidill and Hain, 2008, Rosengren and Kigman, 2013; Naranjo et al. 2015). There were no significant changes in VEMP peak latencies between conditions.

	VEMP Average Peak Latencies				*Muscle Background Activity*	
	1st Peak Latency (ms)		***2nd Peak Latency (ms)***		***BGA (μV)***	
Muscle	***Low***	***High***	***Low***	***High***	***Low***	***High***
IO	10.10 ± 1.73	9.88 ± 1.45	15.75 ± 4.45	15.08 ± 2.53	3.08 ± 1.30	2.93 ± 1.20
SCM	13.76 ± 1.39	14.07 ± 1.78	22.61 ± 1.25	22.70 ± 1.90	30.83 ± 15.30	29.25 ± 14.91
TRP	17.38 ± 2.93	17.03 ± 2.77	26.31 ± 2.32	27.14 ± 2.85	18.53 ± 12.28	16.20 ± 12.15
SOL	76 ± 15.75	69.90 ± 5.35			27.59 ± 24.41	25.04 ± 21.31

Table 1. Mean VEMP peak latencies (for both peaks) and muscle background activity (BGA) with standard deviations for all the muscles investigated in both height conditions. Inferior Oblique (IO), Sternocleidomastoid (SCM), Trapezius (TRP) and Soleus (SOL).

3.1.2 VEMP amplitudes

There was a significant effect of height on VEMP amplitudes with average increases of 17.2% for the TRP ($t(11) = 2.38$, $p = 0.037$), 30.5% for the IO ($t(19) = 3.61$, $p = 0.002$) and 30.2% for the SOL ($t(19) = 2.10$, $p = 0.049$) in the High compared to Low condition (Figure 10). No significant changes were observed for the SCM ptp amplitude ($t(13) = 0.60$, $p = 0.56$) with an average increase of 4% between height conditions.

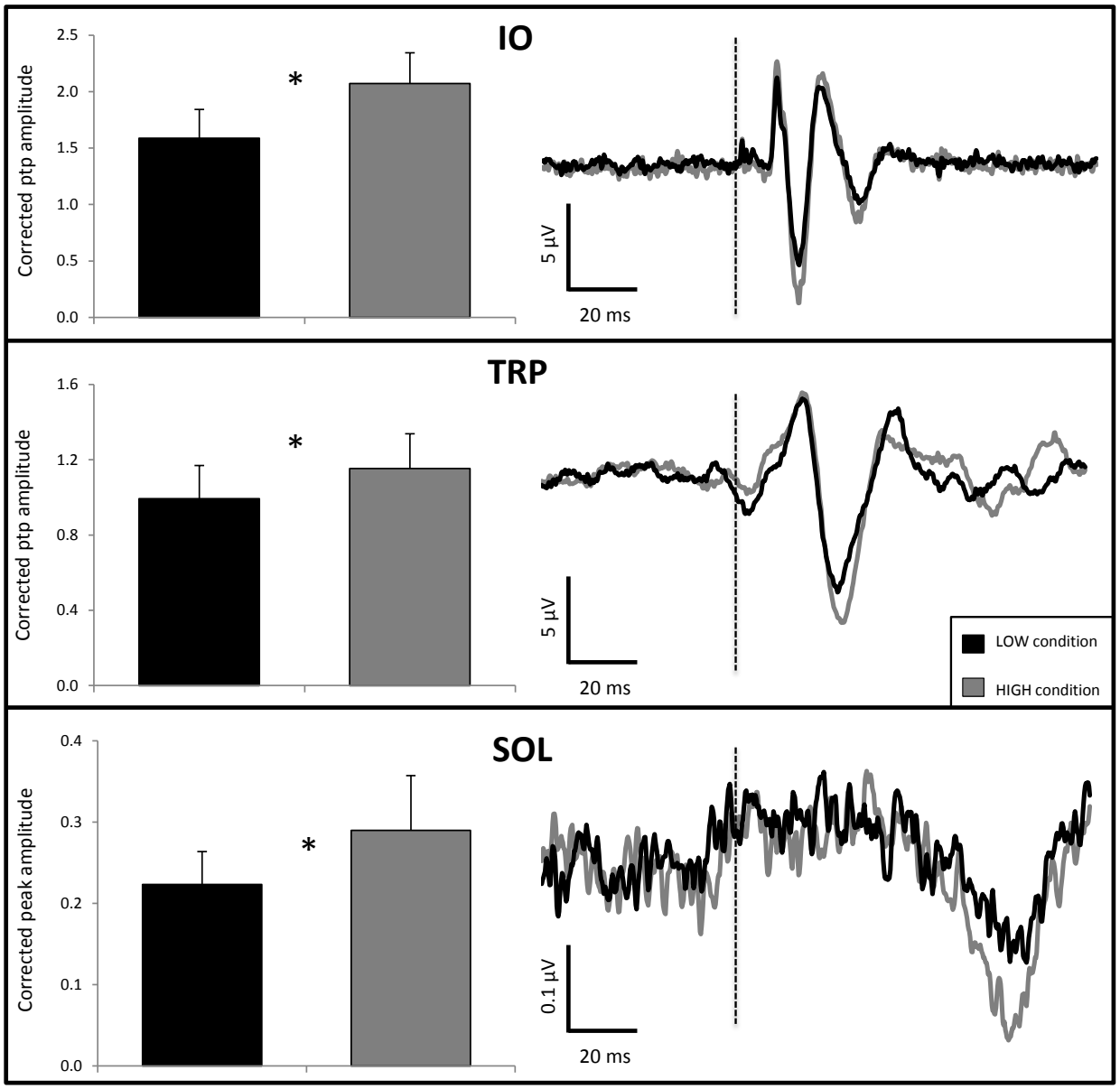

Figure 10. Height effect on vestibular Evoked Myogenic Potentials (VEMPs). Group averages in the 2 height conditions for VEMP responses from Inferior Oblique (IO), Trapezius (TRP) and Soleus (SOL). Black represents the Low condition and grey represents the High condition. The bar graphs in the left set of columns show the group means and standard errors of VEMP amplitudes corrected to background activity. The plots to the right show the averaged VEMP response for both conditions. (*): significant difference ($p < 0.05$).

3.1.3 Correlations between emotional changes and changes in VEMP amplitude with height

Increases in IO ptp amplitude with height were significantly correlated with increases in EDA (*Rho* = 0.44, *p* = 0.028) (Figure 11A), and increases in fear (*Rho* = 0.43, *p* = 0.03) (Figure 11B). Changes in EDA with height were also significantly correlated with changes in SCM ptp amplitude (*Rho* = 0.59, *p* = 0.014), and approached a significant correlation with increases in SOL peak amplitude (*Rho* = 0.37, *p* = 0.058) (Figure 11D).

These correlations do not seem to be related to changes in BGA, as a significant negative correlation (i.e. in the opposite direction) was found between changes in IO BGA and changes in EDA (*Rho*=-0.40, *p*=0.046) (Figure 11C) and changes in fear (*Rho*=0.63, *p*=0.39), and between changes in SCM BGA and changes in EDA (*Rho*=-0.20, *p*=0.23, respectively).

