CONTEXT-DEPENDENT PROCESSING OF VESTIBULAR SIGNALS FOR BALANCE AND ORIENTATION

by

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ABSTRACT

The control of balance and orientation comprises various forms of sensory input, reflexive action and anticipatory mechanisms. An important sensory input is the vestibular system. When a destabilising or disorientating perturbation is sensed by the vestibular apparatus a corrective response is generated. This thesis investigated how the processing of vestibular signals is affected by postural and sensory context. Orientation reflexes evoked by a vestibular signal of head roll were shown to be continuously modulated and even reversed direction during self-generated head pitch movements (Chapter 2). Results also raised the possibility that the direction of a vestibular-evoked balance reflex is automatically rotated following adaptation of motor output (Chapter 3). In addition to modulating the response direction, the context was also found to affect the response amplitude. Passive cutaneous sensory input was shown to attenuate a vestibular-evoked balance reflex (Chapter 4). If, however, such changes in sensory context were anticipated, then response amplitude was unchanged (Chapter 5). Furthermore, the initial balance reflex was not affected by a fear of falling (Chapter 6). The present findings demonstrate that the processing of vestibular signals is indeed context-dependent. However, the modulation of vestibular-evoked reflexes is seemingly automatic, and is not affected by cognition or emotion.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AD……. Angular deviation

CCI……... Co-contraction index

CF……. Contact force

CNS…….. Central nervous system

EMG……. Electromyography/electromyographic data

Fx…….. Mediolateral force vector

Fy…….. Anteroposterior force vector

GVS……... Galvanic vestibular stimulation

RM ANOVA……. Repeated measures analysis of variance

SC…….. Skin conductance

SD…….. Standard deviation

SEM…….. Standard error of the mean

VOR…….. Vestibulo-ocular reflex

ON THE LEVEL

"*We have five senses in which we glory and which we recognise and celebrate, senses that constitute the sensible world for us. But there are other senses – secret senses, sixth senses, if you will – equally vital, but unrecognised, and unlauded. These senses, unconscious, automatic, had to be discovered.*

"The complex mechanisms and controls by which our bodies are properly aligned and balanced in space – these have only been defined in our own century, and still hold many mysteries. Perhaps it will only be in this space age, with the paradoxical licence and hazards of gravity-free life, that we will truly appreciate our inner ears, our vestibules and all the other obscure receptors and reflexes that govern our body orientation.

"For normal man, in normal situations, they simply do not exist."

(Oliver Sacks, 1985)

CHAPTER 1.

GENERAL INTRODUCTION

1.1. Balance and Orientation

1.1.1. Balance

Standing upright, without losing balance, seems very simple and effortless to most humans. However, compared with the majority of other mammals this task is relatively complex. Unlike mammals which predominantly stand on all four limbs, humans walk and stand upright on only two limbs. Evolving in this way has its advantages, but has left humans inherently unstable. In order to maintain this upright posture and avoid toppling over, one must demonstrate ability to balance – the centre of gravity of the body must be kept within the boundaries of the base of support. But adhering to this rule is tricky, as the base of support is relatively small. The centre of gravity of the human body is also relatively high (i.e. positioned a long way from the ground), further reducing stability. Due to these intrinsic characteristics of standing on two limbs, humans must minimise displacement of the body, and hence the centre of gravity, to prevent a fall. Therefore, in order to *effortlessly* stand, systems must be in place which monitor and control body sway.

1.1.2. Orientation

A sense of orientation is also evidently present and seems to be equally effortless in normal situations. An internal representation of the orientation of the body within the environment, or

the spatial relationship between the body and the external world, is constructed. In order to determine which direction one is facing, or to navigate from one location to another, an ability to detect and control whole body orientation must be demonstrated. When walking along a straight corridor this may seem simple, but it is possible to maintain a sense of orientation in more demanding situations. For instance, when completing a maze or exploring an unfamiliar city for the first time, where meaningful visual cues are limited and the body is repeatedly turned relative to the environment. In order to sense and maintain orientation, the relative motion between the body and environment must be monitored and controlled.

1.1.3. Sensory inputs

As the control of body position relative to the external world is required for both balance and orientation, sensory inputs which signal movement of one's body, or self-motion, are of use. We are very aware of some of these inputs, such as vision, touch and hearing – three of the traditional 'five senses'. However, others are seemingly much less apparent.

One of the least apparent senses involves receptors located in the vestibular organs of the inner ears, sensitive to motion of the head. The sensory information derived therefore signals self-motion. In particular circumstances, for example when there is inadequate information from other sensory inputs, stability is dramatically impaired in individuals with complete loss of vestibular function (Martin, 1965;Nashner *et al.*, 1982). In addition, subjects with vestibular lesions demonstrate lateral deviation during target-directed linear walking (Borel *et al.*, 2004). These results establish that vestibular signals of self-motion contribute to balance and orientation.

Vision allows us to 'see' the world around us. Light that has bounced off our surroundings is focused onto light sensitive receptors that cover the retinal surface of the eye. Sensory information is then passed to the brain, where the visual image is processed. This sense is obviously useful for detecting objects and motion in the external world, such as when gathering food, avoiding predators, playing sport and crossing busy streets. The recognition of visual landmarks also clearly contributes to the sense of orientation. But vision is also useful in sensing self motion, as the visual field moves relative to a moving individual (termed optic flow; Gibson, 1958). The rate and direction of optic flow corresponds to the rate and direction of self-motion, and the centre of the flow pattern during locomotion indicates the direction of movement (Gibson, 1958). Lee and Lishman (1975) studied the use of visual information in the control of balance using a 'moving room' paradigm, whereby the walls surrounding a person could be slowly moved. During such movements of the room subjects tended to sway in the same direction, suggesting visual references are used to detect body sway. In addition, postural sway is increased with the eyes closed (Edwards, 1946).

When we touch any object or surface, an array of cutaneous sensory nerve endings and mechanoreceptors in the skin sense the pressure with which we touch it, its texture and any relative motion between it and our skin. If the contact surface is fixed in place, any change in pressure or movement of the skin across its surface must indicate self-motion. During standing, the most obvious points of contact between the skin and the outside world are the soles of the feet. The sensory information derived from receptors that cover the soles of the feet contributes to balance control; by exposing the feet to hypothermia this information is removed and greater sway is demonstrated when the body is perturbed (Magnusson *et al.*, 1990a;1990b). Contact between other areas of skin and external surfaces can also arise during

standing, such as when holding a walking stick or leaning on a wall. In these examples mechanical stabilisation is inevitably provided. However, previous findings also suggest the sensory input derived from light touch contact alone can provide significant stabilisation (Holden *et al.*, 1994;Jeka & Lackner, 1994).

First coined by Sherrington (1906), the 'proprio-ceptive field' is sensitive to changes within the organism itself. Proprioception allows humans a sense of body and limb movement, position and relative orientation, by way of receptors within the musculoskeletal framework. These receptors constantly signal muscle length, velocity of muscle movements, muscle contraction force and joint position. The 'sense of movement' had previously been described as 'kinaesthesis' (Bastian, 1887). Although the terms 'proprioception' and 'kinaesthesia' are often interchanged, they in fact encompass different senses. By Sherrington's (1906) definition the vestibular organs of the inner ear are proprioceptors but cutaneous receptors are not, they are exteroceptors. However, cutaneous receptors do play a role in kinaesthesia (McCloskey, 1978). Thus, for clarity in the current thesis, cutaneous receptors and the vestibular system will both be referred to explicitly. The term 'proprioception' will refer to subcutaneous receptors in the muscle (e.g. spindles, Golgi tendon organs) and joints (e.g. Pacinian corpuscles, Ruffini endings of the joint capsule). Excitation of proprioceptive receptors using muscle vibration induces postural adjustments (Eklund, 1972) and changes in the perceived body orientation (Lackner, 1988), which suggests that the sense is useful in the control of balance and orientation. The effects are most likely a response to an illusion of altered muscle length, as the sensory input from muscle spindles is modulated by vibration (Goodwin *et al.*, 1972).

The auditory system is used to determine the location of audible sounds relative to the head (sound localization; for review see Middlebrooks & Green, 1991). A change in localization may represent motion of the sound source, but if the sound source is static, any relative change in localization must represent self-motion. Thus, auditory cues can be useful in detecting orientation relative to external sound sources. For example, it has been demonstrated that blind subjects use auditory cues to return to their starting position after several changes in orientation (Juurmaa & Suonio, 1975). With regard to balance control, although auditory cues have been demonstrated to improve stability in the absence of vision, the magnitude of this effect was relatively small and required a speaker to be placed adjacent to each ear (Easton *et al.*, 1998). In naturally occurring auditory environments the stabilization provided by the auditory system, if any, is likely to be very small.

The aforementioned sensory inputs are signalled to the central nervous system (CNS), which clearly uses information from these inputs for balance and orientation, as changes in these abilities are evident when these inputs are lost, removed, enhanced or perturbed.

1.1.4. Anticipatory mechanisms

When the body is unexpectedly perturbed, balance and orientation adjustments in response to sensory feedback are relied upon. However, if self-motion is expected, anticipatory mechanisms may also contribute to the control of balance and orientation. When anticipated, as a result of one's own actions or external factors, it is possible to make the necessary adjustments even before destabilising or disorientating circumstances are signalled by sensory inputs.

Anticipatory mechanisms associated with self-generated action have been demonstrated in the control of balance (for review see; Massion, 1992). For example, the centre of gravity is moved forward and upward prior to a rapid bilateral voluntary arm movement which would, if not countered, perturb the centre of gravity backward and downward (Bouisset & Zattara, 1981). Furthermore, a torsional moment about the vertical axis is also demonstrated prior to a unilateral arm movement which would rotate the body if it were not countered (Bouisset & Zattara, 1987). Thus, the command for voluntary movement is preceded by a command for an anticipatory postural adjustment, which counters any forces that would otherwise disturb balance and orientation. Similar anticipatory adjustments are also associated with disturbances that not only involve an individual's own body segments, but also external factors. For example, an anticipatory adjustment precedes the sudden and destabilising removal of an external load from an individual's outstretched arms, even when triggered by a minor action that does not itself cause a postural disturbance (Aruin & Latash, 1995).

These mechanisms, which operate prior to a destabilising or disorientating event, have also been demonstrated when an anticipated perturbation fails to happen. For example, the postural consequences of stepping onto a moving escalator require an appropriate adjustment in order to maintain balance. However, an overshoot of forwards trunk displacement is even displayed when an individual steps on to a broken and therefore stationary escalator (Reynolds & Bronstein, 2003). This suggests that an individual does not solely rely on sensory inputs to detect a moving support surface, but continues to use anticipatory mechanisms operating prior to foot contact.

In addition to anticipation made prior to movement, a prediction of position and orientation can be derived from motor output, during movement. Helmholtz (1866 cited in Carpenter, 2002) first suggested motor output is used in this way, to sense the position of the eye relative to the head during eye movements. When a motor command for eye movement is generated, a prediction of the corresponding change in eye position is made. Ordinarily, during active eye movement, this allows movement of the retinal image to be correctly attributed to the predicted eye movement. However, if the eye is passively moved, for example by displacing it with a finger, movement of the retinal image is incorrectly perceived as movement of oneself relative to the world. This is because, in the absence of motor output, a prediction of eye movement is not made. Von Holst and Mittelstaedt (1950) later termed a copy of motor output as an efference copy, and proposed it as useful not only in the positioning of the eye, but also in the positioning of limbs for posture, locomotion and orientation. That is, with any motor command a copy is used to predict the resulting change in position. It has since been demonstrated that the motor command during active motion contributes to joint position sense (Gandevia *et al.*, 2006).

1.1.5. Integration of information

Previous findings clearly demonstrate that many sensory inputs and anticipatory mechanisms contribute to the control of human balance and orientation. However, most of the time the CNS constantly receives information from many sources.

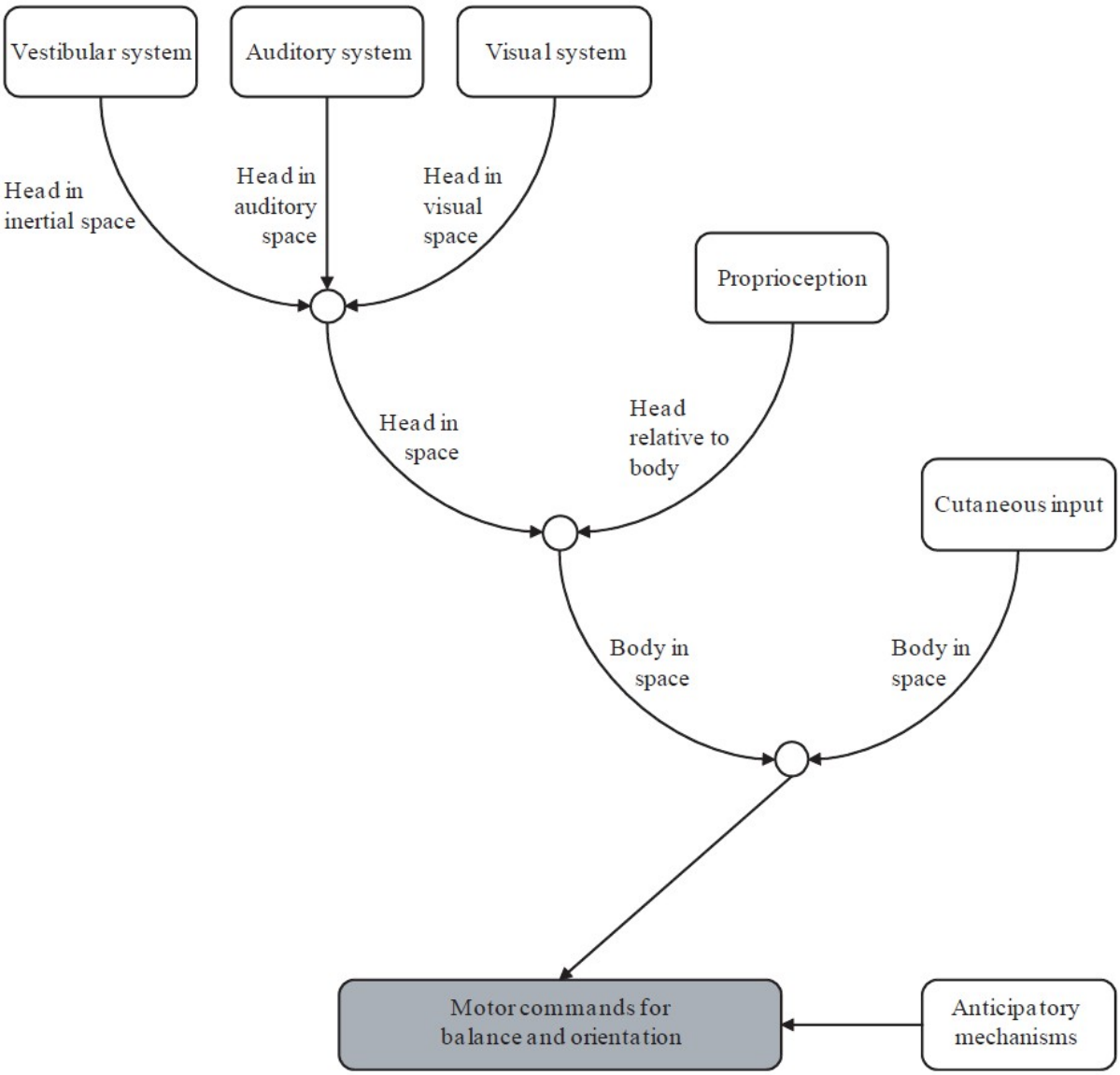

Figure 1.1. Sources of information for balance and orientation

This diagram indicates the many sources of information that are relevant for balance and orientation, including sensory inputs and anticipatory mechanisms. For full description see main text. Adapted from Carpenter (2002).

As Figure 1.1 illustrates, each relevant sensory input is sensitive to subtly different information about head or body position. The vestibular, visual (aided by efference copy), and auditory systems indicate the position of the head in space. However, this is somewhat ineffective in sensing whole body motion, without accurate information regarding the head position relative to the rest of the body. In order to overcome this problem, proprioception –

which is sensitive to the relative position of the head and body – can be used. Therefore, a combination of sensory inputs allows an accurate sense of body position in space. In addition, anticipatory mechanisms can be used to predict self motion. By integrating all sources of information, the appropriate motor commands can be generated in order to control balance and orientation.

Although many inputs are useful for balance and orientation, there is clearly a degree of redundancy. That is to say, not all inputs are required at all times. For example, while reduced stability is demonstrated in blind compared to sighted individuals, they are able to maintain upright stance (Edwards, 1946). Furthermore, blindfolded subjects are able to walk towards a memorised target, suggesting the sense of orientation does not rely on visual inputs (Borel *et al.*, 2004). Cutaneous and proprioceptive inputs are also not essential, as subjects with a loss of sensory input from the soles of the feet (Magnusson *et al.*, 1990a) or feet and ankles (Horak *et al.*, 1990) demonstrate only small increases in postural sway during normal stance. The remarkable case of patient IW also demonstrates that, with time and a large amount of effort, the control of balance and orientation is even possible with no sense of light touch, movement or position below the neck, providing visual inputs are available (Cole, 1995).

Vestibular loss does not largely affect balance and orientation in normal conditions. Individuals with vestibular deficits demonstrate, at most, only small decreases in stability, providing either visual or support surface inputs are available. However, profound instability is evident when both visual and support surface inputs become unreliable (Nashner *et al.*, 1982;Horak *et al.*, 1990). Similarly, following unilateral vestibular loss individuals have no lasting problems with orientation during locomotion, providing visual inputs are available.

But if the eyes are closed, these individuals demonstrate an impaired ability to orientate their locomotion (Borel *et al.*, 2004). These results demonstrate that other sensory inputs may provide sufficient information if vestibular signals for balance and orientation are unavailable.

1.2. The Vestibular System

1.2.1. Vestibular anatomy and physiology

Sometimes referred to as 'balance-organs', the peripheral vestibular organs form the non-auditory part of the inner ears. They are used for balance, orientation, perception of self-motion, reflex eye movements and possibly even the regulation of blood pressure (Yates, 1992). Located bilaterally and fixed within the skull, each vestibular organ comprises three semicircular canals and two otolith organs (see Figure 1.2A), the structure of which makes them sensitive to rotational and linear motion, respectively. Afferent signals pass from the end organs to the CNS along the vestibular afferent nerve, a division of cranial nerve VIII. The vestibular afferent fibres are never silent; even when the head is stationary there is a resting discharge. Although not recorded in humans, in the squirrel monkey the resting discharge is on average ~90 spikes/sec for neurons which innervate the semicircular canals (Goldberg & Fernandez, 1971a;Goldberg & Fernandez, 1971b) and ~60 spikes/sec for those which innervate the otolith organs (Fernandez *et al.*, 1972;Fernandez & Goldberg, 1976). From this resting level, discharge is modulated in either direction as the vestibular organs detect motion (see Figure 1.2D).

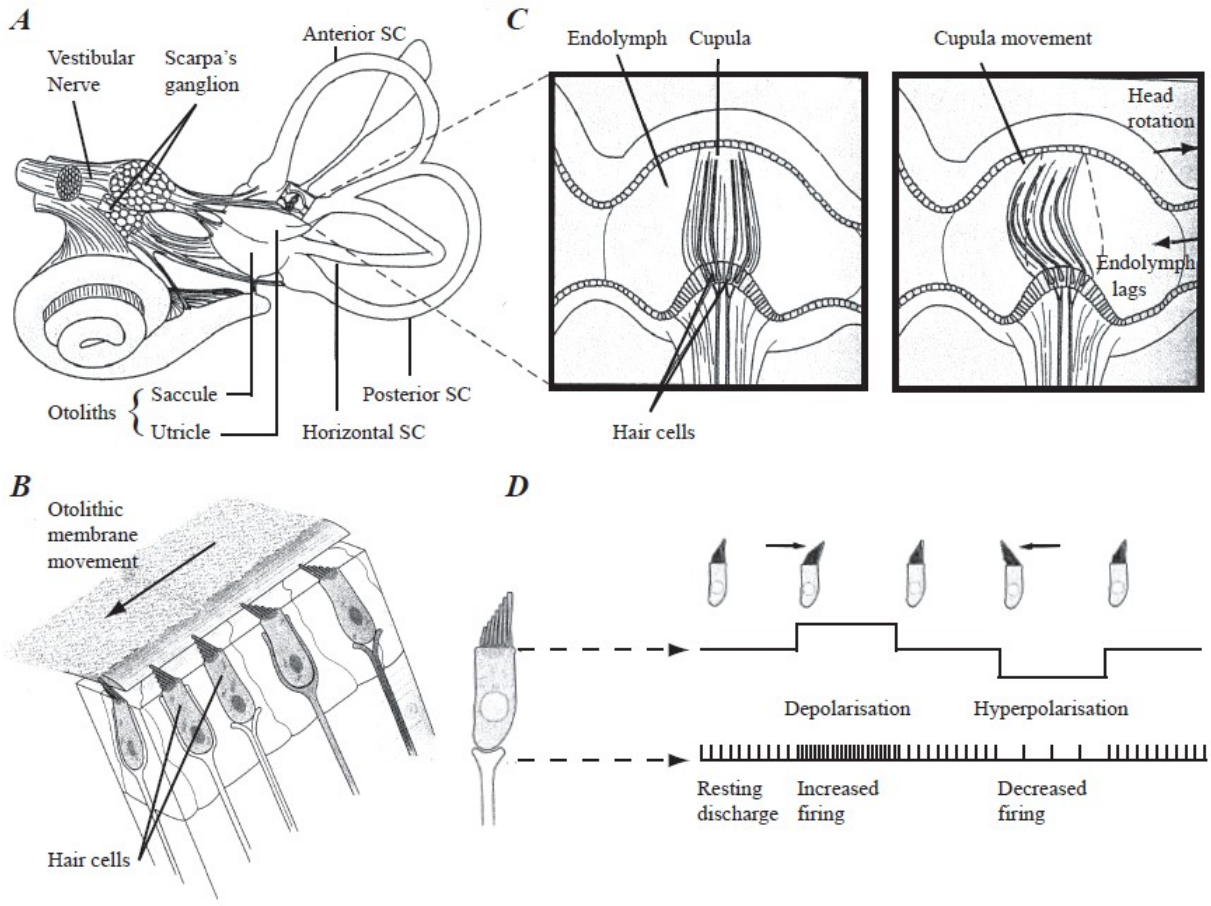

Figure 1.2. The vestibular end organ

A) The vestibular end organ comprises three semicircular canals (anterior, posterior and horizontal SCs) and two otolith organs (saccule and utricle). Motion of the head is detected and signalled along the vestibular afferent nerve. ***B***) The inside surface of the otolith organs are covered with hair cells. During head tilt or linear acceleration, a gelatinous membrane moves across these hair cells causing them to bend. ***C***) Each semicircular canal is filled with endolymph. The cupula (a structure projecting into the canal at its base) is free to move with relative motion of the endolymph. During head rotation, the endolymph lags behind and causes the cupula (and hair cells which extend into it) to bend. ***D***) The discharge rates of afferent fibres are modulated when otolith membrane movement or canal cupula mechanics cause hair cells to bend. When hairs bend towards the kinocilium (the longest hair) the cell is depolarised and the firing rate is increased. When the hairs bend away from the kinocilium the cell is hyperpolarised and firing rate is reduced. Thus, the end organ encodes head acceleration into a neural signal. Adapted from Goldberg and Hudspeth (2000)

The otolith organ consists of a saccule and utricle. A specialised area on the inside surface of each of these components is covered with hair cells, which project into a gelatinous mass weighted with otolith particles (see Figure 1.2B). Each hair cell consists of many shorter

hairs, or sterocilia, and one longer hair, known as the kinocilium. Relative movement between the gelatinous mass and otolith surface bends these hair cells, which in turn, modulate the firing rate of vestibular afferent fibres. The hair cells are depolarised if sterocilia bend towards the kinocilium and hyperpolarised if sterocilia bend away from the kinocilium, leading to an increased or decreased firing rate of afferent fibres, respectively (Fernandez *et al.*, 1972;Fernandez & Goldberg, 1976; see Figure 1.2D). During linear motion (or translation), the gelatinous mass lags behind the otolith surface and hair cells, due to its inertia. During head tilt, gravity acts upon the gelatinous mass, causing it to move across the otolith surface. Hence, both translation and tilt cause relative movement of the gelatinous mass and otolith surface, and both types of motion are detected by the otolith organs. In fact, the effects of tilt and translation on afferent firing are identical, making the otolith signal somewhat ambiguous. For instance, as nose-up tilt and forward acceleration signals are identical, pilots can experience a false climb illusion, if other sensory inputs are insufficient to resolve the tilt-translation ambiguity (Federal Aviation Administration, 2008).

Each semicircular canal is a looped tube filled with endolymph fluid. At a slight swelling at its base, a gelatinous structure known as the cupula projects into the canal (see Figure 1.2C). The semicircular canal cupula is free to move with the endolymph and, as a result, hair cells embedded into its structure also bend. Importantly, the cupula is not weighted by otolith particles, but is the same density as the surrounding endolymph. Therefore, it is not moved by gravity, only by endolymph movement caused by rotation of the head. During such rotation the canal moves but the endolymph lags behind, moving the cupula, and bending its hair cells. Much like the hair cells in the otolith organs, the direction in which they bend determines if they are depolarised or hyperpolarised, which, in turn, determines if the spontaneous

discharge is increased or decreased (Lowenstein & Sand, 1936;Goldberg & Fernandez, 1971a; see Figure 1.2D). Their looped structure means cupula movement predominantly occurs when rotation is about an axis perpendicular to the canal alignment. However, with three semicircular canals on each side of the head, it is possible to detect rotation about three different axes. Due to their alignment, the three canals have been termed the horizontal, posterior and anterior canals (see Figure 1.3A) and excitation of any given canal is mirrored by a bilateral partner. For example, head rotation about a vertical axis to the left depolarizes the left horizontal canal and hyperpolarizes the right horizontal canal (see Figure 1.3B). Bilateral pairings also exist between anterior canals on one side and posterior canals on the other.

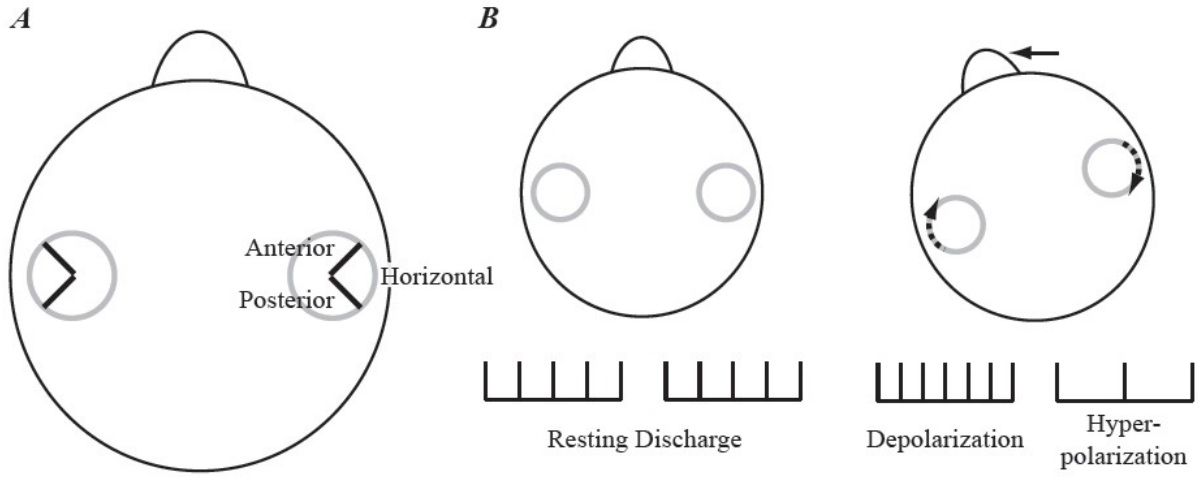

Figure 1.3. Semicircular canal alignment and mirrored bilateral pairings

A) The approximate orientation of the three semicircular canals. Anterior and posterior canals (black lines) and horizontal canal (grey circle) are approximately perpendicular to each other. ***B***) Bilateral pairing of horizontal semi-circular canals. Head rotation about a vertical axis (right hand side), causes endolymph movement in the horizontal canals (dashed arrows). This modulates the resting discharge of afferent fibres. The modulation on one side is mirrored by the bilateral partner. In this case, an increase and decrease are shown in left and right horizontal canals, respectively.

The cell bodies of afferent nerve fibres, which synapse hair cells of the end organs, form the vestibular ganglion (or Scarpa's ganglion). The ganglion has two divisions. The superior division is connected to the anterior and horizontal canals, the utricle, and a portion of the saccule. The inferior division is connected to the posterior canal and the main portion of the saccule. The two divisions, along with afferent fibres from the cochlea, comprise the vestibulocochlear nerve (Cranial nerve VIII). Although some vestibular afferent fibres project directly to the cerebellum, most project to the ipsilateral vestibular nuclei complex of the brainstem (Carleton & Carpenter, 1984) and the processing of vestibular signals largely takes place here (Dickman, 1997). The vestibular nuclei complex, located in the rostral medulla and caudal pons, consists of four nuclei, namely, the medial, lateral, superior and descending nuclei (see Figure 1.4A).

But vestibular afferents are not the sole input to the vestibular nuclei complex (see dashed connections in Figure 1.4B). Afferents from the optic system provide visual information and afferents from the spinal cord provide proprioceptive information (Dickman, 1997;Pompeiano, 1972). The nuclei also share reciprocal connections with the contralateral vestibular nuclei, the reticular formation, other brainstem nuclei and the cerebellum (Carleton & Carpenter, 1983;Balaban, 2002). There are a large number of commissural connections between contralateral vestibular nuclei (Carleton & Carpenter, 1983), which may allow the comparison of vestibular signals from each side, and may be of use for compensation or adaptation following unilateral vestibular loss (Dickman, 1997). Reciprocal links with the cerebellum probably regulate the processing of vestibular signals, or adjust processing under changed conditions (Manzoni, 2005).

There are also other efferent connections (see solid neurons in Figure 1.4B), some of which allow the vestibular nuclei complex to act on motoneurons and hence generate movement. Ascending fibres to the oculomotor nuclei allow compensatory eye movements to be generated (Dickman, 1997). More relevant for balance and orientation are the descending vestibulospinal tracts, originating in the medial and lateral vestibular nuclei (Brodal, 2010; see Figure 1.4C). These descending tracts provide a pathway for the modulation of motor unit firing rates, allowing vestibular signals to evoke whole-body motor responses. Descending fibres either excite motor neurons directly or terminate on interneurons down the spinal cord. The previously mentioned connections with the reticular formation provide an additional pathway to carry efferent commands to the spinal cord, along descending reticulospinal tracts.

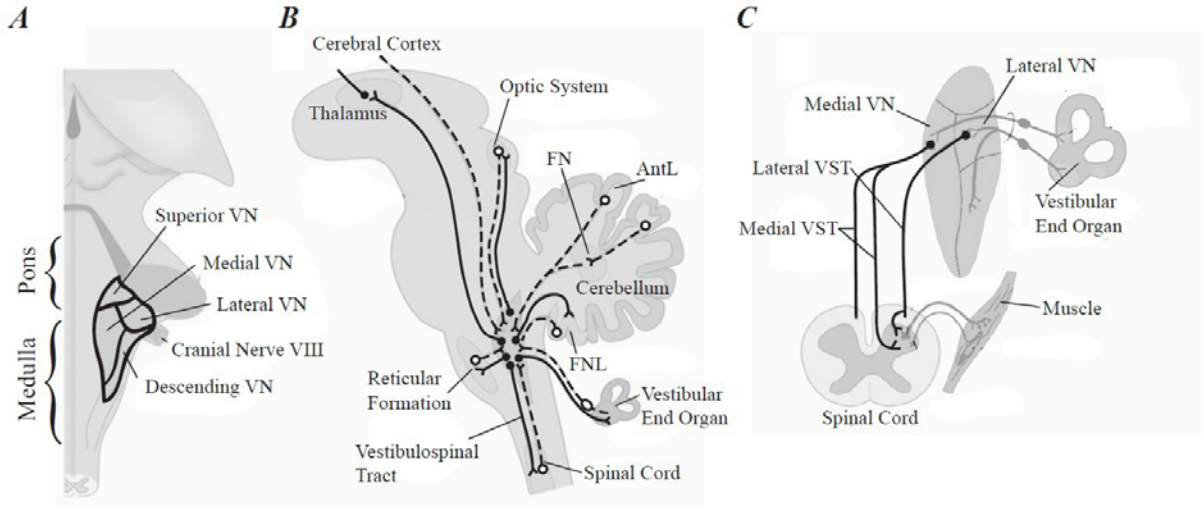

Figure 1.4. Afferent and efferent connectivity of the vestibular nuclei complex

A) Vestibular afferent signals pass along cranial nerve VIII to the vestibular nuclei complex, which is located in the brainstem. It consists of the superior, medial, lateral and descending vestibular nuclei (VN). ***B***) The vestibular nuclei complex shares many afferent (dashed) and efferent (solid) links with other structures of the CNS. These structures include the spinal cord, optic system, reticular formation, cerebral cortex and cerebellum. FNL= flocculo-nodular lobe, FN= fastigial nucleus, AntL= anterior lobe. ***C***) Descending vestibulospinal tracts (VST) form efferent connections with motor neurons which innervate trunk and limb regions, thus allowing modulation of activity in muscles involved in balance and orientation. Adapted from Brodal (2010).

There are also connections from the vestibular nuclei to a range of cortical regions via the thalamus (Corticovestibular interactions; for review see Fukushima, 1997). In animal studies a vestibular cortical system has been proposed, which includes areas 2v, 3a and the parieto-insular vestibular cortex (Guldin & Grusser, 1998). In humans, imaging studies have shown that vestibular stimulation activates analogous cortical areas (Lobel *et al.*, 1998). This vestibular cortical system, along with other sensory inputs to the cerebral cortex, may be involved in the cognitive perception of motion, spatial orientation and spatial memory. Furthermore, in animal studies there is evidence for descending projections from the cortex to the vestibular nuclei complex (Akbarian *et al.*, 1993;1994;Wilson *et al.*, 1999). Such connections potentially affect the processing of vestibular signals.

In summary, the vestibular end organs are structured to sense motion of the head and signal this information to the CNS along the vestibular afferent nerves. Although the processing of these signals takes place mainly in the vestibular nuclei complex, there is a large degree of both afferent and efferent connectivity between the vestibular nuclei complex and other systems within the CNS. Ultimately, projections to the ocular system, cortex and motor system gives rise to eye movements, cognitive perception of self-motion and motor responses, respectively. However, the degree of convergence of many signals suggests any eventual response to a vestibular input is likely to be modulated by other sensory inputs and possibly even by higher level systems. This convergence and integration is required as the afferent vestibular signal, which encodes head motion, is not useful unless interpreted in the current postural and sensory context.

