Psychophysiological markers and the brain processing of visual motion induced nausea in healthy humans.

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Declaration

The work presented in this thesis was done by the author, Kee Seong Ng, at the Wingate Institute of Neurogastroenterology, Barts & The London School of Medicine & Dentistry, Queen Mary University of London. All external sources have been properly acknowledged.

Dr Kenneth Tachi assisted me in some of the experimental work in chapter 2 of this thesis. This was included as part of his M.Sc. thesis submitted to the University of London, 2010.

Vin Fei Ban assisted me in some of the experimental work in chapter 3 and part of chapter 4 of this thesis. He also helped with some of the subsequent data handling. This was included as part of his B.Sc. thesis submitted to the University of London, 2011.

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Abstract

Background: Nausea is a common and complex multi-system sensation however objective psychophysiological markers of nausea that also predict nausea susceptibility in humans are lacking. In addition, the regions of the brain that process the sensation of nausea are unknown.

Aim: To investigate the brain processing of nausea in healthy individuals.

Methods: Study 1 validated the visual motion induced nausea paradigm with autonomic measures. Study 2 preselected nausea susceptible versus nausea resistant subjects using the stimulus with autonomic, electrogastrographic and cortisol monitoring. Study 3 investigated the brain processing of the nausea sensation and Study 4 identified which brain regions were specific to the generation of nausea.

Results: Studies 1 and 2 – The stimulus was validated with stardardised questionnaires and identified nausea susceptible and resistant individuals with those susceptible demonstrating more anxiety; sympathetic arousal, parasympathetic withdrawal; shift from normogastria to dysrhythmia after motion video. Studies 3 and 4 – The inferior frontal gyrus was positively correlated with increasing nausea and the parahippocampus was inhibited. However, nausea resistant subjects demonstrated increased activity in the parahippocampus. The scopolamine study was overall inconclusive due to nausea being induced by the drug itself.

Conclusion: NS subjects decreased parasympathetics, normogastria and increased sympathetics, anxiety and gastric dysrhythmias suggesting these parameters could be used as markers of nausea susceptibility. The inferior frontal gyrus and parahippocampus appears to play a role in nausea genesis and should be investigated further in patients or with other nauseogenic stimulus, newer functional brain imaging modalities, as well as different pharmacological modulations

Abbreviations

$5\text{-}HT_3$ - 5-hydroxytryptamine$_3$

ACTH - Adrenocorticoptropic Hormone

ANCOVA - An Analysis of Covariance

ANS - Autonomic Nervous System

BFI - Big Five Inventory

BMI - Body Mass Index

BP - Blood Pressure

CSB - Baroreflex

CT - Computed Tomography

CV - Control Video

CVT - Cardiac Vagal Tone

DBP - Diastolic Blood Pressure

EGG - Electrogastrogram

FFT - Fast Fourier Transform

fMRI - Functional Magnetic Resonance Imaging

GMA - Myoelectrical Activity

GSR - Galvanic Skin Responses

HADS - Hospital Anxiety Depression Scale

HRRT - High Resolution Research Tomography

HRV -.Heart Rate Variability

ICC - Intraclass Correlation

LVS - Linear Vagal Scale

M1 - Hippocampus Express Type 1

M2 - Muscarinic Type 2

MAP - Mean Arterial Blood Pressure

MBP - Mean Blood Pressure

MEG - Magnetoencephalography

MR - Magnetic Resonance

MSAQ - Motion Sickness Assessment Questionnaire

MSSQ - Motion Sickness Susceptibility Questionnaire

MV - Motion Video

NEO-PI - NEO-Personality Inventory

NK_1 - Neurokinin$_1$

NR - Nausea Resistant

NS - Nausea Susceptible

NTS - Nucleus Tracti Solitarii

PBN - Parabrachial Nucleus

PET - Positron Emission Tomography

S1 - Primary Somatosensory Cortex

SBP - Systolic Blood Pressure

SCR - Skin Conductance Response

SSQ - Simulator Sickness Questionnaire

STAI - Spielberger State and Trait Anxiety Inventory

VAS - Visual-Analogue-Scale

VPpc - Ventroposterior Parvicellular Thalamic Nucleus

WAI - Weinberger Adjustment Inventory

CHAPTER 1

INTRODUCTION

Nausea is a universal human experience and is associated with a range of psychological and physiological responses such as development of anxiety and changes in cardiac autonomic activity, gastric myoelectrical activity and neuroendocrinal hormones. While some knowledge of these psychophysiological responses associated with nausea exists, this information is largely through animal studies and preliminary human studies. In particular, there is a paucity of information available about the brain areas that are involved in the genesis of nausea sensation. There are a variety of novel methodologies for imaging brain function and investigating drug pharmacology (Borsook et al., 2006b) as well as for the induction of nausea by toxins e.g., ipecacuanha (Miller et al., 1996, Minton et al., 1993); and through the motion sickness pathway (Bijveld et al., 2008a). Kowalski (2006) have presented possible approach to the study of nausea using functional brain imaging these include (i) adapting a safe nausea induction method (e.g. visual motion induced nausea), (ii) identifying subjects susceptible to the visual nausea induction and truly experience nausea but who can tolerate nausea without vomiting, (iii) investigation of brain activity using functional magnetic resonance imaging (fMRI).

The following sections will describe nausea, its importance and subsequently propose that fMRI studies are the way forward for nausea research. The current state of knowledge of nausea will be reviewed here and specific aspects reviewed in subsequent chapters.

1.1 Definitions

Nausea is a sometimes difficult-to-describe thoroughly unpleasant sensation usually perceived as being in the stomach (Stern et al., 2011, Quigley et al., 2001) that exists on its own or may sometimes be followed by vomiting (Visser et al., 2001). Meanwhile, vomiting or emesis is the forceful evacuation of gastric contents through the mouth (Steele and Carlson, 2007). The challenge of studying nausea is that it is a dynamic sensation that is very difficult to define with wide variations in the way nausea is reported by each individual e.g. feeling sick, queasy, or butterflies in the stomach (Quigley et al., 2001, Gianaros et al., 2001). The “personal experience” of nausea that is different for every individual was well illustrated by Stern et al., (2011) with his historical list of 30 different descriptions of nausea from the literature. It starts from Galen in the second century, “We also say that the living being becomes nauseous; that is, that the stomach starts to be emptied through vomiting”; and ends with the National Cancer Institute 2009, “Nausea is an unpleasant wavelike feeling in the back of the throat and/or stomach that may or may not result in vomiting”.

In the context of this thesis, the definition of nausea will be the subjective report of nausea on a validated scale that is associated with some or all of the following: increasing levels of anxiety; sympathetic arousal and parasympathetic withdrawal; shift from normal gastric activity to abnormal activity; and also an increase in cortisol.

1.2 Assessing nausea

The experience of nausea is a private sensation and difficult to describe or detect in another person as appearance alone does not reveal the sensation of nausea another human being is feeling (Stern et al., 2011). Thus, it is also not possible to be certain that the “nausea” experienced by one person is the same as another individual.

If the observer is scientific and demands independent evidence before believing what a person is reporting (that is even assuming they are being truthful and can accurately describe their sensations), they are left with three methods: (1) asking the subject, preferably using a validated definition; (2) observing the subject’s behaviour; and (3) obtaining associated psychophysiological data. However, all three methods have limitations. In the chapters that follow in this thesis, it will be pointed out that changes in the autonomic nervous system (Chapter 2 and 3); plasma level of cortisol (Chapter 3); electrogastrogram (EGG) (Chapter 3); and central nervous system (Chapter 4 and 5) are all associated with the sensation of nausea. Appropriate changes measured in these associated markers of nausea would increase the likelihood that an experimenter could conclude that another individual was indeed experiencing nausea.

1.3 Impact of nausea

The socio-economic impact of nausea is considerable and it affects many patients and healthy individuals. In the United States, about 20,000 adults with upper gastrointestinal (GI) disorders were surveyed and nausea was present in 12% of females and 7% of males (Camilleri et al., 2005). The impact of nausea will be explored in a few clinical settings below however as nausea affects so many different conditions, its actual impact is very much larger. About 95% of pregnant women experience nausea during their pregnancy leading to 8.5 million lost working days annually in UK (Gadsby, 1994, Gadsby et al., 1993). It was also reported that post-operative nausea and vomiting was the main reason behind the delayed discharge from hospital of most high-risk patients who had undergone surgery (Fortier et al., 1998) and the costs of caring for such patients were an additional $415 per patient (Gadsby et al., 1993). In addition, it was estimated that nausea and vomiting costs the U.S. economy around 4 and 10 billion dollars per year (Blum et al., 2000). Nausea also impairs the quality of life of affected individuals (Grunberg et al., 1996).

The 2009 European Society of Medical Oncology (ESMO) and the Multinational Association of Supportive Care in Cancer (MASCC) Consensus Conference on antiemetics concluded that the control of vomiting has markedly improved during the last years and therefore attention should shift to control of nausea, at present the greatest remaining emetogenic challenge (Roila et al., 2010).

1.4 Nausea the neglected symptom

Nausea (currently number one fear for cancer patients (Ritter et al., 1998) is typically poorly treated with current management, is more commonly reported than vomiting, and nausea lasts longer and causes more distress overall in comparison to vomiting (Blum et al., 2000). However, most published human (Foubert and Vaessen, 2005) and animal studies of nausea and vomiting have not only reported nausea and vomiting as points on the same continuum of emesis but also mostly ignored the more common and more troublesome symptom of nausea in favour of vomiting leading to our current paucity of knowledge regarding nausea and its possible treatments (Stern et al., 2011). This is also partly due to the lack of a good animal model of nausea even though we have good animal models for vomiting and animal models like the ferret have played pivotal roles in identification of the anti-emetic effects of 5-hydroxytryptamine$_3$ (5-HT$_3$) and neurokinin$_1$ (NK$_1$) receptor antagonists (Andrews and Horn, 2006, Sanger and Andrews, 2006).

In humans, nausea can be more aversive than pain (Pelchat and Rozin, 1982) or vomiting (Morrow et al., 2002b) but is largely neglected due no validated objective markers (Andrews and Horn, 2006, Holmes et al., 2009). In animals, which are unable even to articulate the experience of nausea, the situation is even more critical. Indeed, the experience of nausea and vomiting has so far been an underestimated welfare issue in animal research and in safety studies for new medicines the issue of nausea is not automatically considered (Harrison et al., 1972, Holmes et al., 2009). The result is a paucity of advances in the nausea research area in general.

1.5 Pathways of nausea and vomiting

Traditionally the three inputs inducing nausea and vomiting are the vestibular system via the vestibular nuclei, the area postrema, and the abdominal and cardiac vagal afferents, which all converge in the *nucleus tracti solitarii* (NTS) in the brainstem. This is where vomiting likely diverges from nausea with vomiting pathways confined to the brain stem as retching and vomiting (including prodomata like salivation, swallowing and licking) can be activated in decerebrate dogs, cats, ferrets and *Suncus* (Stern et al., 2011). Meanwhile, the sensation of nausea involves the projection of information rostrally from the brainstem to the forebrain with the vomiting centre integrating vomiting signal and coordinating motor output (Andrews and Horn, 2006).

The vomiting reflex is triggered by activation of the vomiting centre (an inter-related network of neurons rather than a definite anatomical site, first described by (Borison and Wang, 1953) in the medulla oblongata. This involves a complex interaction of receptors and neurotransmitters that are targets of anti-emetic therapy and they include histamine, acetylcholine, dopamine, noradrenaline, adrenaline, 5-hydroxytryptamine and substance P (Lang, 1990, Miller and Leslie, 1994). Vomiting efferent signals are carried via rostral ventrolateral medulla, dorsal vagal motor nucleus, nucleus ambiguous, superior and inferior salivatory nuclei, retrofacial nucleus and ventral respiratory group. These signals produce the complex coordinated set of autonomic responses, muscular contractions and reverse peristalsis seen in vomiting (Lang, 1990, Miller and Leslie, 1994).

1.5.1 Potential cortical pathways of nausea

With few functional brain imaging studies exploring how and where nausea is generated, we can only draw on animal (primarily of rat (lacking an ability to vomit), cat, dog, and to a lesser extent the ferret and nonhuman primates) and human studies of processing visceral and vestibular information and studies of neural pathways of conditioned taste aversion in animals, which has been argued to be similar with nausea (see Andrews 2006 for a detailed review). Comparative studies of fundamental sensations (pain, hunger, and satiety) have identified common features of the processing pathways and as nausea is likely the same then there should be some common features in processing between species where nausea or analogous sensations are present. However, this must be done with the awareness that cerebral cortical anatomy between primates, cetaceans, and other mammals do vary (Craig, 2009b, Craig, 2009a, Dunbar and Shultz, 2007, Marino, 2007, Butler et al., 1996, Craig, 2002).

The vestibular system (crucial for generating visual motion induced nausea (Yates et al., 1998) has projections to the vestibular nuclei and dorsal vagal complex to induce vomiting and its accompanying autonomic changes. There are also direct projections to the cerebellum, spinal cord, and to the extraocular muscles for somatomotor control, postural adjustments to head and neck muscles and limb extensor muscles, and to coordinate the movements of the eyes with those of the head respectively (Felten and Józefowicz, 2003). Thus the vestibular nuclei afferents to the temporoparietal cortex, lateral postcentral gyrus, insular cortex, and thalamus (posterolateral thalamus) may

show that higher cortical regions are likely involved in the genesis of the conscious sensation of nausea.

In a magnetic source imaging study combining magnetoencephalography and MRI structural imaging, nausea induced by head movement during yaw-axis rotation increased activity in the inferior frontal gyrus that was not seen during speech, finger movement, exaggerated breathing or at baseline (Miller et al., 1996). Neuronal activation was also related to the intensity of nausea. The same subject when administered ipecac reported nausea with inferior frontal gyrus activation again that was reversed by 5-HT_3 receptor antagonist (ondansetron). Further exploration is warranted with larger studies utilising newer technology like fMRI and also with stimuli that can be used routinely in the fMRI environment.

Napadow et al (2012b) utilised newer technology to demonstrate that visual motion induced nausea may potentially be used to study nausea mechanisms in fMRI by using a specially designed head coil to present a visual stimulus of alternating black and white stripes with left-to-right circular motion simulating the rotating optokinetic drum to 29 women. There was primary and extrastriate visual cortical activation with the stimulus in all subjects. Increasing nausea was associated with increasing activation in insular, anterior cingulate, orbitofrontal, somatosensory and prefrontal cortices. Moreover, a closer linkage between the anterior insula and midcingulate within the brain areas potentially involved in nausea perception was suggested with anterior insula activation correlating with midcingulate activation ($r = 0.87$). They also showed susceptible

subjects experiencing motion sickness had increasing phasic activity preceding nausea in the amygdala, putamen, and dorsal pons/locus ceruleus. In summary, phasic activations in fear conditioning and brainstem regions may precipitate transition to strong nausea. The multiple dimensions of visual motion induced nausea were then seen with activation of a broader network involving the interoceptive, limbic, somatosensory, and cognitive regions. Unfortunately, his study only utilised female subjects and there was no mention of controlling for their menstrual cycle. The associated psychophysiological correlates of nausea were also not studied and only subjective reports of nausea were used. Furthermore, individual variations were not controlled for with a control stimulus in these studies. In addition, an expensive specially fabricated head coil that was necessary for the stimulus which makes it impractical for other laboratories (verbal communication with a co-author, Professor Braden Kuo).