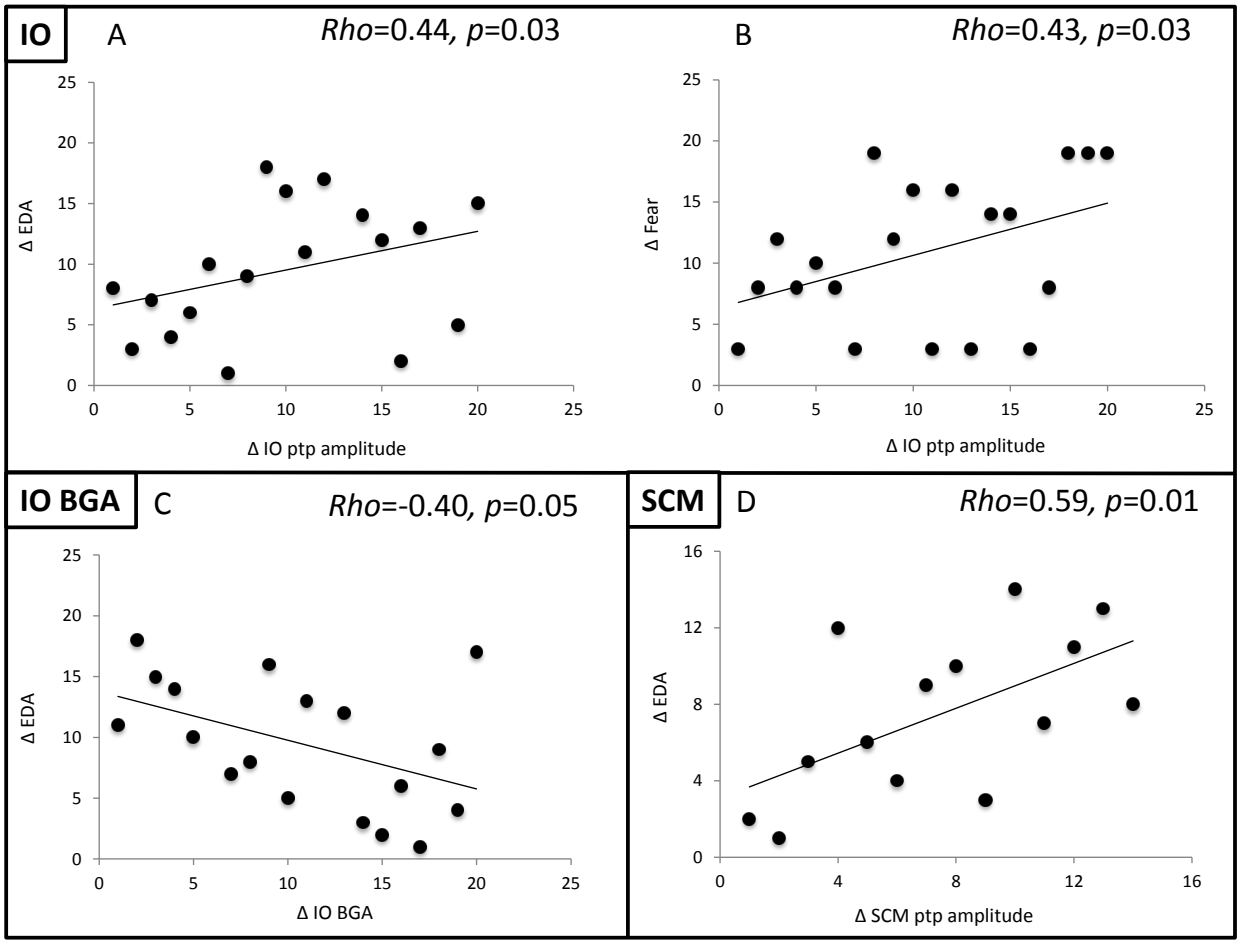

Figure 11. Significant correlations from Experiment 1, Study 1. between changes in corrected Inferior Oblique (IO) VEMP amplitudes and changes in electrodermal activity (EDA) (A) and changes in fear (B), from Low to High. Significant negative correlation between changes in IO muscle background activity (IO BGA) and changes in EDA (C). Significant correlation between changes in corrected Sternocleidomastoid (SCM) VEMP amplitude and changes in EDA (D). All values were ranked.

3.2 Study 1, experiment 2: vHIT

Despite the modifications in the set-up for this second experiment (i.e. having the subjects stand between 2 hand rails and having the experimenter holding the subject's head with his hands), similar changes in psycho-social and autonomic measures were observed between heights in Experiment 2, as observed in Experiment 1. For the horizontal head impulses there was a significant increase in EDA ($p = 0.004$), fear ($p = 0.004$), and anxiety ($p = 0.003$), and a significant decrease in perceived stability ($p < 0.001$) in the High compared to Low condition. Likewise, for the vertical head impulses, there was a significant increase in EDA ($p = 0.01$), fear ($p = 0.002$) and anxiety ($p = 0.008$), and a decrease in stability ($p = 0.005$) observed between height conditions. Confidence in maintaining balance, which was measured before each height condition, decreased from Low to High ($p = 0.007$).

3.2.1 Height effect on the functional VOR gains

All the VVOR and the HVOR gains for each direction are expressed in Table 2. For the combined gains, significant increases with height were observed for the HVOR gains ($t(17) = 2.20$, $p = 0.04$) and VVOR gains ($t(17) = 3.17$, $p = 0.006$) (Figure 12).

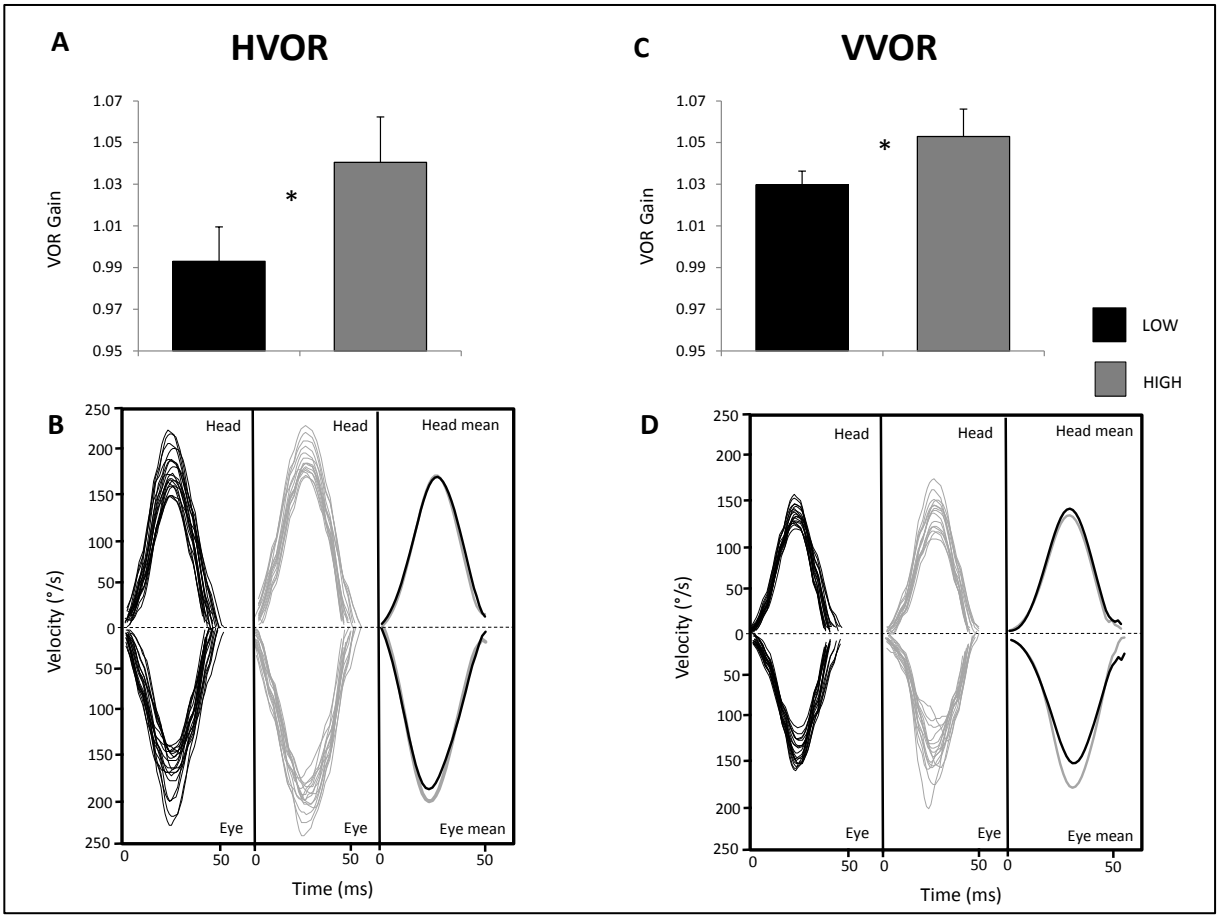

Figure 12. Height effect on video head impulse test (vHIT) gains. Group averages for vestibulo-ocular reflex (VOR) gain responses. Black represents the Low condition and grey represents the High condition. Bar graphs represent the group means and standard errors for the Horizontal VOR (HVOR) (A) and Vertical VOR (C) (VVOR). The plots in the bottom represent a response from a single subject, with the head velocity traces, eye velocity traces and the mean of all traces, at the 2 height conditions (Low at the left in black, High at the right in grey), in the yaw plane (B) and the pitch plane (D). (*): significant difference ($p < 0.05$).

vHIT VOR gains according to head thrust direction				
	Horizontal VOR gain		***Vertical VOR gain***	
Head direction	***Low***	***High***	***Low***	***High***
Positive	1.03 ± 0.11	1.07 ± 0.10	1 ± 0.40	1.03 ± 0.08
Negative	0.95 ± 0.06	1.01 ± 0.10	1.05 ± 0.25	1.08 ± 0.05

Table 2. Vestibulo-ocular Reflex (VOR) gains and standard deviations from the video head impulse test (vHIT) in the horizontal and vertical planes for each head thrust direction, positive and negative, and for each height condition.

3.2.2 Correlations between changes in VOR gains and changes in autonomic arousal

A significant positive correlation was found between changes in EDA and changes in HVOR gain (*Rho* = 0.44, $p = 0.03$) (Figure 13A). In contrast, no significant correlations were found between changes in EDA and changes in the VVOR gain (*Rho* = 0.24, $p = 0.17$), or any other relationships between HVOR or VVOR and other psycho-social outcomes (fear, anxiety, stability and confidence).