1.2.2. Vestibular-evoked balance reflexes

The meaning of the term reflex is open to debate and is therefore hard to define. Describing a movement as reflexive without clarifying exactly what is meant by the term may be confusing (Prochazka *et al.*, 2000). The Oxford English Dictionary Online (2011) defines a reflex, in physiology, as an action "performed independently of the will, as an automatic response to a sensory stimulus". However, sometimes reflexes are assumed to also be invariant, reproducible or simple movements (Prochazka *et al.*, 2000). In this thesis, I will closely follow the Dictionary's definition. That is to say, a movement classified as reflexive may be variable and quite complex, but will always be an automatic stimulus-bound movement which does not appear to have been chosen by the individual.

For example, when cutaneous receptors sense we have touched something hot, a reflex response to withdraw the hand is quickly and automatically generated. Such withdrawal reflexes, as well as many others, involve the spinal cord. Other reflexes involve cranial nerves and the brain stem. In both cases, reflex pathways do not pass through the cortex, thus making the response extremely fast. Through connections between the vestibular end organs and the brainstem, vestibular information is largely used in eliciting fast reflex responses.

An example of a reflex response driven by vestibular information is the vestibulo-ocular reflex (VOR). From the vestibular nuclei of the brain stem, ascending projections to the oculomotor nuclei produce reflex eye movements, as motion of the head is sensed by the vestibular end organs. The reflex eye movements occur at a latency of less than 10ms (Aw *et*

al., 1996;2006;2008), in order to keep the image of the external world stable on the retina during head motion.

Vestibular signals also evoke balance reflexes. From the vestibular nuclei of the brain stem, descending projections to muscles of the trunk and limbs produce reflex muscle responses, as motion of the head is sensed by the vestibular end organs. In the event of a sudden fall of the head and body to one side, these reflexes evoke a pattern of muscular excitation and inhibition, which generates the necessary forces to counter the fall and keep the body upright. Reflexes of this type are sometimes referred to as vestibulospinal reflexes, as they are passed from the vestibular nuclei to the spinal cord. However, the exact reflex pathway remains unknown. Involvement of vestibulospinal, reticulospinal (Britton *et al.*, 1993;Dakin *et al.*, 2007) and corticospinal (Marsden *et al.*, 2005) tracts has been proposed. In this thesis, these reflexes for balance will be referred to as vestibular-evoked balance reflexes.

Evidence of vestibular-evoked balance reflexes was demonstrated by Martin (1965). Blindfolded subjects adopted a number of postures when positioned on a bed that could be tilted from side to side. Subjects with normal vestibular function responded to a tilt of the bed with movement of the trunk and limbs, in order to keep their centre of gravity above their base of support; they were able to prevent a fall. In contrast, subjects with no vestibular function made little or no postural response, and were extremely vulnerable to falling with rapid tilts. However, slower disturbances are unlikely to evoke balance reflexes, as the vestibular system has a relatively high threshold for the perception of motion. When the vestibular system is isolated, so it is the only sensory input signalling self-motion, individuals are unable to report motion at sway velocities of less than ~1 deg/sec (Fitzpatrick &

McCloskey, 1994). Nonetheless, the results of Martin (1965) demonstrate that with greater disturbances vestibular signals clearly evoke balance reflexes. Furthermore, an increased incidence of falls among patients with bilateral vestibular deficits (Herdman *et al.*, 2000) demonstrates the importance of vestibular signals for the control of balance.

It is possible to study vestibular-evoked reflexes using a number of techniques. Firstly, the vestibular end organs can be excited by actual motion. Subjects can be pushed or pulled (Fitzpatrick & McCloskey, 1994) and their support surface can be tilted (Martin, 1965;Nashner *et al.*, 1982) or translated (Nashner *et al.*, 1982;Horak *et al.*, 1990). Alternatively, it is possible to induce a signal of virtual motion by using stimulation techniques. Caloric vestibular stimulation involves irrigating the ear canal with warm or cold water. Although additional effects have been proposed, the stimulus is believed to primarily affect the temperature of the endolymph within the neighbouring region of the horizontal semicircular canal, which in turn, causes a convection current within the canal (Jacobson & Newman, 1997). As a result endolymph movement bends the hair cell of the cupula, thus modulating the firing rate of afferent fibres, mimicking natural movement. Caloric vestibular stimulation has predominantly been used in medical practice for eliciting reflex eye moments (i.e. VOR; Mueller-Jensen *et al.*, 1987), but it is less suitable for evoking balance reflexes. A further stimulation technique, known as galvanic vestibular stimulation (GVS), involves the application of an electrical stimulus in order to induce a virtual signal of self-motion. Although eye movements can be evoked using the technique (Aw *et al.*, 2006;2008), GVS is widely used to evoke balance reflexes (for review see Fitzpatrick & Day, 2004). These techniques have the advantage that they do not affect other sensory inputs relevant to balance and orientation, thus providing a pure vestibular perturbation.

GVS involves applying a small current between two skin surface electrodes. When using a bipolar binaural configuration, the anode is applied over the mastoid process behind one ear, and the cathode applied in the equivalent position behind the opposite ear. Typically, a square wave impulse 1-2mA in amplitude is applied for just a few seconds. The stimulus modulates the firing rate of both semicircular canal and otolith afferents (Lowenstein, 1955;Kim & Curthoys, 2004). Evidence suggests the site of action is the hair cell near the trigger zone of the primary afferent nerve, bypassing the mechanics of the end organ but acting prior to the hair cell afferent synapse (Goldberg *et al.*, 1984;Aw *et al.*, 2008). Although not recorded in humans, in the squirrel monkey an applied cathodal current increases the firing rate of vestibular afferents, an anodal current decreases firing, and the amplitude of the applied current is linearly related to firing rate modulation (Goldberg *et al.*, 1984). In addition, GVS mainly acts on irregular firing afferents, rather than those classed as regular firing (Goldberg *et al.*, 1982;1984).

If one considers only the bilateral pairing of horizontal canals, an applied current induces a pattern of activity (i.e. increased firing on the cathodal side mirrored by decreased firing on the anodal side) signalling rotation about a vertical axis, with the nose moving toward the cathodal side (see *h* vectors, both ears in Figure 1.5A). However, there are 3 pairings of canals to consider and based upon animal data (Goldberg *et al.*, 1982;1984), it is assumed that GVS modulates the firing rate of all responsive semicircular canal afferents equally (Fitzpatrick & Day, 2004). Based upon this assumption, together with anatomical data regarding the orientation of the vestibular system within the skull (Blanks *et al.*, 1975), the semicircular canal signal evoked by binaural bipolar GVS has been predicted by Fitzpatrick and Day (2004). An applied stimulus induces a virtual signal of rotation about a vector orthogonal to

each canal. As the anterior and posterior canals are aligned 45 degrees to the sagittal axis of the head, the induced signal comprises components of both head roll and pitch. For example, with anodal stimulation, the anterior canal signal comprises roll towards the cathode and nose-up pitch (*a* vector, right ear in Figure 1.5A). The posterior canal signal also consists of roll towards the cathode combined with nose-down pitch, when anodal stimulation is applied (*p* vector, right ear in Figure 1.5A). Thus, the oppositely directed virtual pitch rotations effectively cancel each other out, and the resultant vector (*r* vector, right ear in Figure 1.5A) is a summation of both roll components and the yaw induced in horizontal canal afferents. Although the virtual signals of rotation induced by cathodal stimulation on the opposite side are a mirror image of anodal signals, the resultant vector once again is a summation of roll and yaw (*r* vector, left ear in Figure 1.5A). By summing resultant vectors on both sides, a net signal of roll towards the cathode about a mid-sagittal axis directed backward and pitched upwards from Reid's plane by 18.8 degrees was estimated (Fitzpatrick & Day, 2004; see L+R in Figure 1.5A). This axis will be referred to as the GVS rotation vector. Recent evidence has also revealed that the signal evoked is one of angular acceleration about this axis (St George *et al.*, 2011).

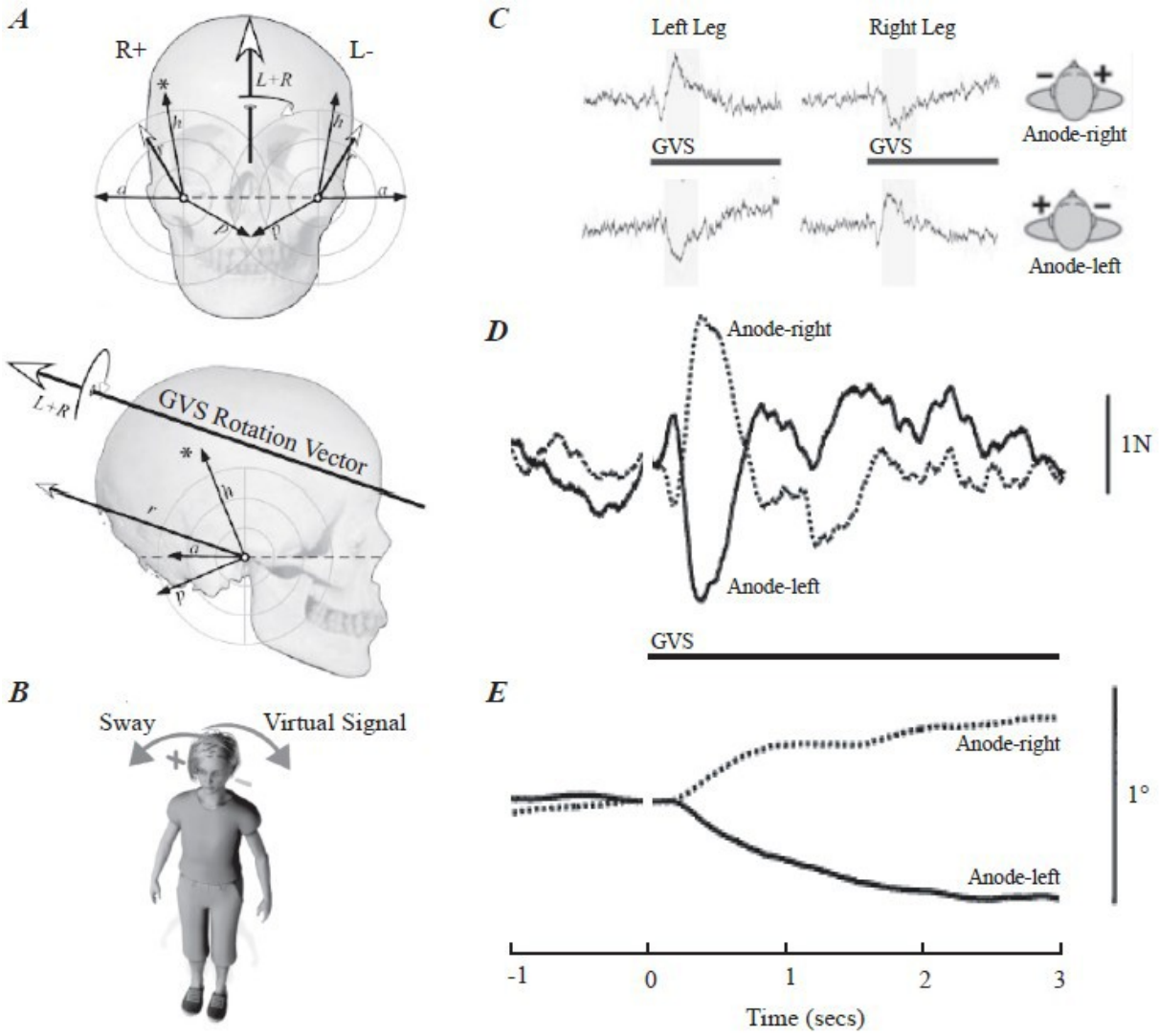

Figure 1.5. Virtual rotation induced by GVS and the evoked balance reflex

A) When GVS is applied, vestibular afferent firing rate is increased on the cathodal side (left ear, L-) and reduced on the anodal side (right ear, R+). This induces a virtual signal of rotation for each semicircular canal, as shown by rotation vectors (*h*=horizontal, *a*=anterior, *p*=posterior). Resultant vectors on each side (*r*) and the net signal for binaural bipolar GVS (L+R) were estimated by summing the virtual signal from all canals. Front and lateral views are shown. All vectors are illustrated according to the right-hand rule. ***B***) In response to an induced virtual signal of roll towards the cathode, standing subjects demonstrate whole-body sway towards the anode. ***C***) A pattern of muscle activation and inhibition shortly follows the onset of GVS. The responses evoked in medial gastrocnemius muscles are shown here. Muscle activity throughout the body generates a ***D***) lateral ground force. In turn, this translates to ***E***) whole body sway. Oppositely directed responses are demonstrated when the position of the anode and cathode are reversed. Adapted from ***A***) Fitzpatrick and Day (2004) ***B***) Day and Fitzpatrick (2005a) ***C***) Day *et al.* (2010) ***D-E***) Marsden *et al.* (2002), positive values represent ground reaction force and body motion in the direction of the anode.

The contribution of otolith afferent stimulation to the evoked response remains open to question. Binaural bipolar GVS stimulation of otolith afferents is predicted to induce a signal corresponding to either linear acceleration toward the cathodal side and/or tilt towards the anode (Fitzpatrick & Day, 2004). As indicated earlier, a tilt-translation ambiguity exists in otolith signals, as both types of movements induce identical patterns of afferent firing. Thus, it is unclear how the GVS-induced otolith signal is interpreted by the CNS. In any case, it has been proposed that this signal is relatively small in magnitude compared to the dominant semicircular canal signal and therefore plays little or no role in the evoked response (Cathers *et al.*, 2005;Mian *et al.*, 2010). Furthermore, the previously mentioned estimation, which only considers semicircular canal afferent responses, has been corroborated by psychophysical findings (Day & Fitzpatrick, 2005b). Responses evoked by GVS have therefore been attributed to the induced semicircular canal signal (Day & Fitzpatrick, 2005b;Reynolds & Osler, 2012).

In standing subjects, a GVS-induced virtual signal of head roll towards the cathode evokes a balance reflex, which ultimately manifests as whole-body sway towards the anode (Figure 1.5B). An early manifestation of this reflex is a pattern of muscle activity in the lower limbs, comprising short and medium latency components (Day *et al.*, 2010; see Figure 1.5C). The short latency component corresponds to a reduction in activity on the cathodal side, beginning around 50ms. The medium latency component corresponds to an increase in activity on the cathodal side, beginning after around 120ms. Opposite effects are seen on the anodal side. Thus, the response is oppositely directed if the position of the anode and cathode is reversed. The short latency component is often small, sometimes completely absent and the pattern of activity is not responsible for the observed sway. However, the larger medium latency

component is congruent with the evoked sway response. This pattern of muscle activity translates to a lateral force applied against the ground after around 250ms, which in turn, translates to movement of the body after 300-400ms (Marsden *et al.*, 2002; see Figure 1.5D-E). The evoked movement is a lean and bend of the body towards the anodal ear (Day *et al.*, 1997). As for the evoked muscle activity, equal and opposite force and sway responses are displayed for anode-left and anode-right GVS configurations.

1.2.3. Vestibular-evoked orientation reflexes

In the control of whole-body orientation, unintended rotations relative to the environment must be countered by an appropriate motor response. Such orientation responses can be evoked by vestibular signals which indicate whole body turn. As GVS can induce changes in perceived body orientation (Day & Fitzpatrick, 2005b;St George *et al.*, 2011), it is possible to evoke vestibular-evoked orientation responses using the technique. For example, a curved walking path can be evoked in blindfolded individuals attempting to walk straight ahead (Fitzpatrick *et al.*, 1999;Fitzpatrick *et al.*, 2006; see Figure 1.6). That is to say, if the GVS-evoked virtual signal indicates a change in whole-body orientation, then a motor response to counter the perceived rotation is demonstrated.

The exact neural pathway for orientation responses remains unknown. Although it is thought that processing of GVS-evoked signals involves subcortical regions of the CNS, it has been suggested that cortical vestibular regions are involved in the perception of body position and movement within the environment (Guldin & Grusser, 1998). Therefore, this raises the possibility that the pathway for vestibular-evoked orientation responses may involve cortical

projections. Nonetheless, by whatever pathway, vestibular signals evoke automatic motor responses which control whole body orientation.

Vestibular-evoked orientation responses have only been demonstrated to manifest as whole body turn. The exact pattern of muscle activity and ground reaction forces has not been reported. However, a vertical torque response (as demonstrated when standing still; see Reynolds, 2011) is presumably required to rotate the whole-body about the support leg, which is likely due to modulated activity in a range of muscles throughout the legs and trunk (Hase & Stein, 1999).

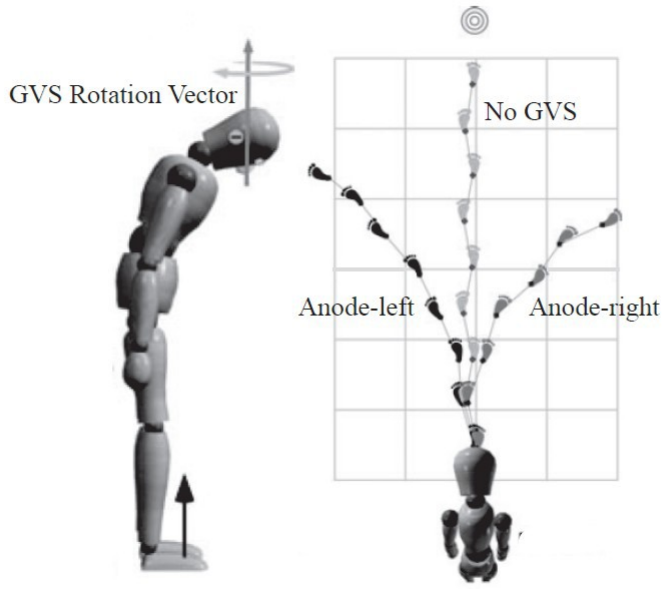

Figure 1.6. GVS-evoked orientation reflexes

If the head is pitched so that the GVS rotation vector is aligned vertical, the virtual signal of rotation induced by an applied stimulus indicates whole-body yaw. When individuals attempt to walk directly forwards with the head in this position and eyes closed, GVS evokes an orientation response. Subjects turn relative to their environment. The direction of turn is opposite for anode-left and right configurations. Head nose-up pitch also reverses the direction of turn compared to nose-down pitch. Adapted from St George and Fitzpatrick (2011).

1.2.4. Modulation of vestibular-evoked reflexes

In general, although human reflexes are automatically generated, there is evidence that they can be modulated. Take the withdrawal reflex, for example. On sensing a damaging stimulus (i.e. pain or heat) the affected limb is withdrawn. However, the response can be voluntarily suppressed, even though the reflex arc only passes to the spinal cord and back. In this case, descending projections from higher centres of the CNS influence the reflex. Although not a spinal reflex, brain stem centres involved in processing vestibular signals for balance and orientation are also linked to other areas of the CNS. Cells of the vestibular nuclei complex receive input from the vestibular organs, the spinal cord, ocular centres, other brainstem nuclei, the cerebellum and the cerebral cortex (see Section 1.2.1). This connectivity may affect the processing of vestibular signals for balance and orientation to suit the current postural context. Such modulation of vestibular-evoked reflexes has been demonstrated using GVS.

When standing, vestibular-evoked balance reflexes modulate muscle activity in the lower limbs (Nashner & Wolfson, 1974;Britton *et al.*, 1993;Fitzpatrick *et al.*, 1994) and trunk (Ali *et al.*, 2003;Ardic *et al.*, 2000). However, if the same vestibular input is applied when sitting down, lower limb activity is no longer modulated (Britton *et al.*, 1993;Fitzpatrick *et al.*, 1994;Ali *et al.*, 2003). The CNS only sends descending motor commands to muscles engaged in balancing the body. Furthermore, if other muscles are being used to support the body, they are recruited. For example, when muscles in the arm are involved in balancing the body, the vestibular-evoked reflex has been demonstrated to manifest as a modulation of triceps muscle activity (Britton *et al.*, 1993;Baldissera *et al.*, 1990). This suggests that the CNS selects which

muscles receive descending motor commands, depending upon the current postural configuration.

Different head orientations also require the processing of vestibular signals to be modulated. As vestibular signals encode head motion, an identical signal does not always indicate identical whole body motion. For an appropriate motor response to be generated to counteract a whole body disturbance, the evoked signal must first be transformed to body coordinates. For example, when facing forwards, with the head in the neutral position, a vestibular signal of motion towards the right ear should be countered with leftward body motion. However, if the head is turned by 90 degrees to face over the right shoulder, an identical vestibular signal should be countered with forward body motion. GVS has been used to demonstrate that the CNS successfully performs such coordinate transformations and modulates vestibular-evoked reflexes accordingly. Such modulation can be explained by the GVS rotation vector. For example, with the head upright the evoked balance response is always directed along the interaural line towards the anodal ear, to counter a virtual signal of motion towards the cathode (Lund & Broberg, 1983;Pastor *et al.*, 1993;Nashner & Wolfson, 1974;Britton *et al.*, 1993). Thus, if the head is turned in yaw, the response will rotate accordingly. Furthermore, a turning or vertical torque response is evoked if the head is pitched up or down (Reynolds, 2011;Fitzpatrick *et al.*, 2006), as the GVS rotation vector is aligned vertically and, therefore, the evoked signal indicates whole-body rotation about a vertical axis (see Figure 1.6). To successfully modulate the response in this way, an estimate of head orientation relative to the rest of the body is required. This estimate is not due to simply neck proprioceptive information, as the same modulation is evident whether a change in head-on-feet orientation is achieved by rotation of the head, trunk or both (Lund & Broberg, 1983). When the eyes are

closed, whole body proprioception, the otolith signal of head orientation with respect to vertical, and efference copy potentially contribute to this estimate.

Non-vestibular sensory inputs have also been demonstrated to modulate the *amplitude* of vestibular-evoked responses as well as their direction. The idea that vestibular reflexes are modulated by other sensory sources was suggested by Martin (1965) and, more recently, demonstrated using GVS. Although GVS is predominantly applied to subjects with vision occluded, when vision is allowed the evoked balance reflexes are reduced in amplitude (Britton *et al.*, 1993;Fitzpatrick *et al.*, 1994;Day *et al.*, 2002) and the orientation response is abolished. Balance reflex magnitude is also attenuated when information from cutaneous receptors is increased by light fingertip contact with a fixed support (Britton *et al.*, 1993). In contrast, reduced cutaneous inputs by anaesthesia of the soles of the feet leads to an increased balance reflex amplitude (Magnusson *et al.*, 1990b). An unstable support surface (Fitzpatrick *et al.*, 1994;Horak & Hlavacka, 2001), or sensory loss (Horak & Hlavacka, 2001;Day & Cole, 2002), both of which reduce the availability of proprioceptive information, also increase balance reflex amplitude. Non-vestibular sensory inputs have been proposed to interact with vestibular inputs by way of two processes (Day & Cole, 2002;Fitzpatrick & Day, 2004). Sensory feedback which conflicts with the virtual vestibular signal arrests the developing response, leading to attenuated peak response amplitude. However, sensory information also appears to modulate the initial response selection through feedforward mechanisms (Day & Guerraz, 2007). That is, the very early stage of the response, reflective of the response gain, is determined by pre stimulus sensory conditions. Day's proportional representation model of sensory interaction (Day *et al.*, 2002;Day & Cole, 2002) proposes that the response to a single sensory input is calculated with reference to the available information from the other inputs.

For example, the gain of one input is increased if other sensory inputs are removed. Thus, the processing of vestibular signals is modulated by the availability of non-vestibular balance-related sensory inputs.

Along with sensory inputs, anticipatory mechanisms may also modulate vestibular-evoked reflexes. When the head is actively moved the vestibular end organ mechanics are no different to those during an unexpected perturbation, and therefore also induce a corresponding vestibular signal termed reafference. However, a reflex is not evoked. It is thought that efference copy is used to cancel out the corresponding reafferent signal during self-generated movement. Evidence from animal studies shows that cells of the vestibular nuclei complex, which encode passive rotation, do not encode self-generated head movement (Roy & Cullen, 2001), but only when congruent proprioceptive input and 'expected consequences' of the motor command are present (Roy & Cullen, 2004). This suggests anticipatory signals modulate the processing of vestibular signals in the CNS, by eliminating vestibular signals corresponding to self-generated motion.

However, merely knowledge of the timing of vestibular input does not suppress the reflex response. Compared to an unpredictable stimulus, GVS-evoked balance reflexes are unaltered even when an individual presses a button to deliver the stimulus, thus making it predictable (Guerraz & Day, 2005). In addition, there appears to be no habituation to the stimulus (Guerraz & Day, 2005) and the initial response is unaffected by voluntary attempts to remain still (Reynolds, 2010). These results suggest vestibular-evoked balance reflexes are not modulated by cognitive processes and likely involve subcortical processing.

That being said, corticovestibular connections have been shown in animal studies (Akbarian *et al.*, 1993;1994;Wilson *et al.*, 1999) and have also been suggested to play a role in the processing of vestibular signals in humans (Marsden *et al.*, 2005). Marsden and colleagues (2005) showed that in generating a balance response to GVS, patients with a middle cerebral artery stroke applied asymmetric ground forces with each leg. The authors suggested that damage to projections from cortical areas to the vestibular nuclei might affect the vestibular-evoked response. The results of two patients with lesions in discrete areas of the brainstem support this explanation. A patient with a lesion in the pons above the vestibular nuclei (see Figure 1.4A for vestibular nuclei location) also demonstrated an asymmetrical response. However, in a patient with a lesion in the medulla below the vestibular nuclei, a normal response was evoked. This suggests that the altered GVS response following stroke is specifically due to damage above the vestibular nuclei. It is therefore possible that corticovestibular connections might be involved in the processing of vestibular signals.

1.3. Summary and Thesis Objectives

1.3.1. Summary

Along with other sources of information, vestibular signals are useful for the control of balance and orientation. Particularly in the event of a large balance disturbance, the vestibular system provides the CNS with information which is clearly used to evoke balance reflexes. In addition, vestibular signals can indicate whole-body rotation and, therefore, be of use in controlling body orientation. However, to use vestibular signals effectively, they must be

integrated with other sources of information by the CNS and the evoked balance or orientation response must be appropriately modulated based upon the current postural context.

The modulation of the reflex responses evoked by a vestibular input, as noted by Fitzpatrick and Day (2004), stimulated the work carried out in this thesis.

> *"These responses are not hard wired but are very sensitive to the task at hand, the balance and orientation of the body, and the information coming from all other sensory sources"*

1.3.2. Aim, objectives and experimental approach

The aim of the current thesis was to further explore how the processing of vestibular signals for balance and orientation depends upon postural context. As the raw vestibular afferent signal encodes motion of the head and is one of many inputs to the control of balance and orientation, considerable processing is evidently required between a vestibular disturbance (input/stimulus) and the evoked whole-body motor response for balance or orientation (output/response).

Initially, I was interested in how vestibular-evoked balance and orientation responses are modulated by other sensory inputs. These inputs also signal self-motion information to the CNS, in addition to vestibular derived information. They are therefore relevant for balance and orientation, and likely influence the processing of vestibular signals, in order to evoke the appropriate response for the current context. As these additional inputs also signal postural

configuration, they are likely involved in estimating head orientation, a process that is crucial for head-referenced vestibular signals to be transformed to body coordinates.

As the brain stem nuclei which process vestibular signals share reciprocal connections with other structures within the CNS, I also became increasingly interested in the function of these links. I wondered whether connections with higher levels of the CNS (such as the cerebellum and cortex), allows these areas to affect the processing of vestibular signals. In particular, I was interested in whether perceptual, anticipatory, cognitive or emotive factors can modulate vestibular-evoked reflexes.

In accordance with the thesis aim and the areas of interest outlined above, the objectives of the current thesis were to answer the following questions:

- Is the direction of a vestibular-evoked orientation reflex continuously updated during self-generated head pitch movements?
- Is the direction of a vestibular-evoked balance reflex affected by illusory changes in body orientation?
- Does passive cutaneous sensory input affect the amplitude of a vestibular-evoked balance reflex?
- Do the anticipated forthcoming sensory conditions affect the amplitude of a vestibular-evoked balance reflex?
- Does a fear of falling affect vestibular-evoked balance reflexes?

To fulfil the thesis aim and objectives, I determined whether vestibular-evoked reflexes were modulated in a number of different postural contexts. In all cases I applied GVS to human subjects with vision occluded, giving rise to vestibular-evoked balance or orientation reflexes. This experimental approach allowed a pure vestibular perturbation to be applied. The evoked response was quantified in terms of lower limb muscle activity, ground reaction forces or body motion, as measured by electromyography (EMG), a force platform and motion tracking, respectively. In some cases a combination of measures was used.

Distinct postural contexts were studied in each chapter. For instance, I began (in Chapter 2) by asking subjects to continuously move their head while I investigated the turning responses evoked by GVS. And by the end (Chapter 6), subjects stood on a narrow beam almost as high as a double-decker bus while GVS was used to induce a virtual signal of motion towards the edge! As a result, each chapter reveals a unique and novel finding. Each provides insight into the context-dependence of vestibular-evoked balance and orientation reflexes. Although termed 'reflexes' by my definition, they could not be termed this way if the most strict definition of an invariant response was followed. As a series of experiments, the combined findings of this thesis document how the processing of vestibular signals for balance and orientation is, or is not, modulated by postural context. Although not the main topic, I have also speculated on the connections within the CNS that are likely involved, in order to explain my findings.

CHAPTER 2.

VESTIBULAR SIGNALS FOR ORIENTATION DURING SELF-GENERATED HEAD MOTION

To indicate whole-body motion, the afferent vestibular signal must be transformed from head coordinates to body coordinates. Although this coordinate transformation process has been demonstrated during static head orientations, it is unclear if it remains effective during self-generated head motion. Here, GVS was used to induce a signal of head roll in blindfolded subjects stepping in place. When subjects maintained static head orientations, whole body turn was demonstrated towards the anode with the head pitched down, and towards the cathode with the head pitched up. In a second experiment, when subjects performed a prescribed head pitch movement profile, turn velocity was continuously modulated and even reversed direction as head pitch progressively changed. The direction of turn was consistent with the pattern observed during static head orientations. Therefore, an identical vestibular signal evoked a motor response which was dynamically modulated by changes in head pitch. However, response gain was significantly reduced compared to during static head orientations. This may represent a partial suppression of vestibular signals during self-generated motion. However, as the orientation response evoked by GVS was systematically modulated according to head orientation in both conditions, these results demonstrate that the coordinate transformation of exafferent vestibular signals remains effective during self-generated head motion.

2.1. Introduction

As the vestibular system is locked within the skull, an identical vestibular input can signal different whole body motion depending upon the orientation of the head. With the head level, a sensation of head roll indicates that a compensatory movement is required in the opposite direction to maintain balance. However, if the head is pitched up or down, the same vestibular input indicates a change in whole body orientation. Therefore, the raw vestibular signal in head coordinates is somewhat meaningless, without first being transformed to body coordinates according to head orientation.

GVS evokes a virtual signal of head roll about a rotation vector that is fixed within the head, specifically a naso-occipital axis elevated from Reid's plane by 18.8 degrees (Fitzpatrick & Day, 2004). This vector was predicted on the basis of anatomical data regarding the orientation of the semicircular canals (Blanks *et al.*, 1975) and on the assumption that firing rates of all canal afferents are equally modulated (Goldberg *et al.*, 1984). Empirical observations support this prediction (Day & Fitzpatrick, 2005b). Previous researchers have used GVS to show that *static* head pitch modulates vestibular-evoked reflexes for posture when standing (Reynolds, 2011) and for orientation during locomotion (Fitzpatrick *et al.*, 2006), thus demonstrating that the CNS successfully performs coordinate transformation of vestibular signals under these conditions. Fitzpatrick (2006) showed that with the head level and, therefore, the GVS virtual rotation vector approximately horizontal, a balance response was evoked. However, a turning response was evoked if the GVS rotation vector was aligned vertically, by pitching the head up or down. These results demonstrate that the vestibular-evoked response is modulated by static changes in head pitch.

But whether the response evoked by an external vestibular input is effectively modulated during self-generated head movement is unclear. This is potentially a complex task. During such movement the total vestibular input comprises signals that are evoked by external influences *and* voluntary action, termed 'exafference' and 'reafference', respectively (Von Holst & Mittelstaedt, 1950). In order to respond to an external perturbation the CNS must therefore distinguish between vestibular exafference and reafference. Neurons in the vestibular nuclei of the monkey have been shown to only encode exafference when passive whole-body rotation is combined with active head rotation (Roy & Cullen, 2001). This suggests a cancellation of vestibular reafference corresponding to self-generated head movement. However, this was not the case when passive *head* rotation (i.e. not whole-body) was combined with active head rotation (Cullen *et al.*, 2009). This raises the possibility that the cancellation of reafference related to self-generated head motion no longer occurs during a simultaneous externally-generated head rotation. Furthermore, if the externally- and self-generated components occur in different axes, then the task becomes more complex. For instance, an identical signal of head roll (as induced by GVS) must be continuously reinterpreted during a concurrent head pitch motion. This requires the estimate of head orientation to be constantly updated.

This chapter studies modulation of vestibular-evoked orientation responses when stepping in place. GVS was used to apply an external vestibular input which was shown to evoke rotary stepping in a direction systematically modulated by head pitch. I then investigated if such modulation continues to take place during self-generated head motion, where the CNS is required to distinguish vestibular exafference from reafference, and to constantly update the estimate of head orientation.

2.2. Methods

Subjects

Twelve subjects (6 male) aged 20 to 24 years (mean±SD: 21±1 years) with no known neurological or vestibular disorders gave informed consent to participate. The experiments were approved by the local ethical review committee at the University of Birmingham and performed in accordance with the Declaration of Helsinki.

Protocol

Blindfolded subjects were instructed to step in place while attempting to maintain body yaw alignment within the laboratory (i.e. continue facing the same direction) for the duration of each 60 second trial. Auditory cues were attenuated by white noise delivered through in-ear headphones. Step cadence was dictated by a metronomic beep played at 80bpm, clearly audible over the white noise. To prevent whole-body translation, subjects kept the medial borders of their feet in contact with a small rubber hemisphere, 7cm in diameter, fixed to the laboratory floor. At the end of each trial subjects were randomly rotated in both directions by the experimenter to ensure they were unaware of any turn that may have occurred when stepping.

