Vestibular, Proprioceptive, and Cutaneous Reflex Modulation Explored Through a Height-Induced Postural Threat

by

Brian Christopher Horslen

M.Sc., The University of British Columbia, 2010

B.Sc. Hon. Co-op, The University of Waterloo, 2007

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSPOHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Kinesiology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

October 2016

Abstract

Context-dependent threats to standing balance have long been known to affect fear and anxiety about falling, as well as static, dynamic reactive, and anticipatory control of upright standing balance. While the neural mechanisms underlying changes in balance behaviours are not yet understood, changes in balance-relevant sensory-motor interactions have been suggested as a possible means to alter balance behaviours with threat. The purpose of this thesis was to understand how different balance-relevant sensory systems are affected by threats to standing balance. Four studies are presented in this thesis which each address the effects of postural threat on a different balance-relevant sensory reflex.

Height-induced postural threat was employed in all studies included in this thesis to manipulate balance threat or challenge; participants stood at, or away from the edge of a hydraulic lift which was elevated to different heights, to create LOW and HIGH threat conditions. The first study revealed that the gain and coupling of balance responses to electrical vestibular stimulation was increased in the HIGH, compared to LOW threat conditions. The second study demonstrated increases in muscle spindle stretch reflexes, and steeper dynamic gain relationships between stretch velocities and short-latency reflex amplitudes with HIGH postural threat. The third study validated a novel technique for probing Golgi tendon organ Ib reflexes in standing, and used this technique to demonstrate reduced Ib inhibition in an ankle plantar flexor muscle with increased threat. The fourth study examined lower-limb muscle reflexes, as well as cortical potentials to cutaneous nerve electrical stimulation. While cutaneous reflexes were not observed to change independently from background muscle activity, cortical

potentials were affected by threat at stages which may represent altered primary and/or secondary somatosensory, as well as posterior parietal processing.

Combined, these studies suggest people respond to height-induced postural threat with a multi-sensory adaptation process where balance-relevant muscular and vestibular senses are tuned to facilitate reactive responses to balance disturbances and/or sensory monitoring of postural state. These novel results provide important insight into neural mechanisms underlying the effects of fear and anxiety on human balance control, and have important implications for clinical balance and neurophysiological testing.

Preface

All data presented in this dissertation were collected by Brian Horslen, with assistance where noted, in the Neural Control of Posture and Movement Lab at the Vancouver-Point Grey campus of The University of British Columbia. The written content and all figures included in this dissertation were drafted by Brian Horslen under the supervision of Professor Mark Carpenter and with guidance from Professors J. Timothy Inglis and Jean-Sébastien Blouin. All methods used were reviewed by The University of British Columbia Clinical Research Ethics Board (IDs: H06-70316 and H12-01698).

Chapter 2 is composed of three articles published in The Journal of Physiology. The first article presents original research [Horslen BC, Dakin CJ, Inglis JT, Blouin JS & Carpenter MG (2014). Modulation of human vestibular reflexes with increased postural threat. *J Physiol* **592**, 3671-3685] and the subsequent articles formed part of a written debate on modulation of vestibular-evoked balance responses with fear and anxiety, and contained no original data [Horslen BC, Dakin CJ, Inglis JT, Blouin JS & Carpenter MG (2015). Crosstalk proposal: Fear of falling does influence vestibular-evoked balance responses. *J Physiol* **593**, 2979-2981; and Horslen BC, Dakin CJ, Inglis JT, Blouin JS & Carpenter MG (2015). Rebuttal from Brian C. Horslen, Christopher J. Dakin, J. Timothy Inglis, Jean-Sébastien Blouin and Mark G. Carpenter. *J Physiol* **593**, 2985]. Brian Horslen (Horslen BC) contributed to study conception and design, and was responsible for data collection and analysis, as well as drafting and revision of the manuscripts and figures. Dakin CJ contributed to experiment design, data collection, analysis and interpretation, and contributed to revisions of the manuscripts and figures. Inglis JT contributed to conception of the experiment, interpretation of results, and critical revisions to the

manuscripts and figures. Blouin JS contributed to design of the experiment, data analysis, interpretation of results, and critical revisions of the manuscripts and figures. Carpenter MG supervised all stages of the project, contributed to experiment conception and design, and critically reviewed manuscripts and figures. All authors contributed to formulation of the arguments for the debate articles.

The study described in Chapter 3 will be submitted for peer review [Horslen BC, Zaback M, Inglis JT, Blouin JS, Carpenter MG]. Horslen BC contributed to conception and design of the experiment, collected and analysed all data, and drafted the written content and figures. Zaback M contributed to data collection and interpretation, and critical review of written content and figures. Inglis JT and Blouin JS contributed to conception of the experiment, interpretation of data, and critical review of written content and figures. Carpenter MG supervised all stages of the project, including conception and design, data collection, analysis and interpretation, and critical revisions to the written content and figures.

The study described in Chapter 4 will be submitted for peer review [Horslen BC, Inglis JT, Blouin JS, Carpenter MG]. Horslen BC contributed to conception and design of the experiment, collected and analysed all data, and drafted the written content and figures. Inglis JT and Blouin JS contributed to conception and design of the experiment, interpretation of data, and critical review of written content and figures. Carpenter MG supervised all stages of the project, including conception and design, data collection, analysis and interpretation, and critical revisions to the written content and figures. Dr Ryan M Peters, who is not listed as a co-author, also provided critical technical assistance with collection of a pilot experiment presented in the discussion of Chapter 4.

The study described in Chapter 5 has not yet been submitted for peer review [Horslen BC, Blouin JS, Inglis JT, Carpenter MG]. Horslen BC contributed to conception and design of the experiment, collected and analysed all data, and drafted the written content and figures. Inglis JT and Blouin JS contributed to conception of the experiment, interpretation of data, and critical review of written content and figures. Carpenter MG supervised all stages of the project, including conception and design, data collection, analysis and interpretation, and critical revisions to the written content and figures. Professor Romeo Chua, not listed as a co-author, also provided invaluable training in electroencephalography recording and analysis methods used in the experiment.

The order in which the chapters are presented does not reflect the order in which the data were collected. Chapter 2 was the first project conducted, followed by Chapters 5, 4, then 3.

Table of contents

List of tables

List of figures

List of abbreviations and symbols

Δ – (Delta) Change
η^2 – Eta Squared – Statistic of effect size
η_p^2 – Partial Eta Squared – Statistic of effect size
AP – Anterior-Posterior body axis
BGA – Background Muscle Activity
COM – Centre of (body) Mass
COP – Centre of (foot) Pressure
EEG - Electroencephalography
EMG – Electromyography
EVS – Electrical Vestibular Stimulation
FoF – Fear of falling
GTO – Golgi Tendon Organ
GVS – Galvanic Vestibular Stimulation
H-reflex – Hoffmann Reflex
HIGH – High height-induced postural threat condition
Ia – Muscle afferent nerve classification type "one-A"
Ib – Muscle afferent nerve classification type "one-B"
II – Muscle afferent nerve classification type "two"
IVN – Inferior Vestibular Nucleus (nuclei)
LGas – Lateral Gastrocnemius muscle
LOW – Low height-induced postural threat condition
LVN – Lateral Vestibular Nucleus (nuclei)
MGas – Medial Gastrocnemius muscle
ML - Medio-lateral body axis
MLP – Medium-Latency Peak – vestibular-evoked balance response
MLR – Medium-Latency (stretch) Reflex
MTJ - Musculo-Tendinous Junction
MVN – Medial Vestibular Nucleus (nuclei)
SD – Standard Deviation
SE – Standard Error
SEP – Somatosensory-Evoked cortical Potential
SLP – Short Latency Peak – vestibular-evoked balance response
SLR – Short-Latency (stretch) Reflex
SOL – Soleus muscle
SVN – Superior Vestibular Nucleus (nuclei)
SVS – Stochastic (electrical) Vestibular Stimulation
T-reflex – Tendon-tap (tendon-stretch) Reflex
TA – Tibialis Anterior muscle
TStim – Tendon-electrical Stimulation
VEMP – Vestibular-Evoked Myogenic Potential
VOR – Vestibulo-Ocular Reflex

Acknowledgments

I would like acknowledge the tremendous support I have received from Professor Mark Carpenter over the course of my graduate education, without whom this work would not have been possible. I am extremely grateful for his endless encouragement, patient mentorship, financial support, and especially his exemplar commitment to high-quality research.

I thank my committee members, Professors Timothy Inglis and Jean-Sébastien Blouin for their support at all stages of the development of this dissertation, and for supporting my general development as a scientist. I would also like to thank Professors Romeo Chua and Parveen Bawa for lending equipment to facilitate my work, and for being generous with their time to support my training.

Financial support for this research was provided in part from the Natural Sciences and Engineering Research Council of Canada.

I am very grateful to all of my lab-mates, past and present, for assisting in my research, providing feedback, providing mentorship, and sometimes just helping me get through the day.

Lastly, none of this would have been possible without the love and support from my parents. I can’t thank them enough for helping me get so far.

Chapter 1: General introduction and literature review

1.1 Falls, fear of falling, and fear-related effects on postural control

Falls, and fall related injuries, are a significant health concern and cause of mortality in the elderly (Sattin, 1992, Li et al, 2003), with an estimated 2.2 million people having experienced a fall causing an injury in the United States in 2002 (Shumway-Cook et al, 2009). Furthermore, people who are fearful of falling are more likely to experience a fall (Li et al, 2003). People with a fear of falling tend to demonstrate more postural sway in a quiet standing scenario (Maki et al, 1991) and have altered reactive balance responses when perturbed (Okada et al, 2001) compared to age-matched non-fearful controls. As such, there is a need to understand how and why a fear of falling influences balance control.

A postural threat can be used to evoke a fear of falling and/or an increase in state anxiety in persons who are otherwise not afraid of falling (Adkin et al, 2002, Carpenter et al, 2006, Davis et al, 2009, Huffman et al, 2009). The most common method of inducing an experimental postural threat is through manipulation of support surface height (i.e. height-induced postural threat; Brown and Frank, 1997, Carpenter et al, 1999b, 2001a, 2006, Adkin et al, 2000, 2008, Davis et al, 2009, 2011, Huffman et al, 2009); although recent studies have also found that threat of postural perturbation (Shaw et al, 2012, Horslen et al, 2013; Lim et al, *in preparation*), or threatening virtual reality scenes can evoke similar effects (Cleworth et al, 2012, 2016). There are stereotyped changes to balance control when people are exposed to a postural threat. People who are asked to stand quietly at the edge of an elevated surface tend to adopt a stiffer control of postural sway, characterized by increased frequency and decreased amplitude of centre of pressure (COP; Carpenter et al, 1999b, Davis et al, 2009) and centre of mass displacements

(COM; Carpenter et al, 2001a). They also lean backward away from the edge (Carpenter et al, 1999b, 2001a, Davis et al, 2009, Huffman et al, 2009) and increase tonic tibialis anterior and decrease tonic soleus muscle activity (Carpenter et al, 2001a). When people unexpectedly have their balance perturbed while standing in a high threat scenario they tend to decrease the amplitude of their COM displacements towards the edge, independent of whether they were perturbed towards (Brown and Frank, 1997, Carpenter et al, 2004) or away from the edge (Carpenter et al, 2004). They also generate larger responses in the muscles used to correct balance (i.e. during the 120-220 ms post-perturbation "balance-correcting" phase; Carpenter et al, 2004). Finally, gait is also influenced by a height-induced postural threat, in that people tend to walk slower (Brown et al, 2002, McKenzie and Brown, 2004, Tersteeg et al, 2012) and spend more time in double-support (i.e. with both feet in contact with the ground; Tersteeg et al, 2012) while traversing an elevated beam. In general, the response to a postural threat is to allow less movement of the body, particularly towards the source of the threat (i.e. the edge).

1.2 Goal of the thesis

While there is now clear evidence to suggest that the control of balance is altered by postural threat across many balance tasks, the mechanisms that underlie these changes are not yet clear. Standing balance is thought to rely on multiple sensory modalities (overviewed by Horak and Macpherson, 1996). As such, it has been proposed that changes to the manner in which balance-relevant sensory information is either acquired or processed may explain the behavioural changes to balance control that occur with a postural threat (Carpenter et al, 2004). The overall goal of this thesis is to understand how the various sensory systems that are known to contribute to balance control are affected when a person stands in a high postural threat scenario. Each study presented here was intended to probe a reflex associated with a specific sensory modality

known to be involved in balance control. Therefore, the purpose of each study was to understand how the specific sensory modality is affected by a height-induced postural threat, as characterized by changes to its reflex. The basic anatomy and physiology of each modality examined, their contributions to control of standing balance, the evidence for modifiability, and the various methods through which each system may be probed are outlined in the introductory chapter.

1.3 Vision

Vision, which undoubtedly can affect balance control, will not be probed in this thesis. The importance of vision in controlling balance in normal healthy adults is still up for debate. For example, a moving visual scene can evoke sway in an otherwise stable subject (Guerraz and Bronstein, 2008); however, standing with eyes closed tends to only produce subtle (Carpenter et al, 1999b, 2001a, Davis et al, 2009) or no (Fitzpatrick et al, 1996) changes in static balance control. Some studies have reported changes in reactive balance control when people stand with a body-referenced visual scene (Nashner and Berthoz, 1978) or with eyes closed (Timmann et al, 1994); while others have failed to demonstrate an effect of eye closure on reactive balance control (Carpenter et al, 1999a). Vision is known to contribute to gait, but it is mainly used for trajectory analysis and planning (Bernardin et al, 2012) or foot placement and/or clearance when traversing an obstacle in the gait path (Patla and Vickers, 1997, Marigold, 2008). Due to the fact that visual control of posture seems uncertain, and the fact that visual control of posture cannot easily be tested without involving other sensory systems (e.g. postural sway with vection generating vestibular and somatosensory cues; Guerraz and Bronstein, 2008), the effects of postural threat on visual control of posture will not be assessed in this thesis.

1.4 Vestibular system and vestibular contributions to balance

1.4.1 Basic anatomy and physiology

The vestibular organs, together with the cochlea, lie in the inner ear labyrinth complex, with the left ear mirroring the right. There are five vestibular organs on each side that can be subdivided into two basic types, otolith organs (two organs) and semicircular canals (3 organs). Hair cells, the basic sensory receptor of the vestibular system are common to all vestibular organs. In this section the basic structure and function of the hair cells will be discussed, and then this model will be applied to demonstrate how each of the different vestibular organs operates in isolation. Finally, the ways in which vestibular organs are coupled across right and left sides of the body to generate complimentary signals will be outlined.

Hair cells are common to all of the vestibular organs, and while each organ is capable of detecting unique sensory events, the basic function of the hair cell, as a sensory unit, is the same for all organs. Each hair cell has a base and a network of hairs. The base is the anchor point for the cell within the epithelial structure of the vestibular organ; it also contains the basic cellular organs and is the principal synaptic contact point for the associated afferent and efferent fibres. Each cell has a network of hairs that protrude from the base out into the vestibular organ and are imbedded into a gelatinous membrane (the macula for otolith organs, or the cupula for the semicircular canals). The hairs are arranged such that there is a single long hair at one end (termed kinocilium), and an array of hairs (called stereocilia) that expand into an approximate wedge shape, where the stereocilia get shorter the further they are from the kinocilium. The stereocilia contain mechanically gated ion channels on their tips and each channel is linked to an adjacent, taller, stereocilium. The gelatinous membranes can be displaced or deformed by different forces acting on the organ. As the membrane, in which the stereocilia are imbedded, is

displaced by some force, the hairs deflect in the direction of the displacement of the medium. Hair cells respond to shearing displacements of the membrane; compressive or tensile forces do not depolarize the cell (Goldberg and Fernandez, 1984). The direction of force with respect to the line of the stereocilia and the kinocilium determines the manner in which the cell responds. Displacements directed from shortest stereocilium to kinocilium cause the tip-link proteins to be strained, which pull open the mechanically gated ion channels and depolarize the cell. If the displacement is in the opposite direction, then the channels either remain closed, or any that happened to be open would close; causing the cell to hyperpolarize. These opposing directions characterize the preferred line of action for the given cell. A force that is not in the preferred line of action causes an intermediate response. Here, the probability of each channel in the cell opening or closing is dictated by the magnitude and direction of the vector component of the actual displacement that lies in the preferred line of action. In this case the cell can be slightly depolarized, slightly hyperpolarized, or not respond if the direction is perpendicular to the preferred line of action. As such, individual hair cells, being either depolarized or hyperpolarized, can only operate in one spatial dimension.

If enough ion channels are opened, then the depolarizing current in the stereocilia can be passively conducted along the cellular membrane to the base and cause neurotransmitter release at the synaptic terminals. There are two basic types of hair cell in the vestibular system, type I hair cells tend to be rounded and the base is surrounded by an afferent nerve chalice (or cup), and type II cells tend to be more rod-like and are connected to afferent nerves through button terminals (Goldberg and Fernandez, 1984). Likewise, there are three basic types of afferent nerve supplying the vestibular hair cells that are named after their fibre diameter and firing properties. Each vestibular afferent neuron is connected to a network of hair cells; the firing probability of

each neuron is dictated by the number of affiliated hair cells that are depolarized, and the relative locations of these cells with respect to the axon hillock. Each thick fibre supplies a small group of adjacent type I cells with nerve chalices and may also contact a few type II cells nearby. Thick fibres generally have an irregular firing pattern and are often referred to as irregular units, meaning they tend not to fire tonically. These fibres adapt to tonic loads by eventually returning to baseline firing, and they likely code for dynamic features such as velocity or acceleration of the medium in which the hairs are imbedded (Goldberg and Fernandez, 1984). Thin fibres spread throughout the host organ to connect with many hair cells, mainly type II, over a broad area; they tend to have a regular firing pattern (regular units). These units have sustained firing rates to tonic loads, and are thought to mainly code for displacement of the host medium. Finally, medium sized afferents tend to connect to both type I and type II hair cells and may demonstrate intermediate firing properties, where some units more closely resemble irregular, and others regular firing units.

The division of vestibular organs into otoliths and semicircular canals is based on the shape of the organs; however, the shape dictates how the organ is influenced by forces and ultimately how the affiliated neurons respond. Generally speaking, the otoliths each consist of a fluid filled sack with a plate of hair cells imbedded into an otherwise free floating gelatinous membrane (macula). The macula floats in endolymph, a specialized extracellular fluid with a relatively positive ionic charge. The macula is also weighted on one surface with a layer of calcium carbonate crystals (called otoconia), which serve to give the structure added mass and rigidity. The macula is subject to displacement by linear accelerations in the plane of the surface of the structure. When the macula is displaced all hair cells attached to it deflect in the same direction. However, the hair cells are arranged such that their preferred lines of action shift slightly across

the surface of the macula. Each hair cell is aligned towards a non-straight line that roughly bisects the macula, called the striola. As such, while all hair cells are deflected, some are depolarized, some hyperpolarized, and many lie in an intermediate state for any given direction within the plane of the macula. As such, some afferents will have an increase in firing rate and other a decrease for any adequate stimulus. This means that each otolith organ can code for forces exerted on it in two spatial dimensions. The problem of coding for the third spatial dimension is solved by having a second two-dimensional organ aligned in a plane orthogonal to that of the first. These two organs are called the utricle and the saccule. The plane of the utricle is aligned approximately 30° above horizontal (anterior tip up) with its long axis approximately in the antero-posterior (AP) axis and short axis in the medio-lateral (ML) axis (Young, 1984). As such, the utricle can detect linear accelerations preferentially along the AP axis and secondarily along the ML axis. The saccule is aligned approximately vertically, with the long axis near vertical and the short axis in the AP direction. As such, the saccule can detect vertically and AP oriented linear accelerations.

There are three semicircular canals on each side of the skull. Each canal is oriented in a plane orthogonal to the other two, and all are attached to the utricle. The canals are filled with endolymph and have an enlarged region at one end of the canal called the ampulla. There is a cluster of hair cells protruding from a mound at the base of each ampulla (called the crista ampullaris) that is embedded into a gelatinous membrane (cupula). Unlike the maculae in the otoliths, the cupula is attached to the canal to create a roughly sealed drum membrane (Goldberg and Fernandez, 1984). Also, all hair cells in the crista ampullaris are oriented in the same direction, as opposed to those in the otoliths, whose preferred line of action changes systematically across the macula. The appropriate stimuli for the semicircular canals are

rotational accelerations. When the head undergoes a rotational acceleration in the plane of a semicircular canal, the endolymph lags (due to inertia) and exerts pressure on one side of the cupula. If the pressure is in the direction of excitation for the hair cells then the affiliated afferents increase their firing rates, but if the pressure is in the opposite direction it causes hyperpolarization and the afferents decrease their firing rate. The three semicircular canals are named the anterior, posterior, and horizontal canals, where the name reflects its relative location and orientation with respect to the other two. The anterior and posterior canals both have vertical axes; the anterior edge of the anterior canal is aligned approximately 45° lateral to the para-sagittal plane, and the posterior canal is orthogonal with the posterior edge 45° from para-sagittal. As such, both anterior and posterior semicircular canals respond (with either increased or decreased firing rates) to both pitch and roll head accelerations. The horizontal canal is roughly aligned with the horizontal plane, yet the anterior end is actually tilted approximately 25° above horizontal (Goldberg and Fernandez, 1984). The horizontal canals respond primarily to head accelerations in the yaw plane.

The vestibular organs are mirrored on the left and right sides of the head. This means each organ has a complimentary organ on the opposite side of the head; both organs will respond (with either excitation or inhibition) to the same stimuli. For the semi-circular canals, a stimulus that excites one canal will decrease the firing rate of the afferents from the antagonistic canal. For example, a nose-left rotational acceleration will excite the left horizontal canal and inhibit the right. Similarly, the left anterior canal is mirrored by the right posterior and left posterior by right anterior canal. This opposing response pattern generates maximally contrasting inputs to the central nervous system, allowing the vestibular nuclei to respond to the relative error between left and right sides (Goldberg and Fernandez, 1984).

Vestibular afferents from the anterior and horizontal canals, as well as the utricle bundle to form the superior division of the vestibular nerve; and afferents from the saccule and posterior semicircular canals bundle to form the inferior division of the vestibular nerve (Goldberg and Fernandez, 1984). The superior and inferior divisions join with the cochlear nerve to communicate with the brainstem as the vestibulo-cochlear nerve (cranial nerve VIII). The vestibular components of this nerve primarily synapse onto neurons in the four vestibular nuclei; which are: superior (SVN), inferior (IVN), medial (MVN), and lateral vestibular nuclei (LVN). The LVN primarily gives rise to the descending lateral vestibulospinal tract, which (indirectly) controls tone in limb muscles and is thought to be heavily involved in balance control (Wilson and Peterson, 1981). The MVN primarily, but also to a lesser extent the IVN, gives rise to the descending medial vestibulospinal tract; which is involved in trunk and axial tone and balance control. There are also ascending projections to oculomotor nuclei, as well as the cortex, from the MVN and SVN.

While the vestibular nuclei are heavily influenced by vestibular afferents, they are also subject to activation or modulation by non-vestibular sources. Each vestibular nucleus has extensive interconnections to the cerebellum and the reticular formation. There are strong inhibitory projections from the cerebellum onto the vestibular nuclei (Goldberg and Fernandez, 1984). A classic example of this is the inhibition of the vestibular-triggered vestibulo-ocular reflex, which can be suppressed to aid focusing on a moving visual target. Likewise, the vestibular nuclei receive indirect somatosensory inputs from the cerebellum, reticular formation, and inferior olive; as well as a few direct inputs from collaterals of the spino-cerebellar pathways (Goldberg and Fernandez, 1984). These inputs may serve to help shape vestibular reflexes (vestibulo-spinal/colic, etc...) to the current posture of the body or segment of interest.

Vestibular nuclei are also extensively interconnected with autonomic and emotional centres of the central nervous system. There are vestibular-evoked autonomic reflexes that aid in blood pressure and heart rate regulation, particularly when changing from laying to seated or seated to standing postures (Olufsen et al, 2008, Carter and Ray, 2008). Likewise, the vestibular nuclei, influenced by visceral inputs, are thought to be involved in nausea related to motion sickness (Arshian et al, 2013). Animal models have revealed modulatory projections onto the vestibular nuclei from autonomic and emotional centres (Balaban, 2002). There is an extensive network involving the vestibular nuclei and: the parabrachial nucleus, dorsal raphe nucleus, locus coeruleus, and the Kölliker-Fuse nucleus (Balaban and Thayer, 2001, Balaban, 2002, Balaban, 2004). This network is thought to serve to excite the vestibular nuclei when the animal is in a fearful or vigilant state, such that the vestibular responses to self- or imposed motion are stronger (Balaban, 2002).

1.4.2 How does the vestibular system contribute to balance control?

The role of the vestibular system in static balance control is not clear. It has been proposed that natural vestibular stimulation from undisturbed postural sway is below vestibular detection thresholds (Fitzpatrick and McCloskey, 1994); this is supported by the fact that humans can balance an upright load while lying down and therefore not using vestibular inputs (Fitzpatrick et al, 1992). However, persons with vestibular deficits (either acute unilateral deficit or cerebellar-pontine-angle tumours) tend to have increased trunk sway while standing quietly, compared to age-matched healthy controls (Allum et al, 2001). Despite the increased sway, persons with vestibular deficits tend to be able to compensate and use other sensory systems to maintain static balance (Allum et al, 2001). The vestibular system is also known to be involved in multiple

aspects of the response to a balance perturbation. Persons with vestibular deficits demonstrate smaller early myogenic balance correcting responses to perturbations (Allum and Pfaltz, 1985, Allum et al, 1994, Keshner et al, 1987, Carpenter et al, 2001b) and therefore have to compensate with larger later responses to regain balance (Carpenter et al, 2001b). Furthermore, biasing the vestibular system with a galvanic current (Galvanic Vestibular Stimulation, GVS) has demonstrated that the vestibular system is involved in orienting the person with respect to gravity while they stabilize their response to a balance perturbation, as GVS causes people to settle into an equilibrium that is not-aligned with vertical (Inglis et al, 1995, Hlavacka et al, 1999). The vestibular system is also thought to contribute to the control of upper body orientation, and perhaps foot placement, during gait, as GVS can cause leaning and wider stepping during steady-state gait (Bent et al, 2000, 2005). This information is thought to be crucial for following an intended trajectory (Deshpande and Patla, 2005), as well as changing trajectory during gait (Kennedy et al, 2005; Fitzpatrick et al, 2006). Another important role for vestibular information during gait is to control anti-gravity muscular activity in the legs, the body achieves this by phasically modulating the connections between the vestibular system and the motor neuron pools of the muscles involved in balance, such that the pools receive the strongest vestibular input at gait stages where the muscles are best able to contribute to the maintenance of balance (Blouin et al, 2011, Dakin et al, 2013).

1.4.3 Modulation of vestibular function

Vestibular function, with respect to balance control, has proven to be tunable across different contexts. There are many examples of state-specific and phasic modulation of vestibular function to suit different tasks and their varying challenges to balance control. Britton

et al (1993) demonstrated several classic examples of state-specific vestibular modulation. They found that soleus EMG responses to discrete galvanic vestibular stimulation (GVS) pulses were observable when subjects were standing, but not when seated, an example of state-specific modulation. Likewise, the soleus EMG responses were scaled to how much the body required vestibular feedback to maintain upright balance. EMG responses were smaller when vestibular information was supplemented by vision (standing with eyes open vs. eyes closed), or by lightly touching a stable surface (providing tactile cues relevant to balance). Furthermore, EMG responses could be evoked in non-traditional balance muscles (e.g. biceps brachii) when the muscle was engaged in a balance control task (by having subject stand on an unstable surface but hold onto a stable hand rail). From these observations, Britton et al (1993) concluded that the magnitude of vestibular-evoked myogenic responses is dictated by the dependence of the body on vestibular information to maintain balance, and the degree to which the observed muscle is engaged in balance control. Luu et al (2012) recently demonstrated that vestibular inputs were correlated to balance control measures when people were balancing through a robotic platform, yet the vestibular-evoked responses were reduced when control of the platform was discretely switched from human to computer control. Modulation of vestibular control of balance, as such, seems to be a continuous and sub-conscious process.

Vestibular control of gait is also known to be continuously modulated. The vestibular system is thought to control anti-gravity muscular activity (Bent et al, 2005) by rhythmically modulating its input to muscles and/or limbs responsible for resisting gravity throughout the different phases of the gait cycle. Vestibular coupling cycles within (Blouin et al, 2011) and across muscles (Dakin et al, 2013) over the course of the gait cycle, such that the vestibular system is constantly involved in balance control, yet the mechanisms (muscles) through which it

acts vary as the different muscles become more or less suited to resist gravity. This modulation is thought to occur at the spinal interneuron level by interaction between descending vestibulo- and/or reticulospinal sources and local propriospinal rhythm generating circuits (Rossignol et al, 2006).

Finally, it is probable that vestibular control of balance is influenced by autonomic and/or emotional stimuli. As outlined earlier, networks between vestibular and autonomic centres have been established in animal models (Balaban and Thayer, 2001, Balaban, 2002, Balaban, 2004). Functionally, there is evidence to suggest that the vestibulo-ocular reflex (VOR) is enhanced by arousal in animals (Crampton, 1961, Crampton and Schwam, 1961, Furman et al, 1981) and by arousal or anxiety in humans (Collins and Poe, 1962, Collins and Guedry, 1962, Kasper et al, 1992, Yardley et al, 1995). However, it is still unknown whether or not these VOR effects reflect general increases in vestibular excitability, or are specific to the VOR, or even oculomotor, pathways. Vestibular-autonomic links have, nonetheless, been implicated in balance deficits in persons with clinical anxiety disorders. Balance control deficits are a common co-morbidity in persons with fear and/or anxiety disorders (Yardley and Redfern, 2001, Balaban and Jacob, 2001, Balaban and Thayer, 2001, Furman and Jacob, 2001). It is thought that these balance issues may stem from abnormal activity in the neural networks normally activated in vigilant states (Balaban and Thayer, 2001, Balaban, 2002).

1.4.4 How can the vestibular system be probed?

One of the simplest and most common methods of probing the vestibular system in humans is measuring the gain of the vestibulo-ocular reflex (VOR). The VOR is an eye stabilizing reflex that allows the eyes to remain relatively stable by counter-rolling in response to head

movements, thereby keeping the visual scene stable and in focus (Goldberg and Fernandez, 1984). When a person is exposed to a prolonged rotational acceleration (or a simulated rotation, such as with caloric stimulation) the VOR eventually pushes the eyes to the limits of their range of motion and a second oculomotor reflex occurs to shift the eyes to a central position, called a nystagmus beat. The nystagmus beat allows the VOR to resume; as such an alternating pattern of eye movements develops where the relatively slow VOR is regularly corrected by the relatively fast nystagmus. In the simplest case, the presence or absence of a VOR and/or nystagmus can be used to determine whether the subject has a functional vestibular system, and can also reveal whether one side is defective or deficient. Common methods for measuring eye movements include: video-oculography which measures eye kinematics; electro-oculography (EOG; sometimes called electro-nystagmography, ENG) which measures changes in local potentials related to different parts of the eye shifting nearer to or further from the electrode leads; and magnetic ocular (scleral) search-coils which measure shifts in local magnetic fields related to shifts in electrical potentials caused by movements of the of the eye. Each of these techniques can be used to measure changes in VOR function. Changes in VOR function have been quantified by measuring changes in the duration of the nystagmus after cessation of acceleration or changes in the speed of the VOR (or slow phase of the nystagmus; Yardley et al, 1995) or by calculating gain and/or phase of the VOR (Ward et al, 2008). While this system is relatively simple to probe and is minimally invasive, there are certain limitations to the method. There are 2 basic reasons why VOR is unsuitable as a probe of how the vestibular system interacts with the balance control system. First, the VOR pathway primarily involves the MVN and SVN, whereas balance and descending vestibulospinal pathways (to the limbs) primarily originate in the LVN and IVN. Second, changes in the VOR might be subject to changes in excitability of the

oculomotor nuclei through which the motor response is evoked. As such, it is important to have a measure of postural control, or a measure from a muscle group involved in postural control, when drawing conclusions about the relationship between the vestibular system and postural systems.

An alternative method for probing the vestibular system is to use vestibular-evoked myogenic potentials (VEMPs). VEMPs are triggered by brief auditory clicks or tone bursts (85-125 dB) that evoke a small muscular twitch that can readily be observed in eye (Todd et al, 2007), neck (Colebatch et al, 1994, Robertson and Ireland, 1995), and some distal limb muscles (Watson and Colebatch, 1998). The tone is thought to stimulate the peripheral organs as the responses can be observed in patients with hearing loss, but not those with vestibular loss (Robertson and Ireland, 1995, Halmagyi and Colebatch, 1995, reviewed by Welgampola and Colebatch, 2005). The response is thought to originate mainly from the otolith organs, however evidence form rat models suggests that the canals may be involved also (Zhu et al, 2011). There is now some evidence to suggest that VEMPs evoked in the lower leg musculature are larger when subjects stand in a high postural threat scenario, compared to a low threat level (Naranjo, unpublished observations). However, as the VEMP is triggered through a peripheral receptor, it is not clear if the change is due to central facilitation or an increase in saccule sensitivity with threat. Furthermore, VEMPs are thought to be related to otolith, and not canal, stimulation (Halmagyi and Colebatch, 1995, Welgampola and Colebatch, 2005), and therefore may not reflect changes in canal excitability. Also, as they incorporate a peripheral receptor, they may not discriminate between central (e.g. nuclei) and peripheral (canal or otolith) changes in vestibular excitation or function.

Galvanic vestibular stimulation (GVS) has frequently been used to probe the effects of vestibular inputs on postural control. The effect of applying a bilateral bipolar galvanic current percutaneously to the mastoid processes (e.g. anode right, cathode left) is to cause reflexive muscular responses (limb and ocular), and a whole-body leaning response towards the anode (reviewed by Fitzpatrick and Day, 2004). GVS has been used to probe vestibular control of balance in static balance (Fitzpatrick and Day, 2004, Britton et al, 1993, Lee Son et al, 2008) and dynamic response scenarios (Inglis et al, 1995, Hlavacka et al, 1999); as well as in gait (Bent et al, 2000, Kennedy et al, 2003, 2005, Deshpande and Patla, 2005). While relatively simple to use, the method is potentially unsuitable for use in a height-induced postural threat paradigm as the leaning response towards the anode is likely a response to the perception of falling (or being perturbed) towards the cathode. Responses to postural perturbations are known to be altered with threat, in that movement is decreased (Brown and Frank, 1997) and myogenic responses are amplified (Carpenter et al, 2004). Responses to postural perturbations are thought to rely on many different afferent sources, including vestibular inputs, but are likely triggered by non-vestibular sources (Allum and Pfaltz, 1985, Allum et al, 1994). Similarly, proprioceptive sensitivity is thought to increase with postural threat (Horslen et al, 2013), which may interact with the (potentially unchanged) vestibular error signal generated by GVS to evoke different postural responses across threat conditions. Despite this limitation, Osler et al (2013) have recently published an investigation into the effects of a height-induced postural threat on postural responses to GVS. As would be expected from the whole-body perturbation work (Brown and Frank, 1997, Carpenter et al, 2004), Osler et al (2013) found attenuated late (>800 ms) body sway responses when subjects stood at height. There were no observed changes in the earlier phases (Osler et al, 2013), where the response is more likely to be of pure vestibular origin

(Fitzpatrick and Day, 2004). However, this finding may be limited by the fact that the study did not explicitly target early responses to GVS, and as a consequence lacked statistical power to properly investigate the small amplitude, non-statistically significant, changes in the early responses that appear in the published data.

Stochastic vestibular stimulation (SVS) has emerged as a viable alternative to GVS. In SVS, a stochastic current (variable in amplitude and direction) is passed across the mastoid processes (Fitzpatrick et al, 1996, Dakin et al, 2007, 2010, 2011, Mian and Day, 2009, Luu et al, 2012). SVS, therefore, stimulates the same structures as bipolar GVS, and can evoke similar responses (Dakin et al, 2007). However, the SVS stimulation pattern can be tuned such that either short or long latency responses are accentuated (Dakin et al, 2010), even allowing the experimenter to evoke myogenic or ground reaction force responses at a high enough frequency that correlated whole-body sway is almost completely eliminated (Dakin et al, 2010). This makes SVS an ideal probe of vestibular function in a postural threat scenario, as the responses of interested can be evoked without worry that the subject will need to respond to, or anticipate, postural perturbations.

Vestibular receptors are known to be served by efferent fibres that are understood to have inhibitory effects on the receptors (Goldberg and Fernandez, 1984). While, SVS is known to bypass the mechanical transduction zone of vestibular receptors, it is thought to act at the vestibular neuroepithelia (reviewed by Fitzpatrick and Day, 2004). As such, SVS may be sensitive to both peripheral and central modulation of vestibular function, which may occur with threat. Furthermore, SVS also has the specific advantage over VEMPs in that it is understood to elicit a reflex response to the error across canal systems (Fitzpatrick and Day, 2004), whereas VEMPs are thought to be otolith mediated (Welgampola and Colebatch, 2005). Ultimately, it

would be ideal to test the effects of postural threat on the vestibular system with both VEMPs and SVS, as both methods potentially reveal different aspects of vestibular functioning. However, the SVS is an ideal starting point, as it tends to be a more robust response in the lower limbs (*cf.* Watson and Colebatch, 1998), and bypasses the potential confound of efferent modulation of peripheral receptors.

1.5 Muscle spindles and stretch reflexes

1.5.1 Basic anatomy and physiology

There are four basic anatomical components that contribute to the reflexive muscle activation pattern in response to muscle stretch. This reflex, also known as the myotatic stretch reflex, relies on muscle stretch receptors (muscle spindles), afferent conduction to the central nervous system (types Ia and II afferent fibres), central projection and modulatory interneuron pathways, and efferent fibres to activate responding muscle tissue. Each component can theoretically be modulated to change the size and/or shape of the resultant muscle activation pattern. The basic structure and function of each component, as well as how it can be modulated to shape the reflex response will be discussed in this section.

Muscle spindles are the first component of the reflex arc. Muscle spindles, as organs, typically contain six to ten specialized muscle fibres surrounded by a fusiform (or spindle-shaped) capsule (intrafusal fibres), that are attached in series to the surrounding muscle tissue (extrafusal fibres) (Matthews, 1981, Prochazka, 1996). The intrafusal muscle fibres each have a non-contractile equatorial region and contractile polar ends. The equatorial region of each fibre is innervated by one or more sensory endings which, depending on the fibre and sensory ending types, attach to one of two afferent neurons. There are three types of intrafusal fibre that are thought to be represented in most muscle spindles; they are dynamic (Bag 1) and static (Bag 2)

bag fibres, and chain fibres. The dynamic bag fibres tend to have shorter and stiffer polar regions compared to static ones, as well as different elastic properties in the equatorial regions (Hulliger, 1984). These differences cause the equatorial regions in dynamic bag fibres to be stretched more in proportion to the loading dynamics (i.e. velocity and/or acceleration) and less to the magnitude of an applied load; whereas the equatorial regions of static bags and chain fibres tend to stretch in proportion to the magnitude of the load. All fibre types are served by spindle primary endings and are innervated by a large diameter type Ia afferent. Chain and static bag fibres are also served by spindle secondary endings, and are innervated by medium-diameter type II afferent fibres. Most muscle spindles will contain one dynamic bag fibre, one or two static bag fibres, and two to eleven chain fibres (Prochazka, 1996); as well as one type Ia and one type II afferent. As such, under passive loading conditions, each type Ia fibre responds to both static (length) and dynamic (velocity and/or acceleration), and each type II fibre responds to static (length) properties of muscle stretch.

All intrafusal fibres are also served by efferent projections to their contractile polar regions. Humans, for the most part, use an independent small diameter gamma (γ) motor neuron system, also known as the fusimotor system, to control spindle fibre tension (Hulliger, 1984). There are two types of fusimotor neurons; one type (dynamic) serves only Bag 1 fibres, whereas the other (static) serves both static Bag 2 and chain fibres. As such, the fusimotor system can be used to tune muscle spindle static and/or dynamic sensitivity to stretch. Work by Prochazka et al (1985, 1988) in cat models has revealed that the fusimotor system can, in certain scenarios, operate independently from the α-motor neuron system to increase the sensitivity of muscle spindles. Furthermore, this work also revealed that static and dynamic fusimotor activity can be, and is, controlled separately, depending on the scenario in which the animal is placed.

Type Ia and type II muscle spindle afferents tend to have different projections within the central nervous system. Type Ia fibres make strong monosynaptic projections to the motor neuron pool of the host muscle, as well as excitatory projections to adjacent heteronymous motor neuron pools and inhibitory projections to antagonist motor neuron pools via Ia inhibitory interneurons (Jankowska, 1992). Type Ia axon collaterals also ascend to the cortex, brainstem and cerebellum via the posterior column medial lemniscus pathway and the spino-cerebellar pathways. Type II afferents tend to have fewer direct monosynaptic connections to homonymous motor neuron pools and yet still have diverse spinal projections. Type II muscle afferents project to ipsilateral homonymous and heteronymous motor neuron pools, as well as ipsi-, contra-, and bilateral spinal interneuron and propriospinal circuits (e.g. multi-segment/limb spinal reflex circuits and central pattern generators; Jankowska, 1992). Type II muscle afferents also project to the brainstem, cerebellum and cortex through the spino-cerebellar and posterior column medial lemniscus pathways.

The most immediate response to an imposed stretch to a muscle is a short latency reflexive contraction of the stretched muscle. The earliest, and most robust, components of the stretch reflexes are thought to be evoked by primarily monosynaptic (with some oligosynaptic) projections from type Ia muscle afferents onto the homonymous motor neuron pool (Burke et al, 1984), with a standard onset latency of approximately 36-40 ms post-stretch (ankle plantarflexors; Burke et al, 1983) and typically lasts for less than 20 ms. This reflex will be referred to as the short-latency reflex (SLR) component of the stretch reflex. Often there is a second, later and longer, burst of reflexive activity in the same muscle (Gottlieb and Agarwal, 1979, 1980), called the medium-latency reflex (MLR) component. There is more debate about the origin of the MLR compared to the SLR. Muscle spindle type II afferents are thought to play

a significant role in this response in the lower limb (Schieppati and Nardone, 1999), however type Ia afferents have also been implicated (Matthews, 1989, Lewis et al, 2005, Schuurmans et al, 2009, Meskers et al, 2010, Klomp et al, 2013). The SLR component is scaled to the velocity of stretch (Gottlieb and Agarwal, 1979), while the MLR is thought to be dictated by stretch amplitude (Gottlieb and Agarwal, 1979) or stretch duration (Lewis et al, 2005, Schuurmans et al, 2009, Klomp et al, 2013), which at a constant velocity reflects amplitude.

1.5.2 How do muscle spindles and stretch reflexes contribute to balance control?

Muscle spindles are thought to play an important role in balance control by monitoring changes in muscle length, particularly about the ankles. Fitzpatrick et al (1992) demonstrated that people could balance an inverted pendulum (based on the Inverted Pendulum model of balance control; *cf.* Winter et al, 1998) with their feet while either seated or strapped in an upright position to a rigid board. This method of balancing does not involve the vestibular system (no body movement), and could be performed without visual inputs (eyes closed). Therefore, it was argued that ankle length proprioceptors (muscle spindles) could be used to maintain balance. These results are in good agreement with observations from persons with peripheral neuropathies (Diabetic, Charcot-Marie-Tooth type II), who tend to sway more than age-matched healthy controls during unperturbed stance (Nardone et al, 2006). Muscle spindles in the ankle musculature, as well as throughout the body, are thought to be involved in detection of balance perturbations, as well as modulation of the amplitude and pattern of reactive balance responses to perturbations (Inglis et al, 1994, Bloem et al, 2000, Bloem et al, 2002, Allum et al, 2001, Nardone and Schieppati, 2004, Nardone et al, 2006). In gait, muscle spindles are thought to be involved in reinforcing anti-gravity muscle tone in ankle extensors, monitoring for

unexpected perturbations, and for transitioning between swing and stance phases (Pearson, 1995). There is now, in fact, strong evidence to demonstrate that the transition from stance phase to swing phase (in cats) is dependent on the length of the hip flexors. The stance phase of gait can be prolonged by limiting flexor stretch and initiated by artificial stretch of the hip flexors (Lam and Pearson, 2001, Lam and Pearson, 2002).

1.5.3 Stretch reflex modulation

There is now mounting evidence to suggest that various components of the stretch reflex pathway can be modulated in different contexts or states. As mentioned earlier, cats use static and dynamic fusimotor activity to tune muscle spindle sensitivity to different tasks (Prochazka et al, 1985, 1988). Likewise, there is now evidence to suggest that humans increase muscle spindle sensitivity when attending to passive movements (Hospod et al, 2007, Ribot-Ciscar et al, 2009), are challenged with high-gain visual feedback in a voluntary task (Nafati et al, 2004), attempt to learn a novel task (Wong et al, 2011), or stand in a high postural threat scenario (Davis et al, 2011, Horslen et al, 2013). In fact, Horslen et al (2013), using the same height paradigm as proposed here, recently demonstrated that there are changes in Achilles tendon-stretch reflexes that cannot be explained by changes in Hoffmann (H-) reflexes, which are an electrical analog of the stretch reflex that bypass the spindle. These stretch reflex changes were interpreted as reflecting an increase in muscle spindle sensitivity with increased postural threat. Likewise, the MLR component of a stretch reflex is known to be influenced by intention (Calancie and Bawa, 1985); or by postural "set", as they are larger when free standing compared to when holding onto a stable surface (Schieppati and Nardone, 1995). This suggests that this phase of the reflex may be subject to supraspinal modulation. Finally, H-reflexes are known to be depressed when people

switch from a seated to standing (Katz et al, 1988, Hayashi et al, 1992) or from prone to standing (Koceja et al, 1993, Koceja et al, 1995) posture. These changes have been attributed to increased pre-synaptic inhibition of Ia afferents in standing compared to seated or prone postures (Katz et al, 1988). Likewise, H-reflexes, and therefore likely stretch reflexes, are known to be modulated phasically throughout the gait cycle (Capaday and Stein, 1987, Llewellyn et al, 1990, Krauss and Misiaszek, 2007). Lower leg extensor H-reflexes are inhibited during the swing phase and dis-inhibited during the stance phase, preventing plantar flexion during swing and assisting in weight-bearing and propulsion during stance.

There is also evidence to suggest that MLR components of stretch reflexes are subject to adrenergic and serotonergic modulation at the spinal interneuronal level (Bras et al, 1989, 1990, Noga et al, 1992, Jankowska et al, 2000). These pathways may even be activated by some of the same arousal and anxiety-related systems that are thought to affect the vestibular nuclei in states of increased vigilance, such as the locus coeruleus, the Kölliker-Fuse nucleus, and the raphe nuclei (Type II afferents: Noga et al, 1992; Vestibular nuclei: Balaban and Thayer, 2001, Balaban, 2002, Balaban, 2004). These models have now been tested in human studies, where small doses of Tizanidine, an α_2-adrenergic agonist that affects muscle type II interneuron pathways, have been used to supress MLR amplitudes (Corna et al, 1995, Grey et al, 2001, Meskers et al, 2010). Together, these studies have revealed that the MLR can be suppressed through suppression of the muscle type II interneurons, but not abolished. This means that type II muscle afferents contribute to, but are not solely responsible for the MLR.

There is now evidence to suggest muscle spindle sensitivity changes with a height-induced postural threat (Horslen et al, 2013). However, the conclusions drawn from this study are inherently limited by the methods used. Tendon stretch reflexes, used by Horslen et al (2013),

preferentially activate muscle spindle primary endings (Burke et al, 1983), most likely from dynamic bag fibres. Dynamic bag fibres have distinct fusimotor innervation from static bag and chain fibres (Prochazka, 1996). And, in cat models, dynamic fusimotor activity has, in certain cases, proven to be tuned independently from static fusimotor activity (Prochazka et al, 1985, 1988).

1.5.4 How can static and dynamic stretch reflex properties be examined?

There are several potential methods that can be used to study stretch reflex function or muscle spindle sensitivity. Microneurography, the direct recording of sensory afferent firing from a peripheral axon (Vallbo et al, 1979), is often held as a gold standard. However, this technique typically requires limb restriction to ensure that the recording electrode is not dislodged from the nerve, and as such, is inappropriate for most free-standing scenarios where postural threat might be manipulated (see for contrast Aniss et al, 1990a, 1990b).

Hoffmann reflexes (H-reflexes), evoked with electrical stimulation of peripheral axons, are a relatively less invasive method of testing the stretch reflex pathway, and are generally considered more suited to use with natural movements, including standing and walking tasks (Capaday, 1997, Zehr, 2002, Misiaszek, 2003). However, this technique is limited in that it 1) bypasses the muscle spindle and therefore cannot be used to investigate changes in receptor function, and 2) preferentially activates large diameter type Ia afferents (Zehr, 2002, Misiaszek, 2003), meaning contributions from medium diameter type II muscle afferents are not accounted for. Tendon-tap stretch reflexes are easy to evoke and even less invasive than H-reflexes. However, like H-reflexes, they preferentially activate spindle primary afferents (Burke et al, 1983), presumably only ones arising from dynamic (bag 1) intrafusal fibres (Hulliger, 1984,

Prochazka, 1996). Despite these limitations, H-reflexes and tendon-tap reflexes have been used to probe the stretch reflex pathway in multiple postural threat scenarios. Sibley et al (2007) in quiet standing at height and Llewellyn et al (1990) in walking across a narrow elevated beam both found that H-reflexes were depressed with height; the cause was likely attributable to pre-synaptic inhibition (Llewellyn et al, 1990). In contrast, Horslen et al (2013) failed to replicate these findings with more stringent ankle angle and background muscle activity controls in either a height or threat of postural perturbation scenario; however, they did find changes in tendon-tap reflex amplitudes, suggesting a change in spindle sensitivity. As such, the Ia muscle afferent component of a stretch reflex may either be inhibited, or unaffected, by a height-induced postural threat. H-reflexes can also be conditioned to bring out oligosynaptic contributions to stretch reflex changes. While H-reflexes are often used to excite Ia afferents, at higher stimulation intensities the stimulus also excites medium-diameter type II muscle afferents. Such contributions to the reflex in the homonymous muscle would be washed out through α-motor neuron post-activation refractory periods related to antidromic axon conduction. However, Marchand-Pauvert et al (2005) described a model where either quadriceps or semitendinosus could be conditioned with type II mediated inputs from tibialis anterior or medial gastrocnemius (TA onto quadriceps). While this method now introduces the opportunity to test changes in muscle type II afferent pathways, it is limited in that it does not test the spindle (and therefore is not sensitive to fusimotor activity), and requires a much more intense, and likely painful, stimulus than un-conditioned H-reflexes. How pain, or fear of pain, might impact the effects of a height-induced postural threat are not known and are beyond the scope of this thesis.

Ischemic block or nerve cooling are effective methods for dissociating muscle type II afferent effects from type Ia in stretch reflex paradigms. In ischemic block a pressure cuff is

inflated around the limb proximal to the muscle of interest. Axon transmission is systematically disrupted as pressure is sustained over prolonged periods, beginning with the largest diameter axons and eventually impacting smaller ones. As such, there is an opportunity window where type Ia fibres are occluded but α-motor neurons remain intact. At this point stretch reflexes can still be evoked (Grey et al, 2001). Here, the SLR is abolished, but the MLR responses remain (Grey et al, 2001). Similarly, limb cooling can be used to cool peripheral nerves, which slows axon conduction velocities. The relative change in velocity is greater for type II afferents than for type Ia. Scheippati and Nardone (1997) found that the delay in MLR was significantly greater than the delay in SLR after cooling. They argue, therefore, that MLR responses in the lower limbs are mediated by type II afferents. Matthews (1989) used the same logic and a different result to argue that MLR responses in the wrist muscles were mediated by type Ia, and not type II afferents, suggesting that MLR in the hands and in the legs may not be the same reflex. While both ischemic block and nerve cooling can be used to dissociate type Ia from II afferent effects, they are both limited in that they can be painful, can have a relatively brief testing window that may change over time, and tend to differentiate type Ia and II afferents but do not necessarily address static and dynamic spindle response properties. A method that could balance the joint goals of dissociating type Ia and II afferent contributions as well as be sensitive to both static and dynamic spindle sensitivity changes would be ideal.

