REFLEXES EVOKED BY ELECTRICAL VESTIBULAR STIMULATION AND THEIR CLINICAL APPLICATION

by

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ABSTRACT

The vestibular system provides vital information about head position and head motion. This information is used for the control of balance through vestibulospinal reflexes. However, as the vestibular system is fixed within the skull, it must first be transformed into body coordinates. Chapter 2 explores this transformation process with and without vision. The results show that when vision is available, the evoked response is paradoxically less precise. Chapter 3 further explores the transformation process before and after 60 days of bedrest. After this period of inactivity, participants spontaneously swayed more, and their EVS-evoked sway response was less precise. This decrement in precision, however, appears to be showing signs of recovery, 6 days post bedrest.

Chapter 4 switches focus from postural reflexes to vestibulo-ocular reflexes. Here electrical vestibular stimulation is used to evoke measurable torsional eye movements. The magnitude of the response is modulated by stimulus frequency. Results also suggest that the CNS interprets electrical vestibular stimulation as a velocity signal rather than a position or acceleration signal. As this technique is an ideal measure of pure vestibular function, Chapter 5 utilised the technique in a clinical environment. Vestibular schwannoma patients, who have a known unilateral vestibular deficit, were tested to identify if the proposed technique can in fact detect this deficit. Results showed that asymmetries could be detected, and, in fact the test may be more sensitive than previously used measures of vestibular asymmetries.

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PUBLICATIONS AND PRESENTATIONS

Chapters presented in this thesis have been published in the following journals;

Chapter 2 - Mackenzie, S. W., & Reynolds, R. F. (2018). Differential effects of vision upon the accuracy and precision of vestibular-evoked balance responses. *Journal of Physiology.* doi:10.1113/JP275645

Chapter 4 - Mackenzie, S. W., & Reynolds, R. F. (2018). Ocular torsion responses to sinusoidal electrical vestibular stimulation. *Journal of Neuroscience Methods, 294*, 116-121. doi:10.1016/j.jneumeth.2017.11.012

Chapter 5 – Under review at Journal of Clinical Neurophysiology.

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- 7th International Posture Symposium 2015 (Smolenice, Slovakia)
- UK Sensory Motor Meeting 2016 (Newcastle, UK)
- MRC-ARUK Student Meeting 2016 (Nottingham, UK)
- Society for the Neural Control of Movement Annual Meeting 2016 (Montego Bay, Jamaica)
- Society for the Neural Control of Movement Annual Meeting 2017 (Dublin, Ireland)
- UK Sensory Motor Meeting 2017 (Bristol, UK)
- International Society of Posture & Gait Research World Congress 2017 (Fort Lauderdale, USA)
- The 30th Bárány Society meeting 2018 (Uppsala, Sweden)

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$\bar{\alpha}$ - Angular direction

F_{rot} – Force rotation

AD – Angular deviation

AL – Anode left

AP – Anteroposterior

AR – Anode right

BBPV – Benign Paroxysmal Positional Vertigo

BD – Balance disturbance

C7 – Cervical spine 7

CL – Cathode left

CR – Cathode right

CNS – Central nervous system

CoG – Centre of gravity

CoP – Centre of pressure

Contr – Contralateral

CPA – Cerellopontine angle

CR – Cathode right

Deg – Degrees

dir – Direction

EMG – Electromyography

ENT – Ear, Nose and Throat department

EVS – Electrical vestibular stimulation

FSR – Functional stretch reflex

Fx – Mediolateral ground reaction force

Fy – Anteroposterior ground reaction force

Fz – Vertical ground reaction force

G - Gravity

GVS – Galvanic vestibular stimulation

HIT – Head impulse test

HL – Hearing loss

Hz – Hertz

IAC – Internal auditory canal

ICD – Intracanalicular diameter

ICL – Intracanalicular length

Ipsi – Ipsilateral

k – Trial

kHz – Kilohertz

M - Mean

mA – Milliamp

min - Minute

ML – Mediolateral

m – Metre

mm – Millimetre

ms – Milliseconds

N – Newton’s

NIRS – Near infrared spectroscopy

nm – Nanometres

PTS – Pure tone Average

$\bar{r}$ - Resultant vector

R – Vector length

r – Vector

RMS – Root mean squared

rSO_2 – Regional cerebral oxygen saturation

s – seconds

SD – Standard deviation

SDS – Speech discrimination score

SE – Standard error of the mean

sEVS – sinusoidal vestibular stimulation

SVS – Stochastic vestibular stimulation

TIN – Tinnitus

VEMPs – Vestibular evoked myogenic potentials

vHIT – Video head impulse test

VNC – Vestibular nuclear complex

VOR – Vestibulo-ocular reflex

VS – Vestibular schwannoma

VSR – Vestibulospinal reflex

x – Horizontal

y – Vertical

z - Torsional

CHAPTER 1

GENERAL INTRODUCTION

Balance

Human posture differs vastly from that of animals. This is largely because humans stand on only two limbs, compared to the majority of animals which stand on all four limbs. This bipedal stance requires the very complex task of maintaining balance to remain upright. Approximately two-thirds of our body mass is located two-thirds of body height above the ground, making humans inherently unstable. However, evolving to walk on two limbs has led to abilities unique to humans. During quiet stance, the centre of gravity (CoG) is located in the middle of the pelvic girdle in the mediolateral (ML) axis. This position is relatively stable, until a force is applied which moves the CoG out of the support base, formed by the feet. Once the CoG is located outside of the support base, the pelvic girdle and knees become extremely unstable. In the anteroposterior (AP) axis, the CoG is positioned in front of the ankle joint leading to the tendency to topple anteriorly. Postural reflexes act to move the body back towards its initial position in response to any disturbances. To ensure humans do not fall forwards, tonic gastrocnemius activity provides counteractive ankle torque (Rothwell, 1994). Usually during quiet stance the knee is locked in hyperextension and the spinal column is naturally curved, with remarkably little electromyography (EMG) activity in the postural muscles (Rothwell, 1994). Given that the CoG is relatively high, further reducing stability, this has led to the human body being described

as an inverted pendulum, pivoted around the ankle joint. To maintain balance in a structure which is inherently unstable, sensorimotor systems are in place to monitor and control body sway.

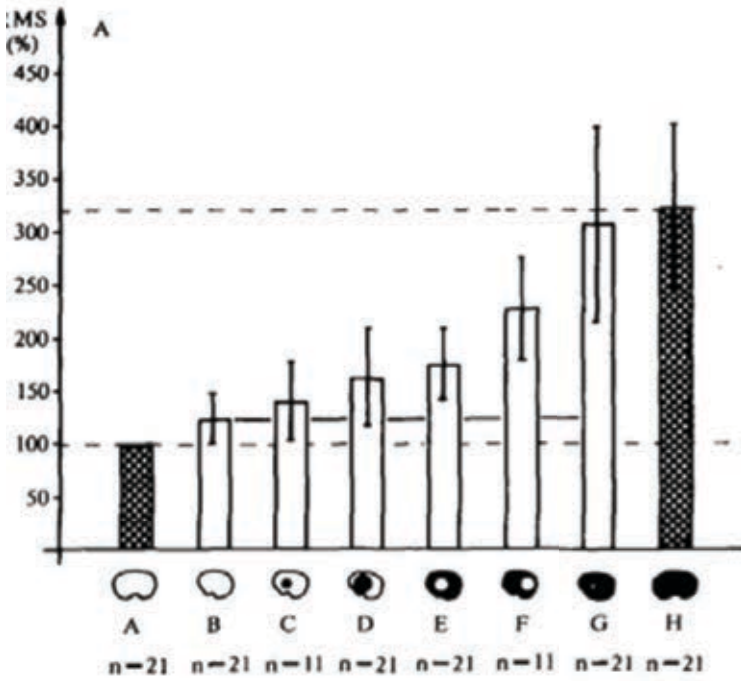
Sensory Inputs

Sensory inputs which signal body movement are used to maintain balance. Some of the traditional 'five senses' are used. However, some are more apparent than others, such as vision and touch. Receptors in the vestibular organs of the inner ears, sensitive to head motion, are one of the least apparent senses used to maintain balance. Nevertheless, signals from the vestibular system play a major role in the maintenance of balance and if vestibular function is completely lost, stability can be dramatically impaired (Martin, 1965; Nashner, Black, & Wall, 1982). During a target-directed linear walk, those with vestibular lesions demonstrate lateral deviations (Borel et al., 2004). Evidence for the importance of vestibular information in the maintenance of balance is vast and will be discussed in more detail in subsequent sections.

Vision

Visual signals are used to maintain balance during slow body movements (<1.0Hz). Visual acuity is extremely important to maintaining balance and it has been shown that as vision becomes increasingly blurry, postural sway increases (Paulus et al., 1984). Sway increases as the visual field is narrowed, suggesting that peripheral vision is critical to maintaining balance (Paulus et al., 1984) as

illustrated by Figure 1.1. It has been shown that when vision is restricted to the fovea, sway is almost as large as when the eyes are closed. Visual information from objects within our environment act as a sway reference. Retinal displacement caused by body sway is larger when an object is closer. If the reference object is situated further away, less retinal displacement occurs and hence there is less information to minimise sway (Guerraz, Sakellari, Burchill, & Bronstein, 2000; Paulus et al., 1984). Vision is an extremely powerful sense and its information is relied upon heavily during balance control. Motion parallax is the optical change of the visual field of an observer which results from a change in viewing position (E. J. Gibson, Gibson, Smith, & Flock, 1959). Displacement of an observer in a three-dimensional visual environment generates spatiotemporal patterns on the retina specific to observer's motion (J. J. Gibson, 1958). Vision is a major contributor to the sense of self motion due to optic flow which is

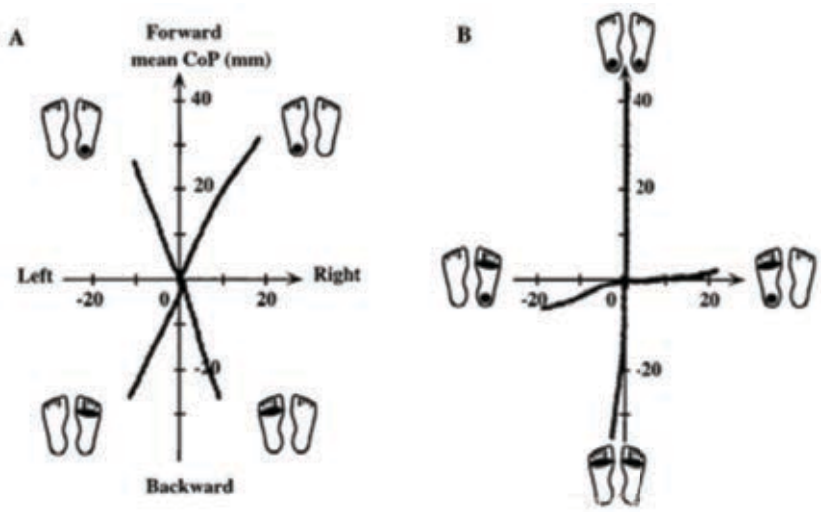


movement of the visual field relative to a moving individual. However, vision is inherently ambiguous and self-motion and world-motion can be confused. The 'moving room' paradigm involved the movement of the four walls around a subject, which resulted in the subject swaying in the same direction, suggesting that vision is used to detect sway. During forward room motion, the brain interprets the visual information as the body moving backwards. This threat to balance is counteracted by producing a compensatory movement to return the body to what is perceived to be its original position (Lee & Lishman, 1975).

Mechanoreceptors

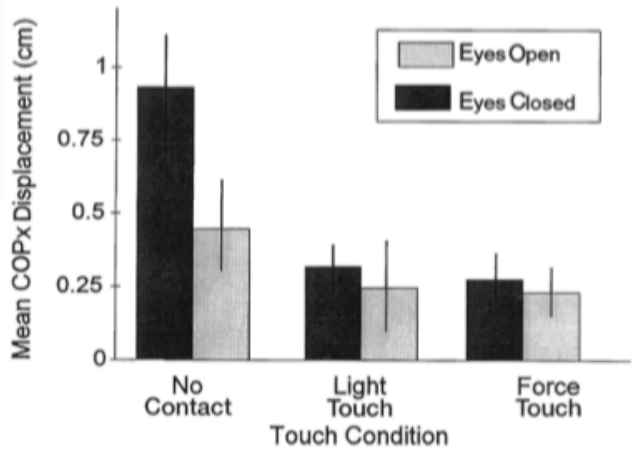
The skin is replete with mechanoreceptors and sensory nerve endings, all of which provide information about texture, motion in relation to the skin and force. The force of an object on the skin can provide information about self-motion if the object is fixed in space. During quiet stance, plantar cutaneous afferents provide valuable feedback regarding ankle torque, weight transfer between the legs and the nature of the support stance. Tangential (shear force) and perpendicular force during stance can be used to estimate CoG location (Morasso & Schieppati, 1999). The role of foot sole receptors has been confirmed by experiments which expose the feet to hypothermia (Magnusson, Enbom, Johansson, & Pyykko, 1990; Magnusson, Enbom, Johansson, & Wiklund, 1990) or anaesthetic (Wu & Chiang, 1997). Both interventions increase sway due to the transient block of exteroceptive afferents. Ischemic block at the ankle results also increases sway when the support surface is moved in a sinusoidal waveform (Diener, Dichgans, Guschlbauer, & Mau, 1984; Mauritz & Dietz, 1980). Furthermore, the nature of

the support surface affects sway. For example, a foam surface which increases contact with the plantar surface and reduces pressure produces a delay in postural response to surface movement (Wu & Chiang, 1997). Conversely, a shotgun ball surface which increases pressure decreases sway (Okubo, Watanabe, & Baron, 1980). The plantar sole has been described a 'dynamometric map' for human balance control. Vibration of certain areas of the plantar surface during bipedal stance results in a corresponding directional sway response, illustrated in Figure 1.2 (Kavounoudias et al., 1998). The skin of the fingertip is also able to determine self-motion when in contact with an earth-fixed object, as any changes in pressure or movement across the skin must be due to self-motion. Even when contact forces are too small to provide mechanical support (<1N), profound reductions in sway are still observed, as illustrated in Figure 1.3.



Proprioception

Proprioception is the sense of the relative position and rates of movement of body parts (Guyton, 1986; Vander, Sherman, & Luciano, 1990). Muscle spindles and Golgi tendon organs (proprioceptors) provide feedback about the status of each muscle which is vital for the control of posture. The length and change in the length of a muscle is monitored by muscle spindles, which are stretch receptors located within the muscle. Muscle spindles comprise of afferent nerve fibre endings wrapped around muscle fibres enclosed in a connective tissue capsule. Golgi tendon organs sense changes in tension of the muscle and are located in the tendon near the junction with the muscle. Both organs send information to the brain which can then be used to determine the necessary action that muscles need to take to remain upright via reflexes. Nashner (1976) suggested that ankle rotation is the most probable stimulus of the functional stretch reflex (FSR). The FSR is the first useful phase of activity in the leg muscle after a change in posture. Muscle spindles sense a stretching of the agonist resulting in signals being sent to contract the muscle to prevent or control sway (Dietz, Horstmann, & Berger,



1989). Muscle vibration can be used to induce postural adjustments (Eklund, 1972) and changes in the control of balance (Lackner, 1988). Muscle vibration is likely to result in a response due to the illusion of altered muscle length (Goodwin, McCloskey, & Matthews, 1972).

Hearing

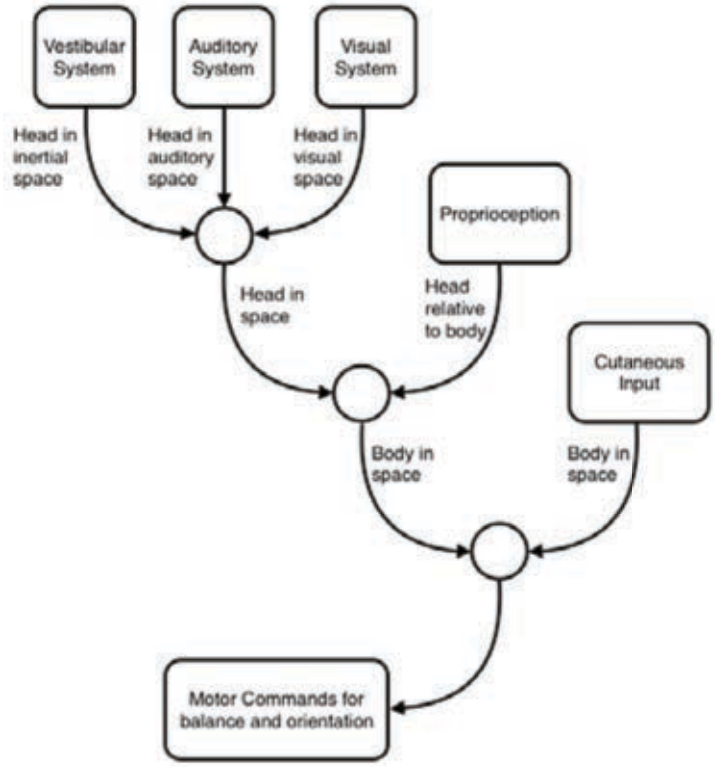
The auditory system is used for localisation of the head in respect to a fixed sound. Sounds waves will reach each ear at differing times depending on the orientation of the head and this time difference allows us to determine location. Auditory cues have been shown to reduce postural instability when vision is not available. However this effect was small and required the sounds to be very close to each ear, which wouldn't happen in a natural environment (Easton, Greene, DiZio, & Lackner, 1998). This leads to the suggestion that the auditory contribution to the control of balance is small, if it makes any contribution at all.

Sensory Integration

Each system is sensitive to different sources of information about the body and its position in space. Vision is used during slow movements (<0.1Hz), whereas the vestibular otoliths are sensitive to even slower movements (<0.05Hz). With so many inputs we need a way to determine which are most important and reliable as well as determining how each relates to one another and whether we need all our senses to remain upright. The vestibular, auditory and visual systems provide information about head position in space. This information is of no use in

determining whole body movement without any sense of head orientation relative to the body. This information is provided by proprioception. The integration of all four senses along with cutaneous input provides information about the body in space, which can in turn be used to determine motor commands for maintaining balance. This integration is as shown in Figure 1.4 (Carpenter, 2002). With so many sensory inputs there is clearly some redundancy between them.

During proprioceptive perturbations, such as vibration of the Achilles tendon, the evoked response is significantly affected by vision. When vision is available, evoked sway magnitude is reduced compared to an eye closed condition (Caudron, Boy, Forestier, & Guerraz, 2008). This effect of vision is also true during vestibular stimulation (Day & Cole, 2002). Many sensory inputs are used



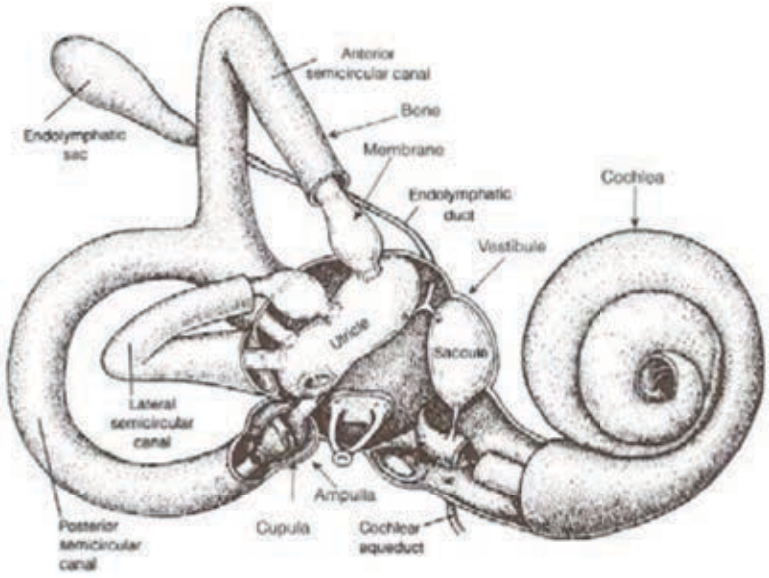
for balance, however there is some redundancy, where not all inputs are required at all times. Large-fibre sensory neuropathy is an extremely rare condition suffered by Patient I.W (Day and Cole, 2002). I.W has no sensations of cutaneous light touch and movement/position sense below the neck. Without vision, he would have no knowledge of the position of his limbs. Although it is a lot of effort I.W is still able to control balance using vision alone.

The loss of vestibular sensation does not have a significant effect on balance during quiet stance; patients with vestibular deficits exhibit only minor instability when vision and/or a support surface are available. Nashner et al., (1982) showed that postural instability in these patients is caused by the inability to suppress unreliable visual and proprioceptive inputs. The redundancy of certain sensory inputs when multiple sources of sensory input are available, means that if one is lost, then the remaining senses can compensate to some extent, although some postural instability will still be present. For example, compensation has been shown in unilateral vestibular neurotomy patients who recovered normal locomotion patterns within 1 month (Borel et al., 2004).

The Vestibular System

The peripheral vestibular organs form the non-auditory part of the inner ear, and are located bilaterally, fixed within the skull. The vestibular organs are sometimes referred to as the ‘balance organs’. In addition to balance (R C. Fitzpatrick & Day, 2004), they are used for orientation (Pastor, Day, & Marsden, 1993), perception

of self-motion (Ivanenko, Grasso, Israel, & Berthoz, 1997) and reflex eye movements (Dieterich & Brandt, 1995). The vestibular organs comprise a bony labyrinth consisting of three semicircular canals and a central chamber called the vestibule. The membranous labyrinth consists of two otolith organs and the membranous portion of the semicircular canals as shown in Figure 1.5. The semicircular canals and otolith organs detect rotational and linear motion respectively. The bony labyrinth is filled with perilymphatic fluid, which is very similar to cerebrospinal fluid, whereas the membranous labyrinth is filled with endolymphatic fluid, which resembles intracellular fluid.



Vestibular Afferents

Afferent signals pass from the end organs to the central nervous system (CNS) along the vestibular afferent nerve, a division of cranial nerve VIII. Vestibular primary afferents innervate the crista and macula, and discharge spontaneously at rest when the head is stationary. This means that, with rate coding, a neuron

can respond to accelerations in both directions. Afferent firing rates have never been recorded in humans. However, the resting discharge of squirrel monkeys is ~90spikes/sec for semi-circular afferent neurons (Fernandez & Goldberg, 1971; Goldberg & Fernandez, 1971a, 1971b) and ~60spikes/sec for otolith afferent neurons (Fernandez & Goldberg, 1976; Fernandez, Goldberg, & Abend, 1972). Primary afferents can be classified as regular or irregular according to their discharge pattern, although this is more for convenience as it is more of a continuum than a discrete classification (Baird, Desmadryl, Fernandez, & Goldberg, 1988; Goldberg, 2000). The degree of regularity is determined by the size of its hyper-polarisation relative to the size and rate of its excitatory postsynaptic potentials. Afferent firing rate increases or decreases depending on the amplitude and direction of an imposed acceleration. Squirrel monkeys have a resting discharge of 65-90s^{-1} with a sensitivity of 2s^{-1}/deg・s^{-2} for semi-circular canals and 33s^{-1}/g for the otolith organs (Fernandez & Goldberg, 1976; Goldberg & Fernandez, 1971b). Irregular primary afferents have a lower tonic rate and are more sensitive to acceleration, with shorter refractory periods.

A single primary vestibular afferent innervates many hair cells (Fernandez, Baird, & Goldberg, 1988; Fernandez, Lysakowski, & Goldberg, 1995). Many secondary vestibular neurons of the vestibular nuclei also receive output from these primaries (Fitzpatrick & Day, 2004). Large secondaries are almost exclusively innervated by irregular afferents, whereas small secondaries are innervated by both types. Regular units have smaller, localised dendritic connection centrally, whereas irregular units have a larger dendritic area (Fernandez et al., 1988;

Fernandez, Goldberg, & Baird, 1990; Sato, Sasaki, & Mannen, 1988). Secondary vestibular neurons of the vestibular nuclei project into many areas of the CNS, including the oculomotor nuclei, spinal cord, and the flocculus of the cerebellum (Highstein, Goldberg, Moschovakis, & Fernandez, 1987), as well as a thalamocortical pathway. There is convergence of afferents, at the level of secondary neurons, from semi-circular canals and otolith organs (Dickman & Angelaki, 2002; Fernandez et al., 1995) from both sides of the striola and both sides of the head (Uchino et al., 1999; Uchino et al., 2001). In a subsequent section, I discuss the effect that electrical vestibular stimulation has on both regular and irregular afferents.

Otolith Organs

The otolith organs, namely the utricle and saccule, sense linear acceleration of the head in space. The macula of each organ contains 20,000 - 30,000 hair cells across a specialised area. Hair cells project into a gelatinous mass weighted with calcium crystals, known as otoconia. A hair cell consists of many shorter stereocilia and one longer kinocilium. During movement, the gelatinous mass lags behind the macula surface, resulting in the deflection of the hair cells, which modulates the firing rate of the vestibular afferent fibres. Deflection of the stereocilia towards the kinocilium results in the depolarisation of the hair cells, increasing the firing rate (Goldberg & Hudspeth, 2000). The opposite is true if the deflection is away from the kinocilium, when hyperpolarisation occurs, decreasing the firing rate (Fernandez & Goldberg, 1976; Fernandez et al., 1972), as illustrated in Figure 1.6. The plane of the utricular macula is inclined backwards

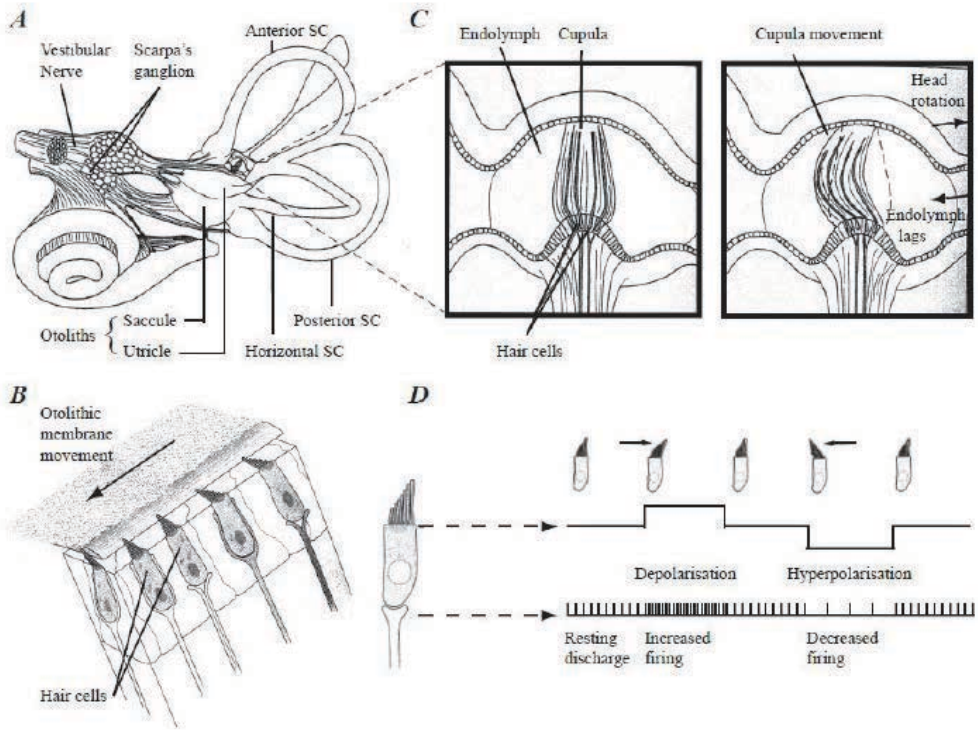
from horizontal by ~30deg and slopes away laterally by ~10deg (Igarashi, 1967; Naganuma, Tokumasu, Okamoto, Hashimoto, & Yamashina, 2003), approximately in the plane of the horizontal semi-circular canal. Utricular afferents are sensitive to lateral and sagittal components. The saccular macula, located on the medial wall is aligned with the sagittal plane and the afferents signal both vertical and anteroposterior components. The macular are ellipsoid in shape with the utricular macula being concave upwards and the saccular macula concave medially (Igarashi, 1967; Naganuma, Tokumasu, Okamoto, Hashimoto, & Yamashina, 2001). This arrangement gives greater sensitivity to different movement directions. The utricular macula, located on the floor of the utricle, is divided into the pars medals (pars internal) and the pars laterals (pars externa). The hair cells of the utricular macula are aligned in a way that means all the kinocilia are closest to the striola, therefore for any movement one side will increase its firing rate and the other will decrease. The hair cells of the saccular macula are aligned with the kinocilia, pointing away from the striola.

Semi-Circular Canals

Three semi-circular canals, located bilaterally, sense rotation of the head which enables reflexes such as the vestibulo-ocular reflex (VOR). The three canals are approximately orthogonal to each other, with the horizontal canals responding to yaw and the anterior and posterior canals, oriented vertically at 45deg to the sagittal axis, both responding to pitch and roll (Gray & Clemente, 1985). The symmetry of the canals on both sides of the head results in an identical inverse signal for any movement. This bilateral arrangement results in a greater

amplitude and directional sensitivity of the entire system and provides sensory redundancy. It also allows the central nervous system to ignore any changes in discharge that may be due to changes in body temperature or chemistry. Each canal is a loop filled with endolymph fluid. The hair cell neuroepithelium, or crista ampullaris, resides within an ampulla at the end of each canal with the cilia embedded in a gelatinous cupola. When the head moves, the hair cells are deflected by the inertial reaction force of the endolymph. Prolonged rotation (>7seconds) at a constant velocity results in an exponential decay of the output. This is due to the tendency of the cupola to restore its resting position once the perilymph no longer moves (Wilson & Jones, 1979).

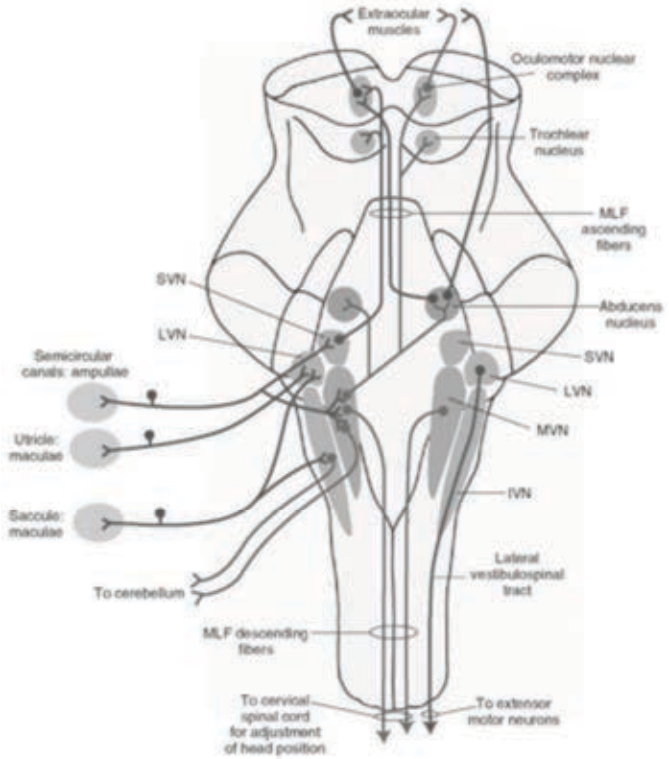
Vestibular Connections



Each hair cell is innervated by an afferent neuron located in the vestibular ganglion (Scarpa's ganglion), which is located close to the ampulla. The ganglion consists of two divisions; 1) the superior division which is connected to the anterior and horizontal canals, the utricle and a portion of the saccule, and 2) the inferior division, which is connected to the posterior canal and the main portion of the saccule. The vestibulocochlear nerve (Cranial nerve VIII) is formed by the central processes of bipolar cells in the vestibular ganglion, located deeply in the internal auditory meatus. This transmits signals from the labyrinths through the internal auditory canal, travelling through the petrous portion of the temporal bone to open into the posterior fossa at the level of the pons. The vestibular nerve enters the brainstem at the pontomedullary junction (Hain & Helminski, 2007). There are two main targets for vestibular input from primary afferents: the vestibular nuclear complex of the brainstem and the cerebellum, suggesting a close functional relation between the labyrinth and the cerebellum (Carleton & Carpenter, 1984). The vestibular nuclear complex consists of four major nuclei; superior (of Bechterew), medial (of Schwalbe), lateral (of Deiters), and descending (inferior spinal) as well as seven minor nuclei. The superior and medial nuclei are relays for the VOR; the medial nucleus is also involved in vestibulospinal reflexes (VSR) and coordinates head and eye movements together, although the lateral nucleus is the principle nucleus for this function (Hain & Helminski, 2007). The descending nucleus is connected to all other nuclei as well as the cerebellum. The two sides of the brainstem are connected via commissures that are mutually inhibitory, allowing information to be shared between the two sides and implement the push-pull relationship of the semi-

circular canals and may be used for compensation after unilateral vestibular loss (Brodal, 1981; Dickman, 1997). Extensive connections between the vestibular nuclear complex (VNC), cerebellum, ocular motor nuclei, and the brainstem reticular activating systems are required to formulate appropriate efferent signals to the VOR and VSR effector organs, the extra ocular and skeletal muscles, respectively (Hain & Helminski, 2007).

