THE EFFECTS OF LOW-FREQUENCY SINUSOIDAL LINEAR ACCELERATION ON SKIN SYMPATHETIC NERVE ACTIVITY IN HUMANS

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STATEMENT OF AUTHENTICATION

I, Monique Foster, declare that this thesis is original and based entirely on my own independent work except if acknowledged and referenced in the text. I declare that I have not submitted this material to gain qualification for this Master of Research, or any other degree at Western Sydney University, and it has not been submitted to any other academic institution.

Monique Foster
ABSTRACT

Motion sickness, caused by actual or apparent motion, is a common disorder that is yet to be fully understood. It is a complex disorder that transcends the most common symptoms of nausea and vomiting, with several of the symptoms or symptom-complexes being under-researched or not well understood. One of these symptom-complexes is centred around the drowsiness, lethargy and irritability associated with typical motion sickness, is known as sopite syndrome. It has previously been shown that sopite syndrome can be caused by exposure to low-frequency motion over a longer duration. Additionally, it is known that the vestibular apparatus, being activated by motion, plays an important role in the pathogenesis of motion sickness, as robust vestibular modulation of skin sympathetic nerve activity (SSNA) has been demonstrated. However, there is currently no research quantifying the physiological changes that occur during sopite syndrome. Therefore, this study aims to quantify the physiological effects of low-frequency motion on SSNA, skin blood flow and heart rate variability (HRV), while also measuring self-reported sleepiness in subjects. The techniques used to obtain these measurements were: microneurography to record SSNA, infrared plethysmography to record skin blood flow, ECG to analyse and determine HRV and the Karolinska Sleepiness Scale to compare sleepiness before and after motion. Subjects were seated upright and exposed to a randomised sequence of five slow, sinusoidal motions on a motorised platform: 0.03 Hz at 0.5 mg, 0.05 Hz at 0.5 mg, 0.1 Hz at 0.5 mg, 0.1 Hz at 5 mg and 0.2 Hz at 5 mg. Cross-correlation analysis was used to calculate the vestibular modulation of SSNA, which was present at all frequencies and ranged from 30.7 ± 3.8 (0.2 Hz) to 37.2 ± 4.4 (0.1 Hz at 5 mg). There was no significant difference in the
modulation indices when the magnitude of vestibular modulation was compared across all frequencies. However, the vestibular modulation of SSNA was greater than cardiac modulation at all frequencies, and the cardiac modulation during each frequency was less than the baseline value. Additionally, the skin blood flow was lower than the baseline at all frequencies and there was no statistical change in any of the HRV indices. Thus, it has been shown that very low-frequency sinusoidal motion can be used to activate the vestibular system causing modulation of SSNA. However, even though subjects reported feeling relaxed or drowsy upon completion of the protocol, with some even falling asleep throughout, it didn’t appear that the motion stimulus applied caused the onset of physiological relaxation.
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CHAPTER 1

INTRODUCTION
1.1 Microneurography

This section will explain the background and developments of microneurography, in particular, the early stages of its conception, the improvements made along the way and the main breakthroughs and discoveries that resulted from the method. It will then discuss the general characteristics of skin sympathetic nerve activity (SSNA) and its related physiological parameters and stimuli. Finally, the manoeuvres affecting skin sympathetic nerve activity will be discussed.

1.1.1 Background and Developments

Microneurography is a nerve recording technique that involves the insertion of a tungsten microelectrode percutaneously into a peripheral nerve. It can be used to record both efferent (from the brain) and afferent (to the brain) responses within either skin or muscle sympathetic nerves (Macefield, 2013, Vallbo et al., 2004).

Prior to the conception of microneurography, the majority of nerve recording data was obtained from reduced preparations, such as animals that were either anaesthetised or decerebrate (lacking a cerebrum – removed for experimental purposes) (Vallbo et al., 2004). It was believed that, due to the nature of the small currents produced by the nerve fibres and the low resistance between the intraneural and reference electrodes, it was not possible to use an extracellular needle in order to record nerve impulses (Vallbo et al., 2004). It was also argued that the risks of damage, trauma, infections and intraneural bleedings from inserting an electrode into the nerve were much too high to justify experiments utilising intraneural microelectrodes in awake human subjects (Vallbo et al., 2004). It is important to note that although, at the time microneurography was conceived, most nerve recordings were obtained from animals, the microneurography technique was
not the first to record normal traffic of nerve impulses from human subjects (Sears, 1959, Hensel and Boman, 1960, Vallbo et al., 2004). Hensel and Boman (1960) obtained high-quality cutaneous nerve recordings by cutting open the skin of young volunteers under local anaesthesia and splitting the nerve until a single fibre was separated (Hensel and Boman, 1960). And, in 1959, Sears explained a non-invasive technique using extracellular electrodes to record compound action potentials (Sears, 1959). However, these techniques were either too invasive and had too many associated risks, or weren’t viable to continue or apply to other areas (Vallbo et al., 2004).

The microneurography technique was developed in 1965-1966 in Uppsala, Sweden, in the Academic Hospital’s clinical neurophysiology department, by two neurophysiologists, Karl-Erik Hagbarth and Åke Vallbo (Vallbo et al., 2004). It was first described by them at a meeting of the Scandinavian EEG Society in Copenhagen, 1966 (Vallbo and Hagbarth, 1967), and was followed up with two articles the next year (Hagbarth and Vallbo, 1967a, Hagbarth and Vallbo, 1967b). It was then further described by them, and their colleagues Wallin and Delius many times in the years to follow (Hagbarth and Vallbo, 1967a, Hagbarth and Vallbo, 1967b, Vallbo and Hagbarth, 1967, Hagbarth and Vallbo, 1968, Hagbarth et al., 1970, Delius et al., 1972a, Delius et al., 1972b, Hagbarth et al., 1972, Vallbo et al., 2004).

The technique was not born overnight, however, it required many months of testing and redesigning to determine what worked best and to finalise the technique (Vallbo et al., 2004). The initial step was performed by Hagbarth, in which he inserted a needle into his own ulnar nerve and was able to distinguish a very faint modulation of noise when a stimulus was applied to an area innervated by the nerve (Vallbo et al., 2004). However, the signal-to-noise ratio was much too low to give clear recordings or be useful at that stage.
This led to many trial-and-error experiments to try and increase the signal to noise ratio, testing electrodes made with a wide range of materials and designs (Vallbo et al., 2004). The final electrode design was made using tungsten, was larger than those previously used in animal studies, with a tip of 5-10µm and was coated with an epoxy-based resin (Vallbo et al., 2004, Macefield, 2013).

While the design and functionality of the electrode was gradually improving, so too was the signal-to-noise ratio of the nerve recording (Vallbo et al., 2004). With the final microelectrode design, as described above, Hagbarth and Vallbo were able to record data of a high enough quality to distinguish afferent modulations of both skin and muscle (Vallbo et al., 2004). Furthermore, during the development of the technique, they performed regular tests assessing any associated risks that may have presented (Vallbo et al., 2004). These tests were performed on Hagbarth and Vallbo themselves to determine the safety of the procedure; they involved stringent observation of signs and symptoms as well as evaluating for any indication of denervation using EMG tests (Vallbo et al., 2004). After this preliminary period, they were satisfied with the efficiency and safety of the procedure – it having only minor side effects documented – and were confident in the ability to recruit young, healthy volunteers in future research (Vallbo et al., 2004).

Microneurography was developed in a time before computers and so, the equipment required to record the nerve signal included the following: amplifiers, oscilloscopes, an analogue tape recorder and cameras to register the events from the oscilloscope and record it onto light-sensitive paper (Vallbo et al., 2004). Because the experimenter was not able to see the screen while the film was running, they often did not
know whether or not the recording was adequate or if they found anything interesting until they were developing the film (Vallbo et al., 2004).

Over many years, and with an ever-growing team of researchers, Hagbarth and Vallbo made many breakthroughs and discoveries in the field. The primary goal was to be able to record from muscle spindles to research the servo control theory – the primary theory of motor control at the time (Vallbo et al., 2004, Macefield, 2013). And, in the beginning, they were more than satisfied with the prospect of only recording multiunit activity (Macefield, 2013, Vallbo et al., 2004). The first experiments targeted human muscle spindles, and it soon became clear that the technique could be used to record activity elicited by muscle stretch and contractions from individual muscle spindle axons and single afferents (i.e. they could record from multiunit as well as single unit afferents) (Hagbarth and Vallbo, 1967a, Hagbarth and Vallbo, 1967b, Torebjork and Hallin, 1974, Vallbo et al., 2004, Macefield, 2013). Although the discovery that it was possible to obtain single-unit recordings was made quite early on (Torebjork and Hallin, 1974), the signal-to-noise ratio was low and the available methods to prove that the signal was from a single unit were inadequate. It was another 20 years before a serious attempt to record single-units was developed, when (Macefield et al., 1994) integrated a much more sophisticated computer program into the methods and analysis. Thus, creating a more efficient technique and leading to a greater understanding of sympathetic drive and its changes in healthy and diseased states (Macefield et al., 2002, Vallbo et al., 2004).

The next discovery was based on a modulation of the noise that could be heard during experiments that sounded like “waves approaching a distant shore” (Macefield et al., 2002, Vallbo et al., 2004). It was originally believed that the only explanation for this sound
was that it was either an artefact or efferent activity from nearby gamma fibres. But after much testing of these hypotheses with only evidence against and not for, as well as a fresh start with more open-mindedness, Hagbarth and Vallbo discovered that the tungsten microelectrode, initially thought to be too large, could be used to record from unmyelinated fibres as well as myelinated fibres – a surprising discovery at the time (Hagbarth and Vallbo, 1967a, Hagbarth and Vallbo, 1967b, Vallbo and Hagbarth, 1967, Delius et al., 1972b, Hagbarth et al., 1972, Vallbo et al., 2004, Macefield, 2013). Following on from this, they found that what sounded like waves on the shore was, in fact, efferent bursts of sympathetic activity – termed “spontaneous” nerve activity (Vallbo et al., 2004, Macefield, 2013). It took a great deal of time to conclude that, using microneurography, they could record sympathetic activity travelling from the brain to the periphery as they did not believe that, in those such early stages, it was possible to obtain (Vallbo et al., 2004, Macefield, 2013).

With this new information, Hagbarth and Vallbo continued to explore the sympathetic nervous system – focusing mostly on muscle and skin nerves. It was in these experiments that they made one of the most important discoveries. They determined that muscle and skin sympathetic activity presented in different ways, have different effectors and, are linked with different external stimuli (e.g. stretch, noise), physiological parameters (e.g. blood pressure, emotions) and reflexes (Hagbarth and Vallbo, 1967a, Hagbarth and Vallbo, 1968, Hagbarth et al., 1972, Vallbo et al., 2004, Macefield, 2013).

This was unexpected because, up until this point, the prevailing theory of the sympathetic nervous system stated that it was diffusely organised and that the nerve traffic was similar for both muscle and skin (Vallbo et al., 2004, Macefield, 2013). Therefore, this
discovery led to a major early achievement for microneurography as it provided the first steps towards disproving the theory of a diffuse sympathetic system and provided strong evidence leading to today’s definition of a highly-differentiated system instead (Vallbo et al., 2004, Macefield, 2013).

The following experiments and descriptions targeted human muscle nerves and the relationship they have with other physiological parameters, such as the baroreflex and blood pressure (Hagbarth and Vallbo, 1967b, Hagbarth and Vallbo, 1968, Delius et al., 1972b). They then described cutaneous nerves and their related physiological parameters, including skin blood flow (skin vasoconstriction) and skin resistance (sweat release) (Delius et al., 1972a, Hagbarth et al., 1972, Macefield and Wallin, 1999). The next step was determining the actions and manoeuvres that affected the frequency of spontaneous bursts (discussed section 1.1.3). Today the majority of experiments utilising the microneurography technique have targeted muscle sympathetic nerve activity and to a lesser extent skin sympathetic nerve activity (Macefield, 2013).

1.1.2 Characteristics of skin sympathetic nerve activity

As described above, the fact that skin and muscle sympathetic nerve activity (SSNA and MSNA) have different characteristics and associated factors was discovered in early experiments utilising the technique. In fact, the two are so different that it is possible to differentiate between spontaneous skin and muscle nerve activity, simply by the character of their patterns and bursts (Delius et al., 1972a, Hagbarth et al., 1972, Vallbo et al., 2004, Macefield, 2013).
It is known that spontaneous bursts of MSNA occur in between intervals of relative silence, in a pulse synchronous manner, and are generally seen as distinct, short, irregular sequences (Delius et al., 1972a, Hagbarth et al., 1972, Macefield, 2013). Bursts of MSNA are also linked with vasoconstriction as well as spontaneous variations in blood pressure, showing that the baroreflex has an inhibitory effect on sympathetic outflow to muscle nerves (Delius et al., 1972a, Delius et al., 1972b, Hagbarth et al., 1972, Macefield, 2013).

However, skin sympathetic nerve activity appears differently, the bursts are not related to the pulse rhythm or blood pressure changes; they occur randomly, irregularly and vary in duration (Delius et al., 1972a, Hagbarth et al., 1972, Vallbo et al., 2004). SSNA is instead coupled with respiration, a relationship that is especially prominent during periods of deep breathing, such as when the subject is relaxed or asleep (Delius et al., 1972a, Hagbarth et al., 1972, Macefield and Wallin, 1999, Vallbo et al., 2004).