One attractive method that would permit interpretations of changes in static or dynamic contributions to stretch reflexes would be to use ramp-and-hold stretch reflexes. The reflex response to a ramp-and-hold stretch is multi-phasic, with short (SLR) and medium (MLR) latency reflex components. The SLR is known to be velocity (Gottlieb and Agarwal, 1979) and not amplitude dependent (Grey et al, 2001, Lewis et al, 2005, Meskers et al, 2010). The SLR is

thought to be the basic Ia-mediated myotatic stretch reflex (Nardone et al, 1996), which is mainly mono-, but with some polysynaptic, contributions (Burke et al, 1984). As such, changes in the amplitude of the SLR when stretch velocity is kept constant reflect changes in either dynamic spindle or motor neuron pool excitability, or pre-synaptic facilitation. In contrast, the MLR response is dependent on stretch amplitude (or possibly duration), but not velocity (Gottlieb and Agarwal, 1979, Gottlieb and Agarwal, 1980, Grey et al, 2001, Lewis et al, 2005, Meskers et al, 2010). The MLR appears to be a complex composite of multiple afferent sources. There is evidence that the MLR can be suppressed, but not abolished, by Tizanidine (Corna et al, 1995, Grey et al, 2001, Meskers et al, 2010), suggesting that type II muscle afferents contribute to the amplitude of (but may not trigger) the MLR. Nerve cooling studies have demonstrated that the MLR is more delayed than the SLR in the legs (Schieppati and Nardone, 1997, Grey et al, 2001), but not in the arms (Matthews, 1989), suggesting that in the legs, at least, the MLR may also be triggered by muscle type II afferents. Changes in the amplitude of the MLR, when stretch amplitude is held constant, might reflect a peripheral change in muscle spindle length-dependent fibre sensitivity, or central effect such as pre-synaptic inhibition/facilitation and/or a change in motor neuron pool excitability. As such, examining the SLR and MLR separately will permit separate interpretations about the effects of a postural threat on static and dynamic properties of stretch reflexes.

It may also be possible to probe the effects of changes in spindle static sensitivity by measuring crossed stretch reflexes (Corna et al, 1996). While type Ia spinal connections tend to be principally unilateral, interneurons activated by type II afferents can have uni- contra- or bilateral projections (Jankowska, 1992). Corna et al (1996) described MLR responses in flexor digitorum brevis that appeared in the left side when the right side was stretched in isolation.

There were no crossed SLR responses, suggesting that the Ia afferents did not make monosynaptic projections to the contralateral motor neuron pool. Since type II afferents only innervate length-sensitive static intrafusal fibres (static bag and chain fibres), any changes in crossed reflexes could be limited to the type II muscle afferent reflex arcs (including spindle, afferent, interneuron, and motor neuron pool). While this method is promising, it is relatively untested, and is not as well understood as the stretch reflex response in a stretched muscle.

1.6 Golgi tendon organs and Ib reflexes

1.6.1 Basic anatomy and physiology

Golgi Tendon Organs (GTOs) are muscle mechanoreceptors that are located in series with muscle fibres at the musculo-tendinous junction (Pearson and Gordon, 2000). The organ is an encapsulated network of collagen fibrils that links a group of 10-20 muscle fibres to the tendon proper, with generally 1 and no more than 6 muscle fibres from a single motor unit (Prochazka, 1996). Each GTO is served by a single Ib afferent that becomes un-myelinated as it enters the capsule and branches into an extensive network of free nerve endings. The nerve endings are entwined in the collagen fibrils, such that when the fibrils are given a tensile load they contract about the nerve endings and depolarize the nerve. While Ib afferents tend to be quiescent when GTOs are unloaded, they tend to have regular firing rates when their GTO is loaded (Prochazka, 1996). GTOs tend to have a high threshold for imposed tension while the parent muscle is relaxed (Prochazka, 1996); however, they are very sensitive to tension applied through active muscle contraction (Houk and Henneman, 1967). As such GTOs are thought to be responsible for monitoring tension developed through active contraction of a muscle. A single GTO is generally not a good indicator of whole muscle force, as each GTO is only sensitive to activation or de-activation of the motor units attached to it (Vallbo et al, 1979) and the fraction of total

muscle force generated by the motor units attached to it may not increase linearly with total force (Prochazka, 1996). However, GTOs as a population are thought to code accurately for total active muscle force (Vallbo et al, 1979, Horcholle-Bossavit et al, 1990, Prochazka, 1996).

Ib afferents have an extensive network of spinal and supraspinal projections. Ib afferents from the legs project to the cortex through the posterior column medial-lemniscus pathway and to the cerebellum and brainstem through the dorsal spinocerebellar pathway. Ib afferents have both inhibitory and excitatory di- and tri-synaptic projections onto homonymous, synergist, and antagonist motor neuron pools (Jankowska, 1992, Baldissera et al, 1981). The most extensively studied Ib spinal reflex is the Ib autogenic inhibitory reflex. In brief, Ib afferents monosynaptically excite Ib inhibitory interneurons, which then inhibit the motor neuron pool of the parent muscle (Prochazka, 1996). Ib neurons are also known to excite antagonist muscles while inhibiting synergists, a reflex that was initially termed the "inverse myotatic reflex", as it has the opposite effect as the myotatic reflex (Baldissera et al, 1981, Yanagawa et al, 1991). There are also excitatory spinal reflexes, likely tri-synaptic, that act on the homonymous and synergist motor neurons that are activated by Ib afferents (Baldissera et al, 1981, McCrea, 2001). While Ib input seems to be the dominant input for each of these pathways, they are not exclusively Ib reflexes as other afferent sources (Ia, cutaneous, joint) can modulate and/or activate these pathways (Jankowska, 1992, Baldissera et al, 1981). Furthermore, Ib reflex pathways are modulated by descending inputs from cortical and brainstem centres (cortico-spinal, rubrospinal, reticulospinal; Baldissera et al, 1981, McCrea, 2001). In cat models, the cortico- and rubrospinal tracts facilitate transmission in both inhibitory and excitatory Ib pathways, while noradrenergic projections from the reticulospinal pathways can depress these reflexes (Baldissera et al, 1981). These effects also seem to be adaptable, as descending

(rubrospinal) inputs (in cats) seem to be able to selectively activate or deactivate different Ib reflex pathways (Hongo et al, 1969). The spinal locomotor circuits are known to have a significant role in selecting which reflexes are activated and when (McCrea, 2001). The Ib autogenic inhibitory reflex and antagonist excitatory reflexes seem to be dominant at rest (Pierrot-Deseilligny et al, 1981), however excitatory force feedback seems to contribute as much as 50% of plantar flexor EMG feedback during the stance phase of walking (Sinkjaer et al, 2000). In conclusion, it is probably inappropriate to refer to "the" Ib reflex, as the reflexive response evoked in any given scenario is likely a composite of the many Ib spinal reflexes.

1.6.2 How do GTOs and Ib reflexes contribute to balance control?

GTOs, as muscle load receptors, are ideally suited to evaluating how gravity is loading the body (Duysens et al, 2000). Afferent feedback from GTOs is thought to interact with other load-relevant information (e.g. skin, muscle spindles) to promote anti-gravity muscle activity (Duysens et al, 2000, Van Doornik et al, 2011). This is particularly true in dynamic scenarios, such as reactive balance responses or gait. Dietz and colleagues have used body immersion in water to study the effects of body loading on responses to balance perturbations. Subjects unloaded by submersion (through buoyancy), and then weight was systematically added to increase body load while they remained submersed. Despite similar ankle kinematic changes across loading conditions, people scaled their muscular responses to body load (Dietz et al, 1989). This means that the response to a perturbation is subject to both the amplitude of perturbation, but also the load of the body when perturbed. GTO load compensation is thought to act through constant online positive force feedback (Grey et al, 2007). This has been demonstrated by monitoring the response to sudden unloading of the ankle plantar flexors during

the stance phase of gait (Sinkjaer et al, 2000, Grey et al, 2007, af Klint et al, 2009). When the plantar flexors are unloaded (e.g. floor is accelerated downward) there is a short-latency decrease in soleus muscle activity (50 ms onset latency; Sinkjaer et al, 2000, Grey et al, 2007); this effect is still present when the ankle is immobilized (af Klint et al, 2009) suggesting that it is not simply antagonist inhibition related to stretch of ankle dorsiflexors. Similar unloading effects have recently been observed in standing, where a sudden downward acceleration of the support surface evokes a brief suppression of soleus EMG (~50 ms latency) prior to a large increase in activity (after 100 ms) that likely serves as a balance correcting response (Van Doornik et al, 2011). The short latency decrease in activity has been attributed to loss of positive force-feedback, likely mediated by GTOs in the ankle plantar flexors (Van Doornik et al, 2011).

1.6.3 Modulation of Ib reflexes

There is considerable evidence to suggest that Ib reflexes are phasically modulated throughout the gait cycle. Pearson and Collins (1993) demonstrated that group I afferent stimulation, that normally would induce inhibition, caused excitation of MGas activity in a fictive locomotor cat model. These results were interpreted as evidence that force-feedback in one plantar flexor could facilitate activity in a synergist, so long as the locomotor spinal circuits were engaged. Similar results have been observed in human gait, in that Ib reflexes in soleus that are inhibitory in the swing phase can become excitatory in the stance phase (Stephens and Yang, 1996). Furthermore, sudden unloading of the ankle plantar flexors during the stance phase of gait is known to reduce plantar flexor EMG at short latencies (~50 ms; Sinkjaer et al, 2000, Grey et al, 2007, af Klint et al, 2009). These results imply that some of the muscle activity in the plantar flexors in the stance phase of gait is related to ongoing positive force feedback, presumably from

Ib afferents (Grey et al, 2007); similar effects have been proposed for quiet standing (Van Doornik et al, 2011). In conclusion, Ib reflexes are not only tunable, but in some cases distinct Ib reflexes may be activated by common input stimuli, depending on the gait phase in which they are triggered.

Ib reflexes are known to be both state- and task-dependent. Ib reflexes that would normally be inhibitory while seated can become excitatory in walking (Stephens and Yang, 1996, Faist et al, 2006). Faist et al (2006) argue that there are, in fact, two independent processes that account for the reflex reversal. Ib inhibition is dictated by muscle loading, as evidenced by the fact that a prone subject who is relaxed demonstrates Ib inhibition in SOL yet when they generate a 300 N isometric load the inhibition disappears (Faist et al, 2006). Second, the Ib excitatory response depends on locomotion, in that reflexes were only ever facilitated during walking (cats: Conway et al, 1987; humans: Stephens and Yang, 1996, Faist et al, 2006).

While inter-task state-specific changes in Ib reflexes have been examined, there has yet to be a thorough investigation into whether intra-task state-specific changes can also occur. There is evidence to suggest that a postural threat can evoke changes in each of the other sensory systems that will be investigated in this thesis. And, there is evidence that supraspinal centres, known to be influenced by threat (e.g. reticulospinal; Balaban and Thayer, 2001), exert control over Ib interneurons (Baldissera et al, 1981, Jankowska, 1992, McCrea, 2001); however, the purpose of these connections has typically been discussed in a locomotor context (Pearson, 2008). As such, the potential for a postural threat to modulate Ib reflexes in a non-locomotor context is still an open question.

1.6.4 How can Ib reflexes be studied in humans?

By far the most common method used to study Ib reflexes has been with Hoffmann (H-) reflex conditioning. Pierrot-Desilligny et al (1979) first demonstrated that soleus (SOL) H-reflexes could be inhibited by stimulating the nerve for the medial gastrocnemius head (MGas) approximately 5 ms beforehand. This effect is explained by the relative strength of projection of Ib fibres from MGas onto SOL, compared to Ia fibres (Pierrot-Deseilligny et al, 1979). Later work expanded on the theme by demonstrating that most leg muscles could be inhibited by stimulating the nerve supplying a synergist (Pierrot-Deseilligny et al, 1981). This method has since been applied to understand the effects of tonic muscle activation on Ib reflexes (Yanagawa et al, 1991), and how Ib reflexes change in different postural or gait tasks (Stephens and Yang, 1996, Faist et al, 2006). A similar H-reflex conditioning technique, first demonstrated in humans by Heckman et al (1984; demonstrated in cats by Coppin et al, 1970), where prolonged muscle vibration (20 min) has been shown to centrally inhibit large diameter Ia afferents while leaving Ib afferents unaffected. Coppin et al (1970) proposed that a current pulse that was previously strong enough to activate both lower-threshold Ia and relatively higher-threshold Ib afferents pre-conditioning should only activate Ib reflexes post-conditioning. These techniques are attractive because they are generally easy to perform, and the stimuli are relatively painless. However, the H-reflex stimulus is known to activate more than Ib fibres, specifically Ia and possibly skin afferents, which are known to converge on the Ib autogenic inhibition pathway (Jankowska, 1992). As such, it would be difficult to conclude which system(s) are responsible for any changes observed with threat.

Another method that has been used to study the Ib pathway is with sudden mechanical unloading of the foot, either by imposing a plantar flexion to the ankle or accelerating the

support surface downward (Sinkjaer et al, 2000, Grey et al, 2007, af Klint et al, 2009, Van Doornik et al, 2011). These techniques have been applied to walking, where there is a characteristic drop in SOL EMG approximately 50 ms after the ankle is plantar flexed in the stance phase. The latency of the response is too early to be explained by the supposed vestibular-evoked response to sudden whole-body drops (~74 ms) described by Mellville-Jones and Watt (1971). Also, Lidocaine block of the common peroneal nerve did not affect this response, suggesting that it arose from afferents in SOL, not tibialis anterior (Sinkjaer et al, 2000). This technique is limited because sudden unloading of one leg during standing is known to perturb balance and evoke dynamic postural responses (Carpenter et al, 1999a). As discussed in other chapters of this proposal, responses to postural perturbations are known to be altered in high postural threat scenarios (Carpenter et al, 2004), and therefore, these effects may confound interpretation of results of a foot-unloading experiment at height.

Tendon electrical stimulation (TES) has emerged as a promising method for probing reflexes evoked by Ib afferents (Burne and Lippold, 1996). Single TES pulses evoke an alternating series of depressions and facilitations in the rectified EMG of a tonically contracting muscle (Burne and Lippold, 1996, Khan and Burne, 2009). The predominant response to TES in a seated or laying posture is inhibitory, as evidenced by suppression of tendon stretch reflexes and magnetic motor-evoked potentials (Khan and Burne, 2010), and decreased single motor unit firing probability and frequency (Rogasch et al, 2011). It was initially unclear whether the response was due to stimulation of Ib afferents or the skin under the electrodes (Burne and Lippold, 1996). However, the response is absent when the skin at the area of stimulation is stretched and is no longer over the tendon (Burne and Lippold, 1996). Furthermore, the pattern and latency of inhibitory and excitatory peaks in the rectified EMG is different for TES

stimulation of the Achilles tendon and sural nerve stimulation (Khan and Burne, 2007, 2009, 2010, Rogasch et al, 2012). Rogasch et al (2012) attempted to clarify the contribution of skin to the response by comparing superficial TES to TES delivered directly to the tendon via needle electrodes. They found that the onset latency of the initial inhibition was the same for both stimulation techniques, however the amplitude was slightly reduced and the excitatory responses were delayed with direct tendon stimulation. As such, it is likely that the initial inhibitory response to TES is related to activation of the Ib autogenic inhibitory pathway via Ib afferents. The source of the later components is, as yet, unresolved. One limitation to this technique is it has only been performed in seated (Burne and Lippold, 1996, Khan and Burne, 2010) or lying subjects (Khan and Burne, 2007, 2009, Rogasch et al, 2011, 2012). As such, it is unclear how the response might be affected by the state-specific modulation of the Ib inhibitory pathways related to standing or walking (Stephens and Yang, 1996, Faist et al, 2006).

1.7 Cutaneous receptors and reflexes

1.7.1 Basic anatomy and physiology

There are four types of cutaneous mechanoreceptor that contribute to the sense of touch. Each receptor is located in a slightly different location in the dermis or epidermis, and each receptor is preferentially activated by a different mechanical stimulus. Merkel cells (disks) are located under epidermal ridges at the border between the epidermis and dermis (Gardner and Johnson, 2013). Merkel cells are preferentially activated by compression, but also respond to lateral stretch of the overlaying skin. Merkel cells are innervated by slow adapting type 1 cutaneous fibres (SA1) and code well for sustained pressure, particularly from edges or points applied to the skin (Gardner and Johnson, 2013). Meissner corpuscles are located in the dermis adjacent to the limiting ridges (indentations) of the epidermal ridges. Shear strain across the skin

surface deforms the epidermal ridges which, in turn, strain the Meissner corpuscles. Meissner corpuscles are innervated by fast adapting type 1 cutaneous afferents (FA1); as such, their code decays rapidly with sustained stimulation, making them ideal for indicating transient skin stretches. Ruffini endings, which are fusiform capsules with lines of collagen anchored to the dermis, are preferentially activated by skin stretch along the axis of their collagen strands (Gardner and Johnson, 2013). They are innervated by slow adapting type 2 cutaneous afferents (SA2), and code well for static skin stretch. Finally, Pacinian corpuscles are multi-layered globular receptors in the dermis that are stimulated by vibrations transmitted through the skin. They are served by fast adapting type 2 cutaneous afferents (FA2), and respond well to the dynamic properties of skin deformations (e.g. onset/offset and rate of indentation or stretch). There are also cutaneous receptors that respond to temperature, pain, and itch. These receptors are known to reflexively evoke movement (scratching, withdrawal responses); however, they will not be addressed in this thesis.

Cutaneous afferents have an extensive network of projections into the central nervous system. Afferents from the cutaneous mechanoreceptors ascend to the cortex via the posterior column medial-lemniscus pathway. This information is used for perception of touch and is also integral to motor control (Johnson, 2001). Cutaneous afferents also project to the cerebellum, the reticular formation, and the vestibular nuclei through the cuneo- and dorsal spinocerebellar pathways (Wilson and Peterson, 1981, Gardner and Johnson, 2013). There is also an extensive network of spinal reflexes that are triggered by cutaneous afferents. Cutaneous afferents can have excitatory and inhibitory effects on motor neurons, which are generally thought to be mediated by propriospinal pathways. The most extensively studied cutaneous-evoked spinal reflex system is the flexor reflex afferent (FRA) system (Baldissera et al, 1981). The FRA network is

extensive, involving multiple muscles and limbs; it is also not purely cutaneous, as Ib, muscle types II and III, and joint mechanoreceptor afferents are all known to access it (Baldissera et al, 1981, Hultborn, 2001). The classic FRA is a generalized flexion of the stimulated limb and extension of the non-stimulated limb, often in response to a painful stimulus (Sherrington, 1910). However, the reflex can be evoked with non-painful tactile stimuli (Forssberg et al, 1975, Forssberg, 1979, Grillner and Rossignol, 1978), or more commonly in humans, with mild electrical stimulation of a cutaneous nerve (likely stimulating larger diameter afferents serving mechanoreceptors, instead of smaller pain afferents; Zehr et al, 1997). The FRA reflex is also not necessarily a "flexor" reflex, because it can evoke general extension in certain tasks or limb configurations (Forssberg et al, 1975, Forssberg, 1979, Grillner and Rossignol, 1978) or in response to certain tactile stimuli (Sherrington, 1910). The neural circuitry responsible for controlling these effects is known to reside in the spinal cord, as chronic spinal cats are known to modulate these reflexes (Forssberg, 1979). Cutaneous afferents are also known to project onto the Ib autogenic inhibitory interneurons (Baldissera et al, 1981). Because cutaneous receptors monitor loads applied to the body, it has been proposed that cutaneous projections onto Ib interneurons serve to compliment proprioceptive load monitoring from GTOs and together they are integrated to control loading (i.e. Ib) reflexes (Duysens et al, 2000). Interneurons serving cutaneous afferents (both excitatory and inhibitory) are facilitated by stimulation of the rubrospinal tract (in cats; Hongo et al, 1969) and depressed by stimulation of the reticular formation (Engberg et al, 1968). These descending pathways are thought to play an important role in directly mediating cutaneous reflexes as well as mediating propriospinal pathways that, in turn, modulate cutaneous reflexes, particularly in locomotion (McCrea, 2001).

1.7.2 Contributions of tactile sensations and cutaneous reflexes to balance control

It has been suggested that the cutaneous system contributes to balance control by monitoring pressure exerted onto the ground through the feet (Duysens et al, 2000) and by detecting obstacles that pose as trip hazards during gait (Forssberg, 1979). Fujiwara et al (2003) provided evidence skin receptors on the foot sole being involved in tracking pressure exerted on the ground by asking subjects to voluntarily match a previously experienced target COP location before and after foot sole cooling. Subjects were less accurate in matching the target position while foot sole tactile acuity was depressed after cooling. Similarly, Maurer et al (2001) demonstrated that vibration applied to the fore foot could evoke fluctuations in COP that were correlated to the vibration stimulation frequency in otherwise quiet standing subjects. While these studies imply a role for cutaneous mechanoreceptors in balance control during quiet standing, they are limited because both cooling and foot sole vibration can affect (inhibit or activate) muscle spindles in the intrinsic foot muscles, which are thought to be involved in balance control in and of themselves (Wright et al, 2012). However, local anesthetic injections into the foot soles also cause an increase in COP movement during quiet standing (Meyer et al, 2004b). There are also changes to reactive balance control with foot sole anesthesia. When the soles of the feet are anesthetised, less plantar flexor EMG is generated in a feet-in-place response to a forward fall (Do et al, 1990), and a shift from an ankle-trunk movement strategy to a hip loading-unloading strategy is observed in response to sideways translations (Meyer et al, 2004a). There are also changes to reactive stepping in response to a balance perturbation when cutaneous information from the feet is altered. Maki and Perry and colleagues have experimented with altering foot sole cutaneous information on reactive stepping to a balance perturbation. When subjects are made more aware of the bounds of the base of support by having them stand on a

small elevated (~1.5 cm) platform that is smaller than their base of support, they are less likely to take a step in response to a balance perturbation (Maki et al, 1999). Likewise, they are more likely to take a step when their feet are cooled to decrease cutaneous sensation (Perry et al, 2000). There are also reductions in SOL EMG and increases in TA EMG during the reactive step when plantar cutaneous information is altered (Do and Roby-Brami, 1991, Thoumie and Do, 1996). Foot sole pressure information, along with muscle load and length information from muscle afferents, is thought to contribute to setting the tone of the leg extensor muscles during the stance phase of gait (Duysens et al, 2000). Tactile information from the legs and feet also plays a role in triggering stumbling reactions during gait, which assist in getting the feet clear of trip hazards (Forssberg, 1979). In conclusion, cutaneous information from the foot soles is crucial for monitoring pressure distribution under the feet, and tactile stimuli are used to detect impending threats to balance (i.e. perturbations) in both standing and walking.

1.7.3 Modulation of cutaneous reflexes

There is now abundant evidence to suggest that cutaneous reflexes can be phasically modulated throughout the gait cycle in cats (Forssberg et al, 1975, Forssberg, 1979, Grillner and Rossignol, 1978) and humans (Yang and Stein, 1990, Van Wezel et al, 1997, Duysens et al, 1990, 2004, Zehr et al, 1997, 1998, 2001a, 2001b, Zehr and Chua, 2000, Komiyama et al, 2000, Haridas et al, 2005, 2006, 2008, Baken et al, 2005, 2006). Forssberg et al (1975) demonstrated that a non-painful tactile stimulus applied to the back of a chronically spinalized cat's paw will evoke limb flexion or extension depending on where the leg is in the gait cycle. These effects are not confined to the stimulated leg, as Grillner and Rossignol (1978) demonstrated similar responses in the non-stimulated hind limb depending on its configuration. Yang and Stein (1990)

were able to demonstrate similar effects in humans; by electrically stimulating the tibial nerve at the ankle they evoked either excitatory or inhibitory responses in TA and SOL that switched polarity in different periods of the gait cycle. In agreement with the literature on cats, phasic modulation of cutaneous reflexes can also be observed in the non-stimulated limb in humans. There is evidence to suggest that reflexes can be evoked in the limb contralateral to that stimulated (Haridas et al, 2005), or in the arms when the legs are stimulated and vice-versa (Zehr and Chua, 2000, Zehr et al, 2001a, 2001b, Haridas et al, 2006). The phasic modulation of cutaneous reflexes is not likely due to the phasic modulation of the lower motor neuron pool onto which the stimulated cutaneous afferents project, as the cyclic modulation of cutaneous reflexes does not follow the changes known to occur in Hoffmann reflexes during gait (Zehr et al, 2001b).

There is now also evidence to suggest that cutaneous reflexes are subject to state-dependent modulation. Cutaneous reflexes differ across postural states. Burke et al (1991) described changes in EMG responses evoked with electrical stimulation of the sural nerve at the ankle. They found that different postural tasks (e.g. sitting, standing normally, standing on an incline, standing on an unstable surface) evoked the same basic reflex pattern, but varied in amplitude across different postural tasks. These differences were mainly discussed in terms of the different muscles engaged in the different tasks. However, the authors noted subtle differences in reflexes evoked in different task conditions with comparable background muscle activation levels (Burke et al, 1991). Likewise, Forth and Layne (2008), using a vibratory tactile stimulus applied to the lateral border of the foot sole, demonstrated that evoked EMG responses in SOL or LGas were larger in standing than when seated. While the authors admit their stimulus likely activated muscle spindles in the intrinsic foot muscles as well as cutaneous

mechanoreceptors (Forth and Layne, 2008), short latency stretch reflexes are typically supressed in standing, compared to sitting (Katz et al, 1988, Hayashi et al, 1992). This indicates the changes observed by Forth and Layne (2008) are not likely attributable to the muscle spindle stretch reflex pathway. Similarly, cutaneous reflexes evoked from the same nerve and measured in the same muscle can differ in both amplitude and pattern when comparing walking to quiet standing (Yang and Stein, 1990, Komiyama et al, 2000).

State-specific changes in cutaneous reflexes are not isolated to different postural tasks. There is evidence to suggest that the manner in which the nervous system uses cutaneous information in a specific task may be subject to the context in which the task is performed. Bouyer and Rossignol (2003) found that otherwise intact cats who had all the cutaneous nerves serving their hind paws severed could regain the ability to walk on a treadmill in 1-3 days, but could not walk on a horizontal ladder (which required precise foot placement) for several weeks. When ladder walking ability did return, the strategy the cats adopted was considerably different from their pre-denervation states. Since the basic mechanics of normal and ladder walking are essentially the same, these data suggest that the role of cutaneous feedback in a motor task may depend on the context in which the task is performed. Humans can also modulate cutaneous reflexes to suit task context. Baken et al (2006) demonstrated that sural nerve reflexes probed during gait were smaller when self-triggered, compared to when computer-triggered with an unpredictable delay. This suggests that these reflexes are subject to anticipation-related modulation. Also, Haridas et al (2005) found that the magnitudes of lower limb reflexes evoked from the sural and tibial nerves were larger when subjects were presented with the threat of an unpredictable forward or backward trunk perturbation while walking on a treadmill, compared to normal treadmill walking. Similar effects were found in arm muscles with sural and tibial nerve

stimulation with the threat of perturbation (Haridas et al, 2006). While Haridas et al (2005, 2006, 2008) did not attempt to quantify the psychosocial or autonomic consequences of their threat of perturbation, Horslen et al (2013) recently demonstrated that the threat of perturbation to quiet standing evokes similar fear, anxiety and arousal effects as the height-induced postural threat that has been used in this thesis. This suggests that cutaneous reflexes, at least during walking, are subject to modulation by a postural threat.

1.7.4 How can the cutaneous system be studied?

There are a number of methods that can be used to study cutaneous inputs to the central nervous system or reflexes evoked by cutaneous inputs. As with any peripheral sensory receptor, microneurography is an ideal technique for determining how a receptor responds to a given stimulus. There are no efferent fibres serving the skin mechanoreceptors, as such, there is no reason to changes in cutaneous receptor function with a postural threat (assuming that the threat does not cause a specific mechanical deformation of the skin). However, microneurographic recordings have been used to demonstrate that natural stimulation of cutaneous afferents can reflexively modulate ongoing EMG activity (McNulty et al, 1999, McNulty and Macefield, 2001, Fallon et al, 2005). While this technique is attractive due to its potential to show gain changes in cutaneous reflexes by demonstrating changes in muscle activity to individual afferent inputs, it is limited by the basic methodological demands of a microneurographic experiment. Specifically, it requires limb stabilization, and therefore is not well suited to a free-standing postural task. Alternatively, changes in perception of cutaneous stimuli can be used to infer changes in input and/or processing of cutaneous information to the nervous system. Methods such as mechanical stimulation with von Frey hairs (*cf.* Perry et al, 2000) or a grating orientation

task (*cf.* Peters et al, 2009) can be used to probe tactile acuity, where changes can then be measured within or across subjects. However, these tasks require perception of a stimulus, which is likely subject to attention to the stimulus. Allocation of attention is known to be altered with a height-induced postural threat (Huffman et al, 2009). Therefore, any method involving perception might be confounded by the attentional shifts that are known to occur at height.

There are forms of cutaneous stimulation that evoke reflex responses, and may therefore avoid some of the confounding effects of changes in attention. Vibratory stimuli applied to the foot sole can evoke short-latency (<60ms) EMG responses (*cf.* Forth and Layne, 2008). However, these stimuli presumably also activate muscle spindles in the intrinsic foot muscles, which may contribute to the EMG response (Forth and Layne, 2008). There is now evidence to suggest that muscle spindle sensitivity is increased with a postural threat (Horslen et al, 2013, Davis et al, 2011). As such, it would be difficult to dissociate cutaneous reflex mediated changes in EMG responses to foot sole vibration from changes related to spindle sensitivity. Similar issues confound the use of electrical stimulation of the foot sole to condition triceps surae stretch reflexes (*cf.* Sayenko et al, 2007, 2009). Electrical stimulation of the skin at low intensities is thought to activate local skin mechanoreceptors (Zehr et al, 1997). Similar to vibration, the method would be confounded in that the conditioned stretch reflexes would likely not be comparable across threat conditions.

Some groups have attempted to study cutaneous contributions to postural control by either adding artificial cutaneous inputs, e.g. with sole vibration (Maurer et al, 2001, Forth and Layne, 2008) or by having subjects stand on an augmented tactile surface (i.e. a frame that outlines their base of support; Do and Roby-Brami, 1991, Maki et al, 1999), or by temporarily removing cutaneous inputs, e.g. by hypothermic anesthesia (Fujiwara et al, 2003, Perry et al, 2000) or

Lidocaine injection (Meyer et al, 2004a, 2004b, Do et al, 1990, Thoumie and Do, 1996), and measuring how postural control changes. All of these methods are limited in that they monitor either postural sway and/or centre of pressure in quiet standing scenarios, or postural responses to perturbations. Postural control in both quiet and dynamic states is known to rely on multiple convergent types of sensory information, including vestibular and proprioceptive sources. Study 1 of this thesis has already established that a postural threat can influence vestibular-postural coupling and gain, and previous height-induced postural threat work suggests that the proprioceptive system (or at least muscle spindles) is influenced by threat (Horslen et al, 2013, Davis et al, 2011). As such, it would be difficult to associate changes to postural control across threat conditions to changes in cutaneous information processing.

Electrical stimulation of the nerves serving the skin of the foot to evoke lower limb muscular responses is a method that is commonly used to probe how cutaneous inputs are utilized in, and how their use is altered across, postural and gait tasks. Electrical stimulation of cutaneous nerves at the ankle with brief pulse trains has frequently been used to evoke reflexes (Yang and Stein, 1990, Burke et al, 1991, Duysens et al, 1990, 2004, Van Wezel et al, 1997, Zehr et al, 1997, 1998, 2001a, 2001b, Zehr and Chua, 2000, Komiyama et al, 2000, Haridas et al, 2005, 2006, 2008, Baken et al, 2005, 2006). The reflexes can be observed in multiple muscles simultaneously, and the responses are typically muscle-specific (Yang and Stein, 1990, Burke et al, 1991, see Komiyama et al, 2000 for contrast). The stimuli required for these responses are not painful, as the ideal stimulation intensity is below the normal pain threshold (Zehr et al, 1997). These reflexes have been demonstrated to be malleable, as they are dependent on the nerve stimulated (Van Wezel et al, 1997, Komiyama et al, 2000), the background activity of the muscle of interest (Burke et al, 1991, Komiyama et al, 2000), and the postural task in which they are

tested (Yang and Stein, 1990, Burke et al, 1991, Komiyama et al, 2000, Haridas et al, 2005). Likewise, changes in these reflexes can occur independently from (although are still subject to) gross changes in motor neuron pool excitability, as demonstrated by different patterns of phasic changes in Hoffmann reflexes and skin reflexes (Zehr et al, 2001b). One potential criticism of this technique is that the superficial peroneal and tibial nerves, commonly chosen for stimulation (Yang and Stein, 1990, Van Wezel et al, 1997, Zehr et al, 1997, Komiyama et al, 2000, Haridas et al, 2005, 2006), are mixed nerves at the ankle. They carry both cutaneous afferent and muscle afferents and efferents (Moore and Dalley, 1999). However, the sural nerve, which is also commonly used (Burke et al, 1991, Van Wezel et al, 1997, Zehr et al, 1998, Baken et al, 2005, 2006), is a purely cutaneous nerve at the level of the ankle. This means that the reflex evoked from stimulation of the superficial peroneal or tibial nerves may contain aspects that are triggered by muscle afferents, and would be subject to any central modulation of muscle afferent pathways. However, this criticism does not apply to the sural nerve. Therefore, stimulation of the sural nerve across threat conditions will be used in this study to probe how cutaneous reflexes from the foot onto the muscles of the lower limb might change with a postural threat.

Chapter 2: Study 1 – Modulation of human vestibular reflexes with increased postural threat

2.1 Preamble

The purpose of this chapter is to determine if vestibular contributions to balance control, as indicated by vestibular-evoked balance responses, are modulated when people are exposed to a height-induced postural threat. This purpose is addressed with the original research presented in section 2.2. However, upon completion of the studies presented in section 2.2 it became apparent that the findings, and conclusions drawn, were in conflict with then-recently published findings from Osler et al (2013). Consequently, both research groups agreed to participate in a published debate on the merits of the research concerning modulatory effects of fear of falling on vestibular-evoked balance responses. The opening argument in support of an effect of fear of falling on vestibular-evoked responses, summary of counter-points, and rebuttal are presented in sections 2.3 and 2.4.

2.2 Original research

2.2.1 Introduction

Converging evidence suggests that the vestibular system can be influenced by emotional factors such as fear and anxiety, or general autonomic arousal. Animal models have revealed excitatory reciprocal projections between the brainstem vestibular nuclei, vestibular cortex, and various neural centres involved with autonomic function and emotion including: the parabrachial nucleus, the dorsal raphe nucleus, and locus coeruleus (Balaban, 2002, Balaban and Thayer, 2001, Staab et al, 2013). These excitatory networks have been proposed to be principally activated in states of fear or vigilance in order to alter vestibular-evoked motor responses to self-

motion, and to increase sensitivity to imposed motion (Balaban, 2002). This hypothesis is supported by several behavioural studies which demonstrated increased vestibulo-ocular reflex (VOR) gain with arousal in both cats (Crampton and Schwam, 1961, Crampton, 1961) and primates (Furman et al, 1981).

Human studies probing the effects of anxiety or arousal on vestibular reflex function normally focus on the VOR (Collins and Poe, 1962, Collins and Guedry, 1962, Kasper et al, 1992, Yardley et al, 1995). Collins and Guedry (1962) revealed that a minimum level of "alertness" is required to evoke a stable nystagmus to a prolonged rotary acceleration. Similarly, drowsy subjects are less likely to produce VOR responses than awake ones (Kasper et al, 1992). Alerting tasks, such as mental arithmetic, are also more effective at increasing VOR gain than arousal-inducing drugs (amphetamine: Collins and Poe, 1962) or exercise (Yardley et al, 1995). As such, Yardley et al (1995) have concluded that VOR gain changes are not mediated by generalized autonomic arousal, but rather are specifically related to anxiety. Fear and anxiety are also known to affect balance control in both humans and animals (Kalueff et al, 2008). Mice that are genetically pre-disposed to anxiety tend to perform poorer on balance and exploration tasks (Lepicard et al 2000). Balance performance in these mice can be ameliorated with anxiolytic treatments (Venault et al, 2001; Lepicard et al, 2003) or worsened with anxiogenic treatment (Lepicard et al, 2000; 2003). Similarly, human studies have shown that fear or anxiety from a potential fall from an elevated support surface (a height-induced postural threat) leads to significant changes to balance control in static (Carpenter et al, 1999b, 2001a), dynamic (Carpenter et al, 2004, Brown and Frank, 1997), and locomotor tasks (Brown et al, 2002, Tersteeg et al, 2012). Since the vestibular system is a significant contributor to standing balance (Fitzpatrick and Day, 2004), altered vestibulospinal function has been proposed as a mediator of

postural threat-related changes to balance control (Carpenter et al, 1999b, 2004). While the effects of anxiety and arousal on VOR and general balance control are in line with predictions from anatomical models, the effects of fear, anxiety, and arousal on vestibular-evoked balance reflexes have yet to be examined directly.

Stochastic vestibular stimulation (SVS) has been proposed as a useful means of probing the human vestibular system during balance (Dakin et al, 2007, 2010, 2011, Mian and Day, 2009, Luu et al, 2012) and locomotor tasks (Blouin et al, 2011, Dakin et al, 2013). SVS evokes ground reaction forces (GRFs, Mian et al, 2010), as well as triceps surae EMG (Dakin et al, 2007), that are comparable to those evoked with discrete galvanic vestibular stimulation (GVS) pulses without inducing prominent whole-body displacements (Dakin et al, 2010) or requiring long stimulation times (Dakin et al, 2007). Certain aspects of the SVS frequency spectrum have been associated with different response features. For example, SVS and body sway are primarily correlated below 2 Hz. As such, the SVS signal can be tailored to produce very little correlated body sway, and yet still produce correlated GRF and EMG responses, by omitting frequencies below 2 Hz (Dakin et al, 2010). Limiting whole-body displacements in a height-induced postural threat scenario is important, as balance responses to whole-body perturbations are known to be altered in these situations (Carpenter et al, 2004, Brown and Frank, 1997).

Here, we present two studies that examined the influence of a height-induced postural threat on the magnitude of vestibular-induced balance responses. While previous work has revealed that long latency stabilizing responses to GVS are affected by a height-induced postural threat (Osler et al, 2013), the purpose of the present studies was to determine whether or not a postural threat modulates the direct relationship between vestibular signals and balance reflexes. Based on the assumption that the vestibular nuclei receive excitatory inputs from emotional

processing areas of the brain (Balaban, 2002, Balaban and Thayer, 2001), we hypothesized that threat-induced changes in the relationship between vestibular inputs and balance control would be reflected by an increase in the coupling between SVS and ground reaction forces and an increase in the gain of the evoked responses.

2.2.2 Methods

2.2.2.1 Study 1

2.2.2.1.1 Subjects and ethical approval

20 subjects were randomly divided into two experimental groups of 10 subjects each (Head Forward: 3 females; mean (SE) age 25.2 (1.6) years; Head Turned: 5 females, 22.5 (0.82) years). No subjects reported any known neurological, vestibular, or orthopaedic impairments that may have influenced their balance, or ability to complete the experimental tasks; also, people who self-reported an extreme fear of heights were excluded from the study prior to participation. All subjects gave written informed consent prior to their participation in the experiments, and all methods were reviewed and approved by the University of British Columbia Clinical Research Ethics Board.

2.2.2.1.2 Stimulation protocol

Bipolar binaural SVS was delivered percutaneously above the mastoid processes to stimulate the nearby vestibular afferents. A unique stimulation profile was generated for each trial using a custom LabVIEW code (National Instruments, USA). To generate the stimulus, random white noise was digitally band-pass filtered around 2-25 Hz. The resulting 2-25 Hz stimulus had a nearly flat power spectrum. The digital signal was then converted to analog (NI PCI-MIO-16E-1 with BNC 2110, NI, USA) and fed to a stimulator (model 2200, A-M Systems,

USA). The SVS stimulus peak amplitude was 4.5 mA with mean (SE) RMS amplitude of 1.1 (0.01) mA. Nominally, the electrodes were positioned such that positive current caused an anode-right cathode-left configuration. In both experiments, subjects experienced two 5-minute trials of continuous SVS stimulation, one in each threat condition.

2.2.2.1.3 Head orientation

Subjects tend to lean away from the edge at height. When subjects stand with their toes at the edge balance changes occur in the antero-posterior (AP) direction, with little to no effect in the medio-lateral (ML) direction (Carpenter et al, 1999b, 2001a, Davis et al, 2009). However, a general excitation of the vestibular system, as hypothesized here, should not be limited to one anatomical plane. Therefore, in order to ensure that any observed changes in the relationship between the vestibular and balance control systems were related to general vestibular excitation and not an effect of edge avoidance, we conducted two separate experiments in Study 1, with two different head orientations. SVS responses (like GVS) are oriented in a craniocentric manner, such that the bipolar binaural stimulation evokes a signal of head rotation occurring around a vector directed posteriorly and pointing approximately 18° above Reid's plane (Fitzpatrick and Day, 2004). In all conditions subjects stood with their head level and fixated on a visual target for the duration of the trial (~9 cm^2 red square on a white wall, 3.7 m from the subject at eye level). In Study 1 - experiment 1, subjects stood with their heads facing forward resulting in a ML perturbation of the body (Fig 2-1A), and in experiment 2 subjects' heads were turned 90° to the right resulting in an AP perturbation of the body (Fig 2-1A; Lund and Broberg, 1983, Pavlik et al, 1999, Mian and Day, 2009). In the head turned experiment, the lift was re-positioned in the room such that the subjects could use the same visual targets, yet also be positioned in the same spot on the lift. If the changes are to be attributed to a general vestibular

excitation, we would expect to see changes in the vestibular-balance control relationship in both the head turned and head forward experiments.

2.2.2.1.4 Experimental protocol

In both experiments subjects stood with their toes at the edge of a force plate (#K00407, Bertec, USA) positioned at the edge of a hydraulic lift (M419-207B10H01D, Penta-lift, Canada). Their foot length was measured and their feet were positioned such that the lateral edges of their 5th metatarsals were spaced equal to their foot length. Subjects were exposed to two postural threat conditions: LOW and HIGH. The first SVS trial was always set in the low threat condition (LOW), where the lift rested in its lowest position (80 cm above ground) and a 60 cm wide table was placed in front of the subjects to provide an extended support surface. This is comparable to standing on the ground (Carpenter et al, 2001a). Subjects were then seated such that their feet stayed in the same place on the force plate, and the lift was elevated to 3.2 m (Fig. 2-1B). They then stood up and completed a second trial in this high postural threat condition (HIGH). Participants wore a safety harness and were attached to a rope system at all times. The rope was kept loose enough to allow free movement of the subject on the platform, but would arrest a fall, if needed. There was also an experimenter within reach of the subject at all times; there were no rails bordering the lift platform to prevent a fall (per Davis et al, 2009). The LOW condition always preceded the HIGH condition as there is a known order effect of presentation (Adkin et al, 2000), and we desired maximum contrast between conditions.

2.2.2.1.5 Measures

Psychosocial measures of fear, anxiety, perceived stability and balance confidence, as well as autonomic arousal were measured. Prior to each trial, subjects evaluated their confidence in maintaining their balance for the stimulation period at that surface height (0: no confidence –

100: completely confident). After each trial (while seated) subjects rated their experienced fear (0: not at all fearful – 100: extremely afraid), perceived stability (0: not at all stable – 100: completely stable), and anxiety (16 questions with a maximum score of 144, higher scores indicate higher anxiety). Each of these questionnaires has been demonstrated to have moderate to high reliability in this threat manipulation paradigm (Hauck et al, 2008). Electrodermal activity (EDA) was also measured from the thenar and hypothenar eminences of the non-dominant hand (model 2501, CED, UK). EDA was sampled at 1000 Hz and averaged across the stimulation period to quantify autonomic arousal (Venables, 1991).

Shear GRFs across the forceplate as well as the SVS drive signal were measured to represent the balance system output and vestibular input, respectively. GRFs were sampled at 100 Hz (Power 1401, CED, UK) and converted to Newtons offline using the plate calibration parameters and a custom MATLAB (MathWorks, USA) script. GRFs were then low-pass filtered at 50 Hz and up sampled to 5000 Hz. The SVS signal was concurrently sampled at 5000 Hz.

2.2.2.1.6 Calculations

The relationship between vestibular stimulation and the balance control system was examined in terms of coupling and gain. Signal coupling reflects the relationship between two signals and in the context of this study reflects the fidelity of transmission of vestibular signals to the balance control system. Signal coupling was examined using 2 separate, but congruent measures: cumulant density and coherence. Coherence is a measure of correlation across frequencies bounded between 0 and 1, and indicates where in the frequency spectrum signals are related. Cumulant density, in contrast, is an indicator of degree and direction of correlation between signals, as well as the relative lead or lag times between them. In keeping with previous studies (Mian and Day, 2009, Dakin et al, 2010), the cumulant density function was calculated

such that a positive value indicates an association between a positive current (anode right-cathode left) and a rightward GRF (acting on the body) or a negative current (anode left-cathode right) and a leftward GRF in the head forward orientation; a positive value would indicate a positive current-forward or negative current-backward association in the head turned orientation. Gain, in turn, reflects the scale relationship between signals; here it reflects the amplitude of the balance response to a given vestibular input. Coherence, gain, and cumulant density estimates between the SVS and GRFs were calculated using the NeuroSpec 2.0 code; a freely available archive of MATLAB code intended for statistical signal processing and based on the methods of Halliday et al (1995). Specific details pertaining to each factor are outlined below.

Coherence and gain estimates were calculated from concatenated data across all subjects for a single condition; the data were not normalized between subjects prior to concatenation. There were 10 subjects in each experiment, with 300 s of data per condition, of which 299.8 s were used. As such, there was just less than 50 min of data for each condition in each experiment. The concatenated data were split into 1830 disjoint segments (183/subject) that were 1.6384 s long from which SVS-GRFs coherence was calculated, giving a frequency resolution of 0.61 Hz (see Dakin et al, 2010 for details). Within-conditions coherence was determined significantly greater than chance when it passed a 95% confidence limit based on the number of disjoint sections used in the analysis (Halliday et al, 1995). Differences in coherence between LOW and HIGH conditions were then assessed with the NeuroSpec 2.0 difference of coherence sub-routine, based on the methods of Rosenberg et al (1989) and Amjad et al (1997). This statistic tests the assumption that the coherence estimates are equal with normally distributed variance. The test compares standardized differences (HIGH-LOW) and 95% confidence limits based on the Fisher transform (tanh-1) of the square root of the coherence values, compared to a

$\chi 2(1)$ distribution (Rosenberg et al, 1989, Amjad et al, 1997); any frequencies where the standardized difference of coherence exceeded the 95% confidence limits were taken to be statistically different.

SVS-GRFs gain magnitude estimates and point-wise 95% confidence limits were also calculated from the concatenated data using the method described by Halliday et al (1995). Point-wise 95% confidence limits were calculated about the gains, and any frequencies where the confidence limits did not overlap were assumed to be statistically different. Ratios between signal gains (HIGH:LOW) were calculated to determine the magnitude of the difference in gain estimates at each frequency where both LOW and HIGH trials had significant within-conditions coherence. Means and standard errors of these ratios were then calculated within these ranges from the grouped data.

Cumulant densities, in contrast to coherence and gain, were calculated on a subject-by-subject basis in both AP and ML directions using a modified version of the NeuroSpec 2.0 software that served to gain-normalize the cumulant density function to provide values of correlation bounded between -1 and +1 (see Dakin et al, 2010). As such, the cumulant density estimates, like coherence, reflected signal coupling; but the subject-by-subject analysis allowed us to ensure that our results were not skewed by outliers. Both coherence and cumulant density provide converging evidence to address our coupling hypothesis in these experiments. Short (SLP) and medium latency peaks (MLP) in the vestibular induced GRF's were identified in plots of the cumulant density using a custom MATLAB script and were visually confirmed by an experimenter. Peak (or trough) amplitudes were recorded for each subject in the relevant direction of stimulation.

2.2.2.1.7 Statistical analyses

Normality was assessed with Kolmogorov-Smirnov tests for all variables; $P<0.05$ was interpreted as a failure to meet the assumption of normality. SLP, MLP, EDA, and ratings of anxiety all met assumptions of normality, and, therefore, paired-samples t-tests were used to identify changes across conditions for these variables. Differences in ratings of fear of falling, balance confidence, and stability were assessed with non-parametric Wilcoxon Signed Rank tests. The criterion for statistical significance was set to $\alpha = 0.05$ for all tests and effect sizes are reported as eta squared (η^2; parametric tests) or r (non-parametric). As reported earlier, difference of coherence tests were used to identify changes in coherence estimates across threat conditions and confidence limits were used to examine differences in gain across threat conditions. In both cases 95% confidence limits were used as thresholds for statistical significance. As expected, there was no significant coherence between SVS and the GRF in the AP direction for the head forward experiment (orthogonal to stimulation) and only minimal coherence in the ML direction for the head turned experiment at either height; as such, effects across height conditions were not evaluated for these planes.

2.2.2.2 Study 2

One potential confound for height effects on vestibular function in Study 1 is the change in peripheral visual cues caused by changes to the distance to the floor and ceiling in raising the platform to the HIGH height. This may affect vestibular-evoked balance reflexes by altering visual-vestibular interactions (Britton et al, 1993, Day and Guerraz, 2007); which are known to be important for balance control (Vidal et al, 1982; Guerraz and Bronstein, 2008). While distance to foveal target was kept constant between height conditions, we recognize the potential

change in peripheral cues in Study 1 as a potential confound. Therefore, a follow up study was conducted to address this issue.

5 subjects (4 males; 27.2 (3.3) years) participated in this control study. The heights, stimulation parameters, measures, and foveal targets were all the same as the head turned group in Study 1. In this study, however, subjects wore a set of goggles fitted with an upper visor and lower cardboard surface extending 19 cm forward (29.5 cm wide, and 5.5 cm separating upper and lower surfaces) that blocked the superior and inferior peripheral visual fields, to ensure the subjects could not see the floor or ceiling in either height condition. Lateral peripheral vision was also kept constant by hanging sheets from ceiling to floor on both sides of the subject (minimum 2.3 m from subject). All measures and calculations described in Study 1 were also used here. Due to the smaller sample, all within-subject differences are described with descriptive statistics; statistical tests were used for concatenated data.

2.2.3 Results

2.2.3.1 Psychosocial state and arousal

Standing at height was effective at inducing significant physiological and psycho-social changes in the participants of these experiments. Participants were more fearful and more anxious in the HIGH versus the LOW condition (Fear: Head Forward: $z = -2.62$, $p = 0.009$, $r = 0.585$; Head Turned: $z = -2.81$, $p = 0.005$, $r = 0.628$; Anxiety: Head Forward: $t_{(9)} = -3.49$, $p = 0.007$, $\eta^2 = 0.575$; Head Turned: $t_{(9)} = -4.54$, $p = 0.001$, $\eta^2 = 0.696$). Participants also felt less confident in their ability to maintain balance at height (Head Forward: $z = -2.72$, $p = 0.007$, $r = 0.607$; Head Turned: $z = -2.41$, $p = 0.016$, $r = 0.538$) and reported feeling less stable (Head Forward: $z = -2.82$, $p = 0.005$, $r = 0.631$; Head Turned: $z = -2.83$, $p = 0.005$, $r = 0.633$). EDA

was significantly increased in both HIGH conditions, indicating that participants were also more aroused in the HIGH compared to LOW conditions (Head Forward: $t_{(8)} = -4.81$, $p = 0.001$, $\eta^2 = 0.743$; Head Turned: $t_{(8)} = -3.15$, $p = 0.014$, $\eta^2 = 0.553$).

2.2.3.2 SVS-GRF relationships

Standing at height modulated the relationship between vestibular inputs and balance control in both experiments. There were significant increases in the peak amplitudes of SVS-GRF cumulant density functions in the HIGH, compared to LOW, condition in both head forward and head turned experiments (Fig. 2-2). There were significant peaks in the cumulant density at lag times of 136.2 ± 5.4 ms (SLP) and 292.9 ± 16.5 ms (MLP) in the LOW condition, and 129.5 ± 6.0 ms (SLP) and 300.3 ± 18.0 ms (MLP) in the HIGH condition with the head facing forward (Fig. 2-2A). SLP and MLP peaks were 39.8 ± 9.5% and 53.7 ± 19.1% larger with height (SLP: $t_{(9)} = 3.34$, $p = 0.009$, $\eta^2 = 0.553$; MLP: $t_{(9)} = -2.59$, $p = 0.029$, $\eta^2 = 0.427$; Fig 2-2B). Similarly, when subjects stood with their heads turned peaks occurred at 150.9 ± 8.9 ms (SLP) and 296.4 ± 22.9 ms (MLP) in the LOW condition and at 122.5 ± 3.6 ms and 260.0 ± 20.9 ms in the HIGH condition (Fig. 2-2C). In this experiment, the SLP amplitudes were 83.4 ± 23.6% larger and the MLP amplitudes were 96.6 ± 34.7% larger in the HIGH, compared to LOW, conditions (Fig. 2-2D); these increases were also statistically significant (SLP: $t_{(9)} = -3.38$, $p = 0.008$, $\eta^2 = 0.559$; MLP: $t_{(9)} = 2.91$, $p = 0.017$, $\eta^2 = 0.485$).