In summary, the structure of the vestibular system allows for the sense of head motion. This information is then passed to the central nervous system (CNS) via vestibular afferent nerves. The majority of the processing occurs in the vestibular nuclei, afferents and efferent connections with other CNS allows for multiple



reflexes to occur. These connections give rise to eye movements, perception of self-motion and motor responses. Vestibular nuclei and their pathways are illustrated in Figure 1.7.

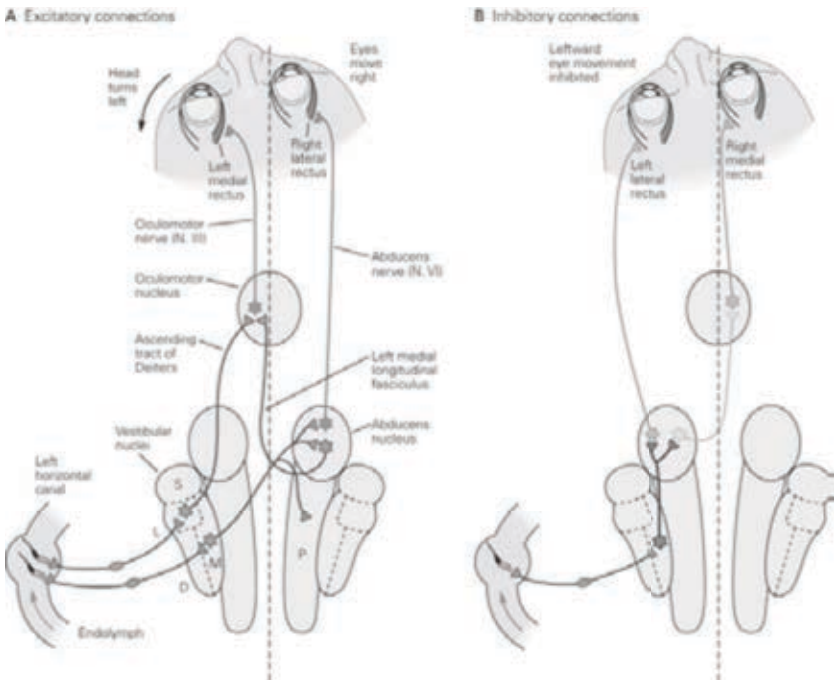
Vestibular Reflexes

The term reflex, in physiology, is defined as an action/response 'performed independently of the will, as an automatic response to a sensory stimulus' and this thesis will refer to this definition when the word 'reflex' is used. For example, when touching something hot, cutaneous receptors evoke a reflex response to withdraw the hand quickly. Reflex signals are sent to the CNS and spinal cord, although some reflexes can involve cranial nerves and the brain stem. In both cases, no reflex signal passes through the cortex in need of a conscious decision. Hence the response is extremely fast. The signals from the spinal cord evokes muscular activity to withdraw the hand.

Vestibulo-Ocular Reflex

For a stable image during head movement, the eyes must produce compensatory eye movements, known as the vestibulo-ocular reflex (VOR). There are two VOR types; 1) rotational and 2) translational. For this thesis, the main VOR of concern is rotational and torsional VOR. The rotational VOR occurs during head rotation and is detected by the semi-circular canals. If the head was to rotate towards the right by 100 deg/s, in an ideal world the eyes would rotated towards the left by 100 deg/s, thus keeping the retinal image stable. This eye movement is known as slow phase. If rotation continues the eyes would eventually reach the limit of

their orbital range and stop moving. To prevent this occurring a rapid eye movement is performed towards the right, known as the quick phase. This moves the eye to a new fixation point and allows further slow phases to occur. This alternating pattern of slow and fast phases is known as nystagmus. If the angular velocity of rotation remains constant, eventually the inertia of the endolymph in the semicircular canals stops moving, thus returning the vestibular afferent firing rate to baseline, ceasing slow phase and nystagmus movements, although the head is still rotating. The nystagmus lasts longer than might be expected given the deflection of the cupula. This is due to a mechanism called velocity storage whereby the brain stem provides a velocity signal to the oculomotor system, although the vestibular nerve is no longer providing information about head motion. If the head was then to stop abruptly, the endolymph will move in the same direction as prior head movement, resulting in a rotational VOR in the



opposite direction. This reflex can occur in complete darkness. If vision is present, optokinetic reflexes also produce nystagmus during head rotation, and can suppress post-rotatory VOR.

The superior and medial vestibular nuclei have many connections with oculomotor centres and the spinal cord. A disynaptic, three-neuron brainstem pathway connects each semi-circular canal to the appropriate eye muscle, as illustrated in Figure 1.8. For leftwards head rotation, the firing rate of the left horizontal canal afferents decrease, while simultaneously the right horizontal canal afferents increase in proportion to head velocity. This modulation is transmitted along the vestibular nerve, influencing the firing rate of both the superior and medial vestibular nuclei as well as the cerebellum. Excitatory impulses transmitted to the oculomotor nuclei evokes contraction of the ipsilateral medial rectus and contralateral lateral rectus muscles. Simultaneously, inhibitory impulses are transmitted to the contralateral medial rectus and ipsilateral lateral rectus, relaxing the muscles. Ultimately, this culminates as a compensatory eye movement to the right. If this eye movement has an error of >2deg/s, the cerebellar projections of the vestibular nuclei modulates the firing rate within the vestibular nuclei, thus reducing the movement error. The entire reflex is completed in less that 10ms (Aw, Todd, & Halmagyi, 2006).

Vestibulospinal Reflex

The purpose of the vestibulospinal reflex is to stabilise the body. During a destabilising event, the head will move and this is detected by both the semicircular canals and otolith organs. Descending vestibulospinal tracts, originating in the medial and lateral vestibular nuclei, excite motor neurons directly or terminate on interneurons in the spinal cord (Brodal, 2010). These descending pathways allow vestibular signals to evoke whole-body motor responses. Extensor activity on the side to which the head is inclined, and flexor activity on the opposite side produce the appropriate forces to produce a movement to stabilise the body.

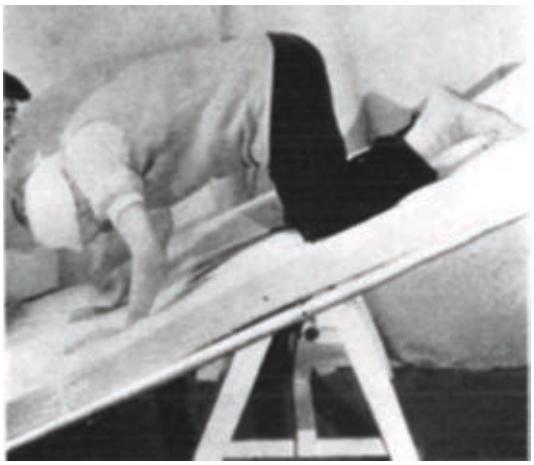
Perturbing Vestibular Reflexes

The vestibular system is clearly important to our everyday lives whether it be via the stabilisation of vision or maintaining balance, hence we need techniques which are able to test vestibular function in clinical scenarios where patients present with vestibular dysfunction. Over the years many techniques have been developed from physical pushes or pulls of the body or via the translation of the support surface, to inducing virtual motion using caloric vestibular stimulation and more recently galvanic vestibular stimulation.

Tilt Table Test

Tilting reactions for the purpose of detecting labyrinth function were first introduced into clinical medicine by Tait (1926). Tilting is defined as angular

displacement of the supporting base relative to the horizontal plane, and in humans it gives rise to large postural adjustments which occur rapidly and usually involve the whole body. Martin (1965) tested blindfolded participants in the 'all-fours' position, with tilting anteriorly or posteriorly on a tilting bed. In a healthy individual a posterior tilt evokes a compensatory anterior body movement via the extension of the hips and knees. This postural adjustment keeps the centre of mass within the support base of the body (Figure 1.9, left). However, an individual without vestibular function would not make any postural adjustments and hence would remain in their original orientation and more than likely fall towards the tilt (Figure 1.9, right). This makes those with little or no vestibular function have a higher risk of falling (Herdman, Blatt, Schubert, & Tusa, 2000). Some have pushed and pulled participants to evoked a balance response (Fitzpatrick & McCloskey, 1994), while others have used the rapid translation of the support surface as a means to evoke a response (Nashner et al., 1982). One disadvantage of clinical tilt tests is that the result is qualitative and requires the examiner to make a subjective decision about the evoked response. The

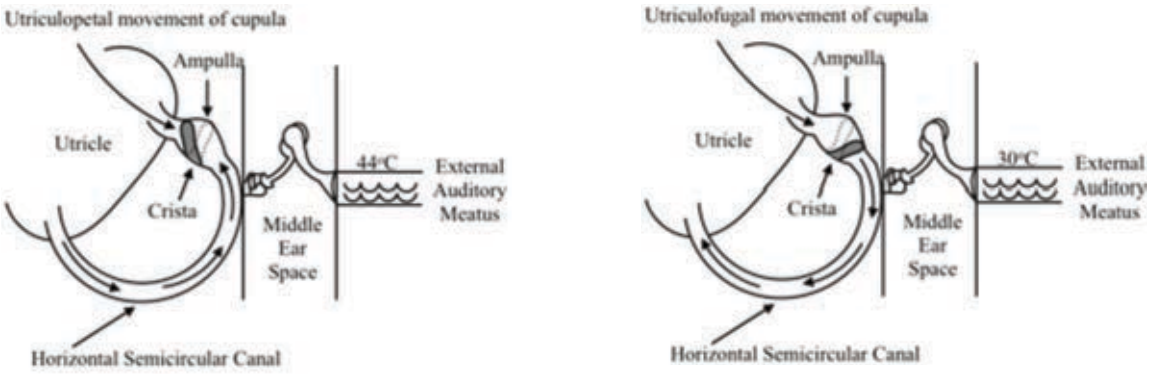


amplitude and force applied to perform the tilt is also not controlled and therefore makes this testing method inheritably variable.

Caloric Reflex Test

While physical methods are useful for testing overall balance reactions, inducing a virtual signal can be useful for investigating various elements of the vestibular system. One technique to induce a virtual sense of rotation is caloric vestibular stimulation, which involves the irrigation of the ear canal with warm or cold water to evoke reflexive eye movements (Jacobson & Newman, 1997; Mueller-Jensen, Neunzig, & Emskotter, 1987). This method was first described by Barany (1906; 1911). A caloric examination evaluates the physiological integrity of a patient's left or right horizontal semi-circular canal. When warm water is used to irrigate the external auditory meatus the skin of the ear canal is heated, resulting in a temperature change which is transmitted to the horizontal semi-circular canal. The endolymph closest to the canal wall is heated, causing it to become relatively less dense than the surrounding endolymph. Less dense fluids rise due to convection, resulting in denser endolymph replacing the space, which is subsequently heated and the process continues. The movement of the endolymph causes the cupola to move and hence results in a signal being produces which replicated that of the head turning towards from the irrigated ear. Activity through the medial longitudinal fascicles results in a slow deviation of the eye in the opposite direction and a fast saccade towards the same side as the irrigated ear. Cold water works in the same way; however, the convection current is reversed and hence the virtual motion is the opposite (figure 1.10). There are

four main disadvantages of caloric testing; 1) the actual stimulation at the end organ may vary greatly depending on the heat transferability of the tissue within the ear canal 2) caloric stimulation is analogous to head rotation of only 0.003 Hz (Hamid, Hughes, & Kinney, 1987), but the vestibular system works over a much larger frequency range of 0.001 to 8 Hz (Gauthier, Piron, Roll, Marchetti, & Martin, 1984; Jell, Guedry, & Hixson, 1982; Vercher, Gauthier, Marchetti, Mandelbrojt, & Ebihara, 1984), 3) caloric stimulation only tests the horizontal canals and isn't representative of the function of the rest of the entire membranous labyrinth, and 4) caloric testing is unsuitable for evoking postural reflexes during standing.



Galvanic Vestibular Stimulation

This thesis explores vestibular-evoked reflexes via three types of electrical vestibular stimulation (EVS): galvanic vestibular stimulation (GVS), stochastic vestibular stimulation (SVS) and sinusoidal electrical vestibular stimulation (sEVS). Although the effect on the firing rate of vestibular afferents is the same for each type of EVS (dependent on polarity), the pattern of delivery differs greatly. GVS involves a constant DC current, whereas SVS involves a random

white noise delivery pattern. sEVS on the other hand delivers EVS in a consistent varying current (sinusoidal in nature). As the difference in each type of EVS is simply the method of delivery, I will only describe the effects of GVS in more detail below.

An alternative technique for evoking vestibular reflexes is Galvanic Vestibular Stimulation (GVS). GVS is considered to be a pure vestibular perturbation as it does not affect any other sensory inputs. In 1790, Alessandro Volta placed electrodes in his ears, which were connected to a battery. He described the sensation as an explosion inside his head, accompanied by spinning and the sound of boiling tenacious matter. The spinning can be attributed to the vestibular system; however, the boiling noise was likely due to the, excessive, ~30V burning the tissue within the ear (Fitzpatrick & Day, 2004). Since this early and extremely dangerous experiment, GVS has now been improved (the voltage is reduced significantly) and is used in many balance-related studies. In 1820, Johann Purkyne reported that a galvanic current passed through the head had a destabilising effect on posture. GVS's ability to evoked ocular movement was discovered by Eduard Hitzig (1871), who noticed nystagmus occurred when applying an electrical current to the brain.

The GVS technique is very simple. Two electrodes are placed on the mastoid processes, behind the ears. Usually a bipolar binaural configuration is used, with an anode behind one ear and a cathode behind the other. A unilateral monopolar configuration is especially useful when testing vestibular function as each ear can

be tested separately by placing one reference electrode on the neck, usually at C7 (Jahn, Naessl, Strupp, et al., 2003; Welgampola, Ramsay, Gleeson, & Day, 2013). GVS usually involves delivering a current of the order of 1-10 mA for a few seconds. It induces a virtual signal of self-motion, evoking compensatory reflexes. The exact site affected by GVS is unknown but it has been shown to be no further central than Scarpa's ganglion (Courjon, Precht, & Sirkin, 1987). GVS has been shown to be effective after labyrinth excision but no response is seen after section of the eighth cranial nerve (Pfaltz & Koike, 1968; Spiegal & Scala, 1943) which led to the conclusion that the site of GVS must be between these two locations. It stimulates irregular primary afferents more readily than regular afferents, which are barely affected even by large stimulus currents (Ezure, Cohen, & Wilson, 1983; Goldberg, 2000; Goldberg, Smith, & Fernandez, 1984). As previously discussed, irregular afferents innervate spinal projecting neurons meaning that the GVS signal is carried to all CNS areas receiving vestibular projections. The body's response to GVS is the same to that of a real head movement in space. Anodal-cathodal GVS affects semi-circular canal afferent discharge in the same way as angular velocity (Lowenstein, 1955).

GVS stimulates the entire population of susceptible afferents, regardless of the alignment of the hair cells that they innervate, for both the semi-circular canals and otolith organs. Therefore GVS has no inherent direction; it is the sensitivity of the semi-circular canals in all three vectors that determines its direction. If we first examine the signal produced by the semi-circular canals we can see that bilateral bipolar GVS will alter the firing rate to produce; 1) yaw towards the

cathodal side by the horizontal canal (h vector Figure 1.11, left), 2) nose-down pitch by the anterior canal (a vector Figure 1.11, left), 3) nose-up pitch by the posterior canal (p vector Figure 1.11 left,) and 4) ipsilateral ear-down roll by the anterior and posterior canals. The anterior and posterior pitch components cancel each other out, thus cathodal GVS will signal rotation with yaw and roll components, relative to the plane of the vestibular apparatus (Fitzpatrick & Day, 2004). Anodal GVS will decrease the firing rate to produce an opposite reaction to that of cathodal GVS. However, due to the mirror symmetry of the canals on either side of the head means the directions are reversed horizontally. We know that the canal structure is tilted backwards by ~30 degrees from the head horizontally. This configuration manipulates the signals produced by each canal. The horizontal canal develops a roll component at the expense of yaw and the vertical canals develop a yaw component at the expense of roll. They do, however, maintain their pitch component. With such a complicated signal, a method of calculating the vector of each canal relative to Reid's stereotactic line (inferior orbital rim to auditory canal, r vector/ L + R, Figure 1.11, left) has been developed (Blanks, Curthoys, & Markham, 1975). During the normal anatomical upright position, Reid's line is nearly horizontal. During bilateral bipolar GVS, summation of the vectors of all the canals will result in a signal of rotation about the mid-sagittal axis directed backwards and pitched slightly upwards (~18.8 degrees) from Reid's line. Thus, bilateral bipolar GVS during normal stance will produce a signal of roll with a small yaw component towards the cathodal electrode, illustrated in Figure 1.11, centre. However, this is not actually the induced signal as the semi-circular canals differ in length and thus in sensitivity,

altering the virtual sensation. The posterior canal is the longest (18-22mm), hence the endolymph will exert greater pressure on the cupula than the anterior (15-20mm) and horizontal (12-15mm) canals (Muller & Verhagen, 1988; Oman, Marcus, & Curthoys, 1987; ten Kate, van Barneveld, & Kuiper, 1970). Therefore, the CNS needs to modulate the semicircular canal signals to produce constant spatial representation (i.e. the shorter horizontal canal will produce a smaller signal, resulting in the representation of yaw to be smaller than other movements). When the lengths of the various canals are considered, the net vector produced is angled backwards by 27.1 degrees from Reid's line, with roll still being the largest component, but the yaw component being slightly larger.

We now must consider the effect that GVS has on the otolith organs, whose contribution to the evoked response is still debated (Cohen, Yakushin, & Holstein, 2011, 2012; Curthoys & Macdougall, 2012). During natural movement, firing rates across the afferent populations differ and we assume that the CNS performs

vector summations to produce a meaningful direction and amplitude of the acceleration. However as previously mentioned we know that GVS affects all susceptible afferents. Hence the vector summation will be determined by the position and alignment of the striola on the saccular surface, as this will determine the direction and amplitude of the response. Therefore, if the hair cell population on each side of the striola is equal, then, anterior left and down vectors would cancel posterior right and up vectors resulting in a zero-net effect of GVS from the otolith organs. It is therefore important to know the distribution and alignment of these populations. Tribukait and Rosenhall (2001) studied 43 human macula utriculi and showed a balance of the pars medialis (47%) and pars lateralis (53%) areas. This imbalance results in a signal of acceleration towards the cathodal side and a smaller signal of acceleration to the anodal side. Overall the summing of these signals produces a small net acceleration towards the cathodal side. Saccule striola population data is not documented and hence we are unable to determine the signal expected from the saccule. However due to the position of the saccule, any net GVS effect would include anteroposterior acceleration but not lateral acceleration. The overall otolith signal is small when compared to the signal from the semi-circular canals. Hence the otolith organs are suggested to play little or no role in the evoked response (Cathers, Day, & Fitzpatrick, 2005; Mian, Dakin, Blouin, Fitzpatrick, & Day, 2010).

Once the stimulus is interpreted by the CNS, this results in a net virtual signal of head roll towards the cathode (Figure 1.11, centre). In standing subjects this evokes whole body compensatory sway towards the anode (Figure 1.11, right).

It also evokes an eye movement, mainly torsional, to ensure a stable image on the retina. In chapter 5 I utilise a monaural stimulus configuration, whereby only one ear is stimulated. As only one side of the vestibular system is stimulated the evoked force vector differs from binaural stimulation, although only marginally. When a cathodal electrode is placed over the right ear, the increase in afferent firing rate of the horizontal signifies a sensation of yaw towards the cathodal side. The combination of anterior and posterior stimulation produces a sensation of roll towards the cathodal as well as ear nose up and nose down sensation. As the pitch components cancel each other out, the overall sensation is mainly one of roll with a smaller yaw component towards the cathodal ear. This contrast with binaural bipolar stimulation, which is exclusively roll.

So, given this knowledge about the virtual sense of movement produced by GVS, and the resulting vestibulospinal and vestibulo-ocular reflexes, we can use it to investigate the physiological properties of these reflexes.

GVS-evoked vestibular reflexes

Ocular response to GVS

GVS evokes eye movements (MacDougall, Brizuela, & Curthoys, 2003; Watson, Brizuela, et al., 1998; Zink, Bucher, Weiss, Brandt, & Dieterich, 1998) with both horizontal (Buys, 1909) and torsional components (Hitzig, 1871). Eye recordings are usually performed in complete darkness as oculomotor responses are suppressed by visual fixation. During stimulation in total darkness, the evoked

eye movement is predominately torsional, with the upper side of the bulbus rotating away from the cathodal electrode (Suzuki, Tokumasu, & Goto, 1969), as illustrated in Figure 1.12. A sustained current step induces two types of torsional eye movement 1) tonic ocular torsion and 2) superimposed torsional nystagmus. Tonic torsion is believed to be a result of the activation of the otolith afferents (Zink et al., 1998), whereas the torsional nystagmus is a result of vertical semicircular afferents (Watson, Brizuela, et al., 1998). However, both tonic and phasic ocular torsion responses to GVS can be reproduced by pure rotational stimuli (Schneider, Glasauer, & Dieterich, 2002). The magnitude of the ocular torsion (0.5-5.4 degrees) increases with current (Zink et al., 1998).

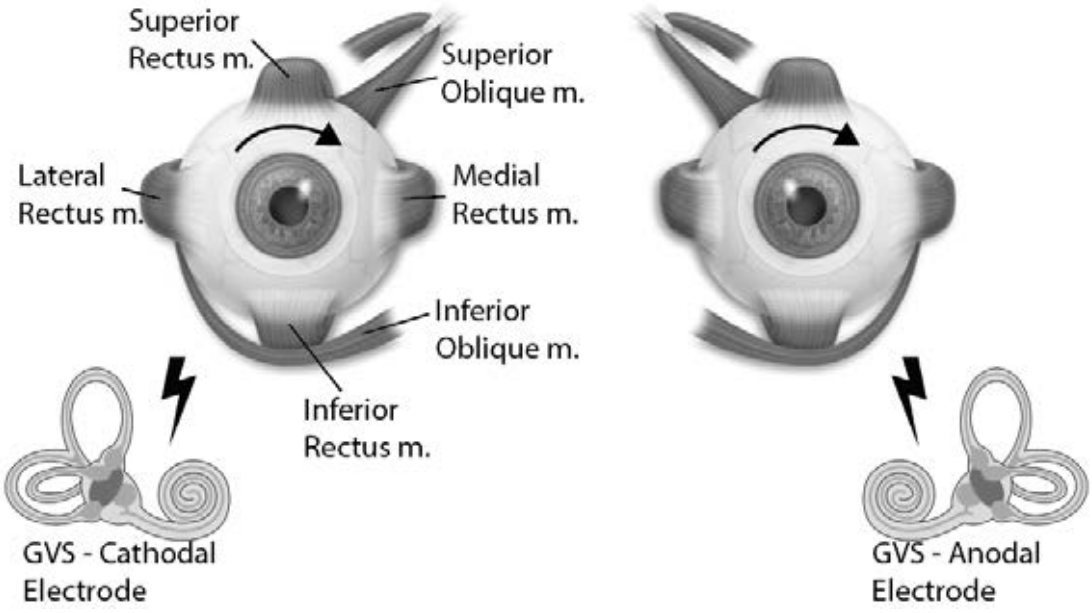


Figure 1.12 GVS evoked ocular torsion. Bipolar GVS evokes a reflex eye movement whose major component is torsion, with the upper side of the bulbus rotating away from the cathodal electrode. This is achieved through activation of the right superior oblique and left inferior oblique, with simultaneous inhibition of the right inferior oblique and left superior oblique.

The relative contribution of the semicircular canals and otolithic pathways to the GVS-evoked ocular torsion response was suggested to be 78% and 22%, respectively. An otolith stimulus of 0.1g is required to produce 1 degree of ocular

torsion (Clarke, Engelhorn, Hamann, & Schonfeld, 1999), modulating the firing rate by 3.72 spikes/s (Fernandez & Goldberg, 1976). Whereas, to produce 1 degree of ocular torsion via semicircular canal stimulation, an angular velocity of 2.7deg/s is required (Seidman, Leigh, Tomsak, Grant, & Dell'Osso, 1995; Tweed et al., 1994) to increase the afferent firing rate by 1.05 spikes/s (Fernandez & Goldberg, 1971). This suggest that an increasing in afferent firing rate by 1spike/s would produce 3.5 times more ocular torsion for semicircular canal stimulation than otolith stimulation. As previously mentioned, GVS is believed to stimulate all afferents, both semicircular canal and otolith, equally (Goldberg et al., 1984), thus semicircular pathways will dominate the GVS evoked eye movement (i.e. torsion). MacDougal and colleagues (2002) examined between-subject and within-subject variability of ocular responses to 5mA rectangular GVS measured by video-oculography. They found high between-subject variability, potentially due to individual differences in afferent susceptibility, but found low within-subject variability. This within-subject repeatability could potentially be utilised to monitor vestibular function over time or during the progression of vestibular degradation.

Balance response to GVS

The GVS-evoked balance response has been investigated in greater depth than ocular reflexes. GVS has a potent effect on whole body motor control resulting in well organised body movement of the trunk and limbs which can be measured using electromyography (EMG), force or body movement. Although the movement is well organised it is not hard wired and is sensitive to many factors.

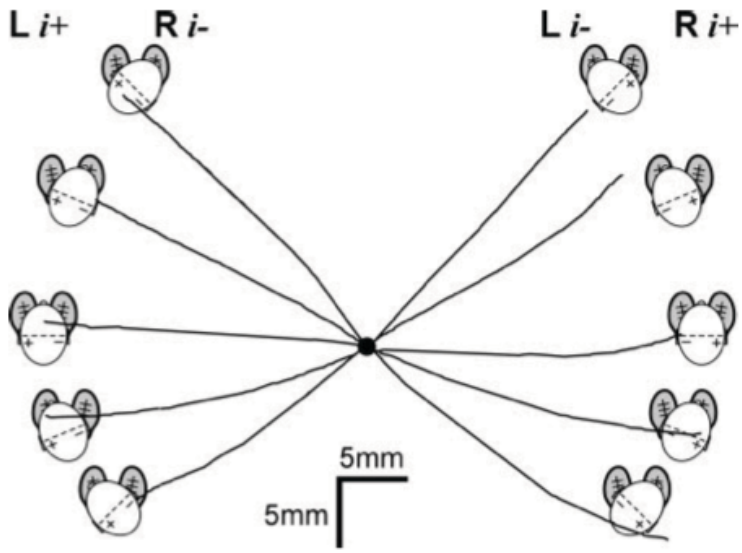
Above all, the muscle being investigated must be involved in the balance task to elicit any response (Britton et al., 1993; Fitzpatrick, Burke, & Gandevia, 1994).

Following the stimulus, both short- (55-65 ms) and medium-latency (110-120 ms) EMG responses are observed in muscles of lower limbs (Britton et al., 1993; R. Fitzpatrick et al., 1994; Nashner & Wolfson, 1974; Welgampola & Colebatch, 2002), the trunk (Ali, Rowen, & Iles, 2003; Ardic, Latt, & Redfern, 2000) and also the upper limbs, assuming they are engaged in the balance task (Baldissera, Cavallari, & Tassone, 1990; Britton et al., 1993). Once the current ceases, equivalent but opposite responses occur, which suggests that the reflexes are driven by the *change* in vestibular nerve discharge rate rather than its absolute level (Watson, Welgampola, & Colebatch, 2003).

Both short and medium latency responses increase in amplitude with increasing stimulus current. However, the short-latency response is far smaller than the medium latency, and therefore greater stimulus intensity is needed for it to appear (Fitzpatrick et al., 1994). The two responses are oppositely directed, with the short-latency response driving the body towards the cathodal side. However, the majority of the sway response can be attributed to the medium latency response due to its much larger size, and it is therefore most frequently measured. EMG responses to GVS have been seen in the legs as early as 55ms for short-latency responses and up to 110-129ms for medium-latency responses (Britton et al., 1993). The physiological origins of the short latency remain unknown.

In a healthy individual facing forwards, the balance response to bilateral bipolar GVS is directed laterally, resulting in whole body sway towards the anodal ear. However, sway direction is always referenced to the head. If the is head turned, the sway response rotates by the same magnitude (Lund & Broberg, 1983; Pastor et al., 1993) as illustrated in Figure 1.13. This phenomenon means that the pattern of muscle activity to maintain balance is very different for a forward facing head orientation (evokes mediolateral sway) compared to 90 deg head yaw (evokes anteroposterior sway), even though the vestibular information is identical.

To explain this, and to ensure an appropriate sway response is produced, a coordinate transformation is needed. This process requires the CNS to combine vestibular and proprioception signals to understand how the body segments are positioned in relation to each other and what muscular activity is needed to remain balanced. The remapping of the GVS response could occur due to the



convergence of proprioceptive afferent axons from the neck onto the second-order vestibular neurons of the vestibular nuclei (Fitzpatrick & Day, 2004). However, Lund and Broberg (1983) showed that the change in head orientation in relation to the feet can be achieved by movement of the neck, trunk or a combination of both, all of which result in the same postural response. Hence, the remapping process must be more complicated and consider the orientation of all segments of the body, not just neck, in order to execute the correct muscle activity to remain upright. The evoked sway response is produced by all body segments (Day, Severac Cauquil, Bartolomei, Pastor, & Lyon, 1997) with the head tilted on the trunk, the trunk tilted on the pelvis and the pelvis tilts with respect to the ground. This response is reversed once the stimulus ceases until all body segments returned to their original position.

However, this transformation can go awry. The Gurfinkel illusion can be used to perturb the sense of head direction by passively holding the head in a 90 degree turn for 15min with the eyes closed (Dalton, Rasman, Inglis, & Blouin, 2017; Gurfinkel, Popov, Smetanin, & Shlykov, 1989). This produces the illusion that the head gradually drifts towards a face-forward position. When perturbed using GVS during this period the sway response was initially appropriate for a 90degree head turn. However, as the illusion grew stronger (i.e. the head drifted closer to a forward-facing position) the evoked sway response was skewed towards the direction of the illusory head orientation. After the 15min period the eyes were opened for 30s, head still in a 90 degree position) before being closed and EVS delivered again. Although the illusion was now abolished by visual information,

the sway response unaffected and remained skewed. Although the underlining mechanisms of this dissociation is unclear, it is only present during passive neck rotation. Proprioceptive inputs from the neck have a strong influence on vestibulo-spinal neurones, whereas visual inputs have a relatively weak influence. Therefore, there may be a bias in favour of proprioceptive information when transforming signals for motor output.

All previous uses of EVS to explore this transformation process have studied the conglomerate response to the stimulus over time. For GVS, this means examining the average response to multiple stimuli (Inglis, Shupert, Hlavacka, & Horak, 1995; Welgampola et al., 2013). Whereas for stochastic vestibular stimulation (SVS), cross-correlation between stimulus and response are calculated for all possible direction over a prolonged period of time ($\geq$ 30s) (Dakin, Son, Inglis, & Blouin, 2007; Mian & Day, 2009). Both techniques miss any transient or trial-by-trial variations in the direction of the sway response, which may be important for understanding the efficacy of balance control in more ethological circumstances. It is therefore important to measure the precision of the response, as well as the accuracy of the evoked response. Chapters 2 and 3 further explore the precision and accuracy of vestibular-evoked sway responses.

GVS as a diagnostic technique

GVS's potential as a diagnostic tool has been the focus of continued research for a number of years. GVS acts upon all susceptible vestibular afferents unlike

caloric irrigation, head impulse test or vestibular myogenic potential tests, which act upon a subset of peripheral organs. Initially, GVS-induced postural responses were the focus of much of this research (Day, Steiger, Thompson, & Marsden, 1993; Welgampola et al., 2013). It has been suggested that GVS might be used to study vestibular function in a clinical setting (Coats, 1973; Watson, Fagan, & Colebatch, 1998) but some have also suggested that the high variability of the response discounts its usefulness (Blonder & Davis, 1936). Blonder and colleagues suggest that GVS was too noxious for subjects, further excluding the technique in a clinical environment. This issue has been eradicated with recent refinements to the delivery method of GVS via the use of large surface area electrodes and conductive electrode gel which reduce current density, thereby reducing unpleasant sensations and the risk of electrical burns. Stimulus waveform and magnitude also have a dramatic effect upon participant comfort. Sharp changes in amplitude and high current amplitude are associated with participant sharp pain at the stimulation site as well as metallic tastes in the mouth (Hlavacka & Njiokiktjien, 1985; Magnusson, Johansson, & Wiklund, 1990; Zink et al., 1998; Zink, Steddin, Weiss, Brandt, & Dieterich, 1997). It is now well established that slowly increasing the current over longer duration is less noxious to the subject than a sharp increase, even when the final amplitude is equal.

Although the precise site of neural activation is uncertain, it is well accepted that GVS bypasses the peripheral vestibular apparatus. This suggests that GVS may only be used to identify *central* vestibular dysfunction. However, Aw et al.,(2008) found that gentamicin-induced vestibular toxicity impairs EVS-evoked eye

movements. Gentamicin kills vestibular hair cells, suggesting that EVS may stimulate the hair cell rather than the primary afferent. However, the loss in hair cell input may conceivably reduce the high firing rate or excitability of vestibular afferents. These gentamicin-induced deficits provide some scope for EVS to be used to diagnose peripheral as cell as central vestibular deficits. Pfaltz (1969) suggest that the location of tumours in conditions such as vestibular schwannoma could be identified using GVS.