GVS was delivered using carbon rubber electrodes (46x37mm) placed in a binaural bipolar configuration. Two electrodes were coated in conductive gel and secured on the mastoid processes using adhesive tape. In each trial, a 60 second trapezoidal signal was passed to a

stimulus isolation unit (AM Systems, Carlsborg, WA, USA). To minimise sensations on the skin, the stimulus was gradually ramped up to 1.5mA over 10s, maintained for 40s, and then ramped down in a symmetrical fashion (see Figure 2.1A). Positive values of current signify an anode-right configuration.

Experiment 1: Static head orientation

Head pitch was aligned to a target level by the experimenter prior to each trial. This was achieved by a combination of verbally directing and manually adjusting head orientation until a head-mounted laser crosshair became aligned with target lines 1m away. Subjects then attempted to maintain this head pitch whilst performing the stepping task. Five target angles (+45, +22.5, 0, -22.5 and -45 degrees) and three conditions of vestibular stimulation (anode-right, anode-left and no GVS) were used. Two trials of each combination of head pitch and vestibular stimulation were conducted, giving a total of 30 trials. Trial order was randomised.

Experiment 2: Self-generated head motion

Subjects performed a prescribed head movement profile over the duration of each trial, while performing the same stepping task. For the first 10 seconds of each trial, subjects maintained their maximum comfortable head up or down pitch, depending upon condition ('start up' or 'start down'). Over the next 20 seconds they performed a smooth movement of the head, until it was maximally pitched in the opposite direction. They then reversed the direction of movement in order to return the head to the start position over the next 20 seconds. The head was then maintained at this pitch for the final 10 seconds of the trial. Three additional 1 sec

tones distinguishable from the metronome were played through the headphones at 10, 30 and 50 seconds to provide an auditory cue of when to begin, reverse direction and stop head movement, respectively. Subjects practised this movement profile prior to the experiment (see Figure 2.3B for example of head kinematics). Two trials of each combination of movement profile and vestibular stimulation were conducted, giving a total of 12 trials. As in experiment 1, trial order was randomised.

Data Acquisition and Analysis

Head orientation was sampled at 50Hz in the form of Euler angles using a Fastrak sensor attached to a welding helmet frame (Polhemus Inc., Colchester, Vermont, USA). Head pitch was expressed as the angle of Reid's plane (line between inferior orbit and external auditory meatus) with respect to the horizontal, with negative values denoting downward head tilt. Any offset between Reid's plane and the sensor orientation was measured using a second sensor and subsequently subtracted.

Sensor yaw was used to measure whole-body turn relative to the starting position in each trial. Yaw data were low-pass filtered to remove step-by-step oscillations (0.2Hz, 4th order, zero-phase Butterworth), then differentiated to derive turning velocity. Averaged data from the control trials (no GVS) was subtracted from GVS data, in order to discount any natural turning bias. Subjects demonstrated approximately equal and opposite turn during anode-right and anode-left configurations (e.g. Figure 2.1C-D). Therefore anode-right and anode-left data were combined so that positive values indicate a turning response towards the anode.

In experiment 1, the relationship between mean head pitch and turning velocity was determined for each 40 second period of constant stimulation (i.e. 10 to 50 seconds). For large head rotations, the effects of GVS upon yaw rotation perception can be estimated as a sine function (see Figure 4 in Day and Fitzpatrick, 2005). However, in the current experiments head pitch is restricted to ±50 degrees, corresponding to a section of the sine wave which can be approximated by a linear function. Therefore, the relationship between head pitch and turning velocity was estimated by linear regression (see Figure 2.1E). The y-intercept and reciprocal of the slope of the regression line provides the point of zero rotation and response gain, respectively. The point of zero rotation is determined by the head orientation corresponding to the virtual GVS rotation vector lying horizontal. A one-sample t-test was used to compare this value to the theoretical value of 18.8° derived by Day & Fitzpatrick (2005).

For experiment 2, time-series data were split into four phases of uni-directional head movement (Start (10-30s) & End (30-50s); head moving Up & Down; see Figure 2.3B). In order to directly compare this data with those of static head pitch, this analysis was initially restricted to the range of head angles observed in experiment 1. Linear regression was then used to determine the relationship between head pitch and turn velocity during each phase (see Figure 2.3E). The point of zero rotation and response gain were calculated as in experiment 1 and analysed using a 2x2 repeated measures analysis of variance (RM ANOVA; SPSS general linear model) (time: start, end; direction: head moving up, down). As there were no significant effects of time, the start and end of trials were subsequently combined. The point of zero rotation and response gain were then calculated for upward and downward movements separately, and compared using paired t-tests.

2.3. Results

Experiment 1: Static head orientation

In subjects attempting to step in place and maintain body alignment within the laboratory, GVS evoked unconscious whole-body turning with a magnitude and direction systematically affected by static head pitch. Data from a representative subject is plotted in Figure 2.1. With the head pitched down -34° and the anode-right (solid black traces), this subject turned rightward with an average velocity of -2.34°/s, resulting in a total rotation of ~94° during the constant stimulation period. With the head pitched up 38° (solid grey traces), turning in the opposite direction with an average velocity of 1.44°/s was demonstrated. Anode-left trials caused a reversal in the direction of turn (dashed traces). For this subject, after combining stimulus polarities, linear regression revealed a significant relationship between head pitch and turning velocity (r^2=0.99, $p<0.001$), a response gain of -0.047°/s per degree of head pitch and a point of zero rotation at 22° upward pitch (see Figure 2.1E).

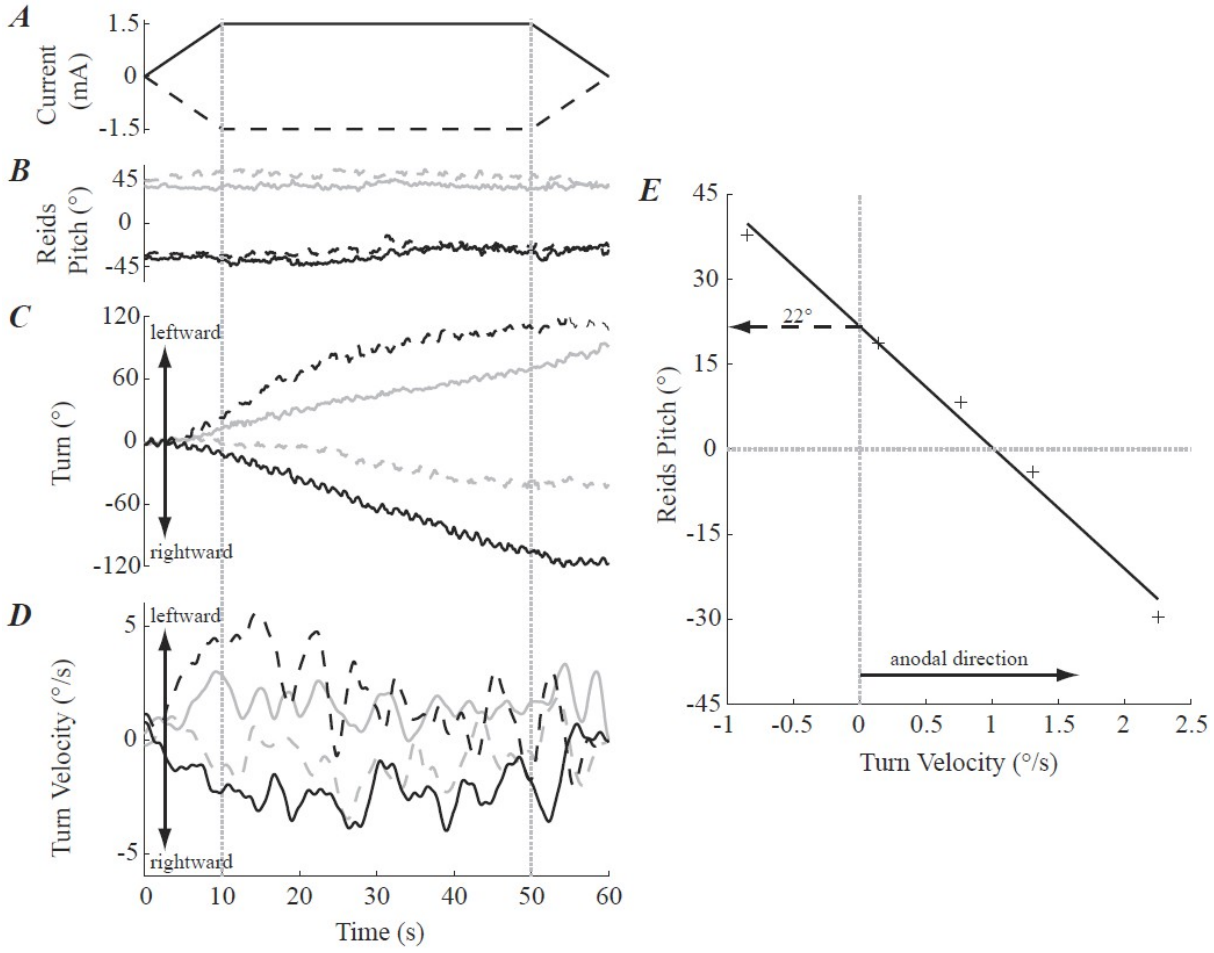

Figure 2.1. Vestibular-evoked orientation responses during static head orientations. Data from a representative subject

A) Electrical current applied in GVS trials. The trapezoidal stimulus ramped up and down over the first and last 10 seconds of each 60 second trial. In ***A-D***, individual anode-right (solid) and anode-left (dashed) trials are plotted. ***B***) Head orientation, measured as pitch angle of Reid's plane. The two extreme conditions are shown; head up +45° (grey) and down -45° (black). ***C***) Whole body turn and ***D***) turn velocity evoked by GVS. The subject turned towards the anode with the head pitched downwards and in the opposite direction with the head pitched upwards. ***E***) Linear regression was used to estimate the relationship between average head orientation and turn velocity. Average measurements were taken over the 40 seconds of constant ±1.5mA stimulation. Positive values indicate turn towards the anode. For this subject, GVS was expected to evoke zero whole-body turn at 22° head upwards pitch.

Anode-left and right trials were combined for all subjects, after confirming stimulus polarity had no significant effect on turn magnitude ($F_{1,11}$=0.31, p=0.59) and point of zero rotation (t_{11}=0.46, p=0.65). Pitch-velocity relationships for all subjects are plotted in Figure 2.2 and the associated data resulting from linear regression are reported in Table 2.1. The goodness of fit (r^2) ranged from very poor to very good. Poor fit may be explained by a low range of turning velocities demonstrated by a subject across conditions, as there was a significant correlation between the range of observed turning velocities and the goodness of fit (r=0.70, p=0.01). Mean response gain and point of zero rotation were -0.051±0.022°/s per degree of head pitch, and 21.7±10.6°, respectively. The point of zero rotation is not statistically different to the theoretical angle of 18.8° predicted by Day & Fitzpatrick (t_{11}=0.96; p=0.36). In the most extreme head up and down conditions mean head pitch angles were 35±5° and -32±8°, resulting in a range of 67±7° (mean±SD is reported throughout).

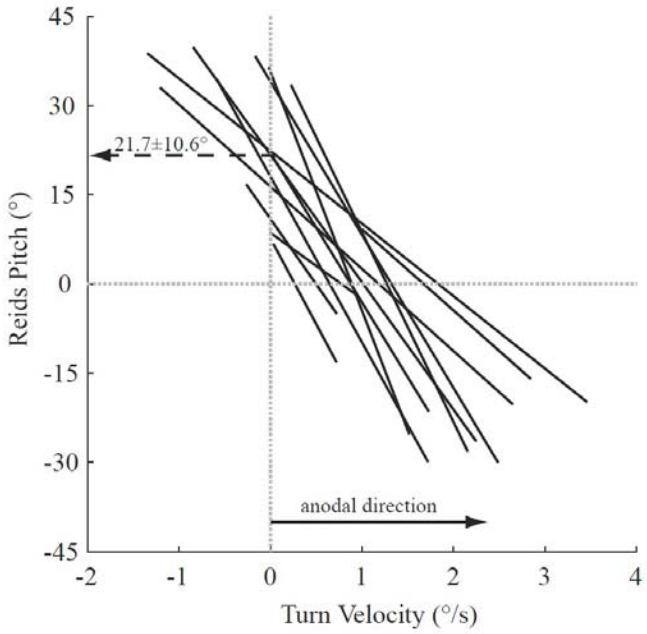

Figure 2.2. Relationship between static head orientation and vestibular-evoked turning velocity. Group data

The estimated relationship between head pitch and turning velocity is plotted for each subject. The linear equations were used to calculate the mean point of zero rotation (+21.7°, dashed arrow) and mean response gain (-0.051°/s per degree of head pitch).

Table 2.1. Relationship between head pitch and vestibular-evoked turning velocity.

The following data is shown for each subject: Angle of head pitch corresponding to zero turning velocity ('Point of zero rotation'),

	Experiment			Experiment 2 - Self-Head Movin			Head Movin		
Subject	**Point of z**	**Resp**	**Fit (r^2)**	**Point**	**Resp**	**Fit (r^2)**	**Point**	**Resp**	**Fit (r^2)**
1	8	-0	0.09	28	-0	0.70	-1	-0	0.29
2	9	-0	0.02	24	-0	0.88	8	-0	0.11
3	11	-0	0.16	12	-0	0.67	0	-0	0.23
4	16	-0	0.94	34	-0	0.78	5	-0	0.84
5	18	-0	0.93	28	-0	0.76	10	-0	0.67
6	22	-0	0.99	32	-0	0.85	15	-0	0.86
7	22	-0	0.56	39	-0	0.86	17	-0	0.10
8	22	-0	0.83	25	-0	0.98	8	-0	0.91
9	23	-0	0.18	15	-0	0.02	42	-0	0.09
10	34	-0	0.83	26	-0	0.67	7	-0	0.34
11	36	-0	0.93	1	0.02	0.76	-15	0.03	0.20
12	41	-0	0.72	36	-0	0.14	35	-0	0.57
Mea	**22**	**-0**	**0.60**	**25**	**-0**	**0.67**	**11**	**-0**	**0.43**
SD	**11**	**0.02**	**0.38**	**11**	**0.02**	**0.29**	**15**	**0.02**	**0.32**

[a] Rows are sorted in ascending order by point of zero rotation in experiment 1

Experiment 2: Self-generated head motion

During trials in experiment 2, all subjects were able to perform smooth and consistent head motion, closely matching the prescribed movement profiles (see Figures 2.3B and 2.4B). Data from a representative subject is plotted in Figure 2.3. As in experiment 1, head pitch modulated the magnitude and direction of the GVS-evoked turn (see Figure 2.3C). As head pitch changed during self-generated motion, the turning velocity was continuously modulated (see Figure 2.3D). When starting with the head pitched down (grey traces), this subject initially turned towards the anode. However, as head pitch was progressively moved into an upwards pitch, turning velocity was attenuated and eventually reversed direction. The opposite pattern is seen when starting with the head up. As in experiment 1, there is an approximately linear pitch-velocity relationship (see Figure 2.3E).

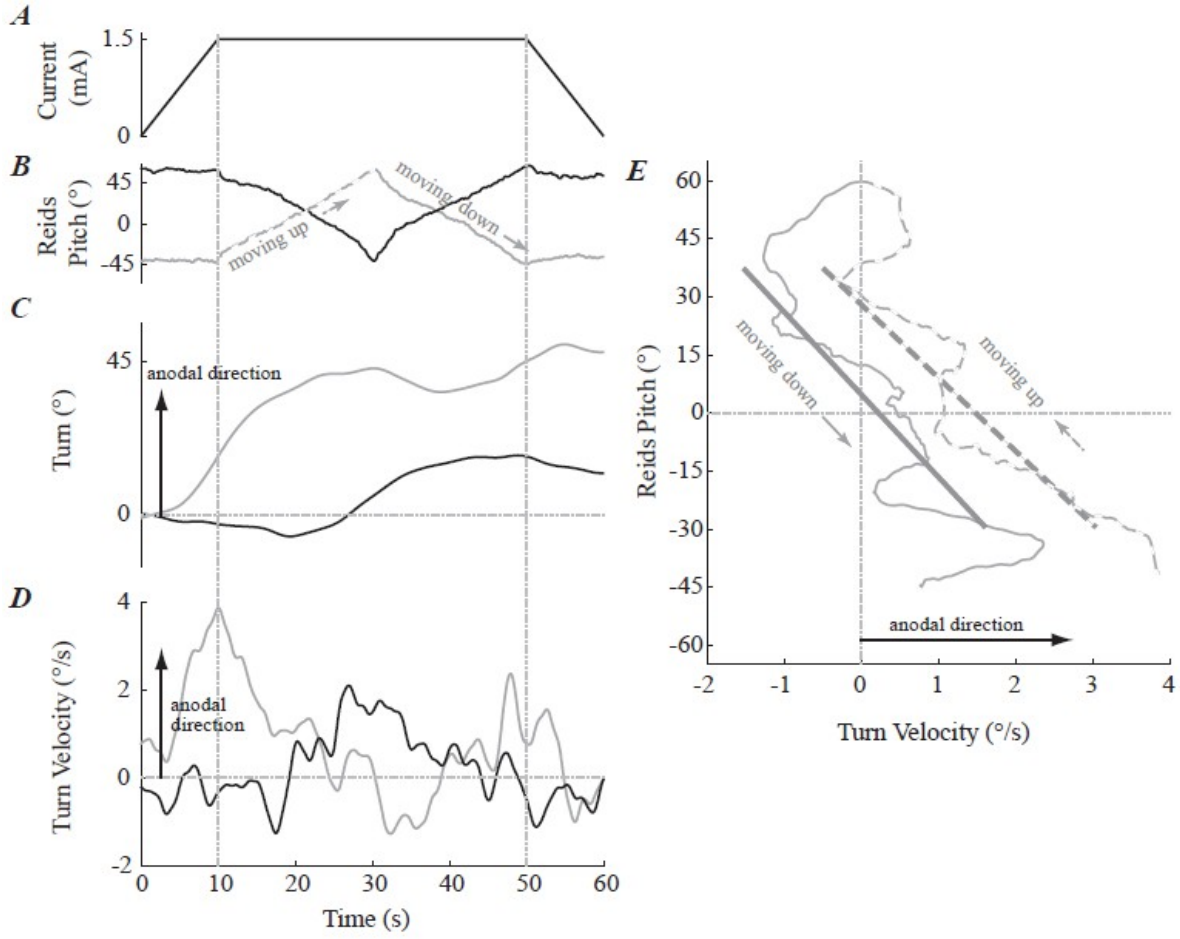

Figure 2.3. Vestibular-evoked orientation responses during self-generated head motion. Data from a representative subject

A) GVS stimulus used. In this case, data from anode-right trials have been inverted and combined with anode-left. ***B***) Mean head orientations of an individual subject. In 'start-up' trials (black traces), subjects began with their head pitched upwards. After 10 seconds they performed a set profile of head pitch motion. They first gradually moved the head to a downwards orientation, before reversing the direction of motion to return to the starting position. In 'start-down' trials (grey traces), the opposite pattern of motion was performed. ***C***) Whole body turn and ***D***) turning velocity evoked by GVS. Positive values indicate turn towards the GVS anode. The velocity and direction of turn was continuously modulated and even reversed direction as head orientation progressively changed. ***E***) Data from 'start-down' condition in ***B*** and ***D*** are plotted against one another to illustrate the relationship between head orientation and GVS-evoked turning. Although the point of zero rotation (intercept) is clearly different between upward and downward phases of movement, the response gain (slope) is similar in both.

Self-generated head motion resulted in similar modulation of GVS-evoked turn in all subjects (for group data see Figure 2.4). Maximum and minimum head pitches achieved during the dynamic head movement were 51±12° and -50±8°, respectively. This range of head movement was greater than experiment 1 (101±14° vs. 67±7°; t_{11}=6.43, p<0.001). Hence, for comparative purposes, the linear regression was restricted to the same range of head angles observed in experiment 1 for each subject.

The four phases of head movement were initially analysed separately (i.e. Direction: head moving up vs. down; Time: start vs. end of trial). The relationship between yaw velocity and head pitch was not significantly affected by the time of the head movement (i.e. start vs. end; gain $F_{1,11}$=3.05, p=0.11; point of zero rotation $F_{1,11}$=0.75, p=0.41). Direction of head movement had no effect on response gain ($F_{1,11}$=0.68, p=0.43), but it did have a significant effect on the point of zero rotation ($F_{1,11}$=29.63, p<0.001). This indicates that although there were no time-dependent effects, there were significant direction-dependent effects. Therefore, phases from the start and end of trials were averaged together, producing a trace for each direction of movement for each subject. Individual subject data resulting from linear regression can be seen Table 2.1, and the average lines of regression between head pitch and yaw velocity are shown in Figure 2.4E. As in static conditions, there was also a significant correlation between goodness of fit and the turning velocity range observed in dynamic conditions (r=0.56, p=0.004). Response gain was not different between each movement direction (t_{11}=0.34, p=0.74) and, on average, was -0.032±0.022°/s per degree pitch. The point of zero rotation was significantly affected by movement direction (t_{11}=3.29, p=0.007). Values of 25±11° and 11±15° were found when moving upward and downward, respectively. When the analysis was extended to include the full range of head movement, this did not affect these

results; i.e. the direction of head movement had an influence upon the point of zero rotation ($F_{1,11}$=16.05, p=0.002), with no other significant effects (p>0.19).

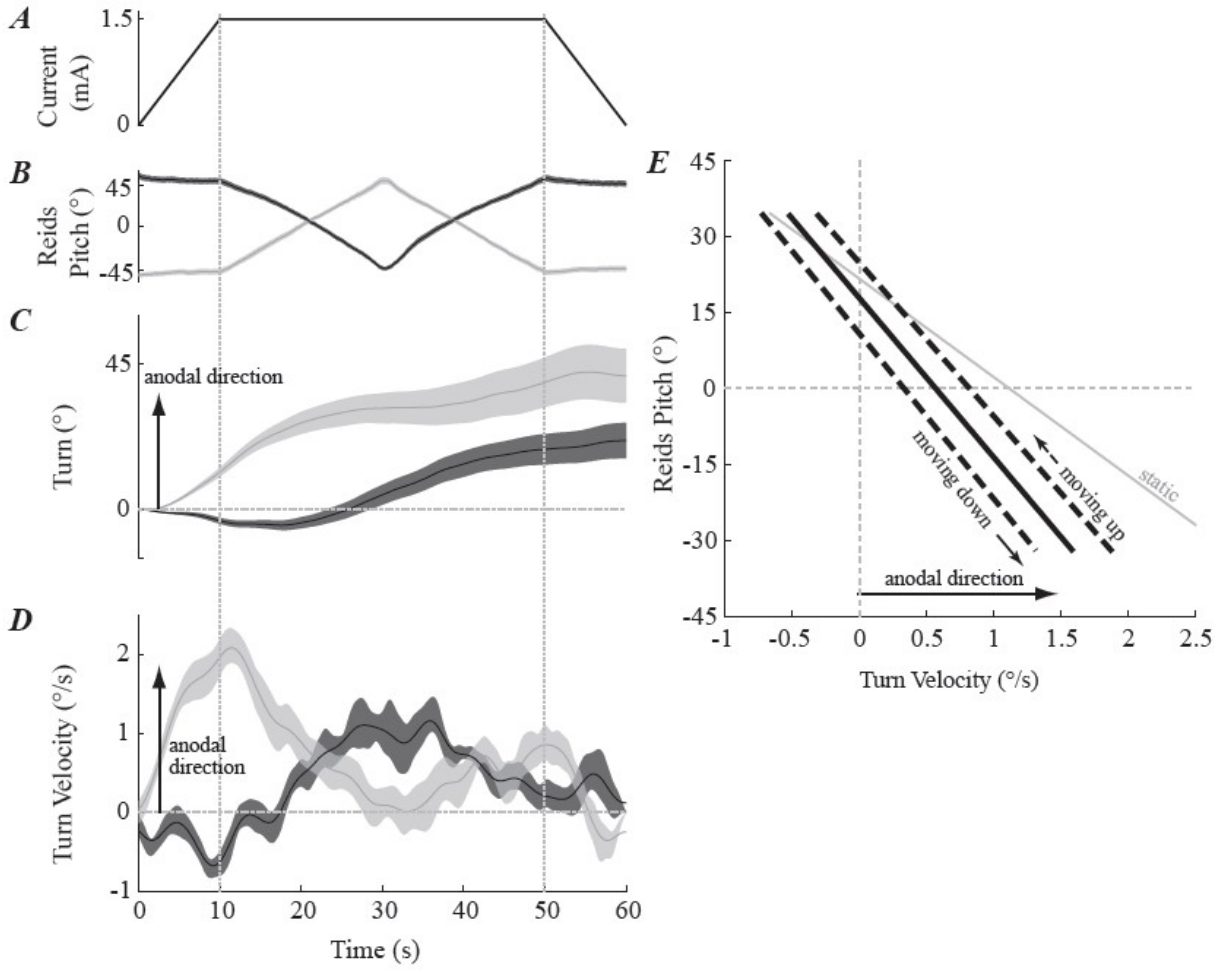

Figure 2.4. Vestibular-evoked orientation responses during self-generated head motion. Group data

A) GVS stimulus. Anode-left and right trials have been combined. ***B***) Group average head orientations during 'start-up' (black) and 'start-down' (grey) trials. ***C***) Whole body turn and ***D***) turning velocity evoked by GVS. Shaded areas in ***B***-***D*** indicate mean±SEM. ***E***) The group average relationship between head orientation (as measured by pitch angle of Reid's plane) and GVS-evoked turning velocity. Separate lines for each direction of head pitch movement (dashed black) and the average of both movement directions (solid black) are shown. For comparison, the group mean relationship in experiment 1 is also plotted (grey). This shows that the point of zero rotation was similar during static head orientations but response gain was greater, as indicated by a less steep slope.

Comparison of static head orientation and self-generated head motion

When the upward and downward head pitch phases were averaged together, there was no significant difference in the point of zero rotation between each experiment (static 22±11°; dynamic 18±11°; t_{11}=0.94, p=0.37). However, response gain was substantially and significantly reduced in dynamic conditions (static -0.051±0.022°/s per degree pitch; dynamic - 0.032±0.022°/s per degree pitch; t_{11}=3.17, p=0.009). This was also the case if the full range of data was used (point of zero rotation: dynamic 27±19°; t_{11}=1.17, p=0.27; response gain: dynamic -0.024±0.015°/s per degree pitch; t_{11}=5.07, p<0.001).

2.4. Discussion

In this chapter GVS was used to evoke whole-body turn in a direction systematically modulated by head pitch. With the head pitched downwards, turn towards the anode was demonstrated. With the head pitched upwards, the direction of turn was reversed (in accordance with Fitzpatrick *et al.*, 2006). The novel finding is that continuous modulation of the evoked turning response occurred during a voluntary head movement, in which head pitch progressively changed. This demonstrates that vestibular input for orientation is continuously reinterpreted in the context of self-generated head motion. However, results also showed that during head motion the magnitude of the evoked turn was significantly reduced.

Coordinate transformation of vestibular signals during static head orientation

The CNS must transform the vestibular afferent signal, which encodes head motion, from head to body coordinates for it to be useful in signalling whole-body motion. To successfully achieve this, an accurate estimate of head orientation with respect to vertical is required. With eyes closed, this is potentially derived by proprioceptive inputs from the neck and trunk and the otolith organs. Previous evidence suggests such coordinate transformation occurs, as changes in *static* head pitch modulate both the turning and postural responses evoked by GVS (Fitzpatrick *et al.*, 2006;St George & Fitzpatrick, 2011;Reynolds, 2011). GVS has also been shown to evoke a perception of whole-body rotation in subjects sitting in a rotating chair, in a direction modulated by head pitch (Day & Fitzpatrick, 2005b). However, the perceptual and motor effects are oppositely directed, which suggests that the latter reflects an attempt to reconcile the former.

In the current study the GVS-evoked turning response was minimal with the head pitched slightly upward. This is in accordance with minimal perception of whole body rotation (Day & Fitzpatrick, 2005b) and vertical torque during standing (Reynolds, 2011) with the head in this position. These results can be explained by the virtual signal that is induced by GVS. The dominant signal is head roll about a naso-occipital axis, which is induced by activation of semicircular canal afferents (Fitzpatrick & Day, 2004). Based upon anatomical data (Blanks *et al.*, 1975), the angle of the virtual rotation vector was predicted as elevated by 18.8 degrees from Reid's plane (Fitzpatrick & Day, 2004). If this virtual rotation vector is aligned horizontally then GVS does not induce a signal of rotation about a vertical axis. The point of zero rotation in the current study reflects where this is the case and, therefore, provides a new estimate of the rotation vector angle based on the evoked motor response. The calculated elevation of 21.7 degrees from Reid's plane is not statistically different to the prediction made

by Fitzpatrick and Day's (2004) model. I will therefore consider their model to adequately predict the effects of GVS in subsequent chapters. However, with power set at 0.80 and α at 0.05, power calculations indicate that a sample size of 12 in a one sample t-test design should be sufficient to detect an effect size of 0.89. This is a large effect and, using the values of variance in the current data, corresponds to a minimal detectable difference of 9.4 degrees. Therefore, small to medium differences to Fitzpatrick and Day's prediction could not be detected with the current sample size. It is also worth noting that if alternative semicircular canal orientation data (Della Santina *et al.*, 2005) are applied, the GVS rotation vector is predicted to be elevated 27.9 degrees (i.e. different to 18.8 degrees) from Reid's plane (Day *et al.*, 2011). Furthermore, although the model assumes that GVS equally modulates afferents from all canals, small adjustments to the gain of each canal also have considerable effects on the predicted angle (Day *et al.*, 2011).

GVS evoked a turning response only when the virtual rotation vector was more vertically aligned. The response was oppositely directed during head up and down conditions (in accordance with Fitzpatrick *et al.*, 2006), because the orientation of the virtual rotation vector relative to the earth referenced vertical was reversed. Furthermore, there was an asymmetry in the turning velocity demonstrated during upward and downward head pitch. As previously discussed, the virtual rotation vector is horizontal when the head is pitched slightly upwards. The angle of the vector is therefore not equivalent during head up and down pitch of equal magnitude. For instance, a 45° downward pitch would align the vector ~65° from horizontal, whereas a 45° upward would only align the vector ~25° from horizontal. For any given downward head pitch the vector is more vertically aligned and, hence, leads to a larger turning response (i.e. body rotation about a vertical axis) compared to the equivalent upward angle.

Coordinate transformation of vestibular signals during self-generated head motion

During head motion, the coordinate transformation process can only be successful if an accurate estimate of head orientation is maintained. When head pitch progressively changed during the course of each trial the GVS-evoked turning response was continuously modulated, reflecting the constantly changing head pitch. This suggests that the coordinate transformation was continuously recalculated according to the current orientation of the virtual rotation vector. As motion was self-generated, a copy of the motor command (or 'efference copy'; see General Introduction) for head movement may have contributed to the required estimate of head orientation, in addition to proprioceptive and otolith inputs.

The *average* head pitch corresponding to zero rotation during self-generated head motion was found to be no different to that during static conditions, although a sample size of 12 may not have been sufficient to detect small to medium differences. Power calculations ($1-\beta = 0.80$ and $\alpha = 0.05$) show that, with this number of subjects in a paired t-test design, it is possible to detect only large effects of 0.89. This equates to a minimal detectable difference of 12.6 degrees. Nonetheless, a significant effect of head movement direction was detected (see Table 2.1 & Figures 2.3E & 2.4E). When the head was pitching upward, it was 25 degrees, but during head-down movement it was 11 degrees. This difference is possibly explained by a delay between vestibular input and motor output. If the actual point of zero rotation lies between 11 and 25 degrees (as estimated in static conditions), and an equal delay in each direction is assumed, then the head moved beyond the actual pitch by a further 7° before the zero-turn point occurred. As average head pitch speed was 4.7°/s, I estimate this delay to be approximately 1.5s. This delay is unlikely to be related to the vestibular transduction

mechanism, as GVS can evoke a VOR at a very short latency (Aw *et al.*, 1996). It is more likely to be due to rotational inertia of the body. This would create a delay between the onset of muscle activation and the point at which the body starts turning. This would explain why turning responses appear to lag behind head position.

Reduced response gain during self-generated head motion

The results of the two experiments show that during self-generated head motion there was a significant reduction in response gain. This was equally true for head-down and head-up movements (see Table 2.1 & Figure 2.4E). Specifically, the change in angular velocity (per degree of head pitch) was reduced from -0.051 (static) to -0.032 °/s/° (dynamic). However, this effect has more than one possible explanation.

Static head orientations were maintained for 60 seconds, whereas during motion each head orientation was encountered for only a short amount of time. There may have been adequate time for a steady state turn velocity to be reached in static conditions but insufficient time during motion. This is unlikely to be due to the apparent sensorimotor delay; given the estimated duration of 1.5s there was presumably sufficient time during the 40s trial for turning velocity to 'catch up' with head position. However, the delay is likely attributable to the inertia of the body resisting changes in its rotational motion. This may effectively act as a low-pass filter between the onset of muscle activity and the observed motor response (Latt *et al.*, 2003;Dakin *et al.*, 2010). As a consequence, there would be an attenuation or removal of any command to turn which is modulated at a frequency greater than the potentially very low

cut-off. This could explain why the velocity of a vestibular-evoked turn was reduced in the context of a continuously modulated head pitch.

Another explanation (not mutually exclusive) for the difference in response gain between conditions is a suppression of vestibular signals during self-generated head motion. Barnett-Cowan and Harris (2011) found that when vestibular input was a consequence of active head rotation the perceived timing was delayed in comparison to passive rotation. They suggested that this delay was because during self-generated motion the reafferent signal was suppressed and, hence, took longer to reach a perceptual level. Such inhibition of sensory reafference was proposed by Von Holst and Mittelstaedt (1950) as a mechanism to distinguish exafference from the total afferent signal. They suggested that, within the CNS, efference copy for voluntary action interacts with the afferent signal to cancel out the corresponding reafference. In terms of vestibular signal processing, this view is supported by evidence demonstrating that, when active head motion and passive whole-body rotation are combined, neurons in the vestibular nuclei of the monkey encode only the passive component (Roy & Cullen, 2001). However, this is only true when the passive component of rotation involves whole-body movement. If active and passive *neck* rotation is combined, then the same neurons can no longer distinguish between exafference and reafference (Cullen *et al.*, 2009). The authors suggested that reafference is not cancelled when neck proprioception signals from *both* the active and passive movement are merged together. Such inhibition of reafference requires proprioceptive input to match the 'expected consequences' of the active movement (Roy & Cullen, 2004). In the current study, however, GVS is an entirely artificial method for evoking sensations of passive head movement, and is not accompanied by a proprioceptive signal of neck rotation. Therefore, by extending the arguments of Cullen *et al.* (2009), it should be

possible for the CNS to successfully cancel reafference during GVS. But is a suppression of vestibular reafference likely to explain the current findings? Would this have any effect on responses evoked by GVS (i.e. an exafferent signal)? As previously discussed, in order to transform a vestibular signal from head to body coordinates, an accurate estimate of head position is required. If the reafferent signal is suppressed then the precise knowledge of head orientation may be compromised, leading to a misinterpretation of the GVS signal.