It is worth noting the inferior frontal gyrus was also activated by galvanic vestibular stimulation (Bense et al., 2001, Stephan et al., 2005) caloric vestibular stimulation e.g., (Fasold et al., 2002) in human fMRI studies. The descending vestibular pathways from the semicircular canals and the otoliths to the dorsal vagal complex activate pathways ascending from the brain stem (Yates et al., 1998). Human fMRI galvanic (Bense et al., 2001, Stephan et al., 2005) or caloric (Fasold et al., 2002) vestibular stimulation studies observed sensations of motion or nystagmus in the subjects as a side effect without any nausea. Galvanic stimulation, activates the basal ganglia, inferior and middle frontal gyrus, parahippocampal gyrus and hippocampus, cerebellum (crus I, vermal lobule IV), anterior and posterior insula and retroinsular regions (interoception and visceral

autonomic response), superior temporal gyrus, temporoparietal cortex, precentral gyrus, thalamus, anterior cingulate gyrus, and the supplementary motor area (Bense et al., 2001, Stephan et al., 2005).

Unpleasant odours (like hydrogen sulphide “rotten egg” smell) and bitter taste (Peyrot des Gachons et al., 2011) may also be used to study nausea but there may be difficulties in separating direct pathway (cf. olfactory, area postrema, vagal afferents) versus learned association induction of nausea. Hospital smells evoking vivid memories of the nausea and vomiting experienced during chemotherapy given at the same time is an example of learned association in anticipatory nausea and vomiting during anticancer chemotherapy (Morrow et al., 2002a). Brain imaging studies exploring real and imagined pleasant (strawberry) and unpleasant (rotten eggs) odours had increased activity in the left frontal piriform cortex (primary olfactory cortex) and the left insula, although activity was also increased in the orbitofrontal cortex in response to the real or imagined unpleasant odour (Bensafi et al., 2007). The pathways involved with unpleasant odours may be more complicated though as there are food in many cultures which smells like vomit (e.g. rancid, acidic, fermented) but are nevertheless eaten, indicating that the revulsion to certain odours can be suppressed probably by observation of conspecifics behaviours (usually parents). These odours have had so much notoriety that airlines in Southeast Asia are reported to have prominent signs to prevent the locally popular Durian fruit (*Durio zibethinus*) being brought into the cabin as when ripe is said to have an odour like stale vomit (Davidson, 1999). Bitter taste has a rational link to nausea because most plant-derived toxins taste bitter and causes

nausea in toxin-induced illness (Peyrot des Gachons et al., 2011) and it has been described that people who are the most sensitive to bitter stimuli are more prone to motion sickness (Benson et al., 2012, Sharma et al., 2008). A functional near-infrared spectroscopy with 6-n-propylthiouracil (bitter tasting) and salt presented to 48 healthy volunteers showed subjects perceiving bitter taste compared with those who don't increased left posterior dorsolateral prefrontal cortex and bilateral ventrolateral prefrontal cortex activity (Bembich et al., 2010).

We may be able to also look at the more readily available information from visceral afferent studies of cortical projections to gain an insight into the nausea pathway(s) with suggestions that the brainstem afferent signals likely evoke a conscious sensation in the cerebrum, which may be interpreted as nausea but this is still being explored with evidence mostly from animal studies and some limited human studies discussed below. On top of that, descending modulation (periaqueductal gray, PAG), anticipation and attention during aversive stimuli (Van Oudenhove et al., 2007) like those seen in human pain pathways studies may also be present in nausea.

Rat electrophysiological studies show vagal afferents going to the primary somatosensory cortex (S1) but in the cat they went to cortical area 3a but not 3b (S1 equivalent) (Ito and Craig, 2003). The 3a, the cingulate, and insular cortex may make up a “visceral afferent cortical network” (Ito and Craig, 2003) and brain imaging studies in humans support the cat data discussed later. In addition, the insular cortex gets afferent projections from the basolateral amygdaloid nucleus and the infralimbic cortex (Loewy

and Spyer, 1990) and initiate and modulate autonomic outflows via efferents to the amygdala, hypothalamus, PAG, and brainstem. Electrical stimulation of the hippocampus, the nucleus ventralis anterior of the thalamus, anterior perforated area, and the amygdala reliably induced vomiting in macaque monkeys (Robinson and Mishkin, 1968). Thus, animal studies show that projections from the brain stem (NTS and parabrachial nucleus, PBN) go to the insular cortex via routes encompassing the ventroposterior parvicellular thalamic nucleus (VPpc) or the hypothalamus (see Stern 2011 for more detailed reviews of animal studies).

Human brain gut pathway studies where nausea is not the primary outcome measure may still give us a preliminary idea of the visceral afferents pathways involved (Stephan et al., 2003, Aziz et al., 2000, Kern and Shaker, 2002, Derbyshire, 2003, Stephan et al., 2005, Dunckley et al., 2005, Lawal et al., 2005, Vandenbergh et al., 2005, Coen et al., 2007, Ladabaum et al., 2007). However there are relatively few stomach stimulation studies (structures commonly associated with nausea induction) with most brain imaging studies investigating either oesophageal or rectal painful and non-painful distension.

In general, the same major nuclei like the parabrachial nucleus, hypothalamus, thalamus, cingulate cortex, and insular cortex are involved in visceral afferent processing in humans as discussed above in animals. The insula, interoceptive cortex, is the major cortical site to which visceral afferent (including vagal) information projects as seen in animal studies and human studies (oesophageal and gastric distension)

including studies (Aziz et al., 2000, Stephan et al., 2003, Stephan et al., 2005, Vandenbergh et al., 2005, Ladabaum et al., 2007) . In addition, thalamic projections are seen in humans to SI/SII somatosensory cortices, cingulate cortex (limbic motor cortex), insular cortex (limbic sensory cortex), prefrontal cortex (orbitofrontal cortex, dorsolateral prefrontal cortex) that are responsible for sensory, emotional, and cognitive responses to painful visceral stimulation in varying degrees (Coen et al., 2007). These areas of the brain have been collectively refered to as the cortical “visceral sensory/ pain neuromatrix” (Van Oudenhove et al., 2007).

Studies stimulating the stomach that may be more associated with nausea induction include a PET study (Ladabaum et al., 2001) where progressive distal stomach distension in healthy volunteers showed activation in the caudate nucleus, anterior cingulate cortex, thalamus, and insula but was not symptom specific because there was strong correlation among the sensations(earlier related study provoked a gradation of bloating, pain, and nausea sensations (Ladabaum et al., 1998). Another fMRI study with painful fundic distension increased activity in the insular cortex, anterior and posterior cingulate cortex, right frontal lobe, and the inferior parietal lobes of the brain (Ladabaum et al., 2007). This is similar to results from fundic distension brain imaging studies with the caveat being primary somatosensory cortex (S1) activation (Vandenbergh et al., 2005, Lu et al., 2004, Ladabaum et al., 2007).

The development of a new treatment for standard therapy resistant epilepsy and depression using electrical stimulation of the vagus nerve has proven fortuitous for brain

imaging studies investigating the vagal pathway. (Dietrich et al., 2008) utilised fMRI and transcutaneous stimulation of the left cervical vagus to show involvement of the left locus coeruleus, left prefrontal cortex, bilaterally in the postcentral gyrus, left posterior cingulate gyrus, thalamus, and the left insula with vagal stimulation. (Narayanan et al., 2002) used implanted electrodes with similar results except for more insular and thalamic activation. (Kraus et al., 2007) also used fMRI and transcutaneous stimulation of the vagus nerve (auricular branch) with increased activation in the insula, precentral gyrus, and thalamus. What may possibly be equally important are areas deactivated by vagal stimulation that includes the hippocampus, parahippocampal gyrus, amygdala, superior temporal gyrus (Kraus et al., 2007), cerebellum, nucleus accumbens, and posterior cingulate gyrus (Henry et al., 1998, Dietrich et al., 2008).

In summary, there are anatomical pathways by which nausea (and vomiting) signals can access the highest level of the brain where we assume they enter our consciousness (Figure 1). This provides a theoretical framework for nausea generation that we can utilise to design future studies investigating the cortical pathways involved in nausea.

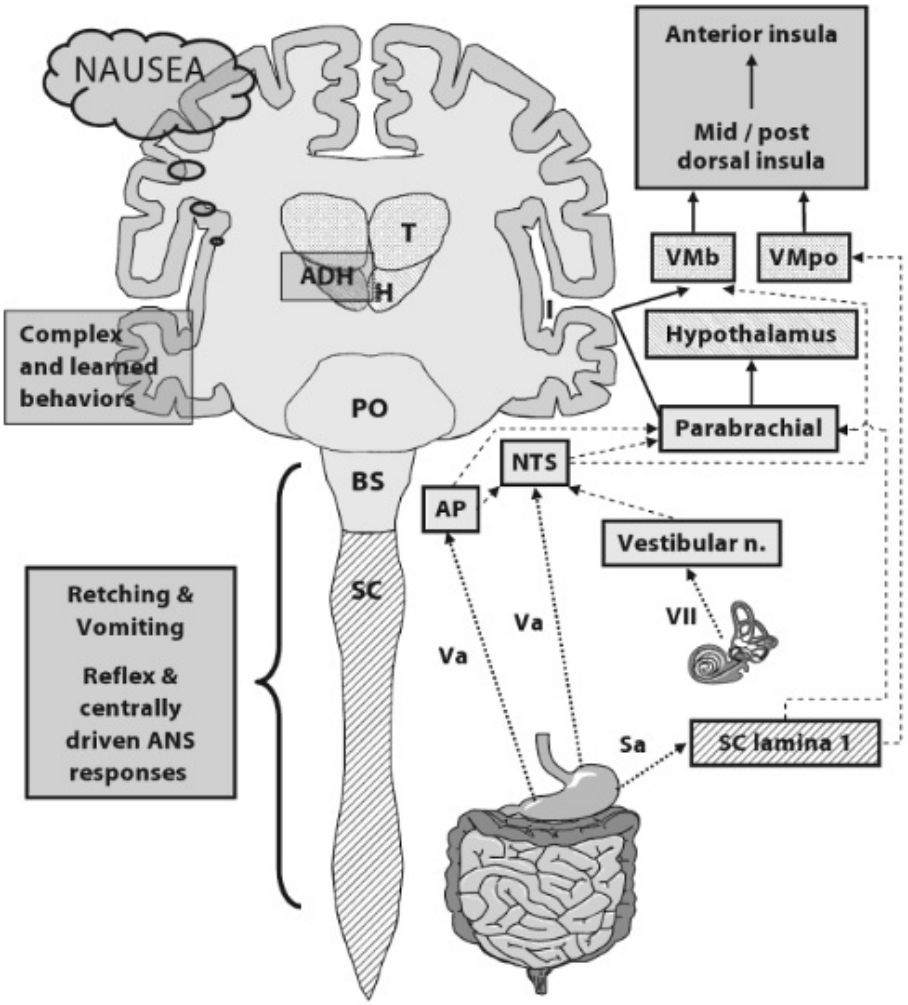

Figure 1. Diagram summarizing major pathway(s) involved in the sensation of nausea. The pathways shown combine Craig's (2002) primate pathways involved in the processing of abdominal vagal afferent information; and projections of the area postrema and vestibular system (Loewy and Spyer, 1990, Yates et al., 1998, Saper, 2002) thus providing a pathway by which nausea could be induced by their activation. It also highlights the hierarchical information processing by shading brain structures with specific structures indicated with a dotted line (...). Second order projections with a dashed line (---) and higher order projections with a solid (__) line. Abbreviations: ANS-Autonomic Nervous System; AP-Area Postrema; BS-Brain Stem; H-Hypothalamus (particularly Posterior hypothalamus, supraoptic and paraventricular nuclei); I-Insular region of the Cerebral Cortex; NTS-Nucleus Tractus Solitarius; PO- Pons; Sa – Greater Splanchnic Nerve Afferent Fibres; SC-Spinal Cord; T-Thalamus; Va-Abdominal Vagal Afferent Nerves; Vestibular n.-Vestibular Nerve Nucleus; VII-Vestibular Nerve; VMb-The basal region of the ventromedial thalamic nucleus; Vmpo-The posterior region of the ventromedial nucleus of the thalamus (Stern et al., 2011)

1.6 Aims

The primary aim of my study was i) to develop a stimulus which could be reliably used to evoke nausea in preselected subjects (with their nauseous response validated by well-defined psychophysiological measurements), ii) to use this stimulus for a functional magnetic resonance imaging (fMRI) study to identify the brain processing of nausea, and iii) to compare brain activity in visual motion induced nausea susceptible and resistant subjects and iv) to perform pharmacological studies to determine that brain areas identified in the above studies were specific to the generation of nausea.

CHAPTER 2

VALIDATION OF A HUMAN MODEL OF NAUSEA

1 Introduction

In identifying a suitable stimulus to study nausea without vomiting in healthy human volunteers, it is important to consider the safety aspects of the stimulus especially since during fMRI studies the subjects will be supine and will need to be able to easily stop the stimulus if necessary and investigators need a gradually increasing severity of the stimulus to have enough warning to prevent volunteers from vomiting.

1.1 The challenge of studying nausea in humans

The challenge of studying nausea is that it is a dynamic sensation that is very difficult to define (discussed in chapter 1) with wide variations in the way nausea is reported by each individual e.g. feeling sick, queasy, or butterflies in the stomach (Quigley et al., 2001, Gianaros et al., 2001, Stern et al., 2011). In addition, there may also be associated symptoms of anxiety and autonomic changes e.g. feeling sweaty, warm, having tachycardia or stomach awareness.

Furthermore, as currently available anti-emetics can be ineffective against nausea, an improved understanding of the pathways unique to nausea will be important in developing pharmacological agents potent against both nausea and vomiting (Herrington et al., 2000, Warr et al., 2005).

The fact that the sensation of nausea represents a complex multi-system overlap of psychological and physiological aspects (Holmes et al., 2009, Morrow et al., 2002b) and

therefore necessitates a comprehensive investigation of all potential psychophysiological measures to fully understand their integration and interaction during the experience of nausea. Visual motion induced nausea provoking the typical psychophysiological markers will allow the identification of subjects who are suitable functional brain imaging studies of nausea genesis (Stern et al., 2011).

The advent of new investigative modalities such as magnetic resonance imaging (MRI), positron emission tomography (PET) and computed tomography (CT) have revolutionized medicine as we now have noninvasive tools for exploring the structural and functional neuroanatomy of the human brain. In addition, other noninvasive methods are now available that allow measurement of outputs from the brain to the body e.g. novel measurements of brain stem mediated beat to beat variations in heart rate and vagal influence on the stomach muscle electrical activity. Furthermore, blood sampling can reveal neurohumoral pathway activity from the brain e.g. cortisol.

Prediction of nausea susceptibility of individuals is important in many situations and not least among aviation trainees. Various motion sickness susceptibility questionnaires (MSSQ) have shown some reliability in predicting individual susceptibility (Golding, 1998). However, they are subject to bias of recall of previous experiences of visually induced motion sickness. A negative history of visually induced motion sickness may be the result of non-encounter with a particular type of motion, lack of recent travel opportunity, reduction in susceptibility with age or lifestyle choice of avoidance

behaviour. Studies to comprehensively define objective biomarkers that accurately predict visually induced motion sickness are necessary.

1.2 Methods of studying nausea.

The first recorded study of nausea may be by Hippocrates two thousand years ago when he wrote '… sailing on the sea proves that motion disorders the body….' following his observation of sailors at sea (Golding, 2006). The origin of the word 'nausea' from the Greek word 'naus' meaning ship may have been formulated from this observation. Following that, there have been many attempts at studying the nausea sensation in humans using various methods.

1.2.1 Ingested or injected agents

Nausea induction using ingested agents e.g. ipecac are effective in humans (Miller et al., 1996) however individual dosage variations to achieve similar levels of nausea and risk of complications are high (Schofferman, 1976), Intragastric irritants such as ipecac, and copper sulphate which cause nausea by stimulating abdominal vagal afferents have also been used as stimuli in nausea studies (Sanger and Andrews, 2006, Andrews and Horn, 2006). Other than that, systemic agents including cytotoxic drugs like cisplatin have been used for chemotherapy patients. There are also absorbed agents (including drugs) acting directly on the *area postrema* e.g. apomorphine, and morphine that have

been utilised in nausea and vomiting research (Andrews and Horn, 2006, Morrow, 1985). They are difficult to control and risk of toxicity is often a major concern.