Welgampola et al (2013) demonstrated GVS's potential as a clinical tool in patients with Vestibular Schwannoma. By utilizing GVS in a monaural configuration they were able to test each ear separately. Vestibular-induced whole-body postural movements were quantified and an asymmetry ratio between the two ears was calculated, providing information about the location of the schwannoma (left or right side) and the level of vestibular loss. Watson et al (1998) studied two patients with Ménière's disease before and after unilateral selective vestibular neurectomy. Here GVS was used to examine evoked EMG responses of the sternocleidomastoid muscle in the neck and they suggest this method could be used clinically to examine vestibulocollic responses.

More recently, GVS-evoked eye movements have been studied in a variety of vestibular disorders. A number of patient case studies showed that different conditions result in systemically altered ocular movements (MacDougall, Brizuela, Burgess, Curthoys, & Halmagyi, 2005). Seven patients with conditions such as vestibular neuritis, absent semi-circular canals and Benign Paroxysmal

Positional Vertigo (BPPV) produced patterns of eye movements which were consistent with a reduction or absence in oculomotor contribution from the specific end-organs implicated in each patient's disease.

Postural reflexes can be utilised to measure behavioural responses to GVS, whereas GVS-evoked eye movements could be seen as a more pure test of vestibular function as it is not affected by vision, proprioception or muscle strength which has been shown to have a profound effect upon GVS-evoked postural responses. Both tests can be utilised in a clinical environment and this thesis will explore viability of GVS as a diagnostic tool.

Summary and Thesis Objectives

Summary

Vestibular information along with other sensory inputs are used for the control of balance and eye movements. When the vestibular system is no longer functioning optimally these previously easy tasks become noticeably more challenging and may need medical intervention. However, for an appropriate intervention to be prescribed, an accurate measure of vestibular function is needed. The ability of EVS to modulate afferent firing rate, induce a virtual sensation of roll and evoke measurable compensatory eye movements and/or sway has led many research groups to investigate its potential as a diagnostic tool.

Aims and Objectives

Initially, I was interested in exploring reflexes evoked by electrical vestibular stimulation. This aim was further spilt as follows: 1) postural reflexes and 2) ocular reflexes. I then turned my attention to the use of EVS-evoked reflexes in a clinical scenario.

Chapter 2 explores the coordinate transformation that takes place when the CNS calculates an appropriate sway response to EVS. Previous research examining the craniocentric nature of this evoked response has focused on the conglomerate response to stimulation over time (Inglis et al., 1995; Lund & Broberg, 1983; Mian & Day, 2009). This has consisted of either averaging many responses to GVS stimuli or calculating the cross-correlation between the SVS stimuli and response over a long period of time. However, this analysis method ignores any transient or trial-by-trial variations in sway direction, which would be important for understanding balance control in more ethological circumstances. Therefore, chapter 2 aims to explore these variations in the direction of evoked sway responses by looking at responses on a trial-by-trial basis (i.e. precision). After developing an analytical method to examine precision, we explored its relationship with response accuracy and how both parameters were affected by vision.

Chapter 3 utilises this measure of response precision in a population who have undergone a prolonged period of inactivity. It is well known that inactivity has negative consequences on cardiovascular and respiratory health as well as

muscle strength and volume. However, little is known about the effects of inactivity on the sensory inputs used for balance control. Chapter 3 addresses this gap in the literature by measuring the effect of prolonged inactivity (60days bedrest) upon spontaneous sway and EVS-evoked sway parameters, such as precision, accuracy and magnitude. There are three main aims of this chapter, to determine: 1) can the dissociable nature of response accuracy and precision found in chapter two be reproduced? 2) how does prolonged inactivity affect the characteristics of the EVS-evoked sway response (i.e. are you less accurate and precise?). 3) Do changes due to prolonged inactivity persist up to 6 days post bedrest?

Chapters 2 and 3 focus on whole-body postural reflexes which are complex behavioural responses which can be modulated by vision, proprioceptive acuity muscular strength and participant volition (Britton et al., 1993; Butler, Lord, Rogers, & Fitzpatrick, 2008). Therefore, the use of EVS-evoked sway responses as a clinical diagnostic tool to measure vestibular would require careful consideration when examining results. A purer test of vestibular function would be preferable in such a situation. This could be achieved by measuring the EVS-evoked VOR, which represents a purer vestibular test due to the small number of neurons involves in the reflex arc.

Chapter 4 switches focus to EVS-evoked ocular responses. As previously mentioned, EVS evokes a torsional eye movement which is inherently more challenging to measure than lateral or vertical eye movements. Torsional

movements have been measured using invasive technical such scleral coils (Severac Cauquil, Faldon, Popov, Day, & Bronstein, 2003) or marking the sclera with a surgical pen to facilitate video tracking (Jahn, Naessl, Strupp, et al., 2003). Therefore, the first aim of this chapter was to develop a simple, reliable and non-invasive method for measuring EVS-evoked ocular torsion responses. The developed technique then allowed for further investigation into how the brain interprets an EVS stimuli (position, velocity or acceleration signal). This was achieved by examining the stimulus-response phase for eye position, eye velocity and eye acceleration.

In chapter 5, our non-invasive techniques developed in chapter 4 for measuring the EVS-evoked VOR, was used to measure vestibular function in vestibular schwannoma patients. This patient population was used as they have a known unilateral vestibular deficit due to the presence of a tumour on the XIII cranial nerve. The use of a monaural configuration for EVS allows each ear to be examined independently, from which an asymmetry ratio can be calculated. The main aim of this chapter was to determine if patients exhibited significantly greater response asymmetry than control subjects, which would indicate a unilateral deficit. Secondly, we compare this measure against two alternative measures of vestibular function, namely the EVS-evoked postural test and the head impulse test (HIT).

Each chapter of this thesis reveals a unique and novel finding. Overall, this thesis provides insight into how vision and head orientation affects vestibular-evoked

balance responses in both healthy and inactive populations. It then determines how the CNS interprets the EVS signal before going on to provide a potentially useful diagnostic tool that could be used clinically to determine vestibular function.

CHAPTER 2

DIFFERENTIAL EFFECTS OF VISION UPON THE ACCURACY AND PRECISION OF VESTIBULAR-EVOKED BALANCE RESPONSES

Highlights

- Effective balance control requires the transformation of vestibular signals from head to foot-centred coordinates in order to move the body in an appropriate direction.
- This transformation process has previously been studied by analysing the directional accuracy of the averaged sway response to multiple Electrical Vestibular Stimuli.
- Here we studied trial-by-trial variability of EVS responses to measure any changes in directional precision which may be masked by the averaging process.
- We found that vision increased directional variability without influencing the mean sway direction, demonstrating that response accuracy and precision are dissociable.
- These results emphasise the importance of single trial analysis in determining the efficacy of vestibular control of balance.

Key words: Balance, Vestibular-motor transformation, Accuracy, Precision.

Abstract

Vestibular information must be transformed from head-to-foot centred coordinates for balance control. This transformation process has previously been investigated using Electrical Vestibular Stimulation (EVS), which evokes a sway response fixed in head coordinates. The craniocentric nature of the response has been demonstrated by analysing average responses to multiple stimuli. This approach misses any trial-by-trial variability which would reflect poor balance control. Here we performed single-trial analysis to measure this directional variability (precision), and compared this to mean performance (accuracy). We determined the effect of vision upon both parameters. Standing volunteers adopted various head orientations (0, ±30 & ±60 deg yaw) while EVS-evoked response direction was determined from ground reaction force vectors. As previously reported, mean force direction was oriented towards the anodal ear, and rotated in line with head yaw. Although vision caused a ~50% reduction in response magnitude, it had no influence upon the direction of the mean sway response, indicating that accuracy was unaffected. However, individual trial analysis revealed up to 30% increases in directional variability with the eyes open. This increase was inversely correlated with the size of the force response. The paradoxical observation that vision *reduces* the precision of the balance response may be explained by a multi-sensory integration process. As additional veridical sensory information becomes available, this lessens the relative contribution of vestibular input, causing a simultaneous reduction in both the magnitude and precision of the response to EVS. Our novel approach

demonstrates the importance of single trial analysis in revealing the efficacy of vestibular reflexes.

Abbreviations: EVS, Electrical Vestibular Stimulation. SVS, Stochastic vestibular stimulation. GVS, Galvanic vestibular Stimulation

Introduction

Since the vestibular system is locked within the skull, the signals it provides must be transformed from head to foot-centred coordinates for balance control (Fitzpatrick & Day, 2004; Hlavacka & Njiokiktjien, 1985; Lund & Broberg, 1983; Mian & Day, 2009; Pastor et al., 1993). For example, when leftward head motion is detected while facing forwards, a compensatory body movement to the right would be the appropriate response to maintain balance. But if the head is turned 90 degrees rightward, the same pattern of vestibular afferent feedback would require a backward body movement. This coordinate transformation process requires an accurate sense of head-on-feet proprioception (Dalton et al., 2017; Reynolds, 2017). Any breakdown in this process would compromise the efficacy of the vestibulo-spinal reflex, which may increase fall risk.

This efficacy of the coordinate transformation process can be investigated using Electrical Vestibular Stimulation (EVS) (Fitzpatrick & Day, 2004). EVS modulates activity of vestibular afferents, leading to a false sensation of body sway towards the cathode electrode. This evokes a compensatory sway response towards the anodal ear. This response is fixed in head coordinates, such that turning the head in yaw produces an equal rotation of the evoked sway direction. Previous studies have demonstrated the craniocentric nature of the EVS response by measuring the direction of the evoked body sway and/or ground reaction force vector at different head angles (Lund & Broberg, 1983; Mian & Day, 2009, 2014). Response direction is typically calculated by averaging sway responses to

multiple EVS pulses of direct current, known as Galvanic Vestibular Stimulation (GVS) (Inglis et al., 1995; Welgampola et al., 2013). More recently, the transformation process has been investigated using Stochastic Vestibular Stimulation (SVS) (Dakin et al., 2007; Mian & Day, 2009). This involves application of a continuous randomly-varying current lasting up to minutes. SVS offers advantages over GVS, including greater signal-to-noise ratio, and the ability to analyse the response in the frequency domain. GVS, on the other hand, allows for the precise determination of response latency in the time domain (Britton et al., 1993; Nashner & Wolfson, 1974).

For both SVS and GVS, previous analysis has involved studying the conglomerate response to stimulation over time. For GVS, this consists of the average response to multiple stimuli. For SVS, cross-correlations between stimulus and response time series are calculated for all possible directions over a prolonged period ($\geq$ 30s). The direction which produces the largest correlation value is then deemed to be the response direction. Both analysis techniques miss any transient or trial-by-trial variations in the direction of the sway response. These variations may be important for understanding the efficacy of balance control under more ethological circumstances. If we suffer a fall due to a transient error transforming vestibular input in motor output, an accurate *average* response is of little consolation. In other words, it is important to measure the precision, as well as the accuracy, of the vestibular-evoked sway response.

Here we address this gap in the literature by measuring variability in the direction of the sway response to GVS and SVS. We ask two related questions. Firstly, is the precision of the vestibular-evoked sway response dissociable from its accuracy? Secondly, how are both parameters affected by vision? We hypothesise that closing the eyes will produce more variable (less precise) sway responses, while accuracy will be unaffected. Our rationale for this prediction is that the absence of vision will negatively affect head-on-feet sensation, and thus the ability to transform vestibular input into motor output for balance (Dalton et al., 2017; Reynolds, 2017). In fact, our results refute this hypothesis. Closing the eyes produced *less* variable responses. This occurred for both GVS and SVS, but was more clearly demonstrated using the latter technique. We discuss this unexpected finding in the context of a multisensory integration process. Accuracy, however, was unaffected by vision, confirming that precision and accuracy are indeed dissociable.

Methods

Ethical Approval

The experiment was approved by the local ethical review committee at the University of Birmingham, and was performed in accordance with the Declaration of Helsinki, except for registration in a database. Informed written consent to participate was obtained from all participants.

Participants

12 participants (9 males) aged 20-30 years (mean±SD; 25±2 years) with no known neurological or vestibular disorder.

Protocol

Participants stood in the centre of a force plate, unshod, with feet together and hands held relaxed in front of them for the duration of each 100 s stimulation period (Figure 2.1). Prior to each trial participants were instructed to face one of five visual targets (±60, ±30 and 0 degrees) located at eye level. This could be achieved through a combination of neck and trunk rotation until a head-mounted laser crosshair became aligned with the target 1 m away.

Electrical vestibular stimulation was delivered using carbon rubber electrodes (46x37mm) in a bipolar binaural configuration. Two electrodes were coated in conductive gel and secured to the mastoid processes using adhesive tape.

Stimuli were delivered from an isolated constant-current stimulator (model 2200; AM Systems, Carlsberg, WA, USA). Two types of electrical vestibular stimulation were used; Galvanic Vestibular Stimulation (GVS) and Stochastic Vestibular Stimulation (SVS). GVS was applied in sequences of twenty 1 s impulses of 1 mA, separated by a 4s gap. Positive values of current signify an anode-right configuration. Each SVS period consisted of a 100s stimulus. The stimulus waveform was generated by passing white noise through a low-pass filter (0-25 Hz; 6th order Butterworth) and then scaling to give an RMS value of 0.6 mA, and a peak amplitude of ±2 mA.

Each target angle (-60, -30, 0, +30 & +60 degrees) and stimulation condition (GVS & SVS) was performed separately with eyes open and closed, giving a total of 20 conditions. Trial order was randomised and participants were allowed seated rest in between trials.

Data Acquisition

Head orientation was sampled at 50 Hz in the form of Euler angles using a Fastrak sensor attached to welding helmet frame (Polhemus Inc, Colchester, Vermont, USA). Sensor yaw was used to calculate head direction (i.e. rotation about the vertical axis). Any offset in yaw or roll angle between head orientation and sensor orientation was measured using a second sensor attached to a stereotactic frame, and subsequently subtracted. A slight head up pitch position was maintained throughout each trial to ensure Reid's plane (line between inferior orbit and external auditory meatus) was horizontal, thus optimising the response

to the virtual signal of roll evoked by vestibular stimulation (Fitzpatrick & Day, 2004). The evoked sway response 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).

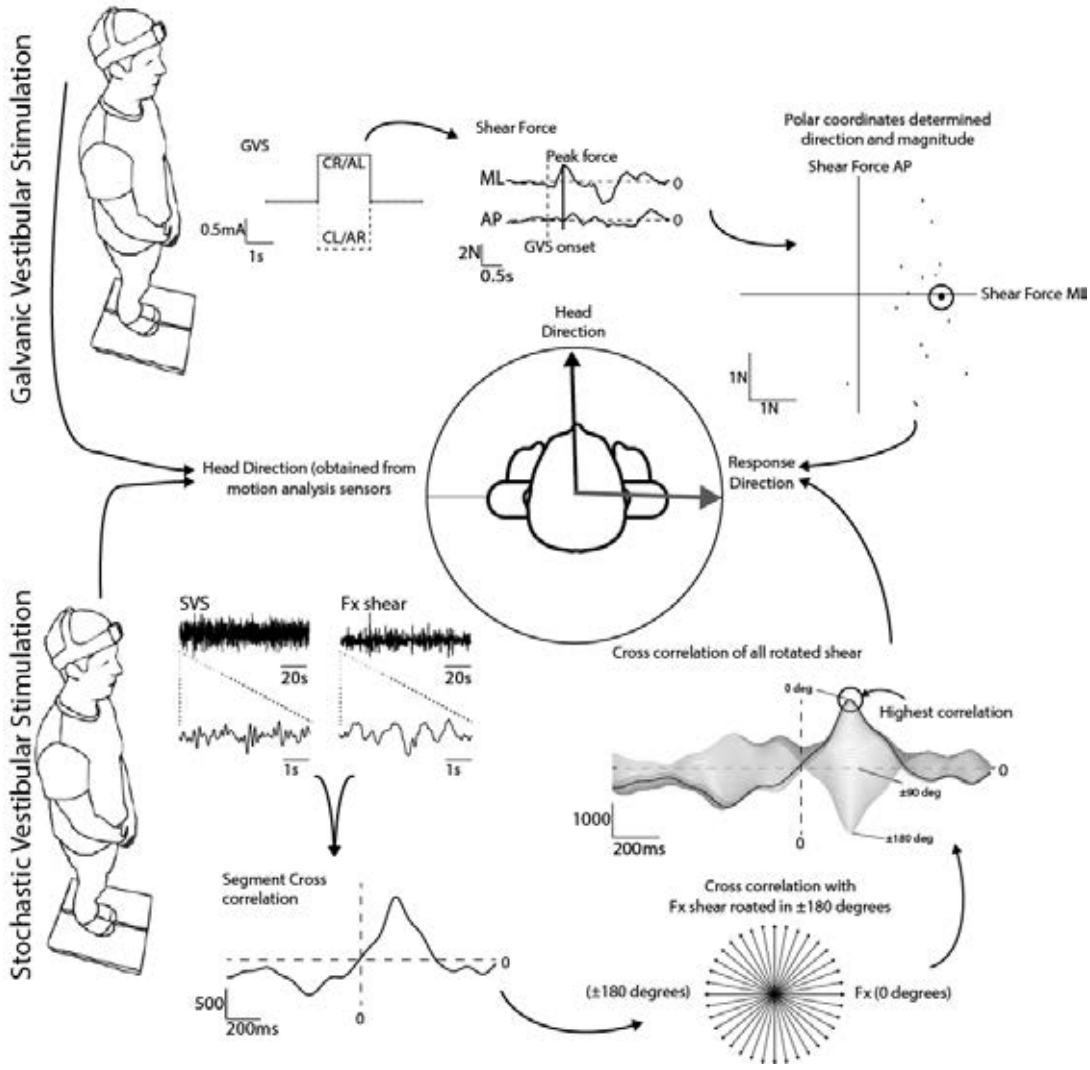


Figure 2.1. Analysis of EVS-evoked postural responses. (Top) GVS was delivered in a binaural bipolar configuration (1mA, 1 s), evoking a reflex sway response that was recorded via a force platform in the form of ground reaction forces. Anode-left data were inverted before combining with anode-right trials. The timing of the peak force vector was first calculated from the averaged forces. Individual trials were then analysed by measuring the direction of the force vector within 200ms of this time point. (Bottom) For SVS, SVS-force cross-correlations were calculated for force vectors directed along all angles of a circle. The largest cross-correlation determined response direction. A Polhemus motion tracker provided head orientation.

Data Analysis

GVS Analysis. Analysis of GVS-evoked shear force is depicted in the top half of Figure 2.1. For each trial, any offset at stimulus onset was first removed from both mediolateral (Fx) and anteroposterior (Fy) force. Prior to individual trial analysis, we first averaged Fx and Fy traces across all trials within each condition. The time of the peak average force vector was then measured, and a window of +/- 200ms either side of this time point was subsequently used to analyse each individual trial. The magnitude and direction (atan Fx/Fy) of the peak force vector within this time window was measured separately for all trials. This resulted in 20 individual trial directions for each condition, from which we could calculate the mean direction (i.e. accuracy) and its variance (i.e. precision) using circular statistics (see below). Response direction was referenced to head orientation, as measured by the Polhemus Fastrak.

After inverting anode-left trials, there was no significant effect of polarity upon response magnitude (M±STD; AL 1.65±1.01, AR 1.62±1.02, $T_{(89)}$=0.39, p=0.70) or direction ($F_{(1,178)}$=0.92, p>0.34). Hence, both polarities were combined.

SVS Analysis. Analysis of SVS-evoked shear force is depicted in the bottom half of Figure 2.1. We used a modified version of the technique described by Mian and Day (2009) whereby the cross-correlation between the SVS stimulus and shear force is calculated. The component of the force vector is first determined for each degree of a circle (±180) to produce 360 separate force traces, using the following formula:

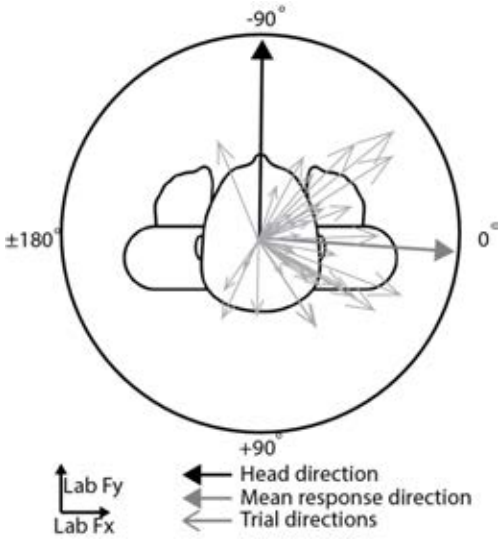
$$\mathrm{F}_{ROT\theta}(\mathrm{s}) = \mathrm{F}_X(\mathrm{s}) \cdot \cos\theta + \mathrm{F}_Y(\mathrm{s}) \cdot \sin\theta$$

The SVS-Force cross-correlation is then calculated for each trace, and the angle which results in the largest cross-correlation value is deemed to be the response direction. Initially we performed this analysis using the entire 100 s stimulation period. This was used to calculate the timing of the peak cross-correlation response. To study response variance, we then split the data into segments and performed the same analysis again, determining peak correlation values at the time point derived from the full 100s. We experimented with segments of differing lengths (1, 5, 10 & 20s) and settled upon 5s since it offered the greatest potential for detecting changes in variance between conditions (see figure 9 in results). As for the GVS analysis, response direction was referenced to head orientation.

To determine response magnitude for SVS data, we measured the peak of the SVS-Force cross-correlation (units in mA·N), and normalised this by dividing it by the peak of the SVS-SVS autocorrelation (units in mA^2). This resulted in a measure of gain that is independent of segment length (units in N mA^{-1}).

Circular Statistical Techniques

For both GVS and SVS, response direction is represented by angular data. Therefore circular statistical techniques were implemented using the CircStat toolbox for Matlab (Berens, 2009). Angular conventions are represented in figure 2, which depicts a representative subjects' responses to GVS during the head-forward/eyes open condition.



To calculate mean directions, individual angles (α_1, α_2 …. α_n) were first transformed to unit vectors in two dimensions (r_1, r_2 …. r_n) by demanding that the circle had a radius of 1. Thus, the magnitudes of the individual subject responses did not affect the analysis of 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:

$$\mathrm{r}_i = \begin{pmatrix} cos\alpha_i \\ sin\alpha_i \end{pmatrix}$$

Vectors (r_1, r_2, … r_n) were then averaged to calculate the mean resultant vector ($\bar{r}$):

$$\bar{r} = \frac{1}{N}\sum_i r_i$$

To compute the mean angular direction $\bar{\alpha}$, $\bar{r}$ is transformed using the four-quadrant inverse tangent function. Angular deviation was calculated as a measure of response variance, as it equivalent to the standard deviation in linear statistics (Batschelet, 1981) where R is the length of the mean resultant vector.

$$AD = \sqrt{-2(1-R)}$$

Statistical Analysis

A 2x5 repeated measures ANOVA (SPSS general linear model) was used to compare head-referenced sway direction, angular deviation and response magnitude across visual conditions and head orientations (Visual condition: eyes open, eyes closed. Head orientation: ±60, ±30, 0 degrees). In all cases, where significant Mauchly's tests indicated violation of the assumption of equal variances, the degrees of freedom were corrected using the GreenHouse-Geisser technique. Response accuracy was determined by a linear fit between response direction and head direction.

We also performed correlations between response direction and head orientation, and between response magnitude and variance. To do the latter, we determined response 'error' for each trial, measured as the angular difference between the individual trial direction and the mean direction. Pearson correlations were used to determine the significance of the direction-orientation and magnitude-error relationship for each condition for each participant (see Figure 2.8).

For all statistical tests, significance was set at $p<0.05$. Mean angle and angular deviation/standard deviation ($\bar{\alpha}$ ± AD (STD)) are reported in text and figures.

Results

Vestibular-evoked sway responses

Figure 2.3 depicts representative ground reaction force responses to vestibular stimulation in a subject standing with the head facing forwards. GVS evoked a polarity-specific response, predominantly in the mediolateral direction (Figure 2.3 A & B). SVS evoked a response in the same direction, as can be seen in the SVS-force cross-correlation (Figure 2.3 C & D). For both GVS & SVS, this subjects' responses were larger with the eyes closed.

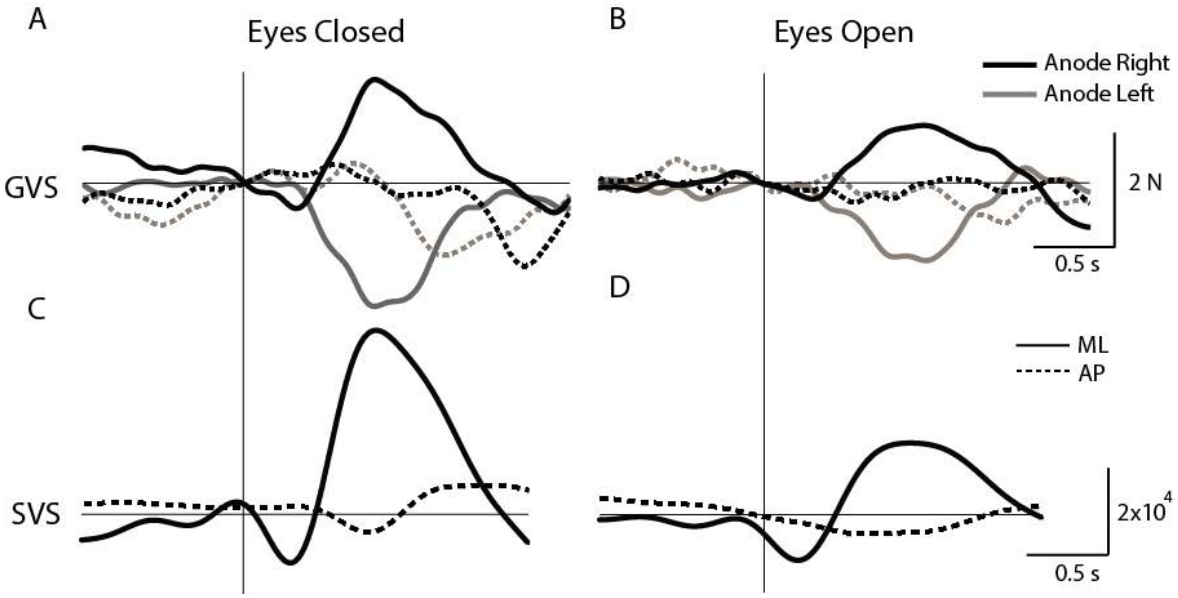


Figure 2.3. Representative EVS-evoked forces with the head forward. A & B show mean GVS-evoked ground reaction forces for a representative subject. Mediolateral and anterioposterior forces are depicted by solid and dashed traces, respectively. C & D show SVS-force cross-correlations for the same subject. Vertical lines depict time/lag zero for all traces. GVS stimuli started at time zero and lasted for 1s.

Assessing response direction

The effect of head orientation upon the direction of the evoked force vector is depicted in Figure 2.4. For all conditions, the mean force response (dashed line) is directed approximately 90 degrees to head orientation (solid line). As the head

is turned between +/-60 degrees, the force vector turns by a similar amount for both GVS and SVS stimuli. The direction of the mean force vector was used to determine response accuracy. In contrast, response precision was determined by analysing the within-subject variability of vector angles taken from individual trials/segments. This variability is depicted by the shaded areas in Figure 2.4 which show angular deviation (circular equivalent of the standard deviation). For SVS, each 100s stimulation period was split into twenty segments of 5s.

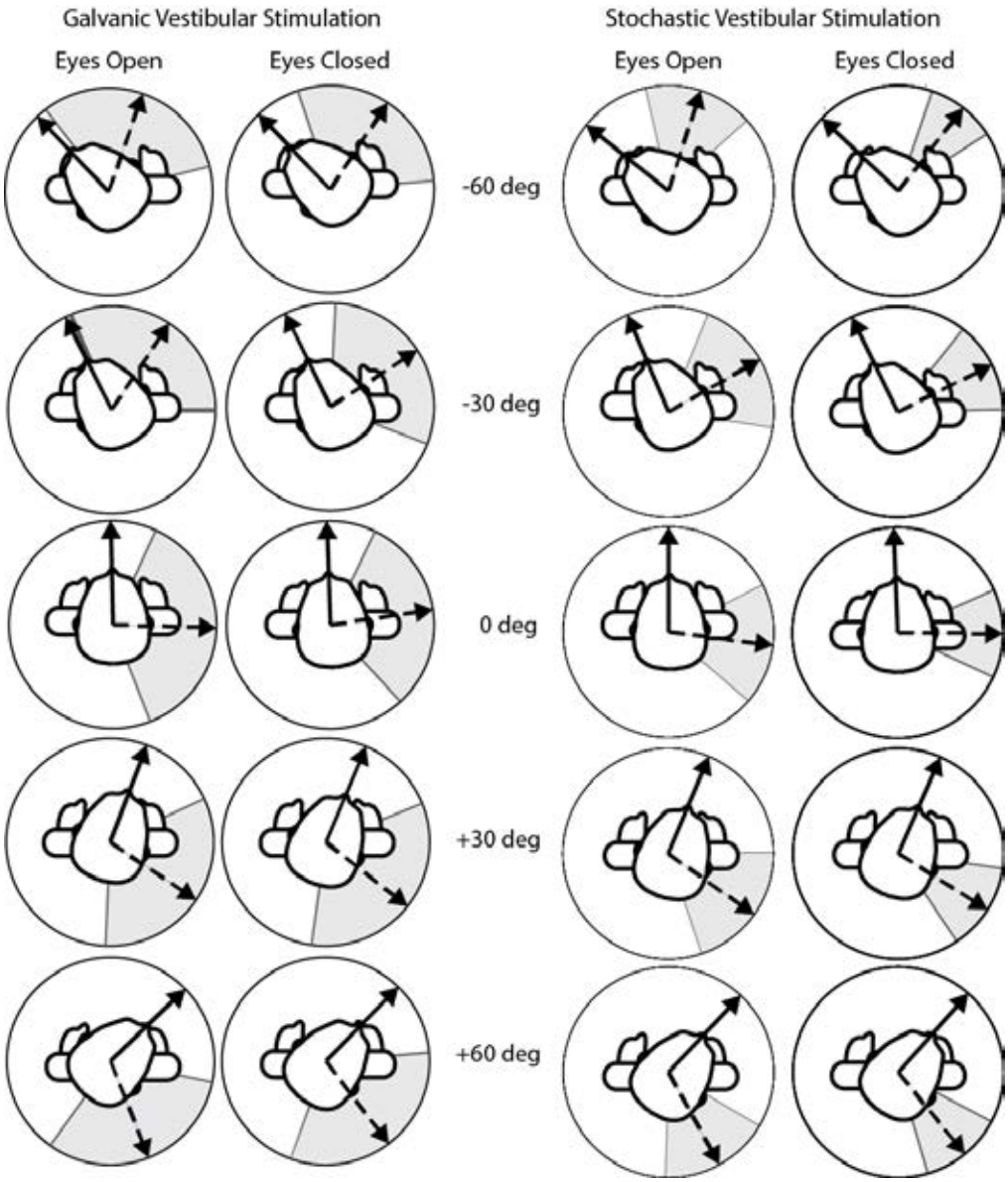


Figure 2.4. Mean and variance of evoked force vectors. Group mean force vectors are shown separately for GVS and SVS. Mean head orientation and evoked force directions are shown by the solid and dashed black arrows, respectively. This response rotated in line with head orientation. The average of the within-subject variability is represented by the grey shaded regions showing ± 1 angular deviation.

Response Accuracy

The effect of head orientation upon mean response direction is shown in further detail in Figure 2.5. GVS-evoked responses exhibited greater between-subject variability than those produced by SVS stimuli (GVS; STD=26.21. SVS; STD=13.56). Furthermore, 3 of 12 subjects showed no significant correlation between head orientation and response direction for GVS stimuli (Eyes closed; $R^2<0.56$. Eyes open; $R^2<0.48$ $p>0.05$). These subjects were removed from subsequent analysis and presentation of GVS responses (although their inclusion did not affect the outcome of any statistical analysis). In contrast, this relationship was significant for *all* subjects when using SVS stimuli (Eyes closed; $R^2>0.90$. Eyes open; $R^2>0.85$, $p<0.01$). One subject was removed due to a malfunctioning of the Polhemus Fastrak system used to record head orientation.

For both GVS and SVS there was a significant linear relationship between head orientation and response direction (GVS $R^2=0.88$ $p=0.03$. SVS $R^2=0.95$, $p<0.01$). However, there was no effect of vision upon this relationship (ANOVA main effect of vision: GVS, $F_{(1,8)}=2.80$, $p=0.13$. SVS; $F_{(1,10)}=0.61$, $p=0.45$. T-test on magnitude of regression slopes: GVS; $T_{(8)}=0.96$, $p=0.364$. SVS; $T_{(10)}=-2.206$, $p=0.07$). This confirms that vision had no influence upon response accuracy, as measured by the direction of the mean force vector.