The exact site of the supposed vestibular reafference inhibition remains unknown. The cerebellum may be involved, as it receives proprioceptive input from the spinal cord and the 'expected consequences' of voluntary movement from cortical structures (Ramnani, 2006). It also receives vestibular signals and projects to the vestibular nuclei (Carleton & Carpenter, 1983;1984;Voogd & Glickstein, 1998), allowing modulation of neurons located here. Furthermore, the analogous suppression of cutaneous sensory information during self-generated tickle is thought to involve the cerebellum (Blakemore *et al.*, 1998;2001). Alternatively, cortical regions may be involved. Akbarian *et al.* (1993;1994) demonstrated projections from cortical regions to the vestibular nuclei complex, and speculated that they may be involved in suppressing vestibular-evoked responses during self-generated movements.

Summary

Vestibular-evoked turning responses in stepping subjects were modulated by changes in head pitch. The direction of the evoked turn reflects the orientation of the GVS virtual rotation vector relative to vertical, suggesting the vestibular signal is transformed from head to body

coordinates and can therefore indicate whole-body turn. When the head was simultaneously moved, the magnitude of the response was reduced. This raises the possibility that vestibular input is partially suppressed during self-generated head motion. Nonetheless, the present findings show that an identical vestibular input was continuously reinterpreted in the context of ongoing changes in head pitch.

CHAPTER 3.

DIRECTION OF VESTIBULAR-EVOKED BALANCE REFLEXES DURING ILLUSORY HEAD ORIENTATION

Previous research has shown that *perceived* head orientation is used when computing the direction of a vestibular-evoked balance reflex. After a prolonged head turn was used to induce an illusory perception of head yaw relative to the trunk, the direction of a vestibular-evoked balance reflex was shown to correspond to the perceived, rather than actual, head orientation. Here, the perception of head yaw relative to the feet was altered using an alternative experimental paradigm. This was achieved by stepping on a platform rotating at 60°/s for 30 minutes, with a cadence of 100steps/min. When subjects were then blindfolded and attempted to stand with all body segments in alignment, they demonstrated an unperceived 15° fixed rotation of head over the feet, in a direction opposite platform rotation. Despite the illusion, the balance reflex evoked by GVS was in a direction appropriate for the *actual* head orientation. This is in contrast to previous research. However, in an additional experiment, a prolonged static body twist was used to achieve a similar unperceived reorientation. In this case, the GVS-evoked balance reflex was appropriate for the *perceived* head orientation. Thus, although the two adaptive periods had a similar effect on posture, the effects on the vestibular-evoked movement were different. The potential reasons for this difference are discussed. An adaptation of motor output following rotary stepping potentially causes the response direction to be rotated by an amount appropriate for the postural reorientation. Nonetheless, the results demonstrate that, in some contexts, the direction of a vestibular-evoked balance reflex is affected by an illusory perception of head orientation.

3.1. Introduction

In the event of a disturbance to balance, the vestibular system detects and signals unintended head motion. However, in order to generate the appropriate motor response, the vestibular signal must be transformed to body coordinates, a process termed coordinate transformation (see General Introduction and Chapter 2). For instance, as head yaw orientation is altered, the response to a vestibular input is modulated accordingly. It has been demonstrated that, with the head in a neutral pitch, the sway response evoked by GVS is always directed along the interaural line towards the anode (Lund & Broberg, 1983;Pastor *et al.*, 1993). This result can be explained by the prediction that an applied current induces a virtual signal of motion towards the cathode (Fitzpatrick & Day, 2004; see General Introduction and Chapter 2).

However, in the case of an inaccurate sensation of head orientation, the direction of the vestibular-evoked sway response has been shown to follow perceived rather than actual head orientation (Gurfinkel *et al.*, 1989). These authors induced an illusion of head orientation relative to the trunk. This was achieved by maintaining a prolonged turn of the head to one side, whilst standing with eyes closed. Over a period of 10 minutes, subjects reported that their perceived head orientation gradually returned to the forward facing position (see Figure 3.1A). The vestibular-evoked response direction was initially anteroposterior, along the actual interaural line, appropriate for the fixed turn of the head (see 2min in Figure 3.1B). However, the response direction gradually changed with time; it became more mediolateral as the minutes passed (see 10min in Figure 3.1B). Thus, the response direction was no longer appropriate for the actual head position, which remained turned to one side, but closely matched the perceived orientation (compare Figures 3.1A and B). This evidence suggests that

the coordinate transformation of vestibular signals is computed in accordance with the perceived, rather than actual, orientation of the head.

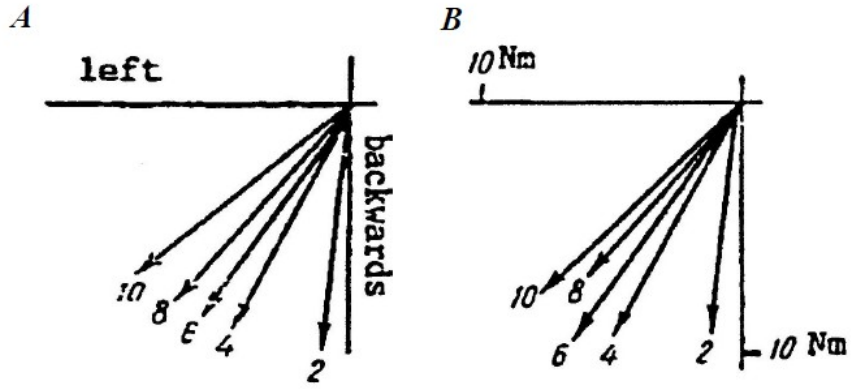

Figure 3.1. Perceived head orientation and direction of the vestibular-evoked balance reflex during prolonged head turn relative to the trunk

A) Perceived head orientation during a fixed rotation of the head relative to the trunk. In this case, although the head was turned to face over the left shoulder for the entire 10 minutes, the perceived head orientation gradually returned to the forward facing position as the minutes progressed (arrows indicate the perceived interaural line at 2, 4, 6, 8 and 10mins). ***B***) Direction of the sway response evoked by GVS during the same adaptive period. This evidence demonstrates the response direction closely matches the *perceived* head orientation (Adapted from Gurfinkel *et al.*, 1989)

An alternative method of inducing illusory changes in body segment orientation involves stepping in place on a rotating platform. Subsequent to an adaptive period of rotary stepping, an unperceived fixed rotation of the upper body over the feet is demonstrated (Hollands *et al.*, 2007;Scott *et al.*, 2011;Osler & Reynolds, 2012). However, unlike passive neck rotation, rotary stepping also has profound effects on motor output. Subjects subsequently demonstrate rotation when they attempt to step in place (Weber *et al.*, 1998), walk directly forwards (Gordon *et al.*, 1995), backwards (Earhart *et al.*, 2001) or hop (Earhart *et al.*, 2002b). A static adaptive period comprising a prolonged body twist, more akin to the method used by Gurfinkel *et al.* (1989), was demonstrated to cause a similar postural after-effect to rotary

stepping, but it did not affect motor output, at least not when stepping in place (Osler & Reynolds, 2012). Thus, the two adaptive periods both alter the perception of head yaw relative to the feet, but appear to have different effects on motor output.

This chapter investigates the direction of vestibular-evoked balance reflexes during illusions of altered head yaw. Under normal conditions, GVS-evoked sway responses were shown to be directed towards the anode, along the interaural line. Dissociation between actual and perceived head orientation was then induced using two types of intervention. In the first, subjects performed a period of stepping in place on a rotating platform. The second intervention consisted of a passive static body twist. In both cases, I investigated whether the direction of the GVS-evoked balance reflex was affected during the induced illusion.

3.2. Methods

Subjects

Ten subjects (6 male) aged 20 to 30 years (mean±SD: 24±3 years) with no known neurological or vestibular disorder gave informed consent to participate. The experiments were approved by the local ethical review committee at the University of Birmingham and performed in accordance with the Declaration of Helsinki.

Protocol

Baseline measures of head-on-feet orientation and vestibular-evoked response direction were established prior to the adaptive period. Blindfolded subjects stood in the centre of a force plate, upright, still but relaxed, with feet together and their arms folded across their chest. They were instructed to face directly forwards with all of their body segments aligned. Subjects wore training shoes to avoid potential foot injury. Four trials were conducted in which binaural bipolar GVS was delivered, as described in Chapter 2. In each trial, 20 impulses (10 anode-left, 10 anode-right) 1.5mA in amplitude and 3 seconds in duration were applied. Impulses were separated by between 4 and 7 seconds. Thus, each trial lasted approximately 3 minutes in total. Although a short period of rest separated trials, seated rest was only permitted after two trials. The blindfold remained in place during all periods of rest. The positions of all subjects' feet were marked on the floor so they could be guided to the same position post-adaptation by the experimenter.

Following baseline trials, the blindfold was removed in order to perform the adaptive period of rotary stepping. Subjects stepped in place for 30 minutes in the centre of a circular platform, 75cm in diameter, rotating at 60°/sec using a DC shunt motor (Parvalux SD12C, Bournemouth, Dorset, UK) and reversing controller (RS Components 425-5254, Corby, Northants, UK). Half the subjects experienced clockwise platform rotation, and half anticlockwise. Step cadence was 100steps/min, as prescribed by a digital metronome (Seiko DM-11, Minato, Tokyo, Japan). Subjects were instructed to fixate on a target positioned straight ahead. Upon completion of the adaptive period, subjects were blindfolded immediately and guided to the force plate to stand with their feet in exactly the same position as during baseline trials. Post-adaptation trials were then completed to determine changes in

head-on-feet orientation and vestibular-evoked response direction induced by the adaptive period.

Following post-adaptation trials, the blindfold was once again removed and subjects stepped in place for 5 minutes at a cadence of 100steps /min. This was performed to negate any existing illusory reorientation. Subjects were then blindfolded and guided to the force plate to stand with their feet in exactly the same position as in previous trials, in order to complete post-washout trials. All instructions in post-adaptation and post-washout trials were identical to those given during baseline trials.

Additional Experiment

Seven subjects returned to participate in an additional experiment. This was conducted to investigate the direction of vestibular-evoked balance reflexes subsequent to an alternative adaptive period; a prolonged static body twist. Following a fixed rotation of the trunk relative to the feet, it has been demonstrated that individuals stand with an offset between their upper body and feet, although they perceive themselves to face directly forwards (Osler & Reynolds, 2012). To achieve this in the current study, the protocol outlined above was repeated, but the adaptive period was changed. Instead of completing an adaptive period of rotary stepping, subjects stood with a fixed rotation of the trunk relative to the feet for 30 minutes. The upper trunk orientation was fixed by strapping subjects around their chest to a fixed support. The feet were then rotated about a vertical axis to achieve the maximum trunk-on-feet twist that could be comfortably maintained. As the upper trunk orientation was fixed, rotation could involve the hips and lower torso. This was designed to ensure the anatomical

site of rotation was similar to the stepping experiment (i.e. predominantly hip rotation). Subjects kept their head aligned with their upper trunk and fixated on a target positioned straight ahead. All instructions in baseline, post-adaptation and post-washout trials were identical to the previous experiment.

Data Acquisition and Analysis

Head orientation in the form of Euler angles was recorded at 50Hz from a motion tracking sensor attached to a welding helmet frame (Polhemus Fastrak, Colchester, VT, USA). As feet positions were constrained, head yaw (i.e. rotation about the vertical axis) during the 1 second prior to each GVS impulse was calculated to give head-on-feet orientation. Orientations were then averaged across the first 20 impulses for each GVS polarity in each block (i.e. the first two trials). Later trials were not included in the analysis because, in some subjects, seated rest taken after two post-adaptation trials considerably reduced the magnitude of the subsequent postural reorientation. In addition, in later post-washout trials there was a tendency for the reorientation to return.

The response to GVS was recorded in the form of ground reaction forces at 1 kHz using a Kistler 9281B force platform (Kistler Instrumente AG, CH-8408 Winterthur, Switzerland). After subtracting force values at the time point of GVS onset, signals were averaged across the first two trials in each block. Mediolateral (Fx) and anteroposterior (Fy) force vectors were calculated between 180-400ms from individual subject averages (for example see Figure 3.3A). This time window was used as it corresponds to the initial rise in the GVS-evoked ground reaction force, which is unaffected by the subsequent destabilising body sway.

Response directions relative to the lab, and thus feet, were calculated as atan(Fx/Fy). Then, head-on-feet yaw was subtracted to calculate head-referenced response directions.

Circular statistical techniques

As head-on-feet orientations and response directions correspond to angular data, a number of circular statistical techniques were implemented using CircStat toolbox for MATLAB (Berens, 2009).

For head-on-feet orientations, an angular convention where zero degrees represent the direction the feet are pointing was used. For GVS response directions, zero degrees represent the direction the head is pointing. In both cases, positive angles increase to 180 degrees in a leftward direction and negative angles decrease to -180 degrees in a rightward direction (see Figure 3.2A).

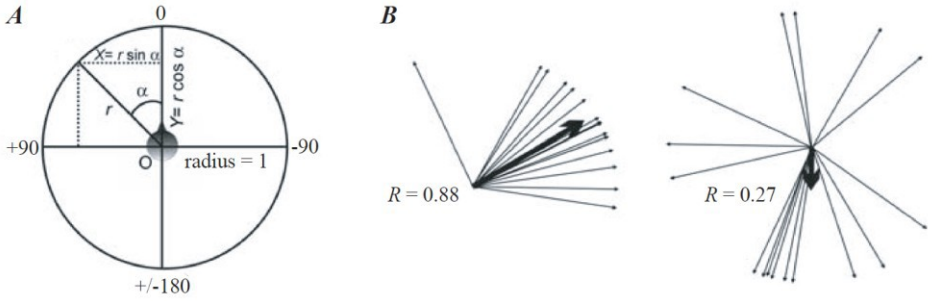

Figure 3.2. Circular data

A) The angular convention, ranging from -180 to +180, is shown. Trigonometric functions were used to calculate the rectangular coordinates (X, Y) of each subject's response direction, after converting each to a unit vector (*r*). ***B***) Individual vectors (thin lines) were then averaged to provide the mean resultant vector ($\bar{r}$; thick lines). The length of the resultant vector (*R*) indicates the concentration of vectors around the mean. Examples of concentrated (*R*=0.88) and nonconcentrated (*R*=0.27) samples are shown. Adapted from Welgampola and Day (2006).

To calculate mean angles, individual angles ($\alpha_1, \alpha_2, \ldots \alpha_n$) were first transformed to unit vectors in two dimensions ($r_1, r_2, \ldots r_n$) by demanding that the circle had a radius of 1 (see Figure 3.2A). Thus, the magnitude of individual subject responses did not affect the mean response direction. Rectangular coordinates of each unit vector were then calculated by applying trigonometric functions, where the sine and cosine of the angle give the x-coordinate and y-coordinate respectively (see Figure 3.2A).

$$r_i = \begin{bmatrix} \sin \alpha_i \\ \cos \alpha_i \end{bmatrix}$$

Vectors ($r_1, r_2, \ldots r_n$) were then averaged to calculate the mean resultant vector ($\bar{r}$; see Figure 3.2B).

$$\bar{r} = \frac{\sum_{i=1}^{n} r_i}{n}$$

The four-quadrant inverse tangent function was used to calculate the angle of the mean resultant vector, thus providing mean angle ($\bar{\alpha}$).

The absolute length of the resultant vector (R) serves as a measure of concentration in unimodal samples, providing a measure of variance (Batschelet, 1981). When this value is close to one, the individual data points are more concentrated around the mean. When it is close to zero, there is no concentration around a single direction (see Figure 3.2B).

$$R = \|\bar{r}\|$$

The Rayleigh test was then used to determine if R was sufficiently large to indicate the group of angles had a preferred direction, and were not from a random distribution. When R is large, the Rayleigh test statistic increases. If this test statistic exceeded critical values (tabled in Zar, 1999), the null hypothesis of 'randomness' was rejected in favour of 'directedness' (Batschelet, 1981).

95% confidence limits ($\theta \pm d$) for mean angles were also calculated (Upton, 1986;Zar, 1999). Overlap between confidence limits and the predicted theoretical direction (e.g. interaural line, ±90deg, for response direction) was considered as statistical evidence that they were not significantly different.

$$d = \arccos\left[\frac{\left[n^2 - n^2 - nR^2 \cdot \exp\left(x^2_{0.95,1} / n\right)\right]^{1/2}}{nR}\right]$$

Although the R statistic and 95% confidence limits can be seen as measures of variance, the angular deviation (AD) was also obtained, as it is equivalent to the standard deviation in linear statistics (Batschelet, 1981).

$$AD = \left(2 - 2R\right)^{1/2}$$

Moore's modification of the Rayleigh test (Moore, 1980;Zar, 1999) was used to test for differences between paired samples of angles. For instance, to test whether a significant

change in head-on-feet orientation was demonstrated in post-adaptation trials compared to baseline. To do this, a single sample of angles was formed by calculating the differences between paired angles ($\theta_1, \theta_2, \ldots \theta_n$). Data following anticlockwise platform rotation were first inverted (in additional experiment, data following anticlockwise rotation of the feet beneath the trunk were inverted). For each calculated angle, the length of the resultant vector was calculated (r). Then, after ranking the resultant vectors from smallest (r_1) to largest (r_n), the test statistic (R') was calculated as follows, where the ranks (i) range from 1 to n.

$$X = \frac{\sum_{i=1}^{n} i\cos\theta_i}{n}$$

$$Y = \frac{\sum_{i=1}^{n} i\sin\theta_i}{n}$$

$$R' = \left(\frac{X^2 + Y^2}{n}\right)^{1/2}$$

If the calculated R' statistic exceeded critical values (tabled in Moore, 1980;Zar, 1999), the null hypothesis of no difference was rejected in favour of a difference between paired angles.

For all statistical tests, significance was taken as $p<0.05$. Mean angle and angular deviation ($\theta \pm AD$) are reported in text. Figures present mean angle and 95% confidence limits ($\theta \pm d$).

3.3. Results

Baseline response direction

GVS evoked a balance reflex as indicated by ground reaction force recordings. Data from a representative subject is plotted in Figure 3.3A. With the head facing forwards, the evoked force response was mainly in a mediolateral direction and was oppositely directed for anode-left and right conditions. The head-referenced direction of the force response for this subject was 78.7deg for anode-left and -81.2deg for anode-right (see thick vectors in Figure 3.3B). Group mean response directions were 75.7±25.6deg and -85.7±24.9deg for anode-left and right respectively. In both cases, individual subject response directions were significantly concentrated to indicate deviation from a random distribution (anode-left, $R=0.900$, $p<0.001$; anode-right, $R=0.906$, $p<0.001$) and 95% confidence intervals overlapped ±90degrees (i.e. interaural line; see Figure 3.3B), indicating response direction did not significantly deviate from the predicted theoretical direction.

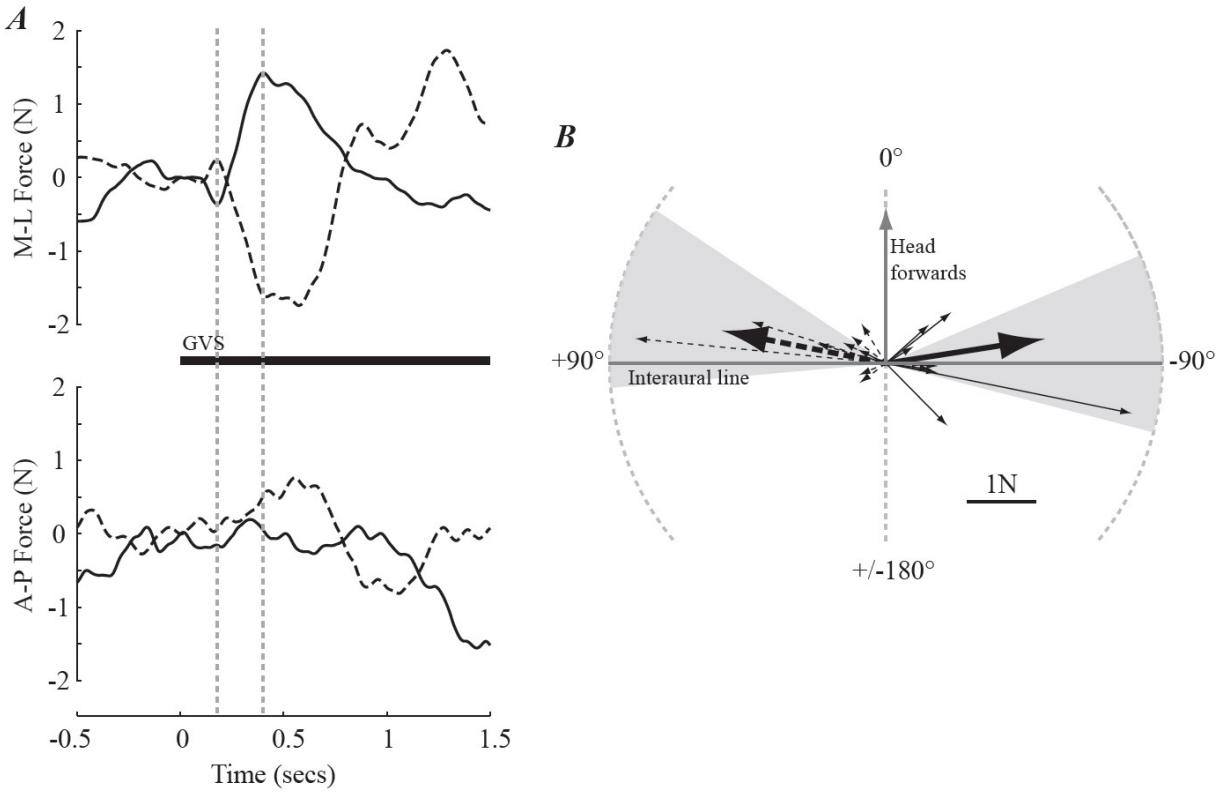

Figure 3.3. The direction of vestibular-evoked balance reflexes under normal conditions

A) Mediolateral (top) and anteroposterior (bottom) ground reaction force traces for an individual subject in anode-right (solid) and anode-left (dashed) trials. In both cases, anteroposterior forces are relatively small, indicating a primarily mediolateral response. However, mediolateral forces are approximately equal and opposite. Positive values indicate a ground reaction force which moves the body to the right. Thus, subjects swayed in the direction of the anode. ***B***) Arrows indicate head-referenced GVS response vectors for individual subjects. Data from part ***A*** are indicated by thicker vectors. 95% confidence intervals (as indicated by shaded grey segments) overlap the predicted theoretical directions of +90deg and -90deg for anode-left and right, respectively.

Effects of rotary stepping

During the adaptive period of rotary stepping, subjects were stable and able to step in time with the metronome. They occasionally looked down at their feet in order to remain in the centre of the rotating platform.

Post-adaptation, the representative subject illustrated in Figure 3.4 demonstrated an unperceived -12.1deg clockwise reorientation of the head over the feet, following anticlockwise platform rotation. The head-referenced direction of the evoked response was 74.9deg and -85.0deg for anode-left and right conditions respectively. Therefore, for this subject, response direction changed by only -3.8deg from baseline. Post-washout, the head orientation of this subject returned to 1.3deg, indicating it was negated by the washout period of stepping. The vestibular-evoked response directions were 79.7deg and -53.7deg. In anode-left trials this was approximately the same as during baseline trials. In anode-right trials, for this subject, this was 27.5° different to baseline. However, group data shows post-washout trials not to be significantly different to baseline (-8.0±16.7°, R'_{10}=0.89, p>0.10).

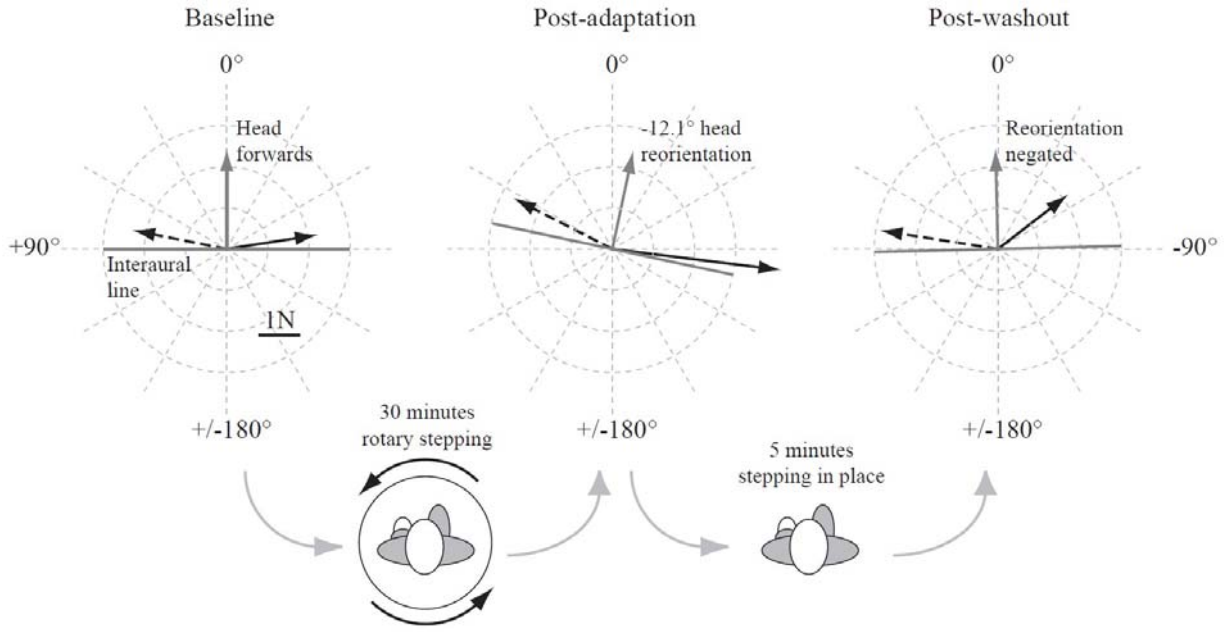

Figure 3.4. Postural orientation and the direction of a vestibular-evoked response following rotary stepping. Data from a representative subject

Following the adaptive period of rotary stepping, an unperceived head-on-feet reorientation was demonstrated (solid grey arrow), in a direction opposite platform rotation. Therefore, dissociation between the actual and perceived head orientation was induced. The balance response evoked by GVS (anode-left=dashed black vector, anode-right=solid black vector) was approximately directed along the interaural line (solid grey line). Thus, it was appropriate for the actual head orientation. Five minutes of stepping in place was sufficient to negate the postural reorientation. Although group results show the response direction was no different to baseline during post-washout trials, for this subject this is apparent for only anode-left vector. In this case the anode-right response was 27.5° different to baseline.

All subjects demonstrated unperceived head-on-feet reorientation in a direction opposite platform rotation, indicating a significant illusion was induced (15.5±6.4°; R'_{10}=1.74, $p<0.001$; see grey arrow in Figure 3.5A). Data from subjects who experienced anticlockwise platform rotations were inverted prior to combining with the remaining subjects, after first confirming the magnitude of reorientation was not significantly different (ACW 12.8±6.0deg, CW 18.3±6.7deg, t_8=1.38, p=0.205). Post-adaptation, during the illusion, the vestibular-evoked response direction was not significantly altered (2.9±13.5°, R'_{10}=0.36, $p>0.50$). This indicates response direction was referenced to the actual head orientation (compare black

vector to solid grey line in Figure 3.5A). Relative to space, response direction was rotated by an amount appropriate for the postural reorientation. Post-washout, postural orientation (-0.8±3.1°, R'_{10}=0.86, p>0.10) and response direction (-8.0±16.7°, R'_{10}=0.89, p>0.10) were not significantly different to baseline (see Figure 3.5B).

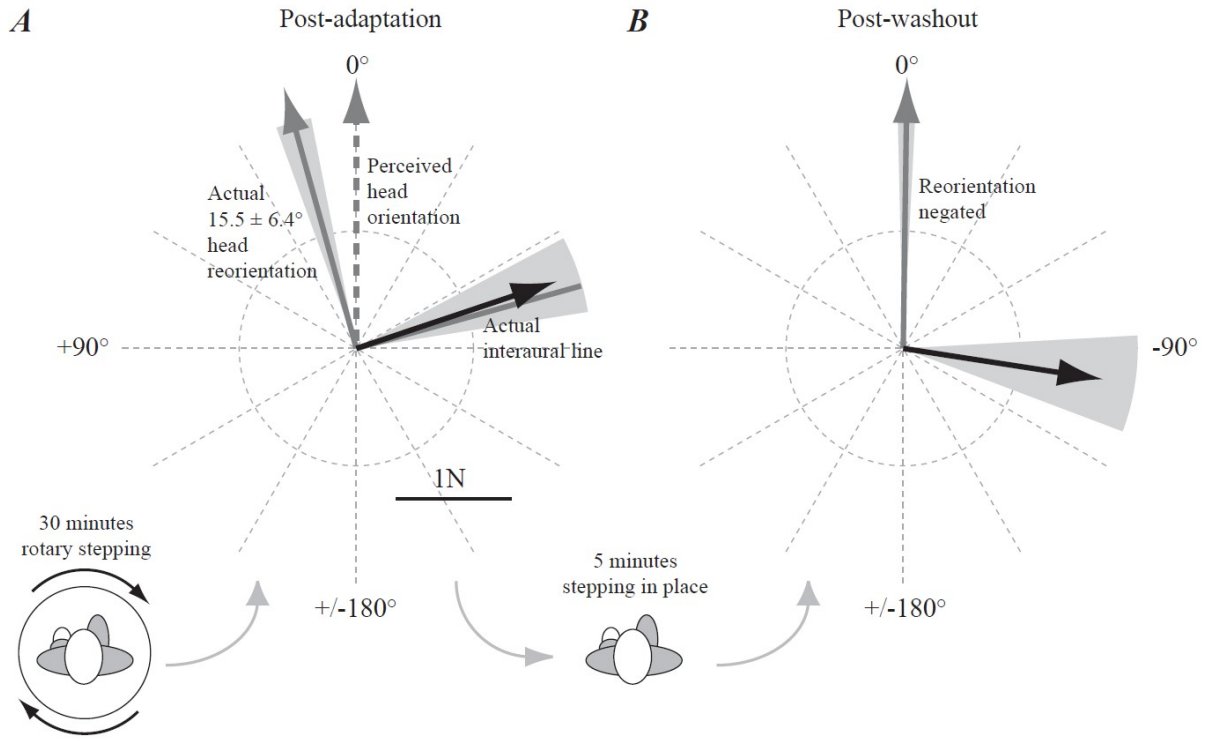

Figure 3.5. Postural orientation and the direction of a vestibular-evoked response following rotary stepping. Group data

A) Following an adaptive period of rotary stepping, an unperceived reorientation of the head relative to the feet was induced, in a direction opposite platform rotation. Thus, there was a mismatch between actual (solid grey arrow) and perceived (dotted grey arrow) head-on-feet orientations. Zero degrees indicate the direction the feet were pointing. The average force vector evoked by GVS is indicated in black. In this case, its direction closely matched the actual interaural line (solid grey line). Anode-left trials have been reversed and combined with anode-right trials ***B***) Following the wash-out period of stepping in place, both head orientation and response direction were no different to baseline. Shaded area indicates 95% confidence limits.

Additional experiment - Effects of prolonged static trunk-on-feet twist

During the static adaptive period subjects maintained a 54±6deg fixed rotation of the upper trunk over the feet (range = 46 to 65deg). All but one subject subsequently demonstrated postural reorientation when attempting to stand straight with all body segments aligned. Data from the subject who did not demonstrate a postural after-effect (-0.04deg head-on-feet rotation) was excluded from subsequent analysis. For all other subjects, a fixed rotation was demonstrated in the same direction as during the adaptive period. As seen after rotary stepping, significant head-on-feet reorientation was demonstrated post-adaptation (10.0±4.4°, R'_6=1.43, p<0.001; see grey arrow in Figure 3.6A), indicating a significant illusion was induced. In this case, data from subjects who experienced anticlockwise rotation of the feet beneath the trunk during the adaptive period were first inverted and combined with the remaining subjects, after confirming reorientation magnitudes were not significantly different (ACW 11.2±3.2deg, CW 8.9±6.7deg, t_4=0.55, p=0.613). In contrast to the GVS response following rotary stepping, where the force vector direction did not change, a significant change in response direction was demonstrated, in a direction opposite postural reorientation (-10.9±12.7°, R'_6=1.42, p<0.001). This indicates it was not referenced to the actual head orientation. As the change was approximately equal and opposite to the head-on-feet reorientation, response direction relative to the lab was approximately unchanged. Thus, the response was approximately referenced to the perceived head orientation (compare black vector to dotted grey line in Figure 3.6A). Post-washout, head-on-feet rotation (2.1±2.6°, R'_6=1.02, p>0.05) and response direction (-3.5±11.6°, R'_6=0.61, p>0.10) were not significantly different to baseline (see Figure 3.6B).