3.3 Comparing physiological and functional measures of the VOR

Although it was not a main goal of this thesis, direct correlations were performed between measures of the VOR response (oVEMPs), and functional VOR outcome (vHIT gains). Even with the 2 measures taking place in different sessions, a strong significant positive correlation

was found between changes in the oVEMP ptp amplitude and changes in VVOR gain with height (*Rho* = 0.55, *p* = 0.02) (Figure 13B).

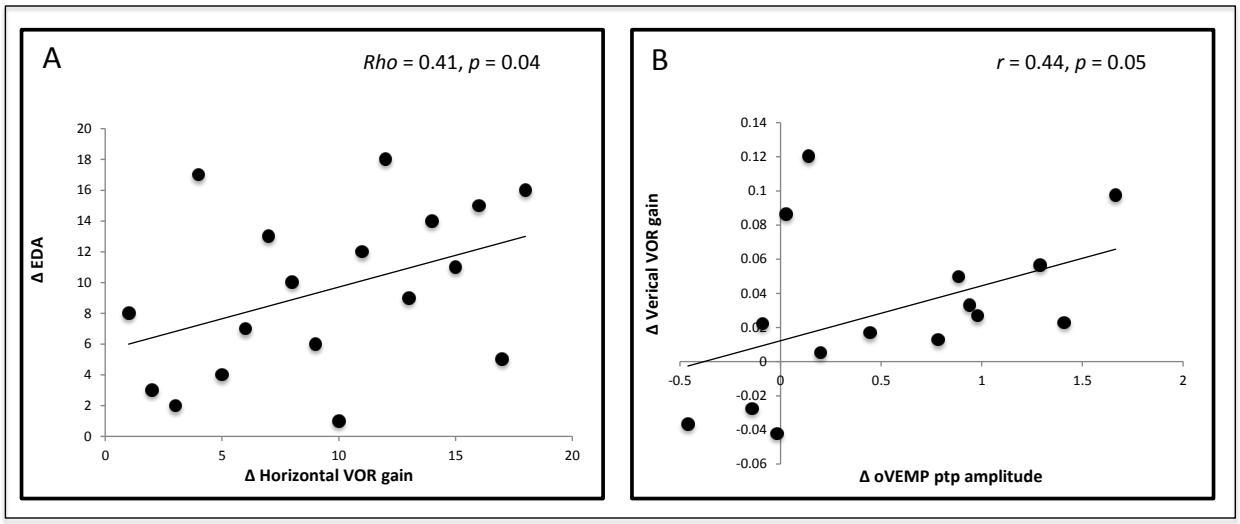

Figure 13. Significant correlations from Experiment 2, Study 1. A) Significant correlation between changes in Horizontal Vestibulo-ocular Reflex (HVOR) gain and changes in electrodermal activity (EDA) from Low to High. B) Significant correlation between changes in corrected Inferior Oblique (IO) VEMP amplitude and HVOR gain from Low to High.

3.4 Study 2

Placing a screen in front of the subjects and conducting the experiment in slight darkness did not reduce the effects of increased surface height on emotions, with significant increases observed in reported fear of falling ($p = 0.001$), anxiety ($p < 0.001$), confidence in maintaining balance ($p <$

0.001) and perceived stability ($p < 0.001$)) in High compared to Low conditions. Although psycho-social measures were pooled across the 3 OMF tests, EDA was obtained during each OMF test independently. EDA significantly increased with height during the eye saccades ($p = 0.001$), smooth pursuit ($p = 0.003$) and OKN ($p = 0.006$) trials.

Two subjects demonstrated an inability to correctly perform the saccade trials in the Low condition, which prevented accurate calibration of the eye position for any OMF test. Therefore, these subjects were removed from the analyses, leaving 18 remaining subjects for Study 2.

3.4.1 Eye saccades

The post-saccadic gain was significantly larger in the High versus the Low condition ($t(17) = 2.14$; $p = 0.047$), with a 4.8% increase. A similar trend, (but with no statistically significant change), was observed in the initial saccade ($t(17) = 2.02$; $p = 0.059$), with an average increase of 4.6% from Low to High. The average saccade peak velocity was also significantly higher in the High condition ($t(17) = 2.34$; $p = 0.03$), increasing from 368.9 to 387.9°/s, with an average 5.1% increase (Figure 14).

A significant correlation was found between changes in the reported levels of fear and changes in the saccade peak velocity ($Rho = 4.02$, $p = 0.05$). The correlation between changes in perceived stability and changes in saccade peak velocity was approaching statistical significance ($Rho = -3.90$, $p = 0.055$) (Figure 17A).

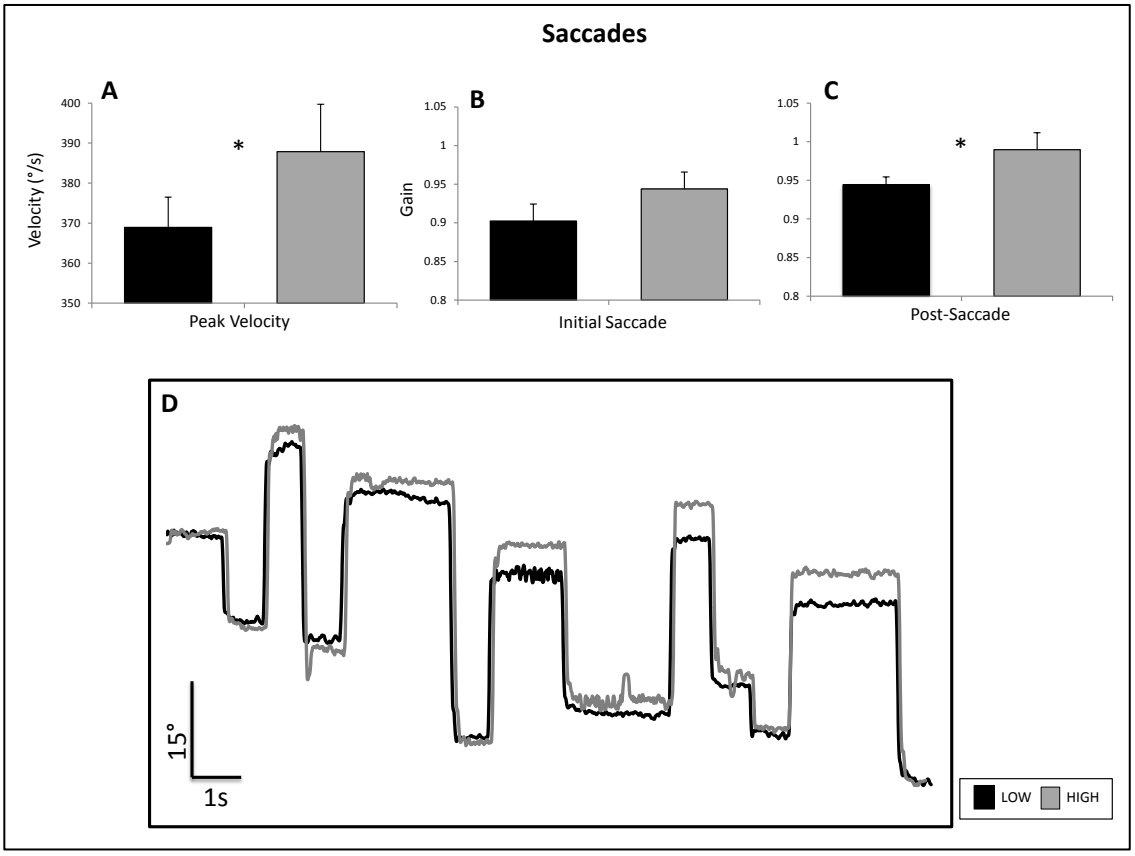

Figure 14. Height effect on eye saccade gains and velocities. Black represents the Low condition and grey represents the High condition. Bar graphs represent the group means and standard errors for the Saccade Peak Velocity (A), Initial Saccade gain (B) and Post-Saccade gain (C). The traces in the bottom (D) represent an individual response for 12 Saccade trials at the 2 height conditions. (*): significant difference ($p < 0.05$).

3.4.2 Smooth pursuit

The smooth pursuit gains are reported for each stimulus direction and height conditions in Table 3. The *t*-Test revealed a significant increase from Low to High in the smooth pursuit gain from 0.91 to 0.99 (an increase of 9.1%) for the 15°/s target speed ($t(17) = 2.65$; $p = 0.02$), and from

0.82 to 0.89 (an increase of 6.8%) for the 20°/s target speed ($t(17) = 2.34$; $p = 0.03$), combining both directions. For the 30°/s target speed, the observed increase in combined eye velocity at height, from 0.81 to 0.87 (7.5% increase) approached statistical significance ($t(17) = 2.03$; $p = 0.058$). The average smooth pursuit gain calculated across the 3 target speeds revealed a statistically significant increase at height from 0.87 to 0.94 (7.8% increase) ($t(17) = 2.63$; $p = 0.02$) (Figure 15).