The coupling observed on a subject-by-subject basis was also demonstrated in the pooled coherence estimates. There was significant ML direction SVS-GRF within-conditions coherence when subjects stood with their heads forward in the LOW condition from 1.8 Hz to 11.0 Hz and in the HIGH condition from 1.8 Hz to 15.3 Hz (Fig. 2-3A). SVS-GRF coherence was

significantly greater at height than when participants were near the ground. This significant increase in SVS-GRF coherence at height was localized to between 4.3 Hz and 6.7 Hz, approximately (Fig. 3B). Likewise, when subjects' heads were turned, significant SVS-GRF coherence was observed in the AP direction between 1.8 Hz and 16.5 Hz in the LOW condition, and 1.8 Hz and 22.5 Hz in the HIGH condition (Fig. 2-3D). There was also greater SVS-GRF coherence in the HIGH, compared to LOW, condition between 5.5 Hz and 17.7 Hz (Fig. 2-3E).

Qualitatively, the signal gain estimates were similar in both experiments. The gains always peaked at the lowest frequency represented (1.8 Hz) and gradually decayed as frequency increased (Fig. 2-3C, F). There were significant increases in signal gain estimates across threat conditions in both head forward and head turned experiments. In the head forward experiment signal gains significantly differed across threat conditions between 1.8 Hz and 8.5 Hz (Fig. 2-3C; except 7.3 Hz). On average, the gain was 81 ± 12% larger in the HIGH, compared to the LOW, condition. This effect was more prominent in the head turned experiment, where signal gains were larger for all frequencies where the SVS and GRF signals cohered in both LOW and HIGH conditions (Fig. 2-3F). Here, the HIGH signal gain was 231 ± 23% larger than the LOW gain.

2.2.3.3 Study 2

Subjects in Study 2, with peripheral vision kept constant, had similar arousal and psychosocial responses to height as those in the experiments of Study 1. EDA (45.7 ± 7.3 %), fear of falling (30 ± 10.5 %), and anxiety (80 ± 27 %Δ) were all larger in the HIGH condition, compared to LOW. Likewise, balance confidence (24 ± 11.2 %) and perceived stability (22.4 ± 9.5 %) were both lower in the HIGH condition, compared to LOW.

SVS-GRF resultant data are plotted in Figure 2-4A. In line with Study 1, SLP amplitudes were 109 ± 59 % larger in the HIGH, compared to LOW, condition. Likewise, MLP amplitudes increased 47 ± 29% in the HIGH condition, compared to LOW. The concatenated SVS and GRF data from the 5 subjects revealed significant within-conditions difference in coherence between 1.8 Hz and 15.9 Hz in the LOW condition and between 1.8 Hz and 20.8 Hz in the HIGH condition. As shown in Figure 2-4A, the magnitude of coherence was generally greater in the HIGH, compared to LOW, condition between 4.8 Hz and 20.8 Hz; the difference of coherence test revealed statistically greater coherence in the HIGH condition at most points in this range. Finally, the gain was significantly larger in the HIGH condition at all frequencies where there was significant within-conditions coherence in both conditions (1.8-15.9 Hz); on average, the gain was 597 ± 136% larger in the HIGH condition.

2.2.4 Discussion

The purpose of these experiments was to determine the effects of a postural threat on the relationship between vestibular signals and balance control. Our results confirmed the first hypothesis that coupling, as indicated by coherence and cumulant density, would increase with increased postural threat. The pattern and timing of cumulant density peaks observed in the current studies are consistent with previous studies that have used SVS under similar quiet standing conditions (Mian and Day, 2009, Mian et al, 2010, Dakin et al, 2010). As hypothesized, both SLP and MLP peaks were increased in the HIGH, compared to LOW, conditions in all experiments. Significant coherence between SVS and GRFs was observed between 1.8-11.0 Hz, and 1.8-16.5 Hz in the LOW conditions of the head forward and head turned experiments of Study 1, respectively. There was significantly greater SVS-GRF coherence in the HIGH,

compared to LOW, conditions between 4.3 Hz and 6.7 Hz in the head forward and between 5.5 Hz and 17.7 Hz in the head turned experiment. Changes in the amount of vestibular-balance coupling reflect changes to the balance correcting response to a known vestibular error. Such changes in coupling have been shown to be influenced by postural state (Mian and Day, 2009, Mian et al, 2010, Reynolds, 2010) and task (Fitzpatrick et al, 1996, Luu et al, 2012). For example, coupling can be unconsciously dis-engaged when people are discreetly stabilized by a robotic support surface (Luu et al, 2012), a state where the subject is not self-balancing. It is also reduced as gait speed and cadence are increased (Dakin et al, 2013), states where a vestibular error evokes smaller step-by-step balance corrections (either by dis-engagement or mechanical stability; Brandt et al, 1999). Likewise, muscle-specific coupling changes throughout the gait cycle. Coupling to individual anti-gravity muscles is modulated by the phase of gait such that coupling rises and falls as the muscle becomes more or less suited to stabilize the body (e.g. early stance versus mid swing for triceps surae; Blouin et al, 2011). Vestibular coupling, in fact, cycles through different muscle groups and across limbs, such that a vestibular error signal evokes balance corrective responses throughout the gait cycle but only phasically in any given muscle (Dakin et al, 2013). Therefore, we interpret the increase in coupling observed in our experiments as state-specific changes in vestibular-motor coupling, whereby the response to a vestibular perturbation is increased, yet the task (standing upright) is essentially unchanged. While these changes in coupling reflect transfer of vestibular error signals to balance responses, they do not reveal changes in the gain of the vestibular-motor relationship; this question is more appropriately addressed using signal gain estimates.

The change in signal gains across threat conditions in all experiments confirms our second hypothesis, that gain would be increased with increased postural threat. The amplitude of evoked

GRF responses steadily decreased as SVS frequency was increased, as indicated by the shape of the signal gain estimate, in both experiments in Study 1 and also in Study 2. This relationship is consistent with previous reports using a 0-25 Hz SVS bandwidth (Dakin et al, 2010); although absolute gain values were on average lower in the study presented here. Compared to the LOW conditions, the signal gain estimates in the HIGH conditions were on average 81% larger in the head forward experiment and 231% larger in the head turned experiment of Study 1. This altered relationship corresponds with previously reported observations of changes in VOR reflex gain in response to anxiety (Yardley et al, 1995) and/or alertness (Collins and Poe, 1962, Collins and Guedry, 1962, Kasper et al, 1992). As a whole, these findings support an increased gain at the level of the vestibular nuclei.

The changes to SVS-GRF gain and coupling observed in our studies may be attributed to greater excitation of the vestibular nuclei. The amygdala, activated by fear inducing stimuli, can influence the vestibular nuclei through two parallel networks (Lang et al, 2000, Balaban and Thayer, 2001, Balaban, 2002, Liddell et al, 2005, Öhman, 2005). In the first network, the amygdala excites the parabrachial nucleus either directly, or indirectly through the locus coeruleus, and dorsal raphe nucleus (Lang et al, 2000, Balaban and Thayer, 2001, Balaban, 2002, Staab et al, 2013). Since the parabrachial nucleus has excitatory (and reciprocal) connections with each of the vestibular nuclei (Balaban, 2004), as well as the vestibulo-cerebellum (Staab et al, 2013), it could potentially facilitate vestibular-evoked reflexes through these pathways. The second network involves amygdalofugal projections to multiple cortical regions (Balaban and Thayer, 2001, Balaban, 2002, Liddell et al, 2005, Staab et al, 2013), including the parieto-insular vestibular cortex and anterior cingulate cortex, which are part of the multi-site vestibular cortex (Dieterich and Brandt, 2008). There is indirect evidence to suggest the vestibular cortex can

modulate the function of vestibular reflexes; presumably via cortico-bulbar projections (Marsden et al, 2005, Doricchi et al, 2002, Ventre-Dominey et al, 2003, Arshad et al, 2013, 2014). For example, middle cerebral artery stroke causes asymmetries in GVS-evoked balance responses, but unilateral pyramidal compression (affecting corticospinal tract in medulla, but not affecting pons) does not (Marsden et al, 2005). Likewise, VOR gain and bias have been shown to be affected by neglect caused by cortical damage (Doricchi et al, 2002, Ventre-Dominey et al, 2003), hemispheric dominance (Arshad et al, 2013), and experimental cortical inhibition with trans-cranial direct current stimulation (Arshad et al, 2014). Therefore, cortical-induced excitation of the brainstem vestibular nuclei could also potentially contribute to the observed facilitation of vestibular reflexes in our studies.

The above explanations for the change in the SVS-GRF relationship all assume that a change in gain occurs at the vestibular nuclei; however alternative mechanisms must be acknowledged. First, the vestibular cortex can evoke a motor response via projections through sensory association cortices to the motor cortex and corticospinal tract (Staab et al, 2013). Therefore, an excitation at the cortical level, independent of the vestibular nuclei, could potentially induce the changes in gain and coherence. However, the evidence from GVS experiments in people with middle cerebral artery stroke suggests cortical contributions to GVS-evoked balance reflexes do not bypass the brainstem (Marsden et al, 2005), making a corticospinal explanation of our results less likely. Similarly, the amygdala has direct excitatory projections to the caudal pontine reticular formation (Lang et al, 2000). Based on extensive reciprocal connections between the reticular formation, vestibular nuclei and vestibulo-cerebellum (Wilson and Peterson, 1981), the reticular formation has been implicated in GVS-evoked reflexes (Britton et al, 1993), and therefore may contribute to the larger SVS-evoked

reflexes observed in this study. Finally, the changes in SVS-GRF gain could potentially be attributed to tonic increases in lower motor neuron pool excitability in the spinal cord. Fear and anxiety based amygdalic activation of autonomic centres, including the locus coeruleus and dorsal raphe nucleus (Balaban and Thayer, 2001, Balaban, 2002), can cause diffuse monoaminergic activation of spinal motor neuron and interneuron pathways (Baldissera et al, 1981, Jankowska et al, 2000, Johnson and Heckman, 2010), which can cause persistent excitation of the motor neuron pool and change the input-output gain of the pool (Johnson and Heckman, 2010). However, it is unlikely that the motor neuron pool is in a general state of excitation in this scenario, as Hoffmann reflexes, which can be used to probe motor neuron pool excitability (Zehr, 2002, Misiaszek, 2003), are either not changed (Horslen et al, 2013) or suppressed (Sibley et al, 2007) while standing at height.

Gaze behaviour, particularly when subjects are not given specific instructions on where to look, can be different when standing at height, compared to standing on the ground. For example, when non-fearful people stand at height they tend to explore their visual scene, whereas fearful people restrict their gaze behaviour to a stable target (reviewed in Brandt and Huppert, 2014), possibly to help stabilize balance. While we attempted to control this effect by providing, and instructing subjects to look at, comparable visual targets in both conditions of the experiments in Study 1, the peripheral visual scene was different between height conditions. Controlling peripheral visual scene in Study 2, by use of blinders that occluded view of the floor and ceiling, provides evidence against a confounding visual effect related to standing at height. Changes in SVS-GRF coupling and gain in Study 2 were similar to those observed in Study 1 (Figure 2-4A). This is in line with other studies that have shown height-related behavioural changes to quiet standing persist when subjects stand at 3.2 m with eyes closed, or while wearing

peripheral vision blinders (Davis et al, 2009). Likewise, height-related changes to gait are still observed when the drop at the edge is obscured by a sheet that would not weight-support (Tersteeg et al, 2012), a situation where the threat of injury persists but the visual scene is similar across threat levels. Therefore, it is unlikely the changes in vestibular-evoked balance reflexes observed here, or the changes in general balance behaviours seen in other studies, can be attributed solely to changes in the visual scene related to standing at height.

By limiting the frequency range of stimulation to avoid the potential confounding effects of correlated body sway, we arguably have focused our stimulus to optimize the SLP component of the response at the expense of the MLP (Dakin et al, 2010). Some researchers argue that the MLP component of the balance response to vestibular stimulation is more likely to be of vestibular origin than the SLP (Britton et al, 1993, Marsden et al, 2002, Fitzpatrick and Day, 2004, Mian and Day, 2009). There is, in fact, some debate as to what the SLP and MLP peaks actually represent. For example, Britton et al (1993) have suggested that the SLP and MLP peaks might be of reticulospinal and vestibulospinal origin, respectively. In contrast, Cathers et al (2005) have suggested that the different peaks reflect otolith and canal inputs; however, Mian et al (2010) tested and failed to support the otolith hypothesis. We cannot contribute to this debate with these data. However, the direction of both peaks is dependent on head orientation (Mian et al, 2010), suggesting vestibular modulation of each. And, recent work from our group has demonstrated that all frequencies in our stimulation band contribute to both peaks (Dakin et al, 2011). As such, it is unlikely that the contributor of the MLP was not stimulated in this study. Finally, both peaks were amplified in HIGH conditions of this study; which implies that they were both modulated by a similar, if not the same, mechanism. However, in anticipation of this potential criticism related to the stimulus profile used in this study, we tested a single case study

subject in the head-forward protocol of Study 1 - experiment 1 with an open 0 Hz (> DC) to 25 Hz stimulation bandwidth to encourage prominent MLP responses. The results from this subject are shown in Fig. 2-4B. In line with the results from the full experiments, this subject had greater coherence, signal gain, as well as larger SLP and MLP amplitudes in the HIGH, compared to the LOW, condition. This further demonstrates that our results are generalizable beyond the SLP, and whatever subsystem(s) it might represent.

Another potential limitation to this study is the directional aspect of the postural threat created by having the edge of the platform always located in front of the subject. Several studies have demonstrated that when subjects stand quietly at the edge of an elevated platform, the changes to balance control only occur in the AP direction (Carpenter et al, 1999b, 2001a, Adkin et al, 2000, Davis et al, 2009). People tend to increase body sway stiffness in the AP direction (Carpenter et al, 2001a), and to lean backward away from the edge (Carpenter et al, 1999b, 2001a, Davis et al, 2009). The purpose of using two different head orientations in this study was to control for potential confounding effects of the edge location. Since the direction of the balance responses to GVS (Lund and Broberg, 1983) or SVS (Pavlik et al, 1999, Mian and Day, 2009) are dictated by head orientation, then we would expect that threat effects should be aligned with the direction of stimulation, not the edge, if the effects relate to an altered vestibular-balance relationship. Since increases in coupling and gain occurred in the both the AP and ML directions, the changes observed in these experiments cannot be attributed entirely to an attempt to avoid the edge or to changes in balance control related to the presence of the edge.

The current results are in conflict with a previous report which suggested early postural responses to square-wave GVS are not altered when people stand on a narrow elevated beam (Osler et al, 2013). Osler et al (2013) found that late (~800 ms) trunk sway responses in the ML

direction were attenuated at height (i.e. subjects swayed less towards the edge), but early responses were not affected. In contrast, our study was only concerned with early latency responses (136 ms for SLP and 296 ms for MLP). While early responses to GVS (or in our case SVS) reflect predominantly vestibular effects, responses occurring 400 ms or more after stimulus onset are thought to be dependent on multi-sensory feedback (Day and Guerraz, 2007); which would be subject to proprioceptive changes thought to occur with threat (Davis et al, 2011, Horslen et al, 2013), and which can be voluntarily attenuated (Reynolds, 2010). Likewise, mechanical filtering is known to reduce high frequency content from SVS-evoked responses in GRFs compared to EMG, and from GRFs to trunk sway (Dakin et al, 2010). In fact, there is negligible correlation between trunk sway and SVS at and above 2 Hz (Dakin et al, 2010), which is where all changes were observed across threat conditions in the current study. Thus, the measures used by Osler et al (2013) are likely insensitive to the early, high frequency, changes in the vestibular-postural relationship observed here.

These results add to a growing body of evidence suggesting that fear, anxiety and arousal can influence vestibular function; and that these effects translate to changes in balance control in humans. Temporary changes in state anxiety and arousal are linked to increased VOR gain (Collins and Guedry, 1962, Collins and Poe, 1962, Kasper et al, 1992, Yardley et al, 1995); suggesting that ascending vestibular reflexes are subject to autonomic or emotional modulation. Similarly, changes in mood and anxiety have been linked to changes in vestibular control of balance (Bolmont et al, 2002) and changes to centre of pressure in frequency bands thought to be under vestibular control (Wada et al, 2001). Furthermore, pathological fear and anxiety disorders have been linked to balance dysfunction (Yardley and Redfern, 2001, Balaban and Thayer, 2001) and vertigo (Balaban and Jacob, 2001, Furman and Jacob, 2001).

Our confirmation that vestibular control of posture is altered with threat sheds new light onto previously unresolved issues related to the effects of fear and anxiety on balance control. Automatic balance responses to perturbations are known to be altered when people stand at the edge of an elevated platform (Brown and Frank, 1997, Carpenter et al, 2004). Specifically, the amplitude of muscle activity in the "balance-correcting" phase (120ms-220ms post-perturbation) is increased with threat (Carpenter et al, 2004). The amplitude of this "balance-correcting" phase is known to be modulated by vestibular inputs (Allum and Pfaltz, 1985, Keshner et al, 1987, Horak et al, 1994, Carpenter et al, 2001b). As such, a functional implication of the increased gain observed here is that the same head acceleration evoked by a perturbation may generate larger vestibular-evoked balance response when the consequences of falling are elevated. Similarly, it has been postulated that continuous postural sway in undisturbed standing might serve to generate a certain quantity or quality of balance-related afferent information that the body can then use to monitor the postural state (Carpenter et al, 2010, Murnaghan et al, 2011, 2013). Undisturbed postural sway is reduced when people stand at the edge of an elevated platform (Carpenter et al, 2001a). We have previously proposed that changes in muscle spindle sensitivity with a postural threat might serve to facilitate this afferent acquisition process, thereby reducing the amount of sway required to meet the afferent demands in a threatening scenario (Horslen et al, 2013). As such, a second implication for these results is that the increased gain, and implied increased excitability, of the vestibular system in a threatening scenario might also facilitate sway reduction without compromising the amount of afferent information available to the central nervous system.

In conclusion, we have demonstrated that the gain and coupling of balance responses to SVS are increased in a high postural threat scenario. These responses are in agreement with the

proposal made by Balaban (2002), that vestibular pathways are excited in states of fear or vigilance to augment vestibular-evoked responses to imposed or self-motion. Furthermore, these data also support the emergent theme of a general sensitization to balance-relevant sensory information in high postural threat scenarios.

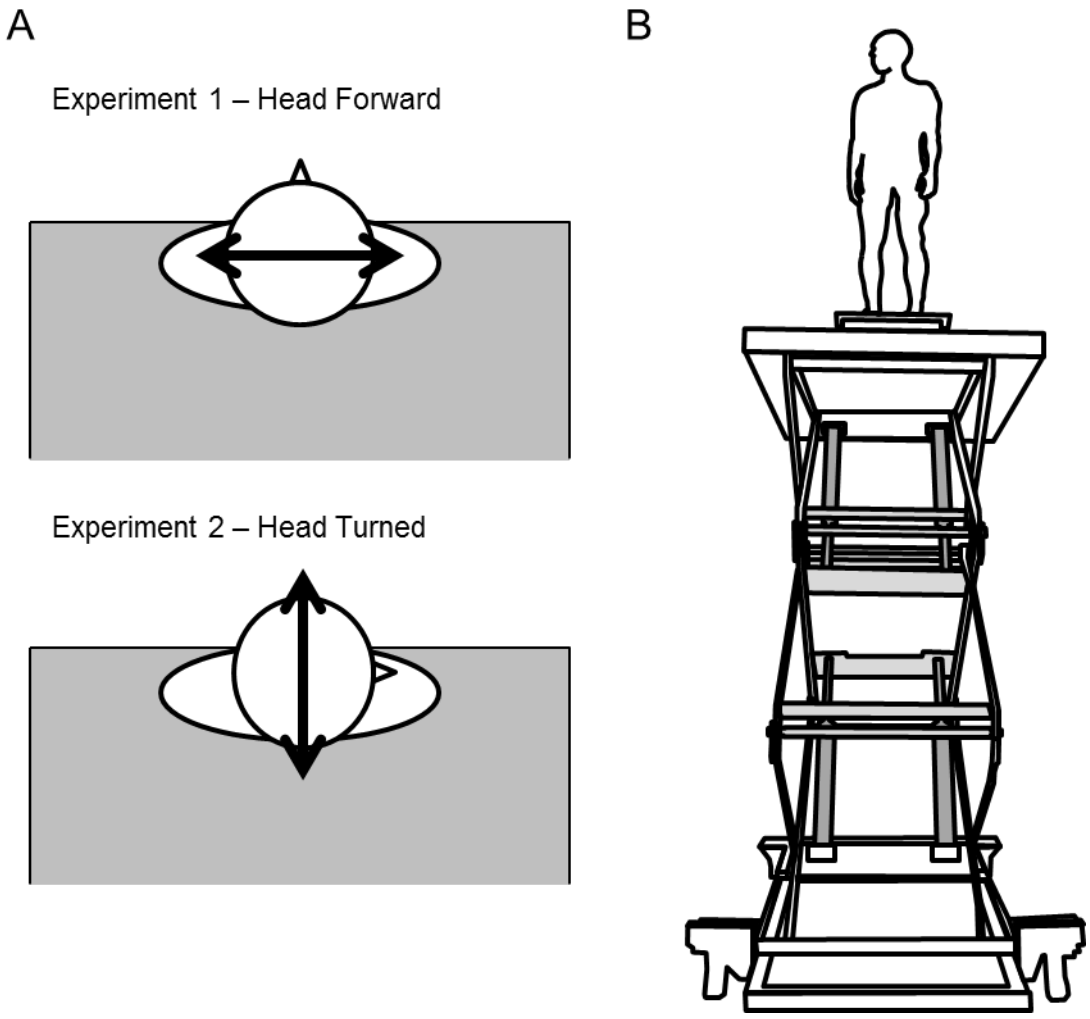

Figure 2-1 – Head orientation and height-induced threat

Participant head orientation with respect to the edge of the platform (part A) and subject standing position at the edge in the HIGH threat condition in the head turned orientation (part B).

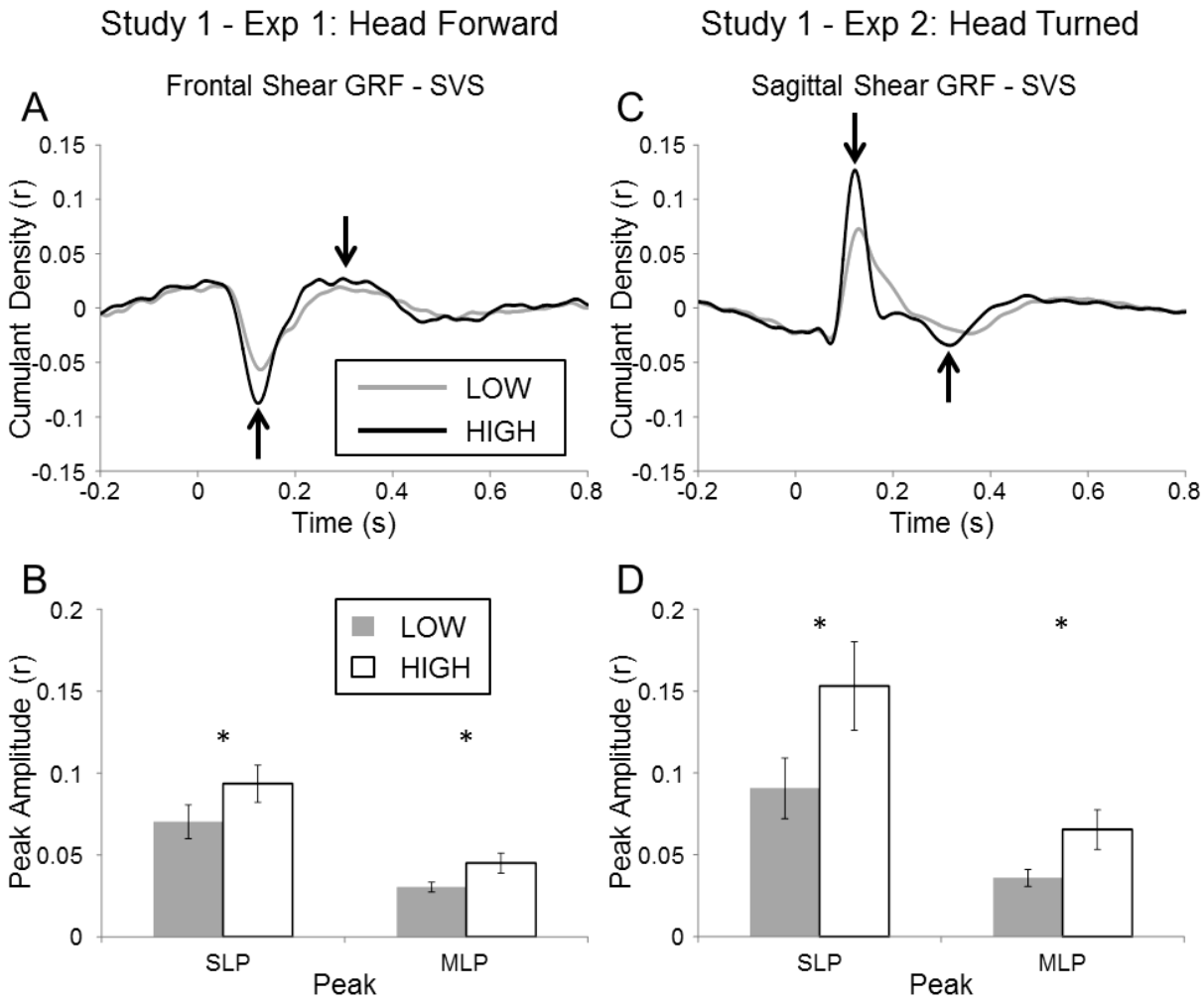

Figure 2-2 – Study 1 – Effects of height on SVS-GRF cumulant density

Mean LOW and HIGH cumulant density plots with peaks marked (arrows) for head forward (A) and head turned (C) experiments of Study 1. A positive deflection in the head forward cumulant density plot (A) means a positive current (anode right) is associated with a rightward GRF acting on the body or a negative current (anode left) causing a leftward GRF; a positive deflection in the head turned (C) trace indicates a positive current is associated with a forward GRF applied to the body. Mean SLP and MLP amplitudes in the head forward (B; n = 10) and head turned (D; n = 10) experiments; bars indicate SE.

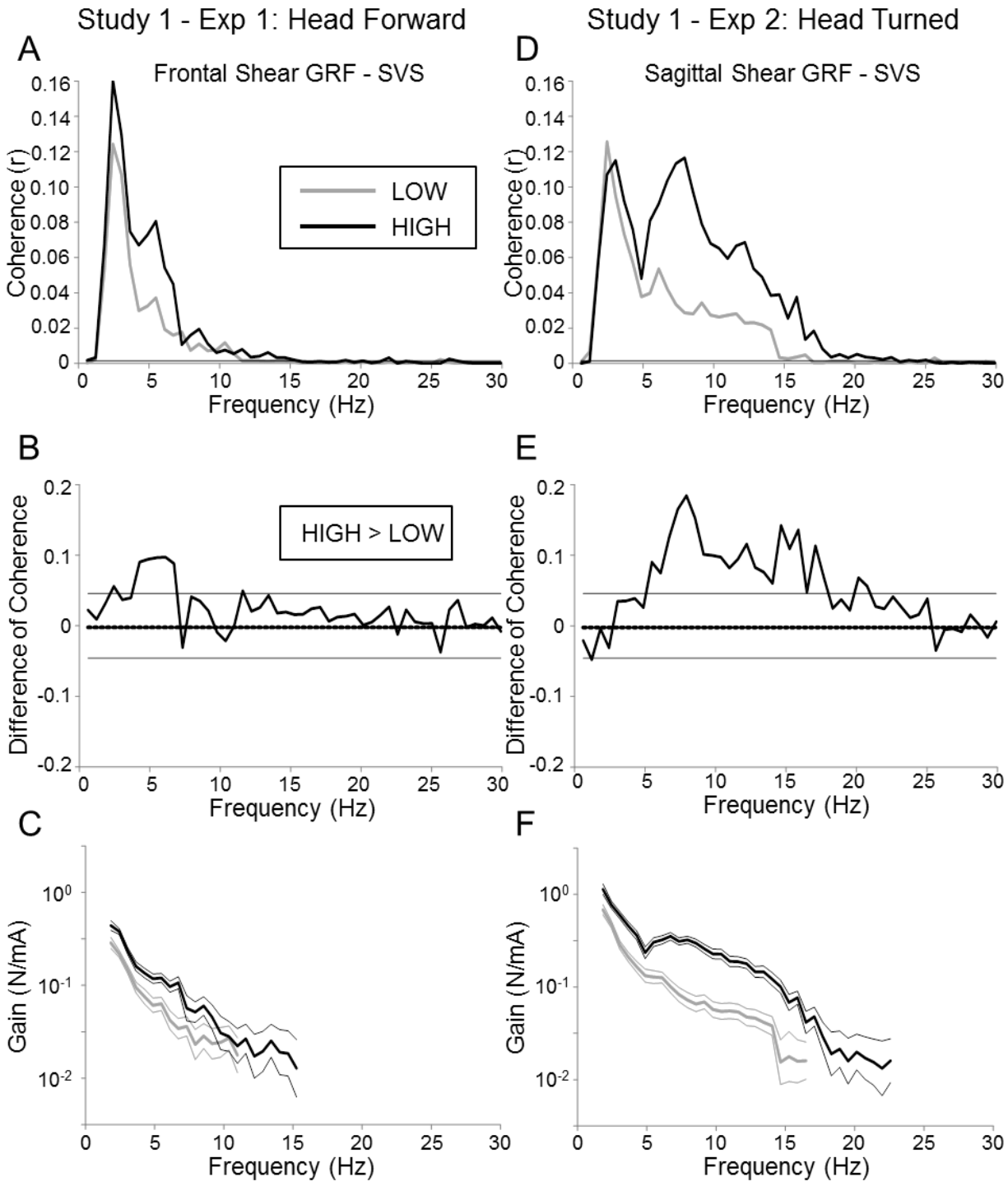

Figure 2-3 – Study 1 – Effects of height on SVS-GRF coherence and gain

Pooled coherence plots for the LOW and HIGH conditions from the head forward (A) and head turned (D) experiments. Note: thin horizontal line above abscissa represents the threshold

for significant within-conditions coherence for both LOW and HIGH conditions. Difference of coherence plotted (thick solid line) for head forward (B) and head turned (E) experiments of Study 1. Thin solid horizontal lines represent the upper and lower 95% confidence limits for the test. Any points where the difference of coherence exceeds the 95% confidence limit are taken to be statistically different. Finally, LOW and HIGH gains (thick lines) of the pooled SVS-GRF data are plotted (on a log scale) for head forward (C) and head turned (F) experiments. Thin lines surrounding the gain traces represent point-wise 95% confidence limits; regions where the confidence limits do not overlap are taken to be statistically different.

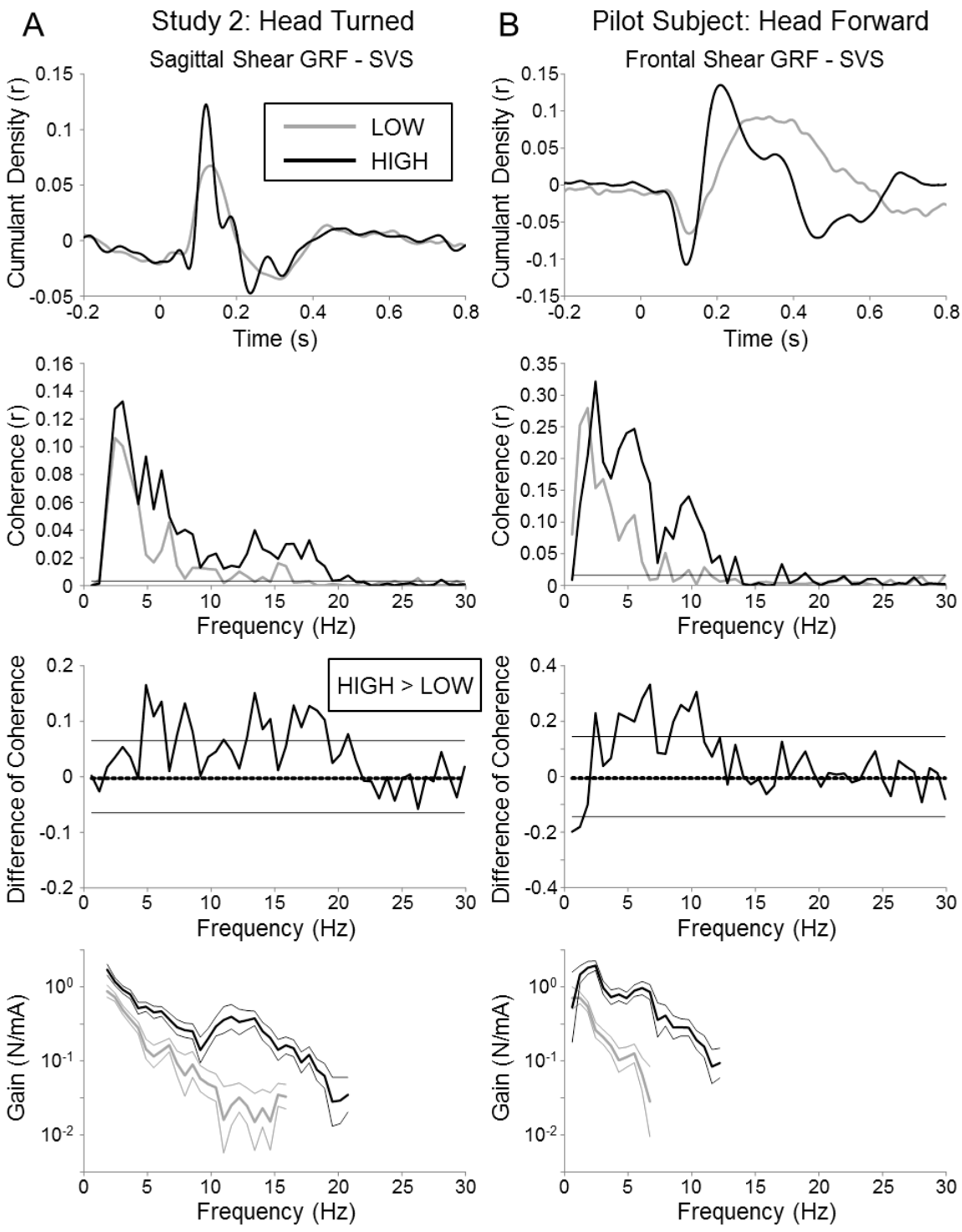

Figure 2-4 – Results of follow-up experiments

Cumulant density, coherence, difference of coherence, and gain are plotted for LOW and HIGH conditions concatenated from a sample of 5 subjects with head right and visual fields

controlled in Study 2 (A), and a single subject standing with head forward and receiving 0-25Hz SVS stimulation (B). Conventions are the same as in figures 2-2 and 2-3.

2.3 CrossTalk proposal: Fear of falling does influence vestibular-evoked balance responses

2.3.1 Introduction

Fear of falling (FoF) is an autonomic, cognitive, and behavioural response to an actual or imminent threat of a fall (Hadjistavropoulos et al, 2011). Given its multi-dimensional nature, the assessment of fear usually relies on self-report and converging evidence from a number of independent, but related, measures such as anxiety (Davis et al, 2010), confidence (self-efficacy) and arousal (Hadjistavropoulos et al, 2011). Recent studies, where young healthy adults have been exposed to a postural threat (e.g. standing on an elevated platform where the consequences of a fall are severe), have shown fear and related factors directly affect control of quiet standing and dynamic balance responses (Carpenter et al, 2001a, 2004, Brown and Frank, 1997, Sibley et al, 2010). However, the mechanisms underlying these fear-related postural changes are yet to be fully understood. Of recent debate is whether FoF-related changes in vestibular function, as evidenced by modulation (or lack thereof) of vestibular-evoked balance responses, might contribute to threat-related adjustments in balance behaviors (Osler et al, 2013, Horslen et al, 2014). Our position is that FoF does influence vestibular-evoked balance responses.

2.3.2 Evidence in support of our position

We would expect to observe changes in vestibular-evoked balance responses with FoF because of the strong excitatory reciprocal projections between all vestibular nuclei and neural regions responsible for fear-related processes, including the amygdala, the parabrachial nuclei (Balaban, 2002) and the histaminergic system (de Waele et al, 1992). These networks have been implicated in the relationship between anxiety and vestibular or dizziness disorders (Staab et al, 2013, Furman and Jacob, 2001). These networks are also thought to be transiently engaged to

limit body movement with threat (Balaban 2002), as part of the "freezing" response to threatening stimuli (Lang et al, 2000). As such, larger vestibular-evoked balance responses may be a result of excitation of the central vestibular system, which might normally serve to limit movement, in the presence of a postural threat (Horslen et al, 2014).

Vestibular-evoked balance responses can be probed with percutaneous electrical stimulation over the mastoid processes bilaterally to modulate vestibular afferent firing rates (Goldberg et al, 1984). This activation of vestibular afferents leads to a virtual head perturbation (Fitzpatrick and Day, 2004). Electrical vestibular stimulation (EVS) evokes patterned activity in axial and appendicular muscles which, when added vectorially, exert a net force onto the ground causing whole-body movement (Britton et al, 1997, Fitzpatrick and Day, 2004, Forbes et al, 2015). The early responses most likely reflect the body's compensation to an isolated vestibular perturbation (Fitzpatrick and Day, 2004). If the stimulation persists then feedback from non-vestibular sources can be used to counteract the evoked balance response (Day and Guerraz, 2007). Continuously varying stochastic electrical vestibular stimulation (SVS; Fitzpatrick et al, 1996, Dakin et al, 2007) evokes muscle and balance responses similar to those elicited with EVS (Dakin et al, 2007). Cross-correlations (between SVS and physiological recordings) can resolve the short- (SL) and medium-latency (ML) responses typically examined in response to EVS (Dakin et al, 2007, 2010, Reynolds, 2010). Likewise, frequency-based analyses can be used to assess the strength of input-output coupling and gain of the relationship (Dakin et al, 2010).

In recent experiments, we showed increased vestibular-evoked balance responses to SVS when subjects stood with their toes at the edge of a 3.2m high platform, compared to standing at ground level (Horslen et al, 2014). Specifically, height-induced threat significantly increased the SL and ML peak force amplitudes (Fig. 2-5a), as well as gain and coherence between SVS and

ground reaction forces. Vestibular-evoked balance responses were also increased in postural muscles when subjects stood under the threat of an unpredictable lateral support surface tilt perturbations (Lim, 2014). Both SL and ML peak muscle responses were larger (Fig. 2-5b,c), and gain and coherence were increased when the threat of perturbation was present, compared to no-threat conditions. Taken together, these findings indicate that the threat of increased consequence, or likelihood, of a fall increases vestibular gain, as measured by vestibular-evoked balance responses. Osler et al, (2013), in contrast, used square-wave EVS to evoke balance responses in subjects who stood with feet in a tandem orientation on an elevated beam and found that trunk kinematic responses were only affected (reduced) in later phases of the response (>800ms). They concluded FoF has no effect on early "feedforward vestibular-evoked balance responses", but "strongly attenuates the feedback" response (Osler et al, 2013). While these results may seem contradictory to the observations of Horslen et al, (2014) and Lim (2014), methodological considerations may account for the reported differences. In particular, the high-frequency threat-related changes observed in ground reaction forces (Horslen et al, 2014) and muscle activity (Lim, 2014), would be less evident in trunk kinematics because of natural low-pass filtering in conversion from muscle activity or force to sway (Dakin et al, 2010, Forbes et al, 2015). Likewise, differences in level of stability due to foot position (tandem vs. side-by-side), threat location/type (both sides vs. front vs. support surface tilt), and/or EVS characteristics (square-wave vs. zero-mean stochastic) may offer additional explanations for the incongruent observations of threat related changes in early vestibular-evoked balance responses between studies (Osler et al, 2013, Horslen et al, 2014, Lim, 2014).

Further evidence supporting fear-related influences on vestibular-evoked responses can be drawn from studies that have used alternative methods to probe vestibular function. Vestibular-

evoked myogenic potentials (VEMPs) use loud auditory tones or clicks to activate the vestibular receptors directly and evoke short-latency reflexes in tonically engaged muscles (Rosengren et al, 2010). Naranjo et al, (2015) observed significant increases in VEMP amplitudes in neck and leg muscles actively involved in stabilizing the body and head when subjects stood at the edge of a high, compared to low surface. Furthermore, changes in VEMP amplitude were positively correlated with changes in both FoF and anxiety. These results are consistent with prior evidence of increased vestibulo-ocular reflex (VOR) gain under conditions of increased anxiety (Yardley et al, 1995), or vigilance (Collins, 1988), that would normally accompany a fear response. The SVS, VEMP and VOR studies all demonstrate anxiety or fear-related excitation of vestibular responses. Combined, this evidence implicates the vestibular nuclei as a likely site for modulation, as vestibular-evoked reflexes in leg, neck and eye muscles all relay through the vestibular nuclei.

2.3.3 Concluding remarks

Based on the evidence reviewed here, we conclude that FoF increases the amplitude of vestibular-evoked balance responses. One question that remains is how (and if) changes in vestibular-evoked balance responses with FoF contribute to the increases in balance-correcting responses to whole-body perturbation with threat (Brown and Frank, 1997, Carpenter et al, 2004, Sibley et al, 2010). Vestibular-evoked balance responses are thought to reflect reactions to virtual head perturbation, and are distinct from balance-correcting responses to whole-body support surface perturbations (Wardman et al, 2003). However, support surface perturbations induce early head accelerations (15-40ms; Carpenter et al, 1999a), and balance-correcting responses are known to be attenuated with vestibular deficits (Horlings et al, 2009). As such, it is

possible the networks responsible for vestibular-evoked responses can contribute, at least in part, to fear-related changes in balance-correcting responses.

2.3.4 Opposing view

The reader is referred to the published Journal of Physiology Cross Talk opposing view and subsequent rebuttal by Reynolds, Osler, Tersteeg and Loram (2015b) for the detailed counter points to the position laid out above. In essence, Reynolds et al (2015a) argue that differences in stimulation techniques, outcome measures, and interpretation underlie the differences between studies. They call into question the relevance of stochastic stimulation patterns, which contain relatively high stimulation frequencies (>5hz), to the understanding of standing balance control, which is dominated by low (<1Hz) frequency sway oscillations. The also argue that kinematic analyses of trunk movements better reflect balance behaviours than ground reaction forces, and that the small amplitude, high-frequency ground reaction force changes observed by Horslen et al (2014), while statistically significant, may lack functional significance to control of standing balance (Reynolds et al, 2015a).

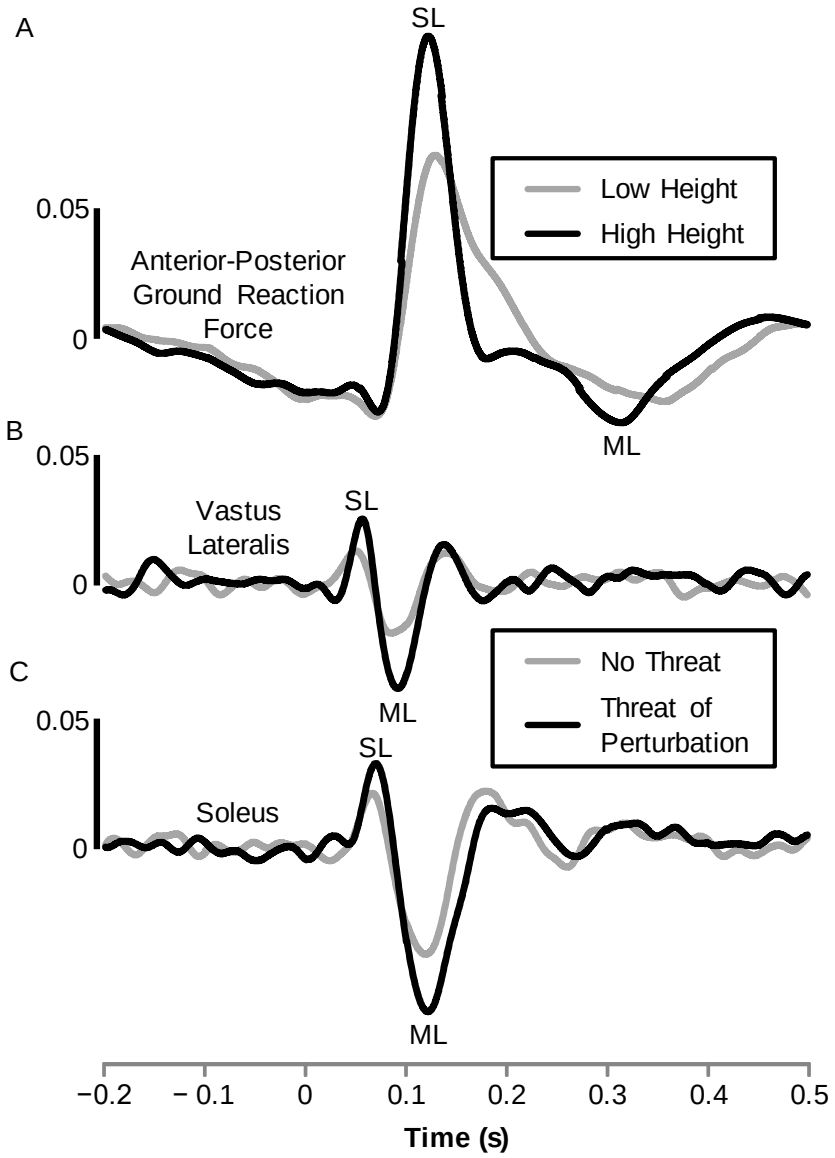

Figure 2-5 – Short- (SL) and medium-latency (ML) vestibular-evoked balance responses with threat

Amplitude-normalized cumulant density plots representing cross-correlation between stochastic electrical vestibular stimulation (SVS) and anterior–posterior ground reaction forces acting on the body (A), vastus lateralis (B) and soleus muscle activity (C). In all cases, positive peaks reflect a positive correlation between SVS and the respective measure [e.g. a positive current caused a forward-directed force (A) or increase in muscle activity (B and C)]. The

ground reaction force trace (A) is reproduced from Horslen et al (2014; their Fig. 2C); SVS (2–25 Hz bandwidth) evoked balance responses while subjects (n = 10) stood at low and high surface heights with feet at the edge and head turned 90 deg to the right. The muscle activity traces (B and C) were reproduced with permission from Lim (2014; her Fig. 3.12); SVS (0–25 Hz) evoked responses while subjects (n = 13) stood with the head facing forward with and without the threat of laterally directed support surface perturbations.

2.4 Rebuttal from Brian C Horslen, Christopher J Dakin, J Timothy Inglis, Jean-Sébastien Blouin and Mark G Carpenter

2.4.1 Rebuttal

The topic of debate is whether fear influences vestibular-evoked balance responses. Opposing arguments from Reynolds et al (2015), rely on the reported lack of changes in the early (<800 ms) response to square-wave EVS, and decreased later (>800 ms) responses. The evidence drawn from the lack of early response change is unconvincing due to an increased likelihood of Type II error caused by a small sample size and poor resolution resulting from a) use of COM position and velocity estimates to detect vestibular-evoked responses in leg muscles, especially since tandem-stance requires multi-joint control, b) limited number of stimulations, and c) small early responses due to low (1mA) stimulus intensity (Ali et al, 2003). Likewise, interpretation of late responses is confounded by the potential influence of fear on reactions to non-vestibular sensory feedback related to the vestibular perturbation (Day and Guerraz, 2007), and conscious strategies (Reynolds 2010) within this time period.

We disagree with Reynolds et al (2015) that fear-related increases in gain and coupling of GRFs to SVS are irrelevant to balance. Early responses are clearly distinguishable using GRFs (Marsden et al 2002), with less mechanical filtering of the neuromuscular response affecting GRFs than kinematics (Forbes et al 2015). In our study, SVS was engineered to highlight changes in early balance responses to vestibular stimulation that are not confounded by later perturbation-induced sensori-motor responses (Horslen et al 2014). The significant SVS gain changes observed between 2-16Hz coincides with natural head frequencies recorded during gait and whole-body perturbations (for review see Forbes et al 2015). After establishing threat-related effects on early vestibular-evoked balance responses, we confirmed these effects in GRFs

and EMG from posturally-engaged muscles using an SVS stimulus that included the 0-2Hz bandwidth, and with vestibular-evoked-myogenic-potentials (Horslen et al 2015).

The notion we have confused statistical and functional significance is false; we view evidence of a true effect, beyond chance or bias, as a necessary pre-requisite to considering functional significance. Furthermore, functional significance shouldn't be discounted because of small absolute changes, which would be expected given the small virtual rotational velocity induced by the stimulus (Peters et al, 2015), and are consistent with magnitudes reported elsewhere (Reynolds 2010, Mian and Day, 2014). In contrast, we believe the relative change in gain of early vestibular-evoked responses provides more meaningful insight into functional significance. Thus, fear-related increases in gain (81-231%) are non-trivial and functionally significant, particularly when translated to real-life head accelerations.

2.4.2 Comments and counter-points

The reader is referred to comments published in the Journal of Physiology by JH van Dieën, JHJ Allum, WR Young and AM Williams, JG Colebatch, and J Duysens for discussion of the debate. The comments are accompanied by Final Word statements from both Horslen et al (2015) and Reynolds et al (2015).

Chapter 3: Study 2 - Changes in stretch reflex dynamic sensitivity with height-induced postural threat.

3.1 Introduction

Threats to standing balance, such as standing at the edge of a high surface, are known to cause changes in balance control behaviours (Carpenter et al, 1999b, 2004), conscious perceptions of body sway (Cleworth and Carpenter, 2016), and reflexes from balance-relevant sensory inputs (Horslen et al, 2013, 2014; Naranjo et al, 2015,2016). Indirect evidence for threat-related changes to spinal stretch reflexes is based on observed increases in tendon-tap reflex (t-reflex) amplitudes (Davis et al, 2011; Horslen et al, 2013), in conjunction with unchanged (Horslen et al, 2013), or decreased (Llewllyn et al, 1990; Sibley et al, 2007) Hoffmann reflex (H-reflex) amplitudes with threat. While both H- and t-reflexes can be modulated centrally (at the synapse/motor neuron pool) in the spinal cord, only t-reflexes are subject to changes in peripheral muscle spindle sensitivity (Pierrot-Deseilligny and Burke, 2012). Therefore, the differential effects of threat on H- and t-reflexes were interpreted as evidence for a change in peripheral muscle spindle sensitivity, possibly due to independent fusimotor drive (Horslen et al, 2013). However, there are technical limitations related to H- and t-reflexes which affect extrapolation to a broad somatosensory response to postural threat. First, it has been argued that differences between H- and t-reflexes in terms of a) shape and duration of afferent volley produced, b) sensitivity to central modulation, c) effects of the afferent volley on the motor neuron pool, and d) population of spindles represented in the reflex (t-reflexes predominantly reflect Ia responses from dynamic bag fibres; Burke et al, 1983), preclude direct comparison of H- and t-reflexes for the purpose of evaluating fusimotor state (*cf.* Pierrot-Deseilligny and Burke,

2012). Therefore, central modulation of t-reflexes cannot be ruled out as an explanation for different H- and t-reflex outcomes at this time. Second, despite using the same neural architecture, increases in t-reflexes conflict with the lack of significant increases observed in stretch reflex amplitudes with height-induced postural threat when triggered with ramp-and-hold support surface tilt balance perturbations (Carpenter et al, 2004). If muscle spindle sensitivity is globally increased with threat, then ramp-and-hold stretch reflexes should also be facilitated with threat. As such, a probe of stretch reflex modulation that is sensitive to changes in peripheral inputs (i.e. spindle sensitization) and which broadly samples spindle responses from the target muscle, is necessary to gain greater insight into threat influences on muscle spindle sensitivity.