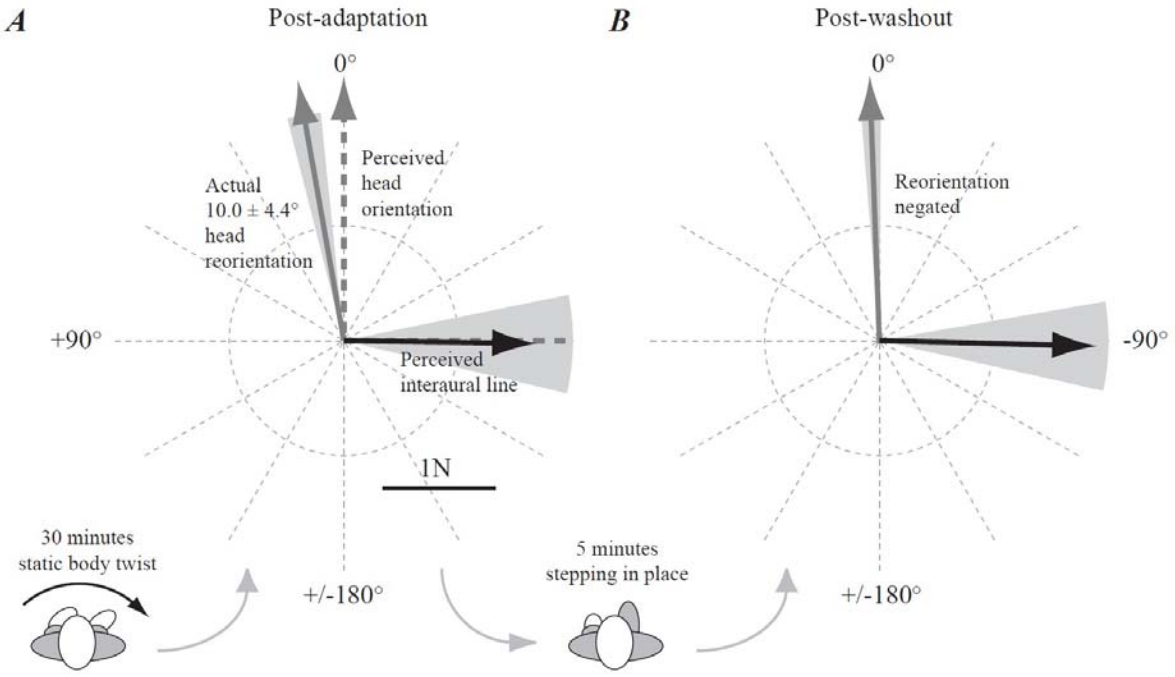

Figure 3.6. Postural orientation and the direction of a vestibular-evoked response following prolonged static body twist. Group data

A) Following static body twist an unperceived reorientation of the head relative to the feet was induced, in a direction opposite to feet rotation during the adaptive period. Therefore, as following rotary stepping, there was a mismatch between actual (solid grey arrow) and perceived (dotted grey arrow) head orientations. Zero degrees indicate the direction the feet were pointing. In this case, the average force vector evoked by GVS (black vector) was in a direction which closely matched the perceived interaural line (dotted grey line). ***B***) Following the wash-out period, both head orientation and response direction were no different to baseline. Shaded area indicates 95% confidence limits.

3.4. Discussion

In this chapter I investigated whether the direction of a vestibular-evoked balance response was affected by illusory changes in head orientation. The perception of head yaw relative to the feet was altered by two separate adaptive periods, namely, stepping on a rotating platform and a prolonged static body twist. GVS was then applied to evoke balance reflexes. The novel finding is that the two adaptive periods had different effects on the direction of the evoked response. The response direction was appropriate for the *actual* head orientation following

rotary stepping. However, following the static adaptive period the response direction was appropriate for the *perceived* head orientation (in accordance with Gurfinkel *et al.*, 1989). Therefore, although the two adaptive periods have similar effects on body posture, they have different effects on the motor response evoked by GVS.

Response direction is appropriate for actual head orientation following rotary stepping

Subsequent to an adaptive period of rotary stepping, an illusory reorientation of body posture was induced. In accordance with previous research (Hollands *et al.*, 2007;Scott *et al.*, 2011;Osler & Reynolds, 2012), this manifested as an unperceived 15 degree fixed rotation of the upper body over the feet during standing, in a direction opposite platform rotation. Although subjects believed they faced directly forwards with all of their body segments aligned, they in fact demonstrated body twist. Thus, the adaptive period induced dissociation between actual and perceived head-on-feet orientation.

Previous researchers have used a static protracted turn of the head to induce mismatch between the actual and perceived orientation of the head (Gurfinkel *et al.*, 1989). Under these conditions, these authors demonstrated the direction of the balance response evoked by GVS to be determined by the *perceived* head orientation. Thus, it was expected that the transformation of vestibular signals from head to body coordinates, which is necessary to generate a balance response, would be determined by the perceived head orientation. However, contrary to this expectation, the current results demonstrate that when illusory head orientation was induced by rotary stepping, the response direction continued to match the

actual head orientation. That is, the motor response was directed approximately along the interaural line, just as it was prior to the adaptive period.

Response direction is appropriate for perceived head orientation following static head-on-feet twist

In an additional study, an adaptive period more akin to the static adaptation adopted by Gurfinkel and colleagues was used. A prolonged static body twist resulted in a 10 degree mismatch between perceived and actual head orientation, when subjects subsequently attempted to stand with all body segments aligned. This can be explained by an altered perception of body segment orientation, as after-effects of this type have previously been shown following prolonged static rotation (Guerraz *et al.*, 2006;Mars *et al.*, 1998). In contrast to rotary stepping, the GVS-evoked response direction was no longer appropriate for the actual head orientation subsequent to the adaptive period. It was rotated by 10 degrees, in a direction opposite to the illusory head-on-feet reorientation. As the magnitude of the induced postural reorientation and the change in GVS-evoked response were approximately equal (both ~10 degrees), this indicates the direction of the vestibular-evoked balance response closely matched the direction expected if the head was facing directly forwards and aligned with the feet (i.e. the perceived orientation). Therefore, in accordance with the results of Gurfinkel and colleagues (1989), response direction was appropriate for the perceived head orientation following prolonged static rotation between body segments.

Rotary stepping and static twist have different effects on the direction of vestibular-evoked balance reflexes

The two adaptive periods used in the current study each induced a similar postural reorientation after-effect, suggesting that a similar illusory rotation of the head relative to the feet was experienced. However, the vestibular-evoked response direction matched the perceived head orientation following static body twist, but was appropriate for the actual head orientation following rotary stepping. But why would the two adaptive periods have similar effects on posture, but different effects on the vestibular-evoked movement?

Adaptation of motor output following rotary stepping may explain the differential effects of the two adaptive periods on the vestibular-evoked response direction. In addition to postural reorientation, subsequent to an adaptive period of rotary stepping a second after-effect termed "podokinetic after-rotation" (PKAR) is demonstrated; individuals unconsciously turn in circles when asked to step in place on a stationary surface without vision (Gordon *et al.*, 1995; Weber *et al.*, 1998). It was initially suggested that PKAR occurs because the signal for rotary stepping is recalibrated to the perception of zero rotation from other sensory channels during the adaptive period (Gordon *et al.*, 1995). However, it has since been shown that sensory conflict during the adaptive period is not a prerequisite for PKAR, as the after-effect is also demonstrated following a period of rotary stepping with naturally occurring perceptions of rotation from other sensory channels (Juergens *et al.*, 1999). Although the exact mechanism for PKAR remains unclear, evidence suggests motor output is modified, as the relative rotation between the feet and trunk during the adaptive period is subsequently preserved (Earhart *et al.*, 2001). It is plausible that the after-effect transfers to balance reflexes, as it has previously been demonstrated to transfer to other tasks, such as walking (Gordon *et al.*, 1995), backward walking (Earhart *et al.*, 2001) and hopping (Earhart *et al.*, 2002b). In contrast to rotary stepping, a prolonged static body twist does not cause PKAR (Osler & Reynolds,

2012), suggesting motor output is unaffected. Therefore, if motor output is affected following only rotary stepping, this might cause the response direction to be rotated subsequent to this adaptive period. If so, it appears the rotation of response direction is similar to the magnitude of postural reorientation, and, hence, it is appropriate for the actual orientation of the head. In contrast, although postural reorientation is induced following static twist, if motor output is unchanged from baseline then this may explain why the response is in a direction appropriate for the perceived orientation.

The complexity of the task performed in each adaptive period may also explain their effects on subsequently evoked balance responses. Morton and Bastian (2004) demonstrated that a visuomotor adaptation of walking using prism spectacles affected subsequent walking and reaching trajectories. However, in the same study, a visuomotor adaptation of reaching did not affect subsequent walking trajectories. Thus, stepping adaptation transferred to reaching, but the reverse was not true. The authors suggested that because stepping involves whole body movements and coordination of many body segments, it may therefore cause a general adaptation that transfers to other tasks. Reaching, on the other hand, involves isolated limb movement and may therefore lead to a more specific after-effect. The period of rotary stepping in the current study, which involved movement of many segments throughout the whole body, may also lead to an adaptation which transfers to many types of movement. However, the static body twist may only lead to a specific after-effect concerning body segment orientation, and therefore not transfer to movement.

Another explanation for the current results is possible differences in muscles and receptors involved in each adaptive period. Rotary stepping involves muscles throughout the body, and

it has been proposed that the subsequent PKAR after-effect is due to adaptation of many lower limb receptors, not simply hip rotation (Wong *et al.*, 2007). On the other hand, static body twist likely involves relatively few muscles. Specifically, the adaptive period would have primarily stretched hip and lumbar rotators, as the majority of body yaw rotation occurs at these joints. It could be that the specific muscles adapted following static twist are simply not involved in generating the vestibular-evoked response. However, previous findings which demonstrate GVS responses in muscles for lateral flexion at the trunk (Ali *et al.*, 2003;Ardic *et al.*, 2000) and hips (Day *et al.*, 1997) suggest otherwise. But whether these muscles are specifically those stretched during static body twist is open to question.

These potential explanations for my results are also compatible with the results of Gurfinkel *et al.* (1989). As the adaptive period used by these authors comprised a static head-on-trunk turn, it was therefore unlikely to induce any adaptation of lower limb motor output. It was an isolated movement of the neck, and was therefore unlikely to lead to an adaptation that transferred to many tasks. Furthermore, it involved only muscles for cervical rotation. Although important for the perception of head orientation, these muscles are not those primarily activated when generating vestibular-evoked balance reflexes.

Is the cerebellum involved in the coordinate transformation of vestibular signals for movement?

In order to evoke the appropriate motor commands for movement, vestibular sensory input must be transformed from head to body coordinates. The cerebellum has been proposed as a key structure in the coupling of sensory inputs and motor responses (Manzoni, 2005;2007).

For example, the adaptation of motor output when visual sensory input is displaced by prism lenses depends upon normal cerebellar function (Weiner *et al.*, 1983;Martin *et al.*, 1996). Manzoni (2005;2007) also suggests the cerebellum allows a coupling of sensory inputs and vestibular-evoked reflex motor responses. With functional inactivation of the cerebellum, vestibular-evoked postural reflexes in the cat are 'frozen' in a head-centred reference frame (Manzoni *et al.*, 1998 cited in Manzoni, 2005). In the monkey, vestibular nuclei cells primarily encode vestibular signals in a head-centred reference frame, whereas cells that encode these signals in a body-centred reference frame exist in the deep cerebellar nuclei (Shaikh *et al.*, 2004). Furthermore, in human patients with cerebellar degeneration, the direction of a vestibular-evoked balance response was not effectively modulated as the head was rotated over the trunk (Kammermeier *et al.*, 2009). Together, this evidence suggests the cerebellum is involved in the coordinate transformation of vestibular signals according to proprioceptive input signalling body segment orientation, which allows the CNS to generate the appropriate motor response, in the appropriate reference frame (both here and in Chapter 2). Projections from the cerebellum to the vestibular nuclei complex (Carleton & Carpenter, 1983;Voogd & Glickstein, 1998) offer potential pathways by which the cerebellum is involved, and may be important for the successful transformation of vestibular sensory signals to motor responses according to head orientation.

It has been suggested that the cerebellum is involved in the PKAR after-effect induced by rotary stepping (Earhart *et al.*, 2002a). As previously discussed, a motor output adaptation potentially affects vestibular-evoked responses following this type of adaptive period. If this is the case, it may also involve the cerebellum. Specifically, modified transformation of sensory input to motor output in this structure may alter the evoked motor response.

Summary

Adaptive periods of rotary stepping and prolonged static body twist both induced unperceived postural reorientation. This took the form of a fixed rotation of the head relative to the feet when standing. The direction of the vestibular-evoked balance reflex was determined by the perceived orientation, but only when the illusion was induced by a prolonged static body twist. In contrast, following rotary stepping the response direction was appropriate for the *actual* head orientation. The reason for this difference remains unclear. However, it may be because rotary stepping, unlike static body twist, leads to an adaptation of motor output, induces an after-effect which transfers to many tasks, or involves the muscles primarily involved in generating the vestibular-evoked balance reflex.

CHAPTER 4.

PASSIVE CUTANEOUS INPUT ATTENUATES VESTIBULAR-EVOKED BALANCE REFLEXES

The amplitude of a vestibular-evoked balance reflex is modulated by non-vestibular sensory input. It has been demonstrated that active touch of the finger with a fixed support reduces the size of the evoked response. However, the effect of passive cutaneous input remains unclear. Here, GVS was applied while subjects stood with their back lightly touching a foam pad. A number of contact forces were used, between 0.5 and 6N. The amplitude of the evoked balance response was significantly attenuated in all conditions, even in the lowest contact force condition (~0.5N), where negligible mechanical stabilisation was provided. This demonstrates that passive cutaneous input attenuates vestibular-evoked balance reflexes. Although the sway response was further reduced when contact force increased to ~6N, results show 0.5N contact provided 74% of the overall attenuation demonstrated. This suggests the majority of the effect was due to sensory input. However, results also showed a significant relationship between baseline sway speed and the response ($R^2 = 0.999$, $p < 0.001$). This suggests that the reduction in the response is largely explained by a reduction in baseline sway. As additional stability was mostly due to sensory input in the 0.5N contact condition, this raises the possibility that passive cutaneous input attenuates the vestibular-evoked response by an indirect mechanism, via a reduction in baseline sway.

4.1. Introduction

In addition to vestibular signals, self-motion information from other sensory inputs contributes to the control of balance (see General Introduction). If balance-relevant information from one of these inputs is removed, the response amplitude to a vestibular input increases. For example, it is well established that closing the eyes augments the response to GVS (Britton *et al.*, 1993;Fitzpatrick *et al.*, 1994;Day & Cole, 2002). Similarly, complete loss of proprioceptive and cutaneous inputs below the neck (Day & Cole, 2002) or severe loss of these inputs from the feet (Horak & Hlavacka, 2001) also lead to larger vestibular-evoked responses.

Cutaneous sensory input arising from contact with the ground or a fixed surface is also relevant for balance. For example, lightly touching a fixed support is just as effective at minimising spontaneous sway as allowing visual input (Jeka & Lackner, 1994;Holden *et al.*, 1994). As for other sensory inputs, if cutaneous inputs are reduced or removed, for example by cooling the soles of the feet, the vestibular-evoked response amplitude is increased (Magnusson *et al.*, 1990b). Conversely, additional input by active light fingertip contact with a fixed support reduces the response amplitude (Britton *et al.*, 1993). However, it is likely that touching a fixed support with the finger does not only provide additional cutaneous sensory information at the fingertip. Proprioceptive information from the muscles and joints of the entire arm may indicate motion of the body relative to a fixed support. Active touch contact with a fixed support may also lead to an improved estimation of body position, as active motion leads to an improved estimation of limb position compared to passive motion (Craske & Crawshaw, 1975;Paillard & Brouchon, 1968), probably because a copy of the efferent

command contributes to position sense (Gandevia *et al.*, 2006). Thus, the results of Britton *et al.* (1993) may be explained in part by additional proprioceptive input and efference copy during active touch. Passive light touch contact has been shown to improve stability (Rogers *et al.*, 2001;Menz *et al.*, 2006) suggesting simply cutaneous input is relevant for balance control, but whether it affects vestibular-evoked reflexes remains unclear.

Progressive modulation of the response amplitude with increasing balance-relevant visual input has been demonstrated (Day *et al.*, 2002;Day & Guerraz, 2007). These authors demonstrated a graded increase in the amplitude of vestibular-evoked balance responses as the visual environment was manipulated to provide less sensory information, by removing coloured LEDs from an otherwise blacked-out visual field. However, the effect of grading other sensory inputs on vestibular-evoked responses has not been investigated.

Here, I study the modulation of a vestibular-evoked balance reflex by light touch contact. GVS was used to evoke balance responses during graded levels of contact force between textured foam padding and the subject's back. The use of very low contact forces (~0.5N), offering negligible mechanical stabilisation, isolated the effect of passive cutaneous input upon the vestibular reflexes.

4.2. Methods

Subjects

Ten subjects (5 male) aged 20 to 29 years (mean±SD = 25±2 years) participated. All gave written informed consent and had no known neurological or vestibular disorder. The study was approved by the University of Birmingham Ethical Review Committee and performed in accordance with the Declaration of Helsinki.

Protocol

Subjects stood upright with barefoot feet together, still but relaxed, with the head facing forwards and hands clasped in front (see Figure 4.1A for diagram of method). Additional cutaneous sensory input was provided by textured foam padding (9x9x6cm) positioned on the midline of the back between the medial borders of the subjects' scapulae. Contact force between the subject and padding was measured by a horizontally mounted load cell and plotted in real time on a visual display, at a distance of 1m and at eye level. Five different target contact forces were used. In three conditions a target contact force for the subject to achieve was indicated on the visual display at the beginning of each trial (conditions: '1N', '3N' and '6N'). In another, subjects were instructed to achieve the smallest force possible whilst maintaining contact; this resulted in forces of approximately 0.5N (condition: '0.5N'). In the final condition, the subject and padding were not in contact (condition: 'No Contact'). In the no contact condition, visual display of contact force was not required. This was also true of the 0.5N condition since this very low level of force was achieved more accurately

simply by asking subjects to lean against the foam pad with the lightest force possible. During both the no contact and 0.5N conditions, subjects were instructed to focus on a fixation point in the centre of the screen at the beginning of each trial in order to keep head orientation the same as in the other force conditions.

Once the requested contact force was achieved, subjects pressed a hand held button to begin each trial. Visual information was immediately occluded by way of PLATO spectacles (Translucent Technologies, Toronto, Canada) and after 2 seconds GVS was applied using methods described in Chapter 2. A square impulse 3 seconds in duration and 1mA in amplitude was used. Vision was restored on completion of each trial, permitting subjects to once again meet contact force requirements for the subsequent trial.

The 5 conditions were each presented in 2 blocks of 10 trials (100 trials in total; 20 per condition). The order in which blocks of trials were presented was randomised and seated rest was permitted between each. Equal numbers of anode-left and anode-right trials were used.

Data Acquisition

Three-dimensional position data were sampled at 50Hz using a Fastrak motion analysis system (Polhemus Inc., Colchester, Vermont, USA). Sensors were placed on the sternum and the most superior point of the head. Ground reaction force data were sampled at 1 kHz using a Kistler 9281B force platform (Kistler Instrumente AG, CH-8408 Winterthur, Switzerland). EMG was recorded at 1 kHz from surface electrodes placed on the medial gastrocnemius muscle of each leg. EMG was amplified (5-10k) and band-pass filtered (10-300Hz) using a

Grass P511 preamplifier (Grass Technologies, West Warwick, USA). Light touch contact between the subject's back and foam padding was also measured at 1 kHz using a horizontally mounted load cell (Novatech Measurements Ltd., St Leonards on Sea, East Sussex, UK).

Data Analysis

Lateral body position and lateral ground reaction force data were averaged with respect to the onset of GVS across all trials in each condition. Baseline values at the time of GVS onset were first subtracted and position data were also first low-pass filtered (5Hz, 4^{th} order, zero phase Butterworth). Anode-left trials were inverted prior to averaging, since there was no significant effect of polarity on response magnitude (mean±SD is reported in the text throughout; initial force, anode-left 4.22±1.27N/sec, anode-right 3.98±1.36N/sec, $F_{1,9}$=0.498, p=0.498; peak sway, anode-left 4.24±1.99mm, anode-right 4.03±1.33mm, $F_{1,9}$=0.106, p=0.752). The maximum gradient between 180-400ms post GVS onset was measured from average lateral ground force traces ('Initial Force Response'; see Figure 4.2A). The rate of force onset was used as it is a very early yet robust measure of response amplitude. The time window was used as it correspond to the initial rise in GVS-evoked ground force and, importantly, it is before the response could be affected by non-vestibular sensory feedback of the resulting body sway. Peak displacement between 180ms-4s post GVS onset was also measured from average lateral body position traces ('Peak Sway Response'; see Figure 4.2C).

Baseline values for contact force, sway speed and EMG were calculated over the 250ms prior to GVS onset in each trial. Baseline contact force between the subject's back and the foam padding was calculated as the average value over this time period. Body position in the

horizontal plane was calculated from low-pass filtered (5Hz, 4^{th} order, zero phase Butterworth) mediolateral and anteroposterior position data and then differentiated to derive velocity. Sway speed was calculated from the two velocity vectors. Rectified EMG traces from left and right legs were averaged, before the area under the curve was estimated by trapezoidal integration. Each subject's baseline contact force, sway speed and EMG were averaged across all trials in each condition. To investigate whether the level of light touch contact changed over the course of the trials, average contact force was also calculated over the 250ms prior to GVS stimulus offset (i.e. 2750-3000ms).

A one-way RM ANOVA (SPSS general linear model) was used to compare initial force response, peak sway response, baseline sway speed and baseline EMG between conditions (condition: 0N, 0.5N, 1N, 3N, 6N). A logarithm (log10) transformation was used for baseline EMG and initial force response to normalise skewed datasets. Light touch contact force was analysed using a 4x2 RM ANOVA (condition: 0.5N, 1N, 3N, 6N; time: baseline, end of stimulus). In all cases, where significant Mauchly's tests indicated violation of the assumption of equal variances, the degrees of freedom were corrected using Greenhouse-Geisser estimates. Where RM ANOVA revealed significant effects, differences were analysed using pair wise comparisons following Bonferroni confidence interval adjustments.

The relationship of baseline contact force (CF) with response amplitude, baseline sway speed and baseline EMG was determined. Linear (1^{st} order polynomial; $f(CF)=aCF+b$) and non-linear (exponential; $f(CF)=ae^{-bCF}+c$) functions were compared. The calculated R^2 values were used to indicate goodness of fit, and thus, the suitability of each function to estimate the relationship. Since Pastor *et al.* (1993) demonstrated that GVS response sway speed is

linearly related to baseline sway speed, I also measured sway speed between 750-1000ms post GVS onset, to study the relationship between baseline and response. Furthermore, in the current results, both measures of sway magnitude were estimated to be similarly related to baseline contact force. Therefore, linear regression was used to estimate their relationship (as per Pastor *et al.*, 1993).

4.3. Results

Light touch contact force

Subjects attained mean baseline contact forces of 0.0±0.00N, 0.5±0.11N, 1.1±0.08N, 3.3±0.20N and 6.1±0.34N in the five conditions (see Figure 4.1B). As expected, there was a strong effect of condition on contact force ($F_{1.7,15.5}$=1300.92, $p<0.001$). All light touch contact conditions were significantly different from each other ($p<0.001$ for all comparisons). However, the level of contact force did not significantly change over the course of the trial (baseline, 2.74±0.12N; end of stimulus, 2.78±0.20N; $F_{1,9}$=1.91, $p=0.200$).

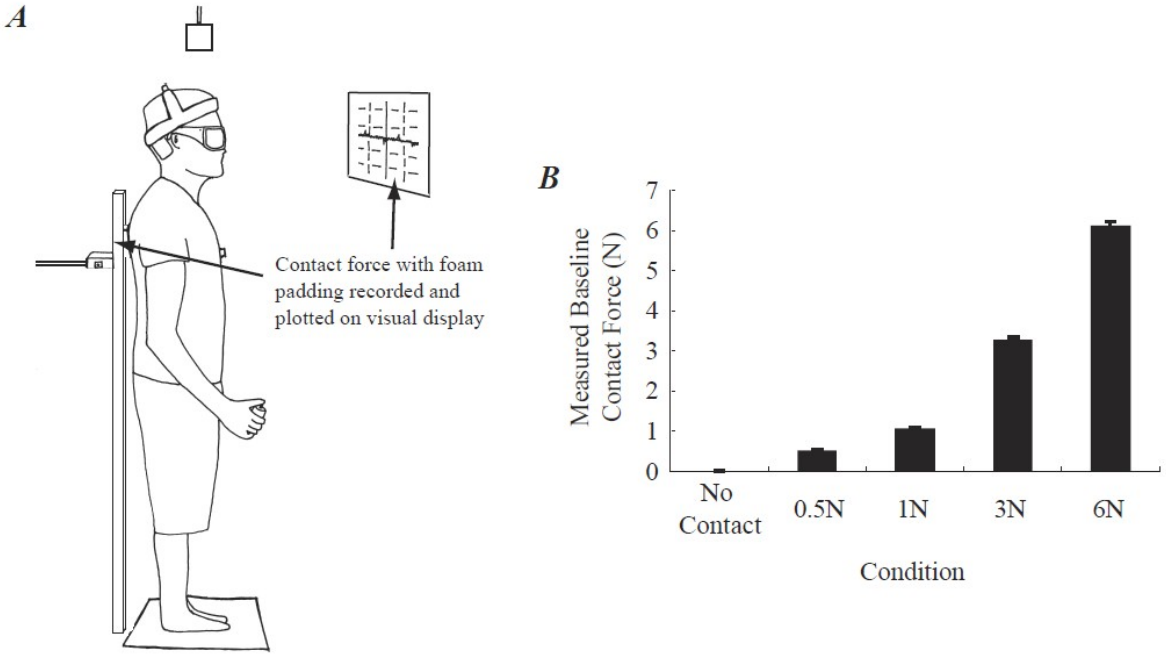

Figure 4.1. Light touch contact force. Measurement technique and baseline data

A) Subjects stood with their back in contact with foam padding attached to a vertical wooden plank. Contact force was registered with a load cell and displayed on a monitor at eye level. Once the required force was achieved a hand held button was pressed by the subject to occlude vision using PLATO specs and start the trial. ***B***) Group mean (±SEM) baseline contact force, measured during the 250ms prior to GVS onset.

Effect of light touch contact on vestibular-evoked response amplitude

Shortly following the onset of GVS a lateral ground force response was evoked (approximately 100ms post-GVS onset; see Figure 4.2A). In the no contact condition the initial force response was 6.80±2.28N/sec. However, this was significantly modulated by light touch contact ($F_{4,36}$=20.92, p<0.001; see Figure 4.2A-B). With power set at 0.80 and α at 0.05, power calculations indicate that a sample size of 10 in a RM ANOVA design is sufficient to detect an effect size of 0.23, which is a medium effect. Even at the lowest force level of 0.5N, the response was attenuated by 49% of that observed during no contact (3.47±1.72N/sec, p=0.028). The rate of force onset was also significantly reduced in all other contact conditions

compared to no contact (1N/3N/6N, $p<0.005$) and was significantly lower with 6N compared to 0.5N contact ($p=0.009$). The highest contact force condition displayed the greatest response attenuation (6N condition, 1.70±0.65N/sec), being 75% lower than no contact.

Following the rise in lateral force, a whole body movement towards the anode was demonstrated (300-400ms post-GVS onset; see Figure 4.2C). The peak sway displacement was 11.16±5.17mm in the no contact condition. However, as seen in the initial force response, light touch contact significantly reduced the peak sway response amplitude ($F_{1.2,10.7}=30.81$, $p<0.001$; see Figure 4.2C-D). With only 0.5N of light touch contact peak amplitude was significantly reduced by 67% of that observed during no contact (3.71±1.77mm, $p=0.020$). It was also significantly reduced in all other contact conditions compared to no contact (1N/3N/6N, $p<0.003$), and peak amplitude in the 0.5N condition was significantly different to all other conditions ($p<0.02$). The peak amplitude was most attenuated in the highest contact force condition (6N condition, 1.16±0.59mm), being 90% lower than no contact.

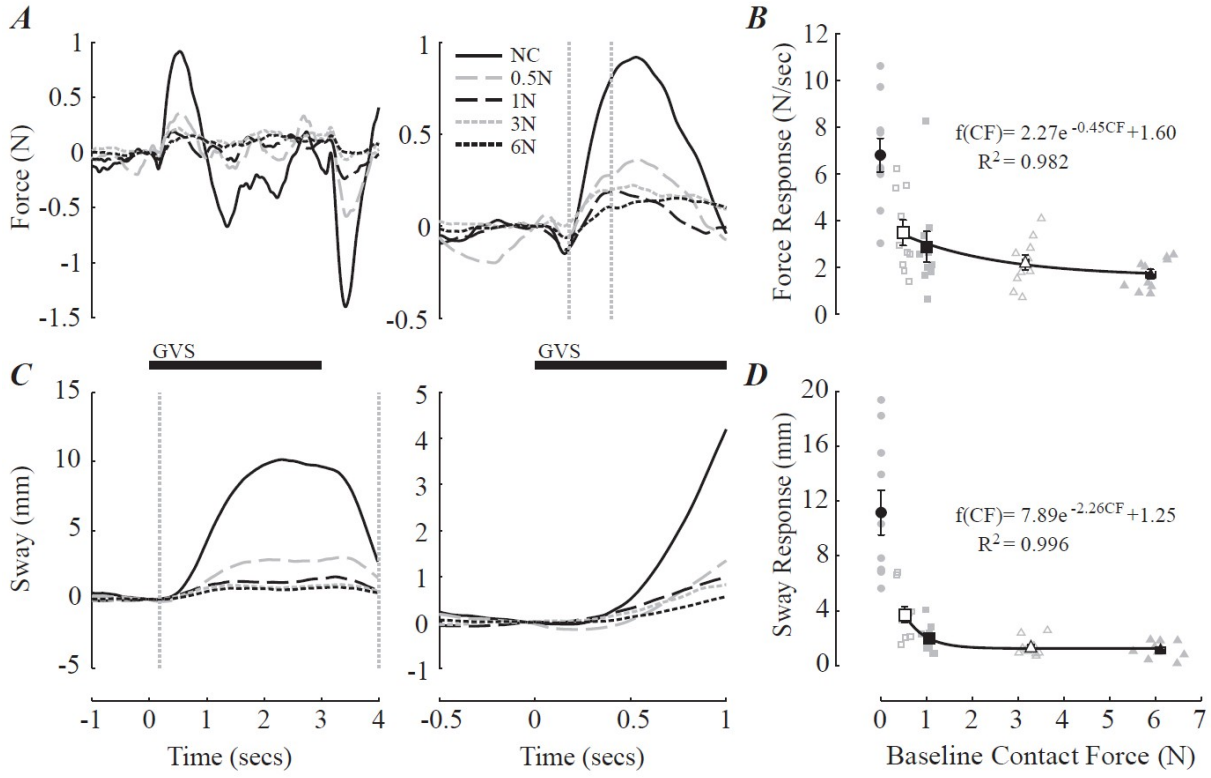

Figure 4.2. Attenuation of GVS-evoked balance reflexes with light touch contact.

A) Shortly following GVS onset a lateral ground force response was evoked, as shown by group average traces. Entire traces are plotted on left hand side, but just the initial 1 second is shown on the right. Here, vertical grey lines indicate the 180-400ms time period, during which the initial force response was measured. NC = No Contact. ***B***) Initial force response in each condition. Individual subject data is plotted in grey and group mean (±SEM) in black. The relationship between baseline contact force and force response was estimated by an exponential function. ***C***) The force response translated into whole body sway. In this case, vertical grey lines indicate 180ms-4s time period, during which peak sway response was measured. ***D***) Sway response in each condition. Once again, an exponential function was used to estimate the relationship between contact force and response.

With an increase in light touch contact force, a non-linear attenuation in both force and sway response amplitude was demonstrated (see Figures 4.2B and D). In both cases, an exponential fit resulted in a higher R^2 value than a linear fit (initial force response: exponential, R^2=0.982; linear, R^2=0.914; peak sway response: exponential, R^2=0.996; linear, R^2=0.611), indicating better goodness of fit and suggesting exponential functions are suitable to estimate the relationship between contact force and the response amplitude.

Effect of light touch contact on baseline conditions

Baseline medial gastrocnemius muscle activity was significantly affected by condition ($F_{4,36}$=6.33, p=0.001). Average baseline activity tended to be lower with contact (average of contact conditions, 2.17±1.57) compared to no contact (2.86±1.62). However, it was significantly reduced in only the 3N condition (2.00±1.54, p=0.011) and the reduction approached significance in the 6N condition (2.15±1.63, p=0.056). This may reflect a more posterior centre of mass (see Horak & Moore, 1993), on average, in order to maintain contact with the foam padding in the 3N and 6N conditions. But as foot position was not strictly controlled, the initial posture and, hence, muscle activity was quite variable in some subjects (individual subject coefficient of variance across the 5 conditions ranged from 5 to 50%). Furthermore, estimating the relationship between baseline contact force and baseline EMG using an exponential or linear function yielded relatively poor goodness of fit (exponential, R^2=0.135; linear, R^2=0.135). This suggests the change in muscle activity is not directly related to the level of contact force. It is probably indirectly modulated by a slight change in initial posture, in some subjects.

Baseline sway speed was 8.49±2.37mm/sec in the no contact condition, but was also significantly affected by light touch contact ($F_{1.9,16.8}$=42.46, p<0.001; see Figure 4.3B). As with response amplitude, baseline sway was significantly lower in all contact conditions compared to no contact (p<0.005 for all comparisons). Baseline sway was lower with 6N compared to 0.5N contact (p=0.024). Also, as with response amplitude, an exponential function provided a good, better than linear fit of the relationship between contact force and baseline sway (exponential, R^2=0.998; linear, R^2=0.612). With an increase in light touch

contact force, a non-linear reduction in baseline sway was demonstrated, to a minimum of 4.34±0.96mm/sec with 6N contact.

Relationship between baseline sway and response amplitude

Previous researchers have demonstrated response sway to be linearly related to baseline sway (Pastor *et al.*, 1993; see Figure 3D). The current results show response amplitude and baseline sway are both significantly reduced in all contact conditions compared to no contact. In addition, when calculated in a similar way to Pastor *et al.* (i.e. sway speed; see Figure 4.3A), baseline and response sway are estimated by similar non-linear functions of baseline contact force (compare Figures 4.3B and C). This prompts the question of whether modulation of response amplitude with light touch contact can be explained by changes in baseline sway. In the current data, there was a significant linear relationship between baseline and response sway speed across contact conditions (R^2=0.980, slope=2.02, intercept=-4.18, p=0.01). A similar significant relationship was estimated when the no contact condition was also included (R^2=0.999, slope=2.17, intercept=-4.84, p<0.001; see Figure 4.3D-E). T-tests to compare the slope and intercept of linear regressions (Zar, 1999) showed the two calculated equations were not statistically different (slope, t_5=0.710, p>0.50; intercept, t_5=0.692, p>0.50).