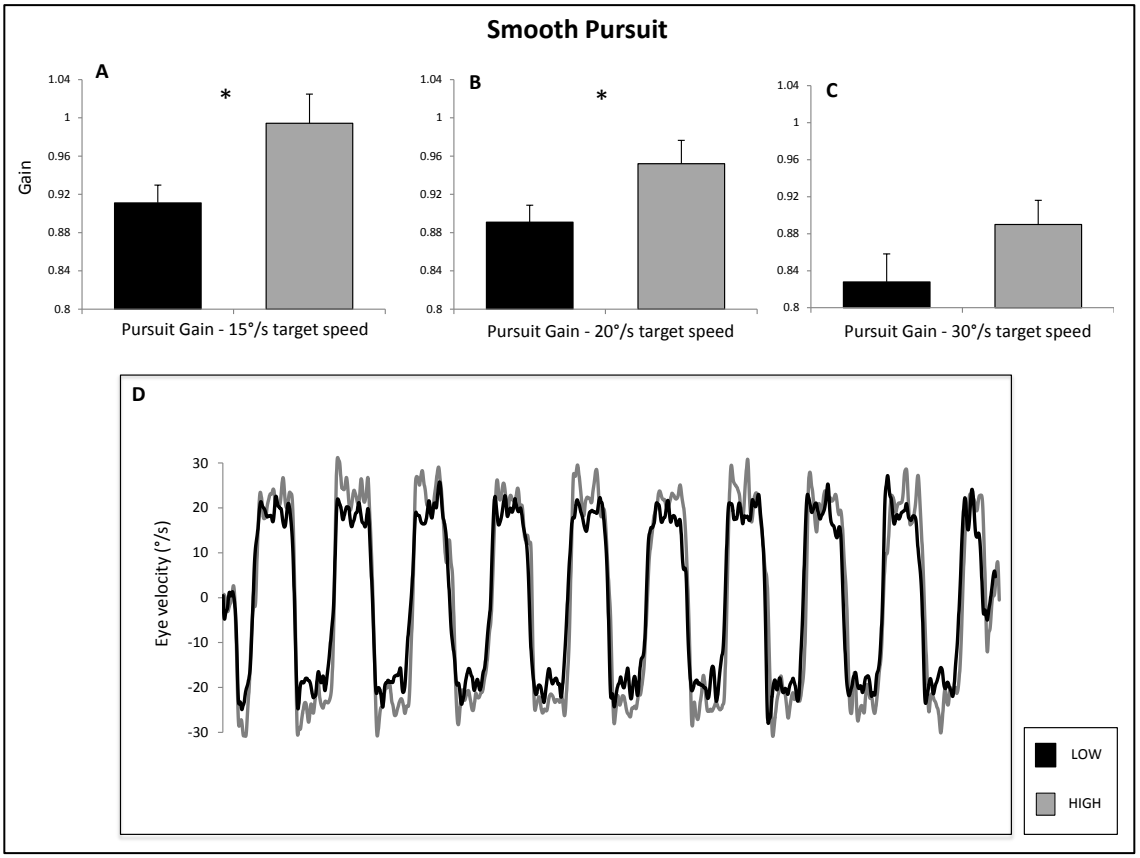

Figure 15. Height effect on smooth pursuit gains. Black represents the Low condition and grey represents the High condition. Bar graphs represent the group means and standard errors for the 3 different target speed trials: 15 (A), 20 (B) and 30°/s (C). The traces in the bottom (D) represent an individual response for a 20°/s trial, comparing the eye velocity traces at the 2 height conditions. (*): significant difference ($p < 0.05$).

Smooth pursuit gains according to stimulus direction (°/s)						
	Low	***High***	***Low***	***High***	***Low***	***High***
Direction	***15 °/s Target speed***		***20 °/s Target speed***		***30 °/s Target speed***	
Left	13.70 ± 1.14	14.92 ± 1.89	17.83 ± 1.54	19.02 ± 2.12	24.92 ± 4.02	26.44 ± 3.40
Right	13.64 ± 1.30	14.90 ± 2.04	17.80 ± 1.52	19.06 ± 2.14	24.75 ± 3.75	26.95 ± 3.40
OKN mean slow phase eye velocities according to stimulus direction (°/s)						
	15 °/s Stimulus speed		***30 °/s Stimulus speed***		***60 °/s Stimulus speed***	
Left	14.21 ± 1.43	15.77 ± 2.40	24.03 ± 2.63	25.60 ± 3.83	27.60 ± 8.84	30.79 ± 7.68
Right	14.54 ± 1.40	16.27 ± 2.80	24.49 ± 2.72	25.89 ± 4.06	31.29 ± 7.89	35.64 ± 8.41

Table 3. Smooth pursuit gains (above) and optokinetic nystagmus (OKN) gains (bottom) with standard deviations for each stimulus velocity investigated and for each height condition.

There was a significant correlation between changes in the eye velocity with a 30°/s target speed and changes in EDA (*Rho* = 0.46, $p = 0.04$) (Figure 17B).

3.4.3 Optokinetic nystagmus

The OKN gains in the Low condition for 15 and 30°/s trials were close to 1, with gain values of 0.95 and 0.81 respectively. In contrast, the OKN gains in the Low condition for the fastest target speed trial (60°/s) were low (0.52) and the velocities showed a high variability. The data was

included in the study as results were similar for the High condition and reduced gains with high stimulus velocities have been reported elsewhere (Koenig et al., 1978).

The average eye SPV for each stimulus direction and height condition are presented in Table 3. The combined OKN gains were significantly increased with the 3 different stimulus speeds, from 0.95 ± 0.08 to 1.07 ± 0.17 for the 15°/s trial ($t(17) = 3.22$; $p = 0.005$), from 0.81 ± 0.08 to 0.86 ± 0.13 for the 30°/s trial ($t(17) = 2.11$; $p = 0.05$) and from 0.52 ± 0.11 to 0.58 ± 0.09 for the 60°/s trial ($t(17) = 3.09$; $p = 0.007$). The percentages of change from the Low to the High condition were of 11.4% for the 15°/s trial, 6.1% for the 30°/s trial and 11.5% for the 60°/s trial, respectively (Figure 16).

A significant correlation was found between changes in the combined OKN gain for the 15°/s stimulus speed and changes in EDA ($Rho = 0.45$, $p = 0.03$). A marginally significant correlation between changes in the combined OKN gain for the 30°/s stimulus speed and changes in EDA ($Rho = 0.35$, $p = 0.08$) was observed (Figure 17C).

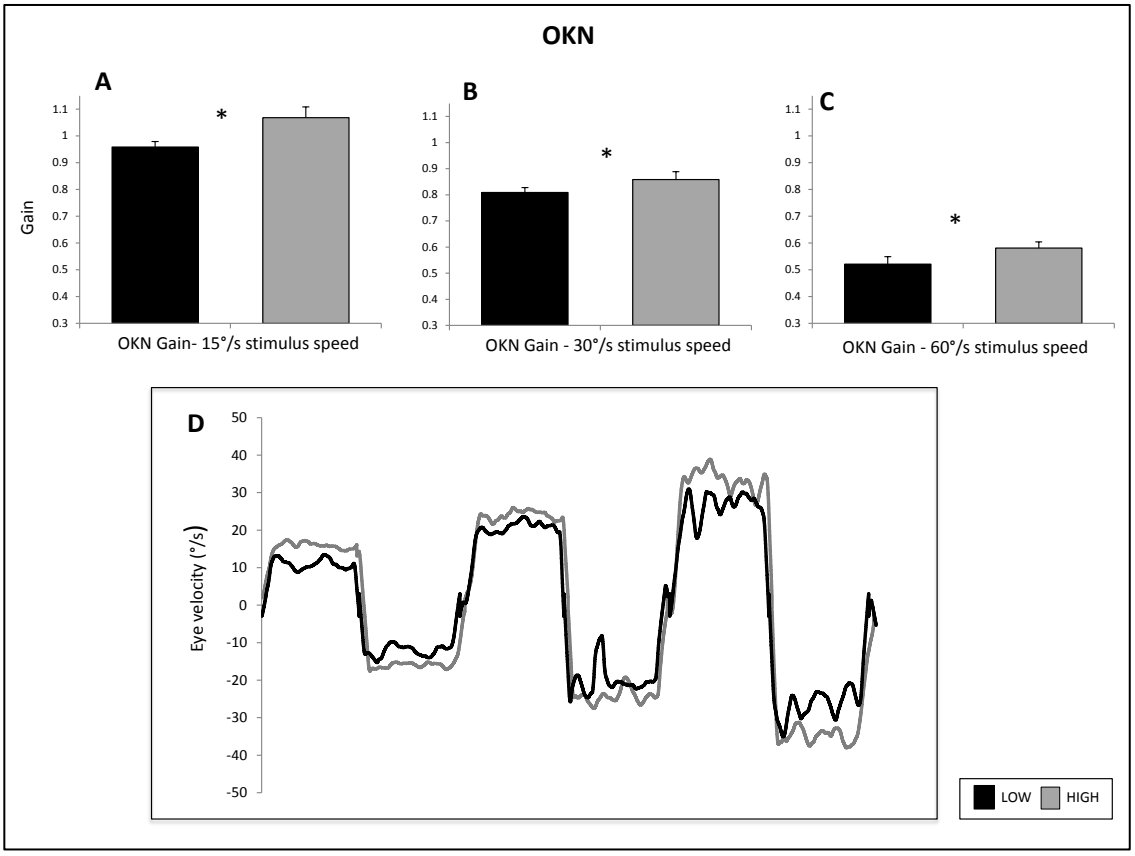

Figure 16. Height effect on the optokinetic nystagmus (OKN) gains. Black represents the Low condition and grey represents the High condition. Bar graphs represent the group means and standard errors for the 3 different target speed trials: 20 (A), 30 (B) and 60°/s (C). The traces in the bottom (D) represent an individual response for an entire OKN test, comparing eye slow phase velocity (SPV) traces at the 2 height conditions. (*): significant difference ($p < 0.05$).