In addition to the different natures of sympathetic outflow, functional differences between the nervous control of the vascular beds that each of the two nerve types, skin and muscle, innervate have been shown using regional blood flow measurements (Delius et al., 1972a, Hagbarth et al., 1972, Macefield and Wallin, 1999, Vallbo et al., 2004). The sympathetic activity recorded from skin nerves mainly consists of impulses from cutaneous vasoconstrictor and sudomotor fibres (Delius et al., 1972a, Hagbarth et al., 1972, Vallbo et al., 2004, Macefield, 2013). Thus explaining why the main purpose of sympathetic outflow to the skin is thermoregulation; the outflow controls the amount of heat lost from the body by controlling the sweat glands and cutaneous blood vessels (Macefield and Wallin, 1999, Macefield, 2013). Additionally, SSNA plays a large part in emotional expression. Therefore, applying arousing stimuli (e.g. loud noises), changing the ambient temperature, and/or
changing the emotional or attentive state (such as with provocative images) can be used to affect the activity of SSNA (Brown and Macefield, 2014, Macefield, 2013).

1.1.3 Manoeuvres affecting skin sympathetic nerve activity

As explained previously, the intensity of sympathetic outflow to skin can be enhanced/decreased by a variety of external stimuli and manoeuvres that act to alter the emotional or attentive state of the subject. The manoeuvres/stimuli that affect skin will be discussed below, and include mental strain and emotional stimuli, thermal stimuli and, sudden changes in respiration.

Multiple studies have shown that skin sympathetic nerve activity is directly related to the arousal state of a subject – both emotional and attentive (Delius et al., 1972a, Brown et al., 2007, Brown and Macefield, 2014). When a subject is experiencing mental stress the activity can be seen to increase, and it decreases during periods of relaxation (Delius et al., 1972a, Hagbarth et al., 1972). Increased sympathetic activity resulting from a stressful situation is also accompanied by increased sweat release (measured by galvanic skin resistance) and skin blood flow (measured by plethysmography) (Delius et al., 1972a, Hagbarth et al., 1972, Brown et al., 2007, Brown and Macefield, 2014). One of the most common means of testing for spontaneous SSNA is performing an action that surprises the subject, for example, a loud clap or shout, or a sudden tap on the head of the subject (Delius et al., 1972a, Hagbarth et al., 1972, Macefield, 2013).

It is important to note that changes in ambient temperature can also affect the intensity of SSNA; if the subject’s body is warmed, the sympathetic activity decreases, and, conversely, the activity distinctly increases when the body is cooled (Delius et al., 1972a,
Hagbarth et al., 1972, Macefield, 2013). As skin activity is coupled with skin blood flow and sweat release, during body cooling, skin blood flow is markedly decreased and sweat release is eliminated (the opposite occurs when the body is warmed) (Macefield, 2013).

As previously stated, SSNA is coupled with the respiratory rhythm, more so with the inspiratory phase than the expiratory phase, therefore, certain respiratory manoeuvres can alter the intensity of SSNA (Delius et al., 1972a, Hagbarth et al., 1972, Macefield and Wallin, 1999, Vallbo et al., 2004, Macefield, 2013). A respiratory capacity apnoea manoeuvre is known to cause a sustained increase in MSNA, however, this is not the case for SSNA (Delius et al., 1972a, Macefield and Wallin, 1999, Vallbo et al., 2004, Macefield, 2013). Instead, the manoeuvres used to test for spontaneous skin sympathetic nerve activity include a brisk sniff through the nose or a sudden deep inspiration (sigh or gasp) (Delius et al., 1972a, Macefield and Wallin, 1999, Vallbo et al., 2004, Macefield, 2013).
1.2 Vestibular System

1.2.1 Structure and physiology

It is known that the vestibular apparatus plays a role in the pathogenesis of motion sickness and that the vestibular system modulates SSNA (Hammam et al., 2014, Klingberg et al., 2015). It has also recently been found that, in participants that report feeling nauseated, vestibular modulation of skin sympathetic nerve activity is greater than in participants who did not (Hammam et al., 2014, Klingberg et al., 2015). Therefore, the structure and physiology of the vestibular system is important for thorough understanding in this study, and will be discussed in this section.

The vestibular system, consists of elaborately organised, fluid filled chambers, and is housed within the petrous part of the temporal bone (Highstein and Holstein, 2012, Purves et al., 2012). It is a sensory system providing important information about equilibrium and spatial orientation – it detects motion of the body, head position, and provides proprioceptive information (Fitzpatrick and Day, 2004, Highstein and Holstein, 2012, Purves et al., 2012). The interconnected chambers of the vestibular system are referred to as the labyrinth; this labyrinth is continuous with and, located postero-lateral to the cochlea of the inner ear (Highstein and Holstein, 2012, Purves et al., 2012).
Figure 1.1. Illustration demonstrating the structural anatomy of the labyrinth of the vestibular system. Each semicircular canal is illustrated, at the base of which the ampullae are highlighted in green. The otolithic organs, the utricle and the saccule within the vestibule, are highlighted in blue. (Figure and legend modified from (Highstein and Holstein, 2012)).

There are two separate structures that comprise the vestibular system: the bony labyrinth and the membranous labyrinth. The bony labyrinth surrounds and protects the membranous labyrinth and is filled with a fluid similar to cerebrospinal fluid, called perilymph (Purves et al., 2012). The bony labyrinth is comprised of three parts: the semicircular canals, the vestibule and the cochlear (Figure 1.1) (Fitzpatrick and Day, 2004, Purves et al., 2012). The membranous labyrinth is housed within these structures, is filled with a different fluid called endolymph, and consists of five receptor organs, known as the semicircular ducts (x 3; within semicircular canals) and the otolith organs (x 2; within the vestibule; Figure 1.1) (Fitzpatrick and Day, 2004, Purves et al., 2012). At the point where the semicircular canals meet the vestibule, i.e. where the semicircular ducts meet the otoliths, there are three small swellings known as ampullae. The semicircular canals are oriented approximately perpendicular to each other and perceive angular velocity or rotational movements of the head, in the direction that they are situated (Fitzpatrick and Day, 2004, Nolte, 2009, Purves et al., 2012). The otolith organs are known as the utricle and saccule...
and they, on the other hand, perceive linear or translational movements of the head as well as the position of the head in relation to gravity.

The vestibular system contains specialised sensory receptor cells within the ampullae and otoliths; these cells detect head position or movement with respect to gravity by converting cell displacement into action potentials, which in turn, leads to neurotransmitter release (Fernandez et al., 1972, Fernandez and Goldberg, 1976, Fitzpatrick and Day, 2004, Nolte, 2009, Purves et al., 2012). In essence, movement of the head leads to displacement of these receptor cells, which then causes a translation of a mechanical stimulus into an electrochemical potential. This electrochemical potential represents the beginning of vestibular sensory information processing.

The sensory receptor cells are known as hair cells, because approximately 60-100 interconnected (via small filaments) stereocilia project from the apex of each cell. Each hair cell also contains one longer hair-like filament, called the kinocilium; the stereocilia are arranged in rows that gradually increase in height until they reach the single kinocilium (Figure 1.2) (Fitzpatrick and Day, 2004, Nolte, 2009, MacNeilage et al., 2010, Purves et al., 2012). Within the semicircular ducts and ampullae, the hair cells are rooted in the crista ampullaris, which lines the base of the ampulla and consists of blood vessels, nerve fibres and supporting tissue (Fitzpatrick and Day, 2004, MacNeilage et al., 2010, Purves et al., 2012). The cupula, a flexible membrane encloses each crista and separates the ampulla from the otoliths. Between the crista and the cupula, the hair cells extend into a gelatinous mass, and when angular motion is detected and the cupula is disrupted, causing movement of the mass and, in turn, stimulating the cells (Fitzpatrick and Day, 2004, MacNeilage et al., 2010, Purves et al., 2012).
Figure 1.2. Cross-sectional view of the hair cells of the vestibular system showing the hair cells. This figure shows the cross-sectional anatomy of the hair cells with associated kinocilia. The arrow at the top signifies which direction the hair cells must be deflected in order to cause depolarisation.

Figure 1.3. Anatomical structure of the macula of either the utricle or saccule. A) Shows an overview of the anatomy including the hair cells, otolithic membrane and supporting cells. B) Shows the way in which the hair cells move as a result of a backwards head tilt. (altered from Purves et al., 2012).
The hair cells of the utricle and saccule are embedded in a structure called the macula; within the vestibule, the hair cells of the utricle line the floor, and those of the saccule line the medial wall (Figure 1.3 A) (Fitzpatrick and Day, 2004, MacNeilage et al., 2010, Purves et al., 2012). The hair cells of the utricle and saccule also project into a gelatinous layer, on top of this layer rests the otolithic membrane – a layer which is embedded with calcium carbonate crystals, called otoconia (Fitzpatrick and Day, 2004, MacNeilage et al., 2010, Purves et al., 2012). The otoconia make the otolithic membrane heavier than the cupula, and are the reason why the otoliths are sensitive to gravity and linear motion. The otolithic membrane moves in relation to the macula as a result of gravity acting on a tilted head (Figure 1.3 B); likewise, linear motion displaces the otoconia, gelatinous mass, and consequently the hair cells in the opposite direction of the movement. The position and direction of the hair cells in the utricle and saccule determine the direction of motion that they perceive (Fitzpatrick and Day, 2004, MacNeilage et al., 2010, Purves et al., 2012). When an individual is standing, or sitting, upright, the utricle is located horizontally and the saccule is located vertically. Therefore, the utricle and saccule, detect motion in the horizontal and vertical planes, respectively. The utricle is particularly sensitive to linear motion in the antero-posterior axis and is the target sensory organ for this study.

Afferent neurons projecting from the vestibular (Scarpa’s) ganglion, found near the ampulla, innervate each hair cell (Fernandez et al., 1972, Fernandez and Goldberg, 1976, Highstein and Holstein, 2012, Purves et al., 2012). The firing rate of the vestibular nerve is increased when hairs are bent towards the kinocilium and decreased when they are bent away from it (Fernandez et al., 1972, Fernandez and Goldberg, 1976, Purves et al., 2012). The depolarisation of the hair cells causes action potentials to be sent through afferents in the vestibular branch of the of the vestibulocochlear nerve (cranial nerve VIII) to four target
vestibular nuclei (medial and lateral on each side) in the medulla and pons (Highstein and Holstein, 2012, Purves et al., 2012). The vestibular nuclei receive input from multiple sources – the contralateral vestibular nuclei, cerebellum, and the visual and somatic sensory systems – and send projections to the spinal cord cerebellum and cerebral cortex of the CNS (Highstein and Holstein, 2012, Purves et al., 2012). This network of nuclei, axons and connections work together to integrate the signals and contribute to the essential functions of the vestibular system, such as, head proprioception, equilibrium and postural stability (Highstein and Holstein, 2012, Purves et al., 2012).

1.2.2. Activation of the vestibular system

The hair cells of the vestibular system are highly sensitive and can be activated both physiologically and electrically by actual motion or galvanic vestibular stimulation (GVS), respectively. As explained, physiological motion in a laboratory using a motorised platform, or in a real world setting can activate different parts of the vestibular apparatus depending on the plane and direction of motion (Grewal et al., 2012, Bolton et al., 2016). For example, the hair cells of the utricle are activated by linear movements in the horizontal plane, while saccular hair cells are activated by linear movements in the vertical plane and the semicircular canals respond to rotational movements.

Another method of activating the vestibular system is known as sinusoidal galvanic vestibular stimulation (sGVS); it involves placing Ag-AgCl surface electrodes over the mastoid process (anode on right mastoid) and to apply an electrical stimulation as sinusoidally modulated galvanic vestibular stimulation (Klingberg et al., 2015). While a motorised platform can be used to target specific vestibular system end-organs, sGVS, being placed on the mastoid process, activates the whole apparatus (Grewal et al., 2012, Bolton et
al., 2016). However, previous research has shown that only activation of the otolithic organs causes vestibular modulation of sympathetic nerve activity (Grewal et al., 2012, Bolton et al., 2016).
1.3. The Autonomic Nervous System

1.3.1. Overview

The human nervous system consists of the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS contains the brain and spinal cord and is the major control centre within the body. The PNS comprises all of the peripheral nerves outside of the CNS; it is divided into the somatic (SNS) for voluntary muscle contractions, and autonomic nervous system (ANS) for involuntary functions.

The ANS requires no conscious management from the CNS to perform the actions needed to control most visceral functions within the body (Pratt et al., 1995, Alexander et al., 2008, Nolte, 2009). The CNS and PNS provide afferent inputs to the ANS, while the divisions of the ANS comprise the efferent outflow (Pratt et al., 1995, Alexander et al., 2008, Nolte, 2009). The ANS is divided into the sympathetic, parasympathetic and enteric subdivisions, each of which are functionally and structurally different from the others (Nolte, 2009). The enteric nervous system is less well researched than the other two and controls the motility and function of the gut (Nolte, 2009). It consists of two interconnected plexuses, the myenteric plexus and the submucosal plexus (Nolte, 2009). The sympathetic and parasympathetic divisions however, are more well-known and the visceral motor neuron activity is controlled by visceral sensory fibres, ascending sensory pathways, reflex arcs, and descending pathways (Pratt et al., 1995, Alexander et al., 2008, Nolte, 2009). The efferents of both the sympathetic and parasympathetic divisions do not have direct contact with their target organs, they instead consist of a two-neuron chain (Alexander et al., 2008, Nolte, 2009). The first neuron originates in the CNS, is thinly myelinated, and is called the preganglionic neuron, the second neuron, on which the preganglionic neuron’s axon terminates, is the unmyelinated postganglionic neuron (Pratt et al., 1995, Alexander et al.,
The postganglionic neuron originates in the autonomic ganglia and relays information to the target effector organ through neurotransmitter release at the synaptic end (Pratt et al., 1995, Alexander et al., 2008, Nolte, 2009). The neurotransmitter released by the postganglionic neuron is different in each system; the parasympathetic division releases acetylcholine, while the sympathetic division releases norepinephrine (except for when relaying messages to the sweat glands) (Nolte, 2009). The parasympathetic nervous system’s overall function is to enhance the storage of energy by shifting into what we call a state of “rest and digest”, characterised by decreased cardiac output and blood pressure, and increased gut motility and salivation (Pratt et al., 1995, Alexander et al., 2008, Nolte, 2009). The sympathetic nervous system on the other hand is related to a state of stress known as the “fight or flight response”, in which it is expected that energy will be expended, rather than saved (Nolte, 2009). While the divisions are functionally and structurally distinct, most organs are oppositely influenced by both divisions of the ANS (Pratt et al., 1995, Alexander et al., 2008, Nolte, 2009). Since the experiments of this thesis will be recording the impulses from sympathetic nerves, I will focus on the sympathetic nervous system for the remainder of this section.