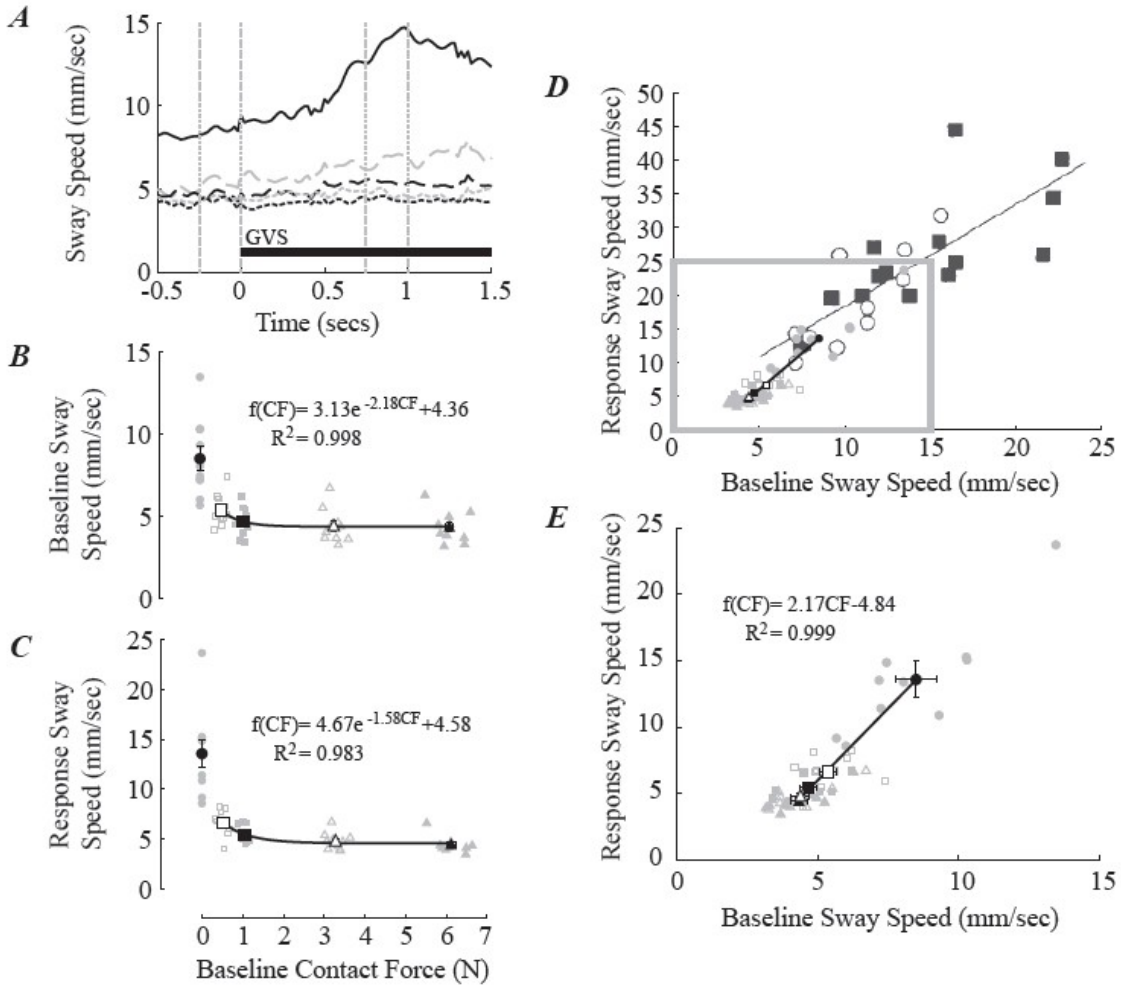

Figure 4.3. Linear relationship between baseline and response sway speed.

A) Sway speed traces in each condition. Baseline and response sway speed were measured between -250-0ms and 750-1000ms, respectively (indicated by vertical grey lines). Same legend as Figure 4.2A. ***B***) Baseline and ***C***) response sway speed were both reduced with light touch contact. The relationships between each variable and baseline contact force were estimated by a similar exponential function. ***D***) Linear relationship between baseline and response sway. Data from current study (small symbols) is superimposed with data from Pastor *et al.* (1993) (large symbols). ***E***) Enlarged section of ***D*** (indicated by grey box), to clearly show the linear relationship between baseline and response sway in the current study. Individual subject data plotted in grey and group mean (±SEM) in black. No Contact: filled circle, 0.5N: open square, 1N: filled square, 3N: open triangle, 6N: filled triangle. Figure 4.3D adapted from Pastor *et al.* (1993).

4.4. Discussion

In this chapter, I have demonstrated that the initial and peak vestibular-evoked balance responses are attenuated by light touch contact. The novel finding is that passive cutaneous sensory input attenuates the response. The attenuation was evident even during the lowest contact force condition, where there was negligible mechanical stabilisation. This suggests that the effect is due to sensory input. Furthermore, the results suggest that a reduction in baseline sway may largely explain the response attenuation observed with graded increases in contact force.

Cutaneous input attenuates vestibular-evoked responses

Attenuation of the vestibular-evoked balance reflex has previously been shown when subjects lightly touch a fixed support with their finger (Britton *et al.*, 1993). However, in this case, proprioceptive inputs from the entire arm and a copy of the efferent command for active touch may provide additional input that contributes to the control of balance. In the current study, the subject's back, not arm, was in passive contact with the fixed support. In addition, the 0.5N contact force condition isolated the effects of passive cutaneous inputs, as it provided minimal mechanical stabilisation. The total torque required to stabilise the body (mgh) has been reported as 11.6Nm/deg, on average (Loram & Lakie, 2002). Alternatively, using conservative estimates of mass (m) and centre of mass height (h) for an individual, total torque is calculated as 8.7Nm/deg (where, m=60kg , gravity=9.8m/s^2, h=0.85m). Although adjusted to the required position, the point of contact between the subject and foam padding was not placed at a height of greater than 1.5m for any subject. A contact force of 0.5N would

have therefore provided no more than 0.75Nm stabilizing torque. This equates to <3% of that required to balance the body when standing with a typical forward lean of around 3 degrees (Loram *et al.*, 2001). Although higher levels of contact force would have provided considerable mechanical stabilisation (6N contact provided up to 36% of stabilizing torque required), the majority of sway reduction occurred at the lowest force condition, in which 0.5N of contact touch force resulted in 67% sway reduction. The maximum force condition (6N) only resulted in a further 23% reduction (see Figure 4.2D). These results therefore suggest that the majority of the effect can be attributed to cutaneous sensory input.

The results demonstrate that in the 0.5N condition, cutaneous sensory input modulates vestibular-evoked responses in two ways. Namely, the rate of initial force application and the peak sway amplitude are both reduced. These two observations are in accordance with modulation of vestibular reflexes by altered visual (Day & Guerraz, 2007) and proprioceptive (Day & Cole, 2002) sensory inputs, and it is proposed they reflect two processes of sensory interaction (Day & Cole, 2002;Day *et al.*, 2002). Modulation of the very early stages of the response, even before any movement has occurred, suggests the gain of the vestibular-evoked reflex is altered. In addition, once movement is underway, the velocity of the developing response is minimised, due to the presence of non-vestibular sensory information which conflicts the virtual signal from vestibular afferents. Hence, both feedforward and feedback vestibular responses are attenuated by passive cutaneous input.

Graded increases in contact force further attenuate vestibular-evoked responses

As light touch contact force was gradually increased from 0.5N to 6N, the force and sway response amplitudes were further attenuated. Results showed this attenuation to be an exponential function of contact force, suggesting that light touch contact does not simply cause a fixed attenuation with all contact forces, but has a graded effect. This is somewhat similar to the graded response demonstrated as the richness of visual sensory input is altered (Day *et al.*, 2002;Day & Guerraz, 2007). However, here, it is not possible to say that the increased level of contact force leads only to a 'richer' sensory input, as it would also have inevitably provided considerable mechanical stabilisation at higher contact forces. Nevertheless, the 0.5N condition provided approximately three-quarters of the total sway response attenuation seen with 6N contact, suggesting that the vast majority of the effect is due to sensory input.

Potential mechanisms of response amplitude modulation

A 'proportional representation' model of sensory interaction (Day & Cole, 2002) proposed that each sensory input is weighted with reference to the other balance-related sensory information available. The balance response evoked by a perturbation to one sensory input is increased if other sensory inputs are providing little or no information about self-motion. In contrast, the response is reduced if other sensory inputs are providing veridical information regarding body motion and position. Thus, when additional cutaneous input is present, the vestibular input to balance control is down-weighted and the response to a given vestibular stimulus is reduced. Although the current results, which demonstrate that cutaneous input reduces the response gain of vestibular-evoked balance reflexes, can be explained by this sensory reweighting hypothesis, the exact underlying mechanism remains unknown.

Previous results demonstrated that the vestibular-evoked response is modulated by the pre-stimulus visual environment even when the post-stimulus onset environment is identical, suggesting a feedforward mechanism is involved (Day & Guerraz, 2007). Thus, the CNS may continuously determine other available balance related inputs and then use this information to compute the response to vestibular stimulation. In addition to receiving vestibular sensory input, the vestibular nuclei receive sensory input from many receptors, including cutaneous receptors, throughout the rest of the body (Jian *et al.*, 2002;Pompeiano, 1972). This connectivity may be through direct spino-vestibular pathways, or indirect pathways involving the cerebellum or reticular formation (Pompeiano, 1972). Thus, sensory integration mechanisms at the level of the vestibular nuclei, or another region of the CNS, may explain feedforward modulation of vestibular-evoked responses.

Alternatively, the results presented here suggest that modulation of vestibular-evoked responses can be largely explained by an indirect effect mediated by alterations in baseline sway. That is to say, as additional balance-related sensory input becomes available, spontaneous sway prior to stimulation is reduced and, hence, the response size is attenuated. In accordance with Pastor *et al.* (1993), the amplitude of a vestibular-evoked balance reflex was shown to be linearly related to baseline sway (see Figure 4.3D-E). Although it is perhaps unsurprising that two identical measures separated by one second are correlated, the linear equation which estimates their relationship is more revealing. A slope of 1 would have been shown if the reduction in response amplitude merely reflected a summation of a fixed response with a reduced baseline sway. But from my results I estimated a slope of ~2 (i.e. greater than 1), suggesting that the response size is not fixed but its amplitude is indeed modulated by changes in baseline sway. It is difficult to say whether, or to what extent,

mechanical stabilisation contributes to the reduction in baseline sway. However, passive light touch contact at forces which would have provided negligible mechanical stabilisation have been shown to lead to significant stabilisation (Rogers *et al.*, 2001;Menz *et al.*, 2006). Furthermore, although arm proprioception and efference copy may contribute, it has been reported that mechanical stabilisation does not contribute to reductions in baseline sway by <1N active fingertip contact (Kouzaki & Masani, 2008). Together, this evidence strongly suggests that passive cutaneous information alone can significantly stabilise the body. Nonetheless, the current results suggest that stability, by whatever means it is achieved, has a strong effect on the amplitude of vestibular-evoked balance reflexes.

Why and how baseline sway has such a potent effect on the vestibular-evoked sway response remains open to question. It was hypothesised by Martin (1965) that balance reflexes are only evoked by vestibular signals if the body is unstable, and that vision and proprioception inform the CNS of the current state of stability or instability. If baseline sway does in fact indicate stability of the body, then this hypothesis provides a feasible explanation. Furthermore, Martin (1965) suggested that, in conditions of stability, vestibular-evoked balance reflexes are suppressed by a "structure in the brain". However, the exact structure remains unknown, and more research is required to determine the exact mechanism which underlies this type of modulation of vestibular-evoked reflexes. It is interesting to note that differences in baseline sway or stability may, in part, explain previous findings which demonstrate vestibular-evoked balance responses are affected by factors such as availability of visual (Day *et al.*, 2002;Day & Guerraz, 2007) or proprioceptive and cutaneous sensory inputs (Day & Cole, 2002;Horak & Hlavacka, 2001;Magnusson *et al.*, 1990b;Britton *et al.*, 1993), stance width (Day *et al.*, 1997) and an unstable support platform (Fitzpatrick *et al.*, 1994;Horak & Hlavacka, 2001).

Summary

When lightly touching a fixed support with a low contact force, vestibular-evoked balance reflexes were attenuated by passive cutaneous sensory input. As contact force increased, response amplitude was further reduced. Although the exact mechanism for this modulation is unknown, the present results show a strong relationship between the evoked response amplitude and baseline sway. Therefore, I propose that modulation of vestibular-evoked balance reflexes by altered sensory input may be largely explained by an indirect effect mediated by changes in baseline stability.

CHAPTER 5.

ANTICIPATED SENSORY CONDITIONS DO NOT MODULATE VESTIBULAR-EVOKED BALANCE REFLEXES

Cognitive processes influence the control of balance, but whether they affect vestibular-evoked balance reflexes remains unclear. GVS-evoked balance responses are identical whether the stimulus is expected or not, suggesting anticipation of the timing of an external vestibular signal has no effect on the response evoked. However, it is unknown whether anticipation of the sensory conditions at the time of stimulus onset has an effect. The results in Chapter 4 clearly show that *actual* light touch contact modulates vestibular-evoked responses. Here, I ask whether *anticipated* light touch contact also has an effect. Subjects initially stood with their back in light touch contact with foam padding, which could be withdrawn using a servo motor. Vision was occluded and subjects pressed a button to trigger GVS. In some trials, they were informed that the foam padding would remain in place following GVS onset ('anticipated contact'). In the remainder, subjects anticipated the foam padding to be withdrawn simultaneous to GVS onset ('no contact'). However, in some 'no contact' trials the foam padding unexpectedly remained in place ('unanticipated contact'). Thus, the only difference between anticipated and unanticipated contact trials was the expected sensory conditions. Although the response amplitude was attenuated in both conditions where light touch contact was present, there was no difference between anticipated and unanticipated contact. These results demonstrate that, unlike *actual* sensory conditions, *anticipated* sensory conditions do not modulate vestibular-evoked balance reflexes. This suggests that cognitive processes do not prepare the systems that process vestibular signals for forthcoming sensory conditions.

5.1. Introduction

It is apparent that cognitive processes play a role in the control of balance. For example, the balance responses evoked by identical support-surface perturbations differ if preceded by trials involving the same or different amplitude perturbations, thus changing the anticipated perturbation characteristics (Horak *et al.*, 1989). The response to a support surface perturbation is also modulated if individuals are instructed to respond with a particular strategy (McIlroy & Maki, 1993) or if they concurrently perform an attention-demanding task (Rankin *et al.*, 2000). However, support surface perturbations activate many different sensory systems and thus evoke many mechanisms of postural control. How specific mechanisms, such as vestibular-evoked balance reflexes, are influenced by cognitive processes remains unclear.

If a moving visual scene is anticipated, the evoked postural response is attenuated (Guerraz *et al.*, 2001). In contrast, even if a proprioceptive perturbation by muscle vibration is self-triggered the response amplitude is not reduced (Caudron *et al.*, 2008). Similarly, anticipation of the timing of a vestibular perturbation does not modulate the postural response; whether GVS is self-triggered, predictable or unpredictable has no effect on the evoked body sway (Guerraz & Day, 2005). In addition, voluntarily following instructions to stand 'still' does not affect the initial response evoked by vestibular stimulation (Reynolds, 2010). This evidence suggests that vestibular reflexes are inflexible to cognitive modulation. This may be because the vestibular system, compared to the visual system, shares few connections with the cortex. However, projections from the cortex to the vestibular nuclei complex have been shown in the monkey (Akbarian *et al.*, 1993;1994). Furthermore, asymmetrical vestibular-evoked reflexes

are demonstrated following damage to brain regions where such projections lie, which suggests that they may be involved in the vestibular control of balance (Marsden *et al.*, 2005).

If cognitive processes are involved, their function may be to prepare the balance control system for forthcoming sensory conditions. During locomotion, both actual and anticipated sensory conditions modify gait characteristics. For example, if a slippery floor surface is anticipated, a cautious gait pattern is observed (Cham & Redfern, 2002;Marigold & Patla, 2002). It is well established that actual sensory conditions modulate vestibular-evoked reflexes; the response evoked by GVS is attenuated by increases in visual (Day *et al.*, 2002;Day & Guerraz, 2007), proprioceptive (Horak & Hlavacka, 2001;Day & Cole, 2002) and cutaneous (Magnusson *et al.*, 1990b;Chapter 4) sensory feedback. Furthermore, a recent study reported that sensory conditions prior to the onset of GVS also affect the response amplitude (Day & Guerraz, 2007). This suggests that vestibular reflexes can be modulated by feedforward processes. But it is unclear whether these reflexes, like gait characteristics, are affected by anticipation. Although vestibular-evoked balance reflexes are unchanged when an individual is able to anticipate the timing of the stimulus (Guerraz & Day, 2005), it is unknown whether anticipation of the sensory conditions at the time of stimulus onset has an effect. If cognitive processes prepare the balance control system for forthcoming sensory environment, and vestibular-evoked balance reflexes can be affected by feedforward processes, then the anticipated conditions may modulate the vestibular-evoked response.

Here, the effect of anticipated sensory conditions on the amplitude of a vestibular-evoked balance reflex was investigated. Subjects pressed a button to trigger GVS, thus making the timing of the stimulus predictable. Foam padding provided cutaneous sensory input at the

start of each trial, but could be withdraw at the time of stimulus onset. A context was engineered where the presence of the foam padding was anticipated in some trials, but unanticipated in others.

5.2. Methods

Subjects

Ten subjects (7 male) aged 20 to 31 years (mean±SD = 24±3 years) participated. Three subjects also participated in the experiment presented in Chapter 4. All gave written informed consent and had no known neurological or vestibular disorder. The study was approved by the University of Birmingham Ethical Review Committee and performed in accordance with the Declaration of Helsinki.

Protocol

Subjects stood upright and still but relaxed, with barefoot feet together, head facing forwards and hands clasped in front. As in Chapter 4, subjects stood in contact with textured foam padding (9x9x6cm) positioned on the midline of the back between the medial borders of their scapulae. In all conditions subjects attained a contact force of 1.5±0.5N at the start of the trial. The target range and a real-time plot of contact force, as measured by a horizontally mounted load cell, were visually displayed at a distance of 1m and at eye level.

Once subjects met the required contact force, they pressed a hand held button to begin each trial. At this point visual information was occluded by way of PLATO spectacles (Translucent Technologies, Toronto, Canada). After two seconds an audible tone was sounded, which informed subjects to press the button a second time in order to self-trigger GVS. A square impulse 0.6 seconds in duration and 2mA in amplitude was delivered using methodology described in Chapter 2.

Although sensory conditions prior to GVS were identical in all trials, the foam padding providing light touch contact could be abruptly withdrawn by way of a servo motor (Copley Controls, Canton, MA, USA) upon the second button press (i.e. simultaneous to GVS onset). In 6 blocks of 30 trials, subjects believed that the simultaneous withdrawal of light touch contact would occur (180 trials in total). In 80% of these trials the withdrawal actually occurred (condition: 'no contact'; see Figure 5.1A), but in the remaining 20% it did not (condition: 'unanticipated contact'; see Figure 5.1B). Unanticipated contact trials were presented at random within each block (6 per block; 36 trials in total). In a further block of 36 trials subjects were aware that no such withdrawal would occur (condition: 'anticipated contact'; see Figure 5.1C). Importantly, as all other instructions were identical in all conditions, the only difference between anticipated and unanticipated contact trials was the anticipated sensory conditions post GVS onset. Therefore, the main comparison of interest is between these two conditions in which everything except subject expectation is identical.

Equal numbers of trials with GVS anode-left, anode-right and no GVS were used. Visual information was allowed at the end of each trial, permitting subjects to once again meet contact force requirements. For half of the subjects the block of anticipated contact trials was

presented first, and for the remainder it was presented last. Seated rest was permitted between blocks.

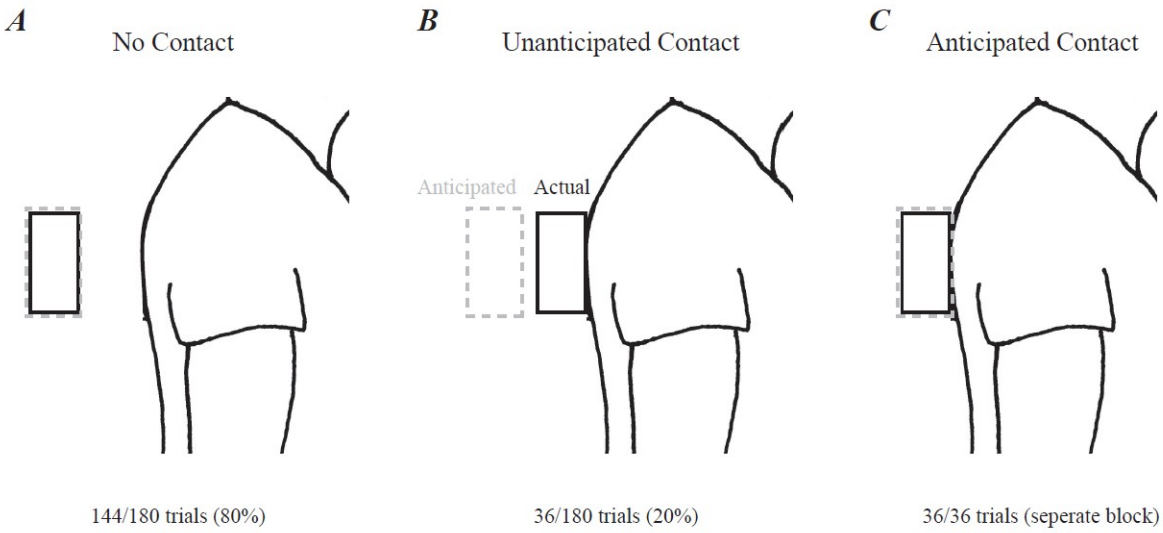

Figure 5.1. Sensory conditions

Illustrations of the three conditions used, ***A***) 'no contact', ***B***) 'unanticipated contact' and ***C***) 'anticipated contact'. Dashed grey and solid black boxes represent the anticipated and actual position of foam padding subsequent to GVS onset, respectively. For instance, in ***B*** the foam padding was anticipated to be withdrawn from contact with the back, but it transpired to in actual fact remain in contact. Therefore, the condition is termed 'unanticipated contact'.

Data Acquisition

Three-dimensional position data were sampled at 60Hz using a Fastrak motion analysis system (Polhemus Inc., Colchester, Vermont, USA). Sensors were placed on the sternum and the most superior point of the head. EMG was recorded at 1 kHz from surface electrodes placed on the medial gastrocnemius muscle of each leg and amplified (10k) using a Bagnoli handheld EMG system (Delsys Inc., Boston, USA). Light touch contact between the subject's back and the foam padding was also measured at 1 kHz using a horizontally mounted load cell (Novatech Measurements Ltd., St Leonards on Sea, East Sussex, UK).

Data Analysis

Position data were averaged with respect to GVS onset (or button press in 'no GVS' trials) across all trials in each condition. Data were first low-pass filtered (5Hz, 4th order, zero phase, Butterworth) and positions at the time of GVS onset were subtracted. Peak lateral body displacement between 180ms-2s post GVS onset was measured from average sway traces (see Figure 5.3A). The magnitude of anode-left and right responses were not significantly different (mean±SD is reported in the text throughout; anode-left, 2.66±1.81mm; anode-right, 2.68±1.63mm; $F_{1,9}$=0.011, p=0.917). Therefore, anode-left trials were inverted and combined with anode-right trials. To determine whether the removal of the foam padding was anticipated by subjects, head and body positions at 100ms post button press were measured in no GVS trials (see Figure 5.2). Baseline contact force, sway speed and EMG were calculated over the 250ms prior to GVS onset in each trial, as described in Chapter 4.

A one way RM ANOVA (SPSS general linear model) was used to compare calculated measures between conditions (condition: no contact, anticipated contact, unanticipated contact). Logarithm (log10) transformations were used to correct skewed datasets. Where significant Mauchly's tests indicated violation of the assumption of equal variances, the degrees of freedom were corrected using Greenhouse-Geisser estimates. Where RM ANOVA revealed significant effects, differences were analysed using pair wise comparisons following Bonferroni confidence interval adjustments

5.3. Results

Anticipation of sensory conditions

As expected, no subject reported being able to predict 'unanticipated contact' trials, as they were randomly presented at a low rate of occurrence (20%) amongst 'no contact' trials. Data from no GVS trials are considered in this section and presented in Figure 5.2, to determine the effects of anticipated sensory conditions. A significant effect of condition on anteroposterior head position at 100ms post button press shows that contact was indeed unanticipated during 'unanticipated contact' trials ($F_{2,18}$=42.830, p<0.001); subjects demonstrated a very small but significant forward head movement compared to anticipated contact trials (unanticipated contact, 0.172±0.114mm; anticipated contact, 0.016±0.157mm; p=0.040; see Figure 5.2A). However, at this time point, there was no significant difference in body anteroposterior movement between these conditions (unanticipated contact, 0.004±0.112mm; anticipated contact, -0.085±0.123mm; p=0.241; see Figure 5.2B). Condition had no effect on the mediolateral position of the head ($F_{2,18}$=0.349, p=0.710; Figure 5.2C) or body ($F_{1.21,10.86}$=1.022, p=0.351; Figure 5.2D) at 100ms post button press. Furthermore, peak lateral body sway was not significantly affected by condition (no contact, -0.46±2.86mm; unanticipated contact, 0.19±1.37mm; anticipated contact, 0.14±0.86mm; $F_{1.28,11.51}$=0.405, p=0.587). Thus, anticipatory postural adjustments were very small, orthogonal to the vestibular-evoked response, and did not interfere with the measure of response amplitude reported in the following section.

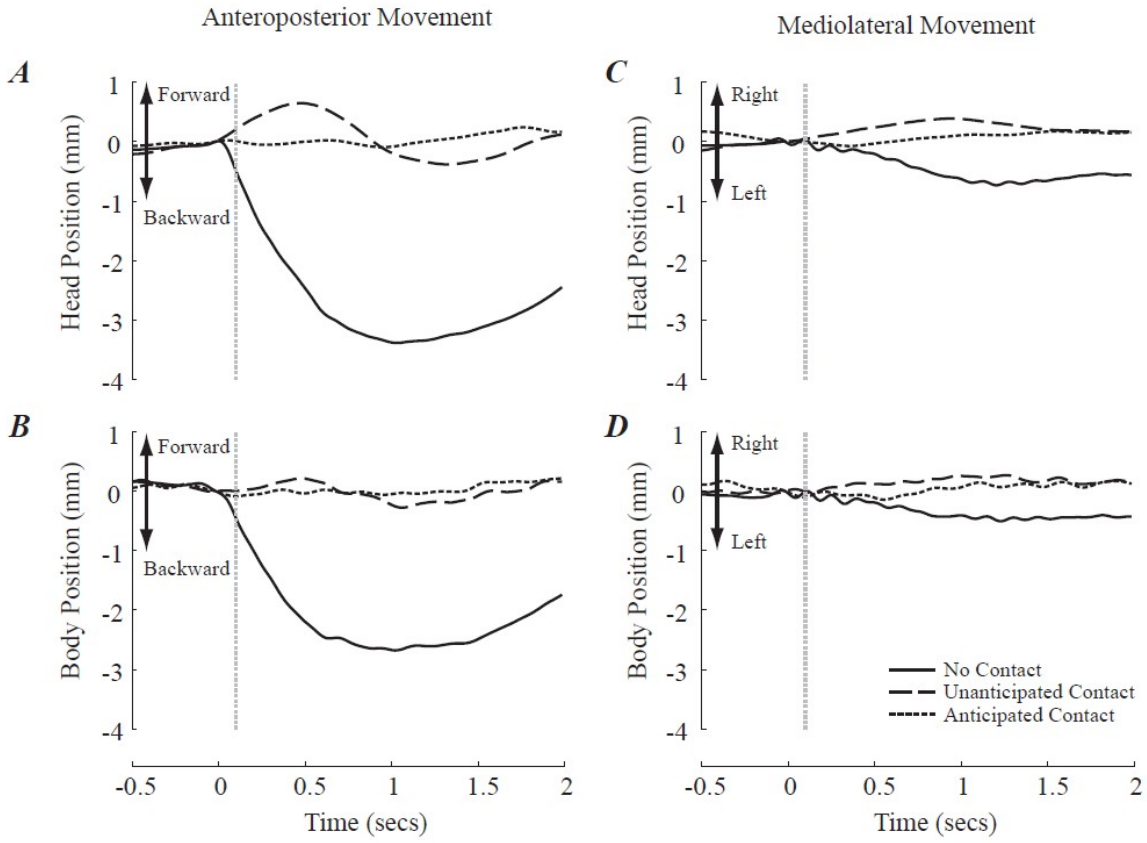

Figure 5.2. Early postural adjustment demonstrates 'unanticipated contact' condition was 'unanticipated'. Data from 'no GVS' trials.

A) Group average anteroposterior head motion traces show that over the initial 100ms after expected removal of foam padding (vertical grey line), a small but significant forward movement was demonstrated in the 'unanticipated contact' condition (dashed trace). This was not demonstrated in 'anticipated contact' trials (dotted trace), suggesting it can be attributed to the subjects' expectations. In no contact trials (solid trace), subjects moved backwards when the foam padding was removed. ***B***) The anticipatory adjustment was not significant at the level of the body. In addition, the anticipated sensory conditions did not have a significant effect on mediolateral sway at the level of the ***C***) head or ***D***) body.

No effect of anticipated sensory conditions on vestibular-evoked responses

Lateral body displacement was evoked 300-400ms following application of GVS (see Figure 5.3A). Peak displacement was significantly affected by condition ($F_{2,18}$=24.016, p<0.001; see Figures 5.3A-B). Although the evoked sway response was significantly attenuated in both

conditions where light touch contact was present compared to no contact (no contact, 4.83±2.95mm; p=0.001 for both comparisons), it was not significantly affected by whether light touch contact was anticipated or not (unanticipated contact, 1.24±1.09mm; anticipated contact, 1.32±1.40mm; p=1.00).

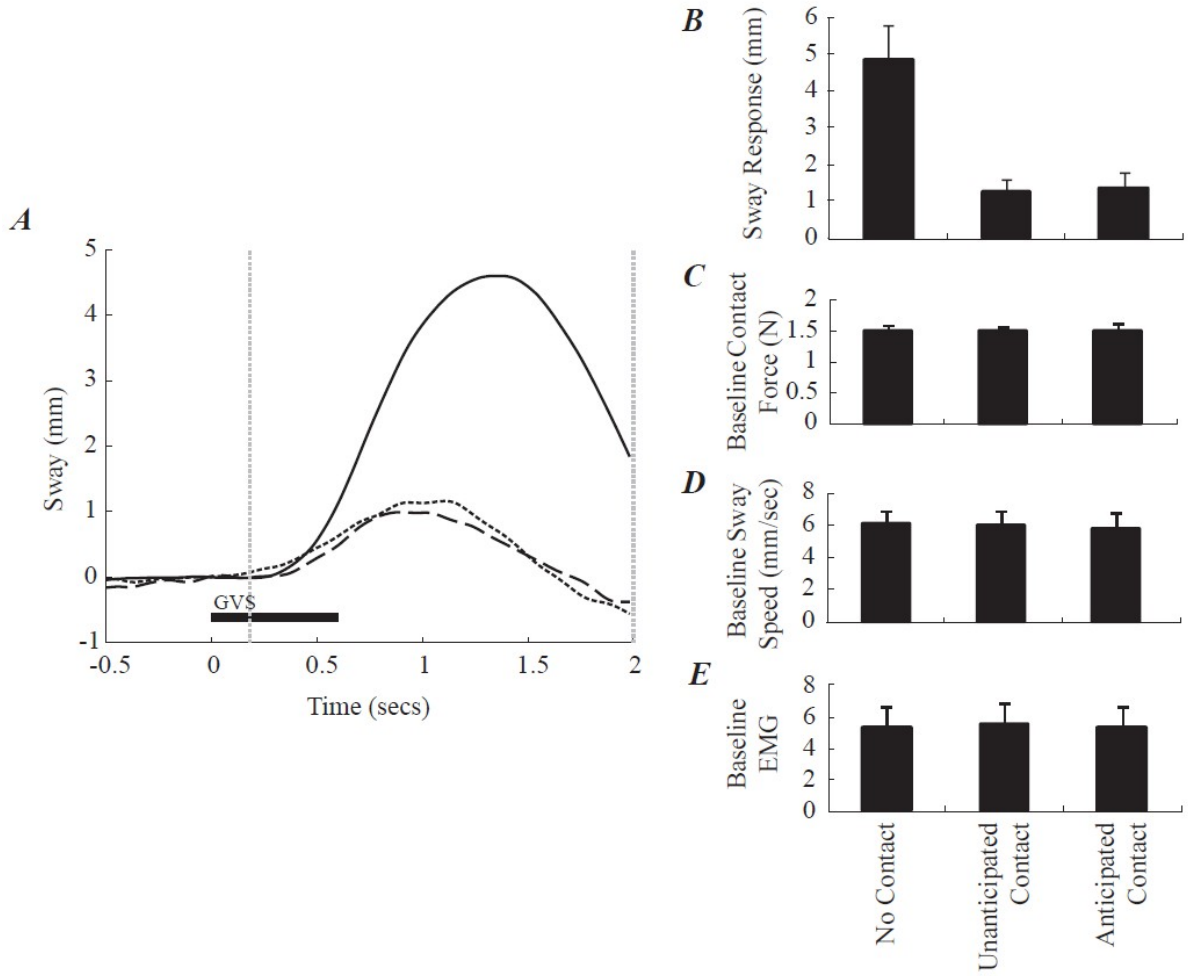

Figure 5.3. GVS-evoked sway response and baseline conditions.

A) Shortly following GVS onset (thick black horizontal line) a lateral sway response was evoked in the direction of the anode. Group mean traces show that the response was largest in the 'no contact' condition (solid trace). Although attenuated in both other conditions, the response was similar in 'unanticipated contact' (dashed trace) and 'anticipated contact' (dotted trace) trials. Sway response was taken as the peak response amplitude between 180ms-2s (vertical grey lines). Same legend as in Figure 5.2. ***B***) Group mean (±SEM) peak sway response. As shown in ***C-E***, the experimental condition had no effect on baseline ***C***) contact force ***D***) sway speed or ***E***) gastrocnemius EMG.

No effect of anticipated sensory conditions on baseline conditions

The finding that GVS-evoked responses were similar in magnitude in both anticipated and unanticipated contact conditions (see Figures 5.3A-B) has more than one possible explanation. If the anticipated sensory conditions induced a change in baseline sway which indirectly modulated the response amplitude by a separate and counteracting mechanism, then the direct effects of anticipation alone would not be apparent. Thus, the baseline conditions, which are known to modulate response amplitude, were quantified. As expected, because pre GVS instructions were identical in all conditions, there were no significant differences in baseline contact force (no contact, 1.50±0.29N; unanticipated contact, 1.48±0.26N; anticipated contact, 1.49±0.37N; $F_{1.06,9.56}$=0.198, p=0.681; see Figure 5.3C), baseline sway speed (no contact, 6.01±2.69mm/sec; unanticipated contact, 5.97±2.59mm/sec; anticipated contact, 5.74±3.25mm/sec; $F_{2,18}$=0.659, p=0.529; see Figure 5.3D) or baseline medial gastrocnemius activity (no contact, 5.37±4.14; unanticipated contact, 5.52±4.26; anticipated contact, 5.30±4.28; $F_{1.01,9.09}$=0.031, p=0.866; see Figure 5.3E). Thus, indirect effects of altered baseline conditions which could have potentially confounded direct effects of anticipation can be ruled out.