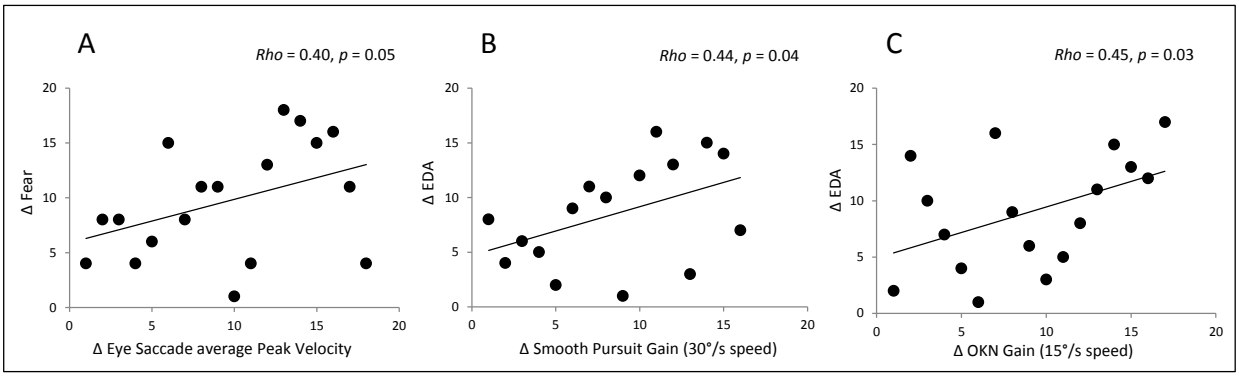

Figure 17. Significant correlations from Study 2. A) Significant correlation between changes in Eye Saccade average Peak Velocity and changes in fear from Low to High. B) Significant correlation between changes in Smooth Pursuit Gain and changes in electrodermal activity (EDA) from Low to High. C) Significant correlation between changes in Optokinetic Nystagmus (OKN) Gain and changes in EDA from Low to High.

Chapter 4: Discussion

The first aim of this thesis was to investigate the effects of height-induced negative emotions such as anxiety and fear on the vestibular reflex response elicited using VEMPs. In addition to replicating prior results of threat-induced changes cervical and leg VEMPS, the first Study of this thesis was designed to extend the investigation to eye reflexes, as well. The second aim was to compare these effects with a more functional measurement of VOR using the vHIT. The third aim of the thesis was to investigate the extent to which the oculomotor nuclei pathways may contribute to the potential increased gain in the physiological and functional VOR outcomes under the same threatening conditions.

The studies within this thesis represent a number of novel elements. To my knowledge, these studies are the first to elicit and record VEMPs simultaneously from eye, neck and lower limb muscles, representing both the VOR and VSR pathways, during a quiet standing task. They are also the first to examine the effects of height-induced states of anxiety, fear and arousal on the VOR, and to conduct a HIT under those induced emotional states. Furthermore, these studies are the first to examine the effects of state anxiety on reflexive and voluntary aspects of OMF (Staab, 2014).

As expected, manipulating surface height had a significant effect on subjects' reported levels of fear and anxiety, as well as on their perceived stability and confidence in maintaining balance at that height. Those experiences were also accompanied by increases in autonomic arousal, measured by EDA. These findings are consistent with prior studies that have used real and

virtual heights (Cleworth et al., 2012) to induce negative emotions in order to investigate the effects of postural threat on balance responses and underlying sensory function (Adkin et al., 2000; Carpenter et al., 2001; Davis et al., 2009; Horslen et al., 2013; Horslen et al., 2014).

4.1 Effects of postural threat on the vestibulospinal reflexes

VEMPs were elicited from 2 neck muscles (SCM and TRP) and from the SOL, under different height conditions. Subjects performed a simple quiet standing task with the head slightly turned to the left and the neck slightly flexed, thus sustaining small levels of tonic activation in order to avoid fatigue and potential saturation effects while VEMPs were recorded (McCaslin et al., 2014). Although levels of BGA were relatively low, VEMP responses were elicited with similar waveforms, amplitude and latencies to those reported previously (Welgampola and Colebatch, 2005; Rosengren et al., 2010; Naranjo et al. 2015).

A significant increase in the normalized VEMP peak amplitude of the SOL was observed, suggesting an effect of postural threat on the gain of the VSR. This finding replicates the results of previous research (Naranjo et al., 2015), in which a significant height effect was observed in the normalized VEMP amplitude in SOL using similar methods, albeit with a slower delivery rate of the ACS stimuli. The slower delivery rate was previously justified based on observed biomechanical effects of VEMPs on antero-posterior ground-reaction moments of force that persisted for approximately 1.2 s. However, the higher stimulus delivery rate (5 Hz) with similar levels of BGA is not a likely confound since the current thesis found similar latencies and amplitudes for the n1 peak in the SOL (Naranjo et al., 2015).

One important addition of this thesis, compared to Naranjo et al. (2015), is the inclusion of the TRP as another measure of the cervical VEMP. Although present in fewer subjects, the VEMPs in this muscle were clearly identified, despite smaller BGA levels than SCM. VEMPs in TRP muscle were first described by Bickford et al. (1964); however prior recordings were from the inion, thus at a higher spinal level than used in the current study. This difference in recording site likely accounts for the slightly earlier peak latencies reported in Bickford et al. (1964) for both the first peak (approximately 12 ms compared to 17 ms) and second peak (approximately 24 ms compared to 26 ms). Previous findings for other neck muscles (Naranjo et al., 2015) were replicated in Study 1 in the TRP, with a significant increase in the VEMP ptp amplitude from Low to High and a significant correlation with changes in reported levels of fear at height.

Ipsilateral SCM VEMP p13 and n23 latencies and ptp amplitudes were similar to those previously described in research and clinical literature (Welgampola and Colebatch, 2005; Rosengren et al., 2010). Although clear responses were elicited in most of the subjects, there were no significant changes in the ptp amplitudes in SCM between height conditions; this result contrasts with prior observations (Naranjo et al., 2015). While significant changes in SCM VEMP amplitudes between heights were not observed, there was however a significant correlation between changes in p13-n23 amplitude and changes in fear of falling, which supports prior results (Naranjo et al., 2015). The lack of significant changes in SCM observed between heights in the current study is likely due to smaller mean differences, and a smaller sample size, in the current study. Alternatively, the differences could be due to the method of activation of this muscle between studies. Subjects added 30° neck flexion to the neck rotation previously

used in order to activate the trapezius muscle in this study. Despite higher levels of BGA in the SCM in Study 1 (38.83 μV), compared to Naranjo et al. (2015) (23.62 μV), this position could potentially have led to a less important role of the SCM in maintaining head posture against gravity. Naranjo et al. (2015) postulated that the effects of height on the VSR gain were only observed in those muscles actively engaged in a postural task. As such, the flexion of the neck and head could have transferred the postural role of head stabilization to other head and neck muscles like the TRP, thereby enhancing the VCR function in TRP and causing the significant increase in VEMP amplitude between conditions. Furthermore, subjects in Study 1 showed a more variable and less robust response to height than in the previous studies, with 35.4% increases in EDA in the present study, compared to the 50.8% in Naranjo et al. (2015). This might explain the absence of significant changes in the SCM between conditions, which would impact statistical *t*-Tests more than correlations.

As hypothesized, changes in VSR gain under threatening conditions were observed in the evoked responses from the ipsilateral TRP and SOL. The significant increases in LcVEMP and legVEMP amplitudes suggest a higher VSR gain in those muscles involved in head stabilization, and body's postural control, with induced state anxiety and fear of falling.

As mentioned above, VEMP amplitude is linearly and positively related to BGA. However, at low activation levels, such as those maintained by the subjects in the first study of this thesis, the relationship is not significantly linear (Bogle et al., 2013), and small changes in BGA could potentially lead to higher increases in VEMP ptp and peak amplitudes. The fact that BGA decreased (significantly, in the case of SOL) from Low to High provides evidence against

changes in VEMP amplitudes being related to height-induced changes in BGA. The relatively small negative changes in BGA could be explained by methods used to keep BGA constant, including: the online control of the subjects' leaning through the medio-lateral moment of force and instructing those subjects who leaned away from the edge to actively lean towards it. Furthermore, changes in SCM BGA showed a negative non-significant correlation with EDA, this is in the opposite direction to the positive significant correlation observed between changes in this muscle's ptp amplitude and EDA.