The preganglionic fibres of the sympathetic nervous system primarily receive excitatory information from the rostral ventrolateral medulla (RVLM) of the brainstem (Dampney et al., 2002, Dampney et al., 2003). They originate from the neurons of the thoracic and upper lumbar section of the spinal cord (T1-L2), thus the information coming from this section is referred to as thoracolumbar outflow (Pratt et al., 1995, Alexander et al., 2008, Nolte, 2009). Myelinated preganglionic sympathetic neurons mainly emerge from the lateral horns or interomediolateral nucleus of the spinal cord; they then exit through the ventral roots to synapse on the soma of the sympathetic postganglionic neurons of either
the paravertebral (sympathetic chain) ganglia or the prevertebral ganglia (e.g. coeliac) (Pratt et al., 1995, Alexander et al., 2008). The target organs, such as the heart, respiratory system, sweat glands, blood vessels and smooth muscles of the gastrointestinal tract, are innervated by the unmyelinated axons projected from the postganglionic neurons of the sympathetic system (Wallin and Charkoudian, 2007, Alexander et al., 2008). It is important to know that the heart and circulation in the periphery are primarily innervated by sympathetic neurons arising from the interomediolateral column in the spinal cord, and the majority of the vascular nerves trigger vasoconstriction (Wallin and Charkoudian, 2007, Alexander et al., 2008). Functionally, blood flow is modulated by these sympathetic vasoconstrictor neurons as they act on the arterioles, causing decreased skin blood flow as a result of vasoconstriction when acting upon cutaneous vessels (Pratt et al., 1995, Wallin and Charkoudian, 2007, Alexander et al., 2008). By using microneurography to target cutaneous fascicles of postganglionic sympathetic axons within human peripheral nerves, it has been shown that skin sympathetic nerve activity (SSNA) is complex, consisting of predominately vasoconstrictor and sudomotor fibres (Wallin and Charkoudian, 2007). The sympathetic outflow to the skin can be influenced by various factors, such as respiration, arousal and mental stress, its central purpose is to control thermoregulation of the body (Delius et al., 1972a, Hagbarth et al., 1972, Wallin and Charkoudian, 2007).

1.3.2. Heart rate variability

Heart rate variability (HRV) is a common and non-invasive technique to measure the autonomic outflow to the heart, it is quantified by measuring the variation in timing of each interval between consecutive R-waves of the ECG (Malliani et al., 1990, Task force of the European Society of Cardiology, 1996, Burton et al., 2010). It can be used as an index of

The pacemaker tissues of the heart intrinsically control the automaticity of the cardiac cycle, however, the autonomic nervous system predominately controls the heart rate and rhythm (Sayers, 1973, Akselrod et al., 1981, Task force of the European Society of Cardiology, 1996, Lombardi, 2000). The parasympathetic influence on the heart rate culminates in the vagus nerve releasing acetylcholine, which in turn activates muscarinic receptors to increase cell membrane potassium conductance (Task force of the European Society of Cardiology, 1996). Alternatively, the sympathetic influence mediates the heart rate by releasing epinephrine and norepinephrine, leading to acceleration of slow diastolic depolarisation (Task force of the European Society of Cardiology, 1996). During a period of rest, the vagal tone of the heart is the predominate modulator, and at all times, the vagal and sympathetic activity are interacting; however, parasympathetic influences are greater than sympathetic influences (Sayers, 1973, Akselrod et al., 1981, Malliani et al., 1990, Task force of the European Society of Cardiology, 1996, Lombardi, 2000).

Variations in the RR intervals at rest are an indication of finely tuned beat-to-beat control mechanisms. A stimulus that activates the vagal afferents will excite vagal efferents and inhibit sympathetic efferents, while activation of sympathetic afferents will cause the opposite excitation-inhibition profile (Task force of the European Society of Cardiology, 1996). Oscillators within the central (vasomotor and respiratory) and peripheral (blood pressure and respiratory) centres can regulate the cardiac synchronous discharge, caused by efferent activity from both the sympathetic and vagal systems to the sinus node (Malliani et al., 1990, Task force of the European Society of Cardiology, 1996). It is these oscillators that
are responsible for the variability in heart rate as they cause the discharge from efferents to have rhythmic deviations (Task force of the European Society of Cardiology, 1996).

HRV can be assessed using a variety of methods, the most common of which (and those that were used during this thesis) being time domain and frequency domain measures (Sayers, 1973, Akselrod et al., 1981, Malliani et al., 1990, Task force of the European Society of Cardiology, 1996, Lombardi, 2000, Burton et al., 2010). These measurements, in comparison to other available measures of HRV, can be successfully used to gain accurate information about the relative sympathetic drive to the heart in recordings of shorter length (less than 24-hours, with 5 minutes or more being ideal) (Sayers, 1973, Akselrod et al., 1981, Malliani et al., 1990, Task force of the European Society of Cardiology, 1996, Lombardi, 2000, Dimitriev et al., 2014). One index that can measure the vagal activity in the time domain is the root mean square successive differences (RMSSD); this measures the short-term variability between consecutive RR intervals that are larger than 50 ms (Task force of the European Society of Cardiology, 1996, Burton et al., 2010, Dimitriev et al., 2014).

However, it is measurements in the frequency domain including the low-frequency (LF; 0.04 – 0.15 Hz) and high-frequency (HF; 0.15 – 0.4 Hz) components that generally provide an easier way to interpret the results as a measure of physiological regulations (Akselrod et al., 1981), Malliani et al. (1990), (Task force of the European Society of Cardiology, 1996, Lombardi, 2000, Burton et al., 2010). Variations in the HF component are indications of efferent input from vagal, primarily parasympathetic activity (Akselrod et al., 1981, Malliani et al., 1990, Task force of the European Society of Cardiology, 1996, Burton et al., 2010). Interpretation of the low-frequency component, however, has been controversial; it was originally believed to be purely an indicator of sympathetic activity, but now it is more widely accepted that it indicates both sympathetic and parasympathetic
modulation (Sayers, 1973, Akselrod et al., 1981, Task force of the European Society of Cardiology, 1996, Lombardi, 2000, Burton et al., 2010, Dimitriev et al., 2014). Additionally, relative sympathetic drive to the heart can be determined through changes in the LF to HF ratio – an increase in the ratio has been suggested to reflect a relative enhancement of cardiac sympathetic drive (Task force of the European Society of Cardiology, 1996, Burton et al., 2010, Dimitriev et al., 2014). The factors that affect the input to the heart and thus, the level of HRV include, the baroreflex, thermoregulatory effects, hormone release, food, activity and arousal or stress. Moreover, the high-frequency component of HRV can be used as an indicator of relaxation as it is known that the HF component increases when an individual is relaxed.
1.4. Motion sickness and sopite syndrome

1.4.1. Motion sickness

Motion sickness is a common disorder that comprises many different symptoms and symptom-complexes (Graybiel and Knepton, 1976, Kiniorski et al., 2004, Lackner, 2014, Matsangas et al., 2014). The multitude of symptoms can present in different ways and at different times but are always the result of some form of motion, whether it be real or apparent (visually-induced, vestibular activation without motion, etc.) (Graybiel and Knepton, 1976, Flaherty, 1998, Kiniorski et al., 2004, Lackner, 2014, Matsangas et al., 2014). The symptoms of motion sickness include uneven breathing, yawning, diaphoresis, confusion, exhaustion, headache, indifference, paleness, cold sweating, salivation, queasiness and vomiting, as well as other symptoms (Kiniorski et al., 2004, Lackner, 2014, Matsangas et al., 2014).

Each individual has a different level of susceptibility to motion sickness and there are multiple scales that have been developed in order to quantify susceptibility as well as severity of symptoms (e.g. MSSQ, MSAQ) (Reason, 1975, Gianaros et al., 2001, Golding, 2006, Golding and Gresty, 2015). Everyone with a functioning vestibular system is potentially susceptible to some degree, with some being more so than others. Some individuals become motion sick as a result of a small amount of motion, while others require a much more provocative stimulus in order to present symptoms (Graybiel et al., 1975, Reason, 1975, Graybiel and Knepton, 1976, Kiniorski et al., 2004, Golding, 2006, Lackner, 2014, Matsangas et al., 2014, Golding and Gresty, 2015). Also, each individual experiences motion sickness in a different way, the level of discomfort and presentation of symptoms
can be wildly different from one person to another (Graybiel et al., 1975, Reason, 1975, Graybiel and Knepton, 1976, Golding, 2006, Lackner, 2014, Matsangas et al., 2014).

Although motion sickness is a very common disorder, with most people being susceptible, it is still not fully understood; this is due the many different symptom-complexes that present in different ways (Reason, 1975, Kiniorski et al., 2004, Lackner, 2014, Matsangas et al., 2014). The “traditional” symptoms that are most commonly attributed to motion sickness are nausea and vomiting. However, motion sickness is a complex disorder that transcends these most common symptoms (Graybiel and Knepton, 1976, Flaherty, 1998, Kiniorski et al., 2004, Lackner, 2014, Matsangas et al., 2014).

It is very common for the symptoms of motion sickness to go undiagnosed or misdiagnosed simply because nausea and vomiting are not present (Graybiel and Knepton, 1976, Flaherty, 1998, Kiniorski et al., 2004, Lackner, 2014, Matsangas et al., 2014). Some of the other common symptoms that are overlooked include drowsiness, yawning, confusion, decreased performance, and disinterest (Graybiel and Knepton, 1976, Kiniorski et al., 2004, Lackner, 2014, Matsangas et al., 2014). When these symptoms present in the absence of those “traditional” of motion sickness, it is often the case that they simply get chalked up to boredom, laziness or unwillingness to participate.

Outside of experimental conditions, it is easy to misinterpret these symptoms as not being the result of a motion environment or motion stimulus. Often the less overt symptoms of motion sickness present as a result of a less provocative motion such as the sway in high-rise buildings (discussed below) or the motion of a vehicle (individuals may not feel nauseous or vomit, but may experience these other symptoms as a result of the motion) (Graybiel and Knepton, 1976, Flaherty, 1998, Lamb et al., 2013).
There is a great deal of literature available on the topic of traditional motion sickness, the main symptoms, and the theories behind the physiological mechanisms involved in the onset of nausea and vomiting. However, there is a considerable gap due to the fact that the less overt symptoms and symptom-complexes are often ignored or overlooked. Many of the symptoms are underrepresented and under-investigated in current literature. Furthermore, due to the complex nature of the physiological mechanisms intrinsic to the provocation and presentation of motion sickness, the mechanisms are still not fully known or understood. Therefore, it is apparent that there is a major gap in the literature when it comes to the less overt, or lesser known, symptoms and symptom-complexes of motion sickness (Graybiel and Knepton, 1976, Flaherty, 1998, Lackner, 2014).

1.4.2. Sopite syndrome

One of the symptom-complexes of motion sickness centres on drowsiness and mood changes; it is called sopite syndrome and was first described by Graybiel and Knepton in 1976 (Graybiel and Knepton, 1976). Sopite syndrome has been researched sporadically, but there is much less information available than for classic motion sickness symptoms.

This syndrome can present itself after a short-lived exposure to high-intensity motion, or a longer-term exposure to low-intensity motion (Graybiel and Knepton, 1976, Lackner, 2014). The resulting drowsiness, lethargy and/or mood changes may last hours, or even days, depending on the length of exposure to a motion stimulus (Graybiel and Knepton, 1976, Lackner, 2014). Other symptoms of sopite syndrome include yawning, apathy and loss of interest in the task at hand, altered mood, interrupted sleep, and minor

The symptoms of sopite syndrome are frequently combined with the other symptoms of motion sickness and so, are not generally separated from it (Graybiel and Knepton, 1976). However, under certain conditions – such as very low-frequency motion – only those symptoms that are associated with sopite syndrome indicate that an individual is experiencing motion sickness (Graybiel and Knepton, 1976, Flaherty, 1998, Kiniorski et al., 2004, Lackner, 2014, Matsangas et al., 2014). In other cases, the symptoms of sopite syndrome can persist long after the other symptoms of motion sickness (e.g. nausea, vomiting, etc.) have subsided (Graybiel and Knepton, 1976, Flaherty, 1998, Kiniorski et al., 2004, Lackner, 2014, Matsangas and McCauley, 2014, Matsangas et al., 2014). Therefore, it appears that sopite syndrome has a different time course than the other symptoms; it may also have a different susceptibility level and mechanism (Graybiel and Knepton, 1976).

It has been shown that sopite symptoms often occur or coincide with the complex set of symptoms of motion sickness (Graybiel and Knepton, 1976, Lackner, 2014, Matsangas et al., 2014). As such, sopite syndrome is commonly overlooked, misinterpreted, or misdiagnosed as its symptoms are those less overt symptoms of motion sickness (Graybiel and Knepton, 1976, Kiniorski et al., 2004, Lackner, 2014, Matsangas et al., 2014).