5.4. Discussion

In this chapter the novel finding is that vestibular-evoked balance reflexes are not modulated by the anticipated sensory conditions. The amplitude of a GVS-evoked sway response was reduced during light touch contact (in accordance with Chapter 4), but whether these sensory conditions were anticipated or not had no effect. There was no difference between the sway

responses observed during anticipated and unanticipated light touch contact. This suggests that cognitive processes of anticipation do not alter the processing of vestibular signals for forthcoming sensory conditions.

Anticipatory postural adjustments

When an anticipated withdrawal of the foam padding did not occur, subjects displayed a small but significant forward head movement. This postural adjustment commenced within 100ms of the anticipated withdrawal, suggesting it was not reactive but predictive of motion. Thus, it is an example of an anticipatory postural adjustment. Just as anticipatory mechanisms operate prior to making a voluntary movement that is likely to disturb posture (for review see Massion, 1992), here, motor commands were generated in anticipation of removal of the foam padding. Although the motor action of pressing the button was not destabilising in itself, when anticipated to bring about a destabilising event even a minor action can be associated with anticipatory postural adjustments (Aruin & Latash, 1995). Even in the absence of sensory inputs signalling that the foam padding had been removed and that the body was in backwards motion, these motor commands were still generated. This result demonstrates the withdrawal of the foam padding was in fact anticipated during 'unanticipated contact' trials.

Actual, but not anticipated, sensory conditions modulate vestibular-evoked balance reflexes

When the foam padding was withdrawn, the amplitude of the vestibular-evoked balance response was increased compared to when the foam padding was present throughout the trial. This is in accordance with the results presented in Chapter 4, which demonstrated that light

touch contact with a fixed support attenuates vestibular-evoked balance reflexes. However, vestibular-evoked balance reflexes were unaffected when the withdrawal of the foam padding, and thus change in sensory conditions, was merely anticipated ('unanticipated contact' trials). Power calculations for a pairwise comparison, with power set at 0.80 and α at 0.167 (corrected for multiple comparisons; 0.05/3), indicate that a sample size of 10 should be sufficient to detect an effect size of 1.12. In the current data this corresponds to a minimal detectable difference of 0.72mm. It is therefore possible that a small effect would not have been detected. Nonetheless, whole-body sway in anticipated and unanticipated contact conditions was numerically almost identical and there was not even a hint of a significant result (difference=0.08±0.64mm; p=1.00). This strongly suggests that anticipation does not modulate the amplitude of a vestibular-evoked balance reflex. This is in contrast to during locomotion, where anticipation of sensory conditions underfoot has been shown to modify gait characteristics (Cham & Redfern, 2002;Marigold & Patla, 2002). Gait characteristics, however, are quite obviously more inclined to modification, as it is possible and sometimes necessary to adjust how and where one steps based upon cognitive processes such as volition, prior knowledge and anticipation. Hence, these results suggest that, although feedback vestibular responses are attenuated by post-stimulus cutaneous input, feedforward responses are unaffected by anticipation.

Vestibular-evoked balance reflexes appear inflexible to cognitive processes

The anticipated forthcoming sensory conditions do not affect the vestibular-evoked balance reflex. However, actual pre stimulus sensory conditions have been demonstrated to affect the subsequent response, suggesting that, in this case, feedforward modulation does occur (Day &

Guerraz, 2007). These researchers showed the GVS-evoked response amplitude to be modulated by pre stimulus visual conditions, even when post stimulus onset visual conditions were identical. This feedforward effect is based upon actual sensory information, thus making it clearly distinct from the effects of anticipation (i.e. a cognitive process) investigated in the current study.

The fact that anticipated sensory input does not modulate the response may be explained by the connectivity within the CNS that would presumably be required for cognitive processes to be integrated with vestibular signals for balance. That is, connections between cortical and sub-cortical systems. In contrast, actual cutaneous inputs are likely signalled from the spinal cord to the vestibular nuclei complex (Jian *et al.*, 2002;Pompeiano, 1972). If the feedforward mechanism based upon actual sensory input reflects a direct modulation, involvement of only sub-cortical systems would be required. Alternatively, the previously established effects of baseline stability (see Chapter 4) may lead to an indirect modulation of response amplitude by changes actual sensory input. The response amplitudes measured by Day and Guerraz (2007) in different visual environments seem to correlate with baseline sway, and could therefore be explained by the indirect effect. Furthermore, since the current results showed that baseline sway was similar in all conditions, the indirect effect may also account for the differences between the present findings and those of Day and Guerraz (2007).

The cerebral cortex can influence postural responses to physical perturbations (for review see Jacobs & Horak, 2007) and the involvement of cortical projections in the vestibular control of balance has been suggested (Marsden *et al.*, 2005). However, although there is evidence that corticovestibular connections exist (Akbarian *et al.*, 1993;1994;Wilson *et al.*, 1999) and brain

imaging techniques have shown vestibular stimulation to activate cortical areas (Lobel *et al.*, 1998), the function of these connections in the control of balance has not been demonstrated. It is evident that these connections do not allow modulation of initial vestibular-evoked balance reflexes by anticipation of forthcoming sensory conditions, anticipation of timing of vestibular disturbance (Guerraz & Day, 2005) or voluntary suppression (Reynolds, 2010). More research is needed to determine the involvement, if any, of cognitive processes, the cerebral cortex and cortical projections in the processing of vestibular signals for balance.

Summary

Light touch contact with a fixed support reduced the amplitude of vestibular-evoked balance reflexes. However, whether light touch contact was anticipated or not had no effect on the evoked sway response. This demonstrates that, unlike *actual* sensory conditions, *anticipated* sensory conditions do not modulate vestibular-evoked balance reflexes. Thus, cognitive processes do not prepare the systems that process vestibular signals for the anticipated forthcoming sensory conditions. It remains unclear whether cognitive processes can modulate vestibular-evoked balance reflexes.

CHAPTER 6.

FEAR OF FALLING HAS NO EFEFCT ON VESTIBULAR-EVOKED BALANCE REFLEXES

Fear of falling occurs when there is a perceived threat to posture, for example when exposed to height. It results from the interaction of many factors including emotion, sensory information and perception. Here, I determine how a fear of falling affects vestibular-evoked balance reflexes. Although previous results suggest they are unaffected by cognitive factors, I created the strongest possible motivation to adopt a modified balance control strategy and therefore influence these reflexes. Nine subjects stood with eyes closed on a narrow walkway elevated 3.85m above ground level. This evoked a fear of falling, as evidenced by a twofold increase in skin conductance. GVS was then used to evoke balance reflexes. The body sway response was significantly and substantially attenuated after ~800ms when standing at height, demonstrating that subjects were highly motivated to avoid body displacement. However, the initial component of the response remained identical whether standing at height or at ground level. These results suggest a fear of falling influences only later integrative balance mechanisms, which modulate the response once the body is in motion. Furthermore, peak displacement was reduced by minimising sway velocity, but the position error was not corrected until after GVS ceased. This suggests the integrative balance mechanisms are sensitive to dynamic rather than static non-visual, non-vestibular information. Nonetheless, as the *initial* vestibular-evoked balance reflex was unaffected even in this highly motivated context, this strongly suggests that it is indeed outside of cognitive and emotive control.

6.1. Introduction

Vestibular-evoked balance reflexes have been shown to be context-dependent. For instance, it is well established that their amplitude varies as a function of balance-relevant input to other sensory channels (Magnusson *et al.*, 1990b;Britton *et al.*, 1993;Horak & Hlavacka, 2001;Day & Cole, 2002;Day & Guerraz, 2007), and their direction is determined by the orientation of the head (Lund & Broberg, 1983;Pastor *et al.*, 1993;Reynolds, 2011). However, there is currently no evidence to suggest they can be modulated by cognitive or emotive factors. In fact, evidence suggests the contrary. The initial sway response evoked by a pure vestibular perturbation is identical whether it is self-triggered, predictable or unpredictable (Guerraz & Day, 2005) and it is not attenuated by voluntary attempts to remain still (Reynolds, 2010). Furthermore, anticipation of forthcoming sensory conditions has no effect on the sway response evoked by GVS (see Chapter 5). These results suggest expectation and volition have no effect on vestibular-evoked balance reflexes. But so far only relatively weak interventions have been used to alter motivation. To truly test if vestibular reflexes are susceptible to modulation by cognitive and emotive factors, the strongest possible motive is required; much stronger than, for instance, merely instructing a subject to voluntarily stand still.

A strong emotional factor particularly relevant to the control of balance is a fear of falling, which occurs when there is a perceived threat to balance. It is induced by the interaction of many components including emotion, sensory information and perception. Maki *et al.* (1991) were the first to make an association between a fear of falling and balance control in the elderly. Furthermore, recent findings suggest a causal link between a fear of falling and falling itself, again in elderly individuals (Delbaere *et al.*, 2010). However, studying postural

control in the elderly is often confounded with other factors such as loss of strength or sensory degradation. To address this limitation many studies have induced fear of falling in young healthy individuals by asking them to stand at height. This has been shown to manipulate cognition (Tersteeg *et al.*, 2012) and to motivate changes in the control of balance which influence spontaneous sway (Carpenter *et al.*, 1999;2001;Adkin *et al.*, 2000;Davis *et al.*, 2009), anticipatory postural adjustments (Adkin *et al.*, 2002) and balance responses evoked by physical perturbations (Brown & Frank, 1997;Carpenter *et al.*, 2004). In response to a physical perturbation in a predetermined direction, body displacements were reduced in amplitude when a fear of falling was induced by standing at height (Brown & Frank, 1997). This is consistent with other results showing that balance-correcting muscle activity increases in response to multi-directional support surface perturbations, also leading to reduced body displacement (Carpenter *et al.*, 2004). One possible explanation is that a fear of falling leads to an increased gain of vestibular-evoked balance reflexes. However, this cannot be concluded on the basis of these results, as the perturbations used did not solely activate the vestibular system. Nonetheless, they *do* suggest that a fear of falling stimulates changes in motivation which, in turn, bring about changes in the control of balance and a strong intent to minimise body displacement. Thus, when fearful of falling, individuals may adopt a strategy of avoiding movement at all costs.

But is a fear of falling likely to affect vestibular-evoked balance reflexes? Does fear motivate changes in the processing of vestibular signals that cannot be achieved by merely expectation and volition? Fear does appear to alter motivation, and is also expressed behaviourally and physiologically. A key structure in the expression of fear is the amygdala (Davis, 1992;Ledoux, 2000;Phan *et al.*, 2002), but many other areas of the cortex and brainstem may

also subserve this function. Balaban (2002) proposed three neural pathways which may link fear and the processing of vestibular signals. Specifically, these were connections to the vestibular nuclei complex from the parabrachial nucleus (Balaban, 2004), locus coeruleus (Schuerger & Balaban, 1999) and raphe nuclei (Halberstadt & Balaban, 2003), which have all been shown in animal studies. Furthermore, projections from the amygdala to all three of these structures have been implicated in the expression of fear (Davis, 1992). Connections to the vestibular nuclei complex have also been shown from the cingulate and insular cortices in the monkey (Akbarian *et al.*, 1993;1994). These regions are thought to play a role in fear expression (Milad *et al.*, 2007;Vogt *et al.*, 2003) and the subjective awareness of fear (Critchley *et al.*, 2002;Morris, 2002), respectively. Thus, any number of these connections may allow a fear of falling to influence the processing of vestibular signals for balance.

This chapter investigates how a fear of falling affects vestibular-evoked balance reflexes. A postural context of standing at height on a narrow walkway was used to induce a fear of falling, as quantified by skin conductance. Thus, a strong motive to influence the control of balance was created. GVS was used to evoke balance reflexes, in order to study the effect of fear of falling on reactions to a pure vestibular stimulus.

6.2. Methods

Subjects

Nine subjects (5 male) aged 23 to 60 years (42±15 years, mean±SD is reported in text throughout), with no known neurological or vestibular disorder, gave informed consent to

participate. The experiments were submitted to, and approved by, the Faculty of Science and Engineering ethical committee at Manchester Metropolitan University and performed in accordance with the Declaration of Helsinki.

Protocol

Vestibular-evoked balance reflexes were studied in two randomly ordered conditions. In one condition subjects stood on a 22cm wide and 2cm high walkway placed on the laboratory floor (Ground). In the other condition subjects stood on a 22cm wide walkway elevated 3.85m from ground level, to induce a fear of falling (Height). In both conditions subjects wore a full body harness attached to a safety system in case of a fall. The safety system consisted of two separate parts: a dynamic rope system which was belayed and an inertial reel. Both were attached to an anchor point positioned directly above the subject. The system created minimal drag on the subject and was attached to the back of the harness so the ropes ran behind the subject outside their visual field. As subjects could not feel or see the safety ropes, they were largely unaware of the safety system. Furthermore, they did not test the system prior to the experiment. Overall, this set up minimised the subjects' sense of security.

Subjects stood still but relaxed, with their head facing directly forwards, eyes closed and hands clasped in front. Their feet were placed together, directed along the anteroposterior axis of the walkway. Binaural bipolar GVS was delivered using the method described in Chapter 2. In each condition 30 impulses (15 anode-left, 15 anode-right, randomly ordered) 1mA in amplitude and 2 seconds in duration were applied. It is important to note that the direction of virtual movement evoked by the stimulus was always in the direction of danger (i.e. towards

the edges of the walkway), irrespective of polarity. To ensure a consistent baseline posture, subjects resumed normal stance position prior to the initiation of each trial. They were permitted to open their eyes after each block of 10 trials.

Data Acquisition

Data acquisition began 1 second prior to and ended 5 seconds following GVS onset. Skin conductance (SC) was recorded from the palmar surface of the left hand at 100Hz using a custom built wireless system based on a standardised method (Lykken & Venables, 1971). Two self-adhesive gel electrodes were placed on the proximal phalanges of the second and fourth fingers. 3D position data were sampled from markers placed around a headband, on each shoulder and the lower trunk (L3) at 100Hz using a CODA mpx30 motion tracking system (Charnwood Dynamics, Rothley, Leicestershire, UK). EMG was recorded from sensors placed on left and right medial gastrocnemius, tibialis anterior and vastus lateralis muscles at 2000Hz using a Delsys Trigno wireless EMG system (Delsys Inc., Boston, USA). EMG data was recorded in the software package EMGworks® (version 3.7). Data collection was synchronized with the motion tracking system via a trigger signal.

Data Analysis

SC was normalised by dividing by the mean level recorded during a 30 second supine period conducted prior to exposure to the fearful stimulus. Baseline SC was calculated as the mean level over the 250ms prior to GVS onset. To investigate SC responses to the GVS stimulus, mean level between 2-5secs following GVS onset was also calculated.

For all recorded muscles, mean-removed EMG data were rectified, lowpass filtered (40Hz, 4th order, zero-phase Butterworth) and normalised by dividing by the mean level recorded during a 30 second standing period prior to exposure to the fearful stimulus. Muscle activities were measured as the area under the curve (estimated using the trapezoidal rule) over three separate 250ms periods of individual subject averages. As in Chapters 4 and 5, baseline EMG was calculated over the 250ms prior to GVS onset. EMG between 120-370ms (Response EMG; see Figure 6.2A-B) and 450-700ms (Later EMG; see Figure 6.4) post GVS onset were also calculated relative to baseline. Co-contraction index (CCI) around the ankle joint was estimated based upon a previously used method (Gontijo *et al.*, 2008). After overlapping the tibialis anterior and gastrocnemius signals, the highest common area under the two traces indicated CCI. Baseline CCI was calculated as the mean level over 250ms prior to GVS onset.

Head, shoulder and lower trunk position data were lowpass filtered (2Hz, 4th order, zero-phase Butterworth). A virtual upper trunk marker was estimated by the midpoint between the two shoulder markers. Marker position in the horizontal plane was calculated from mediolateral and anteroposterior position data and then differentiated to derive sway speed. Baseline sway speed was calculated as the mean value over the 250ms prior to GVS onset. Mediolateral positions at GVS onset were subtracted and sway velocity was derived by differentiation. Mediolateral sway and sway velocity were then averaged across all trials in each condition. Peak sway amplitude during the initial 3 seconds following GVS onset was measured.

Response onset latencies were taken when 95% confidence limits calculated for lateral head velocity first exceeded zero (see Figure 6.3B, solid vertical lines). The latency at which the

sway velocity towards the anode stopped (velocity termination) was taken when the 95% confidence limits first returned to zero (see Figure 6.3B, dashed vertical lines). The latency of motion in the opposite direction, to correct body position, was also measured (position termination). This was taken when the 95% sway velocity confidence limits first dropped below zero, searching in reverse from when the position returned to zero (see Figure 6.3B, dotted vertical lines). In two cases, the search for this latency began at the end of the trace, as position had not completely returned to zero. To investigate for differences in sway response between conditions, the average sway velocity trace for the Height condition was subtracted from that for the Ground condition (see Figure 6.3C). The latency of divergence between conditions was taken as the first point after response onset when the differential trace exceeded 2 standard deviations of the 1 sec prior to GVS onset, as it was not possible to calculate confidence limits. Thus, this measure indicates where the response was significantly attenuated when standing at height (see Figure 6.3C, thick solid vertical line).

A 2x2 RM ANOVA (SPSS general linear model) was used to analyse SC (condition: Height, Ground; time: baseline, post GVS) and EMG data (condition: Height, Ground; leg: anode, cathode). 3x2 and 3x2x2 RM ANOVAs were used to analyse baseline sway speed (body segment: head, upper trunk, lower trunk; condition: Height, Ground) and peak sway response (body segment: head, upper trunk, lower trunk; condition: Height, Ground; polarity: anode-left, anode-right), respectively. Greenhouse-Geisser degrees of freedom adjustments were used to correct for violations of sphericity. Where RM ANOVA revealed significant effects, differences were analysed using pairwise comparisons subsequent to Bonferroni confidence interval adjustments. However, for EMG data, if anodal and cathodal legs were statistically indistinguishable, data from the two legs were combined and a RM ANOVA was replaced by

a paired comparison (condition: Height, Ground). Paired comparisons were also used to analyse CCI, onset latency, velocity termination and position termination. In all cases, a paired t-test was used if the assumption of normality was met. However, a Wilcoxon signed rank test was used if this assumption was violated. Robust regression was used to estimate the relationship between baseline SC and CCI.

6.3. Results

Effects of a fear of falling prior to GVS onset

A significant increase in SC when standing at height demonstrates that a state of arousal indicative of a fear of falling was induced (RM ANOVA: condition, $F_{1,8}$=9.44, p=0.015; see Figure 6.1A). Baseline SC was approximately doubled at height (3.99±1.73) compared to ground level (2.03±0.99). Furthermore, shortly following GVS onset an increase in SC from baseline was demonstrated when standing at height (post GVS onset, 4.09±1.79; pairwise comparison, p=0.038), whereas this response was not observed at ground level (post GVS onset, 2.04±0.98; pairwise comparison, p=0.558) (RM ANOVA: condition x time, $F_{1,8}$=7.10, p=0.029). CCI was not significantly different between conditions; baseline values of 0.67±0.15 and 0.91±0.45 were calculated at ground and height, respectively (Wilcoxon signed rank test, Z=-0.89, p=0.374). However, robust regression was used to estimate a linear relationship between SC and CCI (slope=0.05, intercept=0.57). The slope of the regression line was significantly different from zero (linear hypothesis test on parameter estimate, F=8.99, p=0.003; see Figure 6.1B) suggesting an increase in SC was accompanied by an increase in CCI.

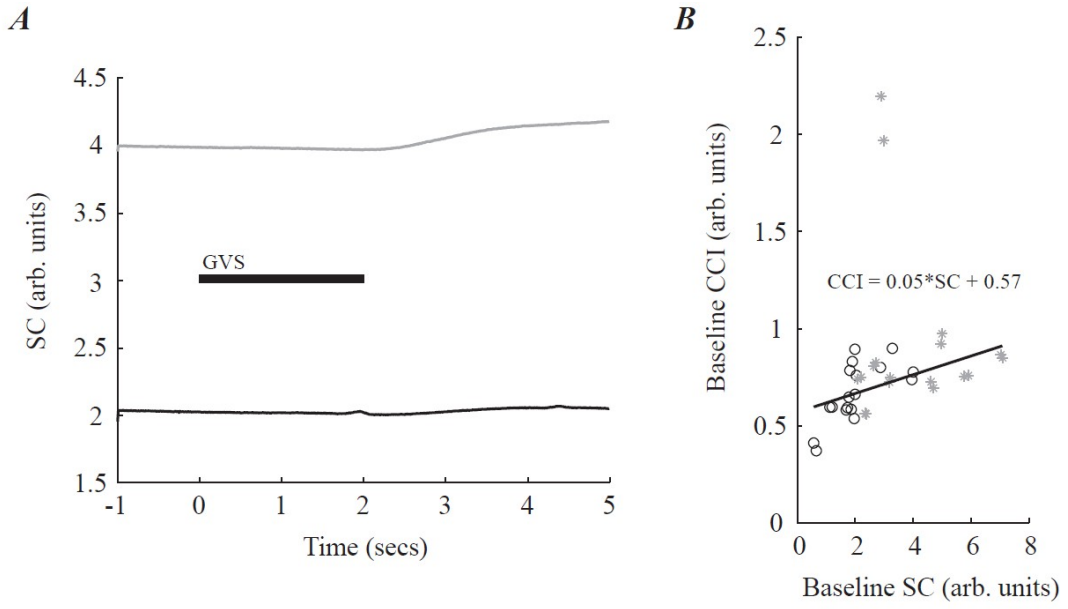

Figure 6.1. Skin conductance and ankle co-contraction.

A) Prior to GVS onset group mean skin conductance was approximately doubled when standing at height (grey trace) compared to ground level (black trace). A rise in skin conductance was also demonstrated 2-5s following GVS onset when standing at height. Thick black line indicates GVS stimulus. ***B)*** Robust linear regression was used to estimate the relationship between baseline skin conductance and ankle co-contraction. Ground: black circles, Height: grey stars.

Baseline medial gastrocnemius activity was significantly elevated when standing at height (H 4.45±1.41, G 3.04±0.85; paired t-test, $t(8)=3.11$, $p=0.014$). In contrast, there was no significant difference in tibialis anterior (H 2.65±1.49, G 1.94±0.60; Wilcoxon signed rank test, $Z=-0.42$, $p=0.678$) or vastus lateralis (H 3.15±1.99, G 1.96±0.39; Wilcoxon signed rank test, $Z=-1.24$, $p=0.214$) muscle activities during this period, although both muscles showed a numerical increase when standing at height. However, these increases were small in comparison to the group variance. A minority of subjects showed a large increase in muscle activity, being more than twice the magnitude at height (Tibialis anterior, 2 subjects; Vastus lateralis, 3 subjects), but the majority of subjects demonstrated a relatively small change in activity.

No effect of a fear of falling on initial muscle response

Modulation of medial gastrocnemius activity was evoked in both conditions shortly following the application of GVS (as shown by EMG recordings, see Figure 6.2A-B). A typical pattern of activity was demonstrated, comprising a short latency component beginning after approximately 50ms followed by a larger oppositely directed medium latency component after approximately 120ms. It is this later component which is responsible for the observed sway response (Britton *et al.*, 1993). Between 120-370ms, an increase in activity was evoked in the cathodal leg and a decrease in activity was evoked in the anodal leg (RM ANOVA: leg, $F_{1,8}$=14.86, p=0.005; cathodal leg 113.41±14.46%, anodal leg 84.40±13.32% of baseline).

The absolute magnitudes of the evoked response were calculated by subtracting baseline from response activity. Although there was a tendency to be greater when standing at height, absolute magnitude of inhibitory responses in the anodal leg (H -0.84±0.83, G -0.44±0.41; pairwise comparison, p=0.090) and excitatory responses in the cathodal leg (H 0.58±0.48, G 0.32±0.48; pairwise comparison, p=0.097) were not significantly affected by condition. Furthermore, when response activity as a percentage of baseline activity was considered, there was no effect of condition (RM ANOVA, $F_{1,8}$=0.03, p=0.864) or condition x leg interaction (RM ANOVA, $F_{1,8}$=0.56, p=0.475). This indicates that the relative effects of GVS were not statistically different between conditions. Average changes in activity relative to baseline in the anodal (H -16.98±13.88%, G; -14.23±14.68%; pairwise comparison, p=0.447) and cathodal (H 14.40±11.10%, G 12.42±19.33%; pairwise comparison, p=0.649) legs were not significantly affected by standing at height.

Between 120-370ms, standing at height had no significant effect on tibialis anterior (RM ANOVA: condition, $F_{1,8}$=3.61, p=0.094; H 102.06±3.74%, G 100.03±4.36% of baseline) or vastus lateralis (RM ANOVA: condition, $F_{1,8}$=0.68, p=0.432; H 103.50±5.15%, G 101.16±5.75% of baseline) muscle activities.

Effect of a fear of falling on sway response

The evoked pattern of activity in medial gastrocnemius muscles contributed to subsequent sway towards the anodal side. As shown in Figure 6.2C-D this comprised a whole-body lean. Although superior body segments were displaced further than inferior segments (Head 12.44±6.30mm, Upper Trunk 10.50±5.39mm, Lower Trunk 7.97±4.05mm; RM ANOVA: segment, $F_{1.04,8.31}$=31.19, p<0.001), all responded in the same way. Head sway traces are presented in Figures 6.2E-F. Sway was similar between conditions for the initial part of the response, but peak displacement was clearly reduced at height (H 6.00±2.69mm, G 14.61±9.16mm; RM ANOVA: condition, $F_{1,8}$=9.18, p=0.016). There was no significant condition x segment interaction (RM ANOVA: $F_{1.02,8.19}$=3.78, p=0.086), indicating that standing at height had a similar effect on the evoked response of all body segments. There was also no significant effect of stimulus polarity on response magnitude (see Figure 6.2E-F, anode-left 10.14±5.81mm, anode-right 10.47±5.20mm; RM ANOVA: polarity, $F_{1,8}$=0.08, p=0.783). Anode-left and right trials were therefore combined in subsequent analysis. Baseline sway speed was not influenced by standing at height (H 10.39±2.97mm/sec, G 9.67±3.08mm/sec; RM ANOVA: condition, $F_{1,8}$=1.20, p=0.306).

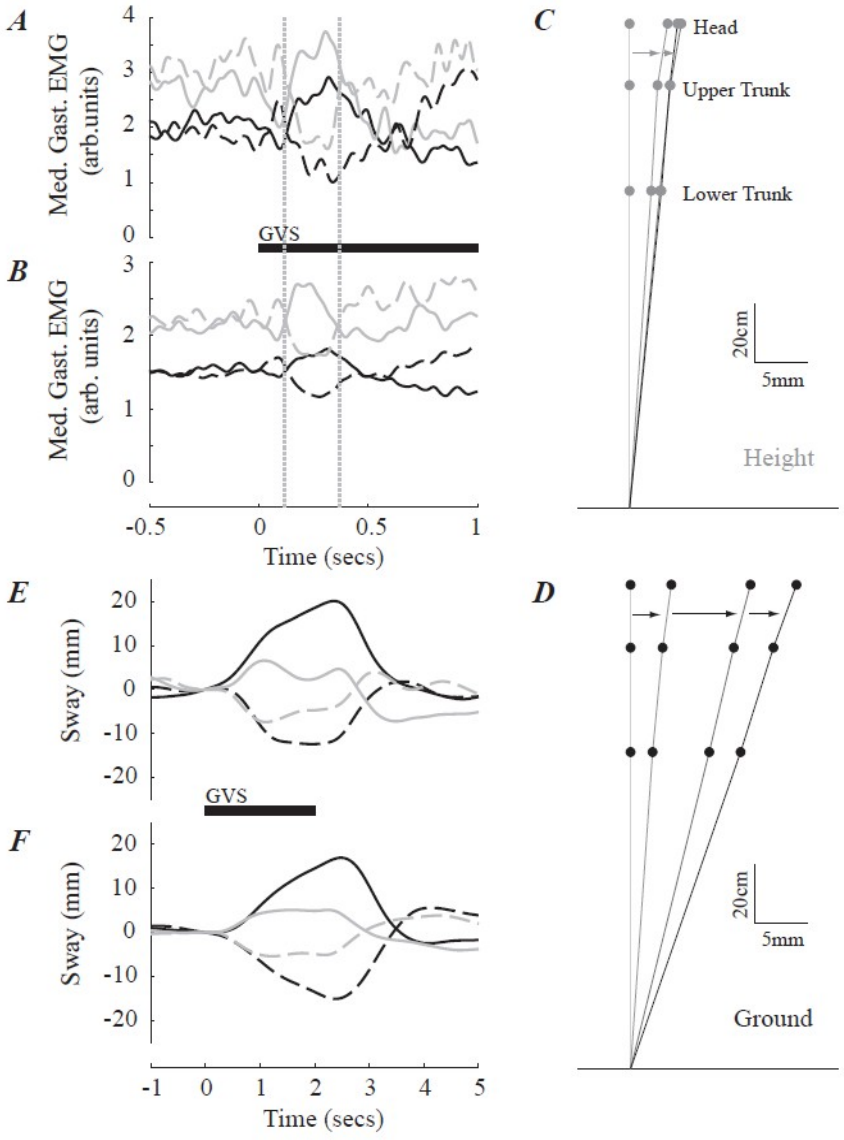

Figure 6.2. Vestibular-evoked balance reflexes when fearful of falling.

A) Representative subject and ***B)*** group average medial gastrocnemius activity evoked by GVS. A reduction was demonstrated on the anodal side (dashed traces) and an increase on the cathodal side (solid traces). Although baseline gastrocnemius activity was greater when standing at height (grey traces) compared to ground level (black traces), the magnitude of the GVS-evoked response was not significantly different. Dotted grey vertical lines indicate 120-370ms, during which the response was quantified. A 10Hz filter was applied for illustration purposes. ***C)*** *and* ***D)*** Group average lateral sway of each body segment at 0, 800, 1600 and 2400 ms (progressing from light grey to black). Anode-left trials have been combined with anode-right such that motion toward the anode is indicated. Positions at 800ms were similar between conditions, but sway was subsequently reduced at height (***C***) compared to ground level (***D***). Please note unequal axis scales. ***E)*** Representative subject and ***F)*** group average lateral head sway traces. Positive values indicate rightward motion. Anode-left (dashed traces) and right (solid traces) have approximately equal and opposite effects, with movement always occurring towards the anode. Notably, the peak sway was reduced at height (grey) compared to at ground (black). However, the initial part of the response is almost identical.

Head velocity traces were used to ascertain when the sway response began, when velocity towards the anode stopped and when the position correction began (response onset, velocity termination and position termination, respectively; see Figure 6.3B). The difference in head velocity was also used to quantify when the sway response in the two conditions first diverged (see Figure 6.3C). In brief, this analysis revealed the following (see Table 6.1 for statistics). Response onset latency was not statistically different between conditions, with an average latency of 382±294ms demonstrated. The sway responses evoked were initially similar in the two conditions until, after 796±358ms, the response was significantly attenuated when standing at height. Sway velocity in the direction of the anode was also terminated 808±716ms earlier at height. However, motion towards the cathode to correct the positional offset did not occur until, on average, after 2 seconds (i.e. after GVS offset) in both conditions and was not significantly affected by standing at height.

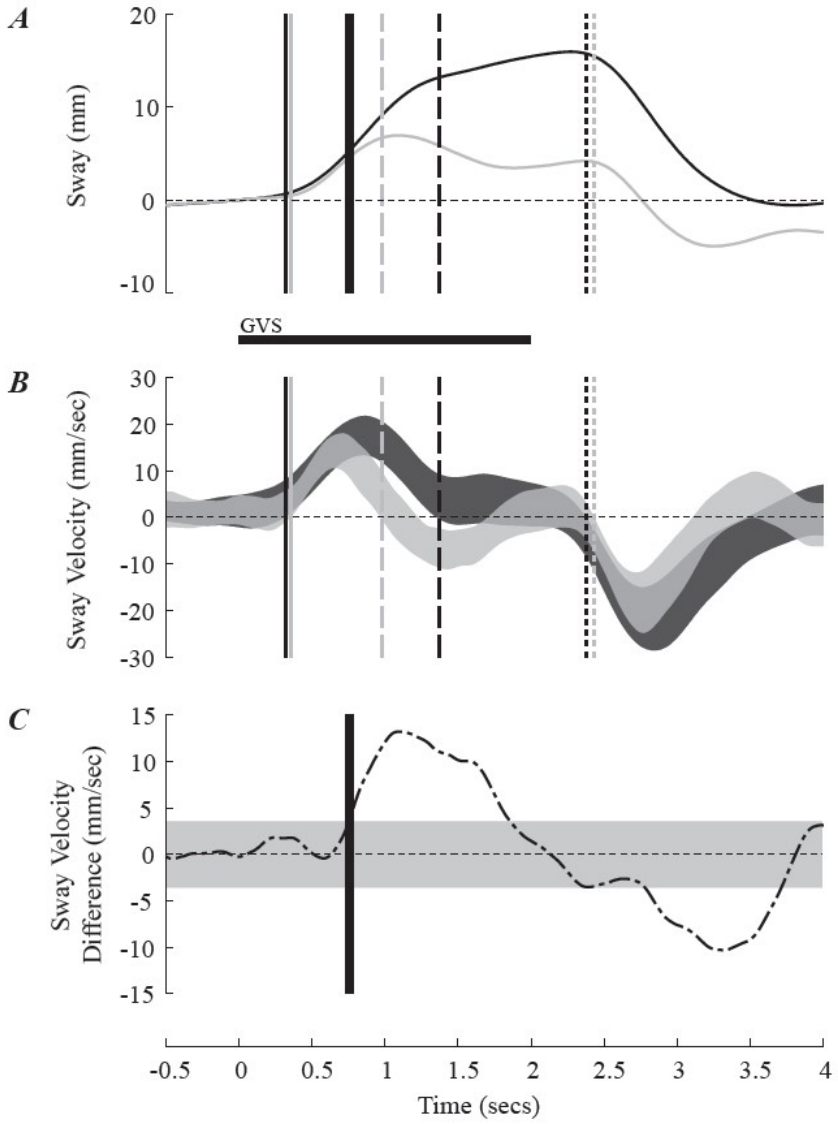

Figure 6.3. Onset and termination latencies of the vestibular-evoked sway response.

A) Sway evoked by GVS in a representative subject when standing at height (grey trace) and at ground level (black trace). Here, head displacements during anode-left trials were first inverted and averaged with anode-right. Vertical lines indicate response onset, divergence and termination latencies, as calculated from traces presented below. ***B)*** Lateral sway velocity was derived by differentiation. 95% confidence limits calculated for sway velocity in each condition are shown here, for the same subject. Vertical lines indicate response onset (solid), velocity termination (dashed) and position termination (dotted). See Methods for description of when these latencies were taken. ***C)*** The difference between sway velocity demonstrated at ground and at height is plotted for the same subject (dash-dot trace). The latency of divergence (thick vertical line) was taken when the differential trace exceeded ±2SD of baseline sway velocity (shaded area).