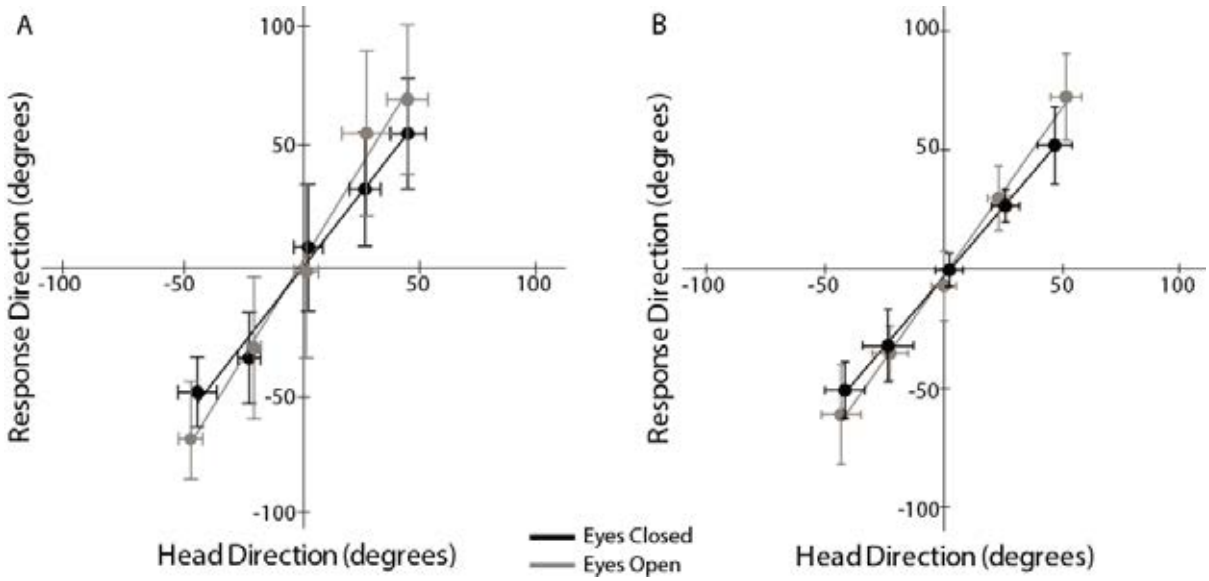
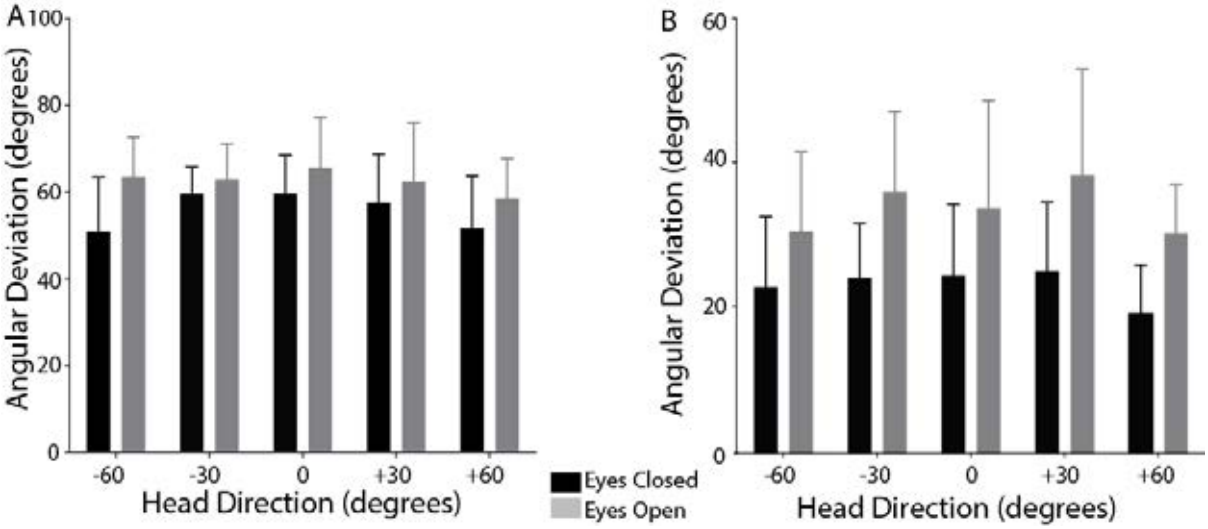


Figure 2.5. Response Accuracy. The effect of head orientation upon mean force vector direction is shown for GVS (A) and SVS (B). Error bars depict between-subject standard deviation.

Response Precision

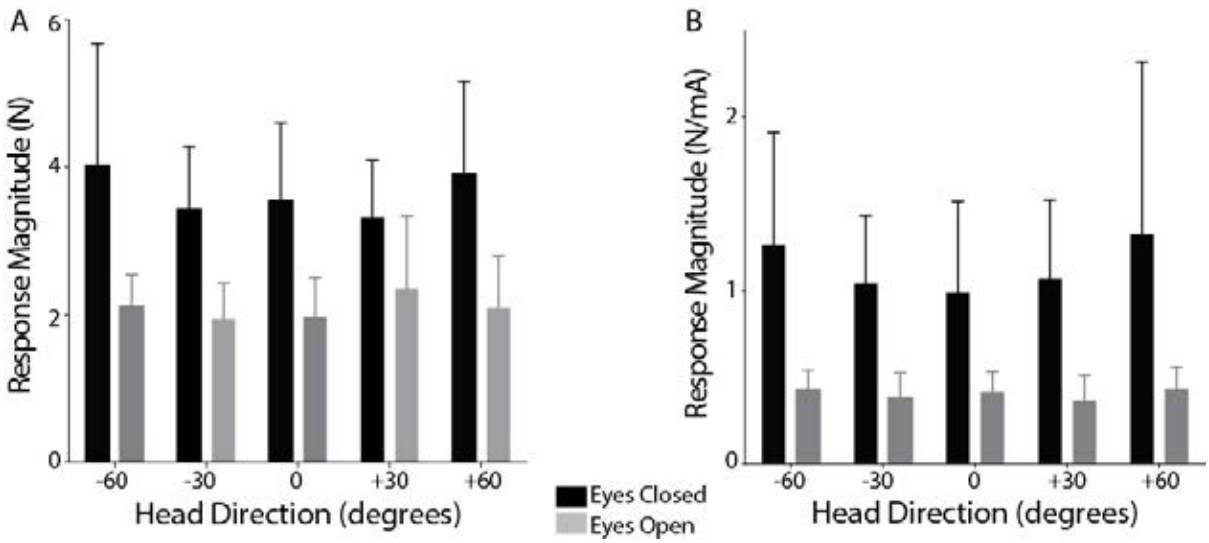
Individual trial/segment analysis was used to determine the variability of the evoked force vector (Figure 2.6). There was a significant increase in angular deviation with the eyes open, both for GVS (11% increase, all head orientations combined; $F_{(1,8)}$=15.16, p<0.01) and SVS (31% increase, all head orientations



combined; $F_{(1,10)}$=26.86, p<0.01), indicating that vision actually *reduced* precision. There was no main effect of head orientation or interaction between head orientation and vision (p>0.05).

Response Magnitude

For GVS and SVS stimuli, response magnitude was determined by the peak force and the stimulus-response gain, respectively (Figure 2.7). With the eyes closed, response magnitude was approximately doubled, both for GVS and SVS (GVS; $F_{(1,8)}$=65.74, p<0.01. SVS; $F_{(1,10)}$=30.32, p<0.01). There was no effect of head orientation upon response magnitude or interaction (p>0.05) (Figure 2.7B).



Relationship between precision and magnitude

To investigate the relationship between response precision and magnitude we calculated both the absolute error and the magnitude of each force vector for individual trials. Absolute error was calculated as the angular difference of

individual force vectors from the mean vector, for each condition (Figure 2.8A). There was a tendency for larger responses to exhibit lower error (Figure 2.8B). This relationship was more consistent for the SVS response, where 9 of 11 participants exhibited a significant inverse correlation between these parameters, for both eyes-open and eyes-closed conditions (Figure 2.8D). For GVS, 4 of 9 participants produced significant inverse correlation for both conditions (Figure 2.8C).

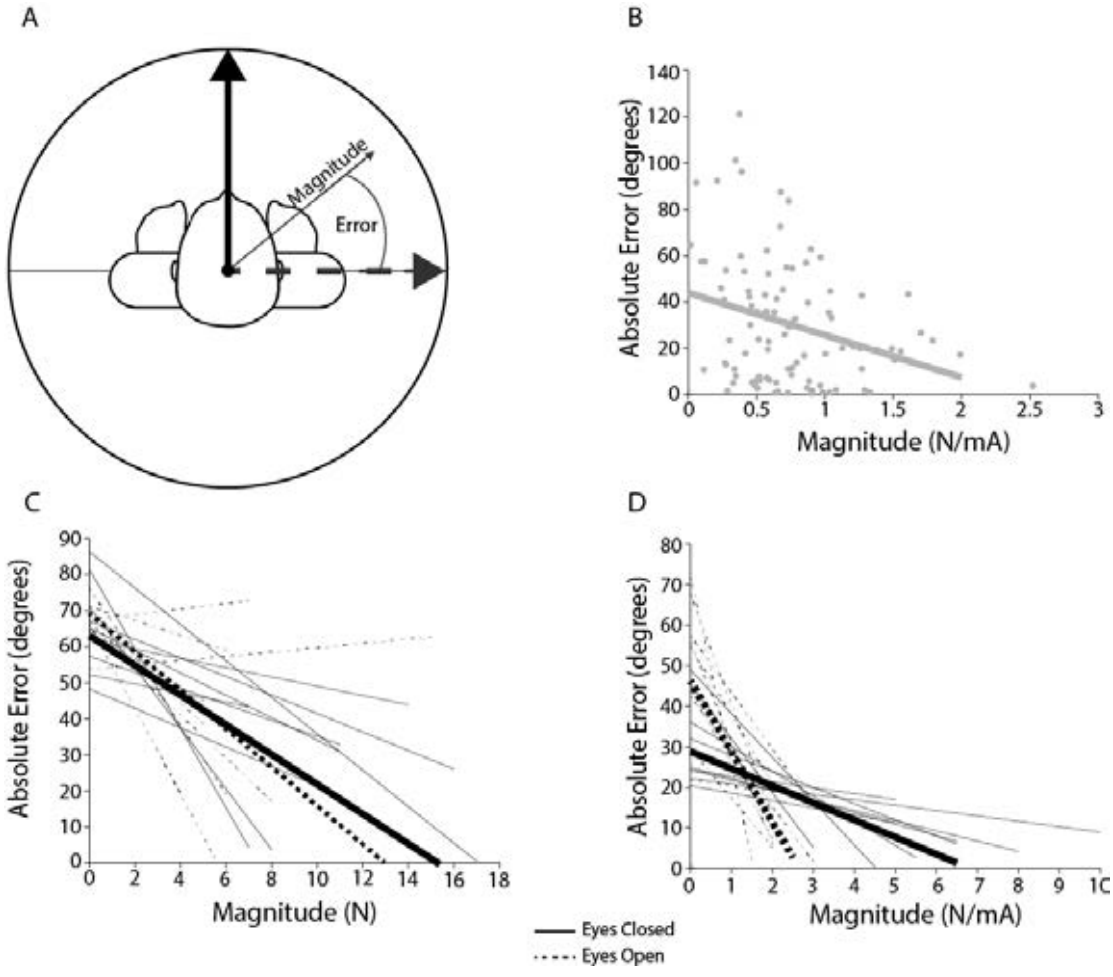
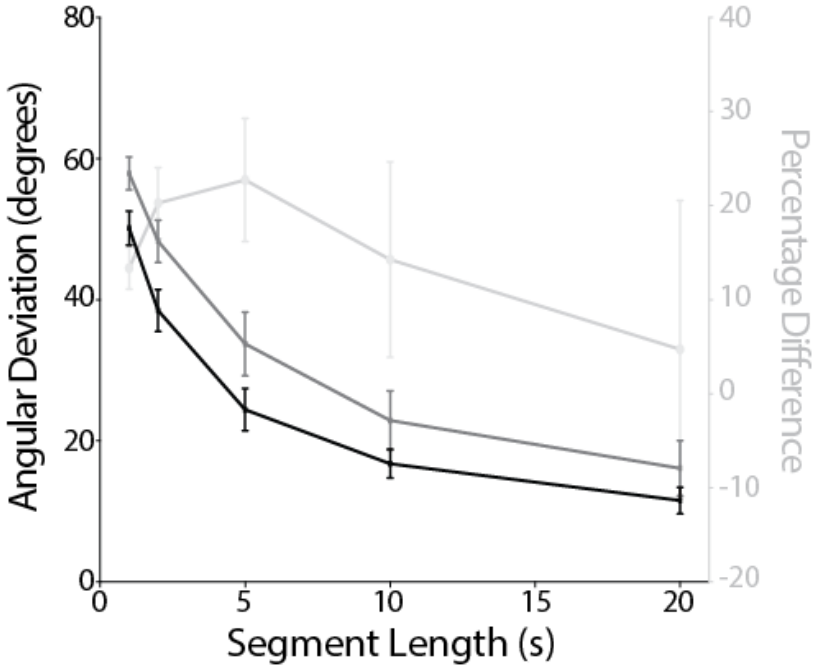


Figure 2.8. Relationship between response error and magnitude. A) The absolute error between individual trial direction (thin grey arrow) and the mean response direction (dashed arrow) was calculated. The corresponding magnitude of each force vector for each trial was also recorded. B) A representative participant's SVS data and linear fit for an eyes open condition. C) and D) show regression lines for all subjects for GVS and SVS, respectively. Mean slopes and intercepts are represented by the thick lines.

Effect of SVS segment length upon response precision

The analysis of SVS responses reported above was obtained by splitting each 100s stimulation period into twenty 5s segments. Figure 2.9 shows the effect of altering segment length upon directional variance for a forward facing orientation. Angular deviation systematically declines as segment length is increased. This may simply be due to the differing numbers of data samples produced by varying segment length. However, the values are consistently higher for the eyes-open condition ($F_{(4,44)}$=318, p<0.01). The largest percentage difference between visual conditions occurred for the 5s segment length (25% increase. M±STD Eyes closed: 24.08±9.53 °, Eyes Open 34.67±13.34 °).



Simulating changes in precision

The above results suggest that vision increases the variability of the vestibular-evoked balance response. However, there was an associated reduction in response magnitude with vision. It is therefore possible that change in variability is a direct consequence of this change in magnitude, rather than sensory reweighting for example (Figure 2.8). To address this possibility, we generated artificial GVS responses where we could systematically modify response magnitude and observe the effect upon angular deviation (Figure 2.10).

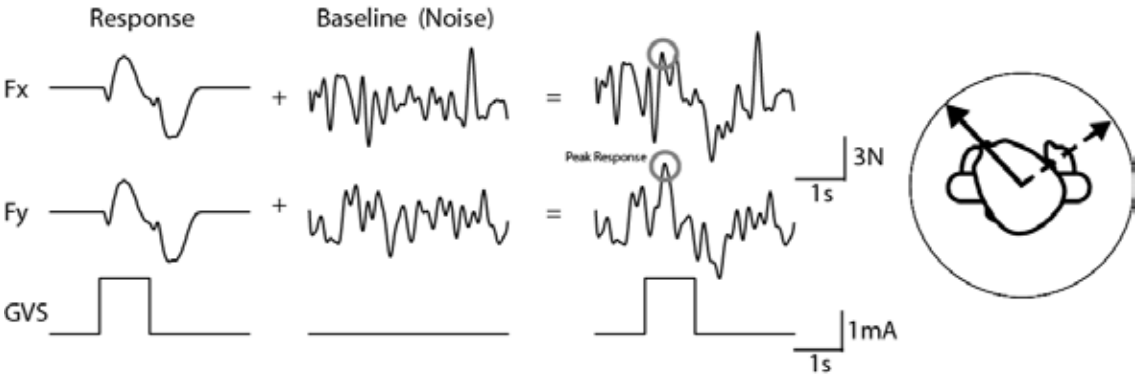
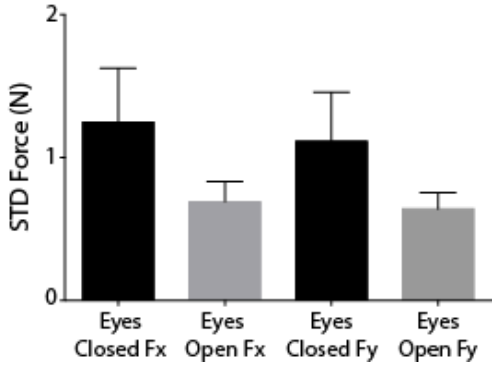


Figure 2.10. Simulating effects of response magnitude upon directional variance. A GVS-evoked force response was generated from averaged empirical data. This archetypal response was then summed with random noise to simulate baseline force variations. The Peak response was used to calculate the direction of the resulting force vector for multiple artificial trials, allowing angular deviation to be calculated. Response magnitude and baseline noise were then independently varied to determine the effect upon angular deviation.

Initial values of response magnitude and baseline noise were set to match the values observed empirically during the eyes-closed GVS condition. We then decreased response magnitude by 42% to replicate the effect of opening the eyes. This caused a 39% increase in angular deviation, suggesting that the change in variance is indeed directly linked to response magnitude. However,

this ignores variations in baseline force which might affect response variance. Analysis of the empirical data shows that baseline force variability decreases by 44% with the eyes open (Figure 2.11). When we simulated this change alone (maintaining a fixed response magnitude), it caused a 27% *decrease* in angular



deviation, opposing the effect of response magnitude.

When we simultaneously implemented the 42% decrease in response magnitude and the 44% increase in baseline force variability, the net effect was a 0.4% increase in response variability (Figure 2.12). This compares to the empirically observed change of 11%. Hence, our simulation suggests that the observed changes in precision are not purely due to changes in response magnitude or baseline variability per se.

Figure 2.11. Baseline force v

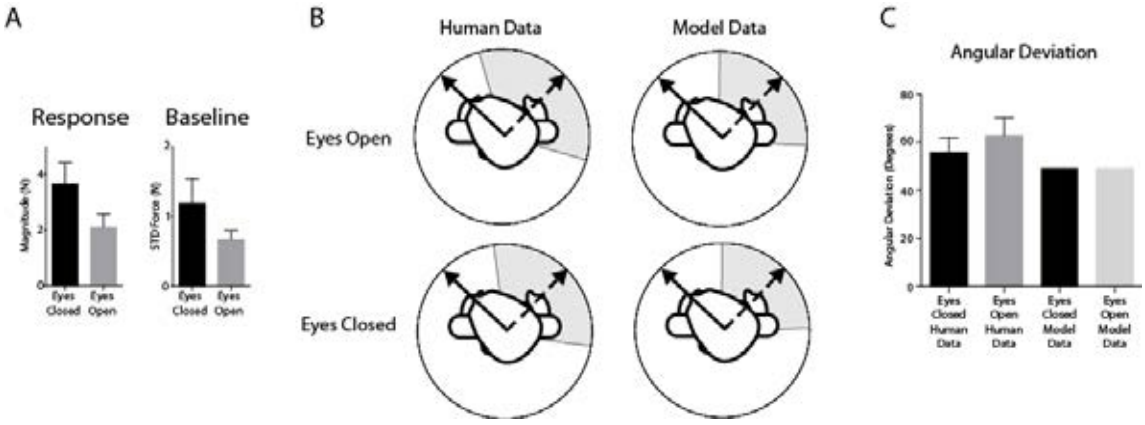


Figure 2.12. Comparison of empirical versus model data. A) The empirically observed effects of vision upon response and baseline force magnitude were simultaneously implemented in the simulation. B) Angular deviation was calculated for comparison against empirical data. C) There was minimal effect of these interventions upon the simulated angular deviation results. This contrasts with the 11% increase in angular deviation observed empirically when the eyes were opened.

Discussion

Our results confirm the craniocentric nature of the vestibular-evoked sway response (Hlavacka & Njiokiktjien, 1985; Lund & Broberg, 1983; Mian & Day, 2009; Pastor et al., 1993). EVS stimuli evoked a ground reaction force directed towards the anodal ear, rotating in line with head orientation. The novel aspect of our study was to analyse the variability of this response in addition to its mean direction. When subjects opened their eyes, mean sway direction was unaffected. However, response variability increased, reflecting a reduction in precision. This demonstrates that the accuracy and precision of vestibular-motor transformations for balance are dissociable. This raises the possibility that a person might exhibit poor balance control at any given instant, while appearing to sway accurately on average. The averaging process may therefore mask any deficits in vestibular control of balance.

We used two different methods of vestibular stimulation. The GVS stimulus consisted of a short-lasting square-wave pulse of direct current, allowing us to measure the direction of the vestibular response at a fixed instant in time. By measuring responses to multiple pulses, variability was readily ascertained. In contrast, SVS involved a continuous, long-lasting and randomly-varying current. To determine variability in this case, we quantified response direction over multiple segments of time ranging from 1 to 20s, using the cross-correlation method described by Mian & Day (2009). We settled upon a segment length of 5s, since it showed the clearest distinction between visual conditions. Despite the

difference in techniques, both GVS and SVS produced essentially the same result; vision had no influence upon the direction of the mean response, while variability *increased* with the eyes open. However, the practicality of both techniques differed. When using GVS, three of 12 subjects exhibited no clear relationship between head angle and response direction, and were thus excluded from further analysis. In contrast, this relationship was significant for *all* subjects when using SVS. Furthermore, the distinction between visual conditions was clearer in the SVS response, which exhibited a 31% increase in angular deviation with the eyes open, versus 11% for GVS. This is supported by previous work demonstrating greater signal-to-noise ratios for SVS-evoked sway responses (Dakin et al., 2007; Reynolds, 2011). Of course, such differences may be partly attributable to the chosen stimulus parameters (Dakin, Luu, van den Doel, Inglis, & Blouin, 2010). Varying the amplitude, number and frequency content of the stimulus current could conceivably alter angular deviation in ways we have not investigated here. Nevertheless, the qualitative similarity in results, regardless of the precise stimulus parameters, supports our assertion that vision increases the directional variability of the vestibular-evoked sway response.

The observed effect of vision refutes our original hypothesis. We had reasoned that the sense of head-on-feet orientation would improve with vision. This would enhance the coordinate transformation of vestibular input into motor output for balance (Dalton et al., 2017; Reynolds, 2017). In contrast to our prediction, however, directional variability *increased* with the eyes open. How could vision reduce the precision of vestibular control of balance in this way? The answer to

this apparent paradox may be sensory reweighting. We found that evoked force responses were ~50% smaller with the eyes open. This concurs with previous findings showing that GVS-evoked sway responses become smaller as additional veridical sensory information becomes available (Day, Guerraz, & Cole, 2002). This has been demonstrated for tactile (Britton et al., 1993; C. P. Smith, Allsop, Mistry, & Reynolds, 2017) and proprioceptive modalities (Day & Cole, 2002), as well as for vision (Day & Guerraz, 2007). The CNS must combine these sometimes divergent sources of information to compute a single estimate of the state of the body. This process has been likened to electoral proportional representation, with each sensory modality providing a vote towards the overall estimate of body orientation (Day et al., 2002). Hence, the relative contribution of any given modality will depend upon how much alternative sensory representation is available. The reduction in EVS-evoked sway size with vision may therefore reflect down-weighting of vestibular information. We also found a negative correlation between response magnitude and directional variability. We confirmed that this correlation was not due to inherent effects of noise in the forceplate sensors (data not shown). Instead, it suggests that reduced precision is a direct consequence of the down-weighting process. In other words, the CNS' estimate of sway direction at any given time is less influenced by vestibular input. Hence there will be a greater influence of veridical visual cues upon sway direction.

Alternatively, it is possible that the changes in precision we observed are not directly attributable to sensory reweighting. The reduction in response magnitude

could conceivably increase the variability of the sway force vectors via changes in signal-to-noise ratio. Specifically, a fixed level of random noise on the shear force signals (Fx and Fy) would evoke greater angular changes for a smaller versus larger force vector. In this case, altered precision would not be caused by sensory reweighting per se. However, the results of our simple model suggest that this is not the case (Figure 2.12). When we recreated the observed reduction in response magnitude, it did cause an increase in angular deviation. But when we simultaneously implemented the empirically observed reduction in baseline force variability, angular deviation stayed constant. This suggests that the effects of vision upon the precision of the vestibular-evoked postural response are not mediated purely by changes in signal-to-noise ratio.

It is important to emphasise that the reduced directional precision that we observed with the eyes open does not reflect impaired balance control overall. Quite the opposite; in the absence of vestibular stimulation, baseline sway was 44% lower with the eyes open. Nevertheless, the analysis that we report here does offer a new method for analysing the efficacy of vestibular control of balance. Any increase in response variability in the absence of any other changes would indeed reflect impaired transformation of vestibular input. Furthermore, as our data demonstrates, it is possible for such changes to occur even when mean response direction remains accurate. This may be important for revealing potential contributions of vestibulo-motor dysfunction towards increased fall risk, caused by age, sensory loss or neurological disease. Analysis of averaged responses may mask such deficits.

In summary, we observed a clear dissociation between the directional accuracy and precision of vestibular-evoked balance responses. The directional variability of the EVS-evoked sway response increased with the eyes open, while its mean direction was unaffected by vision. This paradoxical finding suggests that additional veridical sensory information leads to the down-weighting of vestibular input for balance, resulting in an apparently less precise response.

Additional Information

Competing interests

No conflicts of interest are declared by the authors.

Funding

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CHAPTER 3

EFFECTS OF PROLONGED INACTIVITY ON ELECTRICAL VESTIBULAR STIMULATION EVOKED POSTURAL RESPONSES

Highlights

- Balance control requires the transformation of vestibular signals from head to foot-centered coordinates.
- This transformation process can be studied by analyzing the accuracy and precision of electrical vestibular stimulation-evoked sway responses.
- Prolonged inactivity results in a decrease in muscular strength and volume and has been shown to reduce proprioceptive acuity, which is vital for balance control.
- We found that prolonged inactivity resulted in larger EVS-evoked sway responses which were less precise.
- The inactivity induced changes were seen to be returning to normal levels after 6 days.

Keywords: Bedrest, Vestibular-motor transformation, Accuracy, Precision

Abstract

Vestibular information has to be transformed from head-to-foot centered coordinates to produce appropriate responses balance control. This transformation process has previously been investigated using electrical vestibular stimulation (EVS), which evokes a craniocentric sway response. Investigating individual trial direction and magnitude has allowed for a measure of sway precision to be calculated. Prolonged inactivity has been shown to reduce muscular strength and volume. The weakening of the muscle causes a reduction in proprioceptive acuity due to the saturation of muscle spindles. Here we investigated the effects of 60 day bedrest on spontaneous sway and EVS-evoked sway response characteristics before and after 60 days bedrest. Standing volunteers adopted three head positions (±45 deg and 0 deg) while EVS-evoked response direction was determined from ground reaction forces. The effect of vision upon response precision, magnitude and accuracy were explored. Spontaneous sway was found to increase in both speed and sway area. This was more pronounced in an eyes closed condition. EVS-evoked sway responses were larger and less precise after 60 day bedrest. These changes were seen to be returning to pre bedrest levels after 6 days post bedrest. These observations suggest that a weakening of the muscles result in a higher percentage of muscular voluntary contraction (MVC) for any given contraction. This results in the saturation of muscle spindles and thus a reduction in proprioceptive acuity. Balance control utilizes many sensory inputs. If these inputs conflict (i.e. proprioception and vestibular signals do not indicate the same movement), it can

result in the down-weighting of vestibular contribution, ultimately manifesting as less precise response.

Abbreviations EVS, Electrical vestibular stimulation. SVS, Stochastic vestibular stimulation

Introduction

Human posture is inherently unstable and remaining upright is a very complex task requiring the integration of multiple senses, vision, vestibular and proprioception. If any of these senses deteriorate or are lost, it results in a decline in balance control.

Paulus et al. (1984) showed that a decline visual clarity or a reduction in the field of vision resulting in increased spontaneous sway. A complete loss vision would further destabilise the body. However, even when vision is absent , compensatory postural responses to postural perturbation remain accurate, and actually become more precise (Mackenzie & Reynolds, 2018a). Reductions in proprioceptive acuity have been shown to increase spontaneous sway levels (Butler et al., 2008) and complete proprioceptive loss, as is the case with patient *I.W*, makes standing impossible when visual information is removed (Day & Cole, 2002). Patients with vestibular loss do not exhibit major instability under normal conditions. However, when vision and proprioceptive information is unreliable or unavailable, these patients are unable to maintain balance (Nashner et al., 1982). Postural control may appear normal even when one system is impaired, however if we were to perturb one of remaining sensory inputs we may be able to investigate the effect of this sensory loss on the efficacy of balance control.

The role each sense plays in balance control can be investigated using Electrical Vestibular Stimulation (EVS) (Bent, McFadyen, & Inglis, 2002; R. Fitzpatrick et

al., 1994; Lund & Broberg, 1983; Welgampola & Colebatch, 2001). EVS modulates the firing rate of vestibular afferents, inducing a false sensation of body sway towards the cathode electrode. This evokes a compensatory sway response that drives the body towards the anodal electrode. However, the vestibular system is fixed in the skull and therefore any response is locked in head coordinates and must be transformed into body coordinates to be used for balance control (Fitzpatrick & Day, 2004; Hlavacka & Njiokiktjien, 1985; Lund & Broberg, 1983). This craniocentric nature of the EVS response has previous been shown using EVS (Dakin et al., 2007; Lund & Broberg, 1983; Mackenzie & Reynolds, 2018a; Mian & Day, 2009).

Both vision and proprioception's effect on the magnitude of the EVS response has been widely explored (Bent et al., 2002; Fitzpatrick et al., 1994; Welgampola & Colebatch, 2001). When vision is removed, the sway response is nearly doubled. Patient IW, who suffered from large-fibre sensory neuropathy, had complete loss of cutaneous and proprioceptive sensation below the neck. When tested with EVS he produced responses which were an order of magnitude larger than healthy controls, thus emphasising the role proprioceptive information played in balance control (Day & Cole, 2002). Although response magnitude has previous been examined, response accuracy and response precision has received less attention even though these characteristics could be more important under ethological circumstances (i.e. our average response has little importance if a on a single occasion we are not precise with our response) (Mackenzie & Reynolds, 2018a).

It is well known that prolonged inactivity is detrimental to cardiovascular, respiratory health and muscular volume (Krasnoff & Painter, 1999; Thijssen et al., 2010). However, very little is known about the effects of inactivity on the systems involved in balance control. Prolonged inactivity can occur upon hospital admission, especially in the elderly who fall (Lord, Sherrington, Menz, & Close, 2007). It is therefore important to understand the effects inactivity has on balance control. If as hypothesized, that prolonged inactivity has a detrimental effect on balance, upon discharged, fall risk may be temporarily increased. Previous research investigating prolonged inactivity and balance control has involved measuring spontaneous sway before and after space flight (Fregly, 1974; Homick & Miller, 1975; Homick & Reschke, 1977; Kenyon & Young, 1986; Young et al., 1986). However, inactivity under microgravity cannot be compared to inactivity with gravity. Although both unload the lower limb, microgravity has been shown to alter the firing rates of the vestibular afferents in frogs (Gualtierotti, 1987) increasing the sensitivity of the semicircular canals (Kozlovskaya et al., 1989). Thus, any changes in postural control cannot be examined by inactivity alone and could be due to adaption of the CNS to new otolith firing rates.

Here we address this gap in the literature by measuring the effect prolonged inactivity has on spontaneous sway and the EVS-evoked sway response by examining the accuracy, precision and magnitude of this response. This will be investigated with and without visual information. We ask three related questions. Firstly, can we reproduce the dissociable nature of response accuracy and precision as previous seen (Mackenzie & Reynolds, 2018a). Secondly, how does

prolong inactivity affect the characteristics of the EVS-evoked sway response? We hypothesise that response accuracy will remain unchanged and precision will decrease (more variable). Thirdly, do any changes due to prolonged inactivity persist up to 6 days post bedrest? We hypothesise that any increases in response variability will be returning to pre bedrest levels after 6 days post bedrest. Our rational for this predication is an extrapolation from spaceflight measurements of sway which show a return to baseline after approximately 9 days post spaceflight (Homick & Miller, 1975; Paloski, Reschke, Black, Doxey, & Harm, 1992).

Our results showed that vision did paradoxically increase variability (less precise) while having no effect upon accuracy. This is in keeping with previous research (Mackenzie & Reynolds, 2018a). Prolonged inactivity had a profound effect upon balance control. Spontaneous sway speed and sway area were significantly increased and EVS-evoked sway responses, were larger and more variable. As with vision, response accuracy was unaffected by prolonged inactivity, but response precision declined. We did see a tendency for all changes due to prolonged inactivity to be returning to normal, 6 days after inactivity ceased. We discuss this finding in the context of multisensory integration and balance control.

Methods

Participants

18 male participants aged 20-45 years (mean±SD; 34±9 years) with no known neurological or vestibular disorders gave informed written consent to participate. Participants were non smokers, no alcohol or drug dependencies and were receiving no current medical treatment. Two participants were removed due to poor adhesion to bedrest protocols and the inability to perform protocol post bedrest. The experiment was approved in association with Medes (Institute for space medicine and physiology) part of the European Space Agencies funded project.

Protocol

Participants stood upon a force plate, unshod, with feet 4cm apart (instep to instep) and the hands held relaxed in front of them for the duration of each 40 second stimulation period. Prior to each trial subjects were instructed to face one of three visual targets (±45 and 0 degrees) located at eye level, at a distance of 1m. Verbal instruction from the experimenters guided their head to the correct orientation.