1.3 Optokinetic drum for motion induced nausea

Motion (both real and illusory) provides a stimulus that is relatively easy to control and subjects have complete control in the case of illusory motion that allows for a good safety profile which is important in fMRI studies when the subjects are lying down (Stern et al., 2011). Visual motion induced nausea provides a unique setting for the laboratory study of nausea, because of the observation that persons who are more susceptible are similarly more susceptible to nausea and vomiting from post-operative nausea and vomiting (Morrow, 1985). Laboratory simulation of these have employed means such physical body rotation and the phenomenon of vection (Bonato et al., 2005, Lackner and Dizio, 2006). A revolving chair study investigated the effects of placebo, dimenhydrinate (an antihistamine), and ginger root capsules on gastrointestinal sensations (Mowrey and Clayson, 1982). This psychophysical study reveals the temporal change in the intensity of gastrointestinal sensations and possibly shows the transition from nausea sensory pathways activation to vomiting or its prodromata. Various studies have since used the principles of vection for visual motion induced sickness using an optokinetic drum (Oman, 1998), (Figure 2). Visual field moves in opposite directions during motion and vection is the compelling illusion of self-motion in the opposite direction when a stationary individual observes movement in a large part of

their field of view (Kennedy et al., 1996). This visual input causes visual motion induced sickness on its own (Bubka and Bonato, 2003) likely due to conflicting stimuli from visual, vestibular and somatosensory systems as suggested by the sensory conflict theory (Oman, 1998, Reason, 1978), Although the optokinetic drum is effective in inducing nausea and vomiting but the large and moving metallic structures needed interferes with monitoring equipments and is unsuitable for functional magnetic resonance imaging (fMRI).

Thus a virtual reality projected visual stimulus was developed that was validated against real motion (Bijveld et al., 2008b). With no moving parts and as it is adaptable to projectors already incorporated in standard MRI machines, it is deemed to be the most suitable stimulus available currently to study nausea without vomiting in humans (Figure 3).

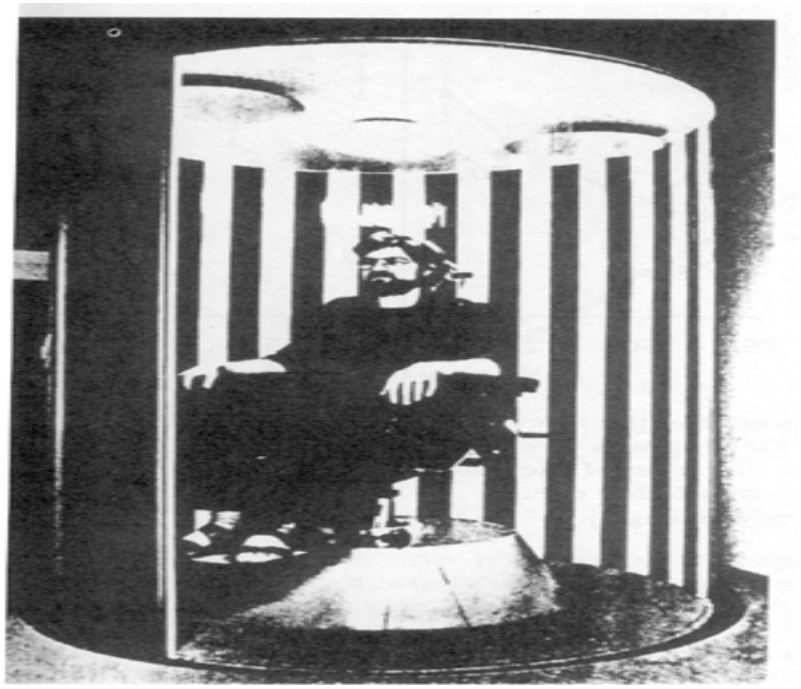

Figure 2. Optokinetic Drum. Subject is in a stationary seat within a painted drum with alternating black and white strips. Subject experiences illusory self-motion opposite to perceived visual motion with rotation of the drum leading to sensory mismatches between the visual, vestibular and kinaesthetic inputs, ultimately causing nausea and vomiting (Image source:http://www.opt.uab.edu, retrieved 8th August 2012).

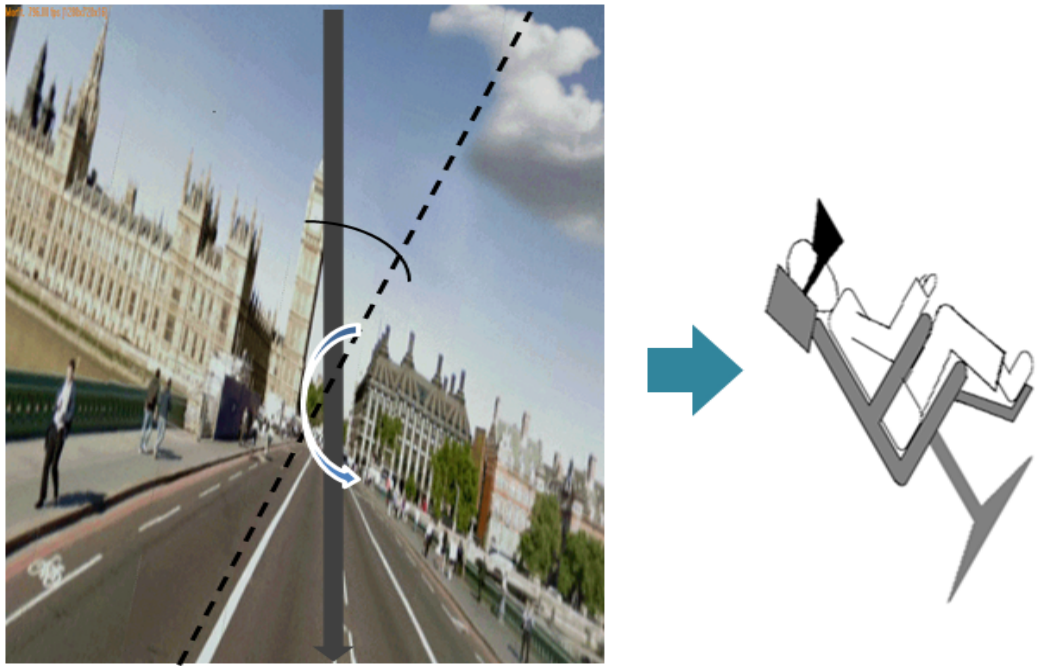

Figure 3. The novel stimulus - projected in front of a subject with goggles to limit their peripheral vision to the stimulus. The off-vertical tilt helps create an illusion that the subject was actually spinning, at an angle which is found to hasten the onset of MSIN (Bijveld et al., 2008b)

1.4 Knowledge gap

Although the motion video has been validated to be an effective stimulus that's similar to real physical motion (Bijveld et al., 2008b) the motion video has not been validated to effectively provoke the related psychophysiological changes. Studies using similar motion videos have generally been effective in provoking physiological changes like autonomic responses to nausea however there are also conflicting results especially when the subjective nausea report was of a mild level (Himi et al., 2004).

1.5 Research aims and hypothesis

The aim of this study was to validate a human model of nausea without vomiting using the visual motion induced nausea method and to determine the psychophysiological changes associated with the development of nausea.

By using the visual motion induced nausea model we can induce nausea in significant proportion of the study population and the induced nausea will be associated with objective changes in the autonomic responses which will act as markers for nausea perception.

2 Methods

2.1 Study design and setting

This was a randomised crossover pilot study i.e. the same subject is exposed to both a control and experimental condition. It was carried out at the Wingate Institute of Neurogatroenterology, Queen Mary University of London (QMUL).

2.2.Ethical approval

The Queen Mary University of London Research Ethics Committee (QMREC2008/37) approved these studies.

2.3 Subjects

Twenty healthy volunteers were recruited. All subjects signed a written informed consent. Volunteers were recruited to meet the following criteria: (i) normal body mass index, (ii) no abnormality on clinical examination, including a history or presence of cardiac, ophthalmologic, gastro-intestinal, hepatic, or renal disease, or other condition known to alter their response to visually induced motion sickness nausea e.g. vestibular disease, (iii) no abnormality on electrocardiogram examination at screening (iv) no abuse of alcohol (defined as an average intake >21 (male) or >14 (female) units per week or 3 units per day); and (v) no history or presence of neurological or psychiatric

conditions (e.g. stroke, traumatic brain injury, epilepsy, space-occupying lesions, multiple sclerosis, Parkinson's disease, vascular dementia, transient ischaemic attack, schizophrenia, major depression, etc) (vi) females during their follicular phase of their menstrual cycle. Subjects with any of the following were excluded: (i) received prescribed medication within 14 days prior to the first visit, which might interfere with the study procedures or compromise safety, (ii) received over-the-counter medicine within 48h before the scanning days, (iii) participated in a trial with any drug within 3 months before the first visit, (iv) had a caffeinated drink within 24 hours of visit.

2.4 Materials and Protocol

Subjects arrived at the institute following a fast of at least six hours. They subsequently underwent the experiment according to the protocol summarised in Figure 4.

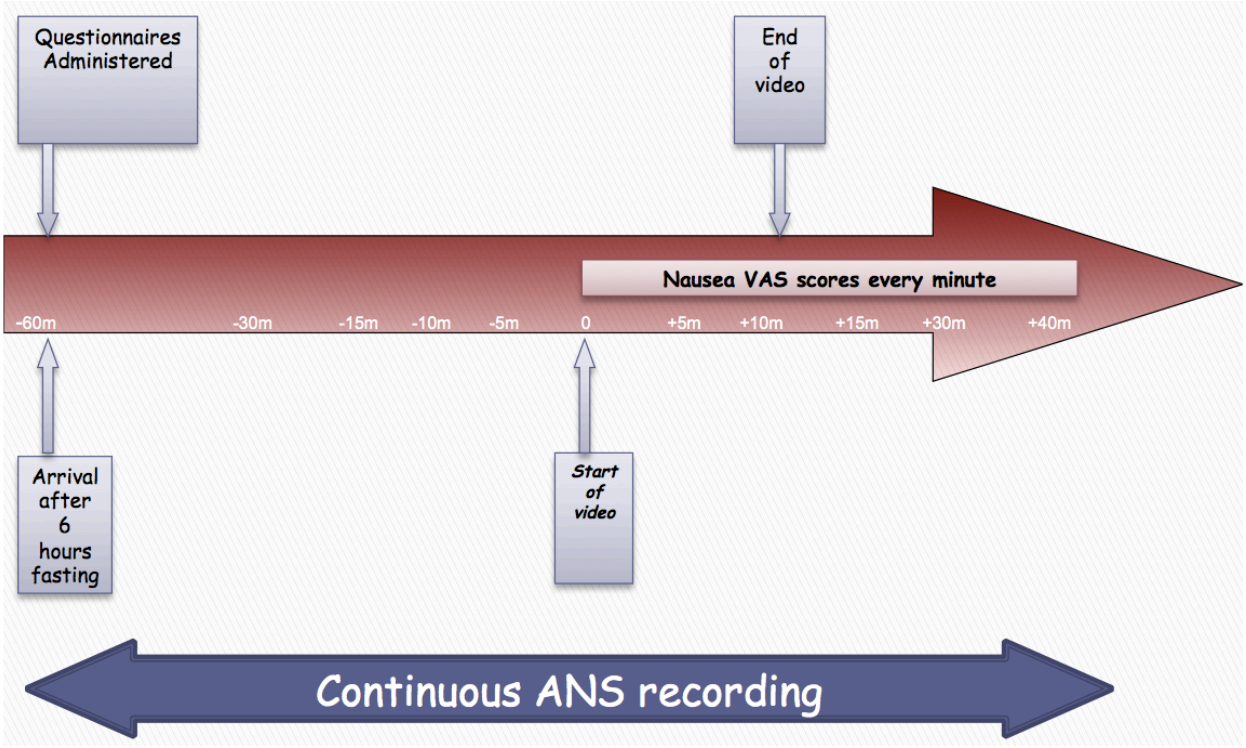

Figure 4. Schematic summary of chapter 2 experimental protocol: After 6 hours of fasting subjects arrived for the study and answered questionnaires which e.g. assess motion sickness sensations and anxiety, reassessed just before starting motion or control video during which minute to minute nausea and anxiety scores were assessed using a VAS and another MSAQ and STAI-S questionnaire done at the end of the video. There were continuous monitoring of cardiac autonomic activity throughout.

2.4.1 *Psychometrics and motion sickness susceptibility questionnaires*

Validated questionnaires were used to assess different aspects of the psychological states and susceptibility to motion sickness of subjects. Psychometric data was analysed by summing individual responses (using formulae provided with each tool) and interpreting the derived sum from excel macro tables provided with a particular tool.

2.4.2 *Big five inventory*

It is known that the usefulness of the NEO-personality inventory (NEO-PI) has been limited due to the large number of items that it contains (240 items), and the big five inventory (BFI) was developed to facilitate the rapid and flexible assessment of McCrae and Costa's five dimensions of personality (McCrae RR, 2003). BFI contains only 44 items, where the respondent agrees or disagrees with a series of statements on a five-point Likert scale. It has been demonstrated that the BFI has substantial reliability and validity and has excellent concordance with the NEO-PI (Soto et al., 2008). The BFI is widely available in the public domain.

2.4.3 Spielberger State and Trait Anxiety Inventory

The Spielberger state and trait anxiety inventory (STAI) is a commonly used instrument for measuring transient and enduring levels of anxiety, respectively (CD, 1983).. The STAI (each scale contains 20 items scored on a four-point Likert scale) has been validated with good test-retest reliability (Rule and Traver, 1983).

2.4.4 Weinberger Adjustment Inventory

The Weinberger adjustment inventory (WAI) is an assessment to measure self-restraint and overall adjustment. It consists of four subscales for Self-Restraint: impulse control, suppression of aggression, consideration of others, and responsibility; and four

subscales for Distress: :anxiety and depression, low self-esteem and low well-being.(Weinberger et al., 1979). Along with these subscales are the measures of self-deception, which is based on the hypothesis that people who deny having negative thoughts and feelings are self-deceptive. In essence, this produces a self-deception or "lie" score and is useful in excluding individuals are responding to self-report questionnaires in a biased manner.

2.4.5 Hospital Anxiety Depression Scale

The hospital anxiety depression scale (HADS) a research and clinical tool which is used extensively to provide clinicians with a reliable, valid and practical tool for anxiety and depression screening (Zigmond and Snaith, 1983). It has been subjected to extensive validation (Herrmann, 1997). It is composed of 14 items, seven of which are related to anxiety and seven to depression, with each item having four possible responses scored 0, 1, 2 or 3. The scale used I a Likert scale and the two subscales of anxiety and depression have been found to be independent measures. Scores on each subscale of less than, or equal to 7, are considered normal (Snaith and Zigmond, 1986).

2.4.6 Assessment of motion sickness susceptibility

The subjects were assessed for motion sickness susceptibility when screened using a validated motion sickness susceptibility questionnaire, MSSQ (Golding, 1998). The

MSSQ predict subjects' likely reaction to visually induced motion sickness nausea by asking for past experiences of nausea in various every-day-life situations. The questionnaire was commonly used for the selection of susceptible subjects [e.g., (Klosterhalfen et al., 2005a, Klosterhalfen et al., 2005b)].