1.4.3. **Causative factors**

Motion sickness and/or sopite syndrome can be triggered by a wide variety of stimuli, such as the typically known stimuli that accelerate and move at frequencies well
above the perceptual threshold, such as vehicles and funfair rides. Relevant to this research, another lesser known and often imperceptible stimulus that can cause motion sickness and, more often, sopite syndrome is the sway of high-rise buildings (Kwok et al., 2009, Kwok et al., 2015). With a constantly expanding population and infrastructure in densely populated areas, it has been necessary to start building upwards rather than outwards. Therefore, there are many high-rise buildings laden with offices and living spaces in all urban areas around the world especially major cities such as Hong Kong and Tokyo.

However, with this necessary shift towards building ever upwards, comes issues and, in recent years, much research has been conducted measuring the levels of high-rise building motion or vibration. It is known that high-rise buildings resonate at frequencies from below 1 Hz, up to 4 or 5 Hz in high-wind areas (Kwok et al., 2009, Lamb et al., 2013, Lamb et al., 2014, Kwok et al., 2015). In some cases, these frequencies are perceptible, while in other cases they are not, and this is where the trouble lies. Due to the impeccable sensitivity of the vestibular hair cells, even frequencies that are below the level of human perception (which considers a variety of factors) can activate the vestibular system and cause physiological responses within the human body (Hammam et al., 2012, Bolton et al., 2016). Particularly in high-rise buildings that are used for office spaces it has been documented that individuals, especially those working in the upper levels of these high-rise buildings, experience a marked decline after a certain time of the day, generally from around midday to three-pm (Kwok et al., 2009, Lamb et al., 2013, Lamb et al., 2014, Kwok et al., 2015). Most people working in these conditions report that at lunchtime they feel the need to go down to the lower levels and even outside to get some fresh air and exercise to wake themselves up (Kwok et al., 2009, Lamb et al., 2013, Lamb et al., 2014, Kwok et al.,
2015). Following on from this, it has been shown that productivity and inclination to do work often decreases (markers of sopite syndrome), which is believed to be a result of the low-frequency resonations of the building (Kwok et al., 2009, Lamb et al., 2013, Lamb et al., 2014, Kwok et al., 2015).
1.5. Aims

This project is focused on providing a better understanding of sopite syndrome - a disorder that is connected with motion sickness - as the knowledge base on both disorders is relatively small. It aims to compare changes in skin sympathetic nerve activity (SSNA) with fluctuating drowsiness levels during very low-frequency sinusoidal motion, as measured by sleepiness scales, skin blood flow and heart rate variability (Kwok et al., 2015). Ideally, sopite syndrome will be induced through the administration of low-frequency motion so that its cause, as well as the body’s physiological reactions can be identified (Kwok et al., 2015).

The motion stimulus, being applied via a motorised platform in the horizontal plane, is aligned with frequencies at which high-rise buildings sway and will target the utricular component of the otolith organs in the vestibular system. Although there is research regarding the role of the vestibular modulation of SSNA that occurs during motion sickness, there isn’t any available information about its modulation during sopite syndrome. And, no studies as of yet have used frequencies ranging from 0.03-0.2 Hz to examine the physiology of sopite syndrome and the extent of vestibular modulation. Therefore, this project will address this gap to determine and quantify how the vestibular apparatus is involved in the pathophysiology of sopite syndrome.

It has been shown that the level of SSNA at rest is representative of an individual’s state of arousal and that vestibular modulation of SSNA increases when an individual is experiencing motion sickness (Bolton et al., 2016, Klingberg et al., 2015). However, the extent of vestibular modulation in sopite syndrome is not yet known. Knowing this link between vestibular modulation of SSNA and arousal state, this project will test the
hypothesis that, in a thermo-neutral environment, as drowsiness increases, then vestibular modulation of SSNA will decrease.
CHAPTER 2

METHODS
2.1. Subjects

Experiments were performed on 15 subjects (6 male, 9 female) who provided informed written consent prior to commencement of the experiment. The majority of subjects were recruited from Western Sydney University and were healthy volunteers. The age of subjects was in the range of 18 to 38 years old. The study was conducted with the approval of the Human Research Ethics Committee (H11010), Western Sydney University, and satisfied the Declaration of Helsinki. All subjects were informed that they could withdraw from the experiment at any time as the microneurography technique can cause discomfort and each individual has a different experience and pain threshold.

It is known that caffeine affects the activity of muscle sympathetic nerve activity (MSNA), causing an increase in activity in individuals that consume caffeine both habitually and non-habitually (Corti et al., 2002). Although, it appears that skin sympathetic nerve activity is not affected by caffeine in the same way (Corti et al., 2002). It is also known that smoking cigarettes has a powerful excitatory influence on sympathetic activity, causing an increase in both skin and muscle sympathetic nerve activity (Narkiewicz et al., 1998, Hering et al., 2010). Additionally, both consuming caffeine and smoking cigarettes have been shown to cause increases in blood pressure and heart rate (Narkiewicz et al., 1998, Corti et al., 2002). Therefore, subjects were asked to abstain from consuming caffeinated beverages or food and smoking cigarettes in the 12-24 hours before the procedure in order to prevent affecting sympathetic activity, and/or, heart rate and blood pressure.
2.2. Protocol

This project utilised microneurography and the recording of physiological parameters obtained from subjects experiencing low-frequency sinusoidal motion on an oscillating platform. Before the onset of platform motion a 10-minute rest period was recorded in order to determine the baseline of all parameters; another 5-minute rest period was recorded at the completion of platform motion. In total, the experiment could last 3-3.5 hours, including time to complete entrance and exit questionnaires, searching for the nerve, and the 1.5 hour-long motion protocol (including baselines). Additionally, it was important to ensure that for the duration of the protocol the laboratory environment was kept calm and quiet, so that the amount of non-protocol related arousal reactions was kept to a minimum. However, in some cases, a loud noise, such as an abrupt clap of the hands was performed in order to determine whether the microelectrode was still within the cutaneous fascicle. Subjects were asked to sit with their eyes closed in a relaxed position for the duration of the motion protocol.
2.2.1. General setup and motion platform

![Figure 2.1. General setup of chair and platform. Showing comfortable chair for subject to sit in, pillow supported by backboard to support head vertically, blue vacuum cushion under thigh to lift and support leg, orange foot/leg support and foam blocks under legs to support legs comfortably. This also shows the subject wearing earmuffs to block out external sounds](image)

The general setup for the experiments, as described below, is shown in Figure 2.1.

For the duration of the experiment, subjects were seated upright in a comfortable chair with legs supported horizontally by a footstool in a relaxed extended position; a backboard with padding was also in place to support the upper back and head in a vertical position. In order to elevate, stabilise and position the leg that was to be recorded from, a vacuum cushion was placed underneath the thigh. The vacuum cushion allowed for the leg to be placed in the best position for finding the nerve, as well as ensuring that the subject was seated comfortably. For additional support and comfort, foam supports were placed under the leg,
ankle, and/or thigh of the recording leg and were also available to be placed underneath the contralateral leg if required. Prior to commencement of the motion protocol, the duration and information about how they may or may not perceive the motion was explained to the subject; they were then asked to put on earmuffs, close their eyes and relax until they were informed that the protocol was complete.

The chair was secured on motorised platform that is driven by two linear motors with a maximum excursion of ±20 cm in the rostrocaudal (X) and mediolateral (Y) directions (Hammam et al., 2013, Bolton et al., 2016). Acceleration was measured by two high-sensitivity accelerometers that have a threshold of <10 μG (QA650, Honeywell, USA); they are fixed to the platform, positioned to record either displacements in the X-axis, or displacements in the Y-axis (Hammam et al., 2013, Bolton et al., 2016). For this project, the motion was only delivered in the X-axis (rostrocaudal), but was delivered as one of five different frequencies.

Previous studies in the Integrative Physiology lab have tested, using both sinusoidal Galvanic Vestibular Stimulation (sGVS) and a motion simulator, the following frequencies: 0.08, 0.13, 0.18, 0.2, 0.5, 0.8, 1.1, 1.4, 1.7 and 2.0 Hz. The frequencies for this experiment were chosen based on those that have already been tested, as well as so that they align with the frequencies that are associated with high-rise building motion (section 1.3.2). The platform delivered low-frequency sinusoidal oscillations at 0.03 Hz at 0.5 mg, 0.05 Hz at 0.5 mg, 0.1 Hz at 0.5 mg, 0.1 Hz at 5 mg and 0.2 Hz at 5 mg in the X direction only, with a 60-second interval between each frequency. The peak-to-peak excursion of the displacement, as well as the number of cycles, differed between frequencies. The excursion and number of cycles respectively was as follows: 276 mm for 25 cycles at 0.03 Hz, 99 mm for 50 cycles at
0.05 Hz, 25 mm for 50 cycles at 0.1 Hz at 0.5 mg, 248 mm for 50 cycles at 0.1 Hz at 5 mg and 62 mm for 100 cycles at 0.2 Hz. The duration of the slowest frequencies of 0.03 Hz at 0.5 mg and 0.05 Hz at 0.5 mg was thirteen minutes each, while the remaining frequencies, 0.1 Hz at 0.5 mg, 0.1 Hz at 5 mg (fastest) and 0.2 Hz at 5 mg, continued for 8 minutes each. As mentioned previously, one of the aims was to induce sopite syndrome and facilitate the onset of drowsiness or a state of relaxation as a result of the low-frequency motion. Therefore, due to the nature of the experiment, the subjects were left undisturbed for the duration of the protocol, except in cases where the site of the microelectrode needed to be readjusted to regain the nerve signal (avoided where possible). Instead, subjects were asked to report their feelings and perceptions upon completion of the protocol; this was both verbal (noted by the experimenter) and written (completion of the exit questionnaire). The onset of each displacement was random as was the order in which the frequencies were delivered; subjects were not informed as to when the motion had started. Upon conclusion of the stimulation sequence – once all frequencies had been completed – subjects were asked to report their perceptions and complete the post-motion section of a questionnaire booklet administered prior to the start of the experiment.
2.2.2. Microneurography

Figure 2.2. Schematic diagram of tungsten microelectrode inserted percutaneously into a cutaneous fascicle. The microelectrode is guided into a cutaneous fascicle using low amplitude electrical pulses (as explained below). The top trace is the RMS-processed (root-mean-square moving average; time constant = 200 ms) raw nerve signal (second trace).

Skin sympathetic nerve activity (SSNA) was obtained from fascicles of the common peroneal nerve by means of a percutaneously inserted tungsten microelectrode (FHC, Bowdoinham, ME, USA; schematic diagram above – Figure 2.2) at the level of the head of the fibula (Macefield, 2013, Klingberg et al., 2015, Bolton et al., 2016). The nerve was first located using a small surface probe that delivers brief pulses of electrical stimuli, and the nerve’s path was traced superficially before inserting the recording microelectrode into the optimal site (Hagbarth et al., 1972, Hagbarth, 2002, Macefield, 2013). Approximately 1cm away from the recording microelectrode, an uninsulated microelectrode was inserted subdermally to act as the reference microelectrode. Both microelectrodes were connected to the input terminals of an isolated amplifier headstage (NeuroAmp EX, ADInstruments, Sydney, Australia). (Shown below in Figure 2.3).
Figure 2.3. Continuous neural recording from a cutaneous (skin) fascicle of the common peroneal nerve. The Amplifier Headstage is used to amplify the raw nerve signal 100 X. The Tungsten recording and sub-dermal reference microelectrodes are shown, connected to the headstage by copper wires. The green lead is connected to the Ag-AgCl surface ground electrode in order to be able to hear the nerve signals. The thigh rests on the blue vacuum cushion which provides support and stabilisation, as well as allowing easier access to the nerve at the fibular head.

The isolated stimulator (Stimulus Isolator, ADInstruments, Sydney, Australia) was used to deliver intraneural stimulation (0.01–1.0mA, 1Hz, 0.2ms pulses) so that the tip of the recording electrode could be guided into the nerve fascicle – determined by a muscle twitch or paraesthesia being elicited at a current amplitude of 20 µA or lower (Macefield, 2013, Bolton et al., 2016). Intraneural stimulation involves using electrical impulses to elicit responses from the muscle and/or skin that the nerve innervates (Hagbarth et al., 1972, Hagbarth, 2002, Macefield, 2013). These responses help to define the fascicle location as well as how deep the electrode must go and at what angle it must be positioned in (achieved by reducing the current amplitude as the microelectrode approaches the fascicle) (Hagbarth et al., 1972, Hagbarth, 2002, Macefield, 2013).
The indications that the microelectrode is in a cutaneous fascicle are: the absence of muscle twitches and the presence of emanating paraesthesia at 20 µA, as well as afferent responses to light stroking of the skin that is innervated by the nerve (Delius et al., 1972a, Hagbarth et al., 1972, Hagbarth, 2002). Additionally, subjects were also encouraged to report any sensations, such as paraesthesia, superficial sensations including pain or burning, elicited in the recording leg by the movement of the microelectrode. The reporting of these sensations was vital in guiding the microelectrode into a cutaneous fascicle rather than a muscle fascicle, as the responses from cutaneous stimulation cannot be observed by the experimenter, only felt by the subject.