While the gold-standard for evaluating muscle spindle sensitivity would be a continuous direct microneurographic recording of Ia afferent activity while participants stand at low and high surface heights, it would be extremely difficult to ensure stable recordings with the position and muscle activity changes known to occur at height (*cf.* Carpenter et al, 2001a). However, changes in scaling of ramp-and-hold plantar flexor stretch reflexes to varying amplitudes and velocities may serve as an indirect probe for changes in muscle spindle sensitivity. Ramp-and-hold stretches evoke short-latency (SLR) and medium-latency reflex (MLR) responses in the stretched muscle. In the lower limb, the SLR is thought to be the basic Ia myotatic stretch reflex (Nardone et al, 1996), which is velocity-, but not amplitude-dependent (Gottlieb and Agarwal, 1979; Allum and Mauritz, 1984; Grey et al, 2001). Conversely, spindle type II afferents, arising from spindle static bag 2 and chain fibres, are thought to make a more significant contribution to the MLR response. This is because the MLR: a) is significantly suppressed with Tizanidine, an α_2-adrenergic agonist which suppresses type II transmission, where the SLR is not (Corna et al, 1995; Grey et al, 2001; Meskers et al, 2010); b) is more delayed with nerve cooling than SLR

responses (Schieppati and Nardone, 1997; Grey et al, 2001), suggesting involvement of smaller-diameter afferent fibres; and c), scales to stretch amplitude (Gottlieb and Agarwal, 1979; Allum and Mauritz, 1984) or stretch duration (Lewis et al, 2005; Schuurmans et al, 2009), while the response to stretch velocity is mixed (*cf.* Allum and Mauritz, 1984; Grey et al, 2001). Therefore, the SLR and MLR responses each contribute unique information about stretch reflex processing, as well as the afferent triggers of the reflex. Both central (e.g. motor neuron pool excitability, pre-synaptic inhibition/excitation, primary afferent post-activation depression) and peripheral factors (i.e. muscle spindle sensitivity or thixotropy) can affect SLR and MLR average amplitudes; it is not possible, using surface EMG, to distinguish between central and peripheral effects in stretch reflex amplitude to any given ramp-and-hold stretch. However, scaling of the SLR to stretch velocity, and of the MLR to stretch amplitude, should primarily reflect changes in receptor sensitivity to stretch velocity and amplitude, respectively. While ramp-and-hold support surface tilts are often delivered with participants standing with both feet on the tilting plate to trigger balance correcting responses (e.g. Keshner et al, 1987; Carpenter et al, 2004), stretch reflexes are inhibited over repeated exposure (Keshner et al, 1987), likely because they act in opposition to the balance corrective response. However, positioning participants with only one foot on the plate can be used to trigger SLR and MLR responses with minimal balance perturbation or corrective response (Corna et al, 1996). To our knowledge, no studies have examined changes to scaling of SLR and MLR responses in lower leg muscles to unilateral ramp and hold stretches of different velocities and amplitudes in standing humans.

The purpose of this experiment was to determine how velocity-dependent SLR and amplitude-dependent MLR responses are affected by height-induced postural threat. Also, the t-reflex protocol from Horslen et al (2013) was replicated to enable comparison with previous

work. It was hypothesized that SLRs, MLRs, and t-reflexes would all be increased with postural threat. Furthermore, it was hypothesized that SLRs would scale linearly to stretch velocity, and that the slope of the SLR-velocity relationship would be steeper with threat, reflecting increased dynamic gain of the SLR. Likewise, it was expected MLRs would scale to stretch amplitude (when velocity is fixed), and that the slope of the relationship would be steeper at height, reflecting increased amplitude-dependent gain.

3.2 Methods

Forty-three young healthy adults were recruited from the community. Two participants withdrew from the study and one participant was excluded due to equipment malfunction. The remaining 40 participants were divided into two experimental groups, with 16 participants in protocol 1 (9 Female/7 Male; mean(SE) Age: 21.4(0.47) years; Height: 1.71(0.02) m; Weight: 68.5(3.14) kg) and 24 participants in protocol 2 (13 Female/11 Male; Age: 20.9(0.63) years; Height: 1.72(0.02) m; Weight: 66.3(1.92) kg). All participants gave written informed consent prior to participation, and the methods used were approved by the University of British Columbia Clinical Research Ethics Board and conformed to the Declaration of Helsinki.

3.2.1 Protocols

In both protocols, participants stood on a custom-built servo-controlled tilting platform surrounded by a non-moving stage mounted at the edge of a hydraulic lift (M419-207B01H01D, Pentalift, Guelph, Canada). Feet were positioned with toes 20cm from the edge of the lift and 25cm apart with right foot on the tilting platform and left foot on the stage (Fig. 3-1A). In the low threat condition (LOW) the platform and stage surfaces were 1.1m above ground (minimum height of lift and stage). An additional 60cm-wide support surface was positioned in front of the

lift, creating an 80cm continuous support surface (Fig. 3-1B). In the high threat condition (HIGH), the additional support surface was removed and platform elevated to 3.5m. Although absolute surface heights in both conditions were higher (to accommodate height of tilting platform and stage), the relative change in height between conditions (2.4m) matches that of previous work (Horslen et al, 2013, 2014). There is a known order effect on changes in balance control behaviours due to height-induced postural threat (Adkin et al, 2000); therefore, the LOW condition was always presented first in order to maximize the contrast between conditions. There were no safety rails bordering the lift, however, participants were harnessed into a safety line and an experimenter was within arms-reach at all times; no participants fell (to ground or required a step to maintain balance) during the experiment.

Foot position, was marked on the stage and platform, and a plumb line was hung from the medial tibial condyle to allow an experimenter to visually monitor that foot position and knee angle were maintained in both conditions. The output of four load cells mounted into the tilting platform (SSB-250 with BSC4A-C14, Interface Advanced Force Measurement, USA) were used to monitor vertical load and AP/ML torque on the plate under the right foot (sampled 1000Hz; Power 1401 with Spike2 software, Cambridge Electronic Designs, UK). Prior to any stimulation, participants stood quietly (at LOW height) for 2 minutes and baseline mean ± SD vertical load, AP and ML torques were calculated. Vertical load was monitored online by an experimenter to ensure weighting between the feet did not change across conditions. Visual feedback of real-time AP and ML torque was projected (MS317, BenQ, Taiwan) on the wall at eye level, approximately 3.7m in front of the participant (display: 1.35m high by 1.83m wide). Participants were instructed to keep the real-time torque position (projected as a green dot on white background) within a target box with dimensions equal to AP and ML mean ± SD from the

baseline trial. The feedback disappeared 10ms prior to each stimulus, and remained hidden for 10s; participants were instructed not to attempt to control their lean while feedback was hidden. Participants were given a practice trial to familiarise themselves with the feedback; the practice trial included a single cued presentation of each stimulus used in the experiment as well as a 5-event session where a random sample of stimuli were presented without cues, similar to the proper experimental trials.

The tilting platform was servo-controlled with an analog input voltage provided through a D/A system (Power 1401 with Spike2 software, Cambridge Electronic Designs, UK). Actual platform tilt angle was measured with a calibrated potentiometer and low-pass filtered offline with a 50Hz 2nd order Butterworth filter passed in the forward and backward direction to remove phase (sampled 5000Hz, Power 1401 with Spike2; filter: Spike2) and differentiated offline to calculate platform velocity (Spike2). In both experiments participants experienced 5 toes-up dorsiflexion (DF) ramp-and-hold stretch profiles (5×/profile) which were presented in a random order. Stretch profiles were grouped into three velocity profiles (used to examine SLR amplitude and SLR-velocity scaling) and three amplitude profiles (to assess MLR amplitude and MLR-stretch amplitude scaling). Preliminary analysis of protocol 1 revealed little difference in amplitudes achieved for MLR-angle scaling, therefore, stretch parameters were changed from protocol 1 to 2 in an attempt to create a larger range of actual angles for MLR comparison. Stimulus parameter details, including velocities and amplitudes demanded of the platform, achieved peak velocities and amplitudes, and velocities and amplitudes achieved within the relevant analysis windows (see below) are reported in tables 3-1 and 3-2. Maximum platform velocity (protocol 2) and angle were dictated by technical limits of the tilting platform under load. Each ramp stretch was followed by a 750ms hold, before returning to baseline position over

500ms. Muscle stretch responses can be affected by previous history (e.g. stiction or thixotropic after-effects; Proske et al, 1993). While a conditioning stretch or contraction prior to each event would be ideal for minimizing these effects (Proske et al, 2014), we did not wish to cue the participant to impending stimuli in case they attempted to voluntarily modify their response. Therefore, the tilting platform was oscillated through the same 2s conditioning pattern of DF and plantarflexion single sine waves (1 cycle at 2Hz ±0.6°, 1 cycle at 1Hz ±1.5° then 2 cycles at 4Hz ±0.5°) after each event to ensure all stimuli ended with similar mechanical effects (Fig. 3-1C). Participants were instructed not to unload the plate, or resist platform movement, during these oscillations.

Within each height condition, the DF stimuli were randomly intermixed with 5 plantarflexion catch trials (5° at 80°/s; not analysed) and 10 Achilles tendon-taps to evoke t-reflexes. Tendon-taps were delivered using a magnetic linear motor (motor: LinMot PS01 – 23x80; controller: LinMot E2000-AT; software: LinMot v.1.3.12; NTI Ltd., USA) stroking ~1cm in 10ms (Davis et al, 2011; Horslen et al, 2013) and tendon-tap impact force was monitored (Isotron Dynamic Force Sensor, Endevco, USA; sampled 5000Hz, Power 1401 with Spike2).

3.2.2 Measures

Psychological and autonomic states were assessed using questionnaires and electrodermal activity (EDA). Prior to each trial, participants rated their confidence they could maintain balance and avoid a fall (0-100%; higher values indicate greater confidence). After each trial participants reported their experienced fear of falling (0-100%; higher values indicate greater fear), perceived stability (0-100%; higher scores indicate greater perceived stability), and

completed a 16-item anxiety questionnaire (1-9 Likert scale for each question summed to a maximum of 144; higher scores indicate greater anxiety). Galvanic skin conductance was measured (model 2501, CED, UK; sampling: 100Hz) to quantify EDA, which indicates degree of sympathetic arousal (Boucsein et al, 2012). Tilting platform movement caused significant artefacts in the EDA (conductance) recording, therefore EDA was clipped to 1s bins immediately preceding each stimulus and these bins were averaged within a condition to establish a condition-wide level (per Horslen et al, 2013).

Muscle activity was recorded with EMG from the right soleus (SOL) and tibialis anterior (TA) muscles (sampled 3000Hz, 10-1500Hz online bandpass filter; Telemyo 2400 G2, Noraxon, USA) and A/D converted at 2000Hz. EMG data were filtered offline (10-500Hz 2nd order Butterworth dual-passed; Spike2) and baseline corrected.

3.2.3 Analyses

SLR and MLR reflex amplitudes, as well as platform displacement angle and velocity parameters were analysed to address the reflex amplitude and scaling hypotheses of this study; reflex latencies were also determined to ensure SLR and MLR responses were properly categorized. A custom algorithm (Spike2, CED, UK) was used to determine onset of platform displacement, reflex latencies and amplitudes, and measure platform displacement parameters. Onset of platform movement for each stretch was determined from the load cell nearest to the participant's toes, and was set to the time where the load exceeded (min 6ms duration) a mean + 3SD threshold from a 100ms period preceding the event trigger; onset was confirmed with visual inspection of load, displacement, and velocity data.

Ramp-and-hold stretch profiles were time-locked to onset of platform movement to create separate waveform averages of rectified SOL EMG for SLR and MLR comparisons. Similar to Corna et al (1995), participant-specific SLR and MLR analysis windows were established from average data and used to assess individual reflex events; the same analysis windows were used within participants between threat conditions. Separate SLR and MLR analysis windows were determined from the waveform averages constructed from the velocity profiles (SLR-velocity), and amplitude profiles (MLR-angle), respectively. As shown in figure 3-2A, SLR onset, peak (amplitude and latency) and end, as well as MLR onset and end were determined for each threat condition. The bounds of the analysis windows were set by the earliest onset and latest end for both SLR and MLR; bounds were confirmed by visual inspection. SLR latencies to peak were averaged across conditions to get a single value for use in platform velocity analyses. While bounds of the analysis windows were participant-specific, the mean±SE (range) of the SLR window (velocity traces) was: start = 40.2±0.47ms (34-46), end = 57.9±0.61ms (49.5-64.5), SLR peak = 48±0.48ms (41.3-54); the bounds of the MLR window (amplitude traces) were: start = 60.4±0.86ms (50.5-73.5), and end = 85.1±2.6ms (67-145.5).

SLR and MLR amplitudes were calculated as the integrated area of rectified SOL EMG activity within the defined analysis windows for individual reflex events (Fig. 3-2A); similar to Corna et al (1995), the area was measured even if no reflex was evoked (SLR or MLR) and reflex areas were not referenced to background muscle activity. Main effects of threat were calculated as the average SLR amplitude (evoked by velocity profiles) and average MLR amplitude (across amplitude profiles) within a condition. Individual reflex amplitudes were also related to corresponding platform movement profiles to examine scaling effects. SLR and MLR onset latencies were measured for each event where a reflex was evoked. Onset latencies were

referenced to a mean +3SD threshold of rectified SOL EMG from a 100ms period ending 10ms before platform onset; onset was defined as the point where the signal remained above threshold for a minimum of 3ms and was confirmed by visual inspection.

Soleus t-reflexes and muscle background activity measures were replicated from Horslen et al (2013). T-reflexes were measured as peak-to-peak amplitudes of unrectified EMG, and screened for changes in tap force. Individual stimuli were excluded post-hoc if they were evoked with peak tap force outside of a mean ±2 SD range of the least variable trial (Davis et al, 2011; Horslen et al, 2013). T-reflex amplitudes from stimuli that passed tap-force screening were averaged within a threat condition. A minimum of 5 tendon-taps per condition were required for the participant's t-reflex data to be included in the final analysis. SOL and TA background muscle activity (preceding both t-reflexes and ramp-stretches) were calculated as the root mean square amplitude of unrectified EMG from a 100ms period ending 10ms before stimulus onset.

Platform movement parameters were measured with respect to significant events in the reflex response. Velocity-dependent scaling of the SLR is likely to be most significant up to SLR peak, before differences in amplitude start to emerge. However, amplitude-dependent scaling of the MLR is potentially possible up to the end of the reflex, as velocity effects have likely stabilized, yet contrast between angles is greatest by this point. We considered the SLR onset latency as the minimum time required for a mechanical stimulus to contribute to the muscle response, based on the assumptions that a) there is no variability in afferent or efferent conduction velocities in the neurons involved in the stretch reflex pathways, b) central processing time does not change over the course of the reflex(es), and c) zero time required for mechanical transduction of the stretch by muscle spindles before an afferent volley leading to a stretch reflex. Therefore, the last possible time where platform movement can contribute to a

given component of the SLR or MLR reflex can be determined by subtracting the SLR latency from the latency of the event of interest. For example, if SLR peak occurs 8ms after SLR onset, then only movement occurring up to 8ms after platform onset can contribute to the SLR peak. Peak platform velocity by SLR peak was related to SLR amplitude to assess SLR-velocity scaling (Fig. 3-2B). Peak platform angular displacement by MLR end was related to MLR amplitude for MLR-angle scaling (Fig. 3-2C). SLR onset, SLR peak and MLR end latencies were based on the analysis windows used to calculate reflex amplitudes (Fig. 3-2A).

Individual SLR reflex amplitudes were plotted against corresponding platform velocities within a condition for each participant and the slope of the line of best fit was calculated to assess the gain of SLR-velocity scaling for that condition (custom Matlab script; Matlab R2012a, MathWorks, USA). Changes in slope across threat conditions were used to assess threat effects on scaling. Since this method assumes the relationship between SLR amplitude and platform velocity is linear, regressions between SLR amplitude and velocity were calculated for each participant and each condition; only participants who had regression values $r>=0.25$ in both conditions were included in the final analysis.

Amplitude-specific scaling of the MLR response could not be properly examined in this study due to tilting platform technical limitations and few observations of reflexes at small tilt angles. On average, there was less than 1° difference between the smallest and largest tilt amplitudes by MLR end for protocol 1 (Table 3-1). While there were larger differences in achieved tilt angle between 2°DF and 5° or 7°DF tilts at 170°/s in protocol 2 (Table 3-2, Fig. 3-2C), 12 of the 24 participants in protocol 2 had no MLRs evoked from 2°DF tilts; only 2/12 participants had more than one MLR per threat condition from the 2°DF tilts. Therefore, only 5° and 7°DF tilts could be analysed. However, like in protocol 1, there was on average less than 1°

difference in achieved tilt angle by MLR end between 5 and 7° (Table 3-2). In fact, only 8 participants, combined across protocols, had differences in achieved tilt angle >=1°, and MLR amplitude did not scale linearly (r<=0.25) to stretch amplitude in these participants.

3.2.4 Statistics

Differences between threat conditions for all variables (SOL/TA background activity, mean SLR amplitude, mean MLR amplitude, SLR-velocity slope, t-reflex amplitude, EDA, psychological state questionnaires) were assessed with paired-samples t-tests (Matlab R2012a, MathWorks, USA); alpha was set to 0.05. Post-hoc correlations between reflex amplitude and background muscle activity were performed with SPSS (IBM Corp, USA).

3.3 Results

Exposure to the HIGH threat condition evoked significant changes in participants' psychological and physiological state. Participants had significantly lower balance confidence ($t_{(39)}$= 7.10, p<0.001, η^2=0. 564) in the HIGH (mean ± SE; 73.5 ± 3.2%), compared to LOW (92.7± 1.8%) condition and felt significantly less stable (LOW: 90.1 ± 2.1%, HIGH: 66.6 ± 3.6%, $t_{(38)}$= 7.62, p<0.001, η^2=0. 605). Participants were also significantly more fearful of falling (LOW: 4.1 ± 1.2%, HIGH: 31.4 ± 4.4%, $t_{(38)}$= -6.79, p<0.001, η^2=0. 549), more anxious (LOW: 29.2 ± 1.9, HIGH: 46.9 ± 3.9, $t_{(39)}$= -5.38, p<0.001, η^2=0.426), and had higher EDA (LOW: 20.61 ± 1.47 µS, HIGH: 27.70 ± 1.87 µS, $t_{(35)}$=-6.27, p<0.001, η^2=0.529), in the HIGH, compared to LOW condition. Four participants were excluded from the EDA analysis due to technical limitations and one participant did not answer the fear of falling or perceived stability questions.

Soleus tendon-tap, short-latency, and medium-latency reflexes were all increased with postural threat. Thirty-one of 40 participants met SOL t-reflex tap-force screening criteria; t-reflex latencies were similar between height conditions (LOW: 39.3 ± 0.6ms; HIGH: 39.6 ± 0.6ms), yet amplitudes were 16.9% larger (Fig. 3-3D) in the HIGH compared to LOW condition ($t_{(30)}$= -2.84, p = 0.008, η^2= 0. 212; Fig. 3-3A). Platform DF ramp-and-hold stretches evoked SLR responses with onset latencies of 42.3± 0.48ms and 42.3± 0.49ms in the LOW and HIGH conditions, respectively, and MLR responses with latencies 63.9± 1.33ms (LOW) and 64.5± 1.42ms (HIGH). Mean SLR amplitudes across all velocity profiles were significantly larger in the HIGH, compared to LOW condition (SLR: $t_{(39)}$= -2.24, p = 0.031, η^2= 0. 114, Fig. 3-3B) with an average increase of 11% (Fig. 3-3D). Likewise, mean SOL MLRs across all amplitude profiles were significantly larger in the HIGH, compared to LOW condition ($t_{(39)}$= -2.35, p = 0.024, η^2= 0.124; Fig. 3-3C), with an average increase of 9.5% (Fig. 3-3D).

SOL and TA background muscle activity significantly increased with height-induced threat. SOL background root mean square amplitude increased on average 1.5 ± 0.4 µV ($t_{(39)}$= -3.77, p < 0.001, η^2= 0. 267), and TA background activity increased 3.2 ± 1.3 µV ($t_{(39)}$= -2.41, p = 0.021, η^2= 0.130) in the HIGH condition, compared to LOW. Correlations between changes in background muscle activity and reflex amplitudes were examined post-hoc to determine if height-induced changes in reflexes were related to changes in background muscle activity. Across participants, changes in SOL background activity with threat were not associated with height-induced changes in SLR area (Pearson's r= 0.259, p= 0.107) or t-reflex amplitude (r= 0.159, p= 0.393), and only weakly correlated with changes in MLR area (r= 0.333, p= 0.036). Likewise, changes in TA background activity were not linked to changes in SLR (Spearman's

rho= 0.194, *p*= 0.231) or MLR area (rho= 0.038, *p*= 0.815), or t-reflexes across threat conditions (rho= 0. 31, *p*= 0.09).

It was possible to investigate velocity scaling of SLR amplitude because the three velocity profiles achieved different actual tilt velocities by the latency of SLR peak in both protocols 1 and 2 (Tables 3-1, 3-2). SLR amplitude increased with increasing platform velocity (Fig. 3-4A), and the relationship scaled (r>=0.25; Fig. 3-4B) for 21 participants (protocol 1 n = 7; mean r = 0.502, protocol 2 n = 14; mean r = 0.54). SLR-velocity slope was significantly steeper in the HIGH compared to LOW condition ($t_{(20)}$= -2.51, p = 0.021, η^2= 0.240); in the HIGH condition SLR-velocity slopes were on average 34.5% steeper than in the LOW condition.

3.4 Discussion

The purpose of this experiment was to determine how soleus SLR and MLR responses to ramp stretch, as well as tendon-tap reflexes, are affected by height-induced postural threat. This was the first study specifically designed to examine threat-effects on SOL SLR and MLR responses to ramp-and-hold stretches of different amplitudes and velocities. The onset and pattern of SOL SLR and MLR responses observed in the low threat conditions were consistent with reflexes previously reported using similar stretch stimuli in sitting (Gottlieb and Agarwal, 1979; Allum and Mauritz, 1984), standing (Corna et al, 1995, 1996; Allum et al, 1993; Carpenter et al, 2004) and walking (Grey et al, 2001). As hypothesized, threat was found to significantly increase SOL SLR and MLR amplitudes by 11% and 9.5%, respectively. Changes with threat were either uncorrelated, or weakly correlated (maximum explained variance = 11%) to changes in background muscle activity in agonist or antagonist muscles. Differences in findings between the current study and a prior study which showed no significant changes in SLR or MLR amplitudes with threat (Carpenter et al, 2004), may be attributed to several methodological

differences. First, Carpenter et al (2004) had participants stand with both feet on the tilting platform, whereas in the current study, just the right foot was tilted and this did not induce whole-body perturbations that required a significant postural response. While unilateral stretches should evoke smaller MLR responses (Corna et al, 1996), the bilateral perturbations used by Carpenter et al (2004) would be more destabilizing and stretch reflexes may have been suppressed because they act in opposition to the protective balance correcting response used to regain balance. Second, Carpenter et al (2004) had a smaller sample size (n=10) and used a slower tilt velocity (50°/s) than the slowest velocity used in either protocol here (60°/s DF). As demonstrated here, the gain of the SLR-velocity relationship is steeper at height, and, since there is more contrast between SLR amplitudes across threat conditions at higher velocities, there may not have been enough power in the Carpenter et al (2004) study to reveal differences in reflex amplitudes at their chosen tilt velocity.

To our knowledge, this is the first study to specifically examine scaling, and changes to scaling, of unilateral SOL SLR responses to stretch velocity in standing humans. Previous studies have manipulated velocity of support surface tilts (Allum et al, 1993) or translations (Diener et al, 1988), applied bilaterally, to demonstrate that balance correcting responses, as well as non-balance correcting SLRs are larger with faster velocities. However, as noted above, the reflexes in these studies may be influenced by the postural set required to counter the de-stabilizing stretch stimuli, whereas unilateral stretches were used here to limit balance disturbances (*cf.* Corna et al, 1996). Nonetheless, mean regression values between SOL SLR amplitude and platform velocity reported here (r=0.502 and 0.54 for protocols 1 and 2, respectively) are comparable to the regressions between medial gastrocnemius SLR and translation velocity (r=0.6319; Diener et al, 1988) or later SOL responses (80-120ms) and tilt

velocity (r=0.77; Allum et al, 1993). The observed SLR-velocity scaling to unilateral stretch of SOL while standing is in good agreement with observations of scaling from seated (Gottlieb and Agarwal, 1979; Allum and Mauritz, 1984) or walking participants (Grey et al, 2001), as well as from studies of upper-limb reflexes (Lewis et al, 2005; Schuurmans et al, 2009; Meskers et al, 2010). Combined, these studies suggest the SLR scales to stretch velocity, and likely originates from Ia afferents originating from spindle dynamic bag fibres.

The current study is also novel because it is the first to show that humans modulate the relationship between stretch parameters and reflex amplitude to suit the context in which they stand. In this case, context changes in the form of a postural threat, had a significant influence on the within-subjects SLR-velocity relationship. On average, there was a 34.5% steeper SLR-velocity slope when participants were exposed to the HIGH postural threat condition (Fig. 3-4). This suggests the dynamic, velocity sensitive component of the stretch reflex is facilitated with postural threat. Unfortunately, technical limitations precluded systemic investigation of amplitude scaling on MLR responses in the current study. As such, it is not possible with the present data to dissociate between central and peripheral modulation of the MLR, or between static or dynamic inputs as a source for modulation of the MLR.

The current observations of increased t-reflexes with threat, is congruent with previous observations (Davis et al, 2011; Horslen et al, 2013). Differences in the magnitude of threat-related changes in the current study (0.07mV or 16.9% increase) compared to prior findings (0.1mV change in Davis et al (2011); and 0.558mV, or 35% in Horslen et al (2013)) are likely due to task differences, as all three studies used a similar height paradigm and induced comparable changes in threat, anxiety and arousal. Only the current study had participants stand unconstrained while actively monitoring visual-feedback of ground reaction torques to control

leaning; the other studies, in contrast, had subject groups that either stood unconstrained (without visual feedback), or stood braced about the ankles to control leaning. The use of online visual feedback in the present study likely lead to more conscious control of balance in both threat conditions, and may have contributed to the increases in SOL and TA background muscle activity, as well as the relatively smaller changes in t-reflexes observed here. However, consistent observations of increased t-reflexes across all studies (Davis et al, 2011; Horslen et al, 2013), and lack of significant correlations between t-reflex changes and background muscle activity suggests that threat-related changes in stretch reflexes are not dependent on changes in background muscle activity.

The combined observations of larger t-reflexes, SLR and MLR amplitudes, and steeper SLR-velocity slopes are all in agreement with previous conclusions that muscle spindle sensitivity is increased with a threat to standing balance (Sibley et al, 2007; Davis et al, 2011; Horslen et al, 2013). Prochazka (1989) suggested "the full power of fusimotion is reserved for novel and/or difficult tasks, where strong dynamic action causes very large increases in spindle primary responses to muscle displacement" (p. 289). Here, participants were threatened with the risk of falling from a 3.5m high platform, where a loss of balance would likely lead to injury. Increases in spindle dynamic sensitivity would lead to an increase in the gain of the stretch velocity-afferent output relationship. The greater afferent responses with greater velocity would lead to larger reflex responses, and a steeper velocity-reflex amplitude relationship. Unfortunately, the inability to examine scaling of the MLR response to stretch amplitude in the current study means we cannot comment on changes in spindle static (amplitude) sensitivity with threat. This limitation is important because models of cat static and dynamic fusimotor actions suggest independent modulation with task, context or challenge (Prochazka et al, 1985, 1988). In

humans, microneurographic recordings of Ia afferent traffic suggests static sensitivity can be increased when people are tasked with attending to limb position, and both static and dynamic sensitivity increased when attending to changes in movement velocity (Hospod et al, 2007; Ribbot-Ciscar et al, 2009). Furthermore, while MLR responses are thought to reflect static, type II afferent responses (Corna et al, 1995, 1996; Schieppati and Nardone,1997; Grey et al, 2001), Tizanidine diminishes, but does not abolish the MLR (Corna et al, 1995; Grey et al, 2001; Meskers et al, 2010) suggesting some type Ia contribution. As such, changes in SLR, MLR and t-reflexes at height could either reflect a global change in spindle sensitivity, or could be specifically linked to a change in dynamic sensitivity in the high threat context.

Other explanations, such as central reflex facilitation or peripheral changes in muscle spindle tension (independent from fusimotor drive) could also explain the larger reflexes observed in the HIGH condition in the present experiment. For example, serotonergic and noradrenergic projections from brainstem centres, including the reticular formation, can cause persistent inward currents (PICs), and subsequent plateau potentials in motor neurons (Johnson and Heckman, 2010), which can amplify and prolong the effects of Ia synaptic input to motor neurons (Lee and Heckman, 2000). Functionally, this may serve to decrease motor neuron firing thresholds and cause units to fire with relatively less synaptic input (Bawa and Murnaghan, 2009), meaning a given stimulus may recruit more motor units and increase the magnitude of reflexes recorded with surface EMG. PICs might be driven by descending inputs from supraspinal regions, such as the reticular formation or vestibular nuclei, which might in turn be excited by fear and/or anxiety networks activated by postural threat (*cf.* Naranjo et al, 2016; Staab et al, 2013). However, PICs could also manifest as a consequence of increased muscle spindle sensitivity. PICs build-up with increasing Ia synaptic input (ElBasiouny et al, 2006), and

Ia afferent activity would be expected to increase with increased muscle spindle sensitivity in unconstrained standing. Therefore, stretch reflexes could be facilitated by both increased muscle spindle sensitivity, and increased motor neuron excitability as a consequence of increased Ia afferent traffic due to increased spindle sensitivity.

Alternatively, the changes in t-reflex, SLR and MLR amplitudes might be explained by changes in muscle spindle sensitivity due to stiction, and not independent fusimotor activity with increased postural threat. Stiction, or passive muscle spindle contractile fibre tension due to formation of stable cross bridges after contraction can lead to larger stretch reflexes independent from central modulation or fusimotor drive (Proske et al,1993). Unfortunately, we could not have participants make a conditioning contraction prior to each stretch to ensure spindles were in a comparable state before each stimulus and between threat conditions (*cf.* Proske et al, 2014). However, the platform was re-set with the same oscillating pattern after each stimulus to break any after effects from the previous stimulus. While this cannot account for changes in muscle spindle state when the participant stood quietly between stimuli, it does ensure the effects cannot be explained by the presentation order of the stimuli.

Increasing the gain of sensory systems under threatening contexts may serve as a protective response to reduce movement and improve detection of imposed movements (Balaban, 2002). While changes to spindle sensitivity have been proposed as a means to increase somatosensory gain to supraspinal centres under challenging/threatening conditions (Llewelyn et al, 1990), the lack of evidence for early increases in evoked cortical responses to tendon-taps with height-induced threat (Davis et al, 2011), suggests increased somatosensory information is used by sub-cortical centres to facilitate balance and other protective reflexes. Increases in muscle spindle sensitivity (as observed here), in conjunction with increased vestibular gain (Horslen et al, 2014;

Naranjo et al, 2015, 2016; Lim et al, *in preparation*), might contribute to larger balance-correcting responses to postural perturbations typically observed with increased postural threat (Brown and Frank, 1997; Carpenter et al, 2004; Cleworth et al 2016) that are thought to involve supra-spinal pathways (reviewed by Horak and Macpherson, 2011). Likewise, increased muscle spindle sensitivity would enable people to maintain, or increase, the volume of muscle spindle afferent feedback despite a reducing actual sway movements with threat. People tend to reduce amplitude and increase frequency of centre of pressure (Carpenter et al, 1999b) and centre of mass displacements (Carpenter et al, 2001a) when standing at the edge of an elevated platform; interestingly, despite actual reductions in amplitude, people perceive themselves to sway just as much in HIGH as in LOW threat conditions (Cleworth and Carpenter, 2016). It has been argued that people gather balance-relevant sensory information from normal postural sway (Carpenter et al, 2010; Murnaghan et al, 2011), and that increasing muscle spindle sensitivity (and other balance-relevant senses; e.g. vestibular) could permit reductions of postural sway without compromising sensory feedback (Horslen et al, 2013, 2014); this may explain why change to perceptions of movement do not follow actual changes in movement.

In conclusion, the study provides novel human evidence of threat-related increases in SLR and MLR to ramp-and-hold stretches, and increased gain of dynamic, velocity-dependent stretch reflexes in a posturally engaged muscle to increased postural threat. These context-dependent changes to stretch reflex amplitude and scaling likely contribute to a broad, multi-sensory strategy to modify balance control in response to threats to standing balance. Furthermore, they support the notion that human sensory function is context-dependant and highly modifiable, suggesting that modulation of sensory processing or acquisition is likely a normal aspect of motor control in healthy individuals.

Table 3-1 – Protocol 1 tilting platform ramp-stretch velocity and amplitude metrics.

		Demand of Platform		Achieved Peak		Analysis Windows	
Reflex Analysis	Threat Condition	Velocity (°/s)	Amplitude (°)	Velocity (°/s)	Amplitude (°)	Peak Velocity by SLR Peak (°/s)	Peak Amplitude by MLR End (°)
MLR	LOW	80	3	91.44 (0.78)	3.34 (0.0025)	N/A	3.06 (0.04)
	HIGH	80	3	91.55 (0.42)	3.33 (0.0019)	N/A	3.06 (0.03)
MLR	LOW	80	7	97.44 (0.33)	7.38 (0.0028)	N/A	3.85 (0.35)
	HIGH	80	7	97.84 (0.46)	7.37 (0.0019)	N/A	3.88 (0.35)
SLR and MLR	LOW	80	5	97.89 (0.65)	5.51 (0.0034)	47.23 (2.23)	3.63 (0.22)
	HIGH	80	5	96.55 (0.44)	5.50 (0.0036)	50.26 (2.18)	3.66 (0.21)
SLR	LOW	60	5	75.56 (0.35)	5.46 (0.0023)	37.09 (2.04)	N/A
	HIGH	60	5	75.41 (0.39)	5.46 (0.0021)	39.68 (2.32)	N/A
SLR	LOW	100	5	115.68 (0.39)	5.54 (0.0041)	58.15 (2.77)	N/A
	HIGH	100	5	114.11 (0.38)	5.53 (0.0037)	57.89 (2.51)	N/A

Values indicate mean (SE).

Table 3-2 – Protocol 2 tilting platform ramp-stretch velocity and amplitude metrics.

		Demand of Platform		Achieved Peak		Analysis Windows	
Reflex Analysis	Threat Condition	Velocity (°/s)	Amplitude (°)	Velocity (°/s)	Amplitude (°)	Peak Velocity by SLR Peak (°/s)	Peak Amplitude by MLR End (°)
MLR	LOW	170	2	110.64 (0.66)	2.24 (0.0020)	N/A	2.10 (0.02)
	HIGH	170	2	110.65 (0.43)	2.24 (0.0017)	N/A	2.10 (0.02)
MLR	LOW	170	7	202.72 (0.55)	7.48 (0.0024)	N/A	5.15 (0.21)
	HIGH	170	7	202.42 (0.55)	7.48 (0.0023)	N/A	5.20 (0.21)
SLR and MLR	LOW	170	5	193.83 (0.52)	5.56 (0.0031)	71.95 (3.78)	4.60 (0.12)
	HIGH	170	5	194.17 (0.35)	5.56 (0.0028)	74.49 (3.80)	4.63 (0.12)
SLR	LOW	60	5	78.37 (0.46)	5.45 (0.0029)	37.02 (1.52)	N/A
	HIGH	60	5	76.07 (0.39)	5.44 (0.0022)	38.49 (1.71)	N/A
SLR	LOW	100	5	117.46 (0.44)	5.52 (0.0031)	55.65 (2.82)	N/A
	HIGH	100	5	115.92 (0.34)	5.51 (0.0028)	56.95 (2.80)	N/A

Values indicate mean (SE).

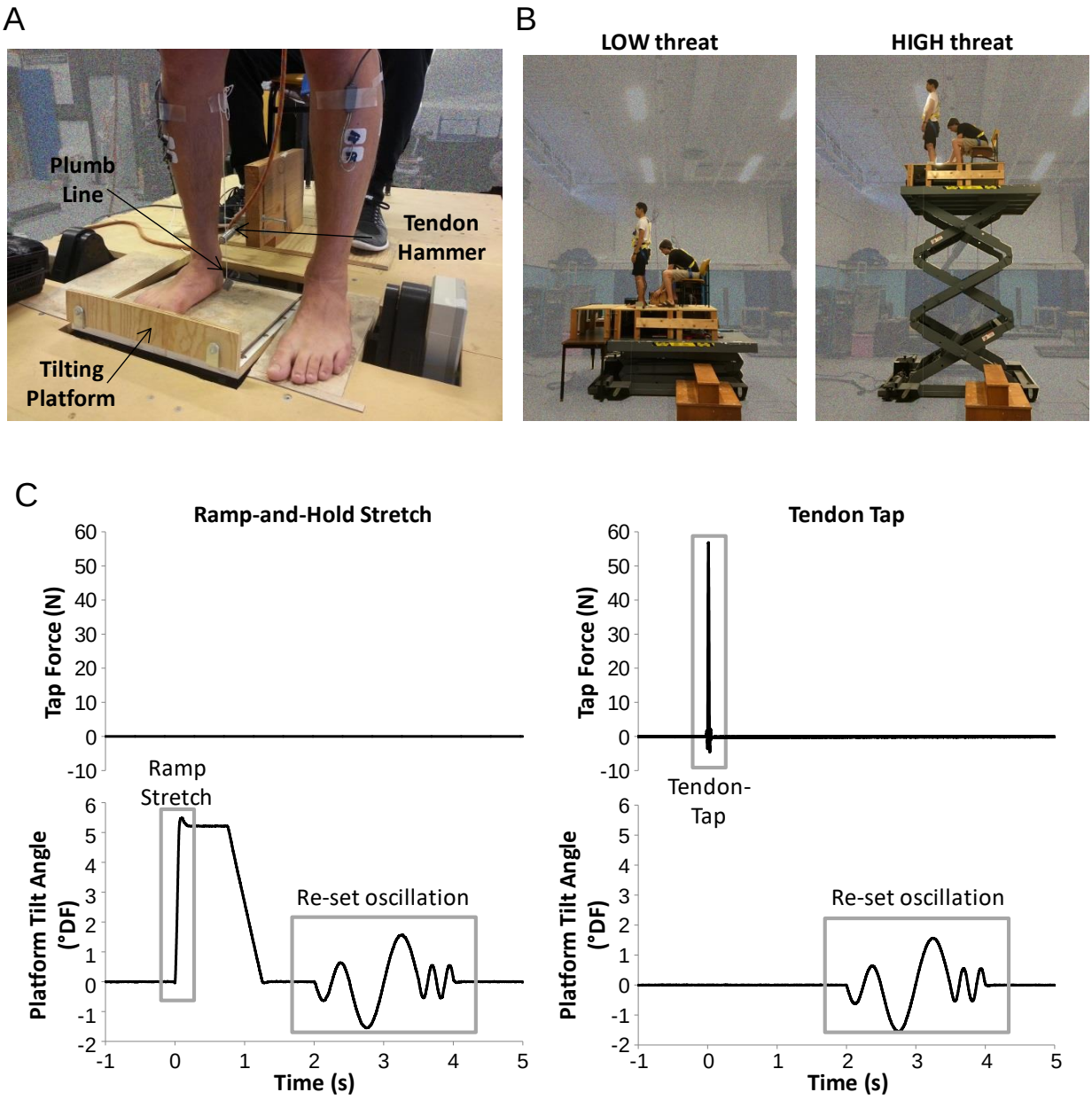

Figure 3-1 – Experiment layout, threat and stimulus presentation

Participants stood with their right foot on a tilting plate and left foot on a stable even surround (3-1A). A linear motor was positioned behind the right Achilles tendon to evoke t-reflexes and a plumb line was hung from the tibial condyle to guide experimenter tracking of knee angle. The tilting plate was mounted at the edge of a hydraulic lift. The lift was lowered to

its lowest level and a 60cm-wide support-surface extension was fixed in front of the participant in the LOW threat condition (3-1B); the extension was removed and the lift elevated for the HIGH threat condition (3-1B). All stimuli began with the tilting plate locked in a horizontal position (0°); at time zero either the plate tilted the foot through dorsiflexion (positive) or plantarflexion (negative) or a tendon-tap was presented (3-1C). Two seconds after stimulus presentation the right foot was oscillated through a re-set pattern (3-1C) to ensure all stimuli ended with a comparable mechanical event.

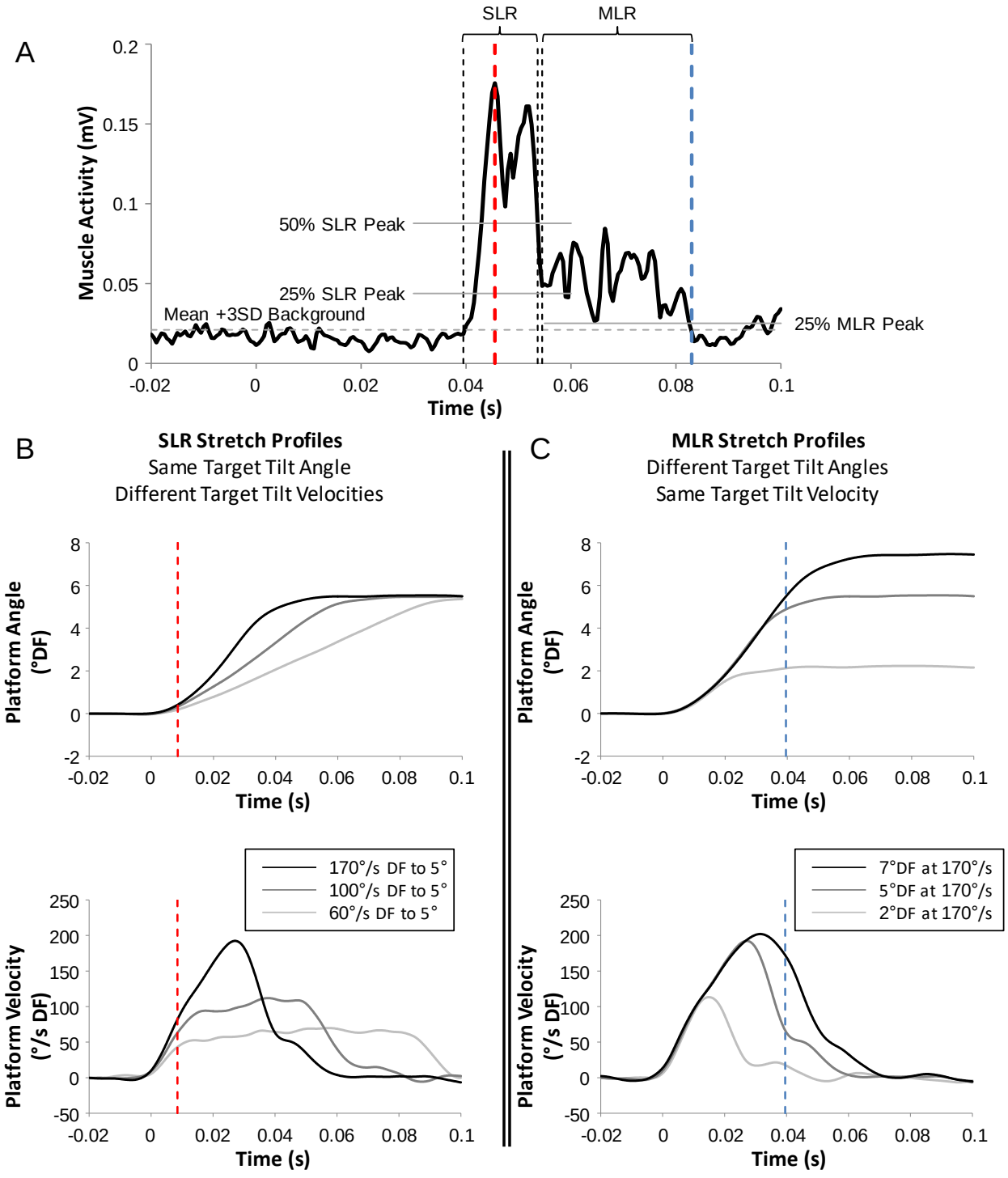

Figure 3-2 – Ramp-stretch reflex muscle activity and platform movement analyses

Soleus waveform averages (3-2A) were used to establish analysis windows and significant points, such as SLR onset, peak and end, as well as MLR onset and end. A mean

+3SD threshold established from a 100ms period ending 10ms before platform onset (dashed horizontal line) was used to determine SLR onset (dashed vertical line). The peak of the SLR waveform (dashed red line) was calculated from a fixed window (35-55ms after platform onset) and used to set 25% and 50% SLR peak thresholds (solid horizontal lines). In some cases, the SLR did not return to baseline before MLR onset. In these cases, the algorithm determined the lowest threshold crossed before 65ms post-platform onset (continuous 3ms; 50% SLR peak in 3-2A) and this value was used to define the end of the SLR (dashed vertical line). SLR onset and end were confirmed by visual inspection and SLR peak (amplitude and latency) were re-calculated within the SLR window. MLR peak was measured from 5ms after SLR end to 150ms after platform onset. The MLR analysis window began (dashed vertical line) at the lowest point between SLR end and MLR peak and adjusted manually if necessary (adjusted here). MLR end (dashed blue line) was defined as the point where the MLR dropped below the highest of either 25% MLR peak (solid horizontal line) or the mean +3SD threshold used to determine SLR onset. SLR and MLR analysis windows were created for both LOW and HIGH trials and the earliest onsets and latest ends were used to set common windows. Peak platform movement parameters for SLR-specific velocity profiles (3-2B) and MLR-specific amplitude profiles (3-2C) were referenced to either SLR peak (dashed red line) or MLR end (dashed blue line). The reference values were calculated by subtracting the latency of SLR onset from the latency of the point of interest (SLR peak or MLR end).

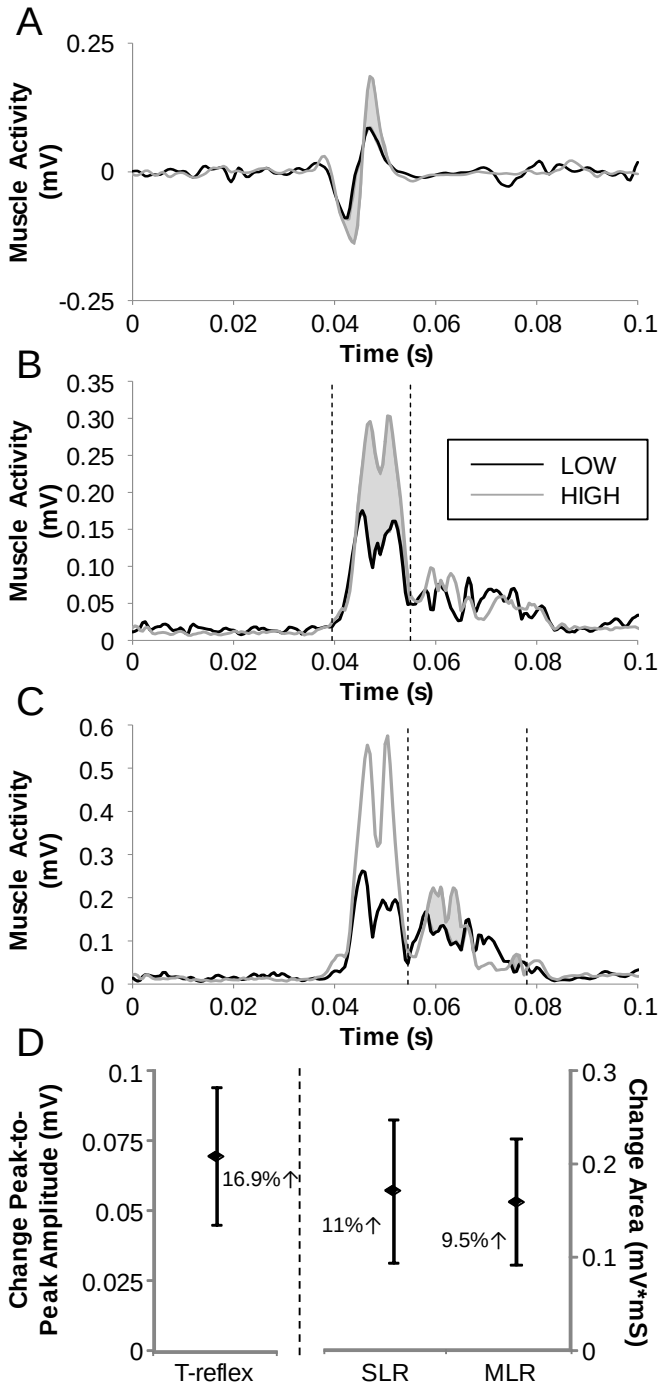

Figure 3-3 – Effects of height-induced threat on tendon-tap and ramp stretch reflexes

Waveform averages of unrectified t-reflexes (3-3A), and rectified SLR (3-3B) and MLR (3-3C) responses from a single representative participant. LOW condition traces are drawn in

black and HIGH in gray, with gray fill highlighting the difference and vertical dashed lines indicating the bounds of the region of interest for SLR and MLR traces. Group-wide mean differences are shown in (3-3D), with error bars indicating standard error and percent change given for reference.

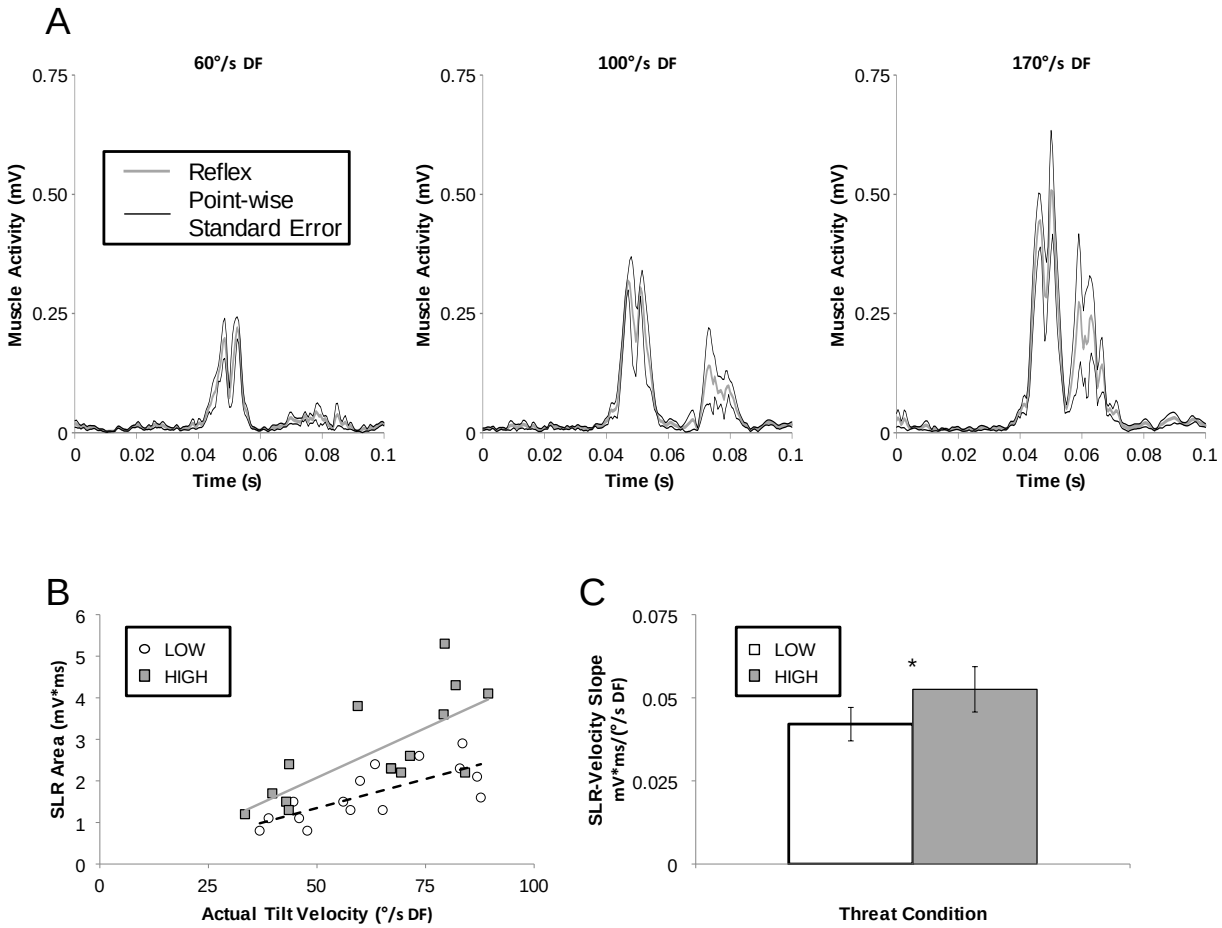

Figure 3-4 – SLR scaling to tilt velocity

SLR responses for a single participant at 60, 100 and 170°/s DF target velocities from a single condition demonstrate larger SLR amplitudes with faster velocities (3-4A). Gray lines indicate mean waveform average and thin black lines indicate point-wise standard error. SLR amplitude scaled linearly with calculated platform velocity for this participant (3-4B), and the slope of the relationship was steeper in the HIGH threat condition. Open circles represent individual reflex responses for this participant in the LOW condition, with dashed line indicating line of best fit; filled squares and gray line represent the HIGH condition. Group-wide, SLR-velocity slopes were 34.5% steeper in the HIGH, compared to LOW threat condition (3-4C);

white and gray columns represent LOW and HIGH conditions, respectively, and error bars indicate standard error about the mean.