2.4.7 Assessment of visual motion induced nausea

The subjects were assessed for motion sickness sensations immediately before and after exposure to the stimulus. Two most commonly used questionnaires to assess motion sickness are the Simulator Sickness Questionnaire (SSQ) and the Motion Sickness Assessment Questionnaire (MSAQ) The SSQ (Kennedy et al., 1993) was validated originally with aircraft simulators but later also with marine vehicle motion sickness (26 sensations scoring none, slight, moderate, severe). Meanwhile, the MSAQ (Gianaros et al., 2001) was validated using rotating optokinetic drum and developed to measure the multiple dimensions of motion sickness with 16 sensations (gastrointestinal (predominantly nausea), central, peripheral and sopite-related) on a visual-analogue-scale (VAS). The MSAQ correlated strongly with other commonly used nausea questionnaires (Pensacola Motion Sickness Questionnaire Diagnostic Index, $r=0.81$, $p<0.01$ (KELLOGG et al., 1965); and the Nausea Profile, $r = 0.92$, $p<0.01$ (Muth et al., 1996). The MSAQ records the subject’s experience of each of 16 descriptors on a visual-analogue-scale of 1 (not at all) to 4 (severe). To score the MSAQ: (1) sum the points scored for GI distress questions (e.g., nausea); (2) sum the points scored for

central distress questions; (3) sum the points scored for peripheral distress questions; (4) sum the points scored for sopite syndrome questions; and (5) sum the total points scored.

A validated nausea VAS questionnaire (Bijveld et al., 2008b) was also used every minute during each video and a detailed MSAQ used just before and after each video documented any sensations reported by the subjects. The VAS was validated with a similar virtual reality video stimulus and correlated with real physical rotations. A scale from 1 to 4 with 1 being, without sensation, and 4 being maximum level of tolerated sensation (e.g., severe nausea) was used. The peak nausea minute-to-minute scores (majority at the last minute of the video) were used for comparisons.

2.5 *Preparation for experiment*

Subjects were comfortably seated in a silent room maintained at a temperature of 25°C and the following electrodes, cuffs and belt attached as indicated below (Figure 5):

Figure 5. A subject with electrodes attached as described (reproduced with subject's consent).

1. Three ECG electrodes to the skin of the left and right infraclavicular areas and the cardiac apex.
2. A photoplethysmographic-cuff (Finapress®, Ohmeda) attached to the middle phalanx of the right middle finger, to measure the systolic, diastolic, and mean blood pressure for each and every heart beat.
3. A piezoelectric plethysmographic belt placed around the chest at the level of the xiphisternum to measure breathing movements.

4. Two dry, bright-plated, bipolar electrodes with Velcro straps were attached to the middle phalanx of the ring and index fingers of the left hand to measure skin conductance response.

2.6 Baseline activity

Subjects were encouraged to relax their muscles, stay still and not talk while baseline recordings were taken over 10 minutes before the start of the stimulus after the subjects have had time to relax for up to 30 minutes before.

2.7 Exposure to stimulus

Subjects watched two different videos consecutively through a black card board designed to limit their field of view to only the screen. The videos consisted of;

- a non-nausea inducing video consisting of a stationary cityscape (control or neutral video) and
- a nausea inducing video consisting of a moving cityscape (nausea video)

The sequence of exposure was assigned randomly by the investigator. All events, including reported sensations were recorded on a data collection sheet.

The first and second videos were separated by a washout period of 20 minutes during which subjects continued to be questioned every minute for sensations of nausea, anxiety or dizziness until no sensations are reported. This was to avoid a carry-over

effect of sensations from one video onto another and allow EGG recording for the exposure period to be complete.

A video of the cityscape rotating as seen from the perspective of a subject standing on Westminster Bridge, London, UK was used. The video was composed of a sequence of digital camera images of the bridge taken from the viewpoint of a subject standing on the bridge. The images were processed on a PC using programs by 3DSTATE to provide a video sequence with a frame resolution of 1024 by 768 pixels at 16-bit color which was projected with an Acer H5360Eco projector at a refresh rate of 60 Hz onto a screen of 2.00 m x 2.00 m placed at a distance of 1.12m from the subject (validated by (Bijveld et al., 2008b). The control video showed a static scene of the above. The lights were turned down and the subject watched the video for 10 minutes or until severe nausea (rating of 4) occurs, whichever happens first. A red target was put in the video at regular intervals to assess the subject's attention on the video.

2.8 Nausea markers

During the videos, subjects were questioned every minute about sensations of nausea, anxiety and any related complaints which they rated on a visual-analogue-scale of 1 (not at all) to 4 (severe). Just before and just after the video subjects also completed the STAI-state anxiety assessment and the MSAQ to assess motion sickness sensations including nausea. Vital signs and skin conductance responses were recorded continuously throughout the experiment. As the autonomic activity is a dynamic one, the

means for the last third of the video is used for comparisons as that is when subjects report their peak nausea levels and the peak associated autonomic changes are expected (Figure 14, Figure 15, Figure 16, Figure 17, Figure 18).

2.9 The autonomic nervous system

Autonomic nervous system (ANS) function can be directly measured with needle recordings of the peroneal nerve, and direct stimulation of vagal nerves via implantable vagal stimulators. However they are invasive and impractical for human experimental studies. Following that, indirect measures of cardiac autonomic function have been developed with the most popular being heart rate variability (HRV).

HRV was first appreciated clinically in 1965 when Lee and Hon demonstrated that alterations in the inter-beat intervals between successive R waves in the ECG preceded foetal distress before any appreciable changes occurred in the heart rate itself (Lee and Hon, 1965). These oscillations in the interval between successive heart beats or “HRV” has been used in preference to crude heart rate in the majority of the more recent autonomic research.

2.9.1 Beat-to-beat measures

Beat-to-beat measures, irrespective of time frame or assumptions of respiratory stationarity, represent direct measures of autonomic tone. The examples include CVT and cardiac sensitivity to the baroreflex (CSB).

2.9.2 Cardiac vagal tone

Cardiac vagal tone (CVT) is a measure of cardiac parasympathetic efferents via the vagus nerve. BP increases momentarily in ventricular systole causing baroreceptor activation in the carotid sinus and pulmonary circulation, which increases their rate of discharge (McAllen and Spyer, 1978). A vago-vagal reflex is then initiated via medullary neurones in the NTS, by stimulating preganglionic neurones of the vagal nerve to increase firing. The increase in cardiac vagal activity reduces the rate of spontaneous depolarisation of the sino-atrial node, widening the RR interval and decreasing heart rate. The humans vagal response to baroreceptor stimulation is around 240ms that is fast enough to delay the subsequent systole (Eckberg, 1976). Notwithstanding the sympathetic influence on heart rate, mainly through changes in peripheral vascular resistance which takes place more slowly, vagal tone can be calculated non-invasively by measuring beat-to-beat changes in RR intervals.

Based on these principles, the Neuroscope^{TM} (MediFit Instruments, Essex, UK) analyses the RR interval from a standard 3 lead ECG (5kHz sampling) to derive the CVT, a real time index of parasympathetic activity is recorded. The acquired QRS complexes are compared to a QRS template generated from the initial recordings. A 1mV pulse is generated by voltage oscillators if there is sufficient similarity between the recorded complex and template with the time between 1mV pulses equivalent to the RR interval on the ECG. This pattern of 1mV pulses is sent to two circuit limbs known as the high pass limb and the low pass limb. The low pass limb produces a damped version of

the signal while the high pass limb tracks the incoming signal without transforming it. The slower the rate of change of the incoming signal, the lower the rate of HRV will be. And the closer the output match is between the high and low pass limbs the lower the CVT will be as well. In reverse, a higher CVT reading is the result of a higher the HRV (the faster the rate of change of the incoming signal) causing more dampening of the low pass circuit output in comparison to the high pass limb (see Figure 6).

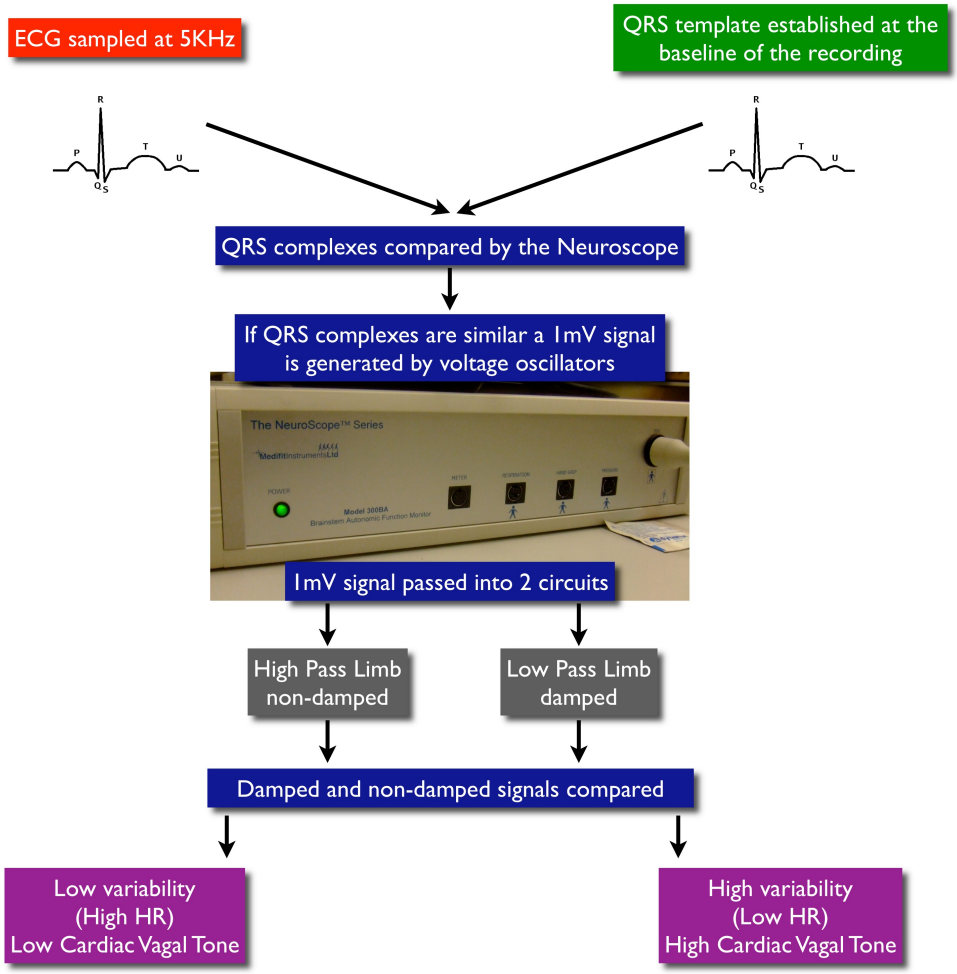

Figure 6 The beat-to-beat measure of cardiac vagal tone as measured by the Neuroscope, using voltage oscillators with high (non-damped) and low (damped) circuit limbs. This diagram is reproduced with permission from Farmer, 2010.

This methodology has been validated in humans and animals and the CVT is measured on an experimentally derived linear vagal scale (LVS) (Julu, 1992). The zero point on the LVS was derived from six fully atropinised healthy volunteers, and 10 units on the LVS established in the same fasting volunteers in the supine position (i.e. maximal vagal activity) (Janig and Kollmann, 1984). Thus, CVT may be considered a validated marker of parasympathetic tone outflow from the brainstem to the heart.

2.9.3 Cardiac sensitivity to the baroreflex

Cardiac sensitivity to the baroreflex (CSB) a validated, non-invasive beat-to-beat measure of parasympathetic afferent activity is measured with a non-invasive continuous blood pressure measurement using the Portapress system (Finapress, Amsterdam, Netherlands). The Neuroscope uses the raw Nexfin waveform to calculate the arithmetic mean of the blood pressure (BP), as opposed to the mean arterial blood pressure (MAP) that is commonly used in clinical settings (MAP = DBP + 1/3(SBP – DBP). The mean blood pressure (MBP) calculated by the Neuroscope is the true arithmetic mean of the BP, i.e. diastolic blood pressure (DBP), dicrotic notch and the systolic blood pressure (SBP). CSB is expressed as a ratio of 'mmHg/'RR interval and calculated as the change in pulse interval per unit change in SBP over a 10-second period by integrating the RR interval data with the BP data (see Figure 7).

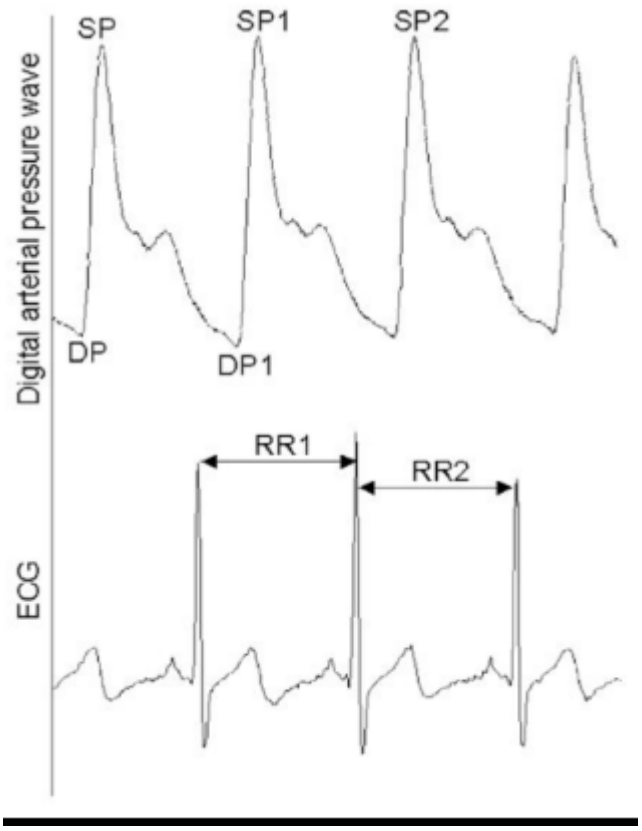

Figure 7 CSB is a beat-to-beat measure of parasympathetic afferent tone derived from changes in BP and expressed as a function of change in RR interval.

2.10 Selective Sympathetic Measures

2.10.1 Vasomotor

Mean arterial pressure was correlated with invasively recorded sympathetic activity via photo-plesythymography (Petersen et al., 1995) that records MBP on a beat-to-beat basis and has been validated against invasive arterial pressure measurements in

humans (Vetrugno et al., 2003). However, vasoconstriction can ensue if the cuff is applied to a subject's finger for a considerable period of time. Thus selecting the wrong finger cuff size can result in large fluctuations in BP readings. The BP cuff was placed on the subjects' left middle finger in this experiment.

2.10.2 Sudomotor

The sudomotor, or skin conductance response (SCR), used for more than 100 years, is a measure of central sympathetic control over sweat gland activity. It is defined as, "...momentary change of the electrical potential of the skin, (it) may be spontaneous or reflexively evoked by a variety of internal or by externally applied arousal stimuli (LOMBARDI and MALLIANI, 1996)." SCR assesses sympathetic cholinergic sudomotor function, and represents a transient change in the electrical resistance of the skin associated with sweating elicited by an arousal or orienting stimulus. Animal studies show efferent sweat fibres originating in the hypothalamic preoptic sweat centre, descend through the ipsilateral brainstem and medulla to synapse with the intermediolateral cell column neurons. The unmyelinated postganglionic sympathetic class-C fibers arise from sympathetic ganglia joining the major peripheral nerves and reaching the sweat glands (LOMBARDI and MALLIANI, 1996). Two interacting types of sweat response are thermal and emotional. Emotional or mental sweating control involves multiple interactions with emotional, cognitive and neuroendocrine functions. It is controlled at multiple levels within the central nervous system, mainly at the anterior cingulate cortex.

One method is to measure spontaneous impedance changes across digits (galvanic skin responses or “GSR”). Another is to pass a small, constant current across the digit and record impedance changes as it crosses the digit (SCR) – with the latter felt to be more reliable. The Powerlab (AdInstruments, UK) biosignals acquisition system can record SCR, which were recorded at baseline and after the videos. The SCR electrodes were placed on the subjects’ left index and ring finger.