Using an isolated amplifier, the neural activity was amplified (gain 20,000, bandpass 0.3–5.0 kHz; NeuroAmp EX, ADInstruments, Sydney, Australia) and the tip of the microelectrode advanced towards an area in the fascicle exhibiting spontaneous oligounitary activity (Hagbarth, 2002, Macefield, 2013). Oligounitary activity is from SSNA if the spikes generated are negative going; a burst can be evoked by a brisk sniff or sharp inhalation (gasp; Figure 2.4 A), an unexpected tap on the nose or a loud shout/clap (Figure 2.4 B); and when an inspiratory-capacity apnoea manoeuvre is performed, no sustained increase in burst amplitude occurs (Delius et al., 1972a, Hagbarth et al., 1972, Hagbarth, 2002, Macefield, 2013).
Figure 2.4. Screenshot of data trains from LabChart, also showing SSNA bursts elicited by manoeuvres known to affect outflow in human skin nerves. The channels are as follows: 1. Raw nerve (blue), 2. Infrared plethysmograph (pink), 3. Blood pressure (red), 4. ECG (yellow), 5. Respiration (light blue), 6. Skin potential (green; large spike at end due to movement), 7. RMS processed nerve data (purple), 8. X-acceleration (flat because platform was not moving). Bursts of activity can be seen in channels 1 (raw nerve) and 7 (RMS nerve) for all manoeuvres as well as in channel 5 (respiration) for the brisk sniff/gasp. A shows two bursts of activity elicited by the performance of a sharp inhalation or gasp. B shows a burst of activity elicited by a loud clap.

2.2.3. Recording procedures

A computer program designed to acquire and analyse data (PowerLab 16SP hardware and LabChart v7 software; ADInstruments, Sydney, Australia; Figure 2.5) was used to store the neural activity recorded from each experiment on a desktop computer (10 kHz sampling). The same hardware and software was used to simultaneously record each of the physiological parameters that were also recorded as described below.

2.2.4. Physiological parameters

Ag-AgCl surface electrodes on the chest sampled at 2 kHz were used to record the electro-cardiogram (ECG; 0.3-1.0 kHz; Figure 2.4 channel 4); the ECG reading was then used
to determine continuous heart rate throughout the experimental procedure. As shown in Figure 2.5 (below), a three-electrode system was used and the surface electrodes were placed as follows: black (reference) over right clavicle, and white (recording) and red (recording) over lowest point of rib cage on right and left sides, respectively.

![Image of electrode placement](image)

*Figure 2.5. Placement of Ag-AgCl surface electrodes for a three-electrode system ECG. The reference electrode (black) is placed on the right-hand side over the clavicle. The white and red electrodes are placed over the lowest point of rib cage on right and left sides, respectively.*
A piezoelectric strain-gauge transducer wrapped around the thorax (Figure 2.6; DC-100Hz; Pneumotrace, UFI, Morro Bay CA, USA) was used to measure respiration (Figure 2.4 channel 5). The piezoelectric transducer measures the circumference of the chest and responds to linear changes in length (UFI, 2016). The respiration rate can be derived from the measurements of inhalation, exhalation and breathing strength (Macknight and Macknight, 2014, UFI, 2016).
Continuous blood pressure was sampled non-invasively at 400 Hz from the fingers using digital arterial plethysmography (Figure 2.6 and 2.7; signal shown Figure 2.4 channel 2; Finometer; Finapres Medical Systems, The Netherlands). The two finger cuffs of the Finometer were connected to the second (index) and fourth fingers of the right hand and were set to alternate inflation every fifteen minutes in order to minimise discomfort (Figure 2.7). The Finometer collects blood pressure data by using the arterial pressure from a finger which allows the determination of systolic, diastolic and mean arterial pressure (Finapres, 2012). In order to correctly sample blood pressure, it also incorporates correction for the height of the hydrostatic column (the difference in position of the finger sensor relative to the position of the heart).

Plethysmography is the recording of skin blood flow; for this experiment an infrared photoelectric sensor (MLT1020PPG, ADInstruments, Sydney, Australia) was used to record changes in tissue blood volume (Brown et al., 2007) using a finger pad attached to the fifth finger of the right hand (Figure 2.7; signal shown in Figure 2.4 channel 2; photoelectric pulse
plethysmograph, ADInstruments, Sydney, Australia; sampled at 400 Hz; (Macknight and Macknight, 2014)). The infrared plethysmograph detects the infrared light that is reflected off the bone in order to record pulsatile changes in cutaneous blood flow and blood volume from the expanding and contracting microvasculature (Macknight and Macknight, 2014).

![Image of surface electrodes]

**Figure 2.8. Attachment of surface electrodes to measure skin potential.** The black (reference) electrode was placed over the head of the ulna, white (recording) on the dorsum of the hand and, red (reference) on the palm of the hand.

Finally, changes in skin potential or electrical conductance measured across the palm and dorsum of the hand by Ag-AgCl surface electrodes (0.1–10 Hz; BioAmp, ADInstruments, Sydney, Australia, sampled at 400 Hz; Figure 2.4 channel 7) indicated sweat release. As indicated in Figure 2.8 the surface electrodes to measure skin potential were placed on the left hand as follows; black (reference) over head of ulna, white (recording) on dorsum of hand and red (recording) on palm of hand.
2.2.5 Questionnaires

During the pre-test procedures, a behavioural checklist (Appendix 1), the ESS (Epworth Sleepiness Scale; appendix 2), KSS (Karolinska Sleepiness Scale; appendix 3), MSSQ (Motion Sickness Susceptibility Questionnaire; appendix 4) and the MSAQ (Motion Sickness Assessment Questionnaire; appendix 5) scales were administered. The KSS and MSAQ were readministered during the post-test procedures – once all frequencies had been delivered and recording was completed. The ESS asks subjects to rate their ease of falling asleep in a multitude of everyday situations in order to determine drowsiness levels (Kiniorski et al., 2004). The KSS is a subjective measure of the subject’s self-reported level of drowsiness at one moment in time, it is a 9-point scale with 1 being “extremely alert” and 9 being “extremely sleepy, fighting sleep” (Johns, 2009). The MSSQ administered was a revised shorter version (MSSQ-short) created by John Golding (Golding, 2006); it is a scale used to measure the subject’s motion sickness susceptibility as reported by them. Finally, the MSAQ is a multidimensional scale that collects data about motion sickness symptoms from four dimensions: gastric, central nervous system, peripheral nervous system and sopite symptoms (e.g. irritability, lethargy) (Gianaros et al., 2001, Kiniorski et al., 2004).
2.3. Analysis

SSNA was displayed as an RMS-processed (root mean square, moving average time constant 200ms) signal as well as the raw neurogram. The raw, negative-going sympathetic spikes were used for analysis. The window discriminator software of the spike histogram module (Spike Histogram for Windows v8, LabChart, ADInstruments, Sydney, Australia) was used to detect the negative-going spikes of the neurogram (with a half-width of 0.2-0.5 ms), the positive-going R-waves of the ECG and the positive peaks of the accelerometer. The same program used the detected spikes and peaks to create cross-correlation and auto-correlation histograms (correlograms). The program plots the times of events (periodicities), making the current event time 0 (zero), events before the current event negative times and those after the current event positive times. An autocorrelation histogram can then be created in order to illustrate these periodicities and used as a comparison against the periodicities of the cross-correlation histograms. Cross-correlation histograms are used to examine the temporal relationship between two physiological variables; in this case we are comparing the spikes comprising the SSNA signal with either the R-waves of the ECG or the positive peaks of the sinusoidal signal in the X direction as recorded from the accelerometers of the motion platform. This may be used in order to determine if one physiological variable impacts the activity pattern of another physiological variable. In order to construct a cross-correlation histogram (cross-correlogram) the following must be selected respectively; each impulse in the analysis channel (SSNA) that coincides with a time defined with respect to events in the reference channel, in this case, the R-waves of the ECG or the positive peaks of the sinusoidal waves. This is followed by allocating all the impulses occurring within a selected analysis period (50 ms bins) to the appropriate bins in the
The discriminator section of the Spike Histogram module (LabChart, ADInstruments, Sydney, Australia) was used to adjust the levels of neural activity in order to show that the negative-going spikes exhibited cardiac modulation; this was shown in the cross-correlation between the SSNA activity and the ECG. Cross-correlograms between SSNA and the positive peaks of the sinusoidal motion were created using the same discriminator settings.

In order for the data to be fit to a mathematical function (smoothed polynomial), it was exported as text to a statistical and graphical analysis program (Prism v7 for Windows, GraphPad Software, USA). The slower vestibular cross-correlation histograms required the fitting of lower-order polynomials (2nd order), whereas the cardiac cross-correlation histograms required higher-order polynomial fitting. Cross-correlations were formed into a smooth graph by adjusting the polynomials. When creating cross-correlation histograms comparing sympathetic nerve activity and platform motion, smoothing must be performed in order to remove any peaks that are cardiac-related. This removal of cardiac-related peaks allows us to more accurately examine the sympathetic nerve activity and its relation to the sinusoidal motion of the platform. Quantification of the cardiac and vestibular modulation of SSNA was obtained by measuring the difference in number of spikes at the peak and the trough of the modulation from the smoothed curve. The modulation index formula – \( \text{MI (\%)} = \left\{ \frac{[\text{peak-trough}]}{\text{peak}} \right\} \times 100 \) – was used in order to express the modulation as a percentage. The mean modulation values of each frequency of platform motion across all individuals were then compared. In order to determine if a significant difference (p < 0.05) was present between values and platform frequencies, a one-way analysis of variance (ANOVA) was applied; this was accompanied by a D’Agostino Pearson omnibus normality
test, and a Wilcoxon signed-rank test (Prism v7 for windows, GraphPad Software, USA). A paired t-test was also performed in order to determine if there were any significant differences between the primary and secondary peaks of the modulation as well as if there were any variances in their latencies.

The HRV (Heart Rate Variability) module in LabChart (HRV for Windows v8, ADInstruments, Sydney, Australia) was used on the ECG data in order to determine the extent of heart rate variability during each baseline and frequency. The HRV module allows for each section to be analysed to determine whether the heart rate variability was in the VLF (very low-frequency; 0-0.04 Hz), LF (low-frequency; 0.04-0.15 Hz), or HF (high-frequency; 0.15-0.45 Hz) band. The band in which HRV is in is an indication of the subject’s emotional state as well as their state of relaxation. It shows all values in the report view, and also allows for the creation of multiple graphs, including the Power Spectrum Plot, which shows a visual representation of whether the heart rate variability was in the LF or HF band for the selected time period. The values that can be analysed included: standard deviation (SD) of the inter-beat-interval (IBI), known as SDRR (RR meaning the R-to-R interval, as in R-wave of the ECG); SD of delta RR; ratio; RMSSD (root-mean-square, standard deviation); and coefficient variation of RR (CVRR). The other required values for analysis were those within the frequency domain, in ms², total power, LF, HF and the LF/HF ratio. All values were exported to GraphPad for (Prism v7 for Windows, GraphPad Software, USA) to perform statistical analysis in the form of a multiple comparisons ANOVA and a Dunnett test.

To analyse skin blood flow, a digital high-pass filter (1 Hz) was applied to the raw plethysmograph signal, then cyclic measurements were used to calculate the pulse
amplitude from this derived signal. The analysis was performed on the pulse amplitude channel; the final sixty seconds for the baseline and each frequency was selected and the mean value calculated. The mean pulse amplitude value of each frequency was then normalised and presented as a frequency of the baseline, and each frequency as a percentage of the baseline (making the baseline value 100%) was compared to the baseline in a multiple comparisons ANOVA.

Since the MSAQ assesses multiple dimensions, the total scores can be used to examine motion sickness overall, or the subscale scores of each dimension can be used to distinguish the experience of each of the dimensions (Gianaros et al., 2001). Therefore, the sopite subscale can be examined specifically to determine if the motion platform causes sopite symptoms to arise. By administering the KSS before and after the onset of motion, a baseline for sleepiness was determined and then could be compared to the post-motion KSS to determine whether the subject experienced a change in drowsiness as a result of the motion. The scores for the MSSQ may be examined to determine if susceptibility to motion sickness also determines susceptibility to sopite syndrome. In addition, the scores from multiple tests (ESS, KSS, and MSAQ) were correlated and contrasted for clarity. The values for these questionnaires were analysed in the aforementioned statistical and graphical analysis program (Prism v7 for Windows, GraphPad Software, USA; unpaired t test).
CHAPTER 3

RESULTS
For this study utilising physiological activation of the vestibular utricle, five frequencies were chosen; with eyes closed, some of these frequencies were generally imperceptible (0.03, 0.05 and 0.1 at 0.5) and others were perceptible (0.1 at 5 mg and 0.2). Some subjects reported feeling some motion – moving “back and forth” or “side to side”, and some reported feeling as though they were moving “around in circles”. However, as the frequencies were administered in succession without pause to administer questionnaires, we didn’t collect data specifically regarding if each frequency was perceptible or not. Upon conclusion of the experiment, most subjects reported feeling relaxed during and at the completion of the motion protocol, and several fell asleep during the motion.

This study collected full protocol data from 15 subjects, however, due to quality issues including loss of signal, too low signal to noise ratio, and non-recording of platform acceleration, nerve analysis was only performed on 10 subjects. However, plethysmograph, HRV, MSSQ and MSAQ data were analysed for all subjects to measure the effects of the motion on other parameters.

The mean values for vestibular modulation are shown as a graphical representation in Figure 3.1. It can be seen in Figure 3.1 and Table 3.1, that with the mean vestibular modulation index ranging from 30.7 (0.2 Hz) to 37.2 (0.1 Hz at 5 mg), there is a robust vestibular modulation, but there was no significant difference between frequencies (ANOVA).
**Figure 3.1. Modulation indices as a function of platform motion.** This graph shows the mean ± SE values of vestibular modulation from 10 subjects that experienced five frequencies of motion. There was robust vestibular modulation of SSNA at all frequencies. However, the modulation was not significant between frequencies.