Stochastic vestibular stimulation (SVS) was delivered using carbon rubber electrodes (46x37mm) in a bipolar binaural configuration. Two electrodes were coated in conductive gel and secured to the mastoid processes using adhesive tape. Stimuli were delivered from an isolated constant-current stimulator (DS5,

Digitimer Ltd., Welwyn Garden City, Herts, UK). Each SVS period consisted of a continuous 40s stimulus. The stimulus waveform was generated by passing white noise through a low-pass filter (0-25 Hz; 6th order Butterworth) and then scaling to give an RMS value of 0.6 mA, and a peak amplitude of ±2 mA.

Near Infrared Spectroscopy (NIRS) was used to monitor real-time regional cerebral oxygen saturation (rSO_2) (INVOS 5100c Cerebral Oximeter, Somanetics Corp, Troy, MI, USA) for safety purposes (indication of fainting). Two surface electrodes were applied to the forehead of the participants using adhesive tape. The two sensors measure the ratio of oxyhaemoglobin to total haemoglobin, with the resulting percentage equal to the value for rSO2. A value of 50% or a 20% reduction from baseline are cause for concern and the aborting of a session (Edmonds, Ganzel, & Austin, 2004; Hongo, Kobayashi, Okudera, Hokama, & Nakagawa, 1995). No participant exhibited such reductions and therefore no trials were stopped and no data has been presented.

Each target angle (-45, +45 and 0 degrees) was performed separately with eye open and closed during SVS stimulation. Spontaneous sway (no stimulation) trials were performed in a forward-facing orientation (0 degrees) with eyes open and closed. Trial order was pseudorandomised and participants were allowed seated rest between trials. The protocol was performed at three time points; pre bedrest (Pre), one day post bedrest (Post 1) and 6 days post bedrest (Post 2).

Intervention

Participants were prescribed 60 days of bedrest in a 6 degrees head down orientation. The head-down bedrest configuration causes a cephalic fluid shift and the restriction to the bed replicates immobilization of space travel. Following bedrest procedures, at least one shoulder had to be in contact with the bed at all time and no torso flexion or exercise was allowed. Participants were monitored throughout the intervention following normal bedrest protocols to ensure the health of all participants. Upon the immediate end of bedrest (Post 0), participants were required to remain out of bed for 7hr/day, although this time could be seated.

Data Acquisition

Head orientation was sampled at 50 Hz in the form of Euler angles using a Fastrak sensor attached to welding helmet frame (Polhemus Inc, Colchester, Vermont, USA). Sensor yaw was used to calculate head direction (i.e. rotation about the vertical axis). Any offset in yaw or roll angle between head orientation and sensor orientation was measured using a second sensor attached to a stereotactic frame. This offset was subsequently subtracted. The evoked sway response to vestibular stimulation 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).

Data Analysis

Spontaneous Sway Analysis. Analysis of spontaneous sway trials were performed in a forward-facing orientation with either the eyes closed or open. Centre of pressure (CoP) displacement in both mediolateral and anteroposterior

directions during 40 seconds of spontaneous sway was used to calculate centre of pressure velocity,

$$\frac{\sum_{i=1}^{k-1}|dir(i+1)-dir(i)|}{\Delta \mathrm{k}}$$

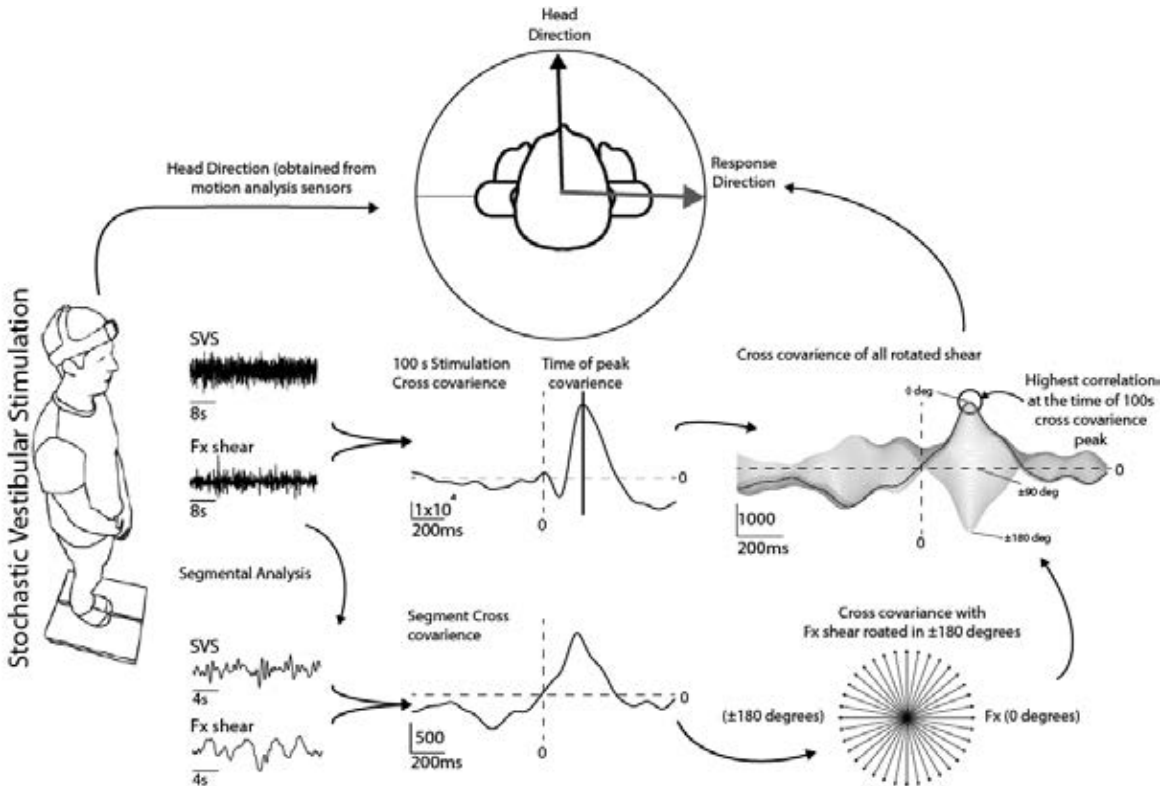
where *k* is trial duration and *dir* is either ML or AP CoP. An ellipse was fitted to CoP path, from which sway area could be determined.

SVS Analysis. Analsyis of SVS-evoked shear force is depicted in Figure 3.1. We used a modified version of the technique described by Mian and Day (2009) whereby the cross-correlation between the SVS stimulus and shear force is calculated. The component of the force vector is first determined for each degree of a circle (±180) to produce 360 separate force traces, using the following formula:

$$\mathrm{F}_{ROT\theta}(\mathrm{s}) = \mathrm{F}_X(\mathrm{s}) \cdot \cos\theta + \mathrm{F}_Y(\mathrm{s}) \cdot \sin\theta$$

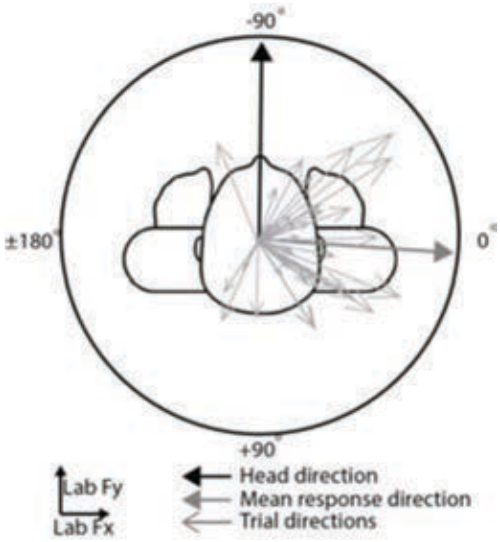
The SVS-Force cross-correlation is then calculated for each trace, and the angle which results in the largest cross-correlation value is deemed to be the response direction. Initially we performed this analysis using the each of the five 40 s stimulation periods. This was used to calculate the timing of the peak cross-correlation response. To study response variance, we then split the data into segments and performed the same analysis again, determining peak correlation values at the time point derived from the full 40s. We experimented with segments of differing lengths (1, 2, 5, 10, 20 & 40s) and settled upon 20s since it offered the greatest potential for detecting changes in variance between conditions (see Figure 3.10 in results). Response direction was referenced to head orientation.

To determine response magnitude for SVS data, we measured the peak of the SVS-Force cross-correlation (units in mA·N), and normalised this by dividing it by the peak of the SVS-SVS autocorrelation (units in mA^2). This resulted in a measure of gain that is independent of segment length (units in N mA^{-1}).



Circular Statistical Techniques

As response direction corresponds to angular data, circular statistical techniques were implemented using the CircStat toolbox for Matlab (Berens, 2009). Angular conventions are represented in Figure 3.2, which depicts a representative subjects' responses to SVS during a pre bedrest head forward/eyes open condition.



To calculate mean directions, individual angles (α_1, α_2 …. α_n) were first transformed to unit vectors in two dimensions (r_1, r_2 …. r_n) by demanding that the circle had a radius of 1. Thus, the magnitudes of the individual subject responses did not affect the analysis of 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:

$$\mathrm{r}_i = \left(\frac{\cos \alpha_i}{\sin \alpha_i}\right)$$

Vectors (r_1, r_2, … r_n) were then averaged to calculate the mean resultant vector $\bar{r}$:

$$\bar{r} = \frac{1}{N}\sum_i r_i$$

To compute the mean angular direction $\bar{\alpha}$, $\bar{r}$ is transformed using the four-quadrant inverse tangent function. Angular deviation was calculated as a measure of response variance, as it equivalent to the standard deviation in linear statistics (Batschelet, 1981) where R is the length of the mean resultant vector.

$$AD = \sqrt{-2(1-R)}$$

Statistical Analysis

A 2x3x3 repeated measures ANOVA (SPSS general linear model) was used to compare centre of pressure, sway area, angular deviation and response magnitude between visual conditions (eyes open and eyes closed), orientation (±45 and 0 degrees) and time points (Pre, Post 1 and Post 2). 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. Response accuracy was determined by a linear fit between response direction and head direction.

We also performed correlations between response direction and head orientation. Pearson correlations were used to determine the significance of the direction-orientation relationship for each condition for each participant.

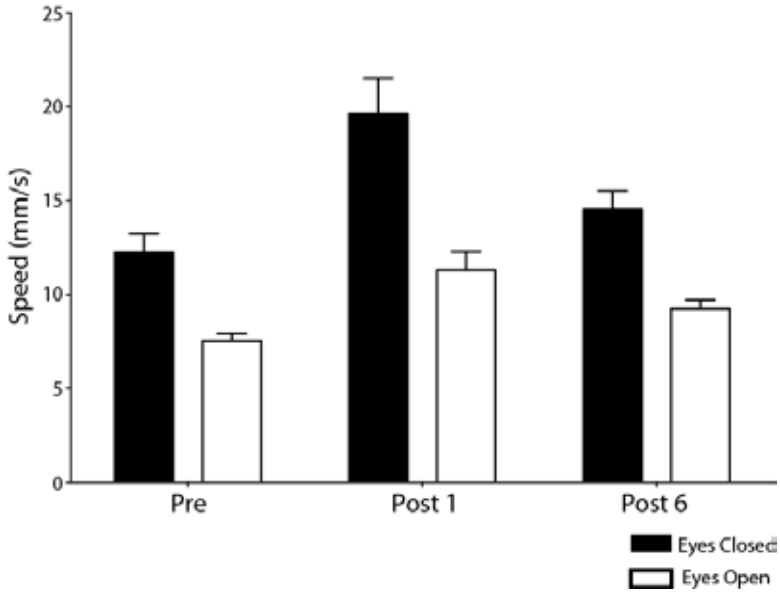
For all statistical tests, significance was set at $p<0.05$. Mean angle and angular deviation/standard deviation ($\bar{\alpha} \pm$ AD (STD)) are reported in text and mean and standard error of the mean ($\bar{\alpha} \pm$ SEM) in figures.

Results

Spontaneous Sway

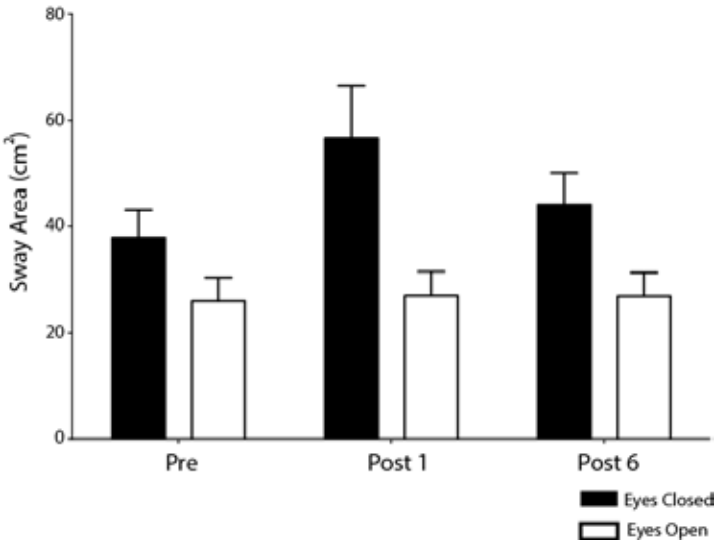
Centre of Pressure Speed

The effect of vision and bedrest on spontaneous sway speed derived from centre of pressure is depicted in Figure 3.3. There was a significant increase in speed with the eyes closed ($F_{(1,17)}$=74.37, p<0.01). There was a significant main effect of time, where speed increased following bedrest but appeared to be returning to pre bedrest speeds after 6 days ($F_{(2,34)}$=19.37, p<0.01). There was also a significant interaction between time point and vision ($F_{(2,34)}$=32.52, p<0.01). Post hoc comparisons showed a significant difference between visual coindition at all time points (p<0.01) as well as significant difference between each time point under both visual conditions (p<0.05).



Sway Area

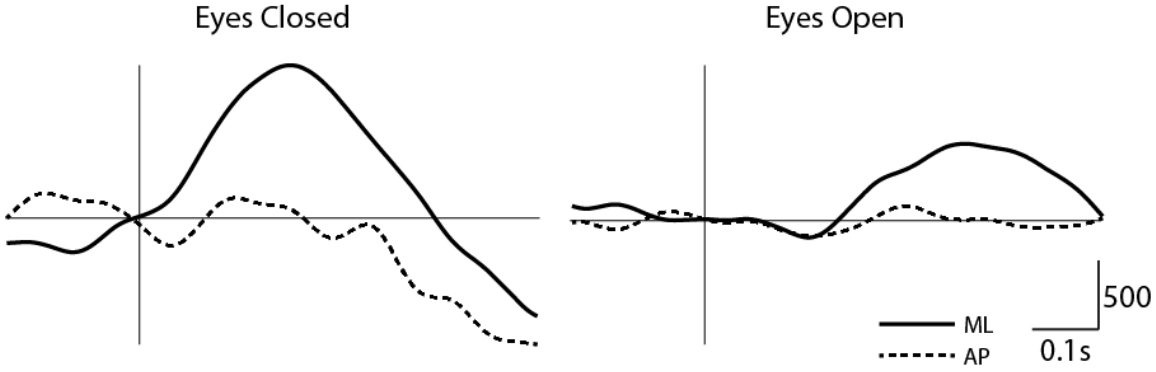
The effect of vision and bedrest on spontaneous sway area is depicted in Figure 3.4. Participants swayed over a significantly larger area with the eyes closed ($F_{(1,17)}$=41.98, $p<0.01$). Immediately following bedrest, sway areas significantly increase with the eyes closed and returned to pre bedrest levels after 6 days. With the eyes open sway area remained unchanged ($F_{(2,34)}$=6.45, $p<0.05$). There was a significant interaction ($F_{(1,34)}$=6.45, $p<0.05$). Post hoc comparisons showed no significant difference between time points for both visual conditions ($p>0.05$), but there was a significant difference between visual conditions at all time points ($p<0.05$)



EVS response

Vestibular-evoked sway responses

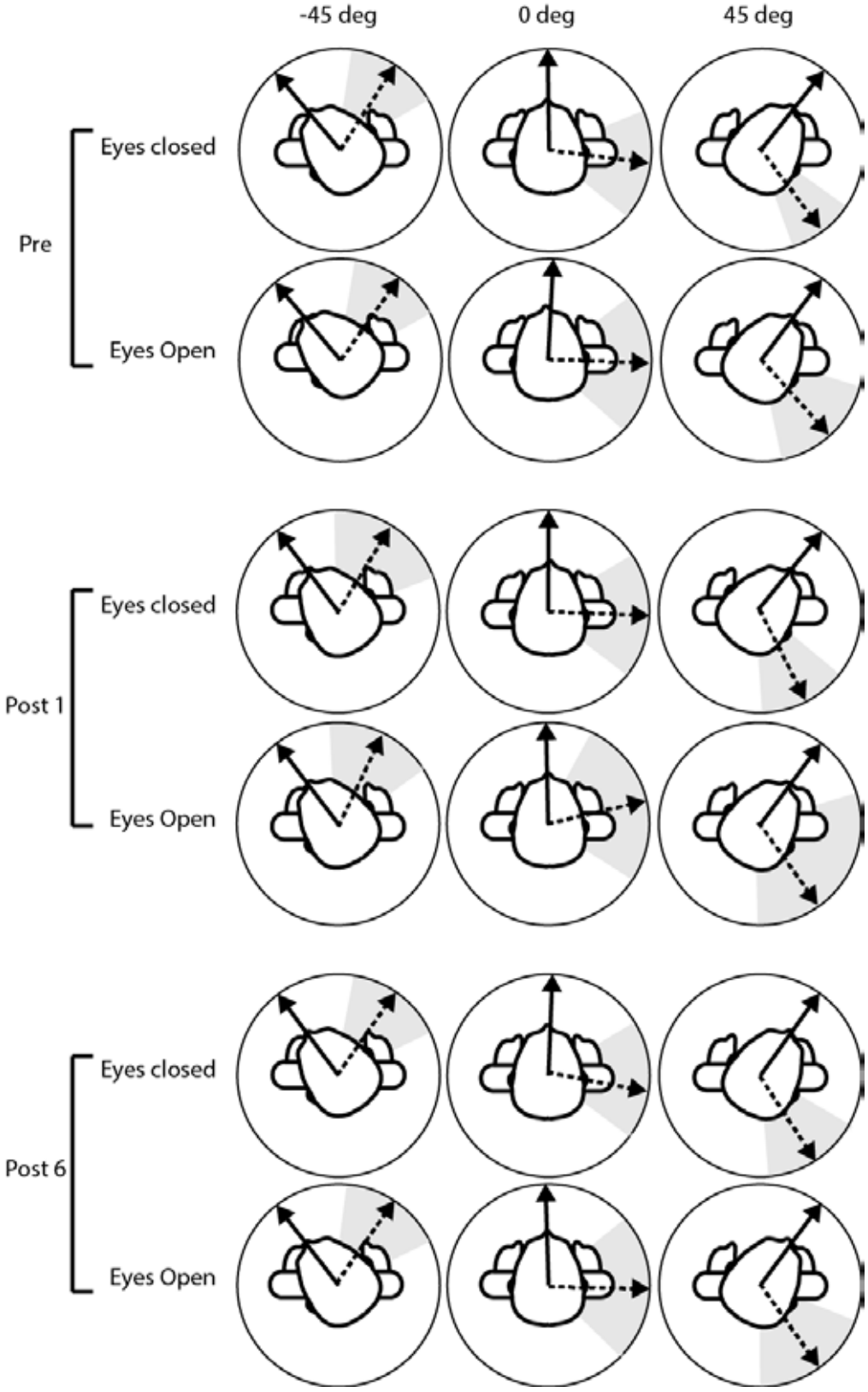
Figure 3.5A depicts representative ground reaction force responses to vestibular stimulation in a subject standing with the head facing forwards. The evoked response was predominately in the mediolateral direction, as can be seen in the SVS-force cross-correlation (Figure 3.5A & B). This subjects' response was larger with the eyes closed.



Assessing response direction

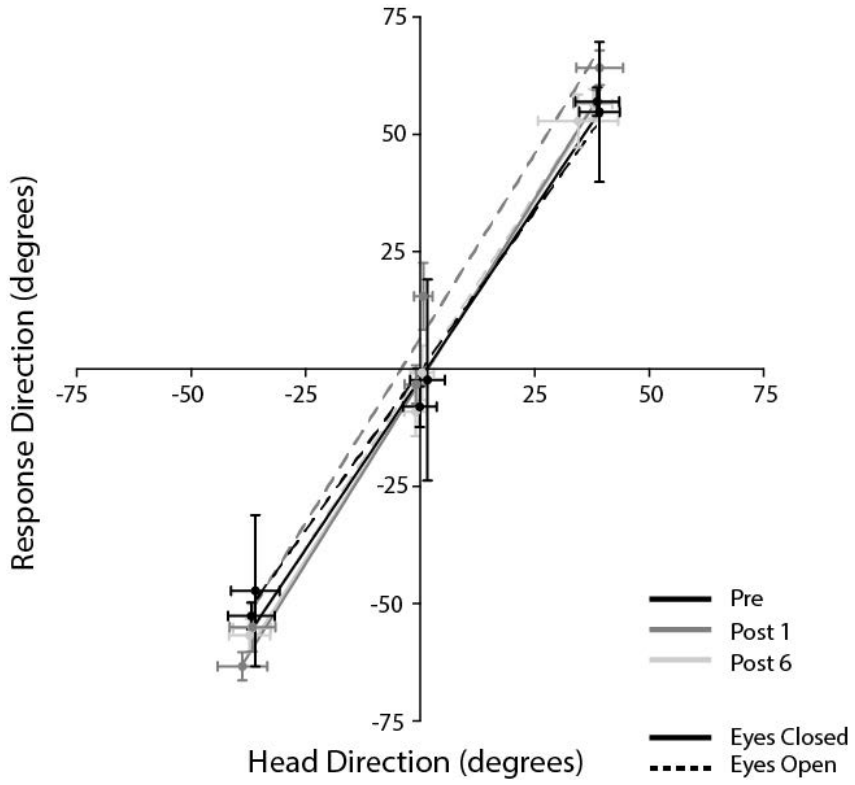
The effect of head orientation upon the direction of the evoked force vector is depicted in Figure 3.6. For all conditions, the mean force response (dashed line) is directed approximately 90 degrees to head orientation (solid line). As the head is turned between ±45 degrees, the force vector also turns by a similar amount. The direction of the mean force vector was used to determine response accuracy. In contrast, response precision was determined by analysing the within-subject variability of the vector angles taken from individual segments. This variability is depicted by the shaded area in Figure 3.6, which show angular deviation (circular equivalent of standard deviation).

Figure 3.5 Representative EV


-45 deg
0 deg
45 deg
Pre
Eyes closed
Eyes Open
Post 1
Eyes closed
Eyes Open
Post 6
Eyes closed
Eyes Open

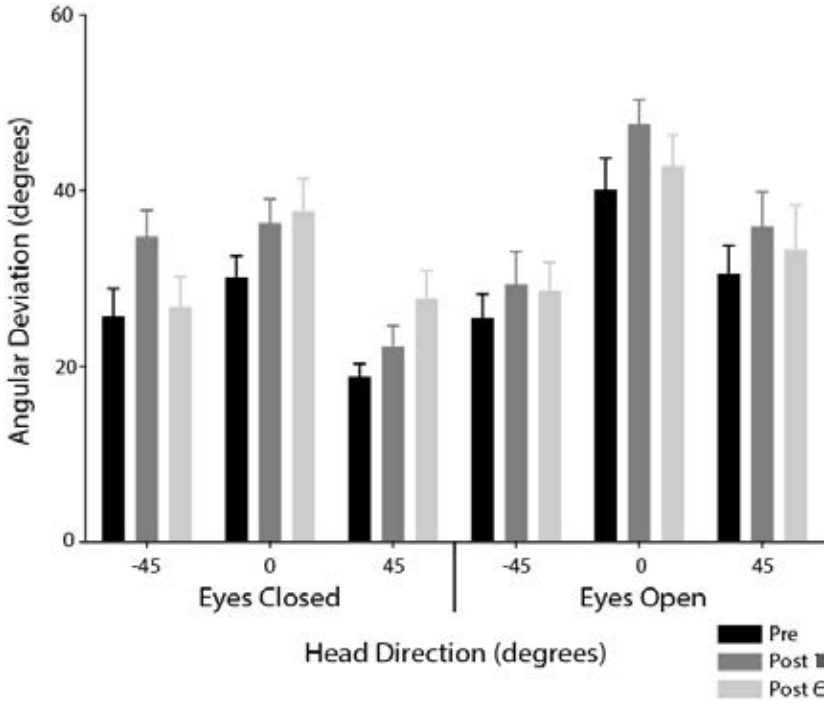
Response Accuracy

The effect of head orientation and bedrest upon mean response direction is depicted in Figure 3.7. There was significant linear relationship between head orientation and response direction (Pre: Closed R^2=0.98, Open R^2=0.99. Post1; Closed R^2=0.99, Open R^2=0.97. Post6; Closed R^2=0.99, Open R^2=0.99). However, there was no effect of vision upon this relationship T-Test on magnitude of regression slopes($T_{(54)}$=0.72, p>0.05), confirming vision had no influence upon response accuracy, as measured by the direction of the mean force vector. This craniocentric response was still present after bedrest ($F_{(2,34)}$=2.995,p>0.05).



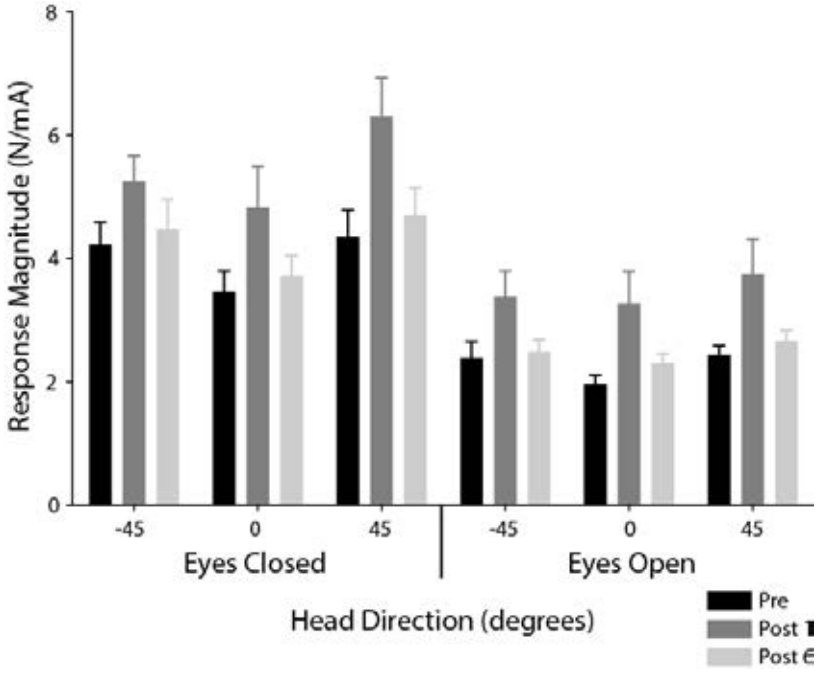
Response Precision

Individual segment analysis was used to determine the variability of the evoked force vector is depicted in Figure 3.8. There was a significant increase in angular deviation with the eyes open ($F_{(1,17)}$=35.41, p<0.01), indicating that vision *reduced* response precision. There was main effect of head orientation ($F_{(2,34)}$=16.351, p<0.01) where a forward facing orientation produced a less precise response. There was a significant increase in angular deviation after bedrest which had a tendency to be returning to pre bedrest levels after 6 days ($F_{(2,34)}$=4.63, p<0.05). A significant vision-orientation interaction showed that responses were significantly more precise with eyes closed when the head was orientated towards 0 or 45 degrees. When the eyes were closed a head orientation of 0 was significantly less precise than -45 and 45 head orientations. When the eyes were open a -45 head orientation was significantly more precise than 0 degree.



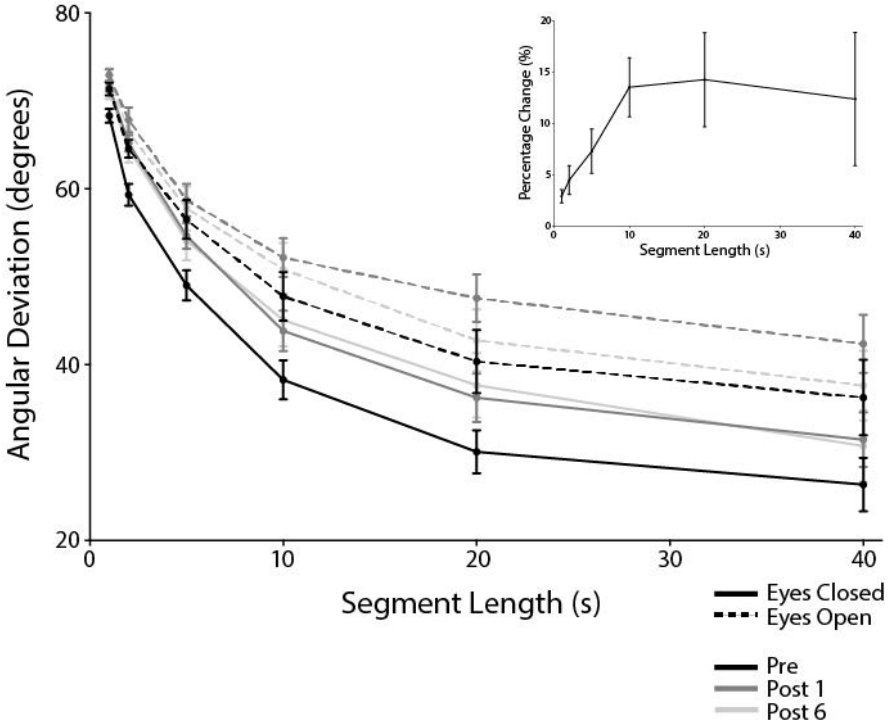
Response Magnitude

Response magnitude was determined by the stimulus-response gain is depicted in Figure 3.9. With the eyes closed, response magnitude was approximately doubled ($F_{(1,17)}$=69.19, p<0.01). Similar to response precision there was a significantly effect of head orientation upon response magnitude ($F_{(2,34)}$=20.97, p<0.01), where larger responses were produced when the head was not in forward-facing orientation. Post bedrest response magnitudes were significantly larger and appeared to be returning to pre bedrest magnitude after 6 days ($F_{(2,34)}$=7.59, p<0.05). A significant vision-orientation effect showed that responses were larger with the eyes closed for all head orientations (p<0.05). When the eyes were closed a head orientation of 0 degrees produced significantly smaller responses than a -45 and 45 orientation (p<0.05). An eyes closed condition produced larger responses during all head orientations. A 45 degree orientation produced the largest responses when the eyes were open.



Effect of SVS segment length upon response precision.

The analysis of SVS responses reported above was obtained by splitting each of the five 40s stimulation periods into two 20s segments. Figure 3.10 shows the effect of altering segment length upon directional variance for a forward facing orientation. Angular deviation systematically declines as segment length is increased ($F_{(6,85)}$=298.42, p<0.01). This may simply be due to the differing numbers of data samples produced by varying segment length. However, the values were significantly higher for the eyes-open condition ($F_{(1,17)}$=20.71, p<0.01). Post bedrest angular deviation were significantly greater than pre and post 6 values ($F_{(2,34)}$=3.32, p<0.05).The largest percentage difference between visual conditions occurred for the 20s segment length, depicted in the insert in Figure 3.10 (14% increase. M±STD Pre:17.30±21.42°, Post 1: 22.13±24.37°, Post 6: 3.26±41.70°).



Discussion

Our results confirm that vision decreases spontaneous sway (Edwards, 1946; Paulus et al., 1984). When visual information is available we see a reduction in sway speed and sway area in both mediolateral and anterioposterior directions. Vision can be used to detect mediolateral sway via the so-called 'efferent movement detection' derived from eye movements (Paulus et al., 1984). Anteroposterior sway can be detected by changes in disparity and target size (Regan & Beverley, 1979). Spontaneous sway was seen to be directionless under both visual conditions. The novel aspect of our study was to examine the effect of prolonged inactivity, achieved via 60 days bedrest, had on spontaneous sway. Immediately after bedrest we see an increase in spontaneous sway speed with the eyes open and closed. However, sway area was only increased under an eyes closed condition. As proprioceptive control of balance is believed to deteriorate after prolonged inactivity, it would suggest that when the eyes are open, any deficit can be compensated for with the use of visual information. All changes due to prolonged inactivity was returning or had returned to pre bedrest levels by 6 days post bedrest.

We used 40s stochastic vestibular stimulation to evoke a postural response directed towards the anodal ear, rotating in line with head orientation. Our results further confirm the craniocentric nature of this response (Hlavacka & Njiokiktjien, 1985; Lund & Broberg, 1983; Mian & Day, 2009). We used the analytical techniques developed in chapter two to examine the accuracy and precision of

the response. Once again, we quantified response direction over multiple segments of time ranging from 1 s to 40 s. We found a 20 s segment length provided the clearest distinction between visual conditions. We found vision had no influence upon response accuracy, as seen in chapter one. Responses are larger when the eyes closed (Smetanin, Popov, & Shlykov, 1990). However, vision increases the variability of the evoked response (i.e. 14% less precise, pre bedrest). This paradoxical finding can be explained by sensory reweighting. Additional veridical sensory information has been shown to reduce the magnitude of vestibular-evoked response (Britton et al., 1993; Day & Guerraz, 2007; Day et al., 2002). The CNS must combine all sources of information to compute a single estimate of the state of the body by weighting each sense. The reduction in response magnitude and increase in angular deviation could be a consequence of the down-weighting of vestibular information.