2.11 Statistical analysis

Psychometric, autonomic cardiac and gastric data and cortisol data had matched-pair t-tests and Wilcoson tests used to compare the means and medians. Pearson’s and Spearman’s correlations were used to determine the relationship between measurements. The ANS axis data was normally distributed. Continuous variables are expressed as mean ± standard error of mean. Independent-measures t-tests and Mann-Whitney tests were used to compare groups. Multi-group comparisons used a one-way ANOVA with Bonferroni correction. Commercially available statistics packages (SPSS, Chicago, IL, USA and GraphPad, San Diego, CA, USA) were used for the analysis. P values <0.05 were considered to be of statistical significance.

3. Results

Twenty healthy volunteers were recruited from among the staff and students of QMUL. All underwent and completed the experiments. No vomiting or unexpected adverse events were recorded.

3.1 Subject characteristics

Ten males and 10 females with age range of 20 - 40 years (mean 27.65 ± 6.98) were studied with the mean BFI personality subclass scores of the subjects shown in Table 1.

Personality subclass	Mean score ± SEM (%)
Openness	70.38 ± 2.79
Conscientiousness	61.81 ± 3.11
Extroversion	52.97 ± 3.41
Agreeableness	74.13 ± 2.44
Neuroticism	43.93 ± 3.96

Table 1. BFI personality subclass mean scores of subjects

Females and males did not have any statistically significant differences as shown in Figure 8.

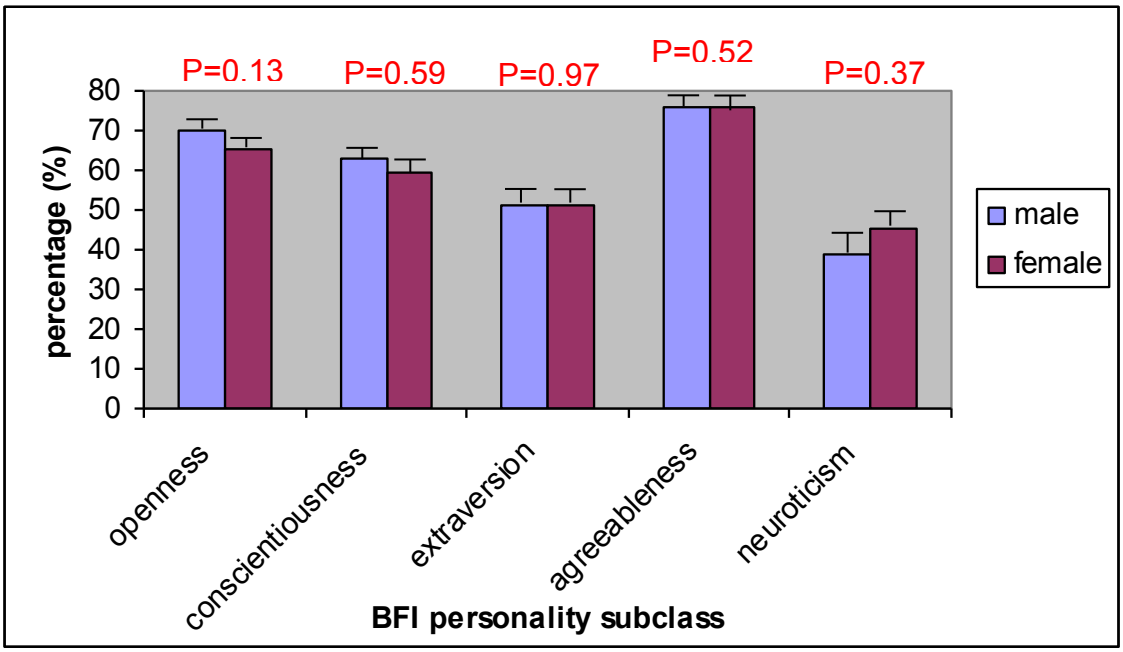

Figure 8. Sex distribution of BFI personality subclass of subjects.

The mean STAI trait anxiety score was 38.7 ± 2.01. The females (44.8) scored higher than males (35.6), but it was not statistically significant. The motion sickness susceptibility questionnaire mean percentage of motion sickness susceptibility was 66.58% ± 29.55 (range 0 - 99).

3.2 Effects of videos on subjective sensations

The GI (mainly nausea) and central (CN) scores were significantly greater after the nausea video compared to control with no significant differences in peripheral (PH) and sophite related (SR) sensation scores between the two videos (Figure 9).

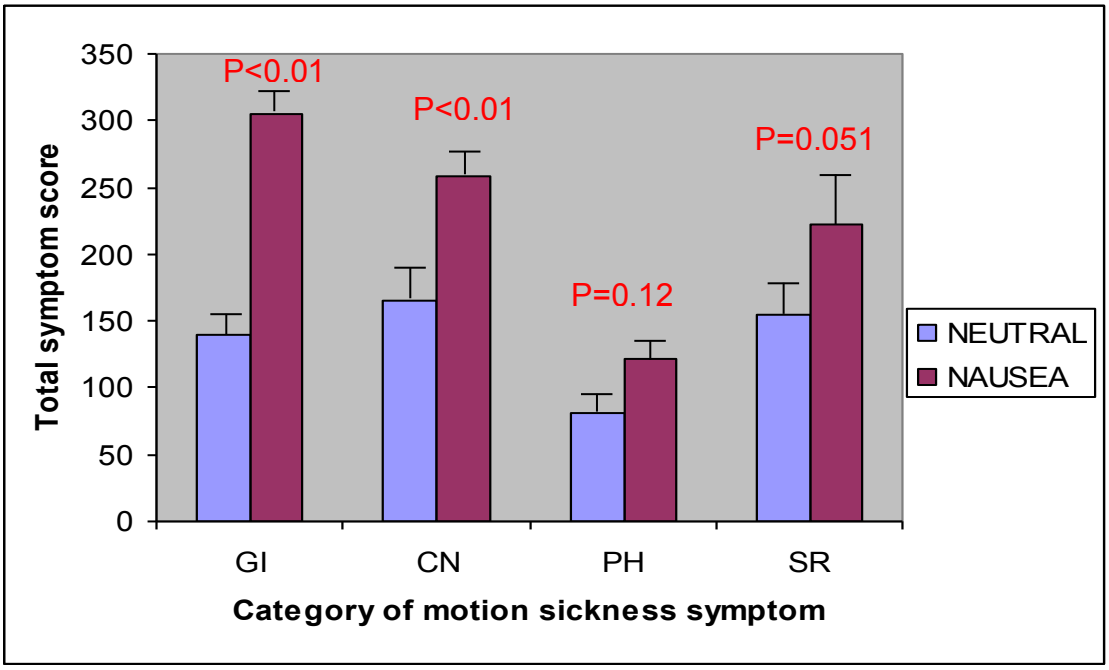

Figure 9. Motion sickness sensation score for the nausea and neutral videos

Fifteen subjects (8 females) had nausea with mean nausea rating of 2.55 ± 0.32 and five (3 females) reported severe nausea that warranted stopping the stimulus with the subjects closing their eyes. The distribution of percentage change in nausea scores of all subjects after the nausea video is shown in Figure 10.

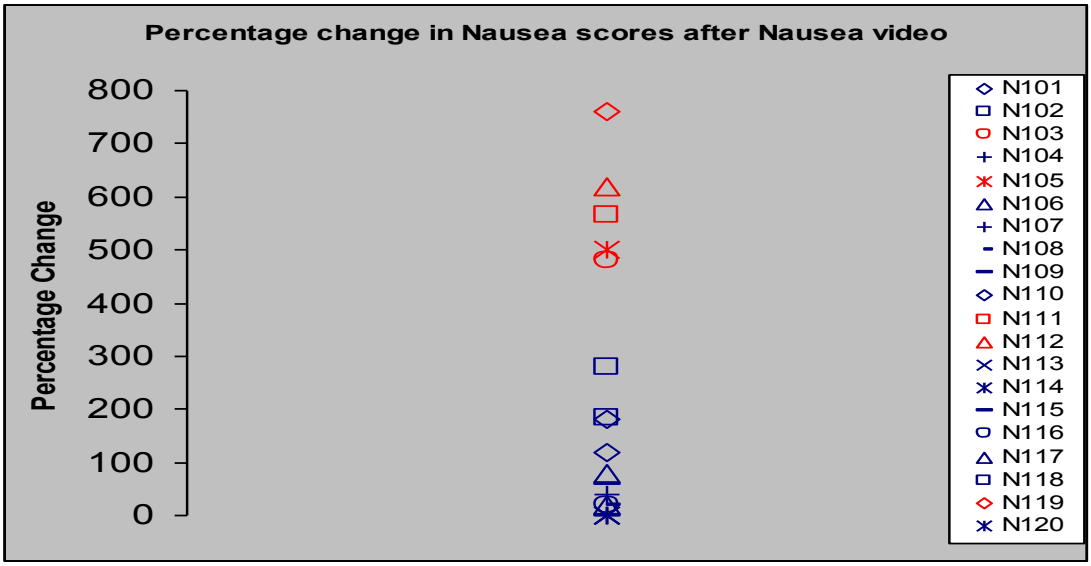

Figure 10. Percentage change in nausea scores for individual subjects after the nausea video with two clusters of susceptible subjects above the midline and resistant subjects at 0%.

The mean percentage change in nausea score from just before each video to the maximum experienced as rated by the subjects on the MSAQ cumulative GI distress scores at the end of each video was significantly higher for the nausea video than for the neutral video (+2.11% vs 0.13% $p<0.01$).

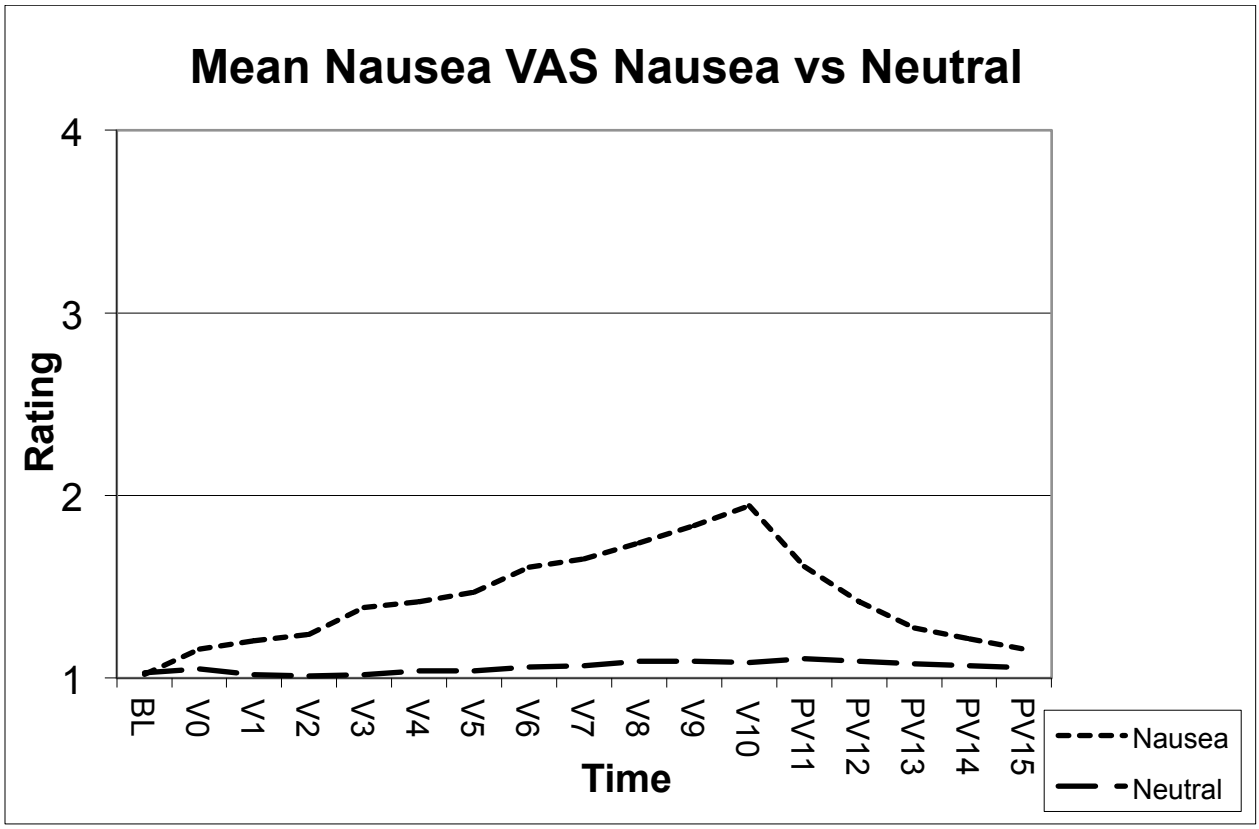

Figure 11. Mean nausea levels during and after the nausea and control videos for all subjects. Mean nausea levels increased gradually throughout the ten minutes duration of the nausea video and reached the peak level at the 10th minute (mean nausea rating =1.73±0.02) compared. There was a steep decrease in mean nausea levels during the five minutes post-video recovery period. Mean nausea levels during the control video remained near baseline values during and after the video. There was a significant difference in mean nausea levels between the nausea and control videos ($p<0.01$). (Ratings - 1=nil; 2=mild nausea; 3=moderate nausea; 4=severe nausea)

As shown in Figure 11, nausea levels increase significantly during the nausea video, peaking at the 10^{th} minute, when compared to the control video. Upon cessation of the nausea video, nausea levels diminished to near-baseline values within 5 minutes.

Nausea levels during the control video remained close to baseline values throughout and after the video.

Mean state anxiety score was higher before the neutral video than before the nausea video, however the change in score was greater during the nausea video (4.7) than during the neutral video (-0.05) (p=0.015). At the end of the videos, the score was greater for the nausea video than for the neutral video, Figure 12.

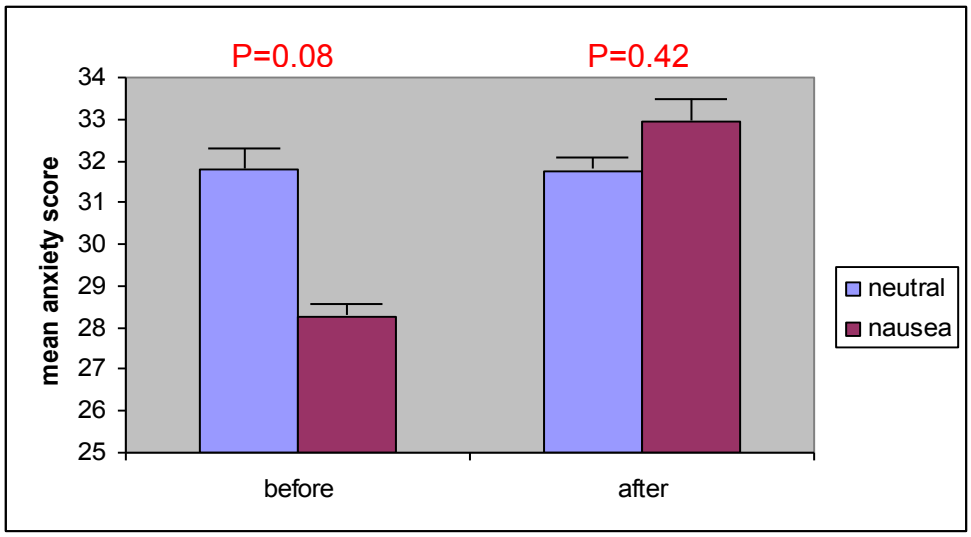

Figure 12. Anxiety scores before and after watching the videos.