The increases in SOL VEMP amplitude in Study 1 of the present thesis and Naranjo et al. (2015) are consistent with previous observations of larger vestibular-evoked balance response gain with height-induced postural threat. In Horslen et al. (2014), subjects were stimulated with SVS and changes in the resultant ground reaction forces between height conditions (0.8m and 3.2 m) were examined; they observed a higher coherence and gain between SVS inputs and ground reaction force outputs. Likewise, Lim (2014) used SVS to investigate the effects of threat on the VSR during the threat of postural perturbation, finding an increased VSR gain when subjects were threatened, compared to standing quietly. In contrast, Osler et al. (2013) used square-wave GVS to stimulate the vestibular system and generated a postural threat by having subjects stand on a beam 3.85m above ground. Osler et al. (2013) concluded that postural threat did not affect the early feedforward component of vestibular-evoked balance responses. Apart from the use of GVS, which might be predictable, one important limitation of this study is the reliance on upper-body kinematics, which may not accurately reflect underlying neuromuscular changes, particularly in the lower-limb. Furthermore, the time period selected for the later corrective

responses (>800 ms) is likely influenced by multisensory feedback of the initial vestibular-evoked body movement.

4.2 Effects of postural threat on the physiological measure of the VOR: oVEMPs

The results from Study 1 also indicate that increases in the VOR gain may occur with threat, as evidenced by the increase in oVEMPs at height. Specifically, a significant increase in the normalized oVEMP ptp amplitude was observed from Low to High. Muscle BGA, n10-p15 ptp amplitudes and peak latencies at the Low condition were consistent with previous work (Rosengren and Kigman, 2013). Multiple significant correlations were found between changes in this amplitude and changes in psycho-social and autonomic measures of EDA, anxiety and fear. These associations were strong and explain up to the 35% of the variance. Other correlations close to significance were observed for decreases in perceived stability and confidence in maintaining balance.

Similar to TRP and SOL, the effects of threat on oVEMP amplitude cannot be explained by changes in tonic BGA, as the averaged IO BGA decreased (non-significantly) between conditions, and the negative correlations between changes in IO BGA and changes in fear, anxiety and arousal are opposite to the positive correlations observed between IO ptp amplitude and the same psycho-social variables.

As mentioned above, this is the first time that a direct physiological measure of the otolithic-originated component of the VOR pathway has been recorded under induced states of anxiety.

However, given the moderate effect size, and different pathways involved, it is not clear whether the observed state-related changes in the physiological vestibular reflex response measured using VEMPS may have any significant impact on the functional output of the VOR.

Therefore, the main goal of Experiment 2 was to confirm the results from Experiment 1 by examining the VOR function in response to a natural stimulus of the vestibular receptors (otoliths and SSC) through fast head movements, involving different pathways.

4.3 Effects of postural threat on the functional measure of the VOR: vHIT gains

The VOR serves the function of adjusting gaze after movements of the head, and therefore the response pathway involves both the vestibular and oculomotor nuclei complexes. A reliable method for evoking the VOR is with the HIT, where a vHIT can be used to measure eye movements and velocities resulting from rapid head thrusts. The vHIT is commonly used to assess SSC function, where HSSCs are stimulated with head impulses in the yaw plane, and the ASSC and PSSC with head thrusts in oblique planes. Functional otolith responses have previously been assessed with OVAR (Hain, 1986), but head impulses in the pitch plane have not been reported. Therefore, a unique aspect of this Study is the investigation of vertical VOR in response to head impulses in the pure pitch plane. An advantage of this technique is that pure pitch impulses will stimulate the otoliths as well as the SSCs, and therefore make results more comparable to the otolithic oVEMP.

A significant effect of height on the VOR gain was observed for the horizontal and vertical head movements. The results show that postural threat had an effect on the VOR gains for both planes, meaning that the compensatory eye movements are modulated in a similar way regardless of the vestibular receptor stimulated. In addition, changes in the horizontal VOR gain were significantly correlated with changes in EDA, suggesting an association between arousal and VOR function.

Overall, the results from Study 1 confirm the hypotheses that induced negative emotions would impact both physiological and functional VOR outcome measures. It is not clear however why changes in the vertical VOR gain did not correlate with changes in EDA, or why changes in neither of both vertical or horizontal VOR gains did not correlated with changes in the other indicators of psycho-social state.

Nevertheless, it is not known if the effects of height on the gain of the normal VOR function measured with vHIT are a result of fear and state anxiety, or of an increased level of vigilance. VOR gain is understood to increase with the level of vigilance or alertness, in which a level of arousal is implied. The horizontal VOR gains showed an association with EDA, and not with other psycho-social measures. Considering arousal as a measure of the state of vigilance (Oken et al., 2006), this effect could be interpreted as the result of a general increase in the state of alertness at height.

This thesis also provided an opportunity to correlate changes in both VOR outcomes for the very first time within subjects, across days. Despite obtaining the oVEMP and vHIT measures in

different sessions, changes in oVEMP amplitudes significantly correlated with changes in the VVOR gain measured through the vHIT, but not with changes in the HVOR. This result was expected because the otolith organs are involved in both tests, making VVOR gains more comparable to oVEMP amplitudes than the HVOR.

The observed effects of threat on VVOR and HVOR gain are consistent with prior results on VOR gain changes associated with trait anxiety (Furman et al., 2006), mental stress (Yardley et al. 1995) and sleep deprivation (Quark et al. 1996). Yardley et al. (1995) observed an increase in the slow velocity phase of both the caloric and post-rotary nystagmus in people with chronic anxiety disorders, compared to healthy controls, interpreted as an increase in the VOR gain. Furman et al. (2007) extended this field with a study investigating off-vertical axis rotation otolith VOR responses in subjects with chronic anxiety that also suffer from space and motion discomfort. Their results also suggested an increase in the gain of the VOR measured through EVAR and OVAR in this type of population. Quarck et al. (1996) investigated VOR gains on subjects deprived of sleep and observed an increased VOR gain when using rotations that abruptly changed velocities, due to the threat to posture generated by this type of stimulation. The observed size of the height effect on the VOR gain from the vHIT tests, ranging from 2.3 to 4.3 %, fall within previously reported ranges of VOR change; there was a 2 to 4% increase in VOR for subjects with trait anxiety compared to healthy controls (Furman et al., 2006), approximately 12% increase in the post-rotatory nystagmus mean slow phase velocity for subjects induced to mental stress compared to minimal alerting (Yardley et al., 1995) and 16% increase in the VOR gain for healthy persons subjected to sleep deprivation (Quarck et al., 2006). Differences may be explained by the differing stimuli; compared to rotary chair tests, the vHIT

induces an VOR in unexpected directions with higher head velocities that are argued to better represent the natural eye compensation required for normal head movements (Allum and Honegger, 2013). Alternatively, the differences may be explained by the type of anxiety; trait anxiety (Yardley et al., 1995; Furman et al., 2006), which refers to a general level of stress that is characteristic of an individual (that is, a trait related to personality), and state anxiety (Yardley et al., 1995; Quarck et al., 1996), as state of heightened emotions that develop in response to a fear or danger of a particular situation (Spielberger, 1985). The higher increase in the oVEMP amplitude (30.5%) at height could be explained by a higher sensitivity of the direct physiological measure provided by this technique.

The fact that similar changes in VOR gain have been observed in subjects with state and trait anxiety, and between different manipulations of arousal through threat, stress, or sleep deprivation, suggests a potential common element involved in the neural modulations of vestibular function.

4.4 Neural modulation of the vestibular reflexes during height-induced postural threat

Horslen et al. (2014) and Naranjo et al. (2015) proposed a model of central modulation of the vestibulospinal reflexes under situations of height-induced state anxiety, based on the described connections between the vestibular system and emotional processing areas of the brain, in which the amplification would be directly localized at the vestibular nuclei complex level. Since the influence of state anxiety on the VOR and its different pathways was never investigated, we can now expand the previous model with results from the 3 different vestibular reflexes.

There is consistent neuroanatomical evidence from animal models showing strong excitatory inputs from neural regions involved in processing emotional and affective responses to the vestibular nuclei and these projections have been proposed as the neural mechanisms for changes under postural threat (Balaban and Thayer, 2001; Balaban, 2002; Staab et al., 2013). The parabrachial nucleus, which processes convergent vestibular, somatic, and visceral information to mediate avoidance conditioning, anxiety and conditioned fear responses, projects to the medial, inferior and superior vestibular nuclei (Staab et al., 2013). The vestibular nuclei also receive noradrenergic projections from the locus coeruleus, via the coeruleo-vestibular pathway, and serotonergic projections from the dorsal raphe nucleus and the nucleus raphe obscurus (Halberstadt and Balaban, 2013). These connections could explain the influence that emotions have on vestibular function, at three potential levels of modulation: the peripheral vestibular receptors via efferents, the vestibular nuclei, and the spinal cord.