As previously stated, the novel aspect of this study lies in examining the effects of prolonged inactivity on postural control. Prolonged inactivity was achieved via 60 day bedrest, during which time participants unloaded the lower limbs and spine. This has previously been linked to a loss of muscle strength and volume. Bedrest had no influence upon response accuracy. This means the mean response direction does not change. Response magnitude, on the other hand, increased immediately after bedrest (Post1), as did response variability. What causes these increases after prolonged inactivity? The answer could be that the reduction muscular strength due to bedrest impairs the proprioceptive control of balance (Butler et al., 2008). Proprioception provides information about body

movement and position (Clark, Burgess, & Chapin, 1986; Clark, Burgess, Chapin, & Lipscomb, 1985; McCloskey, 1973), both of which rely on muscle receptors. Proprioceptive sensitivity and muscle strength are closely related. Small muscular contractions (10% maximal voluntary contraction) improve proprioceptive acuity (Taylor & McCloskey, 1992) and a greater sense of movement is seen during active versus passive stance (Fitzpatrick & McCloskey, 1994). However, with increasing contraction levels (>20% maximal voluntary contraction) proprioceptive acuity reduces (Proske, Wise, & Gregory, 2000). A potential cause for this phenomenon is that during higher contraction levels muscle spindle afferents may saturate (Butler et al., 2008). Therefore, after bedrest our relatively weaker subjects (compared to pre bedrest) have increased muscular contraction levels in order to remain upright. These higher contractions levels would then result in the saturation of muscle spindle impairing proprioceptive acuity, ultimately manifesting as a less precise response direction.

Space travel not only causes physiological changes due to inactivity, there are also other physiological changes due to microgravity, such as increased vestibular afferent firing rates (Gualtierotti, 1987), it also involves long periods of inactivity of the lower limbs. Previous studies have found markedly improved balance control immediately upon return to normal gravity and activity levels, with further increases until approximately 7 days post space flight, when normal function is seen (Homick & Miller, 1975). Our results show a similar trend with many deficits due to bedrest returning partially or fully to pre bedrest levels.

In summary, we observed an increase in the directional variability of the EVS-evoked response with the eyes open, while mean direction was unaffected by vision. This finding suggest that additional veridical sensory information leads to the down weighting of vestibular input for balance resulting in a less precise response. Prolonged inactivity further reduces the precision of the EVS-evoked response. This may be due a loss in strength which starts a cascade of events leading to the saturation of muscle spindles and impaired proprioceptive acuity.

Additional Information

Competing interests

No conflicts of interest are declared by the authors.

Funding

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CHAPTER 4

OCULAR TORSION RESPONSES TO SINUSOIDAL ELECTRICAL VESTIBULAR STIMULATION

Highlights

- We measured ocular torsion responses to sinusoidal Electrical Vestibular Stimulation
- Responses were observed at all frequencies from 0.05 to 20Hz.
- Gain and phase analysis suggest the stimulus is interpreted by the CNS as velocity.
- Our non-invasive method assesses torsional VOR at frequencies impossible with natural stimuli.

Keywords; Ocular torsion, electrical vestibular stimulation, vestibulo-ocular reflex

Abstract

Modulation of vestibular afferent firing rates via electrical vestibular stimulation (EVS) applied to the mastoid processes is interpreted by the brain as a signal of roll. This activates the vestibular-ocular reflex (VOR) evoking a torsional eye movement. Previous methods for measuring torsion eye movements have been invasive and time consuming which are poorly suited to a clinical setting. Here we develop a non-invasive method to measure EVS-evoked torsional ocular responses. Participants received sinusoidal EVS of varying frequencies (0.05, 0.1, 0.2 0.5, 1,2,4,6,8,10,20 Hz) while eye kinematics were recorded using an infrared camera. Ocular torsion responses were observed at all frequencies. The positional gain of the response decreased with increasing stimulus frequency, whereas velocity gain showed a linear increase. Position, velocity and acceleration phases were examined and found that stimulus-velocity response was closest to zero phase lag. Both gain and phase are consistent with EVS-evoked changes in vestibular afferent firing rate being interpreted by the brain as a torsional velocity signal. The stimulation and techniques we describe here offer potential for clinical diagnostic use. To assess the function of each ear individually would simply require a monaural stimulus.

Abbreviations; VOR, vestibulo-ocular reflex. EVS, electrical vestibular stimulation.

Introduction

Electrical vestibular stimulation (EVS) involves currents applied to the mastoid processes. This modulates activity in the vestibular nerve and, when applied in a binaural bipolar configuration, the brain interprets the signal primarily as head roll motion (Fitzpatrick & Day, 2004). This evokes a compensatory whole-body sway response when standing (Lund & Broberg, 1983; Pastor et al., 1993). It also activates the vestibular-ocular reflex (VOR), predominantly in the torsional plane (Hitzig, 1871; Schneider, Glasauer, & Dieterich, 2000; Schneider et al., 2002; Watson, Brizuela, et al., 1998; Zink et al., 1998; Zink et al., 1997). Although some researchers have suggested that the torsional VOR is largely vestigial in humans (Miller, 1962), ocular recordings during natural vestibular stimulation produce eye/head velocity gain values approaching 1 (Peterka, 1992). This is similar to VOR gain in the yaw and pitch axes, suggesting a functional role for the torsional VOR in maintaining gaze. The EVS-evoked eye movement provides a window into this functional reflex.

Clinical studies have shown that EVS has potential as a vestibular diagnostic (Aw et al., 1996; Aw et al., 1995; Aw, Todd, et al., 2013; MacDougall et al., 2005; Welgampola et al., 2013). When applied in a monaural configuration (with a reference electrode distant from the ears), diminished EVS-evoked ocular responses have been demonstrated in the affected ears of patients with a variety of vestibular disorders. This includes unilateral and bilateral dysfunction, canal occlusion, vestibular neuritis, canal hypoplasia and vestibular schwannoma (Aw

et al., 1996; MacDougall et al., 2005). As described above, the primary ocular response to EVS is torsion. This is more challenging to track than lateral or vertical eye movement, which rely upon pupil translation from video recordings (Karlberg, McGarvie, Magnusson, Aw, & Halmagyi, 2000; Quarck, Etard, Normand, Pottier, & Denise, 1998). Previous research has often employed invasive techniques such as scleral coils (Severac Cauquil et al., 2003), or directly marking the sclera with surgical pen to facilitate video tracking (Jahn, Naessl, Strupp, et al., 2003). These techniques are impractical for a routine clinical test of vestibular function. One aim of the current study is to develop a simple, reliable, affordable and non-invasive method for measuring the ocular torsion response to EVS.

In addition to developing a practical method for measuring EVS-evoked ocular torsion, we seek a better understanding of how EVS is interpreted by the brain. As described above, it is well established that the primary EVS sensation is one of head roll motion (Reynolds & Osler, 2012). But whether this motion is position, velocity or acceleration is less well understood. Body orienting responses when stepping on the spot suggest that EVS evokes a sensation of acceleration (St George, Day, & Fitzpatrick, 2011). On the other hand, motion perception when seated in a rotating chair suggests a signal somewhere between position and velocity, depending upon the stimulus frequency (Peters, Rasman, Inglis, & Blouin, 2015). Continuous ocular torsional rotation in response to constant-current GVS suggests a velocity signal, rather than a static position signal (Severac Cauquil et al., 2003). Therefore, our secondary aim is to establish the

kinematic nature of the EVS signal in healthy subjects. Clarifying this issue in healthy participants will aid interpretation of pathological responses.

So, our first aim is to develop a practical recording technique for EVS-evoked eye movement, and our second is to understand the brain's interpretation of the EVS stimulus. To address both aims we applied sinusoidal EVS to healthy volunteers using a binaural bipolar electrode configuration. Eye movements were then tracked off-line using commercially available software (Mocha ©;see Osborne & Lakie (2011)). The use of sinusoidal stimuli at multiple frequencies offers two advantages. Firstly, it allows us to validate the tracking technique, since slow-phase eye movement responses should be observed only at the same frequency as the stimulus. Secondly, analysing stimulus-response gain and phase at different frequencies provides insight into how the brain interprets the EVS signal.

Materials and Methods

Participants

9 male participants aged 20-40 years (mean±SD; 24±6years), with no known neurological or vestibular disorder gave informed written consent to participate. The experiment was approved by the local ethical review committee at the University of Birmingham, and was performed in accordance with the Declaration of Helsinki.

Protocol

Participants were seated with the head restrained (SR Research Ltd. Ontario, Canada) for the duration of each 10 s stimulation period (Figure 4.1). Prior to each trial participants were instructed to focus on the lens of an infrared camera and not to blink before being immersed into darkness. An invisible infrared light (940nm) was used to illuminate the right eye during each trial. No fixation light was provided to ensure that any horizontal and vertical eye movements were not suppressed.

Sinusoidal EVS of varying frequencies (0.05, 0.1, 0.2, 0.5, 1, 2, 4, 6, 8, 10, 20 Hz) were delivered using carbon rubber electrodes (46x37mm) in a bipolar binaural configuration. Two electrodes were coated in conductive gel and secured to the mastoid processes using adhesive tape. Stimuli were delivered from an isolated constant-current stimulator (model 2200; AM Systems,

Carlsberg, WA, USA). Positive values of current signify an anode-right configuration. Current amplitude was ±5 mA.

Each stimulus frequency each was repeated three time giving a total of 33 trials. Trial order was randomised and participants were allowed to rest in between trials.

Data Acquisition

EVS-evoked horizontal (x), vertical (y) and torsional (z) eye movements were sampled at 50Hz using an infrared camera (Grasshopper 3, Point Grey research Inc, Richmond, BC, Canada) from the right eye. Eye movements were tracked off-line using commercially available planar tracking software (Mocha Pro V5, Imagineer Systems Ltd. Guildford, UK). Horizontal and vertical movements were tracked by measuring pupil position. Torsional motion was tracked using iris striations. By using sinusoidal stimuli at various fixed frequencies and observing the response at those frequencies, this allowed us to validate the tracking technique (e.g. Figure 4.3). Mocha V5 has previously quantified changes in muscle fibre length from ultra sound images which are of similar complexity and quality to our iris recordings (Osborne & Lakie, 2011).

Data Analysis

Analysis of the EVS-evoked ocular response is depicted in Figure 4.1. For each trial x, y and z components were quantified in degrees of rotation. Position signals were then differentiated twice to give acceleration signals, from which nystagmus'

could be detected. The nystagmus was removed using an inverted nystagmus algorithm. Briefly, the algorithm detects the presence of a nystagmus within the position signal, generates an equal but inverted artificial compensatory nystagmus which is then added to the position signal.

The magnitude of the eye position response was measured as the peak value of the stimulus-response cross-correlation, using the Matlab XCORR function (units in mA·deg). To normalise this value with respect to the input stimulus, it was divided by the peak of the stimulus autocorrelation (units in mA^2). This resulted in a measure of response gain which was independent of trial length (units in deg mA^{-1}). The lag of the peak cross correlation was then converted to phase in degrees as follows; Phase (degrees) = 360 x frequency (Hz) x lag(s). In addition to measuring the gain and phase of the eye position response, we performed the same analysis for velocity and acceleration. This was done in order to determine if the EVS signal was closest to position, velocity or acceleration at the various stimulus frequencies. However, instead of differentiating eye position twice to obtain a noisy measure of eye velocity and acceleration, for the phase analysis we integrated the EVS stimulus waveform twice, producing a cleaner waveform.

Statistical Analysis

A 1x3 repeated-measures ANOVA (SPSS general linear model) was used to compare response gain between the three axes of eye movement (horizontal (x), vertical (y), torsional (z)). All subsequent analysis was restricted to torsion, since this was the only axis in which eye movements were reliably present. A 3x11

repeated-measures ANOVA compared gain and phase across measures of response (position, velocity & acceleration) and stimulus frequency (0.05, 0.1, 0.2, 0.5, 1, 2, 4, 6, 8, 10, 20 Hz). Following significant interactions, 1x11 repeated-measures ANOVAs were used to investigate effects of frequency separately for position, velocity and acceleration. In all cases, where significant Mauchly's tests indicated violation of the assumption of equal variances, the GreenHouse-Geisser correction was employed. For all statistical tests, significance was set at $p<0.05$. Means and standard deviations are presented in text while means and standard errors of the mean are presented in figures, unless otherwise stated.

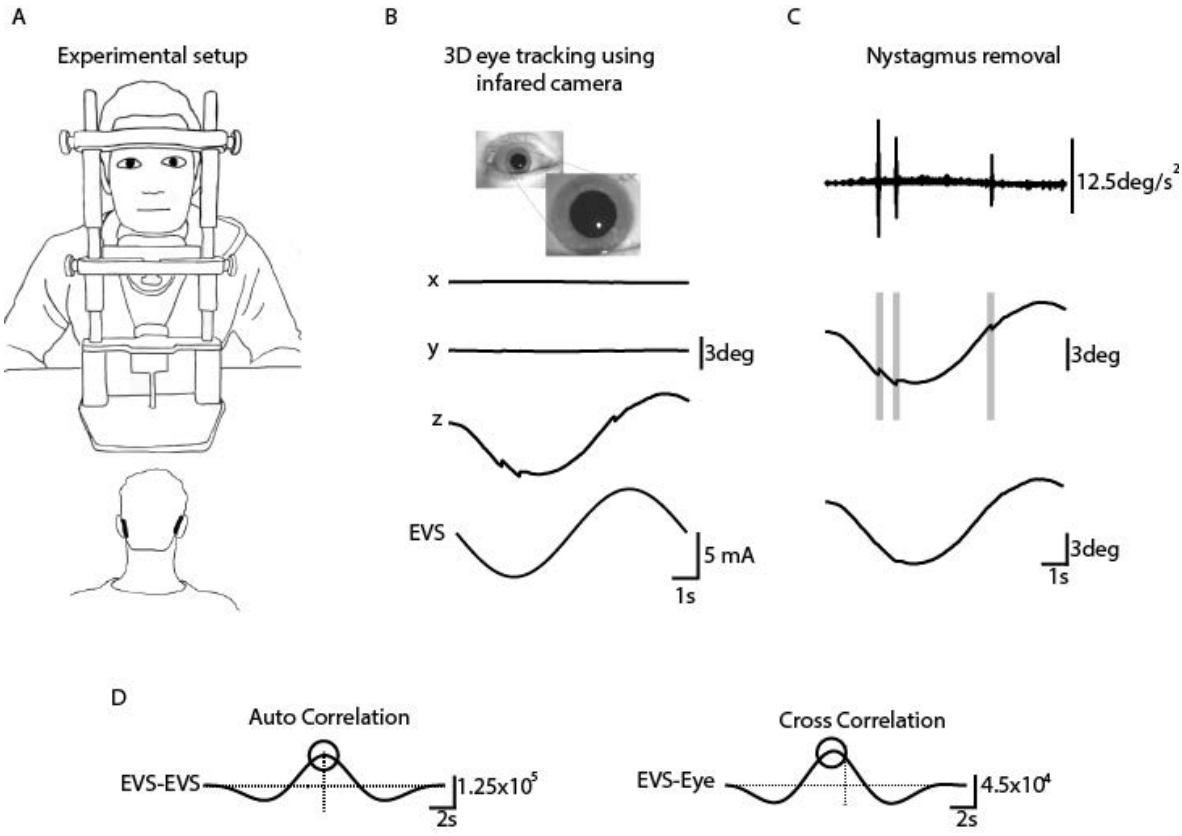
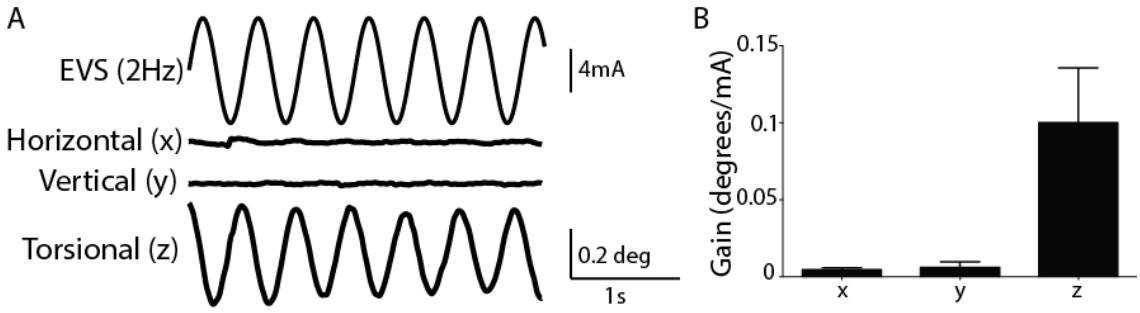


Figure 4.1 Analysis of EVS-evoked ocular responses. A) Subjects sat in darkness with the head fixed while EVS stimuli of varying frequencies (0.05-20 Hz) were delivered in a binaural bipolar configuration (±5mA, 10s), B) The eye was recorded using an infrared camera, and movements in all 3 axes were tracked off-line. C) An eye acceleration threshold procedure was used to detect fast phase movements which were then removed using a compensatory inverse nystagmus algorithm. D) Response gain was determined by the ratio of the peak EVS-eye cross correlation to the peak EVS-EVS auto correlation. Phase was determined from the lag of the cross correlation.

Results

Vestibular-evoked eye movements

Figure 4.2A depicts a representative eye movement response from a subject exposed to 2Hz sinusoidal electrical vestibular stimulation. Horizontal and vertical responses were weak or absent. However, the torsional component was consistently identifiable in all subjects (main effect of axis: $F_{(2,16)}=32.87$, $p<0.001$). Mean response gain for all subjects is shown in Figure 4.2B. All subsequent analysis is restricted to torsional responses.



The ocular torsion response across different stimulus frequencies

The effect of stimulus frequency upon the torsional response is depicted in Figure 4.3 for a representative participant. Across all frequencies, an eye movement response can be seen at the same frequency as the stimulus, validating the tracking technique.

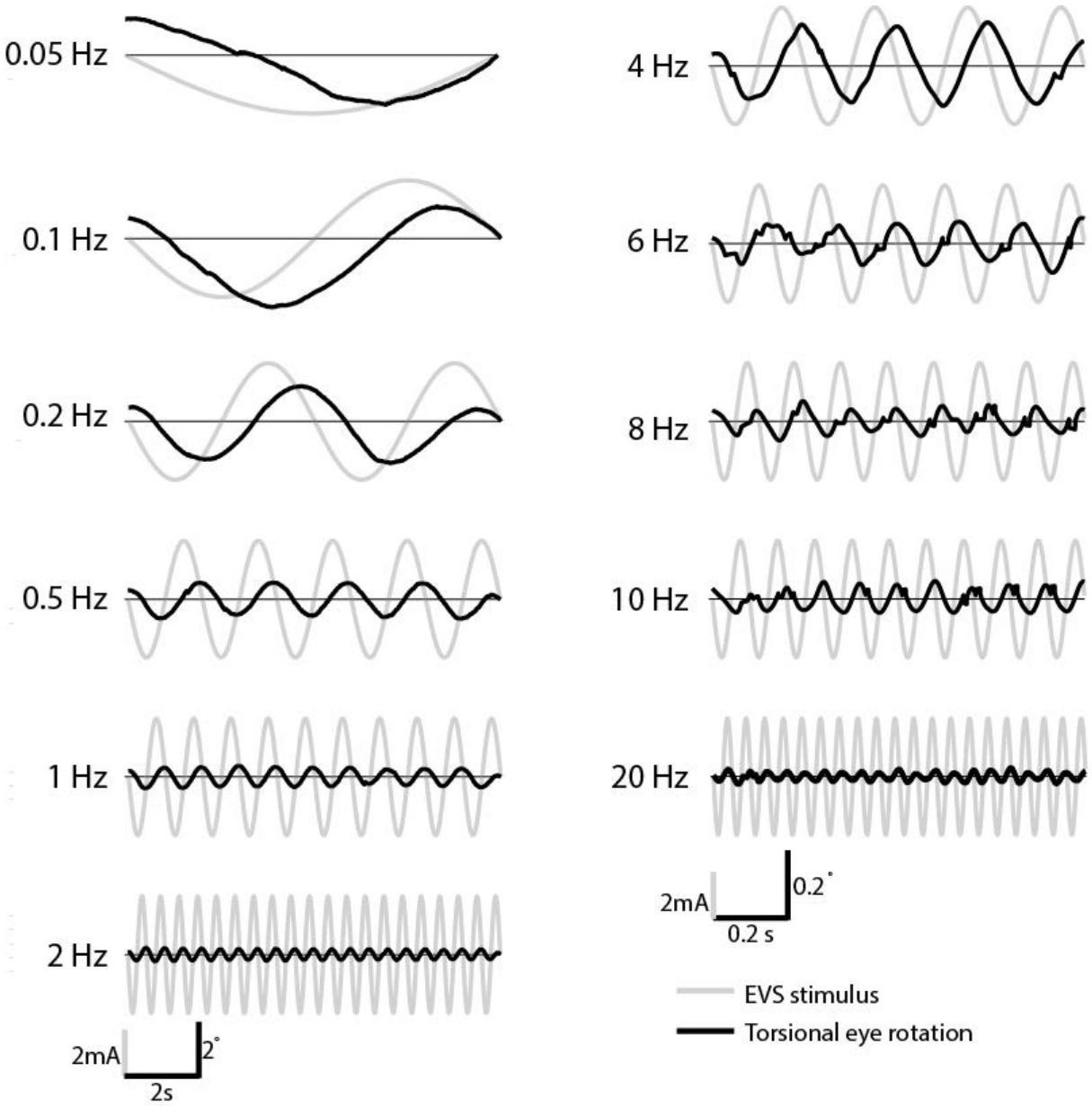


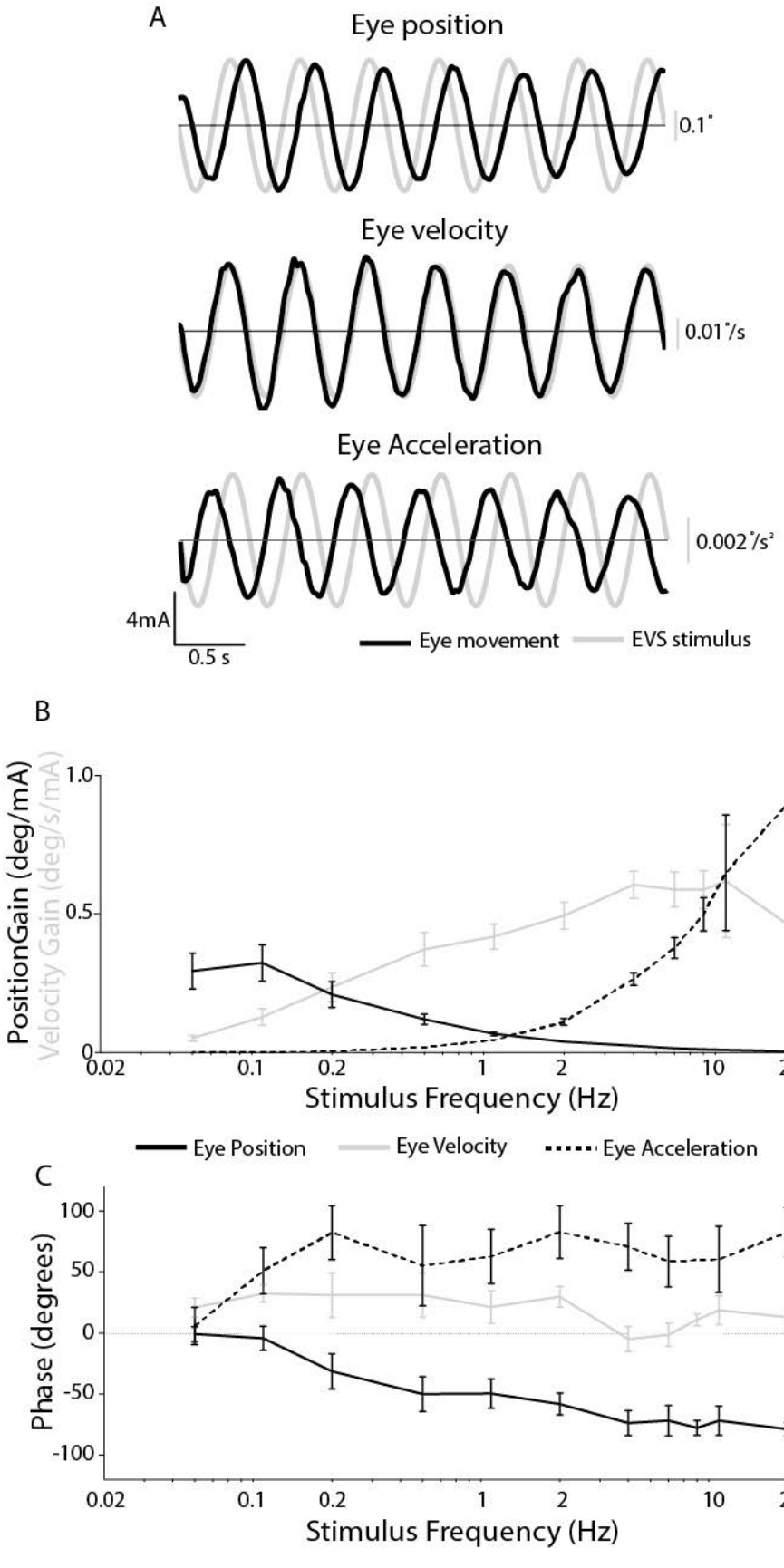
Figure 4.3 Representative EVS-evoked torsional eye movements across frequencies. A compensatory torsional eye rotation was evoked at all EVS frequencies ranging from 0.05Hz to 20Hz. Note the x10 change in eye movement scale between left and right graphs.

Response gain and phase

We analysed the gain and phase between the EVS stimulus and the ocular torsion response. This analysis was performed separately for the three response measures of eye position, velocity and acceleration (see Figure 4.4A for

representative plots). Mean positional gain decreased with frequency ($F_{(10,80)}$=17.3, p<0.001), whereas velocity gain increased ($F_{(10,80)}$=8.5,p<0.001). Acceleration gain also exhibited an increase with stimulus frequency, but with an exponential profile ($F_{(10.80)}$=61.3, p<0.001).

The representative 2Hz data in figure 4A exhibits a phase lag of -107 degrees between the EVS stimulus and eye position. This is not apparent in the eye velocity trace, which is almost in phase with the stimulus (+14 degrees). In contrast, eye acceleration exhibits a moderate phase lead of +106 degrees with respect to the stimulus. These observations are corroborated by the mean data in Figure 4.4C. Positional phase starts around zero degrees for the lowest frequency, increasing to 78 degrees at 20 Hz (main effect of frequency: F(10,80)=10.3, p<0.001). Eye velocity exhibits a flatter phase plot, with a lead of ~18 degrees and no significant effect of frequency ($F_{(10,80)}$=1.2, p=0.29). Eye acceleration shows a progressively increasing phase lead with frequency, from 5 to 82 degrees ($F_{(10,80)}$=2.9, p=0.004).


A
Eye position
0.1˚
Eye velocity
0.01˚/s
Eye Acceleration
0.002˚/s²
4mA
0.5 s
Eye movement
EVS stimulus
B
PositionGain (deg/mA)
Velocity Gain (deg/s/mA)
Acceleration Gain (deg/s²/mA)
1.0
0.5
0
60
40
20
0
0.02
0.1
0.2
1
2
10
20
Stimulus Frequency (Hz)
Eye Position
Eye Velocity
Eye Acceleration
C
Phase (degrees)
100
50
0
-50
-100
0.02
0.1
0.2
1
2
10
20
Stimulus Frequency (Hz)

Discussion

The commercially available software we used to track the eye has previously been shown to be capable of tracking a variety of biological motion images (Osborne & Lakie, 2011). From our video images, it identified an ocular response at all EVS stimulus frequencies from 0.05 to 20Hz. In each case, the observed eye movement occurred at precisely the same frequency as the stimulus. This simple observation validates the tracking technique, and confirms that the software did not generate spurious movements. Hence, a relatively cheap off-the-shelf camera in combination with commercially available software was sufficient for reliable measurement of EVS-evoked eye movements in total darkness.

Small vertical eye movements have been reported in response to EVS when using more sensitive (and invasive) techniques such as scleral coils (Severac Cauquil et al., 2003). Along with the much larger torsional component, these disconjugate polarity-dependent movements are consistent with a virtual sensation of roll. They were not reliably detectable in our video recordings, whereas the torsional component was consistently present in all subjects. A small degree of inter-ocular asymmetry in the magnitude of this torsion response has previously been demonstrated (Severac Cauquil et al., 2003). Given that we recorded the right eye only, we could not have seen this. However, this effect was demonstrated with the use of square-wave Galvanic Vestibular Stimulation (GVS), with the left-right magnitude difference observed when comparing

cathode-right versus cathode-left stimuli. Such differences are not relevant in our study where the use of sinusoidal stimuli negates any such polarity-dependent effects.

The predominantly torsional nature of the eye movement confirms previous findings, and supports the assertion that EVS induces a sensation of roll motion around a naso-occipital axis, due to activation of canal afferents (Fitzpatrick & Day, 2004). For example, Schneider et al (2002) showed that the ocular response to a direct-current EVS stimulus was essentially the same as that evoked by natural head rotation in the roll axis. Both stimuli evoked a fixed torsional offset accompanied by nystagmus. Peterka (1992) systematically examined the torsional VOR evoked by chair rotation at frequencies up to 2Hz, and reported gain values approaching 1. This suggests that the reflex performs a useful function in minimising retinal slip due to head roll, and does not support previous suggestions that it is merely vestigial (Miller, 1962). Hence, by being able to record the EVS-evoked torsional eye movement we gain insight into a functional reflex. Furthermore, it allows us to investigate torsional VOR at frequencies much higher than achievable with a rotating chair.

By analysing response gain and phase as a function of stimulation frequency, we can make inferences about the way in which EVS is interpreted by the brain. When analysed in terms of position, ocular torsion exhibited a steady reduction in gain with frequency. Such low-pass characteristics of EVS-evoked positional responses have previously been demonstrated by Schneider et al, (2000), although they only studied frequencies up to 1.67Hz. Velocity gain, in contrast,

exhibited a steady *increase* with frequency, while acceleration gain showed a much steeper rise. The velocity gain closely resembles the torsional VOR response to natural rotation stimuli, where the ratio of eye velocity to head velocity also exhibits a steady rise with frequency (see Fig. 1 from Peterka 1992). Hence, our gain analysis suggests that EVS current is primarily interpreted as a velocity stimulus. The phase analysis supports this assertion. Eye position exhibited a progressively increasing phase lag with respect to frequency, whereas eye velocity was most in-phase with the stimulus, exhibiting a slight phase lead across all frequencies. Acceleration showed a much larger phase lead, initially increasing with frequency before plateauing. Again, the velocity phase response most strongly resembles the response to natural vestibular stimulation, where eye velocity exhibits a constant small phase lead with respect to rotation velocity, across all frequencies (Fig. 1, Peterka 1992). Hence, both gain and phase are consistent with EVS-evoked changes in vestibular afferent firing rate being interpreted by the brain as a torsional velocity signal.

The stimulation and recording techniques we describe here offer potential for clinical diagnostic use, since it is affordable, non-invasive, comfortable and relatively quick. To assess the function each ear separately would simply require a monaural stimulus, with a reference electrode distant from the ear (Aw, Todd, et al., 2013; MacDougall et al., 2005)

Additional Information

Competing interests

No conflicts of interest are declared by the authors.

Funding

This work was supported by the UK Biotechnology and Biological Research Council (BB/P017185/1 & BB/I00579X/1) and the Ménière's Society. SWM is supported by an MRC-ARUK PhD scholarship.

CHAPTER 5

OCULAR TORSION RESPONSES TO ELECTRICAL VESTIBULAR STIMULATION IN VESTIBULAR SCHWANNOMA

Highlights

- Electrical vestibular stimulation (EVS)-evoked eye movements are trackable with an infrared camera.
- Unilateral vestibular schwannoma attenuated the ocular torsion response to EVS.
- EVS-evoked ocular torsion responses provide a convenient, non-invasive vestibular assessment.

Keywords: Vestibular Schwannoma; Asymmetry ratio; Electrical Vestibular Stimulation; Head Impulse Test.

Abstract

Objectives: We determined if eye movements evoked by Electrical Vestibular Stimulation (EVS) can be used to detect vestibular dysfunction in patients with unilateral vestibular schwannoma (VS).

Methods: Ocular torsion responses to monaural sinusoidal EVS currents (± 2mA, 2Hz) were measured in 25 patients with tumours ranging in size from Koos grade 1 to 3. For comparative purposes we also measured postural sway response to EVS, and additionally assessed vestibular function with the lateral Head Impulse Test (HIT). Patient responses were compared to age-matched healthy control subjects.

Results: Patients exhibited smaller ocular responses to ipsilesional versus contralesional EVS, and showed a larger asymmetry ratio (AR) than control subjects (19.4 vs. 3.3%, $p<0.05$). EVS-evoked sway responses were also smaller in ipsilesional ear, but exhibited slightly more variability than the eye movement response, along with marginally lower discriminatory power (patients vs. controls: AR=16.6 vs 2.6%, $p<0.05$). The HIT test exhibited no significant difference between groups.