Motion sickness sensations resolved in all subjects at the end of the videos. Resolution of nausea occurred in an average of 3.85 minutes (range 0 – 18) and other sensations in 3.75 minutes (range 0 – 14)

3.3 Effects of videos on ANS biomarkers

Levels of ANS biomarkers did not differ significantly between baseline, just before the neutral video and just before the nausea video (p> 0.05). All subjects showed changes in their autonomic biomarkers during the videos. Whereas the mean percentage change in HR, MBP and CSI increased significantly more during the nausea video than during the neutral video, CVT and CSB declined, although the CVT fall was not statistically significant, see Figure 13.

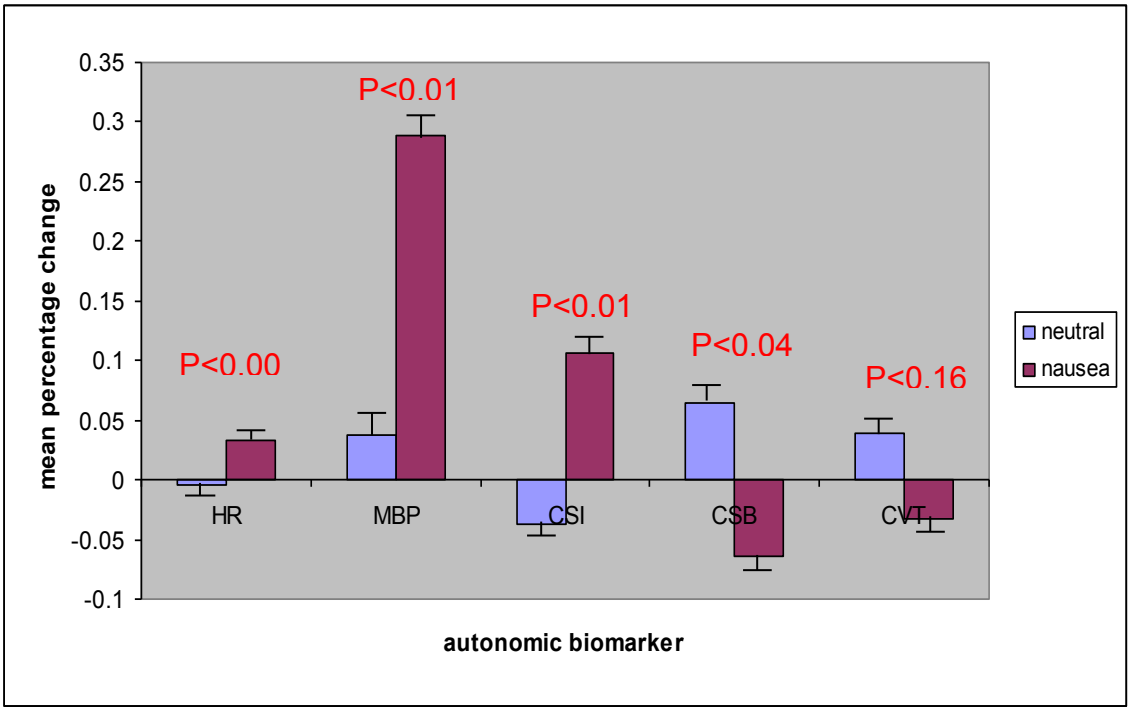

Figure 13. Mean percentage change in ANS biomarkers during neutral and nausea videos.

Also, the differences in the effects of the neutral and nausea videos on the autonomic biomarkers was greatest in the late phase of exposure to the videos, i.e. in the period leading to maximum sensations necessitating premature termination of the video or the full 10 minutes of the video as shown Figure 14 to Figure 18.

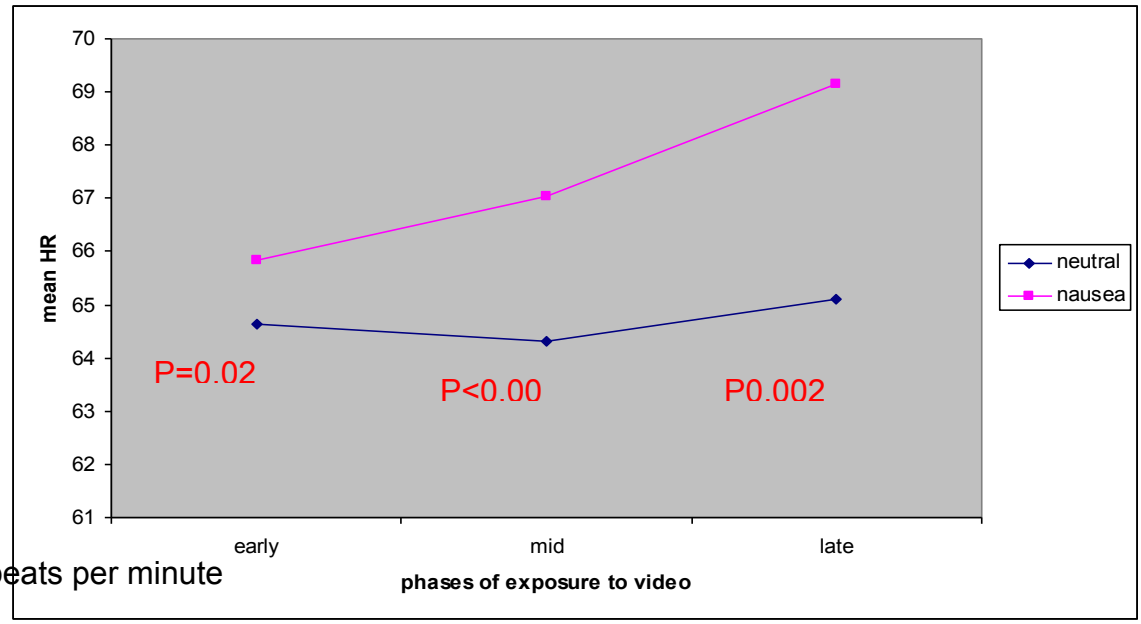

Figure 14. Phasic changes in mean HR during exposure to neutral and nausea videos.

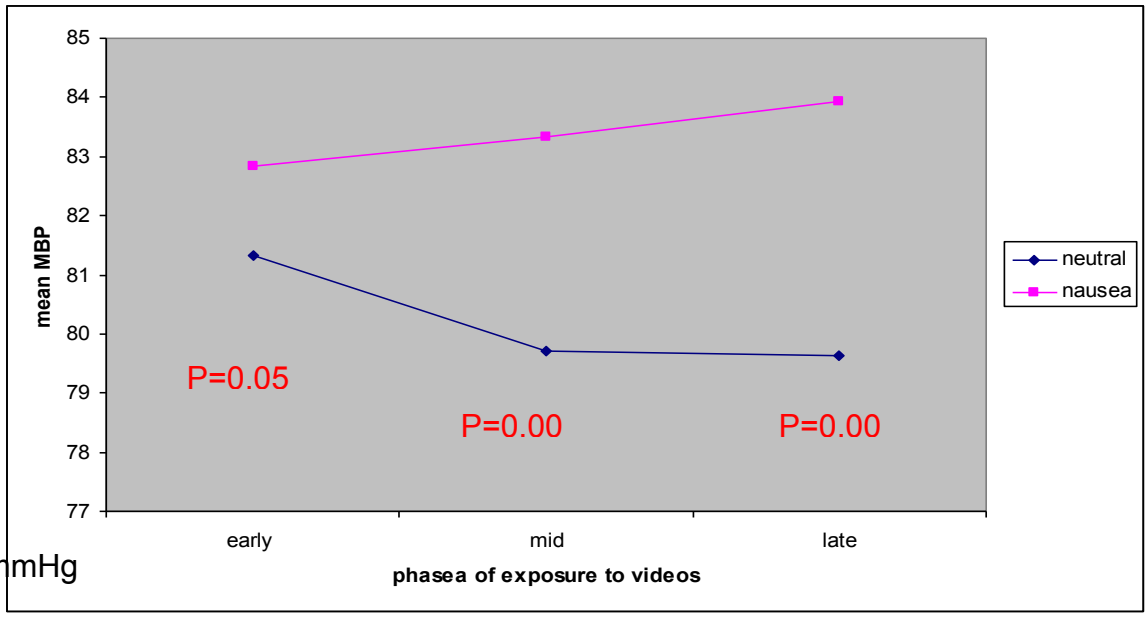

Figure 15. Phasic changes in MBP during the exposure to the neutral and nausea videos

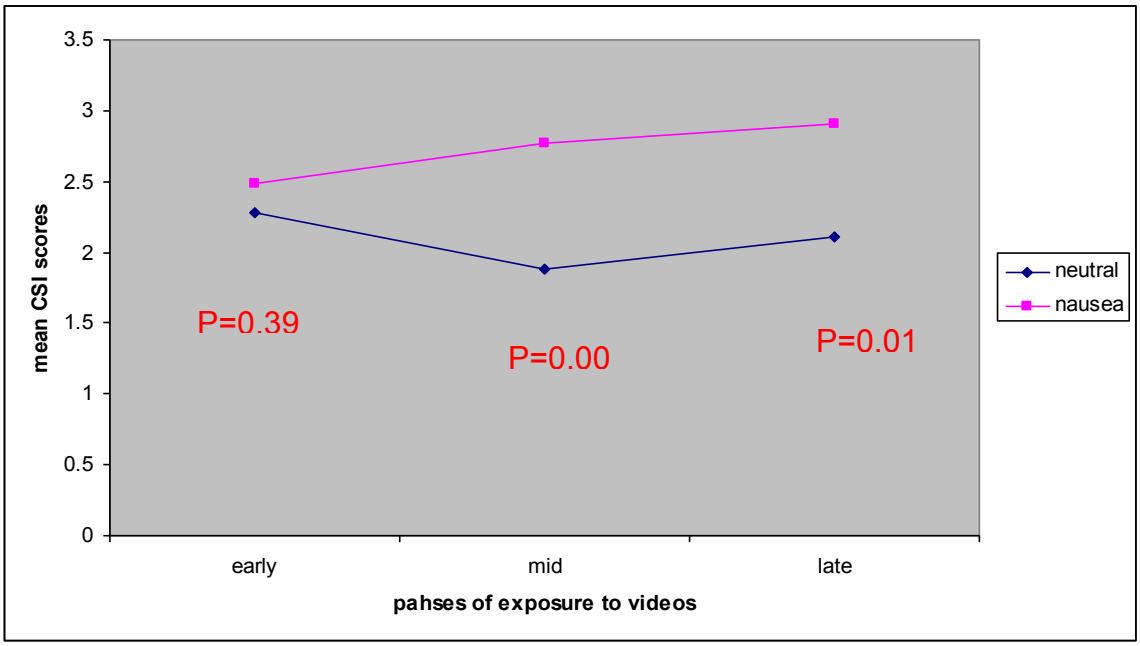

Figure 16. Phasic changes in mean CSI during exposure neutral and nausea videos

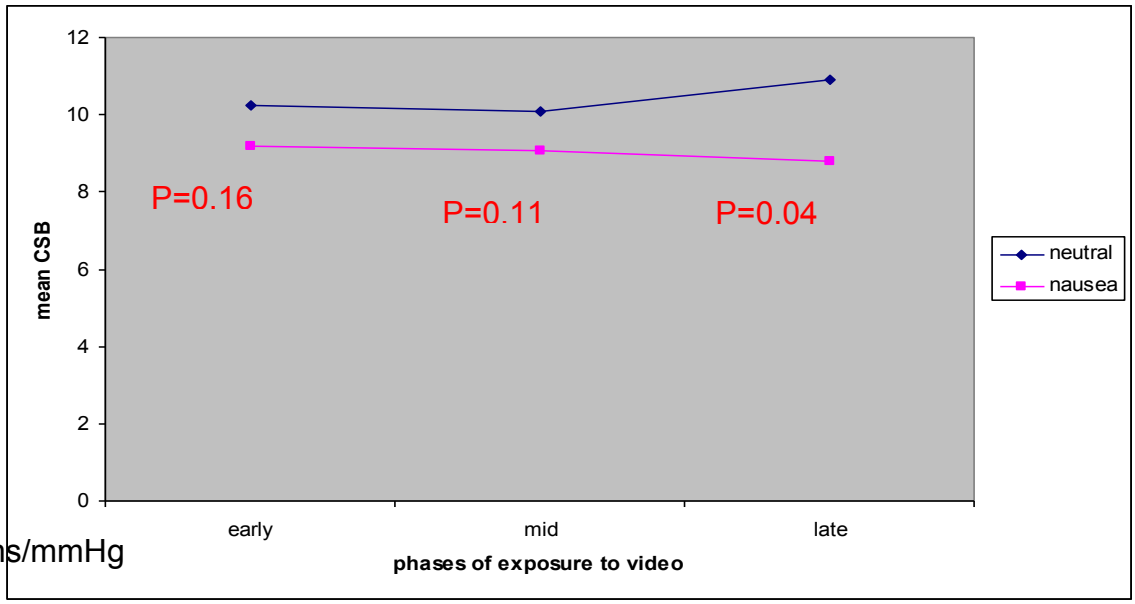

Figure 17. Phasic changes in mean CSB during exposure to neutral and nausea videos

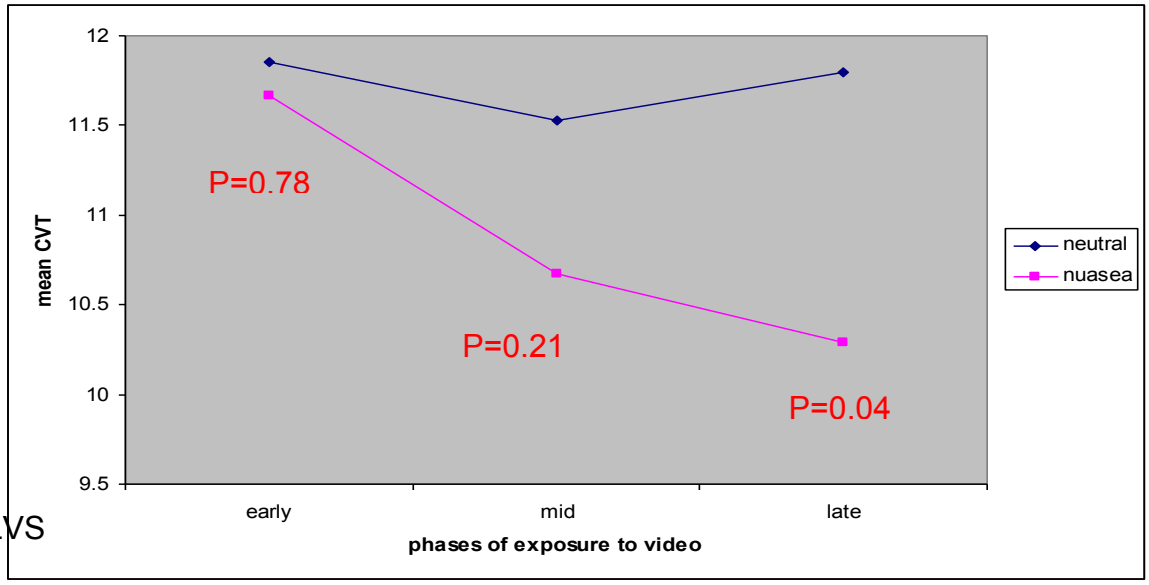

Figure 18. Phasic changes in mean CVT during exposure to neutral and nausea videos.

Skin conductance response (SCR): All subjects experienced changes in their SCR during the videos. However, there was no significant difference in the mean SCR changes during the nausea video and the neutral video, (-5.074 vs -5.462. p= 0.764).

Also, there was no significant correlation between the mean nausea score and the mean ANS biomarker levels of the subjects as shown in Table 2.

ANS biomarker	**Pearson's correlation, r.**	**p-value**
HR (bpm)	-0.139	0.570
MBP (mmHg)	0.094	0.700
CSI	0.000	0.998
CVT (LVS)	0.202	0.407
CSB (∆RR/∆mmHg)	O.257	0.288

Table 2. Correlation between mean nausea score and the ANS biomarker levels.