It has been demonstrated that the peripheral vestibular receptors receive innervation from efferent cell groups located in the brainstem immediately lateral to the abducens nucleus (Rasmussen and Gacek, 1958). These cell groups could at the same time have connections with the anxiety pathways. Although it was thought that this efferent system could modulate the resting firing discharge of hair cells during active head movements, studies on animal models have shown a similar activity in vestibular neurons when comparing responses to active and passive head rotations (Cullen and Minor, 2002), leaving the role of these vestibular efferents still unknown. Recent findings using SVS (Horslen et al., 2014), which likely stimulates the vestibular afferents, also demonstrated an increased gain of the VSR with height. Thus it seems

unlikely that vestibular efferents could be contributing to the observed gain modulation under threatening conditions.

Descending excitatory projections to the spinal cord from fear and anxiety centers could be also modulating the VSR. Serotoninergic projections from the raphe nuclei to the posterior horn mediating in the modulation of pain sensations could also connect with the motorneuron pools via excitatory interneurons (Todd, 2002). Previous findings from investigations of the proprioceptive system at height have suggested a higher sensitivity in muscle spindles during states of fear and anxiety, as demonstrated by a significant increase in tendon tap reflex gain, but not in electrically-evoked H-reflexes (Horslen et al., 2013). Likewise, previous work on VOR gain in chronic anxiety populations (Yardley et al., 1995; Furman et al., 2006; Quarck et al., 2006), argues against significant excitatory contributions from interneurons at the spinal cord because the spinal cord is not involved in the reflex. The results of the present thesis rule out almost completely any involvement of the spinal cord in the modulation of the vestibular reflexes, given the strong height effects observed on oVEMPs, as on the horizontal and vertical VOR gains evoked through head movements, which bypass the spinal cord.

The strong neural links described between the parabrachial nucleus and the vestibular nuclei complex, plus the neurochemical influence of the locus coeruleous and raphe nuclei, could explain the influence of negative emotions on postural control. This neural relationship was used in the past to explain the increased VOR gain in subjects with chronic anxiety disorders, and was supported by the fact that some subjects with evidenced disorders of monoaminergic function,

such as those with migraine-related dizziness (Furman et al., 2005), also show an increased sensitivity of the VOR. Histamine plays an important role as neurotransmitter mediating fear responses, through H1 and H2 receptors. Histamine has been found to excite the inferior and medial vestibular nuclei in rats through activation of those receptors, linking the VN with the histaminergic pathways originated mainly in the hypothalamus (de Waele et al., 1992; Serafin et al., 1993; Yabe et al., 1993; Peng et al., 2013). Since serotonergic, noradrenergic or histaminergic influences converge on the vestibular nuclei complex, there are many possible avenues for excitation of vestibular reflexes with threat.

4.5 Effects of height-induced emotions on the oculomotor function

OMF in the absence of head movements, i.e. inputs to the vestibular system, is also influenced by the anxiety pathways. It is possible that the hypothesized effects of fear and anxiety on VOR gain could be modulated at the level of the oculomotor nuclei complex. In order to verify that oVEMPs and VOR changes rely on anxiety effects on the vestibular nuclei it is necessary to investigate the contributions of other nuclei involved in these pathways, specifically oculomotor. Based on the model derived from previous evidence, I hypothesized that the OKN gain would increase with increased surface height, whereas the eye saccades and smooth pursuit gains would remain unchanged due to a lack of vestibular inputs.

To my knowledge, this is the first time that the OKN was investigated under induced acute states on anxiety and fear. As hypothesized, there was a significant increase in the OKN gain for the 3

rotating stimulus velocities used in Study 2. The OKN gains obtained in the Low condition were consistent with past reports for the first 2 target speeds (15 and 30°/s), but the absolute gain at the highest speed was lower (0.52) than those previously reported by Koenig et al. (1978). This work showed that the OKN gains were close to 1 for target speeds up to 30°/s, but decreased at higher speeds. As mentioned, the OKN gains for the 60°/s target speed in this thesis were low and the OKN velocity showed a much higher variability in the plateaus after the signal processing. Therefore, the values for this fastest speed may not be as reliable. However, comparisons between conditions (with similar results) also revealed a significant increase.

The eye velocities significantly increased with height in both the eye saccades and the smooth pursuit tests. The saccade accuracy, eye saccade peak velocity and the smooth pursuit gains significantly increased at height. The gain and velocity values in the Low condition are consistent with previous research using eye saccades (and anti-saccades) and smooth pursuit in subjects diagnosed with trait anxiety (Derakshan et al., 2009; Kattoulas et al., 2011).

There are no studies so far that have investigated the effects of state anxiety on the voluntary and involuntary eye movements (Staab, 2014). In the few past reports, no difference in the eye saccade gain was found between subjects with chronic anxiety when compared to others with depressive symptoms (Derakshan et al., 2009), whereas saccadic eye movements were more inaccurate in patients with panic disorder compared to healthy controls (Jergelova and Jagla, 2010). Another study revealed an improved predictive smooth pursuit, the ability of maintaining the visual tracking of moving objects with the vision obstructed, in soldiers with high trait anxiety, but no changes in the smooth pursuit gain using similar velocities as Study 2 (Kattoulas

et al., 2011). In addition, poorer gaze stability and less accuracy was previously observed in subjects categorized with high trait anxiety compared to low trait anxiety (Laretzaki et al., 2011), The limited background research led to the hypotheses presented in this thesis that no changes in the gains or peak velocities for the eye saccades and smooth pursuit would be observed. This hypothesis has been rejected by the current results.

Previous work on subjects with chronic anxiety showed an increase in the OKN gains (Levinson, 1989). It is accepted that there is an involvement of the vestibular system and the architecture of the VOR in the OKN responses, also via the vestibulo-cerebellar pathways (Takemori and Cohen, 1974) and receiving contributions from visual areas of the brain (Precth and Strata, 1980). In line with that background, the third hypothesis of Study 2 was confirmed, observing a significant increase of the mean eye SPV with height in the 3 different target velocities of the OKN.

As happened with the different measures of the VOR in this thesis, several correlations were found between changes in autonomic arousal, measured by EDA, and changes in the smooth pursuit gain for the fastest target velocity, and also changes in the OKN gain for the slowest stimulus velocity. A significant correlation was also found between the increase in fear and the increase in the eye saccade average peak velocity at height. All these results suggest an independent effect of fear and arousal, or vigilance, on the voluntary and involuntary ocular motor function, in absence of vestibular input.

Excitatory 5-HT2A receptor subtypes for serotonin, a neurotrasmitter that exerts effects in many brainstem regions, have been found in high proportion in the abducens nucleus of the rat (Fay et al., 2000), and could be responsible of altering the control of both the eye saccades and the smooth pursuit in the horizontal plane under threatening conditions. Tonic and excitatory projections from the ventral portion of the rostral dorsal raphe nuclei, a serotonergic neural area responsible for processing fear and emotional responses, to the trochlear and oculomotor nuclei of the brainstem have been also detailed (Peyron et al., 1998). These neural connections, could cause an increase in the gain of the reflexive and voluntary control of the eye movements, evident in this thesis, and could also be involved in other dysfunctions such as visual height intolerance (Brandt and Huppert, 2014). Serotonin and histamine receptors have been also found in the ventral lateral geniculate nucleus, an important relay centre located in the thalamus for the visual pathways (Harrington, 1997). Just as described for the VOR and VSR reflexes, the histaminergic pathways could be playing an important role in mediating the modulation of the OMF, given the high amount of H1 and H2 receptors found in the brainstem neurons, including the oculomotor nuclei complex (Wada et al, 1991).

Due to the vestibular involvement in OKN, the observed increase in OKN gain could in part be modulated either by the mentioned serotonergic and histaminergic influences on the oculmotor nuclei complex, or the neural connections discussed above between the emotional brain areas and the vestibular nuclei. This fact could explain the relative larger increase in the OKN gain, of 11.4% for the slowest stimulus speed, compared to the smaller increase in the smooth pursuit gain (7.8%) and the eye saccade velocity (5.1%) and accuracy (4.8%).

4.6 Interactions between visual and vestibular function under states of anxiety, fear and arousal

One of the most important findings of this thesis is the evident contribution of the eye movements to the increased gain of the VOR when negative emotional changes occur. When the vestibular receptors are not stimulated, the gains of the OMF increase under those threatening conditions. Considering the strong influence that visual inputs have on vestibular function, mostly through direct projections from vision processing areas of the brain to the vestibular nuclei (Precht and Strata, 1980) and with strong evidence on how the OKN visual stimuli activates the vestibular nuclei neurons (Allum et al., 1976; Waespe and Henn, 1979) and suppress the nystagmus caused by passive head rotations (Koenig et al., 1978), one of the possible explanations for the observed changes in the VSR and VOR gains could be through an increased excitation of the oculomotor system.