Conclusions: These results demonstrate good diagnostic potential for the ocular torsion response to EVS.

Significance: The fast, convenient and non-invasive nature of the test are well suited to clinical use.

Introduction

Electrical Vestibular Stimulation (EVS) is a simple method for activating the vestibular nerve by directly applying cutaneous currents over the mastoid processes (Fitzpatrick & Day, 2004). The resulting change in vestibular afferent firing rate produces a sensation of head roll (Reynolds & Osler, 2012). This, in turn, evokes a variety of motor outputs including sway (Lund & Broberg, 1983) and orienting responses (Fitzpatrick, Butler, & Day, 2006). EVS also activates the vestibular-ocular reflex. The evoked eye movement is primarily torsional, with minimal lateral or vertical component (Jahn, Naessl, Schneider, et al., 2003; Jahn, Naessl, Strupp, et al., 2003; MacDougall et al., 2005; Mackenzie & Reynolds, 2018b; Schneider et al., 2002; Severac Cauquil et al., 2003).

Although EVS has mainly been used as a basic research tool, there is evidence for its clinical diagnostic potential (Dix & Hallpike, 1952). When applied in a monaural configuration, the integrity of each ear can be separately assessed. Using this approach, altered EVS-evoked responses have been reported in a variety of vestibular disorders. For example, the magnitude of ocular torsion responses are significantly reduced following intratympanic gentamicin injections (Aw et al., 2008). This has also been reported for the EVS-evoked sway response following streptomycin toxicity (Dix, Hallpike, & Harrison, 1949). In contrast, responses are *larger* in Meniere's disease (Aw, Aw, Todd, & Halmagyi, 2013). In a series of vestibular case studies MacDougall et al. (2005) reported systematic changes in the 3D orientation of the eye movement corresponding to specific

canal deficits. These studies suggest that the EVS could supplement or even replace existing diagnostic tests. But before it can be useful as a general vestibular diagnostic, it is necessary to establish the normative and pathological responses in a variety of patients. From a practical clinical perspective, it is also desirable to develop a convenient, non-invasive and affordable version of the test for assessing the ocular response to EVS.

Here we measure the ocular response to EVS in patients with vestibular schwannoma (VS), a slow-growing benign tumour arising from the Schwann cells of the vestibulocochlear nerve. Previous research has studied EVS-evoked postural sway in VS, and compared the response to stimulation of the tumour ear to that of the healthy ear (Welgampola et al., 2013). Patients exhibit greater response asymmetry (AR) than control subjects, in terms of their standing sway response. This finding provides valuable diagnostic proof-of-principle for EVS. However, this particular postural test required patients to be capable of standing unaided on a force platform with their eyes closed and feet together. Since balance problems are a common feature of vestibular disorders, this potentially rules out a large minority of patients. In contrast, assessment of the ocular response to EVS can be performed whilst seated. Aw, Todd, et al. (2013) measured the ocular torsion response to brief pulses of square-wave EVS in four unilateral VS patients with large tumours. They reported longer response latencies as well as reduced velocity in the affected ear. Again, while this offers valuable diagnostic proof-of-principle, it is not well suited to routine clinical use due to the invasive nature of the scleral coils which were used. Here we employ

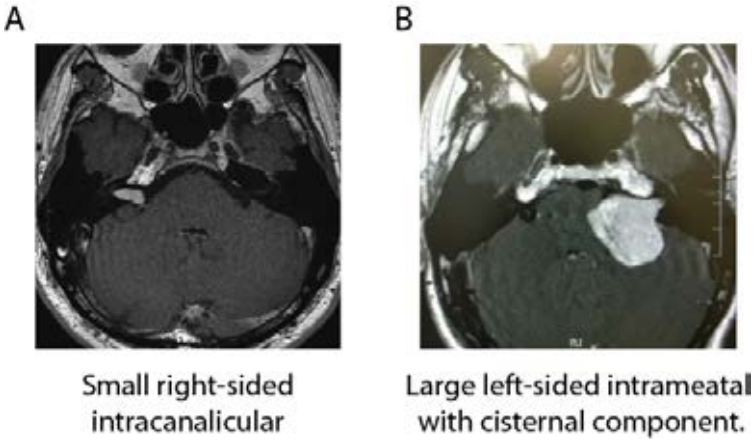
a non-invasive method for recording the ocular response to sinusoidal EVS in darkness using an infrared-sensitive camera. We studied 25 unilateral VS patients with small to moderately sized tumours, and compare them to age-matched controls. Our main aim is to determine whether the patients exhibit significantly greater response asymmetry in terms of the ocular torsion response to sinusoidal electrical vestibular stimulation (sEVS) in each ear. We also performed two additional tests for direct comparison with the sEVS ocular response; firstly, the EVS-evoked postural sway test used by Welgampola et al. (2013), and secondly, the head impulse test (HIT), since reduced HIT responses have previously reported in VS (Taylor et al., 2015; Tranter-Entwistle, Dawes, Darlington, Smith, & Cutfield, 2016). The results show that our sEVS test out-performed the HIT test in terms of discriminatory power and was marginally better than the postural sway test, while being more convenient.

Methods

Participants

25 patients (9 male) aged 30 to 80 (mean±SD; 61±13 years) were recruited from University Hospital Birmingham. The presence of a vestibular schwannoma (VS) was diagnosed by magnetic resonance imaging and quantified using the maximum extrameatal tumour diameter (Kanzaki et al., 2003). 17 healthy controls (9 males) aged 40 to 80 (mean±SD: 68±8 years) with no known neurological or vestibular disorder were studied for the purpose of collecting normative data in a healthy population. All participants gave informed written consent to participate. The experiment was approved by South Birmingham Research Ethics Committee and performed in accordance with the Declaration of Helsinki. Patient's tumour measurements and symptoms are presented in Table 5.1.

Evaluating tumour size



Koos classification and internal acoustic canal filling were assessed by MRI. Koos classification is a four-point grading system based on the size of the tumour (intracanalicular and cisternal) G1 <1 cm, G2 1-2 cm, G3 2-3 cm, G4 >3 cm (Koos, Day, Matula, &

Levy, 1998). Figure 5.1A depicts a small right-sided intracanalicular tumour while figure 1B depicts a large left-sided intrameatal tumour with a cisternal component. Most participants were classified as Koos grade 2, which is partially attributable to the treatment procedure, whereby anyone with a tumour over 2 cm in diameter is offered cyberKnife, ultimately resulting in their exclusion from the study.

Table 5.1. Patient Tumour characteristics and symptoms

ID	VS side	Location	Tumour Type	PTA (dB)	SDS (%)	ICL (mm)	ICD (mm)	Koos Grade	HL	TIN	BD
1	R	IAC/CPA	Solid	50	53	18.2	16.4	2	+	+	-
2	L	IAC	Solid	23	100	9	6.4	1	+	+	+
3	L	IAC/CPA	Solid	48		14.3	10.2	2	+	+	+
4	L	IAC/CPA	Solid	47	60	20.7	16.3	2	+	+	+
5	R	IAC	Cystic	30		10	6	1	+	+	-
6	L	IAC/CPA	Solid	58	20	11.5	13.3	2	+	+	+
7	R	IAC/CPA	Solid	17	87	15.6	12.3	2	+	+	+
8	R	IAC/CPA	Solid	53		20.4	15	2	+	+	+
9	R	IAC/CPA	Solid	3	100	16.2	10.2	2	-	-	+
10	L	IAC/CPA	Solid	23	86	7.5	5.1	1	+	+	-
11	L	IAC	Solid	8	97	4.1	4.3	1	-	-	+
12	L	IAC/CPA	Solid	30	98	8	6	1	+	+	+
13	R	IAC/CPA	Solid	23		17.1	12.2	2	+	+	-
14	L	IAC	Solid	75	40	2.5	4	1	+	+	+
15	L	IAC/CPA	Solid	67	17	20	16	2	+	-	+
16	R	IAC/CPA	Solid	43	90	16	10.9	2	+	-	+
17	L	IAC/CPA	Solid	15	100	22	12.4	2	+	-	+
18	R	IAC/CPA	Solid	7	30	19.7	10.9	2	+	+	+
19	L	IAC/CPA	Solid	50	73	15.7	6.7	1	+	+	+
20	L	IAC/CPA	Solid	35	70	20	18.7	2	+	+	-
21	R	IAC/CPA	Cystic	75	60	16	11.5	2	+	+	+
22	R	IAC/CPA	Solid	37	70	19.3	10.3	2	+	+	+
23	L	IAC/CPA	Solid	30	90	33	35.4	3	+	-	-
24	L	IAC/CPA	Solid	60		27.3	17.1	2	+	+	+
25	R	IAC/CPA	Cystic	72	42	37.8	16.8	2	+	-	-

R = Right, L = Left; IAC = Internal auditory canal, CPA = Cerebellopontine angle; PTA = Pure Tone Average; SDS = Speech Discrimination Score; ICL = intracanalicular length; ICD = intracanalicular diameter; HL = Hearing loss; TIN = Tinnitus; BD = Balance Disturbance, + symptomatic, - non-symptomatic.

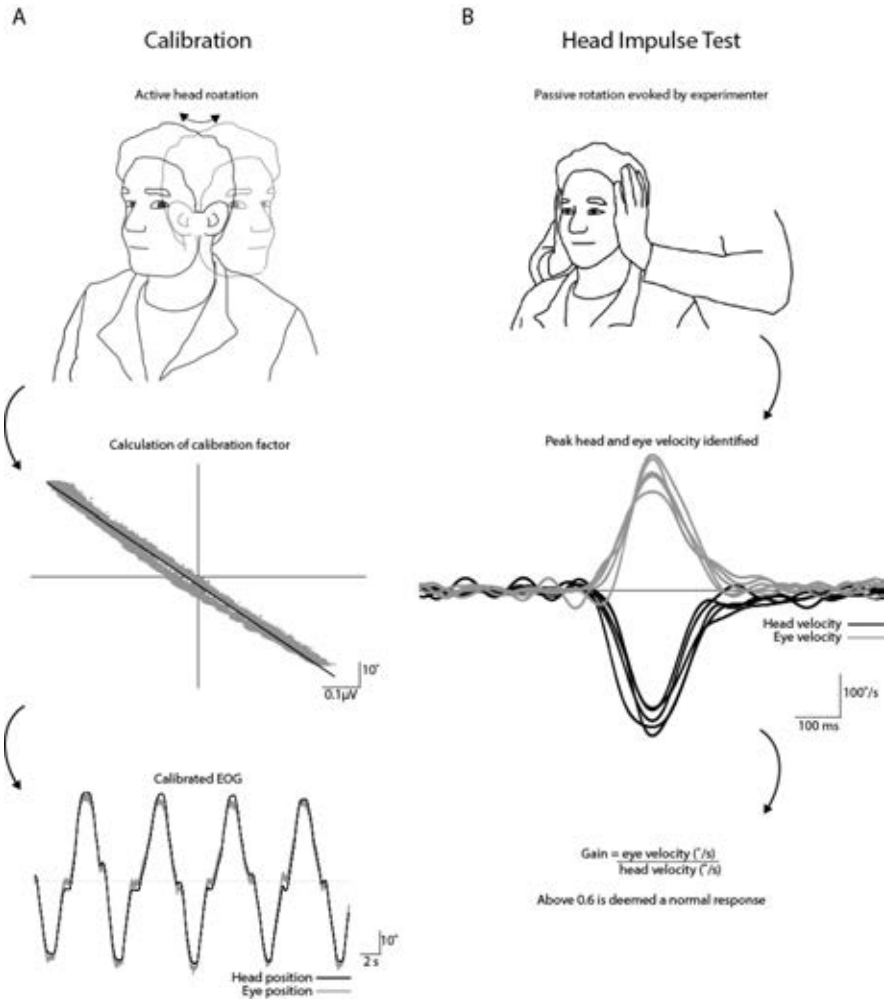
Head Impulse Test (HIT)

Protocol – Participants received 20 (10 right, 10 left) impulses while seated. HIT involves a small (~30 degrees), rapid (50 to 300 degrees/s) head rotation in yaw, evoked by the experimenter. Participants were instructed to fixate on a visual target located 1m in front of them throughout the HIT.

Calibration – Eye kinematics were recorded using electro-oculography (EOG), thus requiring conversion from μV to degrees of rotation. This was achieved by having the participants rotate the head in yaw while keeping the eyes fixated on a target, allowing a regression to be calculated between EOG and degrees of head rotation, measured using a motion tracker (Figure 2A). The calculated calibration was used to calibrate all subsequent EOG signals into degrees. The success of this calibration process can be observed in Figure 5.2A, where head position (black trace) and inverted eye position (grey trace) closely match each other.

Data Acquisition and Analysis - Eye kinematics were sampled at 1 kHz using EOG. Two non-polarizable skin electrodes were applied near the outer canthi and a reference electrode to the forehead. Prior to electrode placement the skin was prepared by rubbing the skin with an abrasive electrode gel, all excess gel was removed before the area of skin was cleaned with an alcohol wipe and left to dry. The calibrated eye position for each head impulse was low pass filtered using a 5th order Butterworth (cut-off 10 Hz), from which eye velocity could be calculated. Head position was sampled at 50 Hz in the form of Euler angles using a Fastrak

sensor attached to a welding helmet frame worn by the participants (Polhemus Inc, Colchester, Vermont, USA). Head velocity during the HIT was sampled at 1 kHz using a gyro sensor located on the welding helmet worn by the participant. Offline analysis of the data was automated using MATLAB software. Peak head velocity and peak eye velocity were automatically selected and used to determine the horizontal gain (eye velocity / head velocity). A gain of 0.68 or greater was deemed normal (MacDougall, Weber, McGarvie, Halmagyi, & Curthoys, 2009). An asymmetry ratio (AR) was calculated for each participant.



EVS evoked postural adjustments

Protocol - Participants stood in the centre of a force plate, unshod, with feet together and hands held relaxed in front of them for the duration of each 60 s stimulation period (Figure 5.3A). Prior to each trial participants were instructed to face a visual target at eye level, 1m in front of them before closing their eyes for the duration of the trial.

Electrical Vestibular Stimulation – EVS was delivered using carbon rubber electrodes (46x37 mm) in a monaural cathodal or anodal configuration. Four electrodes were coated in conductive gel, two were secured to the mastoid processes and two overlying the C7 spinous process using adhesive tape. Stimuli were delivered from an isolation constant-current stimulator (AM Systems, Carlsberg, WA, USA). EVS was applied in sequences of six 3 s impulses of 1 mA, separated by a 6 s gap.

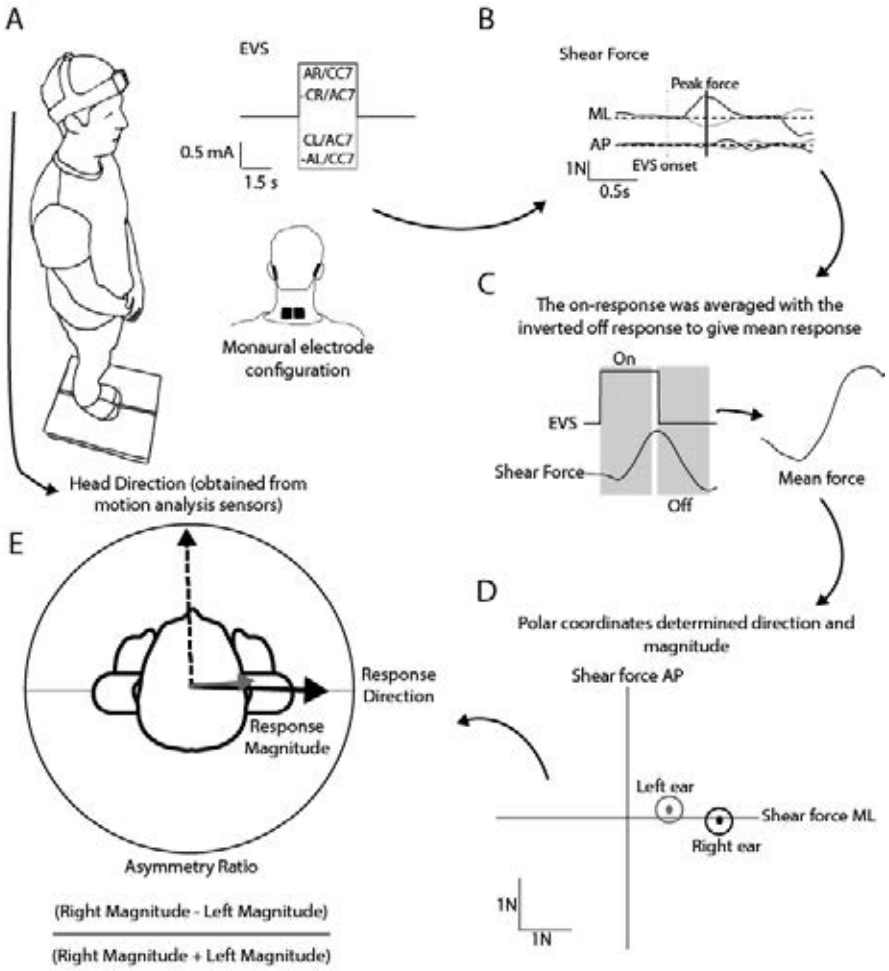
The side of the active electrode (left or right) and the polarity (cathode or anode) was randomised across trials. Two sides and two polarities gave a total of 4 conditions (Anode-Left/Cathode-C7, Anode-Right/Cathode-C7, Cathode-Left/Anode-C7 and Cathode-Right/Anode-C7). Four repeats of each condition resulted in a total of 24 impulses per condition (96 in total).

Data Acquisition and Analysis - Head position was sampled at 50 Hz in the form of Euler angles using a Fastrak sensor (Polhemus Inc, Colchester, Vermont, USA) attached to a welding helmet frame worn by the participants. Any offset in

yaw or roll angle between head orientation and sensor orientation was measured using a second sensor attached to a stereotactic frame, and subsequently subtracted. A slight head up pitch position was maintained throughout each trial to ensure Reid's plane (line between inferior orbit and external auditory meatus) was horizontal, ensuring an optimal response to the virtual signal of roll evoked by vestibular stimulation (R C. Fitzpatrick & Day, 2004). The evoked sway response to vestibular stimulation 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).

Analysis of EVS-evoked shear force is depicted in Figure 5.3. Similar analysis techniques to Welgampola et al. (2013) were used. To increase signal-to-noise ratio of the response, the averages to the two stimulation polarities were combined separately for the mediolateral (Fx) and anteroposterior (Fy) direction. As the two polarities evoked responses in opposite directions, one polarity was inverted before the averaging process took place. For the left ear, the anodal response was inverted where as for the right ear the cathodal response was inverted, this was to ensure both ears resulted in a direction response towards the right. The 'off' response to stimulus cessation was combined with the 'on' response to stimulus onset. Again, the on and off responses are oppositely directed, hence the off response was inverted prior to the averaging process. The force response was quantified as the peak force vector between 200-800 ms after stimulus on/offset. The magnitude and direction (atan Fx/Fy) of the peak force vector within this time window was measured from a participant average. An

asymmetry ratio from stimulation of each ear was calculated using the equation in Figure 5.3E, where R and L represent right and left magnitude respectively.



EVS-evoked torsional eye movements

Protocol - Participants were seated with the head restrained (SR Research Ltd. Ontario, Canada) for the duration of each 10 s stimulation period. Prior to each trial participants were instructed to focus on the lens of an infrared camera and

not to blink before being immersed into darkness. An invisible infrared light (940 nm) was used to illuminate the eye during each trial. No fixation light was provided to ensure that any horizontal and vertical eye movements were not suppressed.

Electrical vestibular Stimulation - Sinusoidal electrical vestibular stimulation (sEVS, 2 Hz, peak ±2 mA) was delivered in a monaural configuration to evoke torsional eye movements. Four conditions (2 sides x 2 polarities) were repeated 3 times giving a total of 12 trials.

Data Acquisition and Analysis - Torsional eye movements were sampled at 50 Hz using an infrared camera (Grasshopper 3, Point Grey Research Inc, Richmond, BC, Canada). Eye movements were tracked and quantified off-line using a commercially available planar tracking software (Mocha Pro V5, Imagineer Systems Ltd. Guildford, UK). Torsional motion was tracked using iris striations. This technique has previously been validated across stimulation frequency range of 0.05-20 Hz (Mackenzie & Reynolds, 2018b). Nystagmus fast phases were automatically identified and removed (Mackenzie & Reynolds, 2018b). The magnitude of the eye response was measured as the peak value of the stimulus-response cross-correlation. Gain was then calculated by dividing this value by the peak stimulus autocorrelation to normalise with respect to the input stimulus. An asymmetry ratio was then calculated from the gains of both ears.

Statistical Analysis

To detect if the patients healthy ear was indeed healthy, it was compared to a random selection of right and left ear responses from the control group using an independent t test (SPSS). Response gain (unitless) was used to quantify both HIT and sEVS-evoked torsional eye movements, whereas peak force (N) was used to quantify the magnitude of the EVS-evoked.

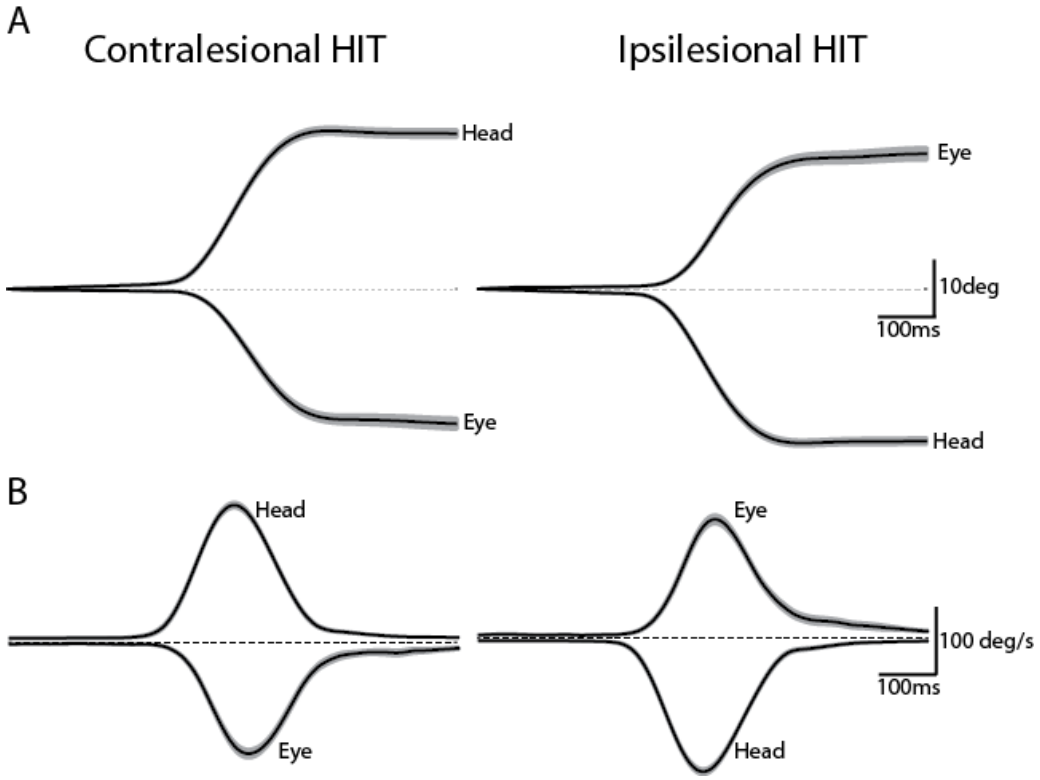
A 1x4 repeated-measures ANOVA (SPSS general linear model) was used to compare response direction between healthy controls left and right ear and patients ipsilateral and contralesional ear. In all cases, where significant Mauchly's tests indicated violation of the assumption of equal variances, the GreenHouse-Geisser correction was employed. An unpaired t test was used to compare asymmetry ratios between controls and patients. We also performed correlations between EVS-evoked postural AR's and sEVS-evoked eye movement AR's. A correlation between tumour size and AR was also performed. Pearson correlations were used to determine significance.

For all statistical tests, significance was set at $p<0.05$. Means and standard deviations are presented in text and figures, unless otherwise stated.

Results

HIT-evoked eye movement responses

Mean head and eye kinematics during the HIT test are shown in Figure 5.4 for schwannoma patients. Mean head rotation amplitude (and peak velocity) was 28° (197°/s) and 27° (200°/s) for contralesional and ipsilesional directions, respectively.



Gain values (eye/head velocity) were approximately 1 in both patients and control subjects, irrespective of head direction (Figure 5.5A). There was no difference in the asymmetry ratio between the patient and control groups ($T_{(36)}$=1.29, p=0.41).

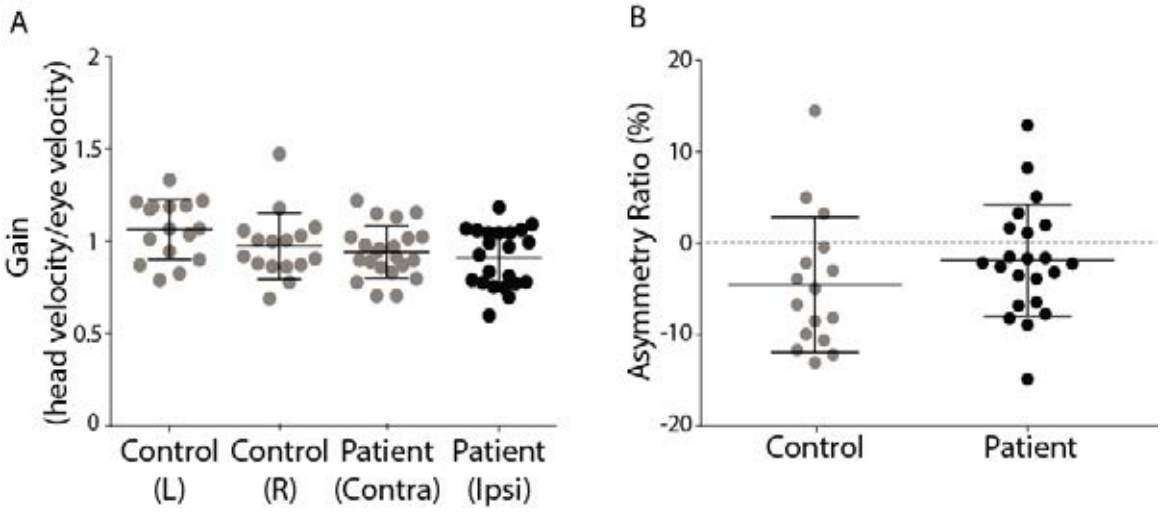


Figure 5.5. HIT Response gains and asymmetry ratios. A) HITs in healthy (towards left or right ear) and VS patients (contralesional or ipsilesional) resulted in response gains of ~1. B) Asymmetry ratios. Mean and SD are presented, along with individual subject data.

EVS-evoked postural responses

Figure 5.6 depicts EVS-evoked ground reaction forces in two schwannoma patients (one left and one right-sided VS) and a control subject standing face-forward. EVS primarily evoked a mediolateral force response, with minimal anterior-posterior response. The control subject showed very similar responses to left and right ear stimulation. In contrast, both patients showed markedly attenuated responses during ipsilesional stimulation.

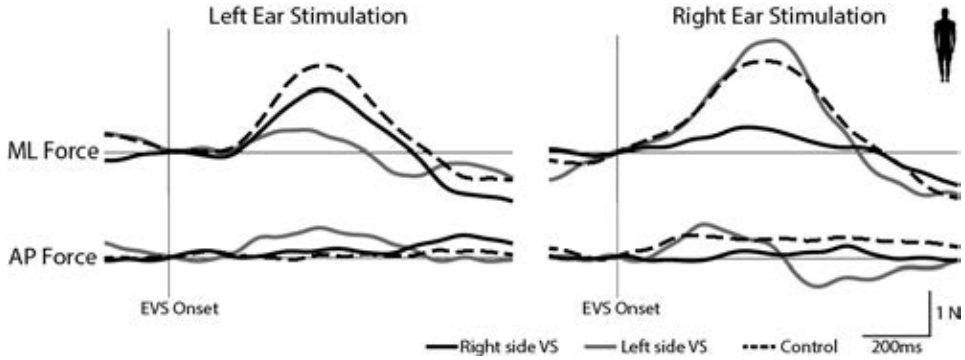
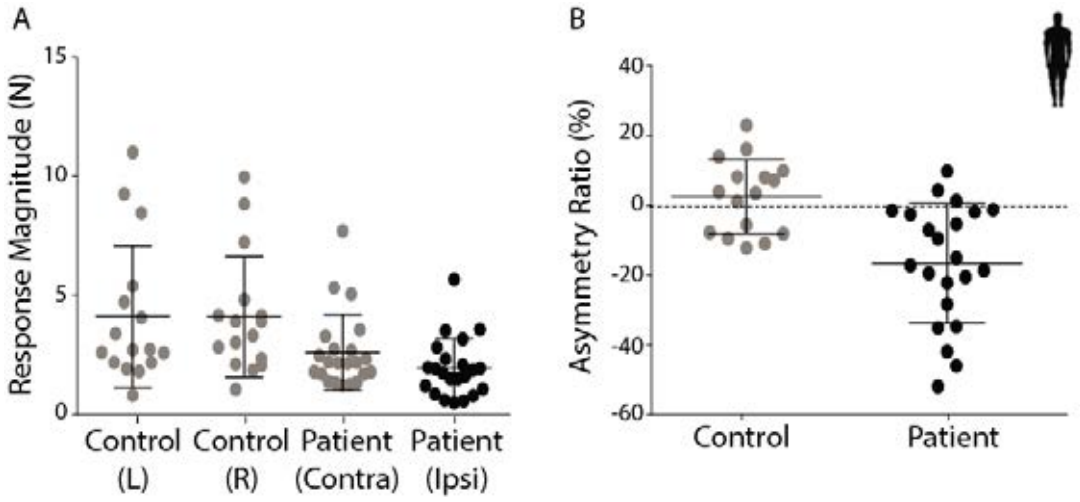
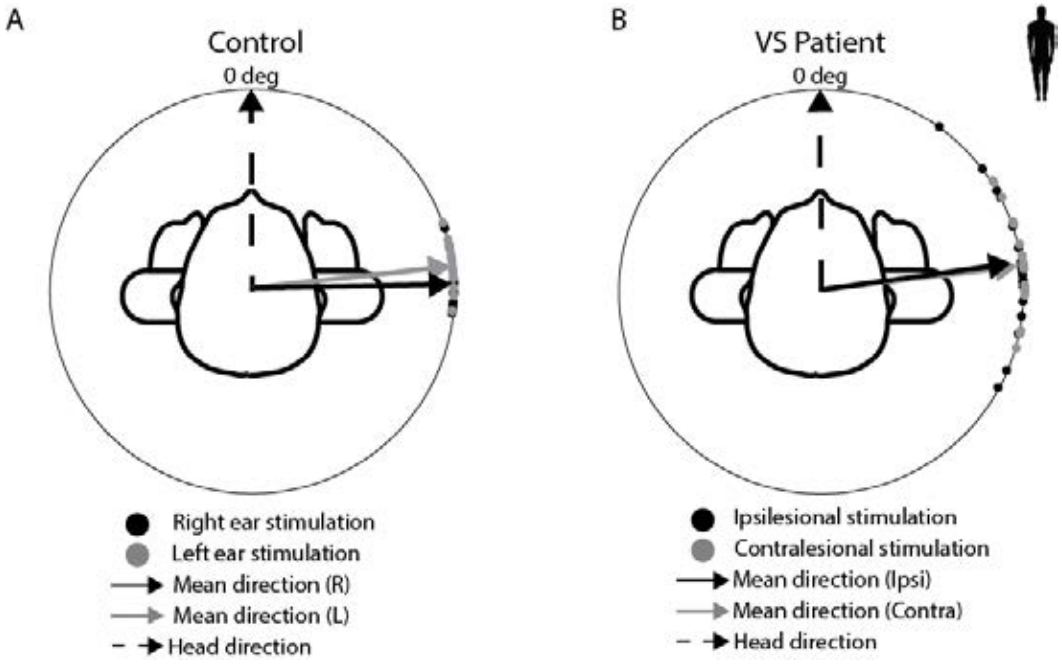


Figure 5.6. EVS-evoked sway response. EVS during a head forward (0 degrees) orientation produces a compensatory sway response as shown by a force increase in the ML force. A healthy individual (black dashed trace) shows as similar response magnitude when either the right or left ear is stimulated. However, the vestibular schwannoma patients show a reduced response magnitude during ipsilesional stimulation (solid black and grey traces).

In control subjects, peak force responses were similar for left and right ear stimulation (Figure 5.7A). In patients, while stimulation of the contralesional ear produced similar responses to control subjects ($T_{(42)}$=1.85, p>0.05), ipsilesional forces were attenuated. This was confirmed by a significant difference in asymmetry ratio between the two groups (Figure 5.7B; Controls = 2.6%, patients = -16.6%; $T_{(36)}$=3.92, p<0.05).