Table 6.1. The effects of a fear of falling on latency of vestibular-evoked sway onset, velocity termination and position termination.

	Ground	Height	p
Onset	412 ± 398	351 ± 193	.778
Divergence	796 ± 358		
Velocity Termination	1802 ± 659	994 ± 293	.010*
Position Termination	2801 ± 671	2398 ± 562	.241

Mean ± SD values are shown. Divergence indicates first time point when sway response at height was significantly attenuated compared to ground level.

Effect of a fear of falling on later muscle activity

At height, increases in tibialis anterior and vastus lateralis muscle activities occurred shortly following the initial muscle response evoked by GVS (see Figure 6.4A-B, grey traces). However, no such increases were demonstrated at ground level (see Figure 6.4A-B, black traces). Here, traces from anodal and cathodal legs were combined, as there was no difference in activity between the two legs in either muscle or condition (paired comparisons, all $p>0.30$). Tibialis anterior activity between 450-700ms was shown to be significantly greater at height (H 120.64±33.06%, G 99.97±8.84%; Wilcoxon signed rank test, $Z=-2.19$, $p=0.028$). Although markedly smaller in magnitude, increases in vastus lateralis activity were also shown to be significantly greater at height during the same time period (H 107.96±7.80%, G 99.47±4.28%; Wilcoxon signed rank test, $Z=-2.43$, $p=0.015$).

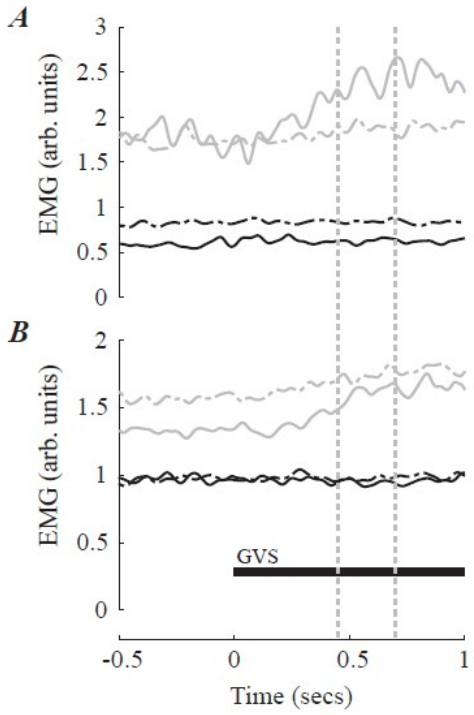

Figure 6.4. Later muscle activities.

A) Representative subject and ***B)*** group average tibialis anterior (solid) and vastus lateralis (dash-dot) EMG traces illustrate the muscle activity subsequent to GVS onset. When standing at height (grey traces), a rise in both muscles relative to baseline levels was demonstrated. At ground level (black traces) activity was not modulated from baseline levels. Dotted grey vertical lines indicate 450-700ms time period, during which 'later' EMG was quantified. Also, it is worth noting that although baseline tibialis anterior and vastus lateralis activities appear to be increased at height, this difference was small compared to group variance and was shown not to be significant. A 10Hz filter was used for illustration purposes.

Between 450-700ms, standing at height had no significant effect on medial gastrocnemius activity (RM ANOVA: condition, $F_{1,8}$=1.68, p=0.231; H 103.05±16.84%, G 95.50±8.01% of baseline). However, in both conditions, a decrease in activity was demonstrated in the cathodal leg and an increase in activity was demonstrated in the anodal leg (RM ANOVA: leg, $F_{1,8}$=9.42, p=0.015; cathodal leg 91.88±10.00%, anodal leg 106.67±14.14% of baseline). This modulation of medial gastrocnemius activity was in an opposite direction to the activity evoked during the initial reflex response (i.e. 120-370ms).

6.4. Discussion

This chapter investigated how a fear of falling affected vestibular-evoked balance reflexes. Subjects stood on a narrow walkway elevated 3.85m above ground level to induce a fearful state, as indicated by a twofold increase in skin conductance. Under these conditions, the initial reflex response evoked by GVS was unaffected, but after ~800ms the evoked body sway was attenuated when standing at height. Thus, although it is clear that individuals were highly motivated to avoid movement when fearful of falling, the initial vestibular-evoked balance reflex was unaffected. This strongly suggests these reflexes are inflexible to cognitive or emotive factors.

Fear of falling

Skin conductance almost doubled when standing on the high walkway compared to ground level. Previous findings demonstrate that in individuals standing at height an increase in this measure of physiological arousal is related to an increased state of anxiety and decreased balance confidence (Adkin *et al.*, 2002). Therefore, although skin conductance can be used to measure a wide range of emotions, this evidence supports the view that it corresponds to a fear of falling in this postural context. Increases in skin conductance were also accompanied by increases in ankle co-contraction, demonstrating that the subjects' initial state was altered when standing at height. Previous studies have reported a phasic increase in skin conductance beginning 2 to 3s following a physical perturbation to upright posture (Sibley *et al.*, 2010). A similar increase could be seen following GVS onset. However, such a response, which resembled that evoked by turning a playing card in a risky gambling task (Figner & Murphy,

2011), was demonstrated only when standing at height. This suggests subjects experienced an increased concern about the potential consequences of the stimulus in this condition. Overall, these results imply that a fear of falling was induced when standing at height.

A fear of falling may have caused a change in initial posture. A significant increase in medial gastrocnemius muscle activity was demonstrated when standing at height. Although there were, on average, increases in baseline tibialis anterior and vastus lateralis muscle activities, they were found not to be significant. An increase in gastrocnemius activity with no change in tibialis anterior activity would suggest a change in initial posture, namely, forward lean of the body (Horak & Moore, 1993). This is in contrast to reduced gastrocnemius activity, increased tibialis anterior activity (Carpenter *et al.*, 2001) and, hence, a more posterior centre of mass (Brown & Frank, 1997;Carpenter *et al.*, 1999;Adkin *et al.*, 2000), when subjects stood at height with their toes on the edge of a platform. In this context, a more upright posture was likely adopted to increase the distance between the centre of mass and the platform edge. In the current study, however, the edges of the walkway were positioned on either side of the body. This likely explains why backwards lean was not demonstrated. Subject lean, if any, was forwards, possibly because it was perceived to be a safer initial posture.

Fear of falling has no effect on initial vestibular-evoked muscle activity

The reflex evoked by GVS was measured in the medial gastrocnemius of both legs, as activity in these muscles contributes to the subsequent mediolateral sway response. In accordance with previous studies, a medium latency response was evoked after approximately 120ms (Britton *et al.*, 1993;Fitzpatrick *et al.*, 1994;Day *et al.*, 1997;2010). This response comprised

an increase in activity on the cathodal side and a decrease on the anodal side, thus generating forces to shift the body towards the anode. Between 120-370ms average medial gastrocnemius activity was modulated by approximately 15% of baseline activity, in accordance with previous findings (see Figure 3B in Day *et al.*, 2010). The magnitude of this response was, on average, ~2% larger when standing at height. However, this increase was small in comparison to the group variance, and results show that the response was not significantly affected. With power set at 0.80 and α at 0.05, power calculations indicate that a sample size of 9 in a pairwise comparison should be sufficient to detect an effect size of 1.07, which is a large effect. Using values of variance from the current data, this corresponds to a minimal detectable difference between height and ground conditions of 13.4% (cathodal side) and 11.0% (anodal side) of baseline activity. The current sample size would not, therefore, be sufficient to detect small to medium changes in medium-latency muscle activity, if they were motivated by a fear of falling.

Fear of falling has no effect on the initial vestibular-evoked body sway response

A fear of falling also had no significant effect on the initial sway response that shortly followed the evoked muscle activity. It is most striking that the average sway traces in height and ground conditions precisely overlap for the early part of the response (see Figure 6.2D). The initial ~800ms following GVS-onset, including the response onset latency, were statistically indistinguishable between conditions.

Sway prior to GVS onset was also similar in both conditions. Previous investigations into how stability is affected by a fear of falling have provided mixed findings. Elderly subjects

self-reported as fearful of falling demonstrate increased anteroposterior sway when blindfolded compared to non-fearful elderly (Maki *et al.*, 1991). In contrast, individuals on the edge of a platform up to 1.6m above ground level demonstrate reduced sway (Adkin *et al.*, 2000;Carpenter *et al.*, 1999;2001). However, other findings suggest increased anteroposterior sway is seen in subjects who report a robust fear response at height (3.2m), whereas a decrease occurs in those who reported a lesser fear response (Davis *et al.*, 2009). Thus, the effects of a fear of falling on spontaneous sway appear to be idiosyncratic, depending upon the level of fear experienced. Baseline spontaneous sway holds a strong relationship with the amplitude of a subsequent GVS-evoked sway response (Chapter 4; Pastor *et al.*, 1993). Therefore, an indirect modulation by changes in baseline sway could have potentially counteracted a direct effect of a fear of falling. However, the current results showed spontaneous sway was not affected when standing at height. The possibility of such indirect modulation can therefore be ruled out. These results show that a fear of falling had no effect on the initial vestibular-evoked sway response.

Initial vestibular-evoked balance reflexes are outside of cognitive and emotive control

A number of neural pathways were identified as possibly linking fear and the processing of vestibular signals. Namely, projections to the vestibular nuclei complex from the parabrachial nucleus (Balaban, 2004), locus coeruleus (Schuerger & Balaban, 1999), raphe nuclei (Halberstadt & Balaban, 2003), cingulate cortex and insular cortex (Akbarian *et al.*, 1993;1994), as these regions have all been implicated in the expression of fear. However, as previously discussed, the present results show that the initial vestibular-evoked balance reflex was unaffected when subjects were fearful of falling. Firstly, this suggests these links do not

allow the processing of vestibular signals for balance to be modulated when fearful of falling. Secondly, this implies that the vestibular control of balance lies outside of cognitive and emotive control. This is probably because it is important for survival that these balance reflexes cannot be interfered with. Visual signals are inherently ambiguous; movement of the retinal image can be due to motion of the head, eye or environment. The semicircular canals, on the other hand, provide an unambiguous signal of head rotation as they are fixed in the skull (GVS predominantly induces a signal from the semicircular canals; Fitzpatrick & Day, 2004). Thus, the unambiguous nature of the vestibular signal may explain why the reflex response was not modulated when standing at height, as the same balance reflex is necessary to prevent a fall, regardless of cognition or emotional state.

In contrast, "balance correcting" muscle activity in response to a physical perturbation – measured at a similar latency to medium latency vestibular-evoked activity in the current study (i.e. beginning at 120ms) – was shown to be significantly increased when standing at height (Carpenter *et al.*, 2004). Although these authors proposed an increased vestibular reflex gain as a potential mechanism, the current results suggest this is not the case. That is not to say all reflexes are inflexible to modulation by a fear of falling. Sibley *et al.* (2007) showed the H-reflex amplitude to be reduced when standing at height. However, this effect was absent if the eyes were closed, and the authors therefore propose visual input is important for the interpretation of postural threat. This suggests that there is a chance the differences between the results here and those of Carpenter and colleagues (2004) may be explained by the availability of visual input. However, postural responses to the two types of perturbation have been shown to be differently susceptible to modification by other factors. For example, repeated exposure to physical platform perturbations leads to a gradual reduction in the

evoked balance correcting muscle activity (Horak *et al.*, 1989), whereas the response to GVS has been reported not to habituate (Guerraz & Day, 2005). This suggests that postural responses evoked by physical perturbations are flexible to cognitive processes, whereas vestibular-evoked responses are not.

Fear of falling attenuates the later body sway response

Although there was no change in the initial reflex, the peak body displacement evoked by GVS was reduced by ~60% at height. This confirms that the context used in this study created a highly motivated state, which stimulated a balance control strategy where individuals tried to avoid body displacement. This is in accordance with previous research which has demonstrated that, in response to a physical perturbation, individuals also displayed reduced body displacement when fearful of falling (Brown & Frank, 1997;Carpenter *et al.*, 2004). Brown and Frank (1997) suggested that under conditions of postural threat the time taken to elicit a control strategy to arrest body sway is reduced. This corresponds to the reduced time taken to stop velocity towards the anode (velocity termination) in the current experiment, suggesting the velocity of the ongoing response is minimised earlier when fearful of falling. A more cautious control of posture, indicated by smaller amplitude body displacement, has also been observed when subjects were asked to rise to their toes when standing on the edge of a high platform (Adkin *et al.*, 2002). These findings suggest a tighter control of body displacement when the consequences of a fall are more severe. Indeed, it has been suggested that fear-induced activation of the locus coeruleus contributes to increased vigilance (Davis, 1992) and more efficient responses in dangerous or threatening circumstances (Liddell *et al.*, 2005). Nonetheless, although subjects were clearly highly motivated to minimise movement,

this had no effect on the early stages of the response. This suggests that the control strategy which is motivated by a fear of falling does not adjust the pre-programmed vestibular reflex in a feedforward manner.

The sway response was only affected once the body had started to move, suggesting feedback mechanisms were modulated by a fearful state. These are most likely mechanisms of sensory integration, which in the presence of conflicting information from non-vestibular and non-visual inputs minimise the response to the vestibular signal. It was not until 796ms following GVS onset that the evoked sway response was first attenuated. This latency corresponds well to a latency of 705ms, after which voluntarily standing "still" reduces the sway response evoked by vestibular stimulation (Reynolds, 2010). Although a reduced body displacement was demonstrated, the response was not entirely abolished until after the GVS stimulus had ceased. In fact, sway velocity was minimised but the body did not return to the pre-stimulus position until after 2 seconds. This suggests that the sensory integration mechanisms which attenuate the response are sensitive to dynamic, but not static sensory information from other inputs. Hence, it is possible that the position offset was neither sensed nor corrected. Alternatively, the position offset was possibly sensed but left uncorrected if it was perceived to be of no consequence. Overall, these results demonstrate that although a GVS-evoked sway response comprises an early component that is inflexible to cognitive and emotive processes, it *is* possible to modify the velocity of its later stages.

Potential involvement of 'later' muscle activity

Individuals demonstrated a rise in tibialis anterior and vastus lateralis activity once GVS-evoked sway was underway (i.e. 450-700ms) when standing at height, but not at ground level. This activity preceded the divergence between height and ground sway traces, which suggests that it contributed to the modified sway response. Although the current data is not conclusive, a bilateral increase in activity of these muscles potentially represents a stiffening strategy. The capability of the tibialis anterior to stiffen the ankle joint is limited by its fairly compliant tendon (Di Giulio *et al.*, 2009). Nevertheless, previous research has shown a significant relationship between activity in this muscle and ankle stiffness, which is thought to reduce body displacement during a relatively static period of standing at height (Carpenter *et al.*, 2001). However, in a dynamic situation, such as when perturbed, it may not be useful to adopt a stiffening strategy. It has been proposed that increased trunk stiffness in the elderly interferes with the ability to compensate for a physical perturbation (Allum *et al.*, 2002). Considering an analogy of a highly stiff mannequin, it clearly has increased stability when upright but, when perturbed, lacks the compliance required in making the appropriate postural adjustment to prevent a fall. Thus, the effects of stiffening on the control of balance are unclear and it is therefore difficult to ascertain whether stiffening would serve as a protective strategy in the current experiment. If the rise in tibialis anterior and vastus lateralis activity is not effective in stiffening the ankle and knee, it may simply reflect another expression of fear or arousal, similar to the increase in skin conductance following GVS onset. Alternatively, it may be explained by less refined motor control when fearful, causing subjects to simply activate all muscles rather than generate fine tuned corrective activity.

Summary

A fear of falling was induced by standing on a narrow walkway elevated 3.85m from ground level. This motivated a control of balance whereby body displacement evoked by GVS was substantially reduced after around 800ms. However, this evidently strong motive to avoid body motion had no effect on the initial stages of the response. These findings therefore strongly suggest that the initial vestibular-evoked balance reflex cannot be modulated by cognitive or emotive factors.

CHAPTER 7.

GENERAL DISCUSSION

7.1. Summary of experimental chapters

This thesis studied the context-dependent processing of vestibular signals for balance and orientation. The results demonstrate that the postural and sensory context, in some cases, modulates balance and orientation reflexes evoked by stimulation of the vestibular system. The main findings can be summarised in three key areas.

7.1.1. Modulation of response direction by changes in postural configuration

Chapters 2 and 3 initially recreated findings from previous experiments, which have demonstrated that static head orientation modulates the direction of both orientation and balance reflexes evoked by GVS (e.g. Lund & Broberg, 1983;Fitzpatrick *et al.*, 2006). When stepping with eyes closed, the direction and velocity of a vestibular-evoked turning response depends upon head pitch. Individuals turned towards the cathode and anode with the head pitched up and down, respectively. With the head in an approximately 22 degree nose-up pitch, the turning response was minimal. When subjects stood with their head in a neutral pitch, a balance reflex evoked by GVS was shown to be directed along the interaural line, towards the anodal ear. These results can be explained by the virtual rotation signal induced by GVS. Crucially, what these experiments demonstrate is that a vestibular signal is transformed from head to body coordinates by the CNS, in order to generate an appropriate response. To achieve this, an estimate of head orientation is required.

Chapter 2 went on to show that the gain of a vestibular-evoked orientation response was reduced during self-generated motion. Nonetheless, the velocity of turn demonstrated was continuously modulated as head pitch progressively changed. This suggests that the estimate of head orientation is constantly updated during self-generated motion. Proprioceptive and otolith sensory inputs likely inform the CNS of head orientation. Furthermore, efference copy also potentially plays a role during self-generated motion.

Chapter 3 also showed that, in some circumstances, the direction of a vestibular-evoked balance reflex does not follow the actual interaural line. This suggests that the perceived estimate of head orientation is important in generating an appropriate response to vestibular input. However, this effect was only apparent following a static adaptive period. Following a period of rotary stepping, a mismatch between perceived and actual head orientation was also induced, but the response direction remained appropriate for actual head orientation. Although the reason for this remains unclear, an additional adaptation of motor output caused by this adaptive period might be involved. It is possible that the direction of a vestibular-evoked balance reflex is modified when the coupling of vestibular input and motor output is altered.

7.1.2. Modulation of response amplitude by non-vestibular sensory inputs

Chapter 4 showed that passive cutaneous input modulates the amplitude of a balance reflex evoked by GVS. Contact between foam padding and the subject's back at very low contact forces reduced the magnitude of the evoked sway. Not only was the peak response attenuated, but the ground force response was also reduced, even before any movement of the body had

occurred. This suggests both feedforward and feedback modulation of the evoked reflex. The effect of light touch contact on the sway response was replicated in Chapter 5.

The results reported in Chapter 4 also show the balance response amplitude to be strongly related to baseline sway. This raises the possibility that cutaneous inputs have an indirect effect on the vestibular-evoked response. That is, additional cutaneous input causes a reduction in baseline sway, which in turn, causes a reduction in vestibular-evoked response amplitude.

7.1.3. No modulation by cognitive or emotive factors

Chapter 5 studied whether vestibular-evoked balance reflexes were modulated by the anticipated forthcoming sensory conditions. The results showed the sway response evoked by GVS to be similar whether light touch contact at the time of stimulus onset was anticipated or not. This suggests that, although the processing of vestibular signals is modulated by actual sensory input, it is unaffected by simply anticipation of altered sensory input.

In Chapter 6, I studied whether vestibular-evoked balance reflexes were modulated by a fear of falling. Subjects stood on a narrow walkway 3.85m above ground level, thus creating a strong motive to avoid body movement. Under these conditions the later part of the vestibular-evoked balance response – after around 800ms – was attenuated. This suggests a fear of falling motivated an altered balance control strategy, which influenced feedback mechanisms. However, this did not affect the pre-programmed reflex response, suggesting no feedforward modulation.

7.2. Thoughts and speculations

When I began this body of work, I thought that vestibular-evoked reflexes - being termed 'reflexes' - were simple and reproducible responses to a stimulus. I also thought they used a neural pathway which involved simply the vestibular afferent neurons, brain stem, spinal cord and motor neurons. However, the use of the term 'reflex' can be a controversial issue (Prochazka *et al.*, 2000). Goldstein (1995) suggested that many so-called reflexes do not fulfil the strict concept of "constant responses to specific stimuli". This also applies to vestibular-evoked balance and orientation reflexes. As demonstrated in the current thesis and in the work of others, an identical vestibular stimulus does not always produce the same response, but it can dramatically change depending upon the context. For instance, the direction of a vestibular-evoked orientation response is reversed by changing head pitch. Nevertheless, I have still considered the balance and orientation responses which I have studied as reflexes. I used a more practical approach to the term. Any automatic response which was bound to the stimulus, in this case GVS, was considered here as a vestibular-evoked reflex. What is clear from the studies reported, however, is that vestibular-evoked balance and orientation reflexes are in some cases modulated (as previously summarised). This is perhaps not that surprising, as vestibular sensory signals form only part of a complex system comprising many inputs for balance and orientation (see Figure 1.1). Furthermore, the structure in the brain stem which is thought to largely process these signals, the vestibular nuclei complex, shares many connections with other structures within the CNS (see Figure 1.4). So my original suggestion of a reflex pathway involving only relatively few structures also seems to have been far too simple. In fact, results in almost every study reported here have suggested that other structures within the CNS might be involved in the processing of vestibular signals.

As Goldstein (1995) put it, there is “no constancy” in many so-called reflexes. Instead, various reactions can be evoked by the same stimulus. If the reflex apparatus were isolated from the rest of the nervous system, constancy would likely be demonstrated. However, when interaction with other parts of the nervous system takes place, variation of a reflex is demonstrated. Here, in some cases, the vestibular-evoked reflex was shown to vary due to a change in context. But, in other cases, the reflex did not vary. Although each chapter was followed by an independent discussion, the collective findings of this thesis provoke further discussion of the following topics, which relate to how and why vestibular-evoked reflexes are, or are not, modulated by context.

7.2.1. Feedforward and feedback modulation

The amplitude of a vestibular-evoked reflex can be modulated in two ways. At the very onset of a stimulus-bound reflex, its amplitude is pre-programmed. Thus, any change in the amplitude at this stage can only be due to changes in the pre-programmed reflex. This has been termed feedforward modulation. In addition, non-vestibular sensory inputs provide self-motion information to the CNS. The reflex might also be modulated if these inputs provide conflicting or opposing information to the vestibular signal, once the response is underway. This has been termed feedback modulation.

In Chapter 4, the initial 400ms of a response was modulated when light touch contact provided cutaneous input (see Figure 4.2). As the reflex was altered from the very onset, this strongly suggests it is an example of feedforward modulation. In addition, the peak sway response was also reduced. By sensing motion of the body relative to a foam pad, known to

be fixed in space, feedback mechanisms likely terminated the developing sway response. However, in this case, it is difficult to distinguish feedforward and feedback effects as they are both operating. Although feedforward modulation can generally be determined by its latency, feedback modulation can only be isolated by using an elegant experimental protocol. Day and Guerraz (2007) studied the effects of visual input richness on vestibular-evoked balance reflexes. To investigate both feedforward and feedback modulation, they switched the visual environment at the time of GVS onset. By comparing trials in which pre-stimulus visual environments were identical but post-stimulus onset environments were different, they were able to distinguish feedback effects.

When there are no feedforward effects, changes in the reflex can only be due to feedback modulation. This was likely the case in Chapters 5 and 6. In both cases, a reduction in the vestibular-evoked response can be attributed to the effects of conflicting self-motion information from non-vestibular and non-visual inputs. In Chapter 6, feedback mechanisms attenuated the response after around 800ms (see Figure 6.2). Here, in standing subjects with eyes closed, the body sway evoked by GVS could have been sensed by proprioceptive input regarding joint and limb positions and cutaneous input regarding pressure changes at the soles of the feet. In Chapter 5, the response was attenuated during light touch contact with a fixed point in space from approximately 500ms post-stimulus onset (see Figure 5.3). Here, slightly faster feedback modulation may be explained by additional self-motion information due to the point-of-contact. This not only provides a larger quantity of information for the CNS to use when computing body position, but it may also provide better quality information. That is, there may be a relative certainty or higher sensitivity in this sensory input compared to those available to a free standing subject in Chapter 6. Incidentally, whether the degree of

modulation is affected by an individual's certainty or belief that a sensory input is veridical would be an interesting question for future research.

7.2.2. Baseline sway

The results reported in Chapter 4 add to previous research which shows an increased availability of non-vestibular sensory input reduces the amplitude of a vestibular-evoked balance reflex (e.g. Britton *et al.*, 1993). The results also demonstrated a linear relationship between baseline sway and the magnitude of a GVS-evoked responses, in accordance with previous work (Pastor *et al.*, 1993). This raises the possibility that non-vestibular sensory input has an indirect effect on the vestibular-evoked response, mediated by baseline sway. Although it remains unclear exactly how baseline sway affects the response, this result does demonstrate the importance of also reporting baseline sway, whenever response sway amplitude is under investigation. Accordingly, I reported that baseline sway was not significantly different across the conditions used in Chapters 5 and 6. In the context of these findings, the supposed influence of sensory conditions upon vestibular reflexes may need re-examination. That is to say, a difference in baseline sway is likely to at least contribute to the modulation of response amplitude by changes in the availability of non-vestibular sensory input. However, more research is required to determine to what extent changes in baseline sway explain effects of this type. Furthermore, the physiological mechanism which underlies the effect of baseline sway on response sway requires further investigation. An increased vestibular reflex gain during higher levels of vestibular activation and increased motor neuron excitability are two candidate mechanisms.

7.2.3. The cerebellum

On numerous occasions the present findings raised the possibility that the cerebellum may be involved in the modulation of vestibular-evoked balance and orientation reflexes. Some vestibular afferent fibres project directly to the cerebellum (Carleton & Carpenter, 1984) and there are also reciprocal connections between the vestibular nuclei complex and the cerebellum (Carleton & Carpenter, 1983). Thus, these neural pathways may permit involvement of this structure in the processing of vestibular signals.

But what could this involvement be? The cerebellum is a key structure for the coupling of sensory input to motor output (Manzoni, 2005). As a result this structure may be involved in the coordinate transformation of vestibular signals (see Chapters 2 and 3). With changes in head orientation, the vestibular sensory input must be transformed from head to body coordinates, before the appropriate motor output is generated. A number of findings in animal studies support the view that the cerebellum is used for this process (Manzoni *et al*., 1998 cited in Manzoni, 2005;Shaikh *et al.*, 2004). In additional, Kammermeier *et al.* (2009) demonstrated that the direction of a vestibular-evoked balance reflex is not correctly rotated with head yaw rotation in human patients with cerebellar degeneration, providing further support for this view. In Chapter 3 I speculated that if, following adaptation, the coupling of sensory input with voluntary motor output is modified, a vestibular-evoked reflex motor response may also be modified. Adaptation can be induced experimentally, but it is also necessary if the relationship between an individual and the environment changes, for instance, during growth. If adaptation of voluntary motor output occurs it seems both plausible and useful for it to also be applied to reflex movements. The cerebellum is also potentially

involved in the cancellation of vestibular input corresponding to self-generated motion, where a motor response is not required (see Chapter 2). The cerebellum seems a suitable candidate structure for this process, as it is thought to be involved in the analogous cancellation of self-generated tickle (Blakemore *et al.*, 1998;2001).

The cerebellum may therefore be involved in a number of computational processes. The thoughts and speculations above provide many hypotheses for future research. To test these hypotheses and to understand how CNS structures, including the cerebellum, influence vestibular-evoked reflexes, it will be valuable to further investigate how particular pathologies, such as cerebellar lesions, alter the evoked response.

7.2.4. Cognitive and emotive factors

The processing of vestibular signals for balance and orientation is complex and vestibular-evoked reflexes are modulated in many ways. However, the results in this thesis, and in the work of others (e.g. Guerraz & Day, 2005;Reynolds, 2010), suggest the processing of these signals is not modulated by cognitive or emotive factors. In Chapter 6, I created the strongest possible motive for subjects to avoid motion of the body. Even so, initial vestibular-evoked balance reflexes were unaffected, leading to the conclusion that these reflexes are placed outside of cognitive and emotive control.

As the vestibular end organs are fixed in the skull, any modulation of afferent nerve activity signals self-motion relative to space. As previously discussed, within the CNS this signal is automatically processed by unconscious mechanisms. These mechanisms include those which

subtract the portion of the signal due to self-generated motion and transform the signal from head to body coordinates. The resultant signal, therefore, indicates unintended motion of the body. The potentially ambiguous otolith afferent signal (i.e. tilt-translation ambiguity) may need to be resolved by non-otolith input (Zupan & Merfeld, 2003;Shaikh *et al.*, 2005). However, there is no ambiguity in the semicircular canal signal. As the GVS-induced sense of rotation is due to semicircular canal activation, a capacity for cognitive processes to modulate the processing of signals of this type is therefore not required. Particularly for the control of balance, if this signal represents a large enough perturbation to upright posture, then a fast and effective reflex response is always necessary. As these reflexes correspond to a first defence against a fall, it is crucial that they are quickly and automatically generated. There may be situations where cognitive modulation of other reflexes is advantageous. For example, the capacity to voluntarily suppress the withdrawal reflex may be useful. However, there is no need to allow cognitive modulation of vestibular-evoked balance reflexes; voluntary suppressing a reflex to prevent a fall, for instance, would be highly counter-productive. Similarly, any given vestibular signal will always indicate the same destabilisation or disorientation regardless of the emotional state of the individual. As a result, the same reflex is required. The combination of an unambiguous signal and a set of fast feedback mechanisms which automatically modulate the response explains why, in my results, anticipation of forthcoming sensory conditions (see Chapter 5) and a fear of falling (see Chapter 6) had no influence on the amplitude of a vestibular-evoked balance reflex.

7.2.5. Cortico-vestibular connections

Direct connections from cortex to the vestibular nuclei complex have been shown in the monkey (Akbarian *et al.*, 1993;1994). Although I initially considered these cortico-vestibular connections as potential pathways for cognitive factors to modulate the processing of vestibular signals, it is apparent that vestibular-evoked balance reflexes are in fact outside of cognitive control. However, these connections possibly serve other purposes. Modulation of vestibular nuclei neuron activity as a result of cortical stimulation (see Fukushima, 1997 for review), suggests that such connections are in some way capable of modulating vestibular reflexes.

The cortex has long been assumed to influence the processing of vestibular signals, as it is possible to voluntarily modify vestibular-evoked nystagmus (Spiegel & Teschler, 1929 cited in Fukushima, 1997). In contrast, initial vestibular-evoked balance reflexes cannot be voluntarily modified (Reynolds, 2010). Similarly, it appears impossible to suppress a vestibular-evoked orientation reflex, as in Chapter 2 subjects were unsuccessful in their attempts to avoid whole body turn when GVS was applied. This difference may be explained by findings which suggest regions of the vestibular nuclei complex associated with ocular outputs are preferred targets of different cortical regions to those associated with vestibulospinal outputs (Akbarian *et al.*, 1994). Cortico-vestibular connections may, therefore, allow voluntary suppression of only vestibular-evoked eye movements. But this does not mean these connections are not at all involved in balance and orientation reflexes. They may serve purposes other than simply voluntary suppression.

The VOR gain is increased in fearful individuals (Yardley *et al.*, 1995) In animal studies, neural links have been shown to the vestibular nuclei complex from the cingulate and insular

cortices (Akbarian *et al.*, 1993;1994), which have been implicated in emotional function. Thus, in Chapter 6, they were identified as connections which could permit emotional factors, such as fear, to influence vestibular-evoked balance reflexes. Although a fear of falling did motivate changes in the control of balance, this did not modulate the initial sway response evoked by GVS. This suggests that these reflexes are inflexible to emotive factors. Hence, the identified connections do not serve this purpose.

Spatial perception is thought to involve cortical vestibular regions, mainly in the parietal cortex (Guldin & Grusser, 1998;Fukushima, 1997). As stated in Chapter 2, vestibular-evoked orientation reflexes reflect an attempt to counteract a GVS-induced perception of turn in the opposite direction. Therefore, conscious perception of turn, which involves the cortex, may be required for a corrective response to be generated. If so, connections from the parietal cortex to the vestibular nuclei complex (Akbarian *et al.*, 1993;1994) might be involved.

There is also a potential role for cortico-vestibular connections that could be relevant to the processing of vestibular signals for eye movements, balance *and* orientation. It has been speculated that links from the premotor cortex to the vestibular nuclei complex are of use in the cancellation of reafference from the total vestibular afferent signal (Akbarian *et al.*, 1994; also see Chapter 2). Together with the previously indicated possibility that cancellation of reafference takes place in the cerebellum, this offers two candidate structures for this process.

It clearly remains open to debate whether the processing of vestibular signals for balance and orientation involves only sub-cortical structures. Previous findings suggest it is more complex; following damage to the cortex or regions where cortico-vestibular connections lie,

an asymmetrical vestibular-evoked balance response was evoked by GVS (Marsden *et al.*, 2005). Interestingly, cortical lesions also cause asymmetries in vestibular-evoked nystagmus (see Fukushima, 1997 for review), although the cortical inputs which influence eye movments and postural responses may differ. My results suggest cognition and emotion are not capable of modulating the processing of vestibular signals for balance and orientation, but whether cortico-vestibular connections allow cortical structures have an effect remains a mystery. Here, I have speculated on some potential functions of these connections. In future, the use of stimulation techniques which excite or suppress the cortex and corticospinal tract during a vestibular-evoked response would help us to further understand the role of these parts of the nervous system in the processing of vestibular signals and, ultimately, in evoking reflexes for balance and orientation.

7.3. Final remarks

It seems fitting to end by referring to the quote at the very start of this thesis. In his popular collection of case studies, 'The man who mistook his wife for a hat' (1985), Oliver Sacks spoke of "complex mechanisms" and of "our vestibules and all the other obscure receptors and reflexes that govern our body orientation". In this thesis, I have demonstrated that signals from the vestibular system evoke balance and orientation reflexes and, therefore, are evidently used for these purposes. Furthermore, I have shown that the processing of these signals is not constant, but is often dependent upon the postural and sensory context. This suggests that the evoked responses are far from the result of simple reflexes, but are indeed part of a complex set of mechanisms, probably involving many different neural pathways and structures, possibly even cortical structures and projections. Nonetheless, the responses are automatic,

and the context-dependent modulation also appears to be carried out automatically by the CNS. I have not, however, presented any evidence that the processing of vestibular signals can be modified by cognition or emotion. I believe the automatic modulation by the CNS is sufficient that one does not need to voluntarily control vestibular-evoked reflexes or, perhaps, even be aware of them. As Oliver Sacks states, “For normal man, in normal situations, they simply do not exist.”

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