Chapter 4: Study 3 - Both standing and postural threat decrease Achilles tendon reflex inhibition from tendon electrical stimulation.

4.1 Introduction

Golgi Tendon Organs (GTOs) are muscle mechanoreceptors located at the musculo-tendinous junction (MTJ) and arranged in series with contractile muscle fibres (Jami, 1992; Pearson and Gordon, 2000). GTOs are sensitive to tensile load applied to the tendon, particularly from active muscle contraction (Houk and Henneman, 1967). Ib afferents, arising from GTOs, project to a wide variety of central nervous system (CNS) targets. While Ib homonymous inhibition is the shortest-latency reflex evoked by GTOs, Ib afferents contribute to a broad array of excitatory and inhibitory spinal and supra-spinal reflexes (Jami, 1992; Jankowska, 1992). GTOs and associated Ib reflexes are thought to contribute to balance control by evaluating body loading (i.e. gravity effects) and contributing to responsive changes in anti-gravity muscle activity (Duysens et al, 2000; Van Doornik et al, 2011). Net GTO-based Ib reflexes are largely inhibitory while people sit or lay prone, yet inhibition is reduced when standing (Faist et al, 2006), and duration of inhibition is reduced while net reflexes can become excitatory when walking (Stephens and Yang, 1996, Faist et al, 2006). Short-latency (<50ms) plantar flexor GTO reflexes are thought to promote triceps surae muscle activity during quiet standing (Van Doornik et al, 2011) and in the stance phase of gait (Sinkjær et al, 2000, Grey et al, 2007), and have been suggested to contribute to the scaling of reactive responses to balance disturbances (Dietz et al, 1992). One important context for balance control is postural threat. Postural threats are environmental factors which impose a challenge to standing balance by increasing the likelihood (e.g. threat of support-surface tilt perturbation; Horslen et al, 2013) or consequence (real or

perceived) of falling (e.g. Height-induced threat by standing at the edge of an elevated platform; Carpenter et al, 1999b; Cleworth et al, 2012), yet do not alter the essential balance task (e.g. stand in place on a stable surface). Height-induced postural threats increase fear of falling and anxiety, increase sympathetic arousal (Carpenter et al, 2006), and affect balance behaviours; typically, postural sway is reduced and people withdraw from the edge while standing quietly (Carpenter et al, 2001a), and move less toward the edge when perturbed (Carpenter et al, 2004). Postural threats have also been shown to influence multiple balance-relevant sensory systems, including vestibular-evoked balance responses (Horslen et al 2014; Naranjo et al, 2015) and 1a monosynaptic reflexes (Llewellyn et al, 1990; Sibley et al, 2007; Davis et al, 2011; Horslen et al, 2013). However, it is currently unknown to what extent postural threat influences GTOs and associated 1b reflexes while engaged in balancing.

Ib reflexes have traditionally been probed with either Hoffmann (H-) reflex conditioning (Pierrot-Desilligny et al, 1979; Stephens and Yang, 1996; Faist et al, 2006) or muscle unloading (Dietz et al, 1992; Sinkjær et al, 2000; Grey et al, 2007; Van Doornik et al, 2011). However, these techniques may be confounded in a postural threat scenario because H-reflexes may be altered (Llewellyn et al, 1990; Sibley et al, 2007) and the perturbations used to unload muscles may trigger balance correcting responses, which are affected by threat (Carpenter et al, 2004). Tendon electrical stimulation (TStim) has emerged as an alternative, more direct, method of evoking Ib reflexes (Burne and Lippold, 1996). TStim involves square-wave electrical stimulation of a tendon at the MTJ and evokes short latency reflexive inhibition in the stimulated muscle (see Fig 4-1C). The reflex has been demonstrated in several upper- and lower-limb muscles (Burne and Lippold, 1996; Priori et al, 1998), but has most extensively been studied in the gastrocnemii muscles (Khan and Burne, 2007, 2009, 2010; Rogasch et al, 2010, 2011).

Percutaneous electrical stimulation of the Achilles tendon near the gastrocnemii MTJ (i.e. over the proximal gastrocnemius aponeurosis; Fig. 4-1D) evokes a short latency (<50ms), multiphasic pattern of negative and positive peaks in rectified surface EMG from both gastrocnemii (Khan and Burne 2007, 2009, 2010). Despite transient increases in EMG activity at approximately 140ms and 240ms post stimulation (Khan and Burne, 2007), the response is thought to be a, possibly singular, inhibitory reflex. This is because tendon stretch (Ia) reflexes and transcranial magnetic stimulation evoked twitches are both inhibited when triggered during the TStim reflex (Khan and Burne, 2010), and single motor unit peri-stimulus frequencygrams do not demonstrate periods of excitation (Rogasch et al, 2011). This reflex is thought to be mediated by Ib pathways because of the short latency (<50ms in gastrocnemii), which is comparable to Ia monosynaptic reflex latencies from the same muscle group (Türker et al, 1997), and the polarity of the response is consistent with Ib autogenic inhibition. However, TStim inhibition has not yet been demonstrated in free standing participants. Ib inhibition is thought to be reduced in standing, compared to sitting or lying (Faist et al, 2006), yet H-reflexes, which were used in the previous studies, are also affected by standing (Cattagni et al, 2014). Therefore, it would be beneficial to replicate changes in Ib inhibition with a complimentary method to ensure Ib reflexes are indeed subject to postural orientation prior to attempting to characterize the effects of postural threat on Ib reflexes.

This paper is intended to address two general aims: first, to determine if TStim is a suitable technique for probing Ib reflexes in standing, with responses mediated in a manner consistent with known changes to Ib reflexes in standing (Exp 1); and second, to understand the effects of height-induced postural threat on GTO-based reflexes (Exp 2). In Exp 1, TStim-evoked reflexes in the medial gastrocnemius were compared between upright standing and lying prone

conditions. It was hypothesized that TStim-evoked inhibition would be observed in both conditions, but the response in standing would be reduced in amplitude and duration, consistent with observations of decreased Ib inhibition in standing from H-reflex conditioning studies (Faist et al, 2006). In Exp 2, TStim-evoked inhibition was compared between conditions of low (LOW) and high postural threat (HIGH) induced by standing at different heights. Since the effect of TStim is to inhibit the stimulated muscle (in this case a plantar flexor that prevents forward falling), and this effect could be more hazardous when standing at height and facing the edge, it was hypothesized that TStim-evoked inhibition would be reduced in amplitude and duration when standing at HIGH, compared to a LOW threat condition.

4.2 Methods

4.2.1 Participants

All participants were young healthy adults recruited from the university community who gave written informed consent. All methods were approved by the University of British Columbia Clinical Research Ethics Board and conformed to the Declaration of Helsinki. Participants were recruited for both experiments (Exp 1 and 2), which took place in a single session (usually less than 2 hours). Of the 41 participants recruited (22 females and 19 males), 29 participants completed the experiments and 12 were excluded from both experiments because they found the stimulus painful or unpleasant (n=2), the stimulus evoked a sural nerve paresthesia or no reflex was observed (n=9), or due to equipment malfunction (n=1). Other participants were excluded post-hoc from one or both experiments due to observations of non-physiological noise, or a lack of measurable TStim response (i.e. response did not return to baseline or no response evoked). As such, 24 people were included in the final Exp 1 sample (12 females, 12 males; age: (mean±SE) 23.7±1.0 years; height: 171.5±2.1cm; weight: 67.8±2.8kg)

and 21 people were included in the final Exp 2 sample (9 females, 12 males; age: 24.1±1.1 years; height: 171.4±2.2cm; weight: 68.8±3.0kg).

4.2.2 Imaging

Ultrasound imaging (MicroMaxx; SonoSite, Inc., USA) was used to locate the MTJ in all subjects as a guide for positioning the cathode stimulating electrode (see Fig. 4-1 for details). This site was targeted because GTOs are precisely located at the junction between contractile muscle fibres and non-contractile tendinous fibres, and not in tendon proper (Jami, 1992), and gastrocnemius TStim effects are optimal when the cathode is positioned near the MTJ (Khan and Burne, 2009).

4.2.3 Muscle activity recordings

Muscle activity was recorded in both experiments with surface EMG from the medial gastrocnemius (MGas) and tibialis anterior (TA) muscles on the left side in all subjects. EMG were pre-amplified ×1000, bandpass filtered online 10-1000Hz (P55 A.C. Pre-amplifier, Grass, USA) and sampled at 2000Hz (Power 1401 with Spike2 software, Cambridge Electronics Design (CED), UK). These experiments focussed primarily on MGas as the muscle of interest because it is: a) an anti-gravity ankle extensor involved in quiet standing; b) accessible and attached to the Achilles tendon (i.e. soleus is deep and lies between cathode and anode, and therefore EMG contained significant artefacts); and c), is active in unperturbed quiet standing, where Lateral Gastrocnemius is often not (Héroux et al, 2014).

4.2.4 Muscle activity matching

Participants performed a 90s quiet standing trial prior to any stimulation, which was used to calculate a baseline mean±1SD of MGas EMG root mean square (RMS) activity. Since

muscle activity must be present to observe inhibition, and some people do not tonically activate MGas while standing quietly (Héroux et al, 2014), some participants were asked to adopt a slight forward lean in standing trials to ensure MGas was engaged. Muscle activity levels were matched between conditions within an experiment (i.e. between prone and standing for Exp 1, and across heights for Exp 2). Muscle activity levels were monitored online by an experimenter and verbal feedback was given in all trials (even if participant was successfully maintaining target activity) to ensure participants remained in the target mean±1SD range.

4.2.5 EMG offline processing and reflex measurement

Raw EMG data were baseline corrected and rectified offline (Spike2, CED, UK) and trigger-averaged to stimulus onset. Two separate techniques were used to build trigger averages: first, averages were constructed from all stimuli in a trial to capture the natural effect of the manipulation. However, the amplitude of TStim-evoked inhibition has been shown to be negatively correlated with background muscle activity level while participants lay on their side (Khan and Burne, 2007) and changes in muscle activity might be expected in both experiments, even with feedback. Therefore, trigger averages were also re-constructed using a more conservative approach where individual stimuli were excluded if they were preceded by background muscle activity outside a pre-specified range, as described below (screened stimuli; Fig. 4-2B, 4-3B; based on Davis et al, 2011). Custom software (Spike2, CED, UK) was used to calculate root mean square (RMS) amplitude of unrectified MGas EMG over a 100ms period ending 10ms before stimulation for each stimulus and a mean and SD were calculated from these values for each condition; The condition with the smallest SD was used to set the mean ± 1SD thresholds for screening. Each trial was then re-evaluated and individual stimuli from either

condition, falling outside the threshold were omitted (i.e. prone or standing trial for Exp 1, between heights for Exp 2).

Four measures of TStim inhibition were calculated offline using custom algorithms (Spike2, CED, UK). A mean - 1SD threshold (calculated over a 100ms period ending 10ms before stimulation onset) was used to calculate onset and duration of inhibition (Fig. 4-1C). Each period of inhibition was then used to calculate a mean level (mean over duration of response, referenced to background) and area of inhibition (based on trapezoid integration) relative to pre-stimulus background EMG activity (see Fig. 4-1D for details).

4.2.6 Height specific measures

In Exp 2, participants' psychological and autonomic responses to threat were also assessed. Self-reported balance confidence (100-point scale where higher values indicate greater confidence) was recorded prior to each trial, and self-reports of anxiety (16-item 1-9 rating questionnaire where higher scores indicate more anxiety; maximum score: 144), fear of falling (100-point scale where higher scores indicate greater fear), and perceived stability (100-point scale where lower scores indicate the participant felt less stable) were recorded after each trial. These questionnaires have previously been demonstrated to have moderate to high reliability in a height-induced postural threat protocol (Hauck et al, 2008). Electrodermal activity (EDA) was measured with galvanic skin conductance (model 2501, CED, UK) over the course of each trial (sampling: 100 Hz; Power 1401, CED, UK) to quantify sympathetic autonomic arousal (Boucsein et al, 2012). EDA data were low-pass filtered offline (5Hz) and averaged over the duration of the trial in each condition (Spike2, CED, UK); mean conductance levels are reported in µS.

4.2.7 Stimulation

TStim stimuli were delivered to the left Achilles tendon with single 0.5ms square-wave pulses (DS7A Constant Current Stimulator, Digitimer, UK) with cathode (9cm² carbon-rubber pad coated with conductive gel; Spectra 360 Gel, Parker, USA) positioned at the MTJ and anode (Kendall H59P Cloth Electrode, Medtronic, Ireland) over the tendon approximately 2cm proximal to the calcaneus (Fig. 4-1D). Stimulation protocols began with detection of perceptual threshold (PT), defined as the lowest current intensity (V_{max} = 300V) where participants could detect a non-specific tingling or tapping sensation on both ascending and descending incremental current changes (mean PT was 4.41±0.36mA). Participants were then presented with individual pulses at multiples of ×2 PT (×2, ×4, ×6, ×8 and ×10) and asked to describe the sensation evoked. Participants typically described a muscular sensation, generally near ×4-6 PT, such as a tugging or pulling sensation at/near the insertion of the Achilles tendon onto the calcaneus, a deep paresthesia or tingling near the cathode or on a line between cathode and anode, or felt a muscle twitch. Participants were excluded at this stage if they felt a paresthesia along the lateral border of the foot, consistent with sural nerve stimulation. Qualitative descriptions were not further analysed.

Next, participants were given 25-pulse trials (random 1-4 s inter-stimulus interval) at a fixed intensity while lying prone with ankle at approximately 90° and contracting against manual resistance provided by an experimenter to activate MGas to determine if a reflex was present. Stimulation intensity was adjusted in ×0.5 PT increments between trials to find the lowest intensity where a response could be observed (group mean = ×4.9±0.3 PT). The criteria used to identify the presence of a response to TStim was rectified MGas EMG dropping below background levels for at least 10ms (width of algorithm detection window, see Fig 4-1 legend)

starting approximately 45ms post-stimulation, determined by visual inspection of a waveform average of each 25 pulse trial.

Participants then did a practice 100-pulse prone trial at the lowest intensity where a reflex could be evoked; during the trial the participant plantar flexed against manual resistance and verbal feedback was used to guide contraction to previously-determined baseline quiet standing levels. Immediately following the practice prone trial, the participant stood up and performed a 100-pulse practice standing trial. During the standing trial the participant faced a blank wall and an experimenter occasionally gave verbal feedback about participant's forward lean to maintain target activation levels. The reflexes from both practice standing and prone trials were assessed online and durations of inhibition (if present) were noted. In most cases, the standing reflexes were found to be less depressed and shorter in duration than the practice prone reflexes; in some cases, no inhibition was initially observed in standing. Subsequently, stimulation intensity was adjusted (×0.5 PT increments) to a level where reflexes comparable to those evoked in the practice prone trial could be evoked in standing (assessed in 25-pulse trials). The purpose of the adjustment was to ensure that a reflex similar to that achievable while lying prone could be achieved in standing, enabling comparison of like responses between conditions. The new stimulation intensity (group mean = ×6.8±0.3 PT) was used for all subsequent trials.

4.2.8 Protocol

Participants performed 2 trials in each experiment. In Exp 1, trials consisted of 100 stimuli presented with a 1-4s inter-stimulus interval, and the standing trial was always performed first. During the standing trial participants stood facing a blank wall and, where necessary, maintained a forward lean to activate MGas; verbal feedback about muscle activity levels was given to all

participants (e.g. lean forward/backward/maintain), even if they successfully maintained target levels. In the prone trial, participants lay prone on a padded table and made an isometric plantar flexor contraction with ankle at approximately 90° against manual resistance. Similar to the standing trial, in all cases an experimenter gave verbal feedback about contraction intensity (e.g. push harder/less/maintain).

After completion of Exp 1, participants were transferred to a hydraulic lift (M419-207B01H01D, Pentalift, Canada) for Exp 2. Participants stood at the edge of the lift first in the LOW condition, where the lift was set to the lowest level (0.8m) and a 0.6m-wide stable support surface was placed directly in front of the participant; this setting is comparable to standing on the ground (Carpenter et al, 1999b). The support surface extension was removed and the lift elevated to 3.2m for the HIGH condition. Since there is a known order effect for this postural threat manipulation (Adkin et al, 2000), and we wished to maximize contrast between conditions, the LOW condition was always presented first. Participants wore a safety harness attached to a safety line and an experimenter was within arms-reach at all times in case the participant lost balance. No participants lost their balance in either experiment. The number of stimuli was reduced to 50 per condition in Exp 2 to limit the amount of time participants had to stand at the edge in the HIGH condition, and because 50 pulses have been shown to be sufficient to evoke TStim inhibition (Miller and Burne, 2014). Otherwise, all methods, including analyses and provision of feedback, were similar to Exp 1.

4.2.9 Statistics

It was hypothesized in Expt 1 that TStim-evoked inhibition would be reduced in amplitude and duration in standing, compared to lying prone. The effects of postural orientation on TStim

inhibition were explored with pre-planned paired-samples t-tests between prone and standing conditions for reflex area, duration and mean inhibition levels (IBM SPSS v23, IBM Corp., USA); paired-samples t-tests were also used to characterize changes in MGas and TA background muscle activity between threat conditions. The effects of height-induced postural threat on TStim inhibition were also explored with pre-planned paired-samples t-tests on area, duration and mean level of inhibition between LOW and HIGH conditions. In Experiment 2, it was hypothesized that TStim-evoked inhibition would be reduced in amplitude and duration when standing at the HIGH, compared to a LOW threat condition. The effects of threat on psychological state (balance confidence, fear of falling, anxiety and perceived stability), sympathetic arousal (EDA), and background muscle activity (MGas and TA) were also explored with paired-samples t-tests. Alpha was set to 0.05 for all statistical tests.

4.3 Results

4.3.1 Experiment 1: prone vs. standing

Clear inhibitory responses to TStim were observed in both prone and standing conditions (Fig. 4-2A). When all stimuli were included in the analysis (Fig. 4-2A,B), onset latency of inhibition in the prone condition (43.96±0.80ms post-stimulus) and standing condition (44.29±0.68ms) were not statistically different ($t_{(23)}$=-0.748, p=0.462, η^2=0.024). However, area of TStim was significantly reduced 42.2% and duration reduced 32.9% when standing, compared to laying prone (area: $t_{(23)}$=3.10, p=0.005, η^2=0.294; duration: $t_{(23)}$=4.70, p<0.001, η^2=0.490; Fig. 4-2C,D). Yet, there was no significant change in mean inhibition when standing, compared to lying prone (0.5% decrease, $t_{(23)}$=-0.068, p=0.946, η^2<0.001; Fig. 4-2E); suggesting the change in area is related to reduced duration of inhibition. There was no significant change in MGas background muscle activity between conditions (Prone: 21.13±3.20μV, Standing:

21.89±3.16μV, $t_{(23)}$=-1.08, p=0.290, η^2=0.049); however, TA background activity was higher in the standing condition (6.60±0.81μV) than in the prone condition (4.73±0.61μV; $t_{(20)}$=-3.62, p=0.002, η^2=0.396). The effects of screening for changes in MGas background EMG on TStim reflexes are demonstrated with data from a representative participant plotted in Figure 4-2B. When controlling for MGas background muscle activity there was a small but statistically significant change in onset latencies between prone (44.31±0.78ms) and standing (45.14±0.81ms) conditions ($t_{(23)}$=-2.49, p=0.021, η^2=0.212). Significant reductions in area (45.7%) and duration (32.7%) of inhibition were still observed (area: $t_{(23)}$=3.34, p=0.003, η^2=0.326; duration: $t_{(23)}$=4.135, p<0.001, η^2=0.426; Fig. 4-2C,D), as were the lack of changes in mean amplitude of inhibition (0.9% increase, $t_{(23)}$=0.130, p=0.898, η^2=0.001; Fig. 4-2E). There was no change in MGas background muscle activity after screening (Prone: 21.22±3.32μV, Standing: 21.61±3.38μV; $t_{(23)}$=-1.20, p=0.242, η^2=0.059), but a statistically significant increase in TA background activity remained in standing (Prone: 4.71±0.61μV, Standing: 6.60±0.83μV; $t_{(20)}$=-3.691, p=0.001, η^2=0.405).

4.3.2 Experiment 2: LOW vs. HIGH threat

Participants were more aroused, and had significant psychological responses to standing at the edge of the elevated platform. Sympathetic arousal, as indicated by EDA, was significantly higher in the HIGH, compared to LOW threat condition (LOW: 18.05±1.83μS; HIGH: 27.70±2.29μS; $t_{(18)}$=-5.71, p<0.001, η^2=0.644). Prior to starting the trial, participants were less confident in their ability to maintain balance in the HIGH, compared to LOW condition (LOW: 95.5±1.7%; HIGH: 68.9±4.1%; $t_{(20)}$=6.69, p<0.001, η^2=0.691). Participants were more anxious at height (LOW: 31.0±2.3/144; HIGH: 54.8±6.2/144; $t_{(20)}$=-4.01, p=0.001, η^2=0.446), more afraid

of falling (LOW: 7.1±2.6%; HIGH: 45.0±6.3%; $t_{(19)}$=-6.69, p<0.001, η^2=0.701), and also felt less stable (LOW: 85.5±2.5%; HIGH: 55.0±5.3%; $t_{(19)}$=7.14, p<0.001, η^2=0.728).

Height-induced postural threat reduced the amplitude and duration of TStim-evoked MGas inhibition (Fig. 4-3A). When all stimuli were included there was a small but statistically significant change in latency of onset of inhibition from 45.19±0.74ms in the LOW condition to 46.5±0.96ms in the HIGH condition ($t_{(20)}$=-2.12, p=0.047, η^2=0.183). There was also a significant reduction in area (32.4%) and duration (16.4%) of inhibition in the HIGH, compared to LOW condition (area: $t_{(20)}$=3.138, p=0.005, η^2=0.330; duration: $t_{(20)}$=3.071, p=0.006, η^2=0.320; Fig. 4-3C,D). There was also a significant 24.8% reduction in the mean amplitude of inhibition when standing at height ($t_{(20)}$=-3.050, p=0.006, η^2=0.317; Fig. 4-3E). However, there was a significant decrease in MGas background muscle activity (LOW: 18.52±2.81µV, HIGH: 15.96±2.80µV; $t_{(20)}$=2.652, p=0.015, η^2=0.260) and trend to increase in antagonistic TA muscle activity (LOW: 4.95±0.80µV, HIGH: 7.13±1.12µV; $t_{(20)}$=-1.807, p=0.086, η^2=0.140) between height conditions when all stimuli were analysed. The effects of screening for changes in background muscle activity on reflex waveform averages in Experiment 2 are demonstrated with data from a representative participant plotted in Figure 4-3B. The differences in background muscle activity between height conditions were controlled by screening (MGas: LOW: 18.53±3.39µV, HIGH: 17.24±3.30µV, $t_{(17)}$=1.743, p=0.099, η^2=0.152; TA: LOW: 4.90±0.89µV, HIGH: 6.29±1.03µV, $t_{(17)}$=-1.38, p=0.185, η^2=0.101), as were differences in onset latency between threat conditions (LOW: 45.78±1.0ms; HIGH: 46.89±1.11ms; $t_{(17)}$=-1.654, p=0.116, η^2=0.139). However, the changes in area (21.1% decrease) and duration (11.4% decrease) remained statistically significant (area: $t_{(17)}$=2.446, p=0.026, η^2=0.260; duration: $t_{(17)}$=2.335,

$p=0.032$, $\eta^2=0.243$; Fig. 4-3B,C) and there was a trend to a decrease in mean inhibition (14.1% decrease, $t_{(17)}=-1.828$, $p=0.085$, $\eta^2=0.164$; Fig. 4-3D).

4.4 Discussion

The purpose of Exp 1 was to determine if TStim is a suitable technique for probing Ib inhibition in a posturally engaged muscle in free standing. To-date, TStim studies have fixed postural orientation (seated or lying) and task as methodological controls. Exp 1 is novel in that it is the first study to use TStim in free, upright standing, and is the first to show changes in TStim-evoked inhibitory reflexes with changes in postural orientation and/or engagement in upright balancing. As hypothesized, TStim-evoked inhibitory reflexes were significantly shorter in duration, and reduced in area, but were not different in latency or mean amplitude in standing compared to lying postures. These results are in line with indirect evidence of decreased Ib inhibition in standing, compared to lying supine or sitting (Faist et al 2006), as indicated by H-reflex conditioning from heteronymous muscles. It is thought the reduction in inhibition reflects a shift from predominantly inhibitory toward excitatory reflexes in standing, which may be important for weight-bearing (Van Doornik et al, 2011) or walking (Duysens et al, 2000). The shorter duration of TStim-evoked inhibition could reflect this shift, as the motor neuron pool took less time to return to activation in Exp 1. Similar changes to Ib reflexes have been observed between sitting and walking with MGas conditioning of soleus H-reflexes; Ib reflexes are inhibitory (and similar in amplitude) in both sitting and walking at short conditioning intervals (1-3ms), however Ib inhibition disappears, and may become excitatory, at longer conditioning intervals (>4ms) in walking (Stephens and Yang, 1996).

While TStim inhibition is thought to arise from Ib afferents or GTOs, other origins, including stimulation of skin overlying the tendon, sural nerve, or stimulation of other muscle or

tendon afferents should be considered. In a replication of a 2-person pilot study by Rogasch et al (2012), we compared reflexes evoked by percutaneous and direct subcutaneous tendon stimulation in a single pilot subject (40 year-old male) in a controlled setting in order to rule out contributions from skin or muscle afferents (see Fig. 4-4 for details). Direct electrical stimulation of the tendon with indwelling TStim evoked an inhibitory response with similar shape and timing to that evoked with percutaneous stimulation in the same participant (Fig. 4-4). The onsets of inhibition to percutaneous and indwelling TStim were 49.35ms and 47.71ms, respectively. The duration of inhibition was longer with percutaneous stimulation; the inhibitory period in this participant lasted 28.44ms with percutaneous stimulation and 17.84ms with indwelling TStim. The current results are similar to those of Rogasch et al (2012) in that both studies found similar patterns of inhibition, which occurred at similar latencies across modalities; the studies differ in that the stimulation intensity used to evoke the reflex was higher, and the duration of the inhibitory period was shorter, with indwelling than with percutaneous TStim in the present study. These discrepancies might be due to methodological differences in terms of stimulation site, criteria for setting stimulation intensity, and small sample sizes in both cases. Furthermore, stimulation of the skin lying over the tendon is not likely the cause of TStim inhibition because the response is abolished when the skin is stretched so that the electrodes no longer lay over the tendon (Burne and Lippold, 1996). Likewise, sural nerve stimulation cannot explain TStim inhibition because it evokes qualitatively different reflex responses from TStim (Khan and Burne, 2009, 2010; Rogasch et al, 2012), and sural nerve conditioning effects on other reflexes differs from TStim in pattern and duration (Khan and Burne, 2010). Furthermore, the TStim-evoked reflex disappears when the tibial nerve is blocked with anesthetic (which supplies the gastrocnemii as well as part of the sural nerve) but is not affected by blocking the sural nerve

(Khan and Burne, 2009). Therefore, the combined observations of reduced inhibition in standing, compared to lying prone (Exp 1; Fig. 4-2), and similar patterns of inhibition from percutaneous and direct tendon stimulation (Fig. 4-4) suggest a Ib reflex of tendinous origin. The exact point of stimulation and origin of the response, be it GTO or peripheral afferent, cannot be determined with these data.

The second purpose of the study (Exp 2) was to characterize the effects of height-induced postural threat on Ib reflexes. In agreement with our hypothesis for Exp 2, TStim-evoked inhibition was reduced in area, duration and amplitude when standing in the HIGH, compared to LOW threat condition. To our knowledge, this is the first example of context-dependent modulation of Ib reflexes without changing the essential postural task (e.g. lying to standing) in humans. The effect of reduced Ib inhibition with threat would be to limit the quiescent period in a posturally-engaged anti-gravity muscle. In the context of the height-induced postural threat, inhibition of MGas would cause the body to fall forward, toward the edge of the lift; therefore, reducing the amount of inhibition would be protective because the person would not sway as far forward. Further work is needed to determine if the observed effects reflect a generalized response to a postural threat (e.g. do not fall down) or a direction-specific response to the forward edge of the platform. This issue might be resolved with a different, non-directionally-specific threat, such as threat of whole-body perturbation (Horslen et al, 2013; Lim et al, *in preparation*), or standing with the direction of threat to either side (Tersteeg et al, 2012; Osler et al, 2013), or behind the subject, in which cases a forward fall would be protective.

Reduced TStim-evoked inhibition might be achieved by either reducing the potency of inhibitory effects (e.g. disinhibition), or by countering inhibition with excitatory influences. Ib reflexes are subject to many spinal (Pierrot-Desilligny et al, 1979, Jankowska, 1992) and

supraspinal modulatory influences (Jami, 1992; Jankowska, 1992). Of particular interest are reticulospinal modulatory projections onto Ib reflex pathways (Jankowska, 1992; McCrea, 2001), which are known to inhibit non-reciprocal inhibition of motor-neurons (i.e. disinhibition; Jami, 1992), and are known to be modulated by fear and anxiety networks (Balaban and Thayer, 2001; Staab et al, 2013). Alternatively, the threat-effects might reflect more central Ib excitation. Ib reflexes in anti-gravity muscles are thought to help excite the motor neuron pool in standing to help resist gravity and changes in muscle loading (Dietz et al, 1992; Sinkjær et al, 2000; Grey et al, 2007; Van Doornik et al, 2011). While Faist et al (2006) distinguished between diminished Ib inhibition in standing and activation of Ib excitatory effects with gait, Van Doornik et al (2011) argued Ib afferent activity contributes to plantar flexor excitation in quiet standing because sudden muscle unloading causes short latency decreases in muscle activity. Unfortunately, the data from the current study do not reveal how the reduction in inhibition was achieved. While surface EMG cannot dissociate between these effects, examining changes in individual motor-unit discharge rates may reveal how threat is reducing Ib inhibition (Rogasch et al, 2011).

Further study is required to understand how changes in Ib reflexes might contribute to altered balance behaviours observed with height-induced postural threat. Typically, people demonstrate smaller amplitude and higher frequency centre-of-pressure (Carpenter et al, 1999b) and centre-of-mass oscillations (Carpenter et al 2001a), as well as less tonic plantar flexor and more dorsi-flexor background muscle activity when standing quietly at height (Carpenter et al, 2001a). They also permit less forward sway in response to whole-body postural perturbations (Carpenter et al, 2004) and adopt a more cautious gait (Tersteeg et al, 2012). Taken together with known changes in muscle spindle somatosensory (Davis et al, 2011; Horslen et al, 2013) and vestibular reflexes (Horslen et al, 2014; Naranjo et al, 2015, 2016) with height-induced postural

threat, the observations from the current study point to a broad, multi-sensory adaptation process to threat. It has been hypothesized that threat-induced changes to sensory function might evoke larger myogenic responses to balance disturbances, as well as permit reductions in postural sway without compromising the fidelity of balance-relevant sensory feedback (Horslen et al, 2013; 2014). The changes in Ib reflexes observed here could lead to more tonic plantar flexor muscle activity, and may contribute to altered scaling of responses to postural disturbances with threat.

There are several limitations to acknowledge for this study. Free, unconstrained standing may have led to changes in muscle activation between prone and standing conditions, over the course of a standing condition, or between threat conditions. The amplitude of TStim-evoked inhibition is known to scale negatively with background muscle activation (Khan and Burne, 2007). We used verbal feedback and post-hoc screening to control for background muscle activations levels, therefore, it is unlikely the changes observed in either Exp 1 or 2 can be explained by changes in muscle activation levels. Likewise, participants may have adopted different ankle angles between threat or postural orientation conditions, and changes in ankle angle can affect the TStim response (Khan and Burne, 2009). Verbal feedback about muscle activity was used as a proxy for feedback about ankle angle in the standing conditions, as participants were instructed to lean forward or backward to compensate for changes in activation levels. Similarly, manual manipulation of ankle angle by an experimenter was used to approximately match prone angle with standing angle. Furthermore, loading the plantar flexors in free-standing may have shifted the location of the MTJ, compared the voluntary contraction used to load the muscle in the prone condition of Exp 1. The location of the cathode with respect to the MTJ is important for evoking the TStim response (Khan and Burne, 2009). While we cannot completely rule out a change in MTJ location between standing and lying trials, it is

unlikely the MTJ moved out of the $9cm^2$ (3cm long) cathode stimulation area. While, to our knowledge, changes in Achilles tendon length between standing and lying prone while contracting isometrically have not been investigated, ultrasound imaging of the Achilles tendon reveals less than 1cm longitudinal displacement of the lateral gastrocnemius MTJ over the course of the gait cycle (Franz et al, 2015). Furthermore, this would not explain changes observed with height, in which subjects maintained a similar forward lean between height conditions. Finally, TStim at the MTJ is likely to only affect a sup-population of all MGas GTOs. Approximately half of all MGas GTOs attach to the aponeurosis of insertion, *vs.* origin (Swett and Eldred, 1960; Jami, 1992); and, due to the pennate orientation of MGas muscle fibres, GTOs on the insertion are distributed from approximately mid-length to distal end of the aponeurosis (Swett and Eldred, 1960). Assuming TStim is stimulating GTOs, then the sample here is limited to the most distal sub-set of MGas GTOs, and the results may not reflect the whole population response.

These experiments make two significant contributions to the study of the contribution of Ib reflexes to standing balance control. First, the results demonstrate that TStim can be used in free-standing participants to evoke Ib inhibition, and confirms that Ib inhibition is reduced when standing, compared to lying prone. The study also provides novel evidence of context-dependent modulation of Ib reflexes within a single task in humans, without modulation of muscle state (e.g. muscle cramp, Miller and Burne, 2014; Khan and Burne, 2007). Finally, these data further support sensory adaptation processes as a likely contributor to altered balance behaviours with threats to standing balance. Future studies should endeavor to reveal how changes in sensory function, or possibly changes in sensory integration, contribute to changes in balance behaviours when humans experience threat.

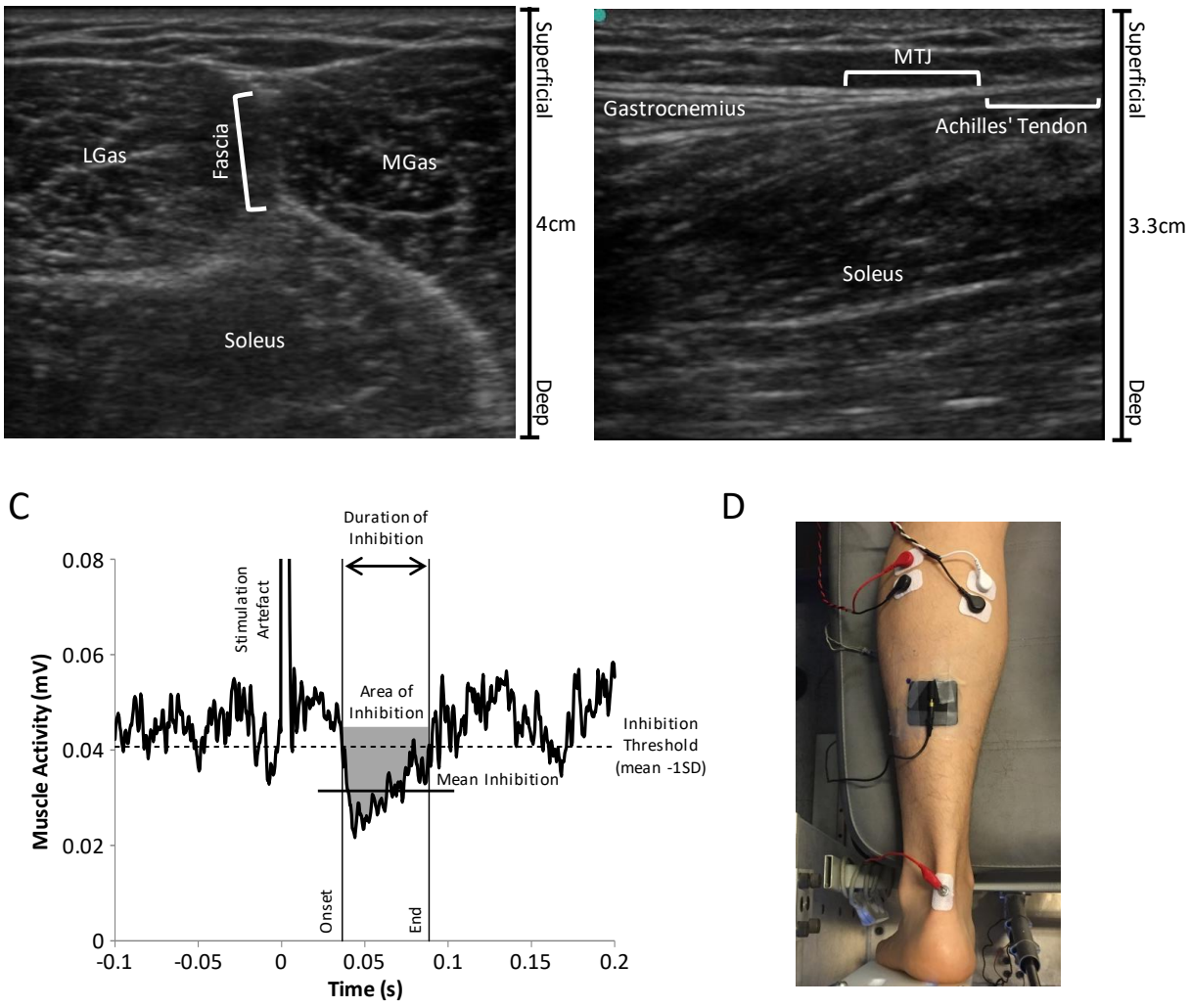

Figure 4-1 – Tendon imaging, stimulating electrode placement and TStim inhibition analyses

Ultrasound images were used to locate the musculo-tendinous junction (MTJ) for placement of the cathode. First, transverse plane ultrasound was used to locate the fascial band between the heads of the gastrocnemii (1A), which was used to mark the centre of the MTJ in the medio-lateral line. Based on the technique of Maganaris and Paul (1999), a sagittal view of the muscle along the marked medio-lateral line was used to identify where muscle fibres terminated and the Achilles tendon began (1B) and the skin was marked accordingly. Figure 4-1C shows a representative trace of TStim-evoked inhibition in a 100-pulse waveform average of rectified

MGas EMG, aligned in time to stimulation. A mean-1SD threshold (dashed horizontal line) was used to determine the onset and end of the response (solid vertical lines); the threshold for detection of both onset and end was the signal remaining below or above threshold for at least 7ms in a 10ms period, only onsets occurring between 35ms and 65ms post-stimulation were accepted. The area of inhibition referenced to mean background activity (gray shaded area), duration of inhibition, and mean amplitude of inhibition (solid horizontal line) were calculated in this window. Finally, Figure 4-1D demonstrates the locations of the cathode (over the MTJ) and anode electrode placements. Note: Lateral gastrocnemius (LGas) is marked on the figure, but no reflexes were analysed for this muscle.

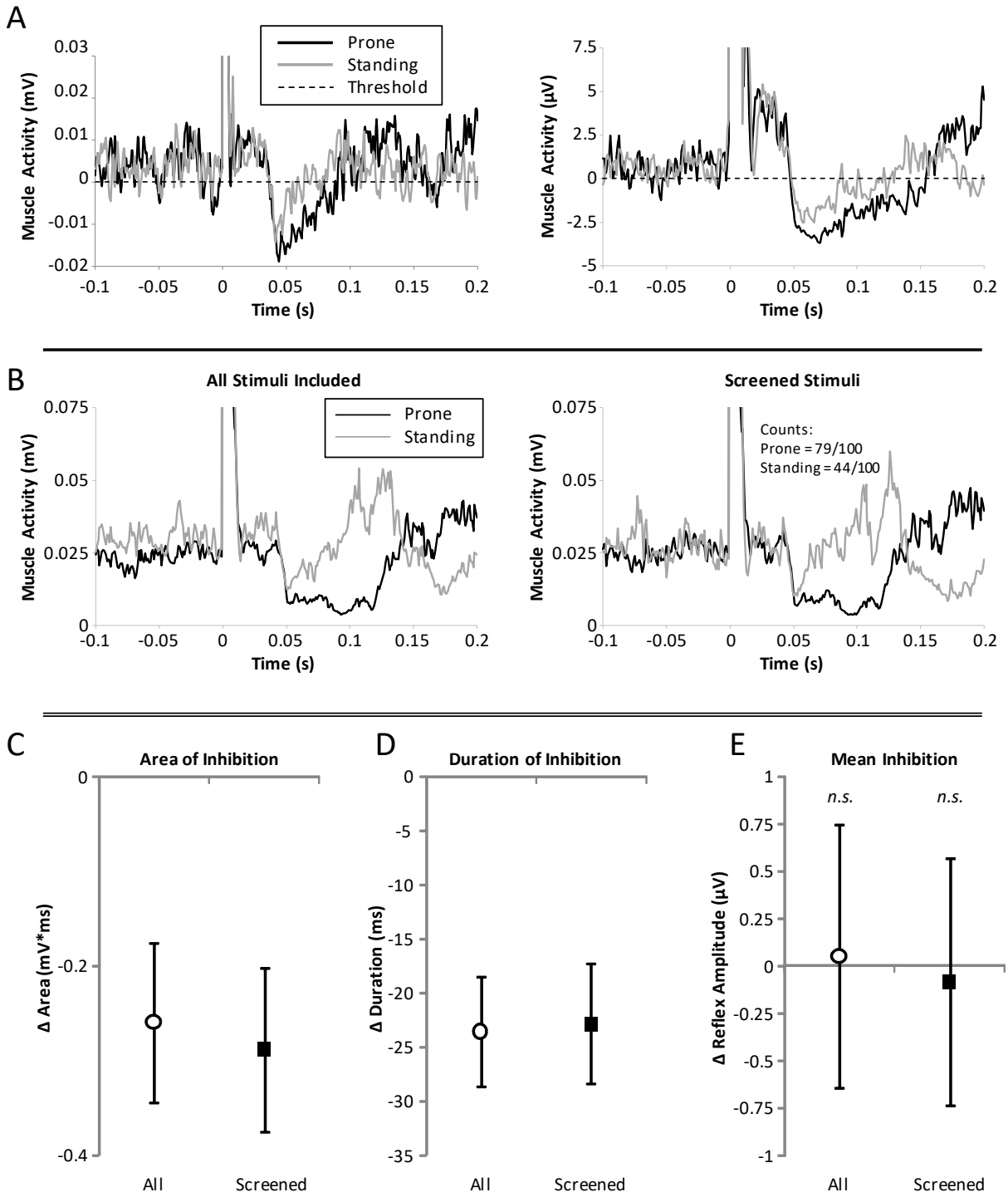

Figure 4-2 – Experiment 1: comparison of prone and standing conditions

Figure 4-2A demonstrates prone (black) and standing (gray) 100-pulse waveform averages from two representative participants. The waveforms have been shifted for display purposes so

that their respective inhibition detection thresholds (dashed horizontal lines) are aligned at zero. In both cases, the inhibition begins at approximately the same point in both prone and standing conditions, but the duration and area of inhibition is longer and larger in the prone condition. The effects of screening for changes in background muscle activity are demonstrated for a third representative participant in part B. The traces in the left column were constructed using all 100 stimuli from each condition, whereas the waveform averages on the right were constructed after individual stimuli outside the screening range were excluded (21 removed from prone, 56 removed from standing condition). Group-wide mean differences in area, duration and mean amplitude of inhibition are shown in Figures 4-2C, D and E. In each figure open circles (n= 24) represent the average effect when all stimuli are included in the analysis and filled squares (n=20; 4 participants excluded because too few stimuli remained after screening) represent effects after screening for changes in background muscle activity; error bars indicate standard error about the mean and *n.s.* indicates effect is not statistically different between postural orientation conditions.

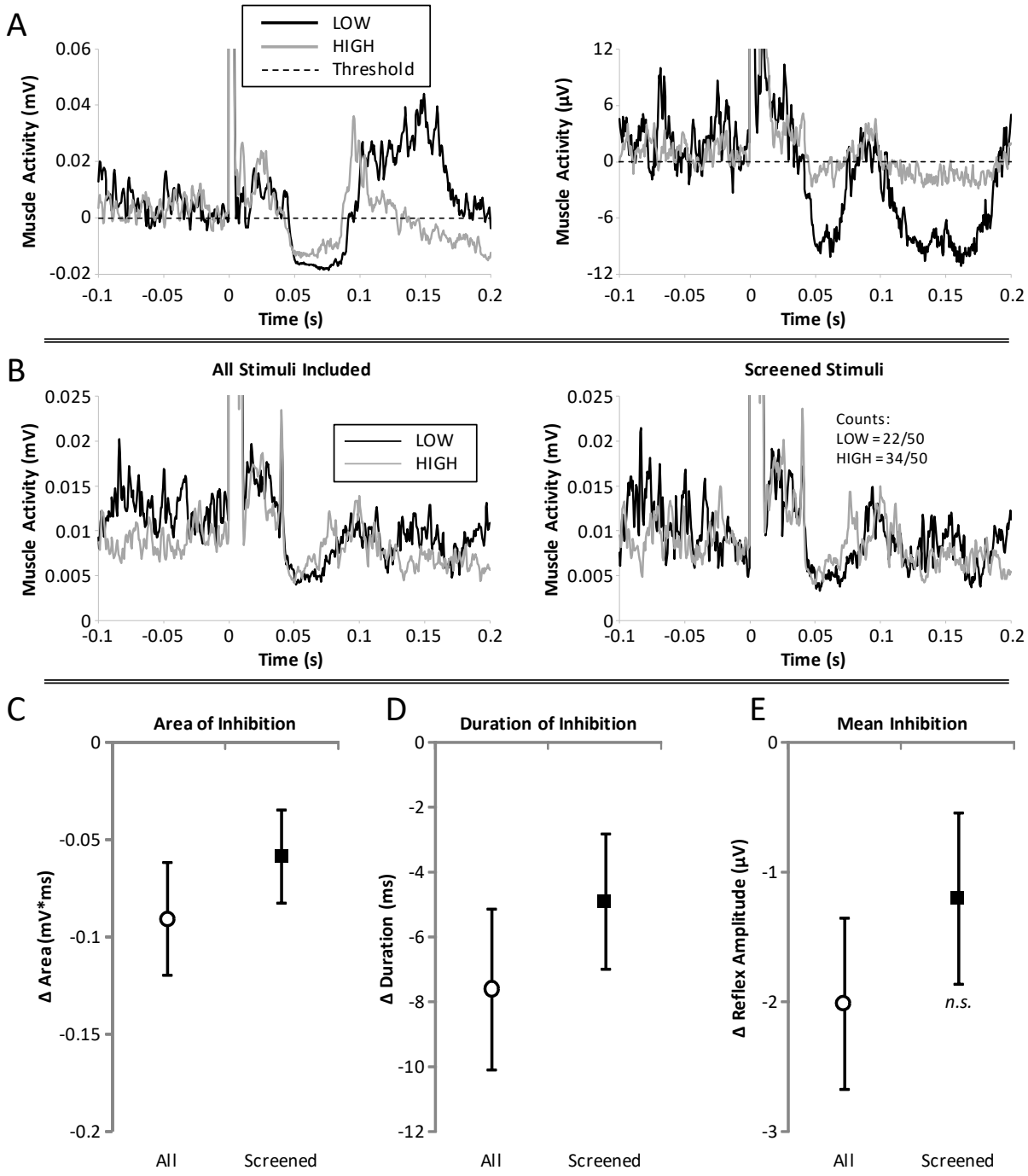

Figure 4-3 – Experiment 2: comparison of LOW and HIGH postural threat conditions

Figure 4-3A represents 50-pulse waveform averages from two representative participants from the LOW (black) and HIGH (gray) postural threat conditions, with amplitude aligned to

inhibition detection thresholds (horizontal dashed line) for display purposes. Similar to Figure 4-2, the effects of background muscle activity screening on TStim waveform averages are shown for a third representative participant in Figure 4-3B. Here, 28 of 50 stimuli were removed in the LOW threat condition, and 16 of 50 removed from the HIGH condition trace in the muscle activity screening process. Figures 4-3C, D and E show mean differences in area, duration and amplitude of inhibition across threat conditions. Similar to Figure 4-2, open circles (n=21) and filled squares (n=18, 3 participants excluded because too few stimuli remained after screening) represent analyses based on all and screened stimuli, respectively. Error bars indicate standard error, and *n.s.* indicates differences between threat conditions are not statistically significant.

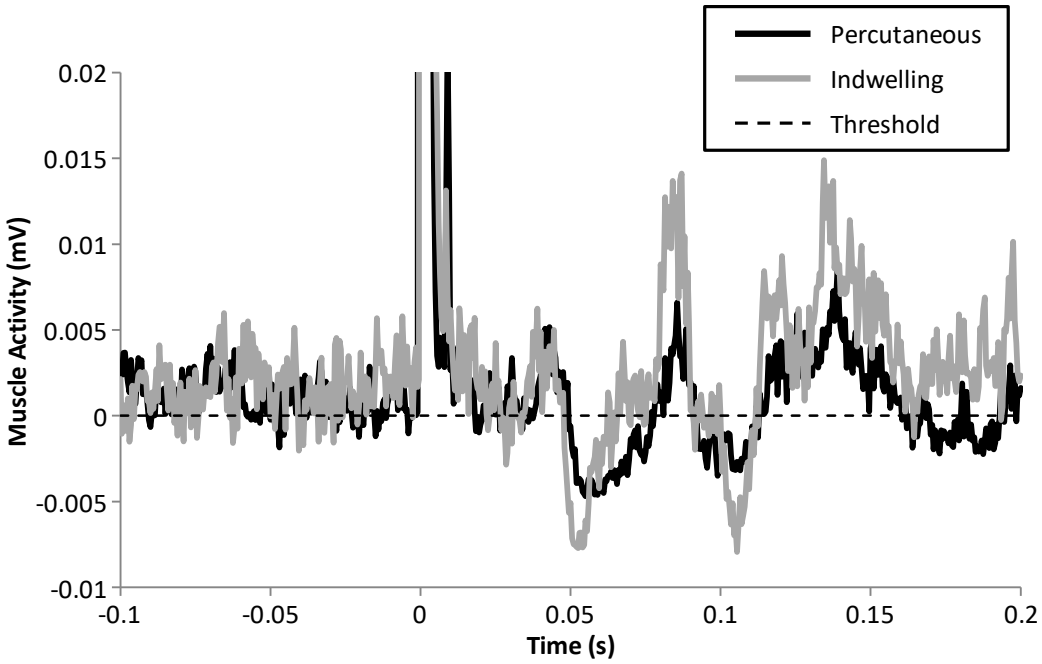

Figure 4-4 – Comparison of indwelling and percutaneous tendon stimulation

Figure 4-4 demonstrates the 100-pulse waveform averages from the percutaneous (black) and indwelling stimulation (gray) techniques in a 40 year-old healthy male lying prone and plantar flexing. The MTJ was identified and marked (see methods), and percutaneous stimulation intensity adjusted to a level where the participant felt a tendinous sensation (e.g. tugging, pulling or tapping) that was subjectively similar to that evoked with percutaneous TStim; stimulation intensity was not the same across conditions (×4 PT with percutaneous and ×10 PT with indwelling TStim). Surface EMG was recorded from the left MGas and TA (Neurolog NL 824 pre-amplified with NL 820 optical isolator, Digitimer, UK) and sampled at 2000Hz (Power 1401 with Spike2, CED). For indwelling stimulation 2 sterile tungsten microneurography needle electrodes insulated to the tip (Impedance: 10 MΩ on insertion; UNA47F0U, Fred Herr, USA) were inserted into the tendon (cathode ~ 1cm distal to the marked MTJ and anode 2cm distal to the cathode). Placement of both electrodes into the tendon was confirmed by having the

participant make a gentle isometric plantarflexion contraction, which caused deflection of both needles toward the foot (i.e. imbedded tips were pulled toward knee). The waveforms have been aligned to inhibition detection thresholds (dashed horizontal line) for display purposes. Both techniques evoke inhibition at approximately 48ms post-stimulation, and the patterns of inhibition are similar; however, the duration of inhibition is shorter in this participant with indwelling stimulation.