It is not possible to know the exact extent of the contributions of the OMF gain on the physiological and functional VOR gain, but it seems unlikely that those changes could be conditioning the VSR gain via direct influence on the LVN and MVN. Davis et al. (2009) found that changes in postural control at height were similar when the subject had their eyes open or closed. Horslen et al. (2014) demonstrated similar coupling between the SVS input and the ground reaction forces output when the visual field was controlled such that subjects could not see the threat.

Finally, the fact that oVEMP and the legVEMP amplitude experienced a similar increase size of 30% from Low to High implies that both evoked reflexes were similarly modulated and that the physiological measure of the VOR gain did not receive strong contributions from the visual structures. However, as a future direction, it would be interesting to examine the VEMP responses at height, recorded from neck and leg muscles, with removal of any visual input (i.e. with eyes closed). It would also be of interest to investigate the interaction of vertical OKN stimulation and VSR evoked through VEMPs.

Chapter 5: Limitations of this thesis

By using a faster delivery rate compared to previous work (*c.f.* Naranjo et al., 2015), we addressed an important limitation that potentially caused a fatiguing effect on the recorded muscles. However, it was difficult to control for other limitations in the first experiment of Study 1. The subjects maintained an upward gaze necessary to elicit oVEMPs from the IO, focusing on the dot marked by the laser pointer, but there was no way to monitor that the gaze was stable or that subjects with high fear of falling were not looking downward occasionally to obtain visual confirmation of their position. This could have contributed to the decrease in the BGA observed in the IO between conditions. While other factors that may have affected muscle BGA, this limitation was addressed by screening BGA levels between conditions and by normalizing the oVEMP response.

Although vigilance was not directly recorded as a variable in this study, it could also relate to the observed changes in VEMP, VOR and OMF responses as it has been shown to influence VOR gain (Collins and Poe, 1962; Collins 1988; Quarck et al., 2006), and is likely sensitive to threatening conditions such as elevated heights (Carpenter et al., 2001). Future studies need to consider vigilance as a potential independent variable, which although related to arousal, has distinct neural pathways (Oken et al., 2006; Samuels and Szabadi, 2008). Likewise there is a limitation in using only EDA as a marker for arousal, as it reflects primarily sympathetic response, and may not capture other aspects of the stress-response, that may be better recorded using measures of heart-rate and blood-pressure.

OMF tests are normally conducted having the subject seated while resting the chin and the forehead on a support, therefore preventing for potential head movements accompanying the visual stimuli. In Study 2, in order to induce emotional changes, subjects were freely standing at the edge of the platform. Although an accelerometer was used to confirm changes in head movements that could affect the results during the OMF trials, small head movements and changes in postural sway during stimulation could have been occurred. However, OMF was not likely affected by more head movement at height, as head and eye movements tend to be restricted with height-induced threat (Brandt and Huppert, 2014). Nevertheless, a deeper analysis and comparisons of the head movements and accelerations in the yaw plane, and of the forceplate data, between conditions could be useful for ruling out any contribution of those factors on the observed effects.

Chapter 6: Implications of this thesis

The findings of the present thesis add supporting evidence to the contributions of the vestibular system to postural threat responses, and of the oculomotor pathways to the increased VOR gain under state anxiety. These findings have other important clinical implications. The methods employed in the two studies are commonly used in clinical settings for testing vestibular and oculomotor function. If, as suggested by the results of this thesis, the outcomes of these tests are affected by the emotional state of the subject, clinicians may need to take that factor into consideration when interpreting the results. As reported previously (Naranjo et al., 2015), changes in VSR gain were modest, but relative changes in oVEMPs from Low to High were approximately one third the size of VSR changes. Being oVEMPs and cVEMPs accepted as standard tests for the otolith function, this effect could have a great impact in the test results. This is especially interesting when tracking changes, as a simple decrease in the patient's anxiety after a first testing could influence the evoked response and be wrongly interpreted as pathology.

Chapter 7: Conclusions

The following *conclusions* can be drawn from this thesis:

1- The previously reported effects of height-induced fear, anxiety and arousal on VSR gain were confirmed and replicated, as reflected by a significant increase in the amplitude of ipsilateral LcVEMPs and legVEMPs.

2- Robust evidence was provided on the facilitatory effect of height-induced emotions on the VOR gains, recorded using both physiological and functional methods.

3- These effects were supported by significant correlations between changes in measures of negative emotional changes and changes in the different multi-segmental vestibular reflex gains.

4- Changes in the physiological and functional VOR tests with otolithic involvement significantly correlated when experiencing similar levels of arousal.

The following *interpretations* can be made from these conclusions:

1- The amplification of the vestibular reflexes during increased threat of falling is likely generated in vestibular nuclei, and mediated by excitatory projections from regions responsible for the processing of affective and emotional responses.

2- The oculomotor nuclei are influenced independently by threatening conditions as suggested by an increased gain in the gaze control and a higher accuracy in the saccade correction.

3- The evident increased VOR gain could receive contributions from the OMF pathways, arguably at a low proportion, as would be suggested from comparing the relative changes in VOR and VSR gains.

I therefore postulate that the increased gain of the vestibular reflexes documented in this thesis is originated at the vestibular nuclei located in the brainstem, where an amplification of the signals arriving from peripheral vestibular receptors occurs as a result of excitatory inputs from anxiety and fear pathways. These pathways could exert also an influence on the vestibular system via other motor areas located in the brainstem, such as the reticular formation, or the cortex. This mechanism appears to affect all excitatory projections from the superior, medial, lateral and inferior vestibular nuclei to the motorneurons of the target eye, neck and lower limb muscles stabilizing the eyes, head and/or body, as reflected by the increased amplitudes of the recorded oVEMPs, LcVEMPs and legVEMPs, and the increased gains of the horizontal and vertical functional VOR gains. I also postulate that the oculomotor regions, and potentially other visual-related areas, are likely influenced by the same anxiety and histaminergic pathways via the cerebellum or the brainstem visual areas. The VOR may receive contributions from the different oculomotor pathways, related at the same time to the anxiety and histaminergic pathways, but the

similar percentage of change observed in oVEMPs and legVEMPs suggest that this contribution may not be strong.

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Appendix

Psycho-social questionnaire

Please answer the following questions about how you honestly feel just after standing at this height using the following scale:

1	2	3	4	5	6	7	8	9
I don't feel at all				I feel this moderately				I feel this extremely

1. **I felt nervous when standing at this height**

1 2 3 4 5 6 7 8 9

2. **I had lapses of concentration when standing at this height**

1 2 3 4 5 6 7 8 9

3. **I had self doubts when standing at this height**

1 2 3 4 5 6 7 8 9

4. **I felt myself tense and shaking when standing at this height**

1 2 3 4 5 6 7 8 9

5. **I was concerned about being unable to concentrate when standing at this height**

1 2 3 4 5 6 7 8 9

6. **I was concerned about doing the balance task correctly when standing at this height**

1 2 3 4 5 6 7 8 9

7. **My body was tense when standing at this height**

1 2 3 4 5 6 7 8 9

8. **I had difficulty focusing on what I had to do when standing at this height**

1 2 3 4 5 6 7 8 9

9. **I was worried about my personal safety when standing at this height**

1 2 3 4 5 6 7 8 9

10. **I felt my stomach sinking when standing at this height**

1 2 3 4 5 6 7 8 9

11. **While trying to balance at this height, I didn't pay attention to the point on the wall all of the time**

1 2 3 4 5 6 7 8 9

12. **My heart was racing when standing at this height**

1 2 3 4 5 6 7 8 9

13. **Thoughts of falling interfered with my concentration when standing at this height**

1 2 3 4 5 6 7 8 9

14. **I was concerned that others would be disappointed with my balance performance at this height**

1 2 3 4 5 6 7 8 9

15. **I found myself hyperventilating when standing at this height**

1 2 3 4 5 6 7 8 9

16. **I found myself thinking about things not related to doing the balance task when standing at this height.**

1 2 3 4 5 6 7 8 9

Please answer the following questions about how you honestly feel just after standing at this height using the following scale:

1. Using the following scale, please rate how stable you felt when performing the balance task:

0……10……20……30……40……50……60……70……80…..90……100

I did not feel stable at all	**I felt moderately stable**	**I felt completely stable**

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2. Using the following scale, please rate how fearful of falling you felt when performing the balance task:

0……10……20……30……40……50……60……70……80…..90……100

I did not feel fearful at all	**I felt moderately fearful**	**I felt completely fearful**

Please use the following scale to rate <u>how confident</u> you are that you can <u>maintain your balance and avoid a fall</u> during the balance task:

0……10……20……30……40……50……60……70……80…..90……100

I do not feel confident at all	**I feel moderately confident**	**I feel completely confident**