**Table 3.1. Modulation indices ± SEM for vestibular and cardiac modulation of SSNA.** Comparison of mean vestibular modulation and mean cardiac modulation. Vestibular and cardiac modulation were compared for each frequency using an unpaired t-test, reaching significance at two frequencies. An unpaired t-test was also used to compare the cardiac modulation at each frequency to the baseline value (significant for three frequencies). * p < 0.05, ** p < 0.01

<table>
<thead>
<tr>
<th>SSNA</th>
<th>Baseline</th>
<th>0.03 @ 0.5</th>
<th>0.05 @ 0.5</th>
<th>0.1 @ 0.5</th>
<th>0.1 @ 5</th>
<th>0.2 @ 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vestibular modulation (%)</strong></td>
<td>--</td>
<td>32.5 ± 3.4</td>
<td>31.6 ± 2.7</td>
<td>32.8 ± 3.2</td>
<td>37.2 ± 4.4</td>
<td>30.7 ± 3.8</td>
</tr>
<tr>
<td><strong>Vestibular vs cardiac significant?</strong></td>
<td>--</td>
<td>Yes*</td>
<td>No</td>
<td>No</td>
<td>Yes*</td>
<td>No</td>
</tr>
<tr>
<td><strong>Cardiac Modulation (%)</strong></td>
<td>37.7 ± 3.9</td>
<td>19.7 ± 3.1</td>
<td>24.8 ± 3.9</td>
<td>23.2 ± 3.3</td>
<td>22 ± 3.5</td>
<td>26.7 ± 3.7</td>
</tr>
<tr>
<td><strong>Cardiac modulation vs baseline significant?</strong></td>
<td>--</td>
<td>Yes**</td>
<td>No</td>
<td>Yes*</td>
<td>Yes*</td>
<td>No</td>
</tr>
</tbody>
</table>
Cardiac modulation of SSNA is represented graphically in Figure 3.2 and numerically in Table 3.1. Cardiac modulation was not significant between frequencies, however, when each frequency was compared to the baseline, it was found that 0.03 Hz, 0.1 Hz at 0.5 and 0.1 Hz at 5 mg were significantly lower than the baseline (ANOVA).

![Cardiac Modulation Graph]

**Figure 3.2. Modulation indices as a function of ECG recording (cardiac modulation).** This graph shows the mean ± SE values of cardiac modulation from 10 subjects that experienced five frequencies of motion. Cardiac modulation was present across all frequencies and the baseline. The modulation was not significant between frequencies, but it was significantly lower between the baseline and three frequencies (0.03 Hz, 0.1 at 0.5 and 0.1 at 5 mg). *p < 0.05, **p < 0.01
The mean values for vestibular and cardiac modulation are graphically presented for all 10 subjects for each frequency in Figure 3.3 and the values are presented numerically in Table 3.1. Vestibular modulation of SSNA was consistently higher than cardiac modulation, being significant during 0.03 Hz at 0.5 mg and 0.1 at 5 mg (two-tailed, unpaired t-test).

![Figure 3.3. Modulation indices as a function of platform motion and ECG. This graph shows a comparison between the mean ± SE values of vestibular and cardiac modulation from 10 subjects that experienced five frequencies of motion. As can be seen, vestibular modulation of SSNA was consistently higher than cardiac modulation (0.03 Hz, 0.1 at 0.5 and 0.1 at 5 mg significant). *p < 0.05](image-url)
As explained previously (Section 2.3), the skin blood flow data was analysed by determining the pulse amplitude of the plethysmography signal. The final sixty seconds of the baseline and each frequency was used to determine the mean pulse amplitude for that period of data, then, means across all subjects for the baseline and each frequency were determined. The mean data of each frequency was then normalised to the baseline and the mean of each frequency (%) was compared to the baseline, as shown in Table 3.3 (ANOVA). The results showed a decrease in skin blood flow at all frequencies, reaching statistical significance in three frequencies – 0.03 Hz, 0.1 Hz at 0.5 mg and 0.1 Hz at 5 mg (Table 3.2).

Table 3.2. Results showing mean pulse amplitude normalised to the baseline, of skin blood flow from plethysmograph recording of 15 subjects. This table shows the results from the plethysmography signal measuring skin blood flow, with each frequency presented as the mean percentage of the baseline. Three frequencies reached statistical significance. *p < 0.05, **p < 0.01

<table>
<thead>
<tr>
<th>Plethysmography</th>
<th>Baseline</th>
<th>0.03 @ 0.5</th>
<th>0.05 @ 0.5</th>
<th>0.1 @ 0.5</th>
<th>0.1 @ 5</th>
<th>0.2 @ 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse Amplitude (mean; %)</td>
<td>100</td>
<td>68.8 ± 9.4</td>
<td>82.5 ± 11.0</td>
<td>64.8 ± 8.2</td>
<td>57.1 ± 5.5</td>
<td>79.6 ± 8.0</td>
</tr>
<tr>
<td>Significant? (y/n)</td>
<td>-</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>P value</td>
<td>-</td>
<td>0.026*</td>
<td>0.381</td>
<td>0.0096**</td>
<td>0.0011**</td>
<td>0.242</td>
</tr>
</tbody>
</table>
Figure 3.4. Mean pulse amplitude values of each frequency normalised to the baseline value. This graph shows a comparison between the mean pulse amplitude ± SE as a percentage of the baseline for all frequencies for 15 subjects that experienced slow sinusoidal motion.

The mean results for heart rate (bpm) and the following indices of heart rate variability (HRV) are shown numerically in Table 3.3; RMSSD, LF (0.04 – 0.15 Hz), HF (0.15 – 0.40 Hz) and LF/HF ratio. There was no significant difference in heart rate or any HRV values between frequencies nor in comparison to the baseline value.
Table 3.3. Results for mean heart rate and heart rate variability (HRV) from 15 subjects that experienced five frequencies of motion. None of the HRV indices showed statistical significance when compared to the value of the baseline.

<table>
<thead>
<tr>
<th>HRV</th>
<th>Base</th>
<th>0.03 @ 0.5</th>
<th>0.05 @ 0.5</th>
<th>0.1 @ 0.5</th>
<th>0.1 @ 5</th>
<th>0.2 @ 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average heart rate (beats/min)</td>
<td>65.9</td>
<td>64.7</td>
<td>64.4</td>
<td>67.1</td>
<td>65.8</td>
<td>65.7</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>55.97</td>
<td>55.26</td>
<td>60.69</td>
<td>57.14</td>
<td>59.3</td>
<td>59.15</td>
</tr>
<tr>
<td>LF (0.04 – 0.15 Hz) (%)</td>
<td>24.98</td>
<td>29.95</td>
<td>27.73</td>
<td>31.58</td>
<td>29.99</td>
<td>29.04</td>
</tr>
<tr>
<td>HF (0.15 – 0.40 Hz) (%)</td>
<td>51.96</td>
<td>43.3</td>
<td>40.45</td>
<td>44.52</td>
<td>41.82</td>
<td>44.43</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>0.5338</td>
<td>0.8078</td>
<td>0.7848</td>
<td>0.8212</td>
<td>0.808</td>
<td>0.8203</td>
</tr>
</tbody>
</table>

The Karolinska Sleepiness Scale (KSS) is a self-reported measure of the level of drowsiness at that specific moment in time (Johns, 2009), it was completed by the subjects before the onset of the motion, and at the completion of it. The mean scores of all subjects for the pre-motion, and post-motion KSS were compared using an unpaired, two-tailed t-test and are shown graphically in Figure 3.5.

Figure 3.5. Mean score for the KSS, before the onset of motion (pre) and upon completion of the protocol (post). This graph shows a comparison between the mean score on the KSS ± SE before and after the protocol for 14 subjects that experienced slow sinusoidal motion. The mean post-motion KSS score was significantly larger than the pre-motion score (5.8 ± 0.46 vs 4 ± 0.41, respectively). ** p < 0.01
The motion sickness assessment questionnaire (MSAQ) scores – including overall, gastric, central nervous system, peripheral nervous system and sopite related indices – for all 15 subjects are shown numerically in Table 3.4. The mean pre-motion MSAQ scores are shown graphically in Figure 3.5, those for the post motion MSAQ are shown in Figure 3.6 and pre- vs post-motion scores are graphically presented in Figure 3.7. An unpaired t-test was used to compare the pre-motion scores with the post-motion scores; it was found that the statistically significant dimensions were; overall, gastric and central (Table 3.4).

There was not a significant difference between the pre- and post-motion scores for the dimension measuring sopite symptoms. However, when the data is separated into subjects that reported increased sopite symptoms (n=7) and those who did not (n=3), as (Hammam et al., 2012, Klingberg et al., 2015) did with nausea and no nausea in their experiments, the data does show a significant difference (Figure 3.8). As comparing those subjects that did experience sopite symptoms showed a significant difference from the pre-motion scores, I also compared the vestibular modulation indices of those who did experience sopite symptoms, against those who did not. There, was no significant difference in the modulation indices of the subjects who reported sopite symptoms when compared with the subjects who did not report sopite symptoms.
Table 3.4. Results showing mean MSAQ scores from 15 subjects before, and after the onset of a motion stimulus. For each dimension, the scores taken before the onset of motion (pre-MSAQ) were compared to those after the motion (post-MSAQ). * p < 0.05

<table>
<thead>
<tr>
<th>MSAQ Scores</th>
<th>Overall</th>
<th>Gastric</th>
<th>Peripheral</th>
<th>Central</th>
<th>Sopite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-MSAQ</td>
<td>13.7</td>
<td>11.3</td>
<td>14.4</td>
<td>12.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Post-MSAQ</td>
<td>17.8</td>
<td>13.0</td>
<td>12.8</td>
<td>20.7</td>
<td>23.3</td>
</tr>
<tr>
<td>Significant? (y/n)</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>P value</td>
<td>0.0422*</td>
<td>0.0470*</td>
<td>0.5056</td>
<td>0.0254*</td>
<td>0.1081</td>
</tr>
</tbody>
</table>

Figure 3.6. Pre-motion MSAQ scores for each measured dimension. This graph shows the mean ± SE values for each dimension of the motion sickness assessment questionnaire for 15 subjects before the experienced the motion protocol. Dimensions are overall (the total score), gastric (G), peripheral (P), central (C), and sopite (S).
Figure 3.7. *Post-motion MSAQ scores for each measured dimension.* This graph shows the mean ± SE values for each dimension of the motion sickness assessment questionnaire for 15 subjects after they completed the motion protocol. Dimensions are overall (the total score), gastric (G), peripheral (P), central (C), and sopite (S).

Figure 3.8. *Pre-motion versus post-motion MSAQ scores for each measured dimension.* This graph shows the mean ± SE values for each dimension of the motion sickness assessment questionnaire for 15 subjects. Each dimension of the pre-motion MSAQ is compared to its equivalent dimension in the post-motion MSAQ. Dimensions are overall (the total score), gastric (G), peripheral (P), central (C), and sopite (S).
Figure 3.9. Pre-motion versus post-motion mean scores for sopite dimension of MSAQ in subjects that reported increased sopite symptoms upon completion of the motion protocol. This graph shows a comparison between the pre-motion sopite score and post-motion sopite score in the 7 subjects that reported increased sopite symptoms, as scored by the MSAQ.
The aim of this project was to examine and quantify the physiological changes that occur, specifically in skin sympathetic nerve activity, skin blood flow and heart rate variability, when low-frequency sinusoidal motion is applied to facilitate the onset of sopite syndrome. As previously explained, sopite syndrome is a symptom-complex of motion sickness, that consists of symptoms such as drowsiness, lethargy and yawning; these symptoms can also lead to, or be accompanied by irritability, disinterest to perform work, depression and/or mood changes. It is known that sopite syndrome can present before the onset of motion sickness in response to provocative motion stimuli, or it can present as the sole manifestation of motion sickness in response to prolonged, low-frequency motion.

The current results, consistent with previous studies performed in the Integrative Physiology lab, show that slow sinusoidal motion delivered by a motorised platform leads to physiological activation of the vestibular system, despite subjects often not feeling any motion. This activation in turn, leads to modulation of skin sympathetic nerve activity (SSNA) in human subjects.

The motion frequencies that were used in this study were selected so that they align with the frequencies that high-rise buildings are known to resonate at. As previously discussed, much research has been performed in recent years measuring the frequencies at which these high-rise buildings sway. This is important because most of the major urban areas around the world, especially in countries such as China and Japan, have made the essential shift towards occupancy, both domestic and professional, in high-rise buildings. With the constant development of better techniques, stronger materials and more advanced structural systems, buildings can be made lighter and more solid, reaching to much greater heights than was previously allowed (Kwok et al., 2009, Lamb et al., 2013,
Lamb et al., 2014). It is even believed that in the not too distant future, we will be able to build up to and beyond 1000 km, while still maintaining structural stability and integrity (Kwok et al., 2009). However, this necessary change into building upwards rather than outwards has not been without issues as wind-induced vibrations can increase occupant perception of motion and lead to stress, discomfort, decreased concentration and even, nausea and dizziness (Kwok et al., 2009). Given that high-rise buildings are known to resonate at frequencies from below 1 Hz up to 5 Hz (especially in high-wind areas), and sopite syndrome, including symptoms such as drowsiness, irritability and loss of productivity, is known to be caused by low-frequency motion, studies such as this are designed to provide greater insight into the physiology and management of such disorders.