Chapter 5: Study 4 - Effects of height-induced postural threat on sural nerve cutaneous reflexes and cortical potentials.

5.1 Introduction

When people are exposed to a threat to balance, such as standing at the edge of a cliff or elevated surface, they alter balance control behaviours; these changes include adjustments to frequency and amplitude of unperturbed postural sway (Carpenter et al, 2001a), larger myogenic responses to balance perturbations to restrict movement (Brown and Frank, 1997; Carpenter et al, 2004), and more "cautious" gait (Tersteeg et al, 2012). Recent evidence suggests postural threat influences balance-relevant muscle spindle somatosensory (Davis et al, 2011; Horslen et al, 2013) and vestibular function (Horslen et al, 2014; Naranjo et al, 2015, 2016; Lim et al, *in preparation*), which may contribute to behavioural changes seen with threat. To date, the effect of threat on balance-relevant cutaneous reflex function has yet to be explored.

Cutaneous information from the foot contributes to balance control by monitoring of pressure distribution under the feet in quiet standing (Duysens et al, 2000; Fujiwara et al, 2003; Maurer et al, 2001; Meyer et al, 2004b), contributes to scaling responses to balance perturbations (Do et al, 1990; Perry et al, 2000; Meyer et al, 2004a) and assists in detecting obstacles or trip hazards during gait (Forssberg, 1979; Zehr et al, 1997, 1998; Haridas et al, 2005, 2008). Cutaneous reflexes, evoked through peripheral nerve stimulation, are highly modifiable; they are phasically modulated over the gait cycle (Forssberg et al, 1975; Grillner and Rossignol, 1978; Yang and Stein, 1990; Duysens et al, 1990) and are sensitive to postural orientation and task (e.g. sitting or standing; Burke et al, 1991). There is also indirect evidence to suggest cutaneous reflexes are affected by postural threat. Cutaneous reflexes elicited by superficial peroneal, tibial

and sural nerve stimulation have been shown to be modulated during gait with threat of anterior-posterior perturbation (Haridas et al, 2005, Haridas 2006) or trip (Haridas et al, 2008). Threat of perturbation has been shown to have similar psychological and sympathetic consequences as height-induced postural threat (Horslen et al, 2013), and observations of changes to spinal stretch reflexes (Horslen et al, 2013) and vestibular-evoked balance responses (Horslen et al, 2014; Lim et al, *in preparation*) in both threat of perturbation and height-induced threat scenarios suggest general responses to threat, instead of task-specific modulation. However, because changes in cutaneous reflexes have only been explored in gait, and only with threats that would interfere with gait (e.g. tripping/stumbling), it is not clear if changes in cutaneous reflexes reflect a broad sensory tuning strategy to threat, or reflect a unique strategy to compensate for the specific risk of tripping/being perturbed during walking (Haridas et al, 2005; 2008).

A second outstanding question is whether or not cortical processing of cutaneous information is altered with postural threat. It has been hypothesized that increasing the gain of muscle spindle somatosensory sensitivity serves to increase the amount of afferent information available to supraspinal centres for monitoring balance control (Llewellyn et al, 1990; Sibley et al, 2007; McIlroy et al, 2003). Although evidence suggests both early and later cortical processing of visual stimuli may be influenced by trait anxiety and panic disorder (Taake et al, 2009; Thomas et al, 2013; Eldar et al, 2010; Pauli et al 2005; Bar-Haim et al, 2005), the effects of acute threat-related factors on cortical processing of somatosensory inputs is less clear. Davis et al (2011) observed no significant changes in early (N40-P50) somatosensory-evoked cortical potentials (SEPs) to muscle spindle Ia afferent stimulation (both electrical and mechanical) with height-induced threat. Davis et al (2011) argued SEPs might be gated because the afferent response to mechanical stimulation would be assumed to be increased due to changes in muscle

spindle sensitivity with threat (*cf.* Horslen et al, 2013; Chapter 3). However, early cortical processing of other somatosensory modalities, including cutaneous information, with threat has yet to be explored. In fact, later N1 perturbation-evoked cortical potentials elicited by unexpected balance disturbances (100-200 ms), which involve multiple balance-relevant somatosensory inputs (Quant et al, 2004), and are related to error detection of unpredictable stimuli (Adkin et al, 2008; Mochizuki et al, 2009), are significantly increased by threat (Adkin et al, 2008; Sibley et al, 2010). SEPs can be evoked with stimulation of cutaneous nerves (e.g. sural), and cutaneous SEPs are known to be affected by postural task (standing vs gait; Altenmüller et al, 1995; Duysens et al, 1995). While the amplitude of the initial N40-P50 component of the sural nerve SEP is similar in standing and during gait (Altenmüller et al, 1995), the amplitude of the subsequent P50-N80 component of the SEP is smaller (Altenmüller et al, 1995; Duysens et al, 1995), and the P110-N140 component is larger while walking than standing quietly (Altenmüller et al, 1995); suggesting cortical processing of cutaneous information is context dependent (Duysens et al, 1995). As such, it is possible that cortical processing of cutaneous afferent information from the lower-limb could be influenced by threat, particularly at later phases known to be sensitive to postural task.

The purposes of this study are to determine if lower-limb cutaneous reflexes and cortical SEPs evoked by stimulation of a lower-limb cutaneous nerve are modulated while people stand quietly under conditions of low and high height-induced postural threat. Reflexes and cortical potentials were evoked with electrical stimulation of the sural nerve at the ankle, which innervates the skin along the lateral border of the foot. It was hypothesized that sural nerve stimulation would evoke larger cutaneous reflexes in all lower-limb muscles tested when postural threat was high, compared to low. Furthermore, it was hypothesized that cortical

potentials would be facilitated by increased threat in both early and later cortical processing periods.

5.2 Methods

5.2.1 Participants

Fifty-one young healthy adults were recruited to participate in this study; all participants gave written informed consent, and the methods used were approved by the University of British Columbia Clinical Research Ethics Board and conformed to the Declaration of Helsinki. No participants reported having any known neurological, orthopedic, muscular or sensory disorder that could affect standing balance, nor did they report an extreme fear of heights. Nine participants were not included in the final analysis; 4 participants withdrew consent, 1 was excluded because no reflex was observed upon stimulation of the sural nerve, and 4 participants were excluded because of equipment malfunction. The remaining 42 participants were randomly divided (coin-flip then counterbalance to equal sample size) into one of two stimulation groups. Twenty-one participants received stimulation to the left sural nerve (Edge Group; 10 females, mean±SE age: 22.3±1.02 years, height: 173.9±1.87cm, weight: 69.5±2.40kg) and 21 received right-side sural nerve stimulation (Inside Group; 8 females, age: 24.2±0.95 years, height: 173.5±1.87cm, weight: 69.7±2.36kg).

5.2.2 Protocol

All participants stood with lateral border of their left foot aligned to the edge of a force plate (#K00407, Bertec, USA) and feet shoulder-width apart (Fig. 5-1A). Participants were aligned in this way because: a) unconstrained standing facing the edge leads to backward leaning and corresponding changes in tonic dorsi- and plantar flexor background muscle activity

(Carpenter et al, 2001a), which may influence cutaneous reflexes (Aniss et al, 1992) independent from threat; and b), it has recently been demonstrated that postural threat-induced changes to psychological state, sympathetic arousal and vestibular reflexes can be evoked with the participant oriented so that they stand with their side to the edge of an elevated lift (Naranjo et al, 2015). The force plate was situated at the edge of a hydraulic lift (M419-207B01H01D, Pentalift, Canada). The lift was positioned at its lowest level (0.8m) and a 0.6m wide level support surface extension was placed at the edge of the lift in the LOW threat condition. The surface extension was removed and the lift was elevated to 3.2m for the HIGH threat condition. The LOW condition was always presented first in order to capitalize on a known presentation order effect of height-induced postural threat (Adkin et al, 2000) and create maximum contrast between threat conditions. There were no barriers between the participant and lift edges, however the participants wore a safety harness secured to a safety line at all times and an experimenter was nearby to provide assistance should a fall occur; no participants fell or stepped away from the edge during the experiment. Participants sat in a chair positioned behind them with their feet in-place on the force plate when the lift was elevated from the LOW to the HIGH level.

5.2.3 Stimulation

The sural nerve was stimulated (V_{max}=300V; DS7A Constant Current Stimulator, Digitimer, UK) using a 2cm-wide bipolar bar electrode positioned along the nerve at the level of the lateral malleolus. The sural nerve was chosen because it is a pure cutaneous nerve, whereas the tibial nerve, which serves the foot sole, contains both cutaneous and muscular afferents. Hoffmann reflexes (muscle spindle type 1a reflex pathway) have been reported to be affected by postural threat (Llewellyn et al, 1990; Sibley et al, 2007); therefore, changes in tibial nerve

cutaneous reflexes might be confounded by changes in non-cutaneous reflex pathways. Half of the participants received stimulation to the sural nerve in the left leg (Edge Group) and half to the right leg (Inside Group); the purpose of the division between groups was to permit identification of side by threat interactions on changes in cutaneous reflex amplitudes, which may have occurred due to potential lateral weight shifts away from the platform edge which was always to the left of the subject. The sural nerve was stimulated with electrical pulse trains (5x 1ms square-wave pulses at 200Hz) with a stimulus intensity set to x2 radiating threshold (RT; Zehr et al, 1998; Haridas et al, 2005). RT was determined as the lowest stimulation intensity where the participant felt a distinct paresthesia radiating into the foot. Each trial (one per threat condition) consisted of 50 stimulus-pulse trains with a random 3-6s inter-stimulus interval.

5.2.4 Measures

Participants' balance confidence, perceived stability, fear of falling and anxiety were measured in both LOW and HIGH threat conditions to assess psychological state. Balance confidence was assessed while the participant was seated before each trial (0%: not at all confident they can maintain balance and avoid a fall; 100%: completely confident). The participant was seated after each trial and reported their experienced fear of falling (0%: not at all afraid of falling; 100%: completely afraid), perceived stability (0%: constantly felt a fall was imminent; 100%; felt completely anchored and at no time felt likely to fall), and anxiety (16-item summed 1-9 Likert scale; higher scores indicate more anxiety). These questionnaires have previously been used in height-induced postural threat studies (Horslen et al, 2013; 2014; Naranjo et al, 2015) and have been demonstrated to have moderate to high reliability in young adults (Hauck et al, 2008). Galvanic skin conductance was recorded (model 2501, CED, UK)

across the thenar and hypothenar eminences (right hand) to quantify electrodermal activity (EDA; sampled: 100Hz; Power 1401 with Spike2 software, CED, UK). EDA levels were averaged over each trial as an indicator of sympathetic arousal (Boucsein et al, 2012) and reported in μS.

Surface electromyography (EMG) was recorded (band passed online: 10-500Hz, digital-to-analog converted at 1500Hz; Telemyo 2400R G2 with 2400T G2, Noraxon, USA) bilaterally from the soleus (SOL), tibialis anterior (TA), medial gastrocnemius (MGas), vastus lateralis and biceps femoris (long head) muscles; in all cases electrodes (Kendall H59P Cloth Electrode, Medtronic, Ireland) were oriented along the presumed muscle fibre path, and spaced approximately 2cm apart (centre-to-centre). EMG data were A/D converted at 1000Hz (Power 1401 with Spike2, CED, UK). Reflex responses were only analysed in the stimulated leg (i.e. left leg for Edge Group, right leg for Inside Group) and, therefore, side is not further reported.

Electroencephalography (EEG) recordings were adapted from previous work in our lab (Davis et al, 2011); EEG was recorded (P511 AC Amplifier, Grass Medical Instruments, USA) from Cz referenced to FPz' (2 cm posterior to FPz, based on the international 10-20 system) and grounded to the right mastoid process (Fig. 5-1A). EEG data were amplified x10,000 and band-pass filtered online (1-1,000Hz) and A/D sampled at 5,000Hz (Power 1401 with Spike2, CED, UK). Recording sites were cleansed (Nuprep gel, Weaver and Company, USA) and impedance between all electrodes, measured before the LOW and after the HIGH trials, was kept below 10kΩ (EZM11 Electrode Impedance Meter, Grass Medical Instruments, USA).

5.2.5 Analyses

EMG data were rectified offline and trigger averaged to the beginning of the stimulus pulse trains within each condition (Spike2, CED, UK). Background muscle activity (BGA) was measured from the trigger averages, and taken as mean rectified EMG over a 75ms period ending 10ms before stimulus onset. Muscle-specific onset and end of the reflex was determined separately for each condition (Fig. 5-1B). Medium-latency, or P2, cutaneous reflexes occurring 75-170ms post-stimulation are reliably observed with sural nerve stimulation (Baken et al, 2005) and were examined in this study to be consistent with other postural threat studies (Haridas et al, 2005, 2008). Reflex onset was determined on a muscle-specific, trial-by-trial basis using a custom detection algorithm (Spike2, CED, UK) as the point where trigger-averaged muscle activity was outside a mean ± 2SD threshold for at least 7ms in a sliding 10ms window, and similar criteria were used to detect the end of the response; these values were confirmed with visual inspection. Reflex amplitudes were calculated as the integrated area between detected onset and end of the response; area was referenced to background muscle activity by subtraction of the mean background multiplied by the duration of the reflex. Note: inhibition (negative sign) responses were not rectified; as such, a positive change between conditions could indicate either a larger magnitude positive (excitation) area or a smaller magnitude negative area. If a reflex was detected in one condition but not the other, then muscle activity was measured in the region where a reflex was detected in the other condition. If no reflex was detected in either condition then that participant was excluded from the analysis for that muscle; as such, sample sizes differ between muscles.

Since changes in background muscle activity may affect reflex amplitude (Aniss et al, 1992), two reflex analyses were performed in this experiment. In the first analysis (‘Full

sample'), all participants who had reflexes were analysed for each muscle. In the second analysis ('Screened sample'), individual participants were excluded if their background activity levels changed by more than 25% from the LOW to HIGH threat conditions. Thus, the Full sample reflects the global effect of threat on cutaneous reflexes, whereas the Screened sample reflects threat-specific scaling of reflexes, independent from background activity.

EEG data were screened for blink artefacts by visual inspection; pulse trains with a blink artefact occurring between 100ms pre and 500ms post-stimulus onset were excluded from analysis. Blink-removed EEG data were band-pass filtered (1-100Hz; 4^{th} order Butterworth; Matlab 2012a; MathWorks, USA), trigger-averaged (Spike2, CED, UK) to stimulus onset to create an SEP for each threat condition. N40, P50, N80, P110 and N140 peaks were identified for each participant in accordance with Altenmüller et al (1995). Peak-to-peak amplitudes were measured between N40 - P50 (per Davis et al, 2011), P50 - N80 (per Altenmüller et al, 1995; Duysens et al, 1995; Mouchnino et al, 2015), and between P110 - N140 (per Altenmüller et al, 1995) for each participant and threat condition.

5.2.6 Statistical analyses

Cutaneous reflex amplitudes and BGA were assessed independently with the Full sample and Screened sample datasets using two (stimulation group: Edge, Inside) by two (threat condition: LOW, HIGH) mixed model ANOVAs for TA, Sol and MGas muscles. Vastus lateralis and biceps femoris were not statistically analyzed as there were too few subjects with responses that surpassed screening for background muscle activity (n=8 (1+7, Inside+Edge) for vastus lateralis; n=9 (4+5) for biceps femoris). Paired-samples t-tests between LOW and HIGH threat conditions for each group were used to explore significant effects. Comparisons between groups

(main effects) were not part of the research question, and thus data normalization to permit valid inter-participant comparisons were not performed. Relationships between changes in reflex amplitudes and BGA across threat conditions were explored post-hoc to characterize relationships between cutaneous reflexes and BGA. Changes in SOL, MGas and TA reflex amplitudes and BGA across height conditions were not normally distributed (per Kolmogorov-Smirnov test); therefore, Spearman's rho correlation coefficients were calculated between the rank scores of the change values (SPSS, IBM, USA). For all statistical tests, alpha was set at 0.05.

5.3 Results

Height-induced postural threat caused significant changes in participants' psychological and autonomic states. As shown in Table 5-1, participants in both groups were significantly less confident, felt less stable, were more fearful of falling, and felt more anxious in the HIGH, compared to LOW condition. Likewise, EDA was significantly higher in the HIGH, compared to LOW conditions (Table 5-1), suggesting greater sympathetic arousal. There were no significant group by threat interaction effects on any psychological or EDA measures (Table 5-1).

When all data, regardless of changes in BGA (Full sample), are considered, standing in the HIGH threat condition lead to significantly larger cutaneous reflexes in SOL and TA. There was a significant main effect of height on both SOL (Table 5-1; Fig. 5-2A, 5-3A) and TA reflex amplitudes (Table 5-1; Fig. 5-2C, 5-3C); there were no significant group by threat interaction effects on SOL or TA reflex amplitudes. While there was no significant main effect of threat on MGas reflex amplitude (Table 5-1; Fig. 5-2B, 5-3B), there was a trend to a group by threat interaction on MGas reflex amplitude (Table 5-1), where MGas reflexes trended to being larger in the HIGH, compared to LOW threat condition for the inside leg group (t=-1.847, p=0.080,

η^2=0.152), but not for the edge leg group (t=1.000, p=0.331, η^2=0.056). However, there were significant increases SOL and TA background activity levels in the HIGH, compared to LOW threat condition (Table 5-1; Fig. 5-3A,C) when all participants were included in the sample. There was also a trend to a group by threat interaction on MGas background activity (Table 5-1), where MGas background activity trended to being larger in the HIGH threat condition for the inside group (t=-2.057, p=0.054, η^2=0.182), but not for the edge group (t=0.482, p=0.636, η^2=0.013). In the Full sample, changes in reflex amplitudes across threat conditions significantly correlated with changes in background muscle activity for SOL and TA (SOL: Spearman's rho = 0.496, p = 0.001, Fig. 5-3A; TA: Spearman's rho = 0.707, p < 0.001, Fig. 5-3C); but not for MGas (Spearman's rho = 0.136, p = 0.422, Fig. 5-3B).

As intended, statistical changes in BGA for all muscles (Table 5-1, Fig 5-3) disappeared when the sample was screened to include only those participants whose BGA changed less than 25% between threat conditions. As demonstrated by the representative traces in Figures 5-2D, E and F, the significant threat effects on reflex amplitudes observed in the Full sample were not observed in the Screened sample (Table 5-1; Fig. 5-3A,B,C).

The sural nerve stimuli evoked cortical SEPs with peaks consistent with those reported by Altenmüller et al (1995). Figures 5-4A and 5-4B show data from a representative participant and the average response from the Full sample, respectively. The sural nerve electrical stimulation-evoked SEP contained a negative-going peak (N40) observed at 38.59±5.79ms in the LOW condition and 38.25±4.72ms in the HIGH condition, followed by a positive peak (P50) at 51.19±6.19ms in the LOW condition and 50.96±5.65ms in the HIGH condition. Subsequent peaks were observed at N80 (LOW: 85.51±8.05ms; HIGH: 83.73±8.56ms), P110 (LOW: 107.97±13.24ms; HIGH: 105.46±15.06ms), and at N140 (LOW: 121.41±21.79ms; HIGH:

125.20±18.20ms). There were no significant interactions or main effects of threat on N40-P50 peak-to-peak amplitudes (Table 5-1). However, there was a significant decrease in P50-N80 peak-to-peak, and a trend to an increase in P110-N140 peak-to-peak amplitudes in the HIGH, compared to LOW threat condition (Table 5-1).

5.4 Discussion

The purposes of this study were to determine if height-induced postural threat affects lower-limb cutaneous reflexes and cortical SEPs during stance. Cutaneous reflexes elicited by sural nerve stimulation were observed in a medium-latency window (75-170 ms) in SOL, MGas and TA in both LOW and HIGH threat conditions. These reflexes were comparable in shape and timing to cutaneous reflexes reported previously during human stance (Burke et al, 1991; Aniss et al, 1992; Komiyama et al, 2000), certain phases of walking (Duysens et al, 1990; Zehr et al, 1998; Baken et al, 2005, 2006), sitting (Burke et al, 1991) and lying supine (Aniss et al, 1992). In the Full sample, where all responses were included irrespective of changes in BGA, cutaneous reflex amplitudes were significantly larger in SOL and TA in the HIGH compared to LOW threat condition, and trended toward being larger in the MGas for the Inside group. These results appear to support our hypothesis that cutaneous reflexes would be larger when participants stood in the HIGH, compared to LOW threat condition. However, consistent with previous work (Aniss et al, 1992; Komiyama et al, 2000), the observed changes in reflex amplitudes across threat conditions were found to be significantly correlated with changes in BGA. After controlling for the confounding effect of BGA activity, no significant threat-related changes in cutaneous reflex amplitudes were observed. Therefore, these results indicate the observed changes in cutaneous reflex modulation with threat are likely due to changes in general motor neuron pool excitation levels.

The current observations of BGA-dependent changes in lower-limb sural nerve cutaneous reflexes with threat may explain differences in findings with prior studies, and provides a new lens through which prior evidence should be viewed. Haridas et al (2005; 2008) observed significant increases in cutaneous reflexes elicited by tibial and/or superficial peroneal nerve stimulation while walking with the threat of a perturbation or trip. Haridas et al (2005; 2008) argued these effects were unrelated to changes in BGA, as statistically significant changes in reflexes did not always coincide with significant changes in background activity in the same muscle or phase of gait. However, to our knowledge, no attempt has been made to specifically control, or account, for the potentially confounding effects of changes in BGA which are known to occur while walking under threatening conditions (Brown et al, 2002). Therefore, it is not yet clear if threat-related changes in cutaneous reflexes occur independent from changes in motor neuron pool excitability that are not directly related to cutaneous afferent inputs (e.g. Johnson and Heckman, 2010; Koutsikou et al, 2014).

Alternatively, the discrepancies between the current result and prior studies by Haridas et al (2005, 2008) might be related to methodological differences in terms of the sources of postural threat (height vs. perturbation), the nerves stimulated (sural vs. tibial or superficial peroneal), and postural tasks (quiet standing vs gait). Based on preliminary evidence by Zaback et al (2016a), the differential effects of threat observed on cutaneous reflexes are not likely to be dependent on the source of threat or nerves stimulated; reflexes elicited in SOL and biceps femoris with sural nerve stimulation were found to be larger when participants view highly arousing pictures during gait. On the other hand, the extent to which gait may be an important determinant in threat-induced modulation of cutaneous reflexes has yet to be determined. Electrical stimulation of the nerves serving the foot while walking is thought to evoke components of a stumbling-corrective

response directed to clear an obstacle or compensate for uneven surfaces contacted by the area of the foot served by the nerve stimulated (Zehr et al, 1997, 1998). Postural engagement is a determinant of whether or not cutaneous reflexes appear in a given muscle while standing (Aniss et al, 1992), and threat-induced modulation of cutaneous reflexes during gait seems to depend on the ability of the target muscle to contribute to maintenance of balance, given the specific threat (Haridas et al, 2005, 2008). Taken together, these observations imply the relevance of the reflex to the balance task may be an important determinant for modulation. Therefore, future studies should examine the effects of threat on cutaneous reflexes during stance under more dynamic conditions when the reflexes may be more germane to maintaining balance.

The second purpose of this study was to determine the effects of height-induced postural threat on sural nerve cortical-evoked potentials. Consistent with previous observations (Altenmüller et al, 1995; Duysens et al, 1995), sural nerve stimulation triggered SEPs at Cz with alternating negative and positive peaks at N40, P50, N80, P110 and N140. The P50 SEP component to tactile stimulation is thought to arise from the primary somatosensory cortex (Hämäläinen et al, 1990), and the N40-P50 peak-to-peak amplitude likely reflects the earliest cortical processing of the cutaneous afferent information. Consistent with our hypothesis, and previous observations using electrical stimulation of the tibial nerve at the popliteal fossa (Davis et al, 2011), peak-to-peak amplitude of the earliest (N40-P50 SEP) response to sural nerve stimulation was not affected by height-induced postural threat. As such, we conclude that cutaneous afferent information is neither muted nor amplified en route to the cortex with postural threat. In contrast to the current cutaneous data, there is indirect evidence to suggest muscle spindle Ia afferent inputs to the cortex may be gated, likely at sub-cortical levels, in a postural threat scenario (Davis et al, 2011). Achilles tendon-tap reflexes are facilitated with threat (Davis

et al, 2011; Horslen et al, 2013; Chapter 3), possibly due to increased muscle spindle sensitivity (*cf.* Horslen et al, 2013), yet tendon-tap evoked SEPs are not altered (Davis et al, 2011). Since afferent volleys from the peripheral receptor are increased and cortical potentials from both tendon-taps and tibial nerve electrical stimulation are unchanged, Ia afferent feedback to the somatosensory cortex may be gated at some point en route to the cortex to account for the lack of change in tendon-tap cortical potentials (Davis et al, 2011).

This study is novel in that it is the first to show modulation of later P50-N80 and P110-N140 sural nerve cortical potentials with increased postural threat. The N80 SEP component to tactile stimulation is also thought to arise from primary somatosensory cortices, although tends to have a bilateral distribution (at least with fingertip stimulation; Hämäläinen et al, 1990). The P50-N80 component, which is thought to be specific to cutaneous cortical input (Mouchnino et al, 2015), was significantly smaller under high compared to low threat conditions. These results are consistent with observations of decreased peak-to-peak amplitudes of the P50-N80 component to fingertip tactile stimulation when conditioned with unpleasant affective pictures (Montoya and Sitges, 2006). Foot tactile and sural nerve-evoked P50-N80 component amplitudes are also decreased with changes in postural task (Altenmüller et al, 1995; Duysens et al, 1995; Mouchnino et al, 2015) and the early negative-polarity (70-110ms) to non-noxious sural nerve stimulation is reduced when people attend to relevant compared to irrelevant stimulus parameters (Dowman, 2007). Therefore, changes in P50-N80 SEP amplitudes in the present study could reflect changes in affect as well as changes in the level of postural engagement or attention to the stimulus with increased threat.

The P110 and N140 SEP components to tactile stimulation are thought to arise from secondary somatosensory, and posterior parietal networks, respectively (Hämäläinen et al, 1990).

The trend for increased amplitude of P110-N140 sural nerve SEPs with threat is similar to previous observations of threat-induced changes in N1 (occurring 100-200ms post-stimulus) cortical potentials elicited by unexpected whole-body balance perturbations (Adkin et al, 2008; Sibley et al, 2010). N1 perturbation-evoked potentials are thought to reflect cortical processing of somatosensory signals related to the whole-body perturbation (Quant et al, 2004), are sensitive to stimulus predictability (Adkin et al 2006, 2008; Mochizuki et al, 2009), and are thought to be related to error detection (Adkin et al, 2008). Similar changes in the 100-200ms post-stimulus latency have been observed with visual event-related potentials (ERPs) as a function of stimulus unpleasantness (Olofsson et al, 2008) and arousal (Rozenkrants et al, 2008). Likewise, later cortical periods (>200 ms) are also increased when people are shown highly arousing pictures (Cuthbert et al, 2000; Rosenkrants et al, 2008) and threatening words (Thomas et al, 2007).

Threat influences cognitive factors such as conscious motor processing (Huffman et al, 2009, Zaback et al 2015) and worrisome-thinking during stance (Zaback et al, 2016b) and increases the perception of whole-body sway during quiet stance (Cleworth and Carpenter, 2016). Although there is strong evidence of cortical influences on posture and gait (Schupert et al, 1997; Taube et al, 2006; Petersen et al, 2009; Tokuno et al, 2009;), it is currently unknown if threat-related changes in cortical processing observed in the current and prior studies (Adkin et al, 2008; Sibley et al, 2010) may be associated with the wide-range of threat-induced changes in static and dynamic balance (Carpenter et al, 1999b, 2001; Brown and Frank, 1997; Carpenter et al, 2004; Cleworth et al, 2016) and gait (Gage et al, 2003; McKenzie and Brown, 2004; Brown et al, 2006; Tersteeg et al, 2012). Further research should explore if and how cortical functions, as well as which specific cortical regions, contribute to the altered balance behaviours with threat.

Likewise, the mechanisms through which perceptual gains are changed, and how perceptual changes contribute to altered behaviours remain to be explored.

In conclusion, reflexive responses to cutaneous stimuli were increased when standing under conditions of height-induced threat only when natural changes in BGA were not accounted for. These data suggest changes in cutaneous reflexes are not specifically related to altered central processing of cutaneous inputs; rather, changes in reflexes generally occurred in people who increased BGA when exposed to the threat. These data also reveal that cortical processing of cutaneous afferent information is altered with height-induced postural threat. Changes to sural nerve SEPs occurred at a latency similar to those seen with unexpected balance perturbations, and paralleled those seen in walking, compared to standing still. As such, these data further support the notion of altered cortical processing of balance-relevant sensory information with increased postural threat.

Table 5-1 – Summary of statistical tests and results.

				Group x Height Interaction			Main Effect of Height			LOW	HIGH
	Variable	Sample Size (Inside, Edge)	df	F	*p*	η_p^2	F	*p*	η_p^2	mean (SE)	mean (SE)
	EDA (µS)	40 (19,21)	1,38	0.63	0.432	0.016	**6.164**	**0.018**	**0.14**	22.357 (1.756)	25.693 (1.923)
	Balance Confidence (%)	42 (21,21)	1,40	0.036	0.85	<0.001	**51.276**	**<0.001**	**0.562**	95.952 (1.112)	78.095 (2.696)
	Fear of Falling (%)	42 (21,21)	1,40	0.03	0.954	<0.001	**43.91**	**<0.001**	**0.523**	1.905 (0.786)	28.81 (4.04)
	Anxiety (sum /144)	42 (21,21)	1,40	0.145	0.705	0.004	**22.405**	**<0.001**	**0.359**	27.143 (1.082)	40.452 (2.986)
	Perceived Stability (%)	42 (21,21)	1,40	1.624	0.21	0.039	**47.363**	**<0.001**	**0.542**	92.262 (1.36)	72.976 (2.908)
All Participants	Soleus Reflex (µV*ms)	41 (21,20)	1,39	1.463	0.234	0.036	**5.387**	**0.026**	**0.121**	139.09 (17.42)	157.89 (17.69)
	Soleus BGA (µV)	41 (21,20)	1,39	2.537	0.119	0.061	**11.058**	**0.002**	**0.221**	8.31 (0.93)	9.54 (0.95)
	MGas Reflex (µV*ms)	38 (20,18)	1,36	***3.521***	***0.069***	***0.091***	0.285	0.597	0.008	69.21 (13.34)	72.92 (11.56)
	MGas BGA (µV)	38 (20,18)	1,36	***2.991***	***0.093***	***0.079***	1.357	0.252	0.037	4.63 (0.59)	4.98 (0.60)
	TA Reflex (µV*ms)	23 (9,14)	1,21	0.923	0.348	0.042	**8.185**	**0.009**	**0.280**	11.04 (2.55)	16.42 (2.87)
	TA BGA (µV)	23 (9,14)	1,22	0.535	0.473	0.025	**4.89**	**0.038**	**0.189**	1.38 (0.062)	1.62 (0.12)
Screen for BGA	Soleus Reflex (µV*ms)	27 (14,13)	1,25	0.744	0.397	0.029	0.016	0.9	0.001	177.30 (20.21)	176.48 (22.76)
	Soleus BGA (µV)	27 (14,13)	1,25	0.427	0.52	0.017	0.96	0.337	0.037	9.98 (1.21)	10.22 (1.34)
	MGas Reflex (µV*ms)	18 (8,10)	1,16	0.557	0.466	0.034	0.426	0.523	0.026	52.79 (12.46)	56.71 (9.68)
	MGas BGA (µV)	18 (8,10)	1,16	0.337	0.569	0.021	0.887	0.36	0.053	4.35 (0.73)	4.18 (0.65)
	TA Reflex (µV*ms)	16 (8,8)	1,14	1.979	0.181	0.124	2.071	0.172	0.129	8.95 (2.25)	12.49 (2.71)
	TA BGA (µV)	16 (8,8)	1,14	2.476	0.138	0.15	1.146	0.302	0.076	1.43 (0.075)	1.47 (0.073)
SEP	N40-P50 (µV)	40 (21,19)	1,38	0.1	0.754	0.003	0.106	0.747	0.003	4.748 (0.644)	4.857 (0.683)
	P50-N80 (µV)	40 (21,19)	1,38	0.238	0.628	0.006	**4.318**	**0.045**	**0.102**	19.408 (2.250)	18.288 (2.174)
	P110-N140 (µV)	40 (21,19)	1,38	1.374	0.248	0.035	***3.181***	***0.082***	***0.077***	2.308 (1.901)	4.548 (1.533)

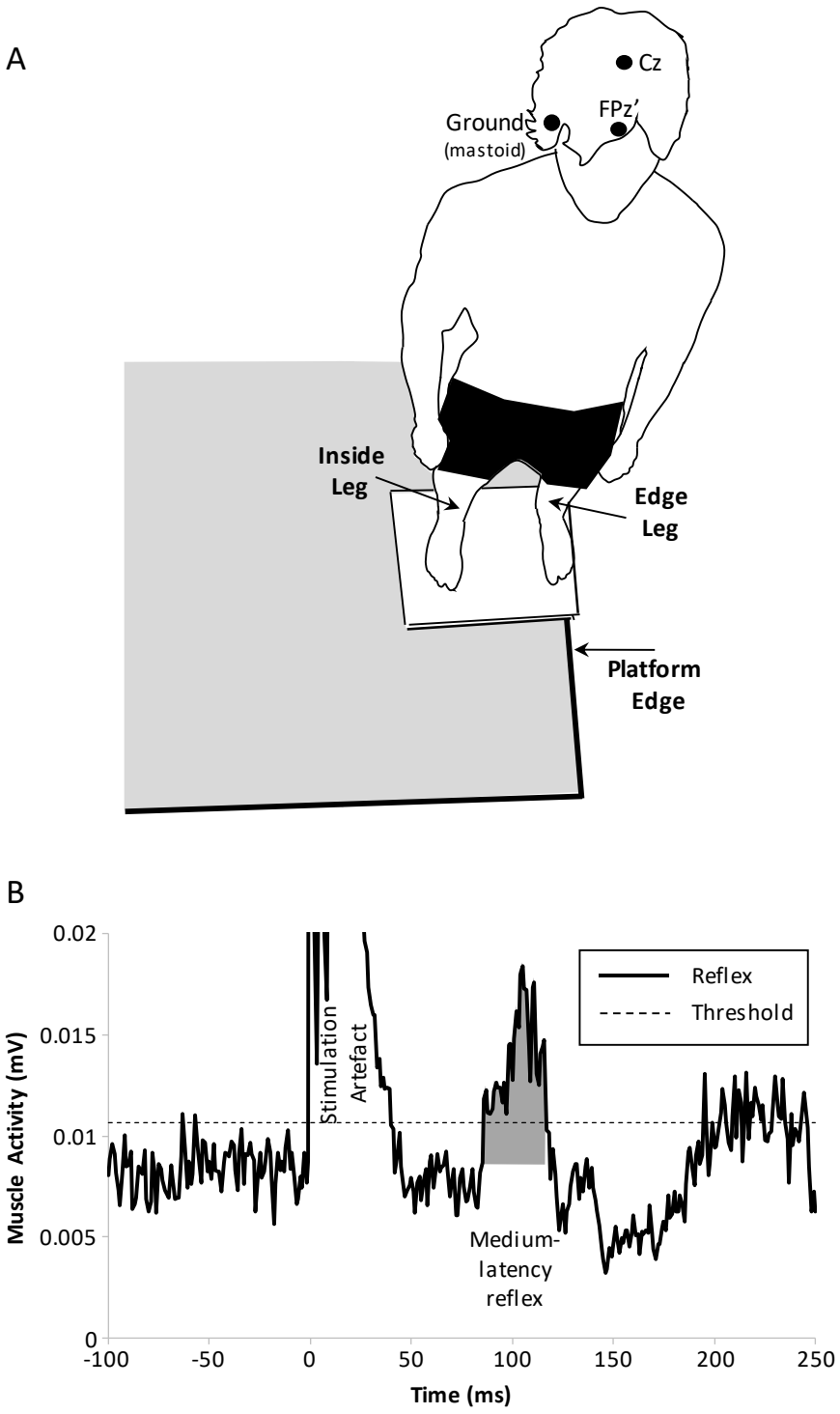

Figure 5-1 – Participant standing orientation and cutaneous Reflex Analysis

A schematic of participant standing orientation on the lift with EEG recording sites marked is shown in Figure 5-1A. Participants were always oriented with their left side to the edge; the

Edge group only received stimuli to their left leg, and the Inside group only received stimuli to their right leg. Figure 5-1B demonstrates a triggered-average of rectified SOL EMG aligned in time to onset of the sural nerve electrical stimulus train (time 0) for a representative participant; 50 stimulus pulse trains were used to construct the waveform average. A mean + 2SD threshold (dashed line) was calculated from a 75ms pre-stimulus period and used to determine the beginning and end of the medium-latency reflex. Reflex amplitude was calculated as the integrated area, referenced to mean background muscle activity, over the detected medium-latency reflex period (shaded in gray).

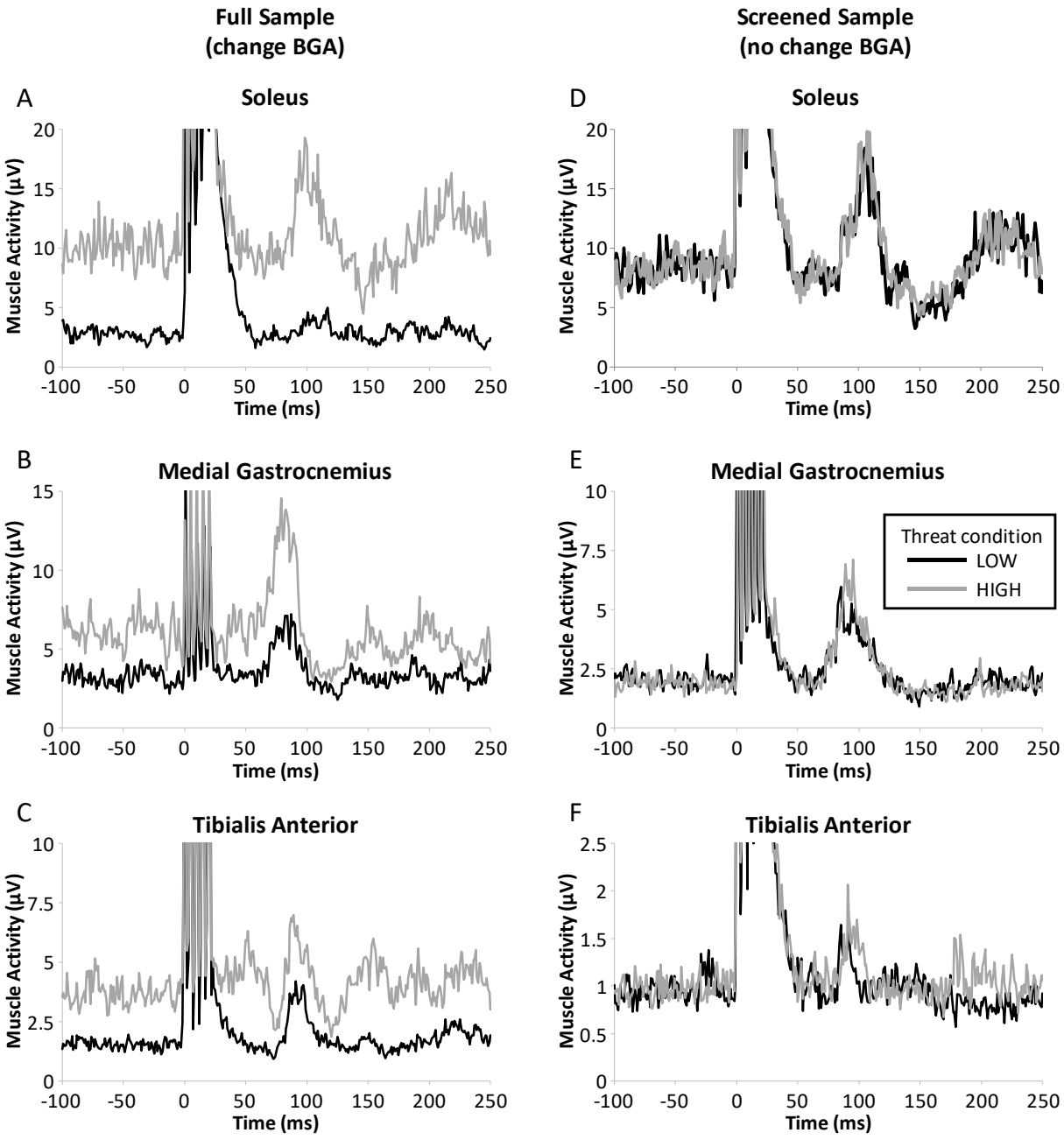

Figure 5-2 – Representative LOW and HIGH threat cutaneous reflexes

Cutaneous reflexes from 6 representative participants are shown to demonstrate the effects of LOW (black lines) and HIGH (gray lines) threat on SOL (A, D), MGas (B, E) and TA (C, F) reflexes. Traces in the left column (A, B, C) represent participants who had significant increases in BGA and were subsequently excluded from the Screened sample. Traces in the right column

(D, E, F) are from participants who did not demonstrate changes in BGA >25%, and were therefore included in both the Full and Screened samples.

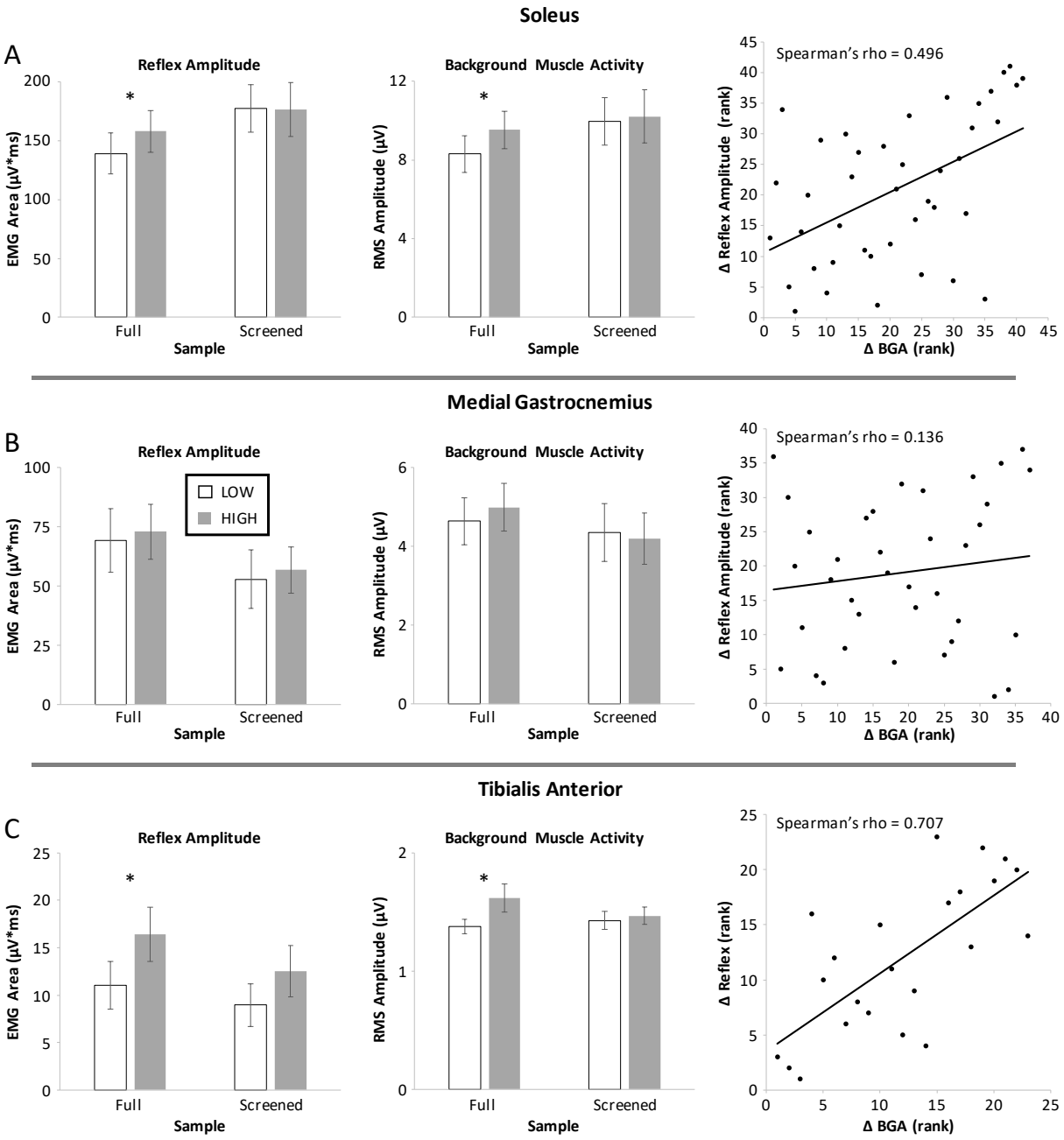

Figure 5-3 – Main effects of threat on cutaneous reflexes and BGA, and correlations between changes in reflex amplitudes and BGA

Main effects of threat on SOL (A), MGas (B) and TA (C) cutaneous reflex amplitudes and background muscle activity both for the Full sample, and after screening for changes in BGA (Screened sample). Also shown are changes in reflex amplitudes plotted against changes in BGA

for the Full sample for each muscle; note: change data were ranked to normalize the distribution of the data. Reflex amplitude and BGA values reflect the group-wide mean and error bars demonstrate SE; * indicates statistical significance at the $p<0.05$ level.

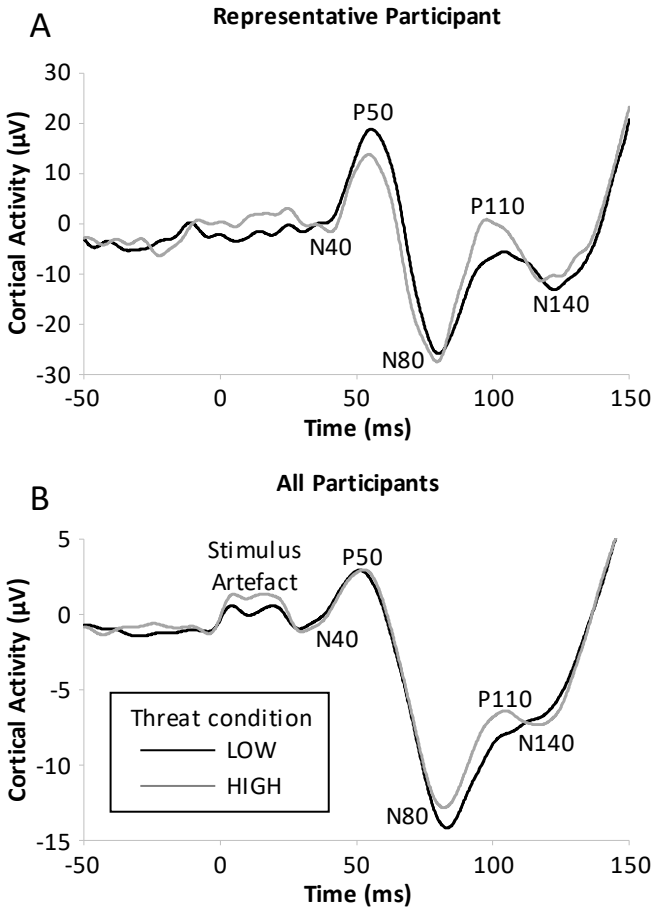

Figure 5-4 – Sural nerve cortical SEPs

Figure 5-4 shows the mean LOW (black) and HIGH (gray) SEPs for a single representative participant (A) and the average across all participants (B). Positive and negative peaks of interest are labelled on both figures.

Chapter 6: Conclusions and general discussion

6.1 Summary of thesis goals and results

6.1.1 Background and aims

It has long been understood that fear of falling leads to altered balance behaviours (Maki et al, 1991; Okada et al, 2001) and increased likelihood of falling (Li et al, 2003). Brown and Frank (1997) were interested in studying fear of falling in a young-healthy adult model and found kinematic characteristics of balance-correcting responses were altered when people stood at surface heights just above the level they could successfully step down from. Subsequent studies revealed that young-healthy adults standing at the edge of an elevated platform adopted stiffer control of COP (Carpenter et al, 1999b; Brown et al, 2006; Davis et al, 2009; Huffman et al, 2009; Cleworth et al, 2012) and COM displacements (Carpenter et al, 2001a) during static, unperturbed balance, and increased balance-correcting myogenic responses to imposed balance perturbations (Carpenter et al, 2004; Cleworth et al, 2016). Height-induced threat is also known to alter walking patterns (Brown et al, 2002; McKenzie and Brown, 2004; Tersteeg et al, 2012) and anticipatory postural adjustments preceding voluntary movements of the body (Adkin et al, 2002; McKenzie and Brown, 2004; Gendre et al, 2016; Zaback et al, 2015). The mechanisms underlying these behavioural changes remain unknown. However, some researchers have hypothesized that changes to balance-relevant sensory function might contribute to altered balance behaviours with threat (Carpenter et al 1999b, 2004; Sibley et al, 2007; Davis et al, 2009, 2011; Tersteeg et al, 2012; Osler et al, 2013; Horslen et al, 2013, 2014; Naranjo et al, 2015, 2016).

Therefore, the purpose of this dissertation was to explore how four senses involved in balance control are affected by threats to standing balance. All studies in this thesis shared the common approach of examining how individual balance-relevant reflexes were affected by a height-induced postural threat. Healthy young adult participants were asked to stand quietly at the edge of a platform elevated to different heights to manipulate threat and state-specific changes in fear, anxiety, confidence and physiological arousal. This consistent approach was employed so that ultimately the independent studies might be compared and amalgamated to reveal a general sensory adaptation response. The subsequent sections will re-cap the principle results and conclusions from each chapter, and incorporate the findings across studies to gain insight into the broader effect of postural threat on balance-relevant sensory function. How these changes might be achieved, how they might be incorporated into the balance control system, clinical implications, and suggestions for future research will also be explored.

6.1.2 Effects of postural threat on vestibular-evoked balance responses

The purpose of the original research presented in chapter 2 was to determine if height-induced postural threat affects the direct relationship between vestibular signals and balance reflexes. Stochastic electrical vestibular stimulation (SVS) was used to stimulate the vestibular system to evoke balance responses, which were recorded as ground reaction forces. Coupling, or correlation, between vestibular input and ground reaction force output was quantified with coherence and cumulant density in the frequency and time domains, respectively; and signal gain was used to assess the scaling of the input-output relationship across heights. Two experiments were conducted to assess changes in vestibular-evoked balance responses in the ML and AP directions, separately. The SLP and MLP components of the time-domain cumulant density

function, amplitude and range of coherence, and magnitude of the signal gain were all increased in the HIGH, compared to LOW threat condition in both experiments. These results suggest that vestibular-evoked balance responses are amplified when people are exposed to a threat to standing balance, possibly through excitation of the vestibular nuclei from fear and/or anxiety CNS processing centres.

The findings of the original research presented in chapter 2 were in conflict with a previous report from Osler et al (2013), and provoked a written debate which is presented in part in the concluding sections of Chapter 2. In contrast to current findings, Osler et al (2013) failed to demonstrate changes in direct (which they called "feedforward") SLP or MLP responses to square-wave GVS when people stood on a high, narrow beam. The crux of the debate was whether or not the vestibular system is affected by threat (or fear of falling), as well as whether or not changes at short latencies, or in higher frequency bands, are relevant and of functional significance to control of standing balance (*cf.* Carriot et al, 2014). Since publication of the original research in Chapter 2, new findings demonstrate that vestibular adaptation is not limited to height-induced threats, as changes in coupling and gain between SVS and EMG from muscles crossing the ankle, knee and hip are modulated in response to a threat of balance perturbation (Lim et al, in preparation). Likewise, vestibular-evoked myogenic potentials (VEMPs) in SOL, sternocleidomastoid, and the inferior oblique ocular muscle are all amplified when people stand at the edge of a 3.2m high platform (Naranjo et al, 2015; 2016). Combined, these studies suggest threat-induced changes in vestibular-evoked reflexes are large enough to be evident in single muscles (Naranjo et al, 2015; 2016; Lim et al, in preparation), and that the adaptations appear with other forms of postural threat where surface height is not manipulated (Lim et al, in preparation), suggesting a general response strategy to threat. Finally, both Naranjo et al (2015;

2016) and Lim et al (in preparation) found vestibular reflexes correlated with changes in anxiety, fear, and arousal (EDA), suggesting the sensory adaptation is related to, and not just concurrent with, participants' experience of the threat.