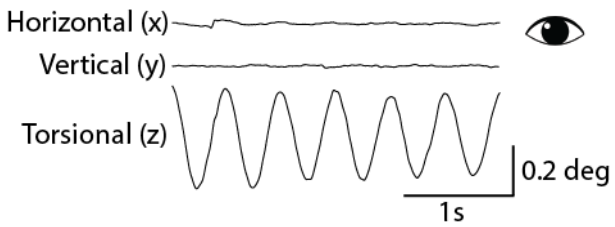


In addition to measuring the magnitude of the EVS-evoked force vector, we also measured its direction (Figure 5.8). With the head facing forwards, anodal EVS over the right ear evoked a postural response directed along the inter-aural axis. Schwannoma had no effect upon the direction of this response, with all controls and patients responses oriented in the same direction ($F_{(4,96)}$=2.13, p>0.05).



sEVS-evoked eye movement

Sinusoidal EVS evoked a strong torsional eye movement, with minimal horizontal or vertical components (Figure 5.9) (Mackenzie & Reynolds, 2018b). Therefore, only torsional eye movements were used in subsequent analysis.



As reported in Mackenzie and Reynolds (2018b), there was a ~90°phase lag between the stimulus and response, with no difference between groups, or between contralesional and ipsilesional stimulation.

Response gain is illustrated in Figure 5.10A. Control subjects exhibited equal gain for left and right ear stimulation. Contralesional stimulation in patients produced similar values to the control group ($T_{(55)}$=0.41, p>0.05). However, ipsilesional stimulation produced an attenuated response. This is apparent in the asymmetry ratios, where the mean values were -3.27% and -19.38% for controls and patients, respectively (Figure 5.10B, $T_{(48)}$=2.53, p<0.05).

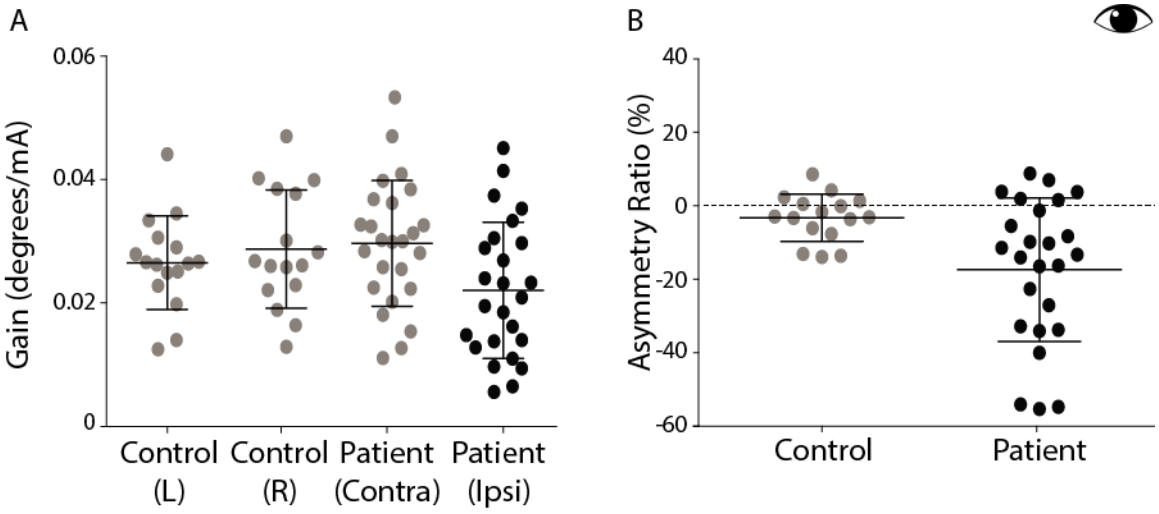
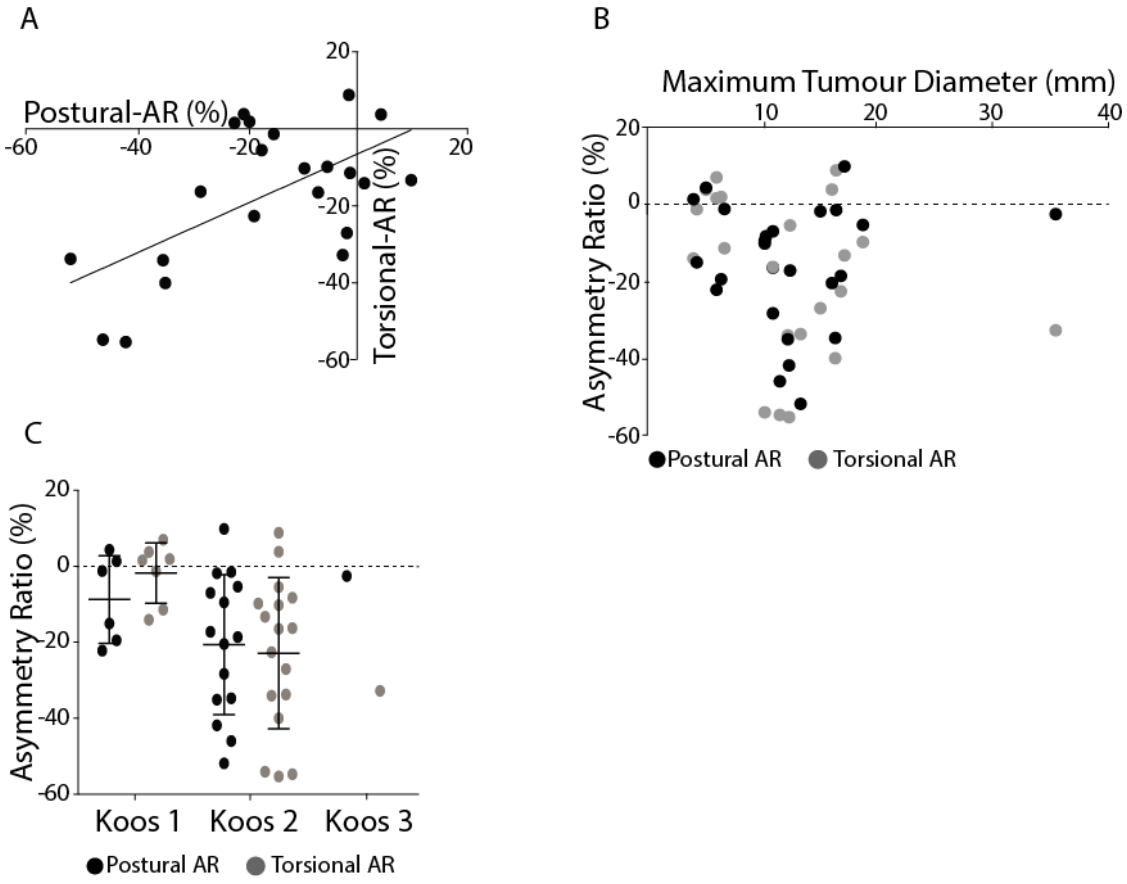


Figure 5.10. sEVS-evoked torsional eye movement response magnitudes and asymmetry ratios. A) Response gains for control's left and right ear stimulation and patient's contralesional ear (grey) and patients ipsilesional ear (black). B) Asymmetry ratio for controls (grey) and patients (black). Mean and SD presented.

Comparison of ocular and postural responses to EVS in Schwannoma patients

Figure 5.11A shows the ocular and postural asymmetry ratios plotted against each other for the patient group. The two methods exhibited a moderate correlation ($r=0.60$, $p<0.05$). Neither ocular nor postural asymmetry exhibited any significant relationship with tumour size (Figure 5.11B). However, when patients were classified according to their Koos grade, those with Koos 1 showed smaller ocular asymmetry than Koos 2 ($T_{(22)}=2.69$, $p<0.05$). There was no effect of Koos grade upon the postural asymmetry ratio ($T_{(19)}=1.46$, $p>0.05$).



Discussion

We measured the ocular torsion response to sinusoidal electrical vestibular stimulation (sEVS) using the same stimulation and recording techniques described in Mackenzie and Reynolds (2018b). The only significant modification was the use of a monaural rather than binaural stimulus, so that each ear could be assessed separately. When we applied this technique to vestibular schwannoma patients we found that the ocular response was significantly reduced in the ipsilesional versus contralesional ear. When combined with the speed, comfort and practicality of the technique, this establishes the potential utility of the sEVS-evoked eye movement as a clinical diagnostic test.

Mean ocular response asymmetry ratio in the VS patients was ~20%, being significantly greater than that of control subjects. This was also true for the EVS-evoked postural response. However, there was considerable overlap between patients and controls for both the ocular and postural tests. This contrasts with the results of Welgampola et al. (2013). They measured the ground reaction force response to EVS in the same way as described here, and found ~40% asymmetry in the patient response and zero overlap with control subjects. However, tumour size in their patient group was more than double that here (27 vs. 12 mm). Therefore, the difference is probably related to the extent of vestibular nerve damage in the two patient cohorts. This suggests that the response variability seen in our patient group reflects genuine differences in vestibular function.

The asymmetry in the patient ocular response was correlated with that of their postural response, suggesting that both results reflect the extent of the underlying vestibular deficit caused by the tumour. The magnitude of EVS-evoked sway responses are affected by numerous factors including head orientation, biomechanics, proprioceptive acuity and baseline sway (Fitzpatrick & McCloskey, 1994; Fitzpatrick & Day, 2004; Mian & Day, 2009; Pastor et al., 1993). The sEVS-evoked eye movement is simpler by comparison, consisting of a tri-neuronal sensorimotor arc combined with the minimal inertia of the eyeball. Hence, the ocular response theoretically constitutes a less variable test of vestibular function. Indeed, we did observe less variability in the ocular asymmetry of control subjects compared to their postural response (6.4 vs 10.7% AR). But perhaps more important than subtle differences in diagnostic efficacy between the two tests is the large difference in practicality. The eye movement recording was performed over a ~10 min period in seated subjects. It is readily applied to patients with a high degree of postural instability and/or physical disability. Indeed, two patients were unable to complete our postural test, while all undertook the ocular recordings. Furthermore, the use of infrared video offers a practical alternative to invasive techniques such as scleral coils or marking the sclera with a surgical pen to aid tracking.

Patients with Koos grade 2 tumours exhibited greater mean asymmetry than those in the smaller grade 1 category, but there was no correlation between tumour size and asymmetry ratio for either test. This tallies with Welgampola et al. (2013) whose data showed no correlation between EVS-evoked force and

tumour size in eight patients with tumours spanning 17-40 mm (see table 1 from Welgampola et al, 2013). The lack of a systematic relationship between tumour size and vestibular deficit is perhaps unsurprising, since limited or absent correlations have also been shown for hearing loss (Mahmud, Khan, & Nadol, 2003; Nadol, Diamond, & Thornton, 1996), although this may not be true for much larger tumours (Schuknecht, 1974). Our data also exhibited no relationship between tumour diameter and hearing loss or speech discrimination (see table 1 above). This absence of a size effect is likely due to the non-uniform manner in which tumour growth impinges upon the auditory-vestibular nerve.

In addition to measuring EVS-evoked postural sway magnitude we also determined sway direction, and found this to be normal in the patient group. Furthermore, the phase lag between the sEVS stimulus and the ocular response was also normal. These findings suggest that sensorimotor transformation processing for vestibular information is entirely normal in VS patients. It is simply the magnitude of the responses which are affected.

In contrast to previous reports, gain values for our HIT test were ~1 for all subjects and directions, with no significant asymmetry in the VS patients, nor any difference between patients and controls. Tranter-Entwistle et al. (2016) reported mean gains of 0.73 and 0.90 during the horizontal canal video HIT test (vHIT) for the ipsilesional and contralesional side, respectively, with 10 of their 30 patients exhibiting < 0.79 (ipsi) gain. Similarly, Taylor et al. (2015) reported vHIT gains of 0.75 (ipsi) and 0.9 (contra) for the horizontal canal. Potential reasons for the null

HIT response here might be differences in head movement kinematics, recording techniques and patient tumour location or size. Regarding kinematics, our peak head displacement (velocity) was ~27° (200°/s), being within most accepted range values for a valid HIT test (Jorns-Haderli, Straumann, and Palla (2007): 20-40°(~300°/s), MacDougall et al. (2009): 5-20° (50-250°/s), Taylor et al. (2015): 10-20° (50-300°/s), McGarvie et al. (2015):(100-200°/s), Tranter-Entwistle et al. (2016):(>150°/s)). Regarding technique, we used electro-oculography rather than video for recording lateral eye movements, but it is not immediately obvious how this would affect gain. Furthermore, any systematic change in gain caused by such technical differences would affect both directions equally so would not influence asymmetry. Regarding tumour location, VS can arise from the superior or inferior branch of the vestibular nerve (Khrais, Romano, & Sanna, 2008). Since the horizontal canal is innervated by the superior branch, a normal HIT test might occur if damage is restricted to the inferior branch. Consistent with this, most studies do indeed show that the superior branch is less commonly affected in VS (Khrais et al. (2008): 76% single nerve involvement with 91.4% inferior and 6% superior, 24% >1 nerve, via surgical identification. Ylikoski, Palva, and Collan (1978): 80% superior, 20% inferior via caloric test. Clemis, Ballad, Baggot, and Lyon (1986): 50% superior via auditory tests. Komatsuzaki and Tsunoda (2001): 84.8% inferior, 8.9% superior via surgical identification). However, this still does not account for the positive results of Taylor et al. (2015) and Tranter-Entwistle et al. (2016) for the horizontal canal. Regarding tumour size, this was 19mm in Taylor et al. (2015) and ~7-13mm in Tranter-Entwistle et al. (2016) which is similar to, or slightly greater than our mean value of 12mm. Hence it is not

immediately apparent why our VS patients exhibited normal HIT gains, but it raises the possibility that the sEVS response is a more sensitive measure of vestibular deficiencies than HIT. Further comparative studies in a larger variety of vestibular disorders are needed to confirm this.

The diagnostic utility of sEVS across a broader range of vestibular disorders may depend upon its precise site of action. While not established beyond doubt, EVS currents most likely alter neural firing rate via the spike trigger zone of the primary afferent (Fitzpatrick & Day, 2004; Goldberg, 2000; Goldberg et al., 1984). This implies that the EVS response can only reveal deficits downstream of the hair cell. Vestibular schwannoma certainly constitutes such a deficit, which explains the impaired responses seen here. However, it has also been reported that gentamicin-induced vestibular toxicity impairs EVS-evoked eye movements (Aw et al., 2008). Since acute gentamicin toxicity kills vestibular hair cells, this could be interpreted as evidence that EVS stimulates the hair cell rather than the primary afferent. However, vestibular afferents have a high resting firing rate, and loss of hair cell input may conceivably reduce their firing rate and/or their excitability. Such a loss of excitability could diminish the response to an externally applied current, analogous to a drop in spinal excitability presenting as a diminished H-reflex (Baldissera, Cavallari, Craighero, & Fadiga, 2001). But irrespective of the precise mechanism of action, the evidence of gentamicin-induced deficits in the EVS-evoked response provides encouraging evidence that it could diagnose peripheral as well as central vestibular deficits, at least if such deficits affect hair cell function. To establish the precise diagnostic scope of EVS

requires a direct comparison against established tests, such as caloric irrigation and chair rotation, in a wider group of vestibular disorders.

In summary, we have demonstrated that sEVS-evoked eye movements can be recorded in a fast, convenient and non-invasive fashion in order to detect asymmetries in vestibular function. Further work is required to validate this technique against existing tests such as caloric irrigation, and in a wider group of vestibular pathologies.

Conflicts of Interest

None of the authors have potential conflicts of interest to be disclosed.

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Thanks to Steve Allen for technical assistance.

CHAPTER 6

GENERAL DISCUSSION

Summary of experimental chapters

This thesis set out to investigate the potential clinical uses of EVS. First EVS-evoked vestibulo-spinal reflexes were examined (Chapters 2 and 3) before focus switched to EVS-evoked vestibulo-ocular reflexes (Chapters 4 and 5). This thesis demonstrates the possible uses of EVS in a clinical environment and highlights considerations which need to be taken into account when interpreting results from each test.

Exploring the vestibular coordinate transformation process using electrical vestibular stimulation

The vestibular system is locked within the skull, therefore vestibular signals provide information about head movement. For vestibular information to be used for balance control they must be transformed from head to foot-centred coordinates (Fitzpatrick & Day, 2004; Hlavacka & Njiokiktjien, 1985; Lund & Broberg, 1983; Mian & Day, 2009). For example, when leftward head motion is detected while facing forwards, a compensatory body movement to the right would be the appropriate response to maintain balance. But if the head is turned 90 degrees rightwards, the same pattern of vestibular afferent feedback would require a backwards movement. In chapters 2 and 3 we have examined this process using EVS to explore the efficacy of vestibular reflexes. We confirmed

that the sway response is craniocentric in nature, with response direction being in line with head orientation (Lund & Broberg, 1983; Mian & Day, 2009). Previously, response direction has been calculated by averaging sway responses to multiple GVS pulses (Inglis et al., 1995; Welgampola et al., 2013) or long lasting SVS (Dakin et al., 2007; Mian & Day, 2009). In chapter 2 we were able to develop an analytical technique to measure response precision. This will potentially inform us about the efficacy of balance control under more ethological circumstances. We investigated the effect of head direction and vision on both response accuracy and precision. We found a clear dissociation between these two parameters. Accuracy was unaffected by vision, with the evoked response being directed 90 degrees to head direction. However, precision was found to decrease with the eyes open. It is well known that vision reduces the magnitude of EVS-evoked responses, as well as causing a reduction in spontaneous baseline sway (Bent et al., 2002; Fitzpatrick & Day, 2004). We confirmed both observations in chapters 2 and 3. However, more interestingly we found a significant relationship between response precision and magnitude. As responses became larger they simultaneously became more precise.

In chapter 2, two types of stimulation were used, SVS and EVS. Three participants were found to have no significant relationship between head angle and response direction during GVS. Therefore, SVS was deemed to have greater potential for measuring response precision in a clinical setting, and therefore led to its use in the bedrest scenario in chapter 3. Here, 60 day bedrest was used to enforce a period of prolonged inactivity. It is well known that inactivity leads to a

loss of muscular volume and strength. Loss of strength has been demonstrated to have a detrimental effect upon balance (Dirks et al., 2016; Ferrando, Lane, Stuart, Davis-Street, & Wolfe, 1996). Furthermore, balance relies on proprioceptive signals from muscle spindles, whose sensitivity is closely related to the mechanical state of the muscle (Fitzpatrick & McCloskey, 1994; Proske et al., 2000; Taylor & McCloskey, 1992). Muscle weakness results in a higher percentage of MVC being produced for any given contraction, causing the muscle spindles to become saturated resulting a reduction in proprioceptive acuity. (Butler et al., 2008). This loss in proprioceptive acuity results in more spontaneous sway (Butler et al., 2008) and we suggest this is the cause of the less precise EVS-evoked response direction we see in chapter 3.

In both chapters 2 and 3, we explore how sensory integration affects balance control. Previously, additional veridical sensory information has been shown to reduce the magnitude of EVS-evoked responses (Day et al., 2002). This has also been demonstrated for tactile (Britton et al., 1993; Smith & Reynolds, 2017) and proprioceptive modalities (Day & Cole, 2002), as well as for vision (Day & Guerraz, 2007). In chapter 2 we showed that additional visual information that conflicted with vestibular information resulted in a smaller and less precise response. In chapter 3 we suggest that a loss in muscular strength and volume will cause a reduction in proprioceptive acuity, explaining the less precise EVS-evoked response. Sensory integration for balance has been likened to electoral proportional representation, with each sensory modality providing a vote towards the overall estimate of body orientation (Day et al., 2002). Hence, the relative

contribution of any given modality will depend upon how much alternative sensory representation is available. In both chapter 2 and 3, the simultaneous reduction in response size and precision with vision may reflect down-weighting of vestibular and proprioceptive information. In chapter 3 we found that a reduction in muscular strength may reduce proprioceptive acuity and found that these divergent sources, vision vestibular and proprioception produce less precise and larger responses. We also found that there was a significant head orientation effect for both response precision and response magnitude in chapter 3, which was not seen in chapter 2. This difference could be explained by the difference in stance width, where a 4cm stance width was used during chapter 3. A wider stance has previously been shown to affect the accuracy of the sway response (Mian & Day, 2014), where a wider stance resulted in the violation of the craniocentric nature of the EVS-evoked sway response direction. It could therefore be conceivable that this wider stance will also affect the response precision and magnitude of the response.

Non-invasive techniques can measure electrical vestibular stimulation evoked torsional eye movements

In chapter 4 we utilized video technology to develop a new, non-invasive method for measuring eye movements. Previously, 3D eye movements have been measured using invasive techniques such as scleral coils (Severac Cauquil et al., 2003), or drawing marks onto the eye surface with a surgical pen (Jahn, Naessl, Strupp, et al., 2003; Schneider et al., 2002). However, these techniques can carry an increased risk of infection, are not practical for wider clinical use as well as

being uncomfortable for the patients. Video-oculography has been widely used when tracking horizontal or vertical motion, as the pupil can be easily tracked, but torsional motion is a much more complicated task. Our use of a high definition infrared camera and tracking of the striations of the iris allowed us to not only recording of horizontal and vertical motion, but also torsion. Being able to measure torsional motion is vital given our knowledge of the induced sensation produced by EVS (Fitzpatrick & Day, 2004). Torsional motion was seen during sinusoidal EVS up to a stimulus frequency of 20 Hz. Although torsional eye movements were measured at all frequencies, they became increasingly smaller as frequency increased.

After demonstrating that we can record EVS-evoked 3D eye movements, we turned our attention to determining the optimal stimulus frequency to use in a clinical environment. There are two variables to consider 1) signal to noise ratio, and 2) patient comfort. Signal to noise ratios were fairly constant across all frequencies with the exception of stimulus frequencies between 2-6 Hz, which were greater. However, participants found that discomfort increased with frequency, especially above 2Hz. Therefore, we settled on using a 2 Hz sinusoidal EVS stimulus in a patient population in chapter 5.

The central nervous system interprets electrical vestibular stimulation a velocity signal

The sinusoidal stimulus evoked a sinusoidal torsional eye movement which allowed for a measure of phase to be calculated. Although postural and ocular

responses to EVS are well documented, we still have a limited understanding about what motion an EVS stimulus represents; does the CNS interpret EVS as a position, velocity or acceleration signal? Others, have used manual tracking tasks (Peters et al., 2015) or 5-point rotation scales (St George et al., 2011) to try to answer this question. However, these techniques rely on subjective judgements. Our EVS-evoked eye movement measures therefore provide a novel technique to investigate this question by measuring a reflexive movement, unaffected by participant volition. After examining the phase graphs for position, velocity and acceleration signals we concluded that EVS represents a velocity signal. Our rationale for this is once eye position was differentiated into velocity we found that eye velocity and stimulus signals were in phase. EVS-evoked postural responses have also led to the conclusion that EVS produces a dynamic continuous sense of rotation (Day & Cole, 2002; Wardman, Day, & Fitzpatrick, 2003). Continuous stimulation has also been found to produce continuous eye rotation (Schneider et al., 2000; Severac Cauquil et al., 2003), further supporting the conclusion that EVS induces a dynamic continuous sense of rotation and is interpreted by the CNS as a velocity signal.

When the head rotates, the lag of the endolymph fluid within the canals displaces the cupula causing a deflection of the stereocilia bundles in the crista. This deflection produces a cascade of events that ultimately results in an increase or decrease in afferent firing (Fernandez & Goldberg, 1971). The canal response has been likened to a overdamped torsional pendulum (van Egmond, Groen, & Jongkees, 1949) and due to fluid dynamics, whereby the internal diameter of the

canals is smaller than their radii of curvature there is high viscous resistance. This results in endolymph movement being proportional to angular velocity. In summary, the canal-cupula mechanism effectively integrates head acceleration signals such that afferents transmit head angular velocity (Dickman & Angelaki, 2002; Mayne, 1950; Shinoda & Yoshida, 1974). It therefore seems logical that electrically stimulating the vestibular afferents would produce the same signal as that produced by the vestibular mechanics.

Methods of measuring vestibular asymmetry in vestibular schwannoma patients

In chapter 5 we further explore the potential use of electrical vestibular stimulation in a clinical environment by utilising a monaural EVS configuration, allowing for separate testing of each ear. We compared our newly developed eye tracking technique with two other methods, in order to measure asymmetry, the head impulse test (HIT) and EVS-evoked postural reflex test. We tested a patient population with vestibular schwannoma, who are known to have a unilateral vestibular deficit, to determine the location of the tumour and the extent of the deficit.

We found that HIT tests did not detect any asymmetry between each vestibular system, even though we know that this patient group did have a diagnosed vestibular schwannoma. Although we met the criteria of a valid HIT we were still unable to detect unilateral deficits. Deficits have been found in unilateral and bilateral deafferentation patients using a HIT (MacDougall et al., 2009; Weber et

al., 2008), however these patient's entire vestibular system is affected whereas, vestibular schwannoma can arise from either superior or inferior vestibular nerve branches. Although unlikely, if all of our 24 patients had tumours affecting only the inferior nerve, a normal HIT response would be observed. As the horizontal canal is innervated by the superior canal, an abnormal response would only be seen if the tumour origination from this branch. This test however, is not performed in a clinical environment using the quantifiable techniques used in chapter 5. Instead consultants make a subjective assessment on the timing of eye movement in relation to head movement. This can lead to varying diagnoses depending on the consultant performing the test and can produce unreliable result even when performed by the same consultant over time. Thus, empathising the need for a more quantifiable and reliable method of measuring vestibular function.

Both EVS evoked postural responses and EVS evoked ocular responses produced significant asymmetry ratios, indicating that stimulation of the side on which the tumour is located produces smaller responses than stimulation of the healthy side. As the tumour grows it slowly compromises the integrity of vestibulo-ocular nerve. Due to its location within the internal auditory canal it compresses the nerve, which produces symptoms such as unilateral hearing loss and dizziness, and impaired balance. In chapter 6 we are able to identify deficits in vestibular function by measuring EVS-evoked postural and ocular reflexes. This now raises the question 'Which test is the best suited for clinical use?'.

Which is better suited for clinical used, EVS-evoked vestibulospinal or vestibulo-ocular reflexes?

In chapter 5 we compare two EVS-evoked reflexes in patients which vestibular schwannoma. Although both tests are to detect vestibular deficits and asymmetries never correlated with tumour diameter. However, when patients were grouped according to their Koos grade it was the ocular reflex test better differentiated between grades, whereas the postural reflex test showed no difference. This may suggest that our EVS-evoked ocular reflex test is more sensitive. A test that can estimate tumour size would be beneficial, however it is not essential. If a clinician wanted information about the location and size of the tumour, the first-choice test would be an MRI with gadolinium contrast as this would provide more information. However, if a patient was claustrophobic and unable to have an MRI the ability to estimate tumour size would be beneficial.

Unlike vestibulo-ocular reflexes, the EVS-evoked postural response constitutes a behavioural response whose magnitude can be modulated by the availability of vision, and proprioception, and musculoskeletal stability. Therefore, the response cannot exclusively represent vestibular function and any response must be interpreted in the context of other sensory modalities. The EVS-evoked VOR however, could be considered as a purer test of vestibular function as the response involves a three neuron arc, and the test is minimally affected by other sensory information. This factor could be the reason for the apparent increased sensitivity when evoking the VOR. We also found that some of the patients were unable to perform the postural tests, due to severe impaired balance control, poor

musculoskeletal strength as well as other medical conditions. All patients, even those unable to perform the postural tests, were able to complete the EVS-evoked ocular test. This could be a critical difference as those who are most affected may not be able to be tested using postural tests.

The time taken to perform each test to achieve the same level of accuracy and reliability differed greatly between each test. The postural tests required approximately 1hr 45 min to completely, during which time multiple rests are needed between trials. Whereas, ocular tests could be completed within 15-20 mins. Although this may seem trivial in an experimental scenario, in a clinical setting where consultants time is stretched and costs need to be reduced, a quicker test may be preferable. Current monitoring at University Hospital Birmingham, involves 6-monthly MRI scans followed by a consultation with a clinician. Prior to meeting the clinician, a hearing test is performed, taking approximately 20 mins, and is instantly available to the clinician when assessing each patient's treatment. We therefore suggest that an EVS-evoked ocular reflex test could also be performed and would provide further information about vestibular function that could be considered when offering treatment. There is also potential for the assessment of 3D eye movements, not just torsional magnitude, to provide even more information about the exact location of any vestibular lesion. MacDougall et al. (2005) applied EVS to 7 patients with various vestibular deficits. These case studies included bilateral impairment of the lateral semicircular canal, superior and inferior vestibular neuritis, benign paroxysmal positional vertigo (BPPV), superior vestibular nerve section, CHARGE

(coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, ear abnormalities), lateral semicircular canal occlusion and delayed endolymphatic hydrops. EVS, in this case a ramped stimulus peaking at 5mA, was used to evoked 3D eye movements. All participants produced varying eye movements determined by the loss of function associated with their condition. Bilateral patients showed an attenuated response compared to healthy controls, whereas unilateral patients produced normal responses to monaural stimulation to the healthy ear and reduced responses in the diseased ear. This is in keeping with the findings in chapter 5; where Vestibular schwannoma patients were tested. MacDougall and colleagues found that the patient with occlusion of the right lateral canal produced a large asymmetry in ocular torsion and the horizontal nystagmus component indicated a larger response from the right side. This augmented response is consistent with the occluded canal. These case studies indicated that EVS has potential to detect central nervous conditions, such as vestibular schwannoma, but also detect end organs deficits with such sensitivity that it could specify which canal is deficient.

Limitations, future studies and scope for EVS as clinical tool

This thesis has provided further support for the use of electrical vestibular stimulation in a clinical environment. Its ability to test pure vestibular reflexes and more behavioural vestibular reflexes provides many options. However, this thesis has only touched on the potential uses of EVS. Clinical tests are not absolutely conclusive and accurate diagnosis from a number of sources is needed. The use

of EVS a clinical tool would have the same issues and other vestibular tests would be required to confirm a diagnosis. However, EVS would be a useful clinical tool which trying to detect asymmetries in labyrinths, or imbalances in canal function. In this thesis, we tested a group of patients who had compromised vestibular nerves and therefore did not test EVS's potential to detect end organ deficits. However, given the finding from the patient case studies in MacDougall et al. (2005) our newly developed technique could be used to non-invasively diagnose patients with a variety of vestibular deficits.

After demonstrating that EVS-evoked ocular response can indeed detect unilateral deficits in vestibular schwannoma patients we now need to compare our results against other NHS used tests such as caloric irrigation. Caloric irrigation has its limitations; it only tests the integrity of the horizontal canals and can be a noxious stimulus for patients. A recent audit performed by the ENT department at the Queen Elizabeth Hospital revealed that 33% of patients referred for caloric testing could not undergo the test for reasons such as heart problems, epilepsy, high blood pressure, wax filled ears and anxiety during the test. Therefore, an alternative test which is more inclusive would be beneficial. Alternative vestibular tests include chair rotation, head impulse test and vestibular-evoked myogenic potentials (VEMPs). The equipment needed for chair rotation is expensive and the test itself cannot detect unilateral dysfunction. The head impulse test requires considerable training and expertise to be able to test all canals. We found HIT unsuccessful in assessing unilateral dysfunction in vestibular schwannoma, for reasons unknown. VEMPs are rarely used tests

otolith function and cannot detect semicircular canal deficits. An alternative test could involve the use of EVS due to its quick application and history of being well tolerated by a variety of patient groups (MacDougall et al., 2005; Morris, Iansek, Smithson, & Huxham, 2000).

In respect of exploring the effects of prolonged inactivity further, a protocol in which both vestibular function and proprioceptive acuity are tested would help discover the cause of the decrease in response precision found in chapter 3. The postural responses evoked in chapter 2 and 3 represent behavioural responses affected by numerous factors, and so provide limited information about the efficacy of vestibular or proprioceptive inputs. A purer vestibular test, such the EVS-evoked torsional eye movement test used in chapter 4 and 5, could be used to measure the effect that prolonged inactivity has on vestibular function. Another benefit of this test is that it could be used throughout the bedrest period as it does not require the participant to stand. This would allow the change in vestibular function over time to be studied, potentially finding linearly decline over time or if there is exponential decrease in function. Pairing this test with a proprioceptive acuity test such as the 2-point discrimination test would also provide a similar time course for proprioceptive function. This would augment the data collected in chapter 3 where changes were only examined pre and post intervention.

Final remarks

The studies which has contributed to this body of work allows a number of general conclusions to be made. Vestibular inputs are used for balance control. However a transformation from head to foot-centred coordination must occur for these inputs to be useful. EVS can be used to investigate this process and it has been shown that visual information has no effect upon response accuracy but does in fact reduce response precision. Proprioception is also vital for balance control and prolonged inactivity could reduce muscular strength and in turn proprioceptive acuity, therefore impair balance control. This reduction in acuity does not affect response accuracy but, as with vision, it does reduce response precision. Both findings can be explained by sensory integration, whereby sensory conflicts produce less precise sway responses.

EVS appears to be interpreted by the CNS as a velocity signal, which is in keeping with literature about the peripheral mechanisms of the vestibular end organs. This conclusion came from the development of techniques used to measure EVS-evoked ocular responses. The same technique was then used along with EVS-evoked postural tests to detect unilateral dysfunction in vestibular schwannoma patients, further supporting the potential use of EVS in a clinical environment. EVS-evoked ocular response appears to have greater potential due as it quicker to perform, more sensitive than postural tests and is less affected by other sensory modalities and patient volition.

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