As explained previously, the vestibular hair cells are acutely sensitive and the way they are activated is well known; much research has been performed to determine the type of motion and/or stimulus and direction that activates the vestibular system. It is known that, within the macula of the otolith organs, each primary afferent innervating a hair cell is morphologically polarised and is most responsive to linear force applied in a specific direction or vector (Purves et al., 2012). The primary orientation of the hair cells of the utricle makes them responsive to linear force in the horizontal plane. The motion applied throughout the current experiment was on subjects that were seated upright with their head vertical; therefore, the utricle was the otolith organ that was targeted. It is important to note that the properties of the stimulus applied, such as frequency, acceleration and number of cycles, can greatly impact the way the sympathetic nerve responses to vestibular stimuli are presented. For this study, the accelerations (0.5 mg and 5 mg) and frequencies that were used were novel, in that the frequencies, accelerations, or the combination of the
two have not previously been tested. Additionally, most frequencies were below the perceptual threshold, especially since specific aspects of sensory input (sight and hearing) were obscured to essentially eliminate proprioceptive inputs. This allowed us to place emphasis on the physiological role of the utricle of vestibular system and how it controls the level of sympathetic outflow to the skin.

As is expected from a series of experiments such as those performed using awake (or semi-awake) human subjects for this thesis, the amount and intensity of inter-individual spontaneous SSNA varied greatly. Being performed in an essentially thermoneutral environment, where there was a lack of thermoregulatory demands, the level of SSNA was reflective of arousal state of the subjects. Subjects were informed of the forthcoming motion, but were also made aware that it was slow motion that was not expected to cause high levels of nausea or dizziness. The level of spontaneous SSNA, cutaneous vasoconstriction, and heart rate, as well as Spielberger’s State and Trait Anxiety index (Appendix 6), were used to conclude that anxiety levels in subjects were not high, despite subjects having a microelectrode inserted into a peripheral nerve. Therefore, it is safe to say that any change in the levels, and vestibular modulation, of SSNA was purely a result of the stimulus applied.

The results from this study revealed a robust vestibular modulation of SSNA, with each frequency showing one large peak of modulation within each cycle of sinusoidal acceleration. The modulation peaked during forward acceleration of the body – which, knowing that the utricle is activated by linear motion in the horizontal plane, means that the vestibular hair cells were moved backwards. It is clear that the motion frequencies that were administered effectively induced vestibular modulation of skin sympathetic outflow as
a result of utricular hair cell displacement. The magnitude of modulation was comparable across all frequencies, as shown by the modulation indices in Table 3.1. There was no significant difference in modulation index between frequencies: 32.5 ± 3.4 (SEM) % during sinusoidal, linear motion at 0.03 Hz, 31.6 ± 2.7 % at 0.05 Hz, 32.8 ± 3.2 % at 0.1 Hz at 0.5 mg, 37.2 ± 4.4 % at 0.1 Hz at 5 mg and 30.7 ± 3.8 % at 0.2 Hz.

Previously, studies have been performed evaluating the effects of either platform motion (physiological activation) or sinusoidal galvanic vestibular stimulation (sGVS; electrical activation) on SSNA and motion sickness in awake human subjects. There have been only two studies in our laboratory that have used the motorised platform to target the vestibular utricle and analyse the effects on SSNA (Bolton et al., 2016, Grewal et al., 2012). Both of these studies tested platform motion at 0.08 Hz, on subjects either sitting upright with head vertical (Grewal et al., 2012), or supine, to individually target either the utricular or saccular components of the otoliths, respectively (Bolton et al., 2016). Studies using sGVS to quantify vestibular modulation of SSNA and its relationship to motion sickness have used the following frequencies: 0.08 Hz, 0.13 Hz, and 0.18 Hz (Hammam et al., 2012, Klingberg et al., 2015), and seven frequencies ranging from 0.2-2.0 Hz (James et al., 2010).

As previous studies have used different techniques and/or frequencies when analysing vestibular modulation of SSNA, it is difficult to compare data from this study with past data, other than to quantify the magnitude of modulation as a function of frequency.

The magnitude of modulation observed in this study was not comparable to either study that utilised physiological activation of the vestibular utricle at 0.08 Hz ± 4 mg, being (as a percentage ± SEM) 96.7 ± 2.5 % in sitting subjects (Grewal et al., 2012) and 27.6 ± 5.0 % in supine subjects (Bolton et al., 2016). Additionally, the one study that did test the
frequency of 0.2 Hz, using sGVS rather than platform motion, calculated a modulation index of 81.5 ± 4.0 (James et al., 2010) – much higher than that of 30.7 ± 3.8 % found in this study.

However, the magnitude of modulation was comparable with the studies that utilised sGVS at frequencies of 0.08, 0.13 and 0.18 Hz, having modulation indices of 34.8 ± 3.5 %, 33.8 ± 3.6 % and 39.9 ± 3.4 % respectively (Hammam et al., 2012, Klingberg et al., 2015).

As sGVS directly targets the whole vestibular system and platform motion in the antero-posterior axis specifically targets the vestibular utricle, it is expected that the magnitude of modulation would be different. However, I cannot account for the large differences in the modulation indices of this study when compared to previous studies using physiological activation of the utricle. Nor can I explain the similarities between the modulation indices reported in this study and those reported in studies that utilised sinusoidal GVS. Further studies testing the same frequencies as used in this study would be required to create a fair comparison in magnitude of modulation.

As previously discussed sympathetic outflow to the skin consists of predominately sudomotor and cutaneous vasoconstrictor fibres, however, the data for this study was recorded in an essentially thermoneutral environment. Therefore, the skin sympathetic nerve activity was primarily associated with activity from cutaneous vasoconstrictor neurons. The results from this study align with current knowledge of skin sympathetic nerve activity’s relationship with the cardiac rhythm, – it is known that cutaneous vasoconstrictor neurons exhibit weak cardiac modulation – as vestibular modulation was greater than cardiac modulation in this study (Delius et al., 1972a, Bini et al., 1981, Macefield and Wallin, 1999, James et al., 2010, Bolton et al., 2016).
The magnitude of cardiac modulation was lower than vestibular modulation at all frequencies, reaching statistical significance at two frequencies (0.03 Hz and 0.1 Hz at 5 mg). However, it is also important to note that cardiac modulation was lower than the baseline value for all frequencies (statistically significant for 0.03 Hz, 0.1 Hz at 0.5 mg and 0.1 Hz at 5 mg). As mentioned above, it is to be expected that the cardiac modulation of SSNA would be less than the vestibular modulation due the weak coupling of SSNA to the cardiac rhythm. However, it is usually the case that cardiac modulation will remain relatively stable throughout application of a stimulus, even though it is lower than vestibular modulation. In the study by James and colleagues (James et al., 2010), quantifying vestibular modulation of SSNA and motion sickness in response to sGVS applied at higher frequencies than those used here, it was found that two frequencies exhibited a significantly higher magnitude of cardiac modulation. These two frequencies (0.5 and 1.4 Hz) were found to elicit a pulse rhythmic entrainment of SSNA which was thought to be because they were more closely related to the cardiac rhythm (James et al., 2010). However, it appears that the findings of this current study, that is, a decrease in cardiac modulation with respect to the baseline during low-frequency sinusoidal motion, is a novel finding. I am unable to explain why the cardiac modulation of SSNA decreased from the baseline level during each frequency of motion, and further experiments would be required to determine the cause and/or physiological basis of this change.

The recording and analysis of plethysmography and heart rate variability (HRV) act as an indirect method of determining how the motion frequencies administered affected sympathetic outflow to the skin, by means other than, or supplementary to, microneurography (Brown et al., 2007). Recording these parameters allows for the indirect
assessment of sympathetic function by recording effector-organ responses (Brown et al., 2007). Furthermore, since skin sympathetic nerve activity is composed of impulses from both sudomotor and cutaneous vasoconstrictor neurons, it has been shown that recording skin vasoconstrictor responses to stimuli, as changes in skin blood flow, is a reliable means of detecting skin sympathetic outflow, (Brown et al., 2007).

Infrared photoelectric plethysmography was used in this study to measure the skin blood flow to a finger; the analysis used involved determining the mean amplitude of the pulsatile blood flow, with pulse amplitude being a measure of cutaneous vasoconstriction. A decrease in the pulse amplitude of the plethysmograph signal is used to infer a decrease in skin blood flow due to increased cutaneous vasoconstrictor drive, while an increase in pulse amplitude represents decreased cutaneous vasoconstriction and therefore, increased skin blood flow (Brown et al., 2007). It is known that, as with SSNA, skin blood flow is associated with arousal state, hence, provocative motion stimuli cause an increase in cutaneous vasoconstriction and thus, decreased skin blood flow (Brown et al., 2007, Brown and Macefield, 2014).

The results from this study showed a decrease in pulse amplitude at all frequencies. As skin blood flow, determined by sympathetically mediated cutaneous vasoconstriction, is related to arousal state, this data can be used to infer the arousal state of the subjects. For all frequencies of motion, the mean percentage of pulse amplitude was lower than the baseline, reaching statistical significance for three frequencies (0.03 Hz at 68.8 %, 0.1 Hz @ 0.5 mg at 64.8 % and 0.1 @ 5 mg at 57.1 %). As we were expecting subjects to progress into a state of relaxation or drowsiness as a result of the low-frequency motion, we expected the pulse amplitude to increase. However, from what we know about the cutaneous
vasoconstrictor response and its relation to arousal state, the results suggest that all frequencies of motion may have caused an arousal or disturbance in subjects. If the motion caused subjects to become physiologically relaxed, skin blood flow comparative to the baseline level would have increased, rather than decreased.

Heart rate variability (HRV) is an index of vagal activity and is used to measure the variation in time between consecutive R-waves of the heart rate, as measured by electrocardiogram (ECG) (Task force of the European Society of Cardiology, 1996, Burton et al., 2010). It can be measured in the time domain using root mean square successive differences (RMSSD) between R-waves, which is an indicator of vagal activity (Task force of the European Society of Cardiology, 1996, Burton et al., 2010, Akselrod et al., 1981, Dimitriev et al., 2014, Lombardi, 2000, Sayers, 1973). HRV can also be measured in the frequency domain (HF and LF); the high-frequency (HF; 0.15 – 0.4 Hz) component is an indicator of vagal, primarily parasympathetic activity and the low-frequency (LF; 0.04 – 0.15 Hz) reflects both sympathetic and parasympathetic activity (Sayers, 1973, Akselrod et al., 1981, Task force of the European Society of Cardiology, 1996, Lombardi, 2000, Burton et al., 2010, Dimitriev et al., 2014). Relative sympathetic drive to the heart can be determined through changes in the LF to HF ratio – an increase in the ratio has been suggested to reflect a relative enhancement of cardiac sympathetic drive (Akselrod et al., 1981, Task force of the European Society of Cardiology, 1996, Burton et al., 2010, Dimitriev et al., 2014). The high-frequency component of HRV can be used as an indicator of relaxation; it is known that the HF component increases when an individual is relaxed (Dimitriev et al., 2014). The results for this study didn’t show significant differences in HRV at any frequencies across any of the measured indices. In particular, the HF component was relatively stable at all frequencies.
Although subjects reported feeling relaxed, this is not reflected in the heart rate variability indices that were compared across frequencies.

The motion sickness assessment questionnaire (MSAQ), administered before and after the motion stimulus, is a multidimensional scale collecting self-reported information about motion sickness symptoms from four dimensions – overall, gastric, peripheral nervous system, central nervous system and sopite related symptoms (Gianaros et al., 2001, Kiniorski et al., 2004). For this study, the most important measure in the MSAQ was the sopite dimension; with this, the sopite score reported before the onset of motion could be compared with the score reported at the completion of the protocol. When the sopite scores from the pre-motion and post-motion MSAQ’s from all subjects were compared, there was an overall increase in the mean value after the motion protocol, however, the difference was not significant.

Previous studies assessing the effects of either physiological or electrical activation of the utricle on motion sickness, SSNA, and other parameters, have divided the data into groups depending on whether they did or did not experience nausea from the stimulus (Hammam et al., 2012, Klingberg et al., 2015). Additionally, the mean score for the pre-motion Karolinska Sleepiness Scale (KSS) was significantly lower than the mean post-motion KSS score in all subjects. Therefore, for the sake of comparison, the sopite dimension of the pre- and post-MSAQ’s were divided into two groups – one for those who reported increased sopite symptoms upon conclusion of the protocol, and another for those who reported either no, or negligible increase in sopite symptoms. When the sopite dimensions of the pre- and post-motion MSAQ were compared based on whether sopite symptoms were experienced or not, the post-motion score was significantly larger than the pre-motion
score. However, the same did not hold true for the HRV indices, the skin blood flow measure, or for the magnitude of vestibular modulation, when these values were separated into groups depending on expression of sopite symptoms.

Although, subjects reported that they felt calm, relaxed and/or tired upon completion of the motion protocol – both verbally and on written questionnaires (MSAQ, KSS) – this is not reflected in the physiological parameters that were measured. Upon examination and analysis of the data, we are not convinced that a physiological state of relaxation was generated. Skin sympathetic nerve activity and its associated parameters (skin blood flow, HRV) are connected to the emotional, attentive and arousal state in human beings. Therefore, the change in each parameter in response to a stimulus that is arousing (increased SSNA, decreased HF in HRV and increased cutaneous vasoconstriction), would be the opposite for a stimulus that causes relaxation. When subjects are aroused or successfully stimulated, for example, in the studies performed observing the physiological changes in motion sickness, the vestibular modulation of SSNA has been found to increase, especially in those subjects that reported nausea (James et al., 2010, Grewal et al., 2012, Hammam et al., 2012, Klingberg et al., 2015, Bolton et al., 2016).