6.1.3 Effects of postural threat on short- and medium-latency stretch reflexes

The purpose of the study presented in Chapter 3 was to determine how velocity-dependent SLR and amplitude-dependent MLR responses in SOL ramp-and-hold stretch reflexes were affected by height-induced postural threat. SOL was stretched with dorsiflexion ramps of differing velocities and amplitudes while participants stood in LOW and HIGH threat conditions; SOL tendon-tap (t-) reflexes were also evoked to enable comparison with previous studies (Davis et al, 2011; Horslen et al, 2013). Mean SLR and MLR amplitudes across stretch velocities or amplitudes, respectively were compared across threat conditions, and amplitudes of the SLR responses were related to stretch velocities to investigate dynamic scaling of the reflex. Unfortunately, technical limitations precluded examination of MLR scaling to stretch amplitude. T-reflex, SLR and MLR amplitudes were found to be increased by 16.9%, 11%, and 9.5%, respectively, when people were exposed to the HIGH threat condition. Changes in t-reflex and SLR amplitudes across threat conditions were not related to changes in background muscle activity (SOL or TA), and changes in MLR amplitude were only weakly correlated to changes in SOL background activity. Furthermore, dynamic scaling, as indicated by the slope of the SLR-velocity relationship, was increased 34.5% in the HIGH, compared to LOW threat condition. These data are interpreted as indirect evidence in support of an increase in muscle spindle dynamic sensitivity when balance is threatened.

6.1.4 Changes to Ib inhibition with postural orientation and postural threat

The study presented in Chapter 4 aimed to understand how Ib reflexes are affected by height-induced postural threat. Electrical stimulation of the Achilles tendon (TStim) was chosen as a method of probing Ib reflexes. Since this method had not yet been used to examine Ib reflexes in standing, the study also aimed to determine the effects of postural orientation on TStim-evoked inhibition. As revealed in Chapter 4-Experiment 1, TStim to the Achilles tendon evoked short-latency (~44ms) inhibition of surface EMG in MGas in both prone and standing orientations. However, area and duration of inhibition were smaller in standing, compared to lying prone. The changes to TStim inhibition with standing were consistent with previous observations of changes in Ib reflexes with changes in postural orientation or task (Stephens and Yang, 1999; Faist et al, 2006). The results from Chapter 4-Experiment 1 revealed that the TStim method can be used to evoke inhibition in people who stand upright, and that TStim inhibition is likely achieved through Ib reflex circuits.

Given these observations, it was possible to use TStim to investigate the effects of postural threat on Ib lower-limb reflexes. Chapter 4-Experiment 2 revealed reduced area and duration of 1b inhibition when participants were exposed to the HIGH threat condition, even after screening for changes in background muscle activity across threat conditions. This experiment provides novel evidence for context-dependent modulation of Ib reflexes in humans, which may contribute to changes in tonic plantar flexor activity, or to scaling of dynamic postural responses with threats to standing balance.

6.1.5 Threat effects on cutaneous reflexes and cortical-evoked potentials

The study presented in Chapter 5 was designed to address two purposes relating to the effects of height-induced postural threat on processing of cutaneous afferent information from the foot. The first purpose was to determine how cutaneous reflexes in ankle dorsi- and plantar flexor muscles are affected by threat during quiet standing, and the second purpose was to determine how cortical potentials evoked by cutaneous nerve stimulation are modulated with threat. SOL and TA reflexes were larger in the HIGH, compared to LOW threat condition for the Full sample, but changes in reflexes were correlated with changes in BGA across threat conditions. The changes in reflexes disappeared when individual participants were removed based on changes in BGA. As such, while reflexes may be larger at height, the changes in reflex amplitude do not appear to reflect specific modulation of cutaneous reflex circuits, but rather general changes in motor neuron pool excitability. The earliest cutaneous cortical potentials were not altered with threat, suggesting there is no change in the sensitivity of the cortex to afferent inputs (*cf.* Davis et al, 2011). However, later potentials, which may reflect primary and/or secondary somatosensory, as well as posterior parietal processing of cutaneous afferent information, are affected by threat. These data suggest integrative cortical processing of balance-relevant sensory information may be altered with threats to standing balance (see also: Adkin et al, 2008; Sibley et al, 2010).

6.2 Evidence for multi-sensory modulation with threat

In-depth analyses of how threats to standing balance affect fear of falling, anxiety and confidence, as well as the scope of autonomic reactive responses to threat, are beyond the scope of this thesis. The reader is referred to other works (e.g. Huffman et al, 2009; Zaback et al, 2015, 2016) for detailed discussion of the psychological consequences of standing at the edge of an

elevated platform. However, changes in participants' assessments of their psychological state, as well as changes in arousal reflected in EDA, can be interpreted as metrics describing the magnitude of the threat manipulation, or psychological perturbation, experienced by the participants in each study. Participants in each study had significant decreases in balance confidence (effect size range: 0.538-0.691), decreases in perceived stability (0.542-0.728), increases in fear of falling (0.523-0.701) and increases in anxiety (0.359-0.699). Likewise, participants were more aroused in the HIGH, compared to LOW threat condition in all studies (Δ EDA range: 3.4-15.9μS; effect size range: 0.14-0.743). While anxiety and EDA responses in the cutaneous study (Chapter 5) tended to be smaller than in the other studies, both variables still significantly increased with threat. It should be noted that Chapter 5 is the only study presented in this dissertation where participants stood with their side to the edge, and height effects on static balance control are generally only seen in the AP direction (when standing facing the edge; Carpenter et al, 2001a; Hauck et al, 2008). Therefore, the smaller, yet still statistically significant, changes in anxiety and EDA in the cutaneous study sample may imply those people were relatively less threatened by height than those who participated in the other studies. Nonetheless, the effects of the threat manipulation appear relatively stable across studies, suggesting the different samples can be compared.

Another avenue to compare across studies is in terms of changes in background muscle activity across threat conditions. Generally, people increase dorsiflexor (TA) and slightly decrease plantar flexor (SOL or MGas) tonic muscle activity in order to facilitate backward leaning (away from the edge) and increased postural stiffness while standing quietly at the edge of an elevated platform (Carpenter et al, 2001a). Of the three studies where EMG was collected in this dissertation, each demonstrated increases in both plantar (SOL: Chapters 3 and 5; MGas:

Chapter 4) and ankle dorsiflexor (TA; Chapters 3-5) background muscle activity across threat conditions; these data suggest participants adopted similar activation changes across studies. As such, it is important to ask whether changes in lower-limb reflexes across threat conditions can be explained by changes in background muscle activity. Correlations between changes in reflexes and muscle activity were not significant for SLR (Chapter 3) or TStim Ib inhibition (Chapter 4). Similarly, recent evidence suggests threat-induced changes in VEMPs occur without changes in background muscle activation (Naranjo et al, 2015). Combined, these studies suggest height-induced postural threat-induced changes in vestibular, SLR stretch, and Ib reflexes occur independently from changes in tonic muscle activation levels. In contrast, cutaneous reflexes were correlated to changes in background muscle activity, and stretch reflex MLRs were weakly correlated to changes in SOL activity. These findings highlight the need to control for BGA when examining threat effects on reflex modulation, and suggest cutaneous reflexes and MLR changes with threat may depend, to some extent, on changes in motor neuron pool excitability.

Therefore, given that all studies included in this dissertation were comprised of similar samples (young-healthy adults, approximately split along gender lines), who had similar psychological, sympathetic, and tonic muscle activation responses to the same height-induced postural threat, I would argue that the "normal" response to height-induced postural threat involves a multi-sensory adaptation process where balance-relevant muscular and vestibular senses are tuned to facilitate reactive responses to balance disturbances and/or sensory monitoring of postural state. Changes in sensory feedback gain have been proposed as a means to: improve sensory acuity (Prochazka et al, 1985, 1988; Hospod et al, 2007; Ribot-Ciscar et al, 2009), particularly for challenging or novel tasks; to facilitate feedforward prediction of future muscle/limb states (Dimitriou and Edin, 2010); or to suppress irrelevant or predictable sensory

feedback during self-initiated (Baken et al, 2006; Mochizuki et al, 2009) or stereotyped movements (e.g. walking or running; Capaday and Stein, 1987; Duysens et al, 1995, 2004). Changes in sensory-motor gain have been proposed as a means to accommodate changes in 'set', or movement readiness, which may depend on motivation or context (Prochazka et al, 1989). It is assumed that standing at, or walking along the edge of an elevated surface changes postural "central set" in order to prepare a response to, and avoid a potential fall (Brown et al, 2002). The multi-sensory adaptation process proposed in this thesis may reflect part of the "set" adaptation to postural threat, serving to monitor balance state and facilitate balance-corrective responses.

6.3 Neural mechanisms contributing to multi-sensory modulation with threat

While these studies, as a whole, demonstrate multi-sensory-motor modulation to height-induced postural threat in healthy young adults, the specific neural mechanisms that could facilitate these multi-sensory changes can only be speculated. People generally respond to threats (postural or otherwise) with a graded response hierarchy, which includes threat identification, orientation and assessment, and response priming and/or initiation (reviewed by Lang et al, 2000; Mobbs et al, 2015). Threat processing involves interactions between medial prefrontal cortical networks; thalamus; amygdala, locus coeruleus and limbic networks; basal ganglia; hypothalamus; and brainstem networks including the periaqueductal gray and parabrachial nucleus (Balaban, 2002, 2004; Staab et al, 2013; Mobbs et al, 2015), which are involved in various aspects of risk assessment and fear, anxiety, and arousal responses (Mobbs et al, 2007, 2009, 2015) commonly observed with height induced threat (see Chapter 2-5, also: Adkin et al, 2002; Carpenter et al, 2006; Huffman et al, 2009; Zaback et al, 2015, 2016b). There are several avenues by which threat processing centres might affect motor output. For instance, direct excitatory connections between the parabrachial nuclei and the vestibular nuclei (Balaban, 2002,

2004; Staab et al, 2013), reticular formation (Noga et al, 1992; Jankowska et al, 1992) or superior colliculus (Paré et al, 2004); histaminergic projections from the hypothalamus to the vestibular nuclei (de Waele et al, 1992); or projections from the locus coeruleus, dorsal raphe nucleus, or nucleus raphe obscurus to the vestibular nuclei (Balaban, 2002; Halberstadt and Balaban, 2003); could each affect vestibulo-, reticulo-, or tecto-spinal motor outputs. Reticular and vestibular nuclei can broadly affect lower-limb motor responses to vestibular inputs (Goldberg and Fernandez, 1984), cutaneous and Ib spinal reflexes (Baldissera et al, 1981; Jankowska, 1992; Jami, 1992), stretch reflexes (Bras et al, 1989, 1990; Noga et al, 1992; Jankowska, 1992), and, possibly, muscle spindle sensitivity (Grillner et al, 1969; Pompeiano, 1972; Hulliger, 1984; see for contrast: Bent et al, 2013; Knellwolf et al, 2016). Similar effects may occur via the rubrospinal and/or tecto-spinal pathways (Noga et al, 1992; Jankowska, 1992), particularly when considering threat effects on neck, shoulder and/or ocular muscles (e.g. Naranjo et al, 2015, 2016). However, these pathways are less likely to contribute directly to changes observed in lower-limb reflex responses observed in this thesis. Alternatively, changes in balance-relevant reflexes might be explained by changes in motor neuron pool excitability. For instance, diffuse spinal adrenergic and/or serotonergic activation from descending motor circuits (Bras et al, 1989, 1990; Noga et al, 1992) could lead to persistent inward current plateau potentials in lower motor neurons (Johnson and Heckman, 2010), thereby affecting the motor unit population response to imposed reflex stimuli. Similar effects could also be achieved with an increase in muscle spindle sensitivity, where increased Ia afferent traffic could depolarize the lower motor neuron pool (ElBasiouny et al, 2006) and increase the response to other reflex stimuli. However, changes in lower motor neuron pool excitability are not thought to be responsible for the changes in reflexes observed here because most reflex changes did not

correlate with changes in background muscle activity, and H-reflex studies suggest the SOL motor neuron pool is either not changed (Horslen et al, 2013) or inhibited with height-induced threat (Sibley et al, 2007). Nonetheless, there is recent evidence to suggest threat-related activation of the ventro-lateral periaqueductal gray (in mice) increases lower-limb muscle H-reflex excitability (Koutsikou et al, 2014) and acts to improve contrast between limb-position proprioceptive and noxious stimuli in afferent feedback to the cerebellum (Koutsikou et al, 2015). These actions are thought to facilitate cerebellar monitoring of balance-relevant sensory inputs to assist in freezing behaviours (Koutsikou et al, 2015), which may serve to alter balance control, and limit movements in threatening environments. It is not yet clear if humans employ similar sensory gating strategies for cerebellar inputs, or if other balance control CNS centres might utilize changes in balance-relevant sensory feedback. As demonstrated in Chapter 5, and previous work (Davis et al, 2011), it does not appear that sensory feedback is amplified en route to the cortex, despite evidence for altered later cortical processing (Chapter 5; Adkin et al, 2008; Sibley et al, 2010). It is not clear how, or if other CNS systems involved in control of standing balance, including propriospinal, brainstem motor, cerebellar, or basal ganglia circuits might use altered sensory feedback to alter balance behaviours.

6.4 How might these changes affect control of standing balance when people are exposed to a height-induced postural threat?

The cumulative changes in sensory function with threat observed in this dissertation might explain some of the behavioural responses to height-induced postural threat. Threatening stimuli, which induce arousal and/or vigilance, are known to cause freezing behaviours in both humans (Azevedo et al, 2005; Stins and Beek, 2007) and animal models (Lang et al, 2000; Balaban, 2002), where movement is minimized to avoid detection by predators. This behaviour is

qualitatively similar to increases in postural stiffness, where static and dynamic balance movements are minimized when people stand at the edge of an elevated platform (Carpenter et al, 1999b, 2001, 2004).

People tend to exhibit higher frequency and smaller amplitude COP (Carpenter et al, 1999b; Davis et al, 2009; Cleworth et al, 2012) and COM (Carpenter et al, 2001a) oscillations during unperturbed quiet standing during high postural threats. If quiet sway is controlled via negative feedback loops (*cf.* Horak and MacPherson, 1996) or is allowed to oscillate until limits of stability are reached (Collins and De Luca, 1993), then increasing the feedback gain of balance responses to sensory stimuli would either increase frequency of reactive responses, or possibly tighten the limits of stability, and thereby reduce amplitude and increase frequency of sway. It has recently been argued that a certain volume of postural sway is desired by the CNS during quiet standing to enable sensory monitoring of postural state (Carpenter et al, 2010; Murnaghan et al, 2011, 2013). In this view, increasing the gain of sensory feedback (e.g. via increasing muscle spindle sensitivity or exciting the vestibular nuclei) might enable people to reduce movements, or freeze at height, without compromising the quality or quantity of balance-relevant sensory feedback available to the CNS.

When balance is physically perturbed while people stand at the edge of an elevated platform, they tend to reduce movement toward the edge (Brown and Frank, 1997; Carpenter et al, 2004) and increase reactive balance-correcting muscle activity (Carpenter et al, 2004; Adkin et al, 2008; Sibley et al, 2010; Cleworth et al, 2016). Increasing gain of vestibular-evoked responses (Chapter 2), muscle spindle stretch reflexes (Chapter 3) and functional cutaneous reflex amplitudes (Chapter 5), as well as reducing Ib inhibition (Chapter 4) would all serve to promote reactive myogenic responses to balance disturbances. Larger balance-correcting

responses would, in turn, serve to arrest imposed movements from the perturbation sooner, and effectively reduce total movement.

There is also new evidence of altered perceptions of balance movements, where the gain of perceived versus actual movement is increased with height-induced threat (Cleworth and Carpenter, 2016). While research into this area is only just beginning, altered muscle spindle sensitivity (Chapter 3) and vestibular gain and coupling (Chapter 2) could serve to increase the amount of sensory information available to the CNS for perception of body movements. Likewise, changes in cutaneous-evoked cortical potentials (Chapter 5) suggest altered higher-level CNS processing of sensory information when people encounter a threat to standing balance. This altered cortical processing might enable changes in the gain of perceived movements at height.

6.5 How these studies inform our general understanding of sensory contributions to balance control, and future directions for research.

6.5.1 Vestibular system

The experiments presented in Chapter 2 were the first to demonstrate changes in vestibular-evoked balance responses with a threat to standing balance. Subsequent studies have confirmed that height-induced postural threat increases vestibular reflex (VEMP) gains in both limb and axial muscles engaged in balance (Naranjo et al, 2015), vestibulo-ocular reflexes (Naranjo et al, 2016), and visual reflexes which engage the central vestibular system (optokinetic nystagmus; Naranjo, 2015). Combined, these studies demonstrate threat-induced modulation of a wide array of vestibular functions, which utilize different vestibulo-motor pathways (medial and lateral vestibulospinal and vestibulo-ocular) and, in some cases, do not directly engage the

peripheral vestibular system. As such, modulation is presumed to occur at the level of the vestibular nuclei, where each of the vestibulo-motor pathways converge.

It would be interesting to examine in follow-up experiments if threat-induced changes in vestibular-evoked balance reflexes are accompanied by changes in psychophysical perceptions of head accelerations or electrical vestibular stimuli. Perception of real and virtual (EVS) head rotations changes as a function of stimulus frequency and modality (Peters et al, 2015) and age (Peters et al, 2016). However, it is not clear if these relationships are also subject to context-dependent modulation of vestibular function. For instance, perception of virtual (EVS) head rotations is harder (higher perception thresholds) at higher stimulation frequencies, yet Chapter 2 demonstrates that the range of coherent frequencies between vestibular-evoked balance responses and SVS increases with height-induced threat (Fig. 2-3). If vestibular-evoked balance responses are taken as evidence of "perception" of vestibular inputs by the balance system, then it may be plausible to assume altered declarative perception as a function of stimulus frequency with threat.

6.5.2 Muscle spindles and stretch reflexes

The changes in SLR-velocity scaling, as well as SLR and MLR mean amplitudes with increased postural threat in Chapter 3 are taken as indirect evidence in support of increased muscle spindle sensitivity independent from general motor neuron pool excitation. This conclusion is significant because the debate about whether or not humans are capable of independent fusimotor modulation of spindle sensitivity is still open. Several studies have attempted, and failed to demonstrate changes in human muscle spindle sensitivity (e.g. Gandevia and Burke, 1985; Gandevia et al, 1997; Macefield et al, 2003; Bent et al, 2013; Knellwolf et al,

2016). While indirect human evidence in favour of independent changes in muscle spindle sensitivity is mounting (Nafati et al, 2004; Wong et al, 2011; Horslen et al, 2013; Chapter 3), there is very little direct microneurographic evidence in support of changes in human muscle spindle sensitivity in the absence of α-motor neuron activity (Hospod et al, 2007; Ribot-Ciscar et al, 2009). Future research should endeavor to replicate the findings from Chapter 3 with more direct measures of muscle spindle sensitivity, such as with changes in Ia afferent firing rates and dynamic indices with microneurography.

The study presented in Chapter 3 failed to meet the stated goal of evaluating length-dependent modulation of muscle spindle medium-latency stretch reflexes with increased postural threat. The limiting factor in adequately probing this research question was the inability of the tilting platform used to reach speeds high enough to both trigger an MLR reflex, and create sufficient separation in achieved amplitudes by MLR end (mean = 85.1ms) under load. It has been argued that minimum 30ms stretch durations (flexor carpi radialis; Schuurmans et al, 2009), or 20ms stretch acceleration durations (triceps surae, seated; Allum and Mauritz, 1984) are required to trigger an MLR; 2°, 170°/s (~12ms) stretches were often insufficient to evoke an MLR in SOL in Chapter 3. As such, a platform which can reach significantly larger speeds, and larger target stretch amplitudes, would be required to properly address the remaining research question.

6.5.3 GTOs and Ib reflexes

The experiments presented in Chapter 4 provide 2 unique contributions to our understanding of sensory-motor control. First, the demonstration that TStim inhibition can be evoked in standing, and that the reflex is modified in a manner consistent with known changes in

Ib reflexes with changes in postural orientation, introduces TStim as a new experimental technique for probing Ib reflexes in balance tasks. This methodological contribution opens the door for further study of the contribution of Ib reflexes to the control of standing balance in humans. These studies might examine how Ib reflexes change with balance task (e.g. standing on one leg, standing in tandem, standing on compliant surfaces), with other forms of postural threat (e.g. threat of perturbation), when the same muscle is engaged versus not engaged in balance control (e.g. arm muscles while holding a handrail), or how Ib reflexes contribute to dynamic balance control (e.g. reactive balance and gait). This technique might also be extended to other, non-balance questions, such as how Ib reflexes contribute to reaching or fine-motor tasks in humans, or maintenance of limb-posture. Likewise, the contribution of GTOs and Ib afferents to proprioception and higher-level somatosensation are not well understood. TStim, especially indwelling direct stimulation of the tendon, might be used in psychometric studies to reveal GTO contributions to proprioception and kinesthesia.

The second contribution to our understanding of motor control from the experiments in Chapter 4 is the observation that people modify Ib reflexes with changes in environmental context. Participants demonstrated less Ib inhibition (shorter duration and smaller area of TStim-evoked inhibition) when standing in the HIGH, compared to LOW threat condition. These changes occurred despite the fact the essential balance task was not changed (stand in place on a stable surface). There has been relatively little investigation of Ib reflex function in humans, and the changes to Ib reflexes which have previously been observed can be attributed to changes in postural task (lying/sitting to standing/walking; Stephens and Yang, 1999; Faist et al, 2006), stimulation parameters (Burne and Lippold, 1996; Khan and Burne, 2009) or muscle cramp (Khan and Burne, 2007; Miller and Burne, 2014). The demonstration of context-dependent

modulation of Ib reflexes with postural threat in Chapter 4 introduces a new potential field of study of context-dependent sensory-motor gain control of Ib reflexes across a wide variety of motor tasks.

6.5.4 Cutaneous contributions to balance

The failure to demonstrate changes in cutaneous reflexes, independent from changes in background muscle activity, in Chapter 5 begs the question, why are cutaneous reflexes unique in that they are not affected by height-induced postural threat during quiet standing? Follow-up investigations with alternative stimulation methods (skin electrical: e.g. Sayenko et al, 2007; mechanical tactile: e.g. Mildren et al, 2016) and/or locations (e.g. foot sole vs lateral border of the foot) will be important to confirm this result and ensure the present null effect is not related to the evoked reflex being "extraneous" to the balance task (Haridas et al, 2008). While afferent information from skin can contribute to proprioception (Proske and Gandevia, 2012), the cutaneous system, and the form of stimulation used here (Zehr et al, 1998), is the only system examined in this dissertation involved in exteroception. It is possible that the CNS preferentially modulates gain of internal, proprioceptive senses with threat over exteroceptive senses. Koutsikou et al (2015) argue in favour of sensory gating of cutaneous over proprioceptive feedback to the cerebellum in mice to enable postural freezing and mute reflexive movements (which would counter the goal of staying still in freezing) to external stimuli. However, since they used nociceptive pinches as their external "cutaneous" stimuli; it is not clear if similar gating would apply to the non-painful stimuli used in Chapter 5. Future research should endeavor to resolve the potentially disparate effects of threat on proprioceptive and exteroceptive senses.

6.6 Clinical significance of the findings

One clinical implication which can be drawn from these studies is that the results of clinical reflex testing might be biased by patient exam-anxiety. Many of the tests used in this dissertation are modified from established clinical reflex tests (e.g. t-reflex, ramp-and-hold stretch reflex), have clinical correlates (VEMPs for vestibular-evoked balance responses), or might be used in clinical neurophysiological exams (e.g. percutaneous electrical nerve stimulation). Arousal or alertness has long been understood to impact clinical reflex tests (e.g. vestibular tests; Collins and Guedry, 1962); and clinical tests, particularly blood pressure tests, are understood to be subject to "white-coat" effects (Pickering et al, 1988). Clinical balance tests are also subject to "white-coat" effects (Geh et al, 2011), possibly because poor performance could consequently impact quality-of-life (e.g. loss of independence, lifestyle or activity changes) for the elderly or certain clinical populations. Anxiety- or threat-mediated changes to balance-relevant reflexes might underscore some of the "white-coat" effects on clinical balance tests in a similar manner to the height-induced threat effects proposed here. Likewise, fear and/or anxiety-related changes to reflexes might also skew results of basic clinical neurophysiological exams, thereby affecting clinical assessments of neurological disorders or injuries. Therefore, it may be important to take into account patients' anxiety about test outcomes when comparing against population standards collected from samples who may not share the same anxiety. Furthermore, there is now mounting evidence to suggest that other forms of postural threat, including threat of perturbation, can affect balance-relevant reflexes (Horslen et al, 2013; Lim et al, in preparation). Therefore, anxiety about the test (perturbation), not just the test outcome, may be sufficient to affect clinical balance or neurophysiological tests. Reactive balance tests (in the laboratory) are known to be subject to "first-trial" effects (Oude Nijhuis et al, 2009; Visser et al,

2010; Allum et al, 2011) and scale with repeated exposure (Keshner et al, 1987; Campbell et al, 2009); it is possible that clinical neurophysiological tests might be subject to similar adaptation effects as anxiety habituates. As such, it may be warranted further investigate "white-coat" effects on neurophysiological tests in the clinic, and to familiarize new patients with clinical techniques prior to actual examination.

The results from the studies presented in this dissertation might also provide insight for design of balance sensory biofeedback models or devices. Balance-relevant sensory biofeedback devices use body-worn (e.g. gyroscopes, accelerometers) or external sensors (forceplates, kinematic sensors) to monitor real-time balance performance and trigger tactile, auditory, and/or visual feedback which the user can use to control walking or standing balance (Dozza et al, 2005; Davis et al, 2010; Anson et al, 2013; Lin et al, 2015; Lim et al, 2016). There is ongoing effort to understand the potential uses of balance-biofeedback as a training aide or wearable sensory prosthetic (Lim et al, 2016). When biofeedback is available (i.e. used as a prosthetic or balance aide), people tend to show greater improvements in balance performance on challenging balance tasks (e.g. standing on an unstable surface, walk with tandem heel-toe gait) than on mundane tasks (e.g. stand in place with eyes open) where feedback may be distracting (Lin et al, 2015; Lim et al, 2016). The suggestion that people benefit most from additional sensory feedback in difficult balancing situations aligns well with the balance-relevant sensory gain changes observed with postural threat in this dissertation. However, the definition of a "difficult" or "challenging" task is likely to vary from person-to-person. For instance, quiet standing COP oscillations, one-legged stance time, and maximum reach distance are all affected by exposure to height-induced postural threat, however the magnitude of these effects for any given person are related to different psychological trait and state factors (e.g. task performance confidence,

anxiety management, state anxiety or perceptions of stability; Hauck et al, 2008). As such, biofeedback designs which only provide feedback in situations where balance is challenged, perhaps by using relatively "loose" feedback activation thresholds and/or thresholds customized to users' psychological traits, might be best suited for use in biofeedback balance aides.

The observations of context-dependent sensory-motor modulation from this dissertation may also impact design and use of sensory prostheses which enable the users to touch or feel limb position. There is an impetus to develop active prosthetics with brain-computer interfacing to enable (prosthetic) limb movement in people otherwise unable to move due to injury or disease (e.g. Collinger et al, 2014). If a neuroprosthesis is to be used like a "normal" limb, then the instrument would require both movement actuators and a system which can detect and deliver touch, movement and/or limb position sensory feedback to the CNS (Prochazka et al, 2001; Lewis et al, 2013; Prochazka, 2015; Ayers et al, 2016). If context-dependent modulation of sensory-motor interactions is a "normal" aspect of motor, or at least balance, control, as suggested in this dissertation, then neuroprostheses and/or brain-computer interfaces should ideally be able to accommodate this function. The threat-induced changes to vestibular (Chapter 2), GTO-Ib (Chapter 4) and cutaneous (Chapter 5) reflexes seen in this dissertation are likely caused by changes in sensory processing inside the CNS, since either the form of stimulation bypasses the peripheral receptor (cutaneous nerve; vestibular afferent, cf. Goldberg et al, 1984), or the receptor is not served by a specific efferent system (GTO, cf. Jami, 1992; Prochazka, 1996). Therefore, a neuroprosthesis serving these systems may not need peripheral gain control to effect threat-induced changes in sensory function. However, neuroprostheses detecting muscle spindle-type sensations of limb position or movement might need to be able to effect peripheral

changes in feedback gain, likely through brain-computer interfacing, if "normal" function is to be achieved (Prochazka, 2015).

6.7 Conclusions

The goal of this dissertation was to understand the modulatory effects of threats to standing balance on balance-relevant sensory function. Vestibular, muscle spindle stretch, and GTO Ib reflexes were all modulated in ways which would either increase muscle activity, or create larger balance responses when participants were exposed to height-induced postural threats. These studies suggest balance-relevant sensory function is tuned with changes in balance context, and it is proposed these changes contribute to altered balance behaviours when people are exposed to threats to standing balance.

Bibliography

Adkin, AL, Campbell, AD, Chua, R & Carpenter, MG (2008). The influence of postural threat on the cortical response to unpredictable and predictable postural perturbations. *Neuroscience Letters* **435**, 120-125.

Adkin, AL, Frank, JS, Carpenter, MG & Peysar, GW (2000). Postural control is scaled to level of postural threat. *Gait Posture* **12**, 87-93.

Adkin, AL, Frank, JS, Carpenter, MG & Peysar, GW (2002). Fear of falling modifies anticipatory postural control. *Exp Brain Res* **143**, 160-170.

Adkin, AL, Quant, S, Maki, BE & McIlroy, WE (2006). Cortical responses associated with predictable and unpredictable compensatory balance reactions. *Exp Brain Res* **172**, 85-93.

af Klint, R, Nielsen, JB, Sinkjaer, T & Grey, MJ (2009). Sudden drop in ground support produces force-related unload response in human overground walking. *J Neurophysiol* **101**, 1705-1712.

Ali, AS, Rowen, KA & Iles, JF (2003). Vestibular actions on back and lower limb muscles during postural tasks in man. *J Physiol* **546**, 615-624

Allum, JH & Mauritz, KH (1984). Compensation for intrinsic muscle stiffness by short-latency reflexes in human triceps surae muscles. *J Neurophysiol* **52**, 797-818.

Allum, JH & Pfaltz, CR (1985). Visual and vestibular contributions to pitch sway stabilization in the ankle muscles of normals and patients with bilateral peripheral vestibular deficits. *Exp Brain Res* **58**, 82-94.

Allum, JH, Honegger, F & Schicks, H (1993). Vestibular and proprioceptive modulation of postural synergies in normal subjects. *J Vestib Res* **3**, 59-85.

Allum, JH, Honegger, F & Schicks, H (1994). The influence of a bilateral peripheral vestibular deficit on postural synergies. *J Vestib Res* **4**, 49-70.

Allum, JHJ, Adkin, AL, Carpenter, MG, Held-Ziolkowska, M, Honegger, F & Pierchala, K (2001). Trunk sway measures of postural stability during clinical balance tests: effects of a unilateral vestibular deficit. *Gait Posture* **14**, 227-237.

Allum, JHJ, Bloem, BR, Carpenter, MG & Honegger, F (2001). Differential diagnosis of proprioceptive and vestibular deficits using dynamic support-surface posturography. *Gait Posture* **14**, 217-226.

Allum, JHJ, Tang, K, Carpenter, MG, Oude Nijhuis, LB & Bloem, BR (2011). Review of first trial responses in balance control: Influence of vestibular loss and Parkinson's disease. *Hu Mov Sci* **30**, 279-295.

Altenmüller, E, Berger, W, Prokop, T, Trippel, M & Dietz, V (1995). Modulation of sural nerve somatosensory evoked potentials during stance and different phases of the step-cycle. *Electroencephalogr Clin Neurophysiol* **96**, 516-525.

Amjad, AM, Halliday, DM, Rosenberg, JR & Conway, BA (1997). An extended difference of coherence test for comparing and combining several independent coherence estimates: theory and application to the study of motor units and physiological tremor. *J Neurosci Methods* **73**, 69-79.

Aniss, AM, Diener, HC, Hore, J, Burke, D & Gandevia, SC (1990a). Reflex activation of muscle spindles in human pretibial muscles during standing. *J Neurophysiol* **64**, 671-679.

Aniss, AM, Diener, HC, Hore, J, Gandevia, SC & Burke, D (1990b). Behavior of human muscle receptors when reliant on proprioceptive feedback during standing. *J Neurophysiol* **64**, 661-670.

Aniss, AM, Gandevia, SC & Burke, D (1992). Reflex responses in active muscles elicited by stimulation of low-threshold afferents from the human foot. *J Neurophysiol* **67**, 1375-1384.

Anson, E, Rosenberg, R, Agada, P, Kiemel, T & Jeka, J (2013). Does visual feedback during walking result in similar improvements in trunk control for young and older healthy adults? *J Neuroeng Rehabil* **10**, 110.

Arshad, Q, Nigmatullina, Y & Bronstein, AM (2013). Handedness-related cortical modulation of the vestibular-ocular reflex. *J Neurosci* **33**, 3221-3227.

Arshad, Q, Nigmatullina, Y, Roberts, RE, Bhrugubanda, V, Asavarut, P & Bronstein, AM (2014). Left cathodal trans-cranial direct current stimulation of the parietal cortex leads to an asymmetrical modulation of the vestibular-ocular reflex. *Brain Stimul* **7**, 85-91.

Arshian, MS, Puterbaugh, SR, Miller, DJ, Catanzaro, MF, Hobson, CE, McCall, AA & Yates, BJ (2013). Effects of visceral inputs on the processing of labyrinthine signals by the inferior and caudal medial vestibular nuclei: ramifications for the production of motion sickness. *Exp Brain Res* **228**, 353-363.

Ayers, CA, Fisher, LE, Gaunt, RA & Weber, DJ (2016). Microstimulation of the lumbar DRG recruits primary afferent neurons in localized regions of lower limb. *J Neurophysiol* **116**, 51-60.

Azevedo, TM, Volchan, E, Imbiriba, LA, Rodrigues, EC, Oliveira, JM, Oliveira, LF, Lutterbach, LG & Vargas, CD (2005). A freezing-like posture to pictures of mutilation. *Psychophysiology* **42**, 255-260.

Baken, BC, Nieuwenhuijzen, PH, Bastiaanse, CM, Dietz, V & Duysens, J (2006). Cutaneous reflexes evoked during human walking are reduced when self-induced. *J Physiol* **570**, 113-124.

Baken, BCM, Dietz, V & Duysens, J (2005). Phase-dependent modulation of short latency cutaneous reflexes during walking in man. *Brain Res* **1031**, 268-275.

Balaban, CD & Jacob, RG (2001). Background and history of the interface between anxiety and vertigo. *J Anxiety Disord* **15**, 27-51.

Balaban, CD & Thayer JF (2001). Neurological bases for balance–anxiety links. *J Anxiety Disord* **15**, 53-79.

Balaban, CD (2002). Neural substrates linking balance control and anxiety. *Physiol Behav* **77**, 469-475.

Balaban, CD (2004). Projections from the parabrachial nucleus to the vestibular nuclei: potential substrates for autonomic and limbic influences on vestibular responses. *Brain Res* **996**, 126-137.

Baldissera, F, Hultborn, H & Illert, M (1981). Integration in spinal neuronal systems. In *Supplement 2: Handbook of Physiology, The Nervous System, Motor control*, ed. Brooks VB, pp. 509-595.

Bar-Haim, Y, Lamy, D & Glickman, S (2005). Attentional bias in anxiety: a behavioral and ERP study. *Brain Cogn* **59**, 11-22.

Bawa, P & Murnaghan, CD (2009). Motor unit rotation in a variety of human muscles. *J Neurophysiol* **102**, 2265-2272.

Bent, LR, McFadyen, BJ & Inglis, JT (2005). Vestibular contributions during human locomotor tasks. *Exerc Sport Sci Rev* **33**, 107-113.

Bent, LR, McFadyen, BJ, Merkley, VF, Kennedy, PM & Inglis, JT (2000). Magnitude effects of galvanic vestibular stimulation on the trajectory of human gait. *Neurosci Lett* **279**, 157-160.

Bent, LR, Sander, M, Bolton, PS & Macefield, VG (2013). The vestibular system does not modulate fusimotor drive to muscle spindles in contracting leg muscles of seated subjects. *Exp Brain Res* **227**, 175-183.

Bernardin, D, Kadone, H, Bennequin, D, Sugar, T, Zaoui, M & Berthoz, A (2012). Gaze anticipation during human locomotion. *Exp Brain Res* **223**, 65-78.

Bloem, BR, Allum, JH, Carpenter, MG & Honegger, F (2000). Is lower leg proprioception essential for triggering human automatic postural responses? *Exp Brain Res* **130**, 375-391.

Bloem, BR, Allum, JH, Carpenter, MG, Verschuuren, JJ & Honegger, F (2002). Triggering of balance corrections and compensatory strategies in a patient with total leg proprioceptive loss. *Exp Brain Res* **142**, 91-107.

Blouin, JS, Dakin, CJ, van den Doel, K, Chua, R, McFadyen, BJ & Inglis, JT (2011). Extracting phase-dependent human vestibular reflexes during locomotion using both time and frequency correlation approaches. *J App Physiol* **111**, 1484-1490.

Bolmont, B, Gangloff, P, Vouriot, A & Perrin, PP (2002). Mood states and anxiety influence abilities to maintain balance control in healthy human subjects. *Neurosci Lett* **329**, 96-100.

Boucsein, W, Fowles, DC, Grimnes, S, Ben-Shakhar, G, Roth, WT, Dawson, ME, Filion, DL & Society for Psychophysiological Research Ad Hoc Committee on Electrodermal Measures (2012). Publication recommendations for electrodermal measurements. *Psychophysiology* **49**, 1017-1034.

Bouyer, LJ & Rossignol, S (2003). Contribution of cutaneous inputs from the hindpaw to the control of locomotion. I. Intact cats. *J Neurophysiol* **90**, 3625-3639.

Brandt, T & Huppert, D (2014). Fear of heights and visual height intolerance. *Curr Opin Neurol* **27**, 111-117.

Brandt, T, Strupp, M & Benson, J (1999). You are better off running than walking with acute vestibulopathy. *Lancet* **354**, 746.

Bras, H, Cavallari, P, Jankowska, E & McCrea, D (1989). Comparison of effects of monoamines on transmission in spinal pathways from group I and II muscle afferents in the cat. *Exp Brain Res* **76**, 27-37.

Bras, H, Jankowska, E, Noga, B & Skoog, B (1990). Comparison of Effects of Various Types of NA and 5-HT Agonists on Transmission from Group II Muscle Afferents in the Cat. *Eur J Neurosci* **2**, 1029-1039.

Britton, TC, Day, BL, Brown, P, Rothwell, JC, Thompson, PD & Marsden, CD (1993). Postural electromyographic responses in the arm and leg following galvanic vestibular stimulation in man. *Exp Brain Res* **94**, 143-151.

Brown, LA & Frank, JS (1997). Postural compensations to the potential consequences of instability: kinematics. *Gait Posture* **6**, 89-97.

Brown, LA, Doan, JB, McKenzie, NC & Cooper, SA (2006). Anxiety-mediated gait adaptations reduce errors of obstacle negotiation among younger and older adults: implications for fall risk. *Gait Posture* **24**, 418-423.

Brown, LA, Gage, WH, Polych, MA, Sleik, RJ & Winder, TR (2002). Central set influences on gait. Age-dependent effects of postural threat. *Exp Brain Res* **145**, 286-296.

Brown, LA, Polych, MA & Doan, JB (2006). The effect of anxiety on the regulation of upright standing among younger and older adults. *Gait Posture* **24**, 397-405.

Burke, D, Dickson, HG & Skuse, NF (1991). Task-dependent changes in the responses to low-threshold cutaneous afferent volleys in the human lower limb. *J Physiol* **432**, 445-458.

Burke, D, Gandevia, SC & McKeon, B (1983). The afferent volleys responsible for spinal proprioceptive reflexes in man. *J Physiol* **339**, 535-552.

Burke, D, Gandevia, SC & McKeon, B (1984). Monosynaptic and oligosynaptic contributions to human ankle jerk and H-reflex. *J Neurophysiol* **52**, 435-448.

Burne, JA & Lippold, OCJ (1996). Reflex inhibition following electrical stimulation over muscle tendons in man. *Brain* **119**, 1107-1114.

Calancie, B & Bawa, P (1985). Firing patterns of human flexor carpi radialis motor units during the stretch reflex. *J Neurophysiol* **53**, 1179-1193.

Campbell, AD, Chua, R, Inglis, JT & Carpenter, MG (2012). Startle induces early initiation of classically conditioned postural responses. *J Neurophysiol* **108**, 2946-2956.

Campbell, AD, Dakin, CJ & Carpenter, MG (2009). Postural responses explored through classical conditioning. *Neuroscience* **164**, 986-997.

Capaday, C & Stein, RB (1987). Difference in the amplitude of the human soleus H reflex during walking and running. *J Physiol* **392**, 513-522.

Capaday, C (1997). Neurophysiological methods for studies of the motor system in freely moving human subjects. *J Neurosci Methods* **74**, 201-218.

Carpenter, MG, Adkin, AL, Brawley, LR & Frank, JS (2006). Postural, physiological and psychological reactions to challenging balance: does age make a difference? *Age Ageing* **35**, 298-303.

Carpenter, MG, Allum, JH & Honegger, F (1999a). Directional sensitivity of stretch reflexes and balance corrections for normal subjects in the roll and pitch planes. *Exp Brain Res* **129**, 93-113.

Carpenter, MG, Allum, JH & Honegger, F (2001b). Vestibular influences on human postural control in combinations of pitch and roll planes reveal differences in spatiotemporal processing. *Exp Brain Res* **140**, 95-111.

Carpenter, MG, Frank, JS & Silcher, CP (1999b). Surface height effects on postural control: a hypothesis for a stiffness strategy for stance. *J Vestib Res* **9**, 277-286.

Carpenter, MG, Frank, JS, Adkin, AL, Paton, A & Allum, JHJ (2004). Influence of Postural Anxiety on Postural Reactions to Multi-Directional Surface Rotations. *J Neurophysiol* **92**, 3255-3265.

Carpenter, MG, Frank, JS, Silcher, CP & Peysar, GW (2001a). The influence of postural threat on the control of upright stance. *Exp Brain Res* **138**, 210-218.

Carpenter, MG, Murnaghan, CD & Inglis, JT (2010). Shifting the balance: evidence of an exploratory role for postural sway. *Neuroscience* **171**, 196-204.

Carriot, J, Jamali, M, Chacron, MJ & Cullen, KE (2014). Statistics of the vestibular input experienced during natural self-motion: implications for neural processing. *J Neurosci* **34**, 8347-8357.

Carroll, JP & Freedman, W (1993). Nonstationary properties of postural sway. *J Biomech* **26**, 409-416.

Carter, JR & Ray, CA (2008). Sympathetic responses to vestibular activation in humans. *Am J Physiol Regul Integr Comp Physiol* **294**, R681-688.

Cathers, I, Day, BL & Fitzpatrick, RC (2005). Otolith and canal reflexes in human standing. *J Physiol* **563**, 229-234.

Cattagni T, Martin A & Scaglioni G (2014). Is spinal excitability of the triceps surae mainly affected by muscle activity or body position? *J Neurophysiol* **111**, 2525-2532.

Cleworth, TW & Carpenter, MG (2016). Postural threat influences conscious perception of postural sway. Neurosci Lett **620**, 127-131.

Cleworth, TW, Chua, R, Inglis, JT & Carpenter, MG (2016). Influence of virtual height exposure on postural reactions to support surface translations. *Gait Posture* **47**, 96-102.

Cleworth, TW, Horslen, BC & Carpenter, MG (2012). Influence of real and virtual heights on standing balance. *Gait Posture* **36**, 172-176.

Colebatch, JG, Halmagyi, GM & Skuse, NF (1994). Myogenic potentials generated by a click-evoked vestibulocollic reflex. *J Neurol Neurosurg Psychiatry* **57**, 190-197.

Collinger, JL, Kryger, MA, Barbara, R, Betler, T, Bowsher, K, Brown, EH, Clanton, ST, Degenhart, AD, Foldes, ST, Gaunt, RA, Gyulai, FE, Harchick, EA, Harrington, D, Helder, JB, Hemmes, T, Johannes, MS, Katyal, KD, Ling, GS, McMorland, AJ, Palko, K, Para, MP, Scheuermann, J, Schwartz, AB, Skidmore, ER, Solzbacher, F, Srikameswaran, AV, Swanson, DP, Swetz, S, Tyler-Kabara, EC, Velliste, M, Wang, W, Weber, DJ, Wodlinger, B & Boninger, ML (2014). Collaborative approach in the development of high-performance brain-computer interfaces for a neuroprosthetic arm: translation from animal models to human control. *Clin Transl Sci* **7**, 52-59.

Collins, JJ & De Luca, CJ (1993). Open-loop and closed-loop control of posture: a random-walk analysis of center-of-pressure trajectories. *Exp Brain Res* **95**, 308-318.

Collins, WE & Guedry, FE, Jr (1962). Arousal effects and nystagmus during prolonged constant angular acceleration. *Acta Otolaryngol* **54**, 349-362.

Collins, WE & Poe, RH (1962). Amphetamine, arousal, and human vestibular nystagmus. *J Pharmacol Exp Ther* **138**, 120-125.

Collins, WE (1988). Some effects of sleep loss on vestibular responses. *Aviat Space Environ Med* **59**, 523-529.

Conway, BA, Hultborn, H & Kiehn, O (1987) Proprioceptive input resets central locomotor rhythm in the spinal cat. *Exp Brain Res* **68**, 643-656.

Coppin, CML, Jack, JJB & MacLennan, CR (1970) A method for the selective electrical activation of tendon organ afferent fibres from the cat soleus muscle. *J Physiol* **210(suppl)**, 18P-20P.

Corna, S, Galante, M, Grasso, M, Nardone, A & Schieppati, M (1996). Unilateral displacement of lower limb evokes bilateral EMG responses in leg and foot muscles in standing humans. *Exp Brain Res* **109**, 83-91.

Corna, S, Grasso, M, Nardone, A & Schieppati, M (1995). Selective depression of medium-latency leg and foot muscle responses to stretch by an alpha 2-agonist in humans. *The J. Physiol* **484**, 803-809.

Crampton, GH & Schwam, WJ (1961). Effects of arousal reaction on nystagmus habituation in the cat. *Am J Physiol* **200**, 29-33.

Crampton, GH (1961). Habituation of vestibular nystagmus in the cat during sustained arousal produced by d-amphetamine. *Rep US Army Med Res Lab* **488**, 1–15.

Cuthbert, BN, Schupp, HT, Bradley, MM, Birbaumer, N & Lang, PJ (2000). Brain potentials in affective picture processing: covariation with autonomic arousal and affective report. *Biol Psychol* **52**, 95-111.

Dakin, CJ, Inglis, JT & Blouin, JS (2011). Short and medium latency muscle responses evoked by electrical vestibular stimulation are a composite of all stimulus frequencies. *Exp Brain Res* **209**, 345-354.

Dakin, CJ, Inglis, JT, Chua, R & Blouin, JS (2013). Muscle-specific modulation of vestibular reflexes with increased locomotor velocity and cadence. *J Neurophysiol* **110**, 86-94.

Dakin, CJ, Lee Son, GM, Inglis, JT & Blouin, JS (2007). Frequency response of human vestibular reflexes characterized by stochastic stimuli. *J Physiol* **583**, 1117-1127.

Dakin, CJ, Luu, BL, van den Doel, K, Inglis, JT & Blouin, JS (2010). Frequency-specific modulation of vestibular-evoked sway responses in humans. *J Neurophysiol* **103**, 1048-1056.

Davis, JR, Campbell, AD, Adkin, AL & Carpenter, MG (2009). The relationship between fear of falling and human postural control. *Gait Posture* **29**, 275-279.

Davis, JR, Carpenter, MG, Tschanz, R, Meyes, S, Debrunner, D, Burger, J & Allum, JHJ (2010). Trunk sway reductions in young and older adults using multi-modal biofeedback. *Gait Posture* **31**, 465-472.

Davis, JR, Horslen, BC, Nishikawa, K, Fukushima, K, Chua, R, Inglis, JT & Carpenter, MG (2011). Human proprioceptive adaptations during states of height-induced fear and anxiety. *J Neurophysiol* **106**, 3082-3090.

Davis, M, Walker, DL, Miles, L & Grillon, C (2010). Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology* **35**, 105-135.

Day, BL & Guerraz, M (2007). Feedforward versus feedback modulation of human vestibular-evoked balance responses by visual self-motion information. *J Physiol* **582**, 153-161.

de Waele, C, Serafin, M, Khateb, A, Vibert, N, Yabe, T, Arrang, JM, Mulhethaler, M & Vidal, PP (1992). An in vivo and in vitro study of the vestibular nuclei histaminergic receptors in the guinea pig. *Ann N Y Acad Sci* **656**, 550-565.

Deshpande, N & Patla, AE (2005). Dynamic visual-vestibular integration during goal directed human locomotion. *Exp Brain Res* **166**, 237-247.

Diener, HC, Dichgans, J, Bootz, F & Bacher, M (1984). Early stabilization of human posture after a sudden disturbance: influence of rate and amplitude of displacement. *Exp Brain Res* **56**, 126-134.

Diener, HC, Horak, FB & Nashner, LM (1988). Influence of stimulus parameters on human postural responses. J Neurophysiol **59**, 1888-1905.

Dieterich, M & Brandt, T (2008). Functional brain imaging of peripheral and central vestibular disorders. *Brain* **131**, 2538-2552.

Dietz, V, Gollhofer, A, Kleiber, M & Trippel, M (1992). Regulation of bipedal stance: dependency on "load" receptors. *Exp Brain Res* **89**, 229-231.

Dietz, V, Horstmann, GA, Trippel, M & Gollhofer, A (1989). Human postural reflexes and gravity — An under water simulation. *Neurosci Lett* **106**, 350-355.

Dimitriou, M & Edin, BB (2010). Human muscle spindles act as forward sensory models. *Curr Biol* **20**, 1763-1767.

Do, MC & Roby-Brami, A (1991). The influence of a reduced plantar support surface area on the compensatory reactions to a forward fall. *Exp Brain Res* **84**, 439-443.

Do, MC, Bussel, B & Breniere, Y (1990). Influence of plantar cutaneous afferents on early compensatory reactions to forward fall. *Exp Brain Res* **79**, 319-324.

Doricchi, F, Siegler, I, Iaria, G & Berthoz, A (2002). Vestibulo-ocular and optokinetic impairments in left unilateral neglect. *Neuropsychologia* **40**, 2084-2099.

Dowman, R (2007). Neural mechanisms of detecting and orienting attention toward unattended threatening somatosensory target stimuli. II. Intensity effects. *Psychophysiology* **44**, 420-430.

Dozza, M, Chiari, L & Horak, FB (2005). Audio-biofeedback improves balance in patients with bilateral vestibular loss. *Arch Phys Med Rehabil* **86**, 1401-1403.

Duysens, J, Bastiaanse, CM, Smits-Engelsman, BC & Dietz, V (2004). Gait acts as a gate for reflexes from the foot. *Can J Physiol Pharmacol* **82**, 715-722.

Duysens, J, Clarac, F & Cruse, H (2000). Load-regulating mechanisms in gait and posture: comparative aspects. *Physiol Rev* **80**, 83-133.

Duysens, J, Tax, AA, Nawijn, S, Berger, W, Prokop, T & Altenmuller, E (1995). Gating of sensation and evoked potentials following foot stimulation during human gait. *Exp Brain Res* **105**, 423-431.

Duysens, J, Trippel, M, Horstmann, GA & Dietz V (1990). Gating and reversal of reflexes in ankle muscles during human walking. *Exp Brain Res***82**, 351-358.