3.4 Differences between nausea susceptible and resistant subjects

The 5 subjects who did not experience any nausea and the other 5 who experienced severe nausea were grouped as 'nausea resistant' (NR) and 'nausea susceptible' (NS)

respectively for these comparisons. There was no significant difference between the mean percentage visually induced motion sickness nausea susceptibility, assessed by MSSQ, of the NS (68.75%) and NR subjects (65.82%) (p= 0.864).

Mean personality subclass scores also did not differ significantly between nausea susceptible and resistant subjects (Figure 19).

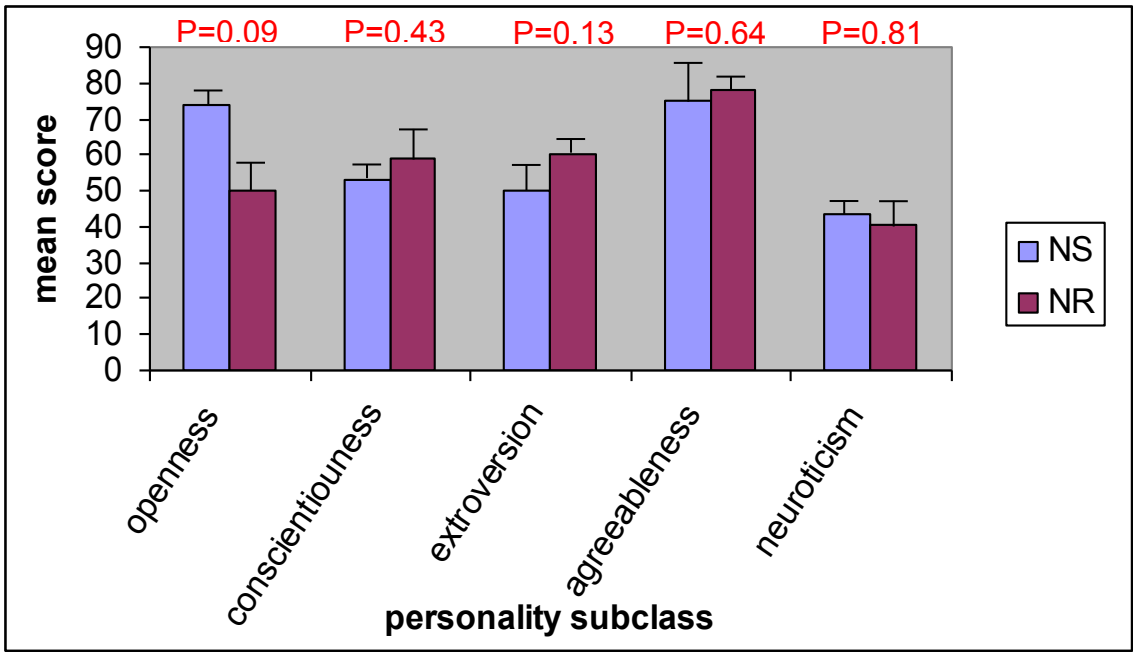

Figure 19. Personality subclass mean scores of nausea susceptible and nausea resistant subjects

There was also no significant difference between mean trait anxiety score for NS and NR subjects. However, state anxiety increased to a significantly higher score for NS subjects at the end of the nausea video (Figure 20).

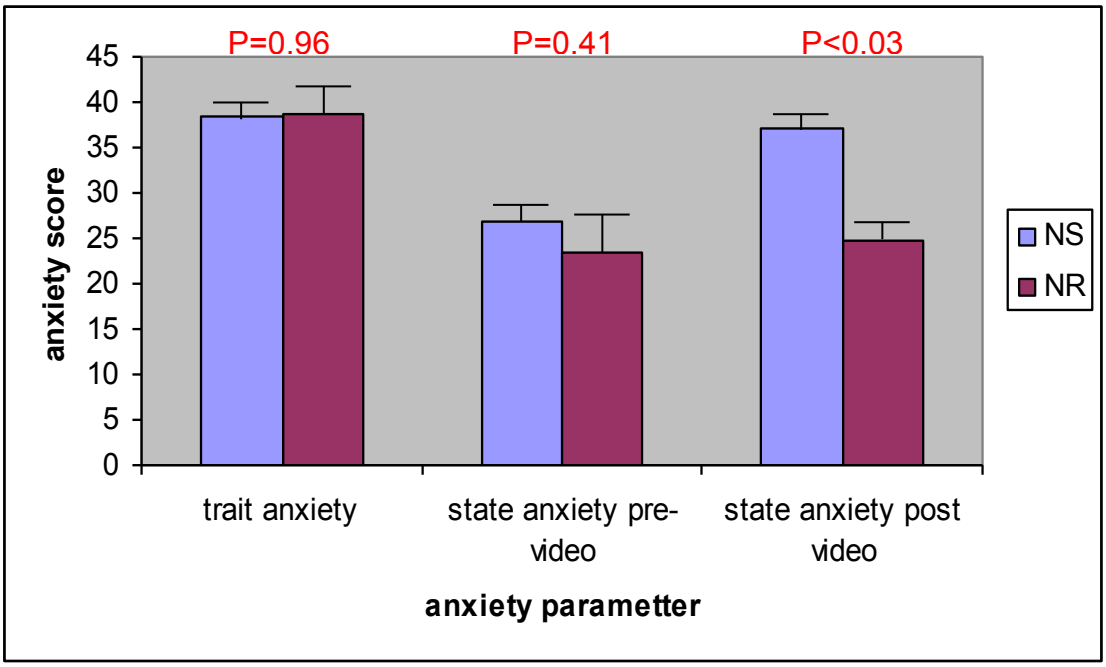

Figure 20. Trait anxiety scores and changes in state anxiety for nausea susceptible (NS) and nausea resistant (NR) subjects after watching the nausea video.

The mean score for maximum GI (mainly nausea), CNS and sopite related sensations experienced as rated by subjects at the end of each video was significantly greater for the NS subjects than the NR group. The difference in peripheral sensation score was however not statistically significant as shown in Table 3.

Motion sickness category	NR	NS	p-value
GI (nausea)	5.00	35.60	<0.01
CNS	6.40	31.60	<0.01
SOPITE-RELATED	7.40	17.00	<0.03
PERIHERAL	3.00	11.80	0.074

Table 3. Mean scores of the different motion sickness sensation categories for nausea resistant and nausea susceptible subjects.

There were no differences in the baseline autonomic marker levels of the NS and NR groups (Table 4). During the nausea video these markers including SCR showed greater change in the nausea susceptible than nausea resistant subjects, the difference was not statistically significant (Figure 21).

ANS BIOMARKER	NR	NS	p-value
HR (bpm)	65.55 ± 2.12	62.04±2.05	0.58
MBP (mmHg)	78.65±3.56	81.96±4.02	0.51
CSI	2.37±0.17	2.47±0.25	0.86
CVT (LVS)	12.97±1.10	13.62±1.02	0.88
CSB (ΔRR/ΔmmHg)	11.98±1.55	12.98±1.26	0.82

Table 4. Mean baseline ANS biomarker values for nausea resistant and nausea susceptible subjects.

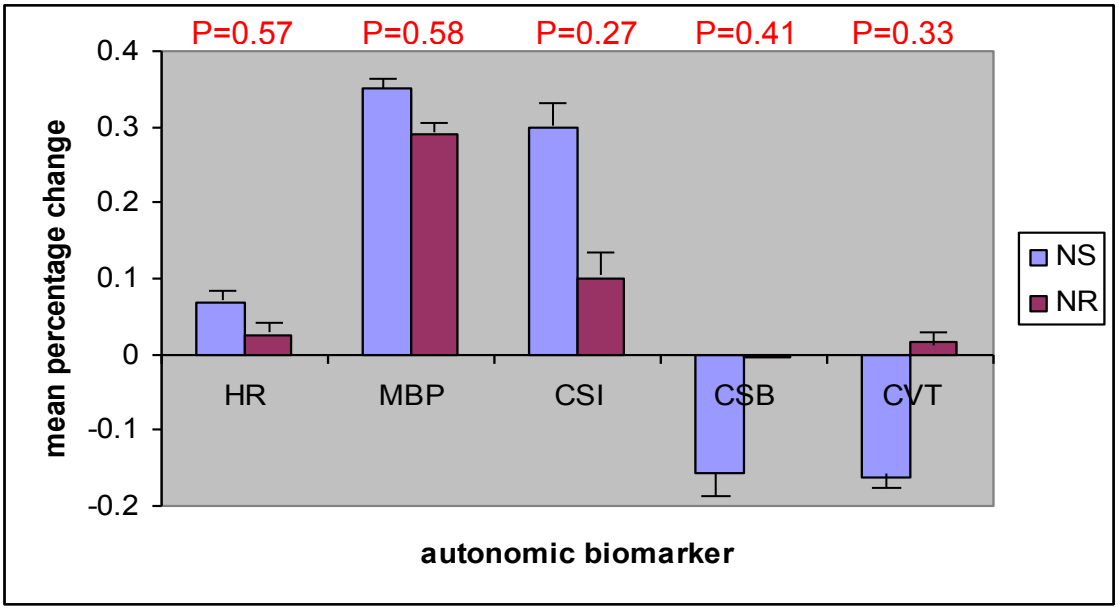

Figure 21. Mean percentage change in ANS biomarker values of nausea susceptible (NS) and nausea resistant (NR) subjects.

4 Discussion

This was a pilot study to validate the motion and control video and the results herein discussed must be viewed with this in mind.

4.1 Visual motion induced nausea

Visual motion induced nausea is the result of sensory conflict in inputs from the visual versus vestibular systems (Lackner and Dizio, 2006) and leads to gastrointestinal (e.g. nausea) and central sensations in healthy volunteers in this study. Nausea in 75% of the subjects compares favourably with 50% reported for a rotating optokinetic drum (Kiernan et al., 1997). The safety profile that is crucial for the adaptation of a nausea stimulus to fMRI studies was met with no volunteers retching or vomiting for all visits.

4.2 Nausea associated psychophysiological measures

There was significantly increased state anxiety after watching the motion video consistent with the unpleasant nature of nausea causing much anxiety and discomfort with increased anxiety states and anticipation reportedly leading to more severe nausea responses (Morrow et al., 2002b).

There were also significant increases in heart rate, mean blood pressure and cardiac sensitivity index consistent with the classical increased sympathetic activation in vection

experiments (Cowings et al., 1986, Himi et al., 2004). Skin conductance response, the other marker of sympathetic activity, did not show similar changes and possibly may indicate poorer findings with finger measurements compared to metopic or forehead skin conductance response (Golding, 1992).

Contrary to previous reports (Cowings et al., 1986, Himi et al., 2004), the CSB withdrawal was significant with CVT lower but not significantly during the nausea video. When divided into 3 phases; early, mid and late significant decreases in both components of are noted in the late phase of video exposure which follows the maximal nausea ratings at the late third of the video and this was used for the rest of the experiments. Both SNS and PNS markers were not correlated with nausea severity and probably due to the small sample size. Interestingly, a study of the ANS response to a similarly subjective and distressing sensation, pain, rather showed co-activation of PNS and SNS, a phenomenon referred to as 'tonic freeze' (Paine et al., 2009). The reasons for this complexity in ANS response requires further exploration.

4.3 Limitations and the way forward

It is encouraging that with such small numbers we were able to show significant differences with nausea induction as well as its associated psychophysiological measures. However a much larger study is needed to assess if these results still hold true for a larger population with a separate reproducibility study performed to assess

reliability of the repeat study. Furthermore, the study of the psychological factors was largely under powered as such studies usually require large sample size.

CHAPTER 3

PSYCHOPHYSIOLOGICAL RESPONSE TO VISUAL MOTION INDUCED NAUSEA

1 Introduction

1.1 Individual variability to motion sickness nausea

There are considerable individual differences in the development of motion induced nausea (Golding, 2006). According to transport surveys, the individual susceptibility to motion sickness appears to change with age. It starts sometime during childhood, peaks at around puberty and slightly declines through adulthood (Turner and Griffin, 1999). Females also appear to be more susceptible to nausea and vomiting (Quigley et al., 2001, Klosterhalfen et al., 2005a, Flanagan et al., 2005) with ferry passengers having a 5 to 3 female to male risk ratio for vomiting (Lawther and Griffin, 1988). This may be partly due to the effect of female hormones (Golding and Gresty, 2005) as some changes in susceptibility motion induced nausea are noted with the menstrual cycle (Matchock et al., 2008) and also during pregnancy (Walsh et al., 1996).

It is suggested that about half of motion sickness susceptibility is determined by genetic variation and that the improvement in adulthood is because of individual differences in habituation, exposure to and avoidance of motion (Reavley et al., 2006). A large scale survey of female twins found greater sensitivity to motion induced nausea in monozygotic twins (0.56, n=702) in comparison with dizygotic twins (0.16, n=727) and susceptibility decreased with age in both groups. This supports the age-old clinical anecdote that motion sickness ‘runs in families’. There also appears to be slightly higher

susceptibility to motion sickness amongst people of Chinese origin (Stern et al., 1993, Klosterhalfen et al., 2005a).

Psychological factors can also influence nausea susceptibility (Morrow et al., 2002b). Haug et al. (2002) demonstrated that anxiety and depression are associated with nausea susceptibility. Patient expectation of nausea during treatment has also been shown to increase the severity of nausea (Roscoe et al., 2000).

Smokers are also more susceptible to motion sickness when at their normal level of cigarette use than when they are nicotine deprived (Golding et al., 2011). Greater aerobic fitness also reportedly makes an individual more susceptible to motion sickness (Rawat et al., 2002).

Acute vestibular disorder cause intense vertigo, nausea, and imbalance. In chronic vestibular disorders patients can become susceptible to vection and visually induced imbalance as they become over-reliant on visual cues for orientation (Pavlou et al., 2004, Guerraz et al., 2001). Spatial disorientation appears to play a role in these patients and in those suffering from visual vertigo; this is evident from the abnormally strong nausea that develops to disorienting visual environments (Guerraz et al., 2001).

Physiological factors that worsen motion sickness usually involve a ‘conflict’ between sensory inputs (Rainford et al., 2006). A classic example is reading in a moving vehicle

where the vestibular ocular reflexes (stabilizes eyes on external stationary objects) must be suppressed by visually guided eye movements to maintain scanning fixation on the text which is moving with the protagonist. Similar 'conflicts' arise in 'tilting trains' or an airplane making coordinated turns when landing or taking off which are particularly nauseogenic if the passenger feeling completely upright inside views the external landscape that appears to swing dramatically up and down (Neimer et al., 2001).

Last but not least, these various traits may not be independent e.g. patients undergoing chemotherapy treatment who are susceptible to visual motion induced nausea have more post-chemotherapy nausea than patients who aren't susceptible (Morrow, 1985).

Possible Factors	**Possible Causes of Increased Susceptibility to Nausea**
Physiology	High aerobic fitness
Lifestyle	Past experience of motion-induced nausea, alcohol abuse, smoking cigarettes
Psychology	Spatial disorientation, depression, anxiety, expectation, anticipation, fear
Neuroendocrinology	Cortisol, vasopressin
Genetics	Oriental ethnic origin, α2-adrenergic receptor genes single nucleotide polymorphism, familial history
Gender	Female
Disease	Migraine, vestibulopathy
Age	Young children, peaking at puberty

Table 5. Factors possibly contributing to nausea susceptibility in healthy individuals.

1.2 Psychophysiological markers of nausea

The autonomic nervous system (e.g. cardiac autonomic systems, stomach autonomic activity and skin conductance responses), neuro-endocrinal systems and psychological state like anxiety are currently known markers associated with nausea.

Anxiety is part of the generalised response to a aversive stimulus like nausea and plays an important role in the susceptibility to nausea and and its severity (Haug et al., 2002). A standardised method of assessing anxiety is the Spielberger state and trait anxiety inventory (STAI; chapter 2 section 2.4.3).