Additionally, a decrease in the amplitude of pulsatile blood flow in the finger is caused by a decrease in the diameter of the cutaneous blood vessels; it is known that, when humans are aroused, or cold, cutaneous vasoconstriction occurs, leading to decreased skin blood flow (Wallin, 1990). Since the mean pulse amplitude for skin blood flow was lower than the baseline at all frequencies, this seems to show that subjects were not physiologically relaxed, as this would instead be reflected as an increase in skin blood flow. Furthermore, when an individual is relaxed, this is shown as an increase in the high-
frequency component of heart rate variability. However, in this study, HRV and particularly the HF component remained relatively stable, with no significant difference in any of the HRV indices measured.

Since subjects were kept in an essentially thermoneutral environment, it cannot be said that these responses, or lack thereof, were caused by thermoregulatory demands. It also cannot be said that the motion was a novel arousal response, as the duration of each frequency was long enough that if habituation to a novel stimulus were to occur, it would have done so much earlier. Therefore, it is safe to say that the modulation that was seen was purely due to activation of the vestibular system and its effects on sympathetic outflow.

**Limitations**

The number of subjects studied in this project was relatively low (n=10), so it is possible that statistical significance could be found when comparing the values using a larger pool of data from more subjects. Moreover, the order at which the frequencies and magnitudes of stimulation were applied was randomised. This had the advantage of allowing us to examine the effects of acceleration frequency and amplitude independently, and rule out any order effects. However, it is likely that a longer period of sinusoidal linear acceleration, given at only one frequency, would result in greater relaxation and, perhaps, evidence of a decrease in cutaneous vasoconstriction and an increase in the high-frequency component of heart-rate variability, both of which would indicate that the subjects were entering a state of relaxation. Nevertheless, some subjects certainly did drift off to sleep,
and it may be that the vestibular modulation of SSNA over- rode any of the predicted physiological markers of sleepiness.

**Methodological Considerations**

We believe that the stimulation was limited to the utricle because subjects were seated upright with their head vertical and eyes closed for the duration of the procedure. Additionally, most of the frequencies were generally imperceptible and, even though the motors were audible for some of the frequencies, with 0.1 @ 5 mg being especially loud, this did not provide the subjects with any information regarding the direction of the motion. And, most subjects, upon completion of the motion reported that they usually could not tell if they were moving, and when they could, they were unable to discern the direction of motion. The motion protocol performed utilised very slow motion with accelerations of 0.5 and 5 mg and previous studies used different frequencies, accelerations, number of cycles and/or techniques (sGVS or motion platform). Therefore, it is difficult to compare the results of a study with a long duration and very low-frequency motion such as this, with other studies utilising different techniques, of shorter duration and/or with substantially higher frequencies.

As mentioned above, it is not possible for the vestibular modulation of SSNA in this study to be attributed as a response to a novel stimulus causing an arousal response, as habituation would have occurred within seconds if this were so. Similarly, it was shown previously that SSNA cannot be entrained to the respiratory or cardiac cycles (Bent et al., 2006), therefore, the modulation of SSNA shown in this study could not be the indirect
result of respiratory coupling. Skin sympathetic nerve activity, is dissimilar to MSNA in multiple ways, one of which being that SSNA is not entrained to the cardiac cycle and only exhibits weak levels of pulse-linked rhythmicity (Grewal et al., 2012). And, although the recordings, in a thermoneutral environment, were dominated by activity from cutaneous vasoconstrictor neurons, the results lined up with previous research, showing weak levels of cardiac rhythmicity (Grewal et al., 2012).

Furthermore, future studies, using the same (or slightly altered methods), could employ several techniques to less subjectively measure drowsiness and sleep in subjects. Drowsiness and loss of focus, for example, could be assessed using pupillometry – a technique that uses an infrared signal trained to the eyes – to monitor the diameter of the pupil as well as frequency and duration of eyeblinks. It is known that pupil diameter fluctuates and decreases overall as drowsiness increases and focus is lost; these symptoms also lead to increased frequency and duration of eye blinks (Laeng et al., 2012, Larson and Behrends, 2015). Recent research has also discovered that variation in attention can affect pupil diameter; when a person is focused, the pupil is dilated, however, when they are drowsy and/or losing focus, the diameter fluctuates and decreases over time (Laeng et al., 2012). Infrared pupillometry as a method has been utilised in a range different studies to assess a multitude of variables (Laeng et al., 2012, Larson and Behrends, 2015). Thus, since the main markers of sopite syndrome are drowsiness and loss of concentration, and the pupil, as well as frequency and duration of eye blinks, provides indications of both of these factors, pupillometry could provide a great deal more insight on the topic. Moreover, sleep, or the transition from wakefulness into sleep, can be measured by recording electrical activity of the scalp using an electroencephalogram (EEG). The EEG mainly measures in
power distribution in the frequency bands Alpha, Beta, Delta and Theta (Piryatinska, 2013). When humans drift from being awake into sleeping, the power of the EEG shifts from higher frequencies to lower frequencies, and the frequency band shows how deep the individual is sleeping (Piryatinska, 2013). As this is a concrete way to quantify if subjects transition into sleep during the protocol, future studies could use EEG instead of or supplementary to, the observational method used in this current study to.
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APPENDIX
APPENDIX 1

Behavioural checklist

I. Subject information

1. Age: ______

2. Sex: ______

3. Which eye do you use when using only one? (Please circle the appropriate option.)
   Left       Right

4. Any vision problems? (Please circle the appropriate option.)
   Normal vision       Short-sighted       Long-sighted       Astigimatisms

5. Are you colour blind? (Please circle the appropriate option.)
   Yes       No

6. How many hours did you sleep last night?
   __________________________

7. When was your last meal?
   __________________________

8. When was your last caffeine (such as coffee, tea, soda, etc.) intake?
   __________________________

9. How often do you use computer? (Please circle the appropriate option.)
   Daily       Weekly       Occasionally

10. Which hand do you use when using the mouse? (Please circle the appropriate option.)
    Left       Right
APPENDIX 2

Epworth Sleepiness Scale (ESS)

Name: ___________________________ Today’s date: _________________
Your age (Yrs): _______________ Your sex (Male = M, Female = F): ________

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?

This refers to your usual way of life in recent times. Even if you haven’t done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the **most appropriate number** for each situation:

- **0** = would never doze
- **1** = slight chance of dozing
- **2** = moderate chance of dozing
- **3** = high chance of dozing

*It is important that you answer each question as best you can.*

<table>
<thead>
<tr>
<th>Situation</th>
<th>Chance of Dozing (0-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting and reading</td>
<td></td>
</tr>
<tr>
<td>Watching TV</td>
<td></td>
</tr>
<tr>
<td>Sitting, inactive in a public place (e.g. a theatre or a meeting)</td>
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<tr>
<td>As a passenger in a car for an hour without a break</td>
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<tr>
<td>Lying down to rest in the afternoon when circumstances permit</td>
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<tr>
<td>Sitting and talking to someone</td>
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<tr>
<td>Sitting quietly after a lunch without alcohol</td>
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<tr>
<td>In a car, while stopped for a few minutes in the traffic</td>
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</tbody>
</table>
APPENDIX 3

Pre-Motion Karolinska Sleepiness Scale (KSS)

Here are some descriptors about how alert or sleepy you might be feeling right now. Please read them carefully and CIRCLE the number that best corresponds to the statement describing how you feel at this moment.

1. Extremely alert
2. Very alert
3. Alert
4. Rather alert
5. Neither alert nor sleepy
6. Some signs of sleepiness
7. Sleepy, but no difficulty remaining awake
8. Sleepy, some effort to keep alert
9. Extremely sleepy, fighting sleep

Post-Motion Karolinska Sleepiness Scale (KSS)

Here are some descriptors about how alert or sleepy you might be feeling right now. Please read them carefully and CIRCLE the number that best corresponds to the statement describing how you feel at this moment.

1. Extremely alert
2. Very alert
3. Alert
4. Rather alert
5. Neither alert nor sleepy
6. Some signs of sleepiness
7. Sleepy, but no difficulty remaining awake
8. Sleepy, some effort to keep alert
9. Extremely sleepy, fighting sleep
APPENDIX 4

Motion sickness susceptibility questionnaire short-form (MSSQ-short)

This questionnaire is designed to find out how susceptible to motion sickness you are, and what sorts of motion are most effective in causing that sickness. Sickness here means feeling queasy or nauseated or actually vomiting.

**Your CHILDHOOD Experience Only** (before 12 years of age), for each of the following types of transport or entertainment please indicate:

As a CHILD (before age 12), how often you **Felt Sick or Nauseated** (tick boxes):

<table>
<thead>
<tr>
<th></th>
<th>Not Applicable – Never Travelled</th>
<th>Never Felt Sick</th>
<th>Rarely Felt Sick</th>
<th>Sometimes Felt Sick</th>
<th>Frequently Felt Sick</th>
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<tr>
<td>Cars</td>
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<td>Buses or Coaches</td>
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<td>Trains</td>
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<td>Aircraft</td>
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<td>Small Boats</td>
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<td>Ships, e.g. Channel Ferries</td>
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<td>Swings in playgrounds</td>
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<td>Roundabouts in playgrounds</td>
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<tr>
<td>Big Dippers, Funfair Rides</td>
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Your Experience over the LAST 10 YEARS (approximately), for each of the following types of transport or entertainment please indicate:

Over the LAST 10 YEARS, how often you **Felt Sick or Nauseated** (tick boxes):

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<thead>
<tr>
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<th>Not Applicable – Never Travelled</th>
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<th>Rarely Felt Sick</th>
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<tr>
<td><strong>Big Dippers, Funfair Rides</strong></td>
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</tbody>
</table>
APPENDIX 5
Motion Sickness Assessment Questionnaire (MSAQ)

Instructions.
Using the scale below, please rate how accurately the following statements describe how you feel right now.

<table>
<thead>
<tr>
<th>Statements</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I feel sick to my stomach</td>
<td></td>
</tr>
<tr>
<td>2. I feel faint-like</td>
<td></td>
</tr>
<tr>
<td>3. I feel annoyed/irritated</td>
<td></td>
</tr>
<tr>
<td>4. I feel sweaty</td>
<td></td>
</tr>
<tr>
<td>5. I feel queasy</td>
<td></td>
</tr>
<tr>
<td>6. I feel lightheaded</td>
<td></td>
</tr>
<tr>
<td>7. I feel drowsy</td>
<td></td>
</tr>
<tr>
<td>8. I feel clammy / cold sweat</td>
<td></td>
</tr>
<tr>
<td>9. I feel disoriented</td>
<td></td>
</tr>
<tr>
<td>10. I feel tired/fatigued</td>
<td></td>
</tr>
<tr>
<td>11. I feel nauseated</td>
<td></td>
</tr>
<tr>
<td>12. I feel hot/warm</td>
<td></td>
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<tr>
<td>13. I feel dizzy</td>
<td></td>
</tr>
<tr>
<td>14. I feel like I was spinning</td>
<td></td>
</tr>
<tr>
<td>15. I feel as if I may vomit</td>
<td></td>
</tr>
<tr>
<td>16. I feel uneasy</td>
<td></td>
</tr>
</tbody>
</table>
## Appendix 6

### Spielberger’s State and Trait Anxiety Inventory

**DIRECTIONS:** A number of statements which people have used to describe themselves are given below. Read each statement and then blacken in the appropriate circle to the right of the statement to indicate **how you feel right now, that is, at this moment.** There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

<table>
<thead>
<tr>
<th></th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Agree</th>
<th>Strongly agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I feel calm...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>2. I feel secure...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>3. I feel tense...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>4. I am regretful...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>5. I feel at ease...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>6. I feel upset...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>7. I am presently worrying over possible misfortunes...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>8. I feel rested...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>9. I feel anxious...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>10. I feel comfortable...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>11. I feel self-confident...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>12. I feel nervous...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>13. I am jittery...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>14. I feel “high strung”...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>15. I am relaxed...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>16. I feel content...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>17. I am worried...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>18. I feel over-excited and “rattled”...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>19. I feel joyful...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>20. I feel pleasant...</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
**DIRECTIONS:** A number of statements which people have used to describe themselves are given below. Read each statement and then blacken in the appropriate circle to the right of the statement to indicate how you generally feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

<table>
<thead>
<tr>
<th></th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Agree</th>
<th>Strongly agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.</td>
<td>I feel pleasant.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.</td>
<td>I tire quickly.</td>
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<tr>
<td>23.</td>
<td>I feel like crying.</td>
<td></td>
<td></td>
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<tr>
<td>24.</td>
<td>I wish I could be as happy as others seem to be.</td>
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<tr>
<td>25.</td>
<td>I am losing out on things because I can’t make up my mind soon enough.</td>
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<tr>
<td>26.</td>
<td>I feel rested.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27.</td>
<td>I am “calm, cool, and collected”.</td>
<td></td>
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<tr>
<td>28.</td>
<td>I feel that difficulties are piling up so that I cannot overcome them.</td>
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<tr>
<td>29.</td>
<td>I worry too much over something that really doesn’t matter.</td>
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<tr>
<td>30.</td>
<td>I am happy.</td>
<td></td>
<td></td>
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<tr>
<td>31.</td>
<td>I am inclined to take things hard.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32.</td>
<td>I lack self-confidence.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.</td>
<td>I feel secure.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34.</td>
<td>I try to avoid facing a crisis or difficulty.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35.</td>
<td>I feel blue.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36.</td>
<td>I am content.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.</td>
<td>Some unimportant thought runs through my mind and bothers me.</td>
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</tr>
<tr>
<td>38.</td>
<td>I take disappointments so keenly that I can’t put them out of my mind.</td>
<td></td>
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<tr>
<td>39.</td>
<td>I am a steady person.</td>
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</tr>
<tr>
<td>40.</td>
<td>I get in a state of tension or turmoil as I think over my recent concerns and interests.</td>
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</tbody>
</table>