Elbasiouny, SM, Bennett, DJ & Mushahwar, VK (2006). Simulation of Ca2+ persistent inward currents in spinal motoneurones: mode of activation and integration of synaptic inputs. J Physiol **570**, 355-374.

Eldar, S, Yankelevitch, R, Lamy, D & Bar-Haim, Y (2010). Enhanced neural reactivity and selective attention to threat in anxiety. *Biol Psychol* **85**, 252-257.

Engberg, I, Lundberg, A & Ryall, RW (1968). Reticulospinal inhibition of interneurones. *J Physiol* **194**, 225-236.

Faist, M, Hoefer, C, Hodapp, M, Dietz, V, Berger, W & Duysens, J (2006). In humans Ib facilitation depends on locomotion while suppression of Ib inhibition requires loading. *Brain Res* **1076**, 87-92.

Fallon, JB, Bent, LR, McNulty, PA & Macefield, VG (2005). Evidence for strong synaptic coupling between single tactile afferents from the sole of the foot and motoneurons supplying leg muscles. *J Neurophysiol* **94**, 3795-3804.

Fitzpatrick, RC & McCloskey, DI (1994). Proprioceptive, visual and vestibular thresholds for the perception of sway during standing in humans. *J Physiol* **478**, 173-186.

Fitzpatrick, RC, Burke, D & Gandevia, SC (1996). Loop gain of reflexes controlling human standing measured with the use of postural and vestibular disturbances. *J. Neurophysiol* **76**, 3994-4008.

Fitzpatrick, RC, Butler, JE & Day, BL (2006) Resolving head rotation for human bipedalism. *Curr Biol* **16**, 1509-1514.

Fitzpatrick, RC & Day, BL (2004). Probing the human vestibular system with galvanic stimulation. *J Appl Physiol* **96**, 2301-2316.

Fitzpatrick, RC, Gorman, RB, Burke, D & Gandevia, SC (1992). Postural proprioceptive reflexes in standing human subjects: bandwidth of response and transmission characteristics. *The Journal of Physiology* **458**, 69-83.

Forbes, PA, Dakin, CJ, Vardy, AN, Happee, R, Siegmund, GP, Schouten, AC & Blouin, JS (2013). Frequency response of vestibular reflexes in neck, back, and lower limb muscles. *J Neurophysiol* **110**, 1869-1881.

Forbes, PA, Siegmund, GP, Schouten, AC & Blouin, JS (2015). Task, muscle and frequency dependent vestibular control of posture. *Front Integr Neurosci* **8**, 94.

Forssberg, H (1979). Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion. *J Neurophysiol* **42**, 936-953.

Forssberg, H, Grillner, S & Rossignol, S (1975). Phase dependent reflex reversal during walking in chronic spinal cats. *Brain Res* **85**, 103-107.

Forth, KE & Layne, CS (2008). Neuromuscular responses to mechanical foot stimulation: the influence of loading and postural context. *Aviat Space Environ Med* **79**, 844-851.

Franz JR, Slane LC, Rasske K & Thelen DG (2015). Non-uniform in vivo deformations of the human Achilles tendon during walking. *Gait Posture* **41**, 192-197.

Fujiwara, K, Miyaguchi, H, Asai, A, Toyama, H, Kunita, K & Inoue, K (2003). Perceived standing position after reduction of foot-pressure sensation by cooling the sole. *Percept Mot Skills* **96**, 381-399.

Furman, JM & Jacob, RG (2001). A clinical taxonomy of dizziness and anxiety in the otoneurological setting. *J Anxiety Disord* **15**, 9-26.

Furman, JM, O'Leary, DP & Wolfe, JW (1981). Changes in the horizontal vestibulo-ocular reflex of the rhesus monkey with behavioral and pharmacological alerting. *Brain Res* **206**, 490-494.

Gage, WH, Sleik, RJ, Polych, MA, McKenzie, NC & Brown, LA (2003). The allocation of attention during locomotion is altered by anxiety. *Exp Brain Res* **150**, 385-394.

Gandevia, SC & Burke, D (1985). Effect of training on voluntary activation of human fusimotor neurons. *J Neurophysiol* **54**, 1422-1429.

Gandevia, SC, Wilson, LR, Inglis, JT & Burke, D (1997). Mental rehearsal of motor tasks recruits alpha-motoneurones but fails to recruit human fusimotor neurones selectively. *J Physiol* **505**, 259-266.

Gardner, EP & Johnson, KO (2013). Touch. In *Principles of Neural Science*, 5th edn, ed. Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA & Hudspeth AJ, pp. 498-529. McGraw-Hill Companies, Inc, United States of America.

Geh, CLM, Beauchamp, MR, Crocker, PRE & Carpenter, MG (2011). Assessed and distressed: White-coat effects on clinical balance performance. *J Psychosom Res* **70**, 45-51.

Gendre, M, Yiou, E, Gelat, T, Honeine, JL & Deroche, T (2016). Directional specificity of postural threat on anticipatory postural adjustments during lateral leg raising. *Exp Brain Res* **234**, 659-671.

Goldberg, JM & Fernandez, C (1984). The vestibular system. In *Supplement 3: Handbook of Physiology, The Nervous System, Sensory Processes*, ed. Darian-Smith I, pp. 977-1022.

Goldberg, JM, Smith, CE & Fernandez, C (1984). Relation between discharge regularity and responses to externally applied galvanic currents in vestibular nerve afferents of the squirrel monkey. *J Neurophysiol* **51**, 1236-1256.

Gottlieb, GL & Agarwal, GC (1979). Response to sudden torques about ankle in man: myotatic reflex. *J Neurophysiol* **42**, 91-106.

Gottlieb, GL & Agarwal, GC (1980). Response to sudden torques about ankle in man. II. Postmyotatic reactions. *J Neurophysiol* **43**, 86-101.

Grey, MJ, Ladouceur, M, Andersen, JB, Nielsen, JB & Sinkjaer, T (2001). Group II muscle afferents probably contribute to the medium latency soleus stretch reflex during walking in humans. *J Physiol* **534**, 925-933.

Grey, MJ, Nielsen, JB, Mazzaro, N & Sinkjaer, T (2007). Positive force feedback in human walking. *J Physiol* **581**, 99-105.

Grillner, S & Rossignol, S (1978). Contralateral reflex reversal controlled by limb position in the acute spinal cat injected with clonidine i.v. *Brain Res* **144**, 411-414.

Grillner, S, Hongo, T & Lund, S (1969). Descending monosynaptic and reflex control of gamma-motoneurones. *Acta Physiol Scand* **75**, 592-613.

Guerraz, M & Bronstein, AM (2008). Mechanisms underlying visually induced body sway. *Neuroscience Letters*, **443**, 12-16.

Hadjistavropoulos, T, Delbaere, K & Fitzgerald, TD (2011). Reconceptualizing the role of fear of falling and balance confidence in fall risk. *J Aging Health* **23**, 3-23.

Halberstadt, AL & Balaban, CD (2003). Organization of projections from the raphe nuclei to the vestibular nuclei in rats. *Neuroscience* **120**, 573-594.

Halliday, DM, Rosenberg, JR, Amjad, AM, Breeze, P, Conway, BA & Farmer, SF (1995). A framework for the analysis of mixed time series/point process data—Theory and application to the study of physiological tremor, single motor unit discharges and electromyograms. *Prog Biophys Mol Biol* **64**, 237-278.

Halmagyi, GM & Colebatch, JG (1995). Vestibular Evoked Myogenic Potentials in the Sternomastoid Muscle Are Not of Lateral Canal Origin. *Acta Otolaryngol* **115**, 1-3.

Hamalainen, H, Kekoni, J, Sams, M, Reinikainen, K & Naatanen, R (1990). Human somatosensory evoked potentials to mechanical pulses and vibration: contributions of SI and SII somatosensory cortices to P50 and P100 components. *Electroencephalogr Clin Neurophysiol* **75**, 13-21.

Haridas, C, Zehr, EP & Misiaszek, JE (2005). Postural uncertainty leads to dynamic control of cutaneous reflexes from the foot during human walking. *Brain Res* **1062**, 48-62.

Haridas, C, Zehr, EP & Misiaszek, JE (2006). Context-Dependent Modulation of Interlimb Cutaneous Reflexes in Arm Muscles as a Function of Stability Threat During Walking. *J Neurophysiol* **96**, 3096-3103.

Haridas, C, Zehr, EP & Misiaszek, JE (2008). Adaptation of cutaneous stumble correction when tripping is part of the locomotor environment. *J Neurophysiol* **99**, 2789-2797.

Hauck, LJ, Carpenter, MG & Frank, JS (2008). Task-specific measures of balance efficacy, anxiety, and stability and their relationship to clinical balance performance. *Gait Posture* **27**, 676-682.

Hayashi, R, Tako, K, Tokuda, T & Yanagisawa, N (1992). Comparison of amplitude of human soleus H-reflex during sitting and standing. *Neurosci Res* **13**, 227-233.

Heckman, CJ, Condon, SM, Hutton, RS & Enoka, RM (1984). Can Ib axons be selectively activated by electrical stimuli in human subjects? *Exp Neurol* **86**, 576-582.

Héroux, ME, Dakin, CJ, Luu, BL, Inglis, JT & Blouin, JS (2014). Absence of lateral gastrocnemius activity and differential motor unit behavior in soleus and medial gastrocnemius during standing balance. *J Appl Physiol (1985)* **116**, 140-148.

Hlavacka, F, Shupert, CL & Horak, FB (1999). The timing of galvanic vestibular stimulation affects responses to platform translation. *Brain Res* **821**, 8-16.

Hongo, T, Jankowska, E & Lundberg, A (1969). The rubrospinal tract. II. Facilitation of interneuronal transmission in reflex paths to motoneurones. *Exp Brain Res* **7**, 365-391.

Horak FB & Macpherson JM (2010). Postural Orientation and Equilibrium. In Comprehensive Physiology. John Wiley & Sons, Inc.

Horak, FB & Macpherson, JM (1996). Postural orientation and equilibrium. In *Handbook of Physiology, Section 12: Exercise: Regulation and integration of multiple systems*, ed. Rowell LB & Shepherd JT, pp. 255-292. Oxford, New York, USA.

Horak, FB, Shupert, CL, Dietz, V & Horstmann, G (1994). Vestibular and somatosensory contributions to responses to head and body displacements in stance. *Exp Brain Res* **100**, 93-106.

Horcholle-Bossavit, G, Jami, L, Petit, J, Vejsada, R & Zytnicki, D (1990). Ensemble discharge from Golgi tendon organs of cat peroneus tertius muscle. *J Neurophysiol* **64**, 813-821.

Horlings, CG, Carpenter, MG, Honegger, F & Allum, JH (2009). Vestibular and proprioceptive contributions to human balance corrections: aiding these with prosthetic feedback. *Ann N Y Acad Sci* **1164**, 1-12.

Horslen, BC & Carpenter, MG (2011). Arousal, valence and their relative effects on postural control. *Exp Brain Res* **215**, 27-34.

Horslen, BC, Dakin, CJ, Inglis, JT, Blouin, JS & Carpenter, MG (2014). Modulation of human vestibular reflexes with increased postural threat. *J Physiol* **592**, 3671-3685.

Horslen, BC, Dakin, CJ, Inglis, JT, Blouin, JS & Carpenter, MG (2015). Crosstalk proposal: Fear of falling does influence vestibular-evoked balance responses. *J Physiol* **593**, 2979-2981

Horslen, BC, Dakin, CJ, Inglis, JT, Blouin, JS & Carpenter, MG (2015). Last words on fear of falling does influence vestibular-evoked balance responses *J Physiol* (online) http://onlinelibrary.wiley.com/store/10.1113/JP270269/asset/supinfo/tjp6714-sup-0002-Carpenter.pdf?v=1&s=b15fa6c9f7f3371b43c28ff6b4cc8943411ebac8 (as of Aug 4, 2016)

Horslen, BC, Dakin, CJ, Inglis, JT, Blouin, JS & Carpenter, MG (2014). Modulation of human vestibular reflexes with increased postural threat. *J Physiol* **592**, 3671-3685.

Horslen, BC, Murnaghan, CD, Inglis, JT, Chua, R & Carpenter, MG (2013). Effects of postural threat on spinal stretch reflexes: evidence for increased muscle spindle sensitivity? *J Neurophysiol* **110**, 899-906.

Hospod, V, Aimonetti, J, Roll, J & Ribot-Ciscar, E (2007). Changes in Human Muscle Spindle Sensitivity during a Proprioceptive Attention Task. *J Neurosci* **27**, 5172-5178.

Houk J & Henneman E (1967). Responses of Golgi tendon organs to active contractions of the soleus muscle of the cat. *J Neurophysiol* **30**, 466-481.

Huffman, JL, Horslen, BC, Carpenter, MG & Adkin, AL (2009). Does increased postural threat lead to more conscious control of posture? *Gait Posture* **30**, 528-532.

Hulliger, M (1984). The mammalian muscle spindle and its central control. *Rev Physiol Biochem Pharmacol* **101**, 1-110.

Hultborn, H (2001). State-dependent modulation of sensory feedback. *J Physiol* **533**, 5-13.

Inglis, JT, Horak, FB, Shupert, CL & Jones-Rycewicz, C (1994). The importance of somatosensory information in triggering and scaling automatic postural responses in humans. *Exp Brain Res* **101**, 159-164.

Inglis, JT, Shupert, CL, Hlavacka, F & Horak, FB (1995). Effect of galvanic vestibular stimulation on human postural responses during support surface translations. *J. Neurophysiol* **73**, 896-901.

Jami, L (1992). Golgi tendon organs in mammalian skeletal muscle: functional properties and central actions. *Physiol Rev* **72**, 623-666.

Jankowska, E (1992). Interneuronal relay in spinal pathways from proprioceptors. *Prog Neurobiol* **38**, 335-378.

Jankowska, E, Hammar, I, Chojnicka, B & Hedén, CH (2000). Effects of monoamines on interneurons in four spinal reflex pathways from group I and/or group II muscle afferents. *Eur J Neurosci* **12**, 701-714.

Johnson, KO (2001). The roles and functions of cutaneous mechanoreceptors. *Curr Opin Neurobiol* **11**, 455-461.

Johnson, MD & Heckman, CJ (2010). Interactions between focused synaptic inputs and diffuse neuromodulation in the spinal cord. *Ann NY Acad Sci* **1198**, 35-41.

Jones, GM & Watt, DG (1971). Muscular control of landing from unexpected falls in man. *J Physiol* **219**, 729-737.

Kalueff, AV, Ishikawa, K & Griffith, AJ (2008). Anxiety and otovestibular disorders: linking behavioral phenotypes in men and mice. *Behav Brain Res* **186**, 1-11.

Kasper, J, Diefenhardt, A, Mackert, A & Thoden, U (1992). The Vestibulo-Ocular Response during Transient Arousal Shifts in Man. *Acta Otolaryngol* **112**, 1-6.

Katz, R, Meunier, S & Pierrot-Deseilligny, E (1988). Changes in presynaptic inhibition of Ia fibres in man while standing. *Brain* **111**, 417-437.

Kennedy, PM, Carlsen, AN, Inglis, JT, Chow, R, Franks, IM & Chua, R (2003). Relative contributions of visual and vestibular information on the trajectory of human gait. *Exp Brain Res* **153**, 113-117.

Kennedy, PM, Cressman, EK, Carlsen, AN & Chua, R (2005). Assessing vestibular contributions during changes in gait trajectory. *Neuroreport* **16**, 1097-1100.

Keshner, EA, Allum, JH & Pfaltz, CR (1987). Postural coactivation and adaptation in the sway stabilizing responses of normals and patients with bilateral vestibular deficit. *Exp Brain Res* **69**, 77-92.

Khan SI & Burne JA (2007). Reflex inhibition of normal cramp following electrical stimulation of the muscle tendon. *J Neurophysiol* **98**, 1102-1107.

Khan SI & Burne JA (2009). Afferents contributing to autogenic inhibition of gastrocnemius following electrical stimulation of its tendon. *Brain Res* **1282**, 28-37.

Khan SI & Burne JA (2010). Inhibitory mechanisms following electrical stimulation of tendon and cutaneous afferents in the lower limb. *Brain Res* **1308**, 47-57.

Klomp, A, de Vlugt, E, Meskers, CGM, de Groot, JH, Arendzen, JH & van der Helm, FCT (2013). Reduction of the linear reflex gain explained from the M1–M2 refractory period. *IEEE Trans Biomed Eng.* **60**, 1721-1727.

Knellwolf, TP, Hammam, E & Macefield, VG (2016). The vestibular system does not modulate fusimotor drive to muscle spindles in relaxed leg muscles of subjects in a near-vertical position. *J Neurophysiol* **115**, 2529-2535.

Koceja, DM, Markus, CA & Trimble, MH (1995). Postural modulation of the soleus H reflex in young and old subjects. *Electroencephalogr Clin Neurophysiol*, **97**, 387-393.

Koceja, DM, Trimble, MH & Earles, DR (1993). Inhibition of the soleus H-reflex in standing man. *Brain Res* **629**, 155-158.

Komiyama, T, Zehr, EP & Stein, RB (2000). Absence of nerve specificity in human cutaneous reflexes during standing. *Exp Brain Res* **133**, 267-272.

Koutsikou, S, Crook, JJ, Earl, EV, Leith, JL, Watson, TC, Lumb, BM & Apps, R (2014). Neural substrates underlying fear-evoked freezing: the periaqueductal grey-cerebellar link. *J Physiol* **592**, 2197-2213.

Koutsikou, S, Watson, TC, Crook, JJ, Leith, JL, Lawrenson, CL, Apps, R & Lumb, BM (2015). The Periaqueductal Gray Orchestrates Sensory and Motor Circuits at Multiple Levels of the Neuraxis. *J Neurosci* **35**, 14132-14147.

Krauss, EM & Misiaszek, JE (2007). Phase-specific modulation of the soleus H-reflex as a function of threat to stability during walking. *Exp Brain Res* **181**, 665-672.

Lam, T & Pearson, KG (2001). Proprioceptive modulation of hip flexor activity during the swing phase of locomotion in decerebrate cats. *J Neurophysiol* **86**, 1321-1332.

Lam, T & Pearson, KG (2002). Sartorius muscle afferents influence the amplitude and timing of flexor activity in walking decerebrate cats. *Exp Brain Res* **147**, 175-185.

Lang, PJ, Davis, M & Öhman, A (2000). Fear and anxiety: animal models and human cognitive psychophysiology. *J Affect Disord* **61**, 137-159.

Lee Son, GM, Blouin, JS & Inglis, JT: (2008). Short-duration galvanic vestibular stimulation evokes prolonged balance responses. *J Appl Physiol* **105**, 1210-1217.

Lee, RH & Heckman, CJ (2000). Adjustable amplification of synaptic input in the dendrites of spinal motoneurons in vivo. *J Neurosci* **20**, 6734-6740.

Lepicard, EM, Venault, P, Negroni, J, Perez-Diaz, F, Joubert, C, Nosten-Bertrand, M, Berthoz, A & Chapouthier, G (2003). Posture and balance responses to a sensory challenge are related to anxiety in mice. *Psychiatry Res* **118**, 273-284.

Lepicard, EM, Venault, P, Perez-Diaz, F, Joubert, C, Berthoz, A & Chapouthier, G (2000). Balance control and posture differences in the anxious BALB/cByJ mice compared to the non anxious C57BL/6J mice. *Behav Brain Res* **117**, 185-195.

Lewis, G, Perreault, E & MacKinnon, C (2005). The influence of perturbation duration and velocity on the long-latency response to stretch in the biceps muscle. *Exp Brain Res* **163**, 361-369.

Lewis, RF, Haburcakova, C, Gong, W, Lee, D & Merfeld, D (2013). Electrical stimulation of semicircular canal afferents affects the perception of head orientation. *J Neurosci* **33**, 9530-9535.

Li, F, Fisher, KJ, Harmer, P, McAuley, E & Wilson, NL (2003). Fear of falling in elderly persons: association with falls, functional ability, and quality of life. *J Gerontol B Psychol Sci Soc Sci* **58**, P283-P290.

Liddell, BJ, Brown, KJ, Kemp, AH, Barton, MJ, Das, P, Peduto, A, Gordon, E & Williams, LM (2005). A direct brainstem-amygdala-cortical 'alarm' system for subliminal signals of fear. *Neuroimage* **24**, 235-243.

Lim, SB (2014). Modulation of stochastic vestibular stimulation-induced reflexes within a dynamic balance paradigm: the effect of response phase and emotional state (T). University of British Columbia. Retrieved from https://open.library.ubc.ca/cIRcle/collections/24/items/1.0167596 (Aug. 11, 2016; Original work published 2014).

Lim, SB, Cleworth, TW, Horslen, BC, Blouin, JS, Inglis, JT & Carpenter, MG (*in preparation*). Postural Threat Influences Vestibular-Evoked Muscular Responses.

Lim, SB, Horslen, BC, Davis, JR, Allum, JH & Carpenter, MG (2016). Benefits of multi-session balance and gait training with multi-modal biofeedback in healthy older adults. *Gait Posture* **47**, 10-17.

Lin, CC, Whitney, SL, Loughlin, PJ, Furman, JM, Redfern, MS, Sienko, KH & Sparto, PJ (2015). The effect of age on postural and cognitive task performance while using vibrotactile feedback. *J Neurophysiol* **113**, 2127-2136.

Llewellyn, M, Yang JF, & Prochazka, A (1990). Human H-reflexes are smaller in difficult beam walking than in normal treadmill walking. *Exp Brain Res* **83**, 22-28.

Lund, S & Broberg, C (1983). Effects of different head positions on postural sway in man induced by a reproducible vestibular error signal. *Acta Physiol Scand* **117**, 307-309.

Luu, BL, Inglis, JT, Huryn, TP, Van der Loos, HF, Croft, EA & Blouin, JS (2012). Human standing is modified by an unconscious integration of congruent sensory and motor signals. *J Physiol* **590**, 5783-5794.

Macefield, VG, Sverrisdottir, YB & Wallin, BG (2003). Resting discharge of human muscle spindles is not modulated by increases in sympathetic drive. *J Physiol* **551**, 1005-1011.

Maki, BE, Holliday, PJ & Topper, AK (1991). Fear of falling and postural performance in the elderly. *J Gerontol* **46**, M123-31.

Maki, BE, Perry, SD, Norrie, RG & McIlroy, WE (1999). Effect of facilitation of sensation from plantar foot-surface boundaries on postural stabilization in young and older adults. *J Gerontol Med Sci* **54**, M281-M287.

Marchand-Pauvert, V, Nicolas, G, Marque, P, Iglesias, C & Pierrot-Deseilligny, E (2005). Increase in group II excitation from ankle muscles to thigh motoneurones during human standing. *J. Physiol* **566**, 257-271.

Marigold, DS (2008). Role of peripheral visual cues in online visual guidance of locomotion. *Exerc Sport Sci Rev* **36**, 145-151.

Marsden, JF, Castellote, J & Day, BL (2002). Bipedal distribution of human vestibular-evoked postural responses during asymmetrical standing. *J Physiol* **542**, 323-331.

Marsden, JF, Playford, ED & Day, BL (2005). The vestibular control of balance after stroke. *J Neurol Neurosurg Psychiatry* **76**, 670-678.

Matthews, PB (1989). Long-latency stretch reflexes of two intrinsic muscles of the human hand analysed by cooling the arm. *J Physiol* **419**, 519-538.

Matthews, PBC (1981). Muscle spindles: Their messages and their fusimotor supply. In *Supplement 2: Handbook of Physiology, The Nervous System, Motor Control*, ed. Brooks VB, pp. 189-228. American Physiological Society, Bethesda, USA.

Maurer, C, Mergner, T, Bolha, B & Hlavacka, F (2001). Human balance control during cutaneous stimulation of the plantar soles. *Neurosci Lett* **302**, 45-48.

McCrea, DA (2001). Spinal circuitry of sensorimotor control of locomotion. *J Physiol* **533**, 41-50.

McIlroy, WE, Bishop, DC, Staines, WR, Nelson, AJ, Maki, BE & Brooke, JD (2003). Modulation of afferent inflow during the control of balancing tasks using the lower limbs. *Brain Res* **961**, 73-80.

McKenzie, NC & Brown, LA (2004). Obstacle negotiation kinematics: age-dependent effects of postural threat. *Gait Posture* **19**, 226-234.

McNulty, PA & Macefield, VG (2001). Modulation of ongoing EMG by different classes of low-threshold mechanoreceptors in the human hand. *J Physiol* **537**, 1021-1032.

McNulty, PA, Turker, KS & Macefield, VG (1999). Evidence for strong synaptic coupling between single tactile afferents and motoneurones supplying the human hand. *J Physiol* **518**, 883-893.

Meskers, CM, Schouten, A, Rich, ML, Groot, J, Schuurmans, J & Arendzen, JH (2010). Tizanidine does not affect the linear relation of stretch duration to the long latency M2 response of m. flexor carpi radialis. *Exp Brain Res* **201**, 681-688.

Meyer, PF, Oddsson, LI & De Luca, CJ (2004a). Reduced plantar sensitivity alters postural responses to lateral perturbations of balance. *Exp Brain Res* **157**, 526-536.

Meyer, PF, Oddsson, LI & De Luca, CJ (2004b). The role of plantar cutaneous sensation in unperturbed stance. *Exp Brain Res* **156**, 505-512.

Mian, OS & Day, BL (2009). Determining the direction of vestibular-evoked balance responses using stochastic vestibular stimulation. *J Physiol* **587**, 2869-2873.

Mian, OS & Day, BL (2014). Violation of the craniocentricity principle for vestibularly evoked balance responses under conditions of anisotropic stability. *J Neurosci* **34**, 7696-7703

Mian, OS, Dakin, CJ, Blouin, JS, Fitzpatrick, RC & Day, BL (2010). Lack of otolith involvement in balance responses evoked by mastoid electrical stimulation. *J Physiol* **588**, 4441-4451.

Mildren, RL, Strzalkowski, ND & Bent, LR (2016). Foot sole skin vibration perceptual thresholds are elevated in a standing posture compared to sitting. *Gait Posture* **43**, 87-92.

Miller, KC & Burne, JA (2014). Golgi tendon organ reflex inhibition following manually applied acute static stretching. *J Sports Sci* **32**, 1491-1497.

Misiaszek, JE (2003). The H-reflex as a tool in neurophysiology: its limitations and uses in understanding nervous system function. *Muscle Nerve* **28**, 144-160.

Mobbs, D, Hagan, CC, Dalgleish, T, Silston, B & Prevost, C (2015). The ecology of human fear: survival optimization and the nervous system. *Front Neurosci* **9**, 55.

Mobbs, D, Marchant, JL, Hassabis, D, Seymour, B, Tan, G, Gray, M, Petrovic, P, Dolan, RJ & Frith, CD (2009). From threat to fear: the neural organization of defensive fear systems in humans. *J Neurosci* **29**, 12236-12243.

Mobbs, D, Petrovic, P, Marchant, JL, Hassabis, D, Weiskopf, N, Seymour, B, Dolan, RJ & Frith, CD (2007). When fear is near: threat imminence elicits prefrontal-periaqueductal gray shifts in humans. *Science* **317**, 1079-1083.

Mochizuki, G, Sibley, KM, Cheung, HJ & McIlroy, WE (2009). Cortical activity prior to predictable postural instability: Is there a difference between self-initiated and externally-initiated perturbations? *Brain Res* **1279**, 29-36.

Montoya, P & Sitges, C (2006). Affective modulation of somatosensory-evoked potentials elicited by tactile stimulation. *Brain Res* **1068**, 205-212.

Moore, KL & Dalley, AF (1999). *Clinically Oriented Anatomy*. Lippincott Williams & Williams, USA.

Mouchnino, L, Fontan, A, Tandonnet, C, Perrier, J, Saradjian, AH, Blouin, J & Simoneau, M (2015). Facilitation of cutaneous inputs during the planning phase of gait initiation. *J Neurophysiol* **114**, 301-308.

Murnaghan, CD, Horslen, BC, Inglis, JT & Carpenter, MG (2011). Exploratory behavior during stance persists with visual feedback. *Neuroscience* **195**, 54-59.

Murnaghan, CD, Squair, JW, Chua, R, Inglis, JT & Carpenter, MG (2013). Are increases in COP variability observed when participants are provided explicit verbal cues prior to COM stabilization? *Gait Posture* **38**, 734-738.

Nafati, G, Rossi-Durand, C & Schmied, A (2004). Proprioceptive control of human wrist extensor motor units during an attention-demanding task. *Brain Res* **1018**, 208-220.

Naranjo, EN (2015). Modulation of vestibular-evoked reflexes and oculomotor function when standing under height-induced states of fear, anxiety and arousal (T). University of British Columbia. Retrieved from https://open.library.ubc.ca/cIRcle/collections/24/items/1.0166145 (Aug. 11, 2016; Original work published 2015)

Naranjo, EN, Allum, JH, Inglis, JT & Carpenter MG (2015). Increased gain of vestibulospinal potentials evoked in neck and leg muscles when standing under height-induced postural threat. *Neuroscience* **293**, 45-54.

Naranjo, EN, Cleworth, TW, Allum, JH, Inglis, JT, Lea, J, Westerberg, BD & Carpenter, MG (2016). Vestibulo-spinal and vestibulo-ocular reflexes are modulated when standing with increased postural threat. *J Neurophysiol* **115**, 833-842.

Nardone, A & Schieppati, M (2004). Group II spindle fibres and afferent control of stance. Clues from diabetic neuropathy. *Clin Neurophysiol* **115**, 779-789.

Nardone, A, Grasso, M & Schieppati, M (2006). Balance control in peripheral neuropathy: Are patients equally unstable under static and dynamic conditions? *Gait Posture* **23**, 364-373.

Nardone, A, Grasso, M, Giordano, A & Schieppati, M (1996). Different effect of height on latency of leg and foot short- and medium- latency EMG responses to perturbation of stance in humans. *Neurosci Lett* **206**, 89-92.

Nashner, L & Berthoz, A (1978). Visual contribution to rapid motor responses during postural control. *Brain Res* **150**, 403-407.

Noga, BR, Bras, H & Jankowska, E (1992). Transmission from group II muscle afferents is depressed by stimulation of locus coeruleus/subcoeruleus, Kölliker-Fuse and raphe nuclei in the cat. *Exp Brain Res* **88**, 502-516.

Öhman, A (2005). The role of the amygdala in human fear: automatic detection of threat. *Psychoneuroendocrinology* **30**, 953-958.

Okada, S, Hirakawa, K, Takada, Y & Kinoshita, H (2001). Relationship between fear of falling and balancing ability during abrupt deceleration in aged women having similar habitual physical activities. *Eur J Appl Physiol* **85**, 501-506.

Olofsson, JK, Nordin, S, Sequeira, H & Polich, J (2008). Affective picture processing: an integrative review of ERP findings. *Biol Psychol* **77**, 247-265.

Olufsen, MS, Alston, AV, Tran, HT, Ottesen, JT & Novak, V (2008). Modeling heart rate regulation--part I: sit-to-stand versus head-up tilt. *Cardiovasc Eng* **8**, 73-87.

Osler CJ, Tersteeg MC, Reynolds RF & Loram ID (2013). Postural threat differentially affects the feedforward and feedback components of the vestibular-evoked balance response. *Eur J Neurosci* **38**, 3239-3247.

Oude Nijhuis, LB, Allum, JHJ, Borm, GF, Honegger, F, Overeem, S & Bloem, BR (2009). Directional Sensitivity of "First Trial" Reactions in Human Balance Control. *J Neurophysiol* **101**, 2802-2814.

Paré, D, Quirk, GJ & Ledoux, JE (2004). New vistas on amygdala networks in conditioned fear. *J Neurophysiol* **92**, 1-9.

Patla, AE & Vickers, JN (1997). Where and when do we look as we approach and step over an obstacle in the travel path? *Neuroreport* **8**, 3661-3665.

Pauli, P, Amrhein, C, Mühlberger, A, Dengler, W & Wiedemann, G (2005). Electrocortical evidence for an early abnormal processing of panic-related words in panic disorder patients. *International Journal of Psychophysiology* **57**, 33-41.

Pavlik, AE, Inglis, JT, Lauk, M, Oddsson, L & Collins, JJ (1999). The effects of stochastic galvanic vestibular stimulation on human postural sway. *Exp Brain Res* **124**, 273-280.

Pearson, KG & Collins, DF (1993). Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *J Neurophysiol* **70**, 1009-1017.

Pearson, KG & Gordon, J (2000). Spinal Reflexes. In *Principles of Neural Science*, 4th edn, ed. Kandel ER, Schwartz JH & Jessell TM, pp. 713-736. McGraw-Hill, New York, NY, USA.

Pearson, KG (1995). Proprioceptive regulation of locomotion. *Curr Opin Neurobiol* **5**, 786-791.

Pearson, KG (2008). Role of sensory feedback in the control of stance duration in walking cats. *Brain Research Reviews*, **57**, 222-227.

Perry, SD, McIlroy, WE & Maki, BE (2000). The role of plantar cutaneous mechanoreceptors in the control of compensatory stepping reactions evoked by unpredictable, multi-directional perturbation. *Brain Res* **877**, 401-406.

Peters, RM, Blouin, JS, Dalton, BH & Inglis, JT (2016). Older adults demonstrate superior vestibular perception for virtual rotations. *Exp Gerontol* **82**, 50-57.

Peters, RM, Hackeman, E & Goldreich, D (2009). Diminutive digits discern delicate details: fingertip size and the sex difference in tactile spatial acuity. *J Neurosci* **29**, 15756-15761.

Peters, RM, Rasman, BG, Inglis, JT & Blouin, JS (2015). Gain and phase of perceived virtual rotation evoked by electrical vestibular stimuli. *J Neurophysiol* **114**, 264-273.

Petersen, N, Christensen, LO, Morita, H, Sinkjaer, T & Nielsen, J (1998). Evidence that a transcortical pathway contributes to stretch reflexes in the tibialis anterior muscle in man. *J Physiol* **512**, 267-276.

Petersen, TH, Rosenberg, K, Petersen, NC & Nielsen, JB (2009). Cortical involvement in anticipatory postural reactions in man. *Exp Brain Res* **193**, 161-171.

Pickering, TG, James, GD, Boddie, C, Harshfield, GA, Blank, S & Laragh, JH (1988). How common is white coat hypertension? *JAMA* **259**, 225-228.

Pierrot-Deseilligny, E, Katz, R & Morin, C (1979). Evidence for lb inhibition in human subjects. *Brain Res* **166**, 176-179.

Pierrot-Deseilligny, E, Morin, C, Bergego, C & Tankov, N (1981). Pattern of group I fibre projections from ankle flexor and extensor muscles in man. *Exp Brain Res* **42**, 337-350.

Pierrot-Desseilligny, E & Burke, D (2012). The circuitry of the human spinal cord spinal and corticospinal mechanisms of movement. Cambridge University Press, Cambridge, UK.

Pompeiano, O (1972). Vestibulospinal relations: vestibular influences on gamma motoneurons and primary afferents. *Prog Brain Res* **37**, 197-232.

Priori A, Berardelli A, Inghilleri M, Pedace F, Giovannelli M & Manfredi M (1998). Electrical stimulation over muscle tendons in humans. Evidence favouring presynaptic inhibition of Ia fibres due to the activation of group III tendon afferents. *Brain* **121**, 373-380.

Prochazka, A (1989). Sensorimotor gain control: A basic strategy of motor systems? *Prog Neurobiol* **33**, 281-307.

Prochazka, A (1996). Proprioceptive feedback and movement regulation. In *Section 12: Handbook of Physiology, Exercise: Regulation and Integration of Multiple Systems*, ed. Rowell LB & Shepherd JT, pp. 89-127. Oxford, New York, United States of America.

Prochazka, A (2015). Sensory control of normal movement and of movement aided by neural prostheses. *J Anat* **227**, 167-177.

Prochazka, A, Hulliger, M, Trend, P & Durmuller, N (1988). Dynamic and static fusimotor set in various behavioural contexts. In *Mechanoreceptors: Development, structure, and function*, ed. Hnik P, Soukup T, Vejsada R & Zelena J, pp. 417-430. Plenum Press, New York, NY.

Prochazka, A, Hulliger, M, Zangger, P & Appenteng, K (1985). 'Fusimotor set': new evidence for α-independent control of γ-motoneurones during movement in the awake cat. *Brain Res* **339**, 136-140.

Prochazka, A, Mushahwar, VK & McCreery, DB (2001). Neural prostheses. *J Physiol* **533**, 99-109.

Proske, U & Gandevia, SC (2012). The proprioceptive senses: their roles in signaling body shape, body position and movement, and muscle force. *Physiol Rev* **92**, 1651-1697.

Proske, U, Morgan, DL & Gregory, JE (1993). Thixotropy in skeletal muscle and in muscle spindles: A review. *Prog Neurobiol* **41**, 705-721.

Proske, U, Tsay, A & Allen, T (2014). Muscle thixotropy as a tool in the study of proprioception. *Exp Brain Res* **232**, 3397-3412.

Quant, S, Adkin, AL, Staines, WR & McIlroy, WE (2004). Cortical activation following a balance disturbance. *Exp Brain Res* **155**, 393-400.

Reynolds, RF (2010). The effects of voluntary sway control on the early and late components of the vestibular-evoked postural response. *Exp Brain Res* **201**, 133-139

Reynolds, RF, Osler, CJ, Tersteeg, MCA & Loram, ID (2015a). CrossTalk opposing view: Fear of falling does not influence vestibular-evoked balance responses. *J Physiol* **593**, 2983-2984

Reynolds, RF, Osler, CJ, Tersteeg, MCA & Loram, ID (2015b). Rebuttal from Raymond Reynolds, Callum Osler, Linda Tersteeg and Ian Loram. *J Physiol* **593**, 2987

Reynolds, RF, Osler, CJ, Tersteeg, MCA & Loram, ID (2015c). Last words on fear of falling does not influence vestibular-evoked balance responses. *J Physiol* (online) http://onlinelibrary.wiley.com/store/10.1113/JP270269/asset/supinfo/tjp6714-sup-0003-Loram.pdf?v=1&s=d3c371e6eb85815139686d7dccb25851e5ffe2f8 (as of Aug 4, 2016)

Ribot-Ciscar, E, Hospod, V, Roll, J & Aimonetti, J (2009). Fusimotor Drive May Adjust Muscle Spindle Feedback to Task Requirements in Humans. *J Neurophysiol* **101**, 633-640.

Robertson, DD & Ireland, DJ (1995). Vestibular evoked myogenic potentials. *J Otolaryngol* **24**, 3-8.

Rogasch, NC, Burne, JA & Turker, KS (2012). Comparison of the inhibitory response to tendon and cutaneous afferent stimulation in the human lower limb. *J Neurophysiol* **107**, 564-572.

Rogasch, NC, Burne, JA, Binboğa, E & Türker, KS (2011). Synaptic potentials contributing to reflex inhibition in gastrocnemius following tendon electrical stimulation. *Clin Neurophysiol* **122**, 1190-1196.

Rosenberg, JR, Amjad, AM, Breeze, P, Brillinger, DR & Halliday, DM (1989). The Fourier approach to the identification of functional coupling between neuronal spike trains. *Prog Biophys Mol Biol* **53**, 1-31.

Rosengren, SM, Welgampola, MS & Colebatch, JG (2010). Vestibular evoked myogenic potentials: past, present and future. *Clin Neurophysiol* **121**, 636-651.

Rossignol, S, Dubuc, R & Gossard, JP (2006). Dynamic sensorimotor interactions in locomotion. *Physiol Rev* **86**, 89-154.

Rozenkrants, B, Olofsson, JK & Polich, J (2008). Affective visual event-related potentials: arousal, valence, and repetition effects for normal and distorted pictures. *Int J Psychophysiol* **67**, 114-123.

Sattin, RW (1992). Falls among older persons: a public health perspective. *Annu Rev Public Health* **13**, 489-508.

Sayenko, DG, Vette, AH, Kamibayashi, K, Nakajima, T, Akai, M & Nakazawa, K (2007). Facilitation of the soleus stretch reflex induced by electrical excitation of plantar cutaneous afferents located around the heel. *Neurosci Lett* **415**, 294-298.

Sayenko, DG, Vette, AH, Obata, H, Alekhina, MI, Akai, M & Nakazawa, K (2009). Differential effects of plantar cutaneous afferent excitation on soleus stretch and H-reflex. *Muscle Nerve* **39**, 761-769.

Schieppati, M & Nardone, A (1995). Time course of 'set'-related changes in muscle responses to stance perturbation in humans. *J Physiol* **487**, 787-796.

Schieppati, M & Nardone, A (1997). Medium-latency stretch reflexes of foot and leg muscles analysed by cooling the lower limb in standing humans. *J Physiol* **503**, 691-698.

Schieppati, M & Nardone, A (1999). Group II spindle afferent fibers in humans: their possible role in the reflex control of stance. *Prog Brain Res* **123**, 461-472.

Schieppati, M, Nardone, A, Siliotto, R & Grasso, M (1995). Early and late stretch responses of human foot muscles induced by perturbation of stance. *Exp Brain Res* **105**, 411-422.

Schubert, M, Curt, A, Jensen, L & Dietz, V (1997). Corticospinal input in human gait: modulation of magnetically evoked motor responses. *Exp Brain Res* **115**, 234-246.

Schuurmans, J, de Vlugt, E, Schouten, AC, Meskers, CG, de Groot, JH & van der Helm, FC (2009). The monosynaptic Ia afferent pathway can largely explain the stretch duration effect of the long latency M2 response. *Exp Brain Res* **193**, 491-500.

Shaw, JA, Stefanyk, LE, Frank, JS, Jog, MS & Adkin, AL (2012). Effects of age and pathology on stance modifications in response to increased postural threat. *Gait Posture* **35**, 658-661.

Sherrington, CS (1910). Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *J Physiol* **40**, 28-121.

Shumway-Cook, A, Ciol, MA, Hoffman, J, Dudgeon, BJ, Yorkston, K & Chan, L (2009). Falls in the Medicare population: incidence, associated factors, and impact on health care. *Phys Ther* **89**, 324-332.

Sibley, KM, Carpenter, MG, Perry, JC & Frank, JS (2007). Effects of postural anxiety on the soleus H-reflex. *Hum Mov Sci* **26**, 103-112.

Sibley, KM, Mochizuki, G, Frank, JS & McIlroy, WE (2010). The relationship between physiological arousal and cortical and autonomic responses to postural instability. *Exp Brain Res* **203**, 533-540.

Sinkjaer, T, Andersen, JB, Ladouceur, M, Christensen, LO & Nielsen, JB (2000). Major role for sensory feedback in soleus EMG activity in the stance phase of walking in man. *J Physiol* **523**, 817-827.

Staab, JP, Balaban, CD & Furman, JM (2013). Threat assessment and locomotion: clinical applications of an integrated model of anxiety and postural control. *Semin Neurol* **33**, 297-306.

Stephens, MJ & Yang, JF (1996). Short latency, non-reciprocal group I inhibition is reduced during the stance phase of walking in humans. *Brain Res* **743**, 24-31.

Stins, JF & Beek, PJ (2007). Effects of affective picture viewing on postural control. *BMC Neurosci* **8**, 83.

Swett, JE & Eldred, E (1960) Distribution and numbers of stretch rceptors in medial gastrocnemius and soleus muscles of the cat. *Anat Rec* **137**, 453-460.

Taake, I, Jaspers-Fayer, F & Liotti, M (2009). Early frontal responses elicited by physical threat words in an emotional Stroop task: Modulation by anxiety sensitivity. *Biol Psychol* **81**, 48-57.

Taube, W, Schubert, M, Gruber, M, Beck, S, Faist, M & Gollhofer, A (2006). Direct corticospinal pathways contribute to neuromuscular control of perturbed stance. *J Appl Physiol* **101**, 420-429.

Tersteeg, MCA, Marple-Horvat, DE & Loram, ID (2012). Cautious gait in relation to knowledge and vision of height: is altered visual information the dominant influence? *J Neurophysiol* **107**, 2686-2691.

Thomas, SJ, Gonsalvez, CJ & Johnstone, SJ (2013). Neural time course of threat-related attentional bias and interference in panic and obsessive-compulsive disorders. *Biol Psychol* **94**, 116-129.

Thomas, SJ, Johnstone, SJ & Gonsalvez, CJ (2007). Event-related potentials during an emotional Stroop task. *Int J Psychophysiol* **63**, 221-231.

Thoumie, P & Do, MC (1996). Changes in motor activity and biomechanics during balance recovery following cutaneous and muscular deafferentation. *Exp Brain Res* **110**, 289-297.

Timmann, D, Belting, C, Schwarz, M & Diener, HC (1994). Influence of visual and somatosensory input on leg EMG responses in dynamic posturography in normals. *Electroencephalogr Clin Neurophysiol* **93**, 7-14.

Todd, NPM, Rosengren, SM, Aw, ST & Colebatch, JG (2007). Ocular vestibular evoked myogenic potentials (OVEMPs) produced by air- and bone-conducted sound. *Clin Neurophysiol* **118**, 381-390.

Tokuno, CD, Carpenter, MG, Thorstensson, A, Garland, SJ & Cresswell, AG (2007). Control of the triceps surae during the postural sway of quiet standing. *Acta Physiol (Oxf)* **191**, 229-236.

Tokuno, CD, Garland, SJ, Carpenter, MG, Thorstensson, A & Cresswell, AG (2008). Sway-dependent modulation of the triceps surae H-reflex during standing. *J Appl Physiol* **104**, 1359-1365.

Tokuno, CD, Taube, W & Cresswell, AG (2009). An enhanced level of motor cortical excitability during the control of human standing. *Acta Physiol (Oxf)* **195**, 385-395.

Turker KS, Yang J & Scutter SD (1997). Tendon tap induces a single long-lasting excitatory reflex in the motoneurons of human soleus muscle. *Exp Brain Res* **115**, 169-173.

Vallbo, AB, Hagbarth, KE, Torebjork, HE & Wallin, BG (1979). Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiological Reviews* **59**, 919-957.

Van Dieën JH, Allum JH, Young WR, Williams AM, Colebatch JG and Duysens J (2015) Comments on CrossTalk 22: Fear of falling does influence vestibular-evoked balance responses. *J Physiol* (online) http://onlinelibrary.wiley.com/store/10.1113/JP270269/asset/supinfo/tjp6714-sup-0001-Comments.pdf?v=1&s=5b6b48e9d0932a38bb95ac0152d08ec1d6cfc207 (as of Aug 4, 2016)

Van Doornik, J, Azevedo Coste, C, Ushiba, J & Sinkjaer, T (2011). Positive afferent feedback to the human soleus muscle during quiet standing. *Muscle Nerve* **43**, 726-732.

Van Wezel, BM, Ottenhoff, FA & Duysens, J (1997). Dynamic control of location-specific information in tactile cutaneous reflexes from the foot during human walking. *J Neurosci* **17**, 3804-3814.

Venables, PH (1991). Autonomic activity. *Ann N Y Acad Sci* **620**, 191-207.

Venault, P, Rudrauf, D, Lepicard, EM, Berthoz, A, Jouvent, R & Chapouthier, G (2001). Balance control and posture in anxious mice improved by SSRI treatment. *Neuroreport* **12**, 3091-3094.

Ventre-Dominey, J, Nighoghossian, N & Denise, P (2003). Evidence for interacting cortical control of vestibular function and spatial representation in man. *Neuropsychologia* **41**, 1884-1898.

Vidal, PP, Berthoz, A & Millanvoye, M (1982). Differene between eye closure and visual stabilization in the control of posture in man. *Aviat Space Environ Med* **53**, 166-170.

Visser, JE, Oude Nijhuis, LB, Janssen, L, Bastiaanse, CM, Borm, GF, Duysens, J & Bloem, BR (2010). Dynamic posturography in Parkinson's disease: diagnostic utility of the "first trial effect". *Neuroscience* **168**, 387-394.

Wada, M, Sunaga, N & Nagai, M (2001). Anxiety affects the postural sway of the antero-posterior axis in college students. *Neurosci Lett* **302**, 157-159.

Ward, BK, Redfern, MS, Jennings, JR & Furman, JM (2008). The influence of cognitive tasks on vestibular-induced eye movements in young and older adults. *J Vestib Res* **18**, 187-195.

Wardman, DL, Taylor, JL & Fitzpatrick, RC (2003). Effects of galvanic vestibular stimulation on human posture and perception while standing. *J Physiol* **551**, 1033-1042.

Watson, SRD & Colebatch, JG (1998). Vestibular-evoked electromyographic responses in soleus: a comparison between click and galvanic stimulation. *Exp Brain Res* **119**, 504-510.

Welgampola, MS & Colebatch, JG (2005). Characteristics and clinical applications of vestibular-evoked myogenic potentials. *Neurology* **64**, 1682-1688.

Wilson, VJ & Peterson, BW (1981). Vestibulospinal and reticulospinal systems. In Supplement 2: Handbook of Physiology, The Nervous System, Motor Control, ed. Brooks VB, pp. 667-702.

Winter, DA, Patla, AE, Prince, F, Ishac, M & Gielo-Perczak, K (1998). Stiffness Control of Balance in Quiet Standing. *J Neurophysiol* **80**, 1211-1221.

Wong, JD, Wilson, ET & Gribble, PL (2011). Spatially selective enhancement of proprioceptive acuity following motor learning. *J Neurophysiol* **105**, 2512-2521.

Wright, WG, Ivanenko, YP & Gurfinkel, VS (2012). Foot anatomy specialization for postural sensation and control. *J. Neurophysiol* **107**, 1513-1521.

Yanagawa, S, Shindo, M & Nakagawa, S (1991). Increase in Ib inhibition by antagonistic voluntary contraction in man. *J Physiol* **440**, 311-323.

Yang, JF & Stein, RB (1990). Phase-dependent reflex reversal in human leg muscles during walking. *J Neurophysiol* **63**, 1109-1117.

Yardley, L & Redfern, MS (2001). Psychological factors influencing recovery from balance disorders. *J Anxiety Disord* **15**, 107-119.

Yardley, L, Watson, S, Britton, J, Lear, S & Bird, J (1995). Effects of anxiety arousal and mental stress on the vestibulo-ocular reflex. *Acta Otolaryngol* **115**, 597-602.

Young, LR (1984). Perception of the body in space: mechanisms. In *Supplement 3: Handbook of Physiology, The Nervous System, Sensory Processes*, ed. Darian-Smith I, pp. 1023-1066.

Zaback, M, Carpenter, MG & Adkin, AL (2016b). Threat-induced changes in attention during tests of static and anticipatory postural control. *Gait Posture* **45**, 19-24.

Zaback, M, Cleworth, TW, Carpenter, MG & Adkin, AL (2015). Personality traits and individual differences predict threat-induced changes in postural control. *Hum Mov Sci* **40**, 393-409.

Zaback, M, Horslen, BC, Cleworth, TW, Collings, L, Langlet, C, Inglis, JT & Carpenter, MG (2016a). The influence of physiological arousal on lower-limb cutaneous reflexes during treadmill walking. *SFN 2016* (Abstract - accepted), ID#: 10134.

Zehr, EP & Chua, R (2000). Modulation of human cutaneous reflexes during rhythmic cyclical arm movement. *Exp Brain Res* **135**, 241-250.

Zehr, EP (2002). Considerations for use of the Hoffmann reflex in exercise studies. *Eur J Appl Physiol* **86**, 455-468.

Zehr, EP, Collins, DF & Chua, R (2001). Human interlimb reflexes evoked by electrical stimulation of cutaneous nerves innervating the hand and foot. *Exp Brain Res* **140**, 495-504.

Zehr, EP, Hesketh, KL & Chua, R (2001). Differential regulation of cutaneous and H-reflexes during leg cycling in humans. *J Neurophysiol* **85**, 1178-1184.

Zehr, EP, Komiyama, T & Stein, RB (1997). Cutaneous Reflexes During Human Gait: Electromyographic and Kinematic Responses to Electrical Stimulation. *J Neurophysiol* **77**, 3311-3325.

Zehr, EP, Stein, RB & Komiyama, T (1998). Function of sural nerve reflexes during human walking. *J Physiol* **507**, 305-314.

Appendix 1 – Psychological Questionnaires

The following questionnaires were used in all studies included in this dissertation to assess participants' self-report of balance confidence, fear of falling, perceived stability and state anxiety.

Balance Confidence:

Please use the following scale to rate how confident you are that you can maintain your balance and avoid a fall 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

Fear of Falling:

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

Percieved Stability:

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

State Aniexty:

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

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

3. I had self doubts when standing at this height

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

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

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

7. My body was tense when standing at this height

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

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

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

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

12. My heart was racing when standing at this height

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

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

15. I found myself hyperventilating when standing at this height

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