The autonomic nervous system (ANS) appears to have an important role in nausea with generally sympathetic activation and parasympathetic withdrawal occurring during nausea (Cowings et al., 1986, Himi et al., 2004, Hu et al., 1991). Furthermore, the ANS may also have a role in predicting nausea susceptibility (Muth, 2006) with high resting sympathetic tone (Parker and Wilsoncroft, 1978a), low resting parasympathetic tone (Rawat et al., 2002), and parasympathetic activation in response to nausea (Uijtdehaage et al., 1992) being protective against nausea.

Monitoring the electrical activity of the gut via electrogastrography (EGG) shows the final outcome of autonomic nervous system influence on the GIT during nausea.

1.3 Electrogastrography

Electrogastrography (EGG) measures gastric myoelectrical activity (GMA) that regulates gastric motility (Chang, 2005) with slow waves from interstitial cells of cajal (gastric pacesetter potentials) modulating maximum frequency of spike potentials that initiate gastric muscles contraction (Koch, 2001). The frequency presumed to be of gastric origin and at which the power in EGG power spectrum peaks in the range of 0.5–9.0 cycles per minute (cpm) is the EGG dominant frequency while the dominant power is the power during that dominant frequency. Simultaneous mucosal (Stern, 2000, Stevens LK, 1974) or cutaneous and serosal (Tumpeer and PHILLIPS, 1932a, Brown et al., 1975, Smallwood, 1978, Linkens and Datardina, 1978) recordings of GMA have shown that the dominant frequency of the EGG accurately represents the gastric slow wave frequency. The amplitude and regularity of gastric slow waves reflects the dominant power.

There is no established definition for the normal range of the gastric slow wave but generally the normal dominant frequency of the EGG in asymptomatic healthy subjects is accepted to be between 2.0 and 4.0 cpm (Chen et al., 1994, Chen and McCallum, 1992, Chen et al., 1993a, Parkman et al., 2003). The abnormal frequencies may be divided further into tachygastria if its frequency is >4.0 cpm, but <9.0 cpm, bradygastria if its frequency is <2.0 cpm and arrhythmia if there is a lack of a dominant frequency (Chen et al., 1995). This can be quantitatively assessed further to determine the percentage of time during which normal slow waves (% EGG in normogastria) are

observed in the EGG. In contrast to normogastria, the percentage of gastric dysrhythmia is defined as the percentage of time abnormal gastric rhythm (includes tachygastrias, arrhythmias and bradygastrias) is observed in the EGG (% EGG in dysrhythmias).

Dr. Stern wrote in 2000, ‘‘the history of EGG can be described as three beginnings, a length period of incubation, and a recent explosion’’ (Stern, 2000). Historically, the first human electrogastrography was first performed by a gastroenterologist Walter Alvarez back in the early 1920s (Alvarez, 1922, Stern, 2000), by placing two electrodes on the abdominal surface of ‘‘a little old woman’’ connected to a galvanometer. Meanwhile I. Harrison Tumpeer, a pediatrician performed the first EGG in children (Tumpeer IH, 1926, Tumpeer and PHILLIPS, 1932b)(Tumpeer IH, 1926) with limb leads to record the EGG from a 5 week old child suffering from pyloric stenosis. Thirty years later EGG was recovered by R.C. Davis, a psychophysiologist, with validation of the EGG using simultaneous recordings from needle electrodes and a swallowed balloon (DAVIS et al., 1957, DAVIS et al., 1959). This stimulated EGG research, with Dr. Stern working in Davis’ lab in 1960 (Stern, 2000) and Stevens and Worrall who were probably the first ones applying spectral analysis to EGG (Stevens LK, 1974). England also joined with studies on frequency analysis of the EGG signal e.g. fast Fourier transform (FFT) (Brown et al., 1975), phase-lock filtering (Smallwood, 1978), and autoregressive modeling (Linkens and Datardina, 1978). They also reconfirmed there was no 1:1 correlation between the EGG and the contractions (Nelsen and Kohatsu, 1968) so the

frequency of the contractions can be determined but not when they were occurring (Chen and McCallum, 1991). Running spectral analysis method using FFT was introduced (van der Schee and Grashuis, 1987) to extract the frequency of EGG and time variations of the frequency (Stern et al., 1987b, Pfister et al., 1988, Stern et al., 1987a) that is still used today (Chen JZ, 1994). Chen (Chen, 1989, Chen et al., 1990) improved it with an adaptive autoregressive moving average model (avoiding averaging effect by FFT block processing) to detect gastric dysrhythmia in short durations (Chen et al., 1993b).

The EGG during optokinetic drum rotation period showed a decrease in normogastria, which was accompanied with an increase in tachygastria with increasing reports of nausea (Imai et al., 2006). GMA also shows a dominant frequency of 3 cpm (cycles per minute) during fasting periods with an increase in frequency during nausea (Stern et al., 1985) (Holmes and Griffin, 2000) and (Miller and Muth, 2004). The same was seen between subjects susceptible to motion sickness versus subjects resistant to motion sickness (Muth et al., 1995).

Muth (2006) argued that in trying to piece all of these markers of nausea together the variations in susceptibility seen are unaccounted for by autonomic nervous system changes alone and may likely be accounted for by the gastric and neuroendocrinal system changes.

1.4 Neuroendocrinal System influence on nausea

The HPA axis is central in orchestrating the body's response to stress. Cortisol, a glucocorticoid hormone, is the final effector of this axis and can be assayed in biological fluids including peripheral blood, saliva and urine (Figure 22). Approximately 3-5% of cortisol is in its bioactive, unbound form with the majority of cortisol bound to the corticosteroid binding globulin or albumin while in the blood, preventing it from penetrating the membrane of the target cell.

Acutely occurring nausea and vomiting releases "stress hormones" (Drummond, 2005, Kohl, 1992, Klosterhalfen et al., 2000) and thus raise the question of whether the development of motion sickness or nausea in general involves the neuroendocrinal systems. Nausea is correlated with an increase in serum cortisol (Eversmann et al., 1978) and vasopressin levels, likely a response to a stressful nauseous event (Otto et al., 2006, Grigoriev et al., 1988). Although how cortisol is synthesised and released into the blood is known (Kohl, 1985, Kohl, 1992), however, it is still unclear whether the cortisol release is the direct result or cause of nausea in humans (Otto et al., 2006).

Stress hormone profiles of cortisol and vasopressin during nausea currently appear to be the result of nausea rather than the cause, however further investigations during different experimental nausea stimulations and using newer neuroendocrinal system markers might clarify whether gastrointestinal peptides acting as neurotransmitters and

stress hormones play a specific role in the development of acute nausea and vomiting (Otto et al., 2006).

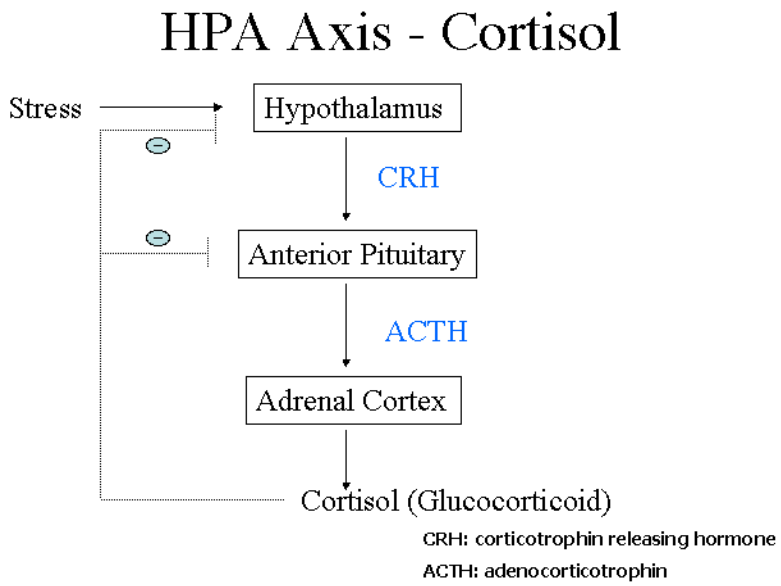

Figure 22 The hypothalamic-pituitary-adrenal axis. Corticotropin-releasing hormone is secreted from the hypothalamus following a stressful stimulus, which then stimulates the anterior pituitary to release adrenocorticoptropic hormone (ACTH). ACTH is carried via the blood stream to the adrenal glands and stimulates the production of cortisol. (adapted from www.ahs.uwaterloo.ca, retrieved 8th August 2012)

1.5 Identifying Suitable Study Subjects

Subjects need to be screened before they are selected for fMRI studies as there are wide individual variations to visual motion induced nausea. This is related to individual differences in susceptibility that will be explored in more detail later. With regards to selecting suitable individuals for fMRI studies, only those with at least moderate to severe nausea compared with those who don't experience nausea have the best chance of discovering the differences between nausea susceptible (NS) and nausea

resistant (NR) individuals. On top of that, self-reported nausea should be validated with associated psychophysiological measures to ascertain when an individual reports increased visual motion induced nausea levels there is a corresponding change in the psychophysiological markers as well.

1.6 Knowledge gaps

The understanding of the neuropsychophysiology of nausea is imperative for the development of effective treatment against nausea. However, the comprehensive understanding of the mechanisms of nausea is still lacking. The lack of validated models of nausea as well as objective biomarkers of nausea has significantly hampered the research of this complex sensation (Holmes et al., 2009). Furthermore, nausea research can be advanced further using a novel objective human nausea model whilst reducing the need for unnecessary animal studies.

1.7 Aims

The aims of this study were to identify individuals who are susceptible and resistant to develop nausea without vomiting using a human model of visual motion and to identify the psychophysiological markers for nausea.

1.8 Hypothesis

By using the human model of visual motion we can induce nausea in a significant proportion of the study population which can be objectively measured by observing changes in the autonomic nervous system, neuroendocrinal system, EGG and fMRI. This will help the identification of nausea susceptible and resistant individuals .

2 Methods

2.1 Study design and setting

This was a randomised crossover study carried out at the Wingate Institute of Neurogastroenterology, Queen Mary University of London (QMUL).

2.2 Ethical approval

The QMUL Research Ethics Committee (QMREC2008/37) approved these studies.

2.3 Subjects

Ninety-eight healthy volunteers completed the studies. All subjects signed written informed consent. Volunteers were recruited to meet the following criteria: (i) normal body mass index, (ii) no abnormality on clinical examination, including a history or presence of cardiac, ophthalmologic, gastro-intestinal, hepatic, or renal disease, or other condition known to alter their response to visually induced motion sickness

nausea e.g. vestibular disease, (iii) no abnormality on electrocardiogram examination at screening (iv) no abuse of alcohol (defined as an average intake >21 units per week or 3 units per day); and (v) no history or presence of neurological or psychiatric conditions (e.g. stroke, traumatic brain injury, epilepsy, space-occupying lesions, multiple sclerosis, Parkinson's disease, vascular dementia, transient ischaemic attack, schizophrenia, major depression, etc). Subjects with any of the following were excluded: (i) received prescribed medication within 14 days prior to the first visit, which might interfere with the study procedures or compromise safety, (ii) received over-the-counter medicine within 48h of the study, (iii) participated in a trial with any drug within 3 months before the first visit, (iv) had a caffeinated drink within 24 h of visit.

2.4 Psychometrics and motion sickness susceptibility questionnaires

Validated questionnaires were used to assess different aspects of the psychological state and susceptibility to motion sickness of the subjects. The big five inventory (BFI; chapter 2 section 2.4.2), the Spielberger state and trait anxiety inventory (STAI; chapter 2 section 2.4.3), the Weinberger adjustment inventory (WAI; chapter 2 section 2.4.4), hospital anxiety depression scale (HADS; chapter 2 section 2.4.5), motion sickness susceptibility questionnaire (MSSQ; chapter 2 section 2.4.6), motion sickness assessment questionnaire (MSAQ; chapter 2 section 2.4.7) and the validated nausea VAS questionnaire (chapter 2 section 2.4.7) were used as previously described.

2.5 Preparation for experiment

After fasting for 6 hours and refraining from drugs, caffeine, alcohol and smoking a day before, subjects were studied between 0800 to 1400 hours (both visits performed at approximately the same time for each individual). They were prepared as described in the previous chapter with the addition of electrogastrography and intravenous access for blood sampling. Subjects were seated comfortably in a silent room at 25^{o}C ambient temperature and administered questionnaires that assessed motion sickness sensations and anxiety. After starting motion or control video, minute-to-minute nausea and anxiety scores were monitored using a visual analogue scale. Blood was collected for cortisol at baseline and post video 5, 15, 30 minutes. (Figure 23 and Figure 24)

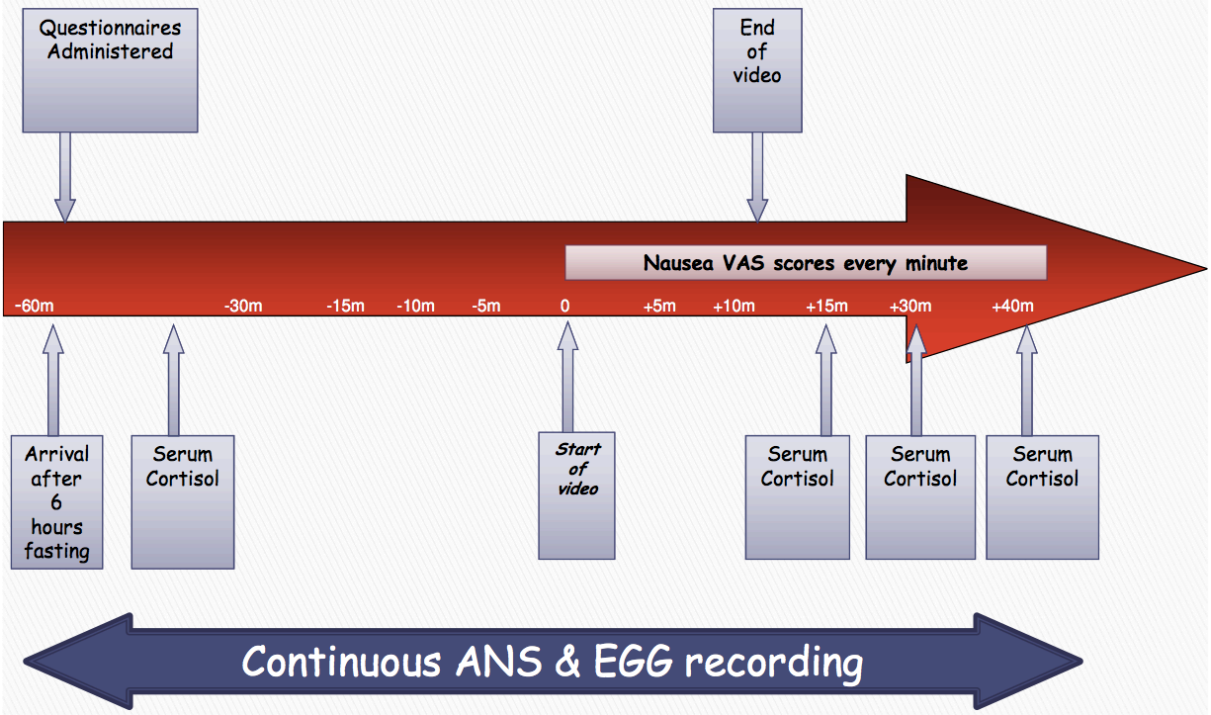

Figure 23 Schematic summary of chapter 3 experimental protocol: After 6 hours of fasting subjects answered MSAQ & STAI-S questionnaires, reassessed just before starting each video during which minute-to-minute nausea and anxiety scores were assessed using a VAS were recorded. There were continuous monitoring of cardiac autonomic activity and gastric myo-electrical activity throughout. Bloods were also taken for cortisol at baseline and post video 5, 15, 30 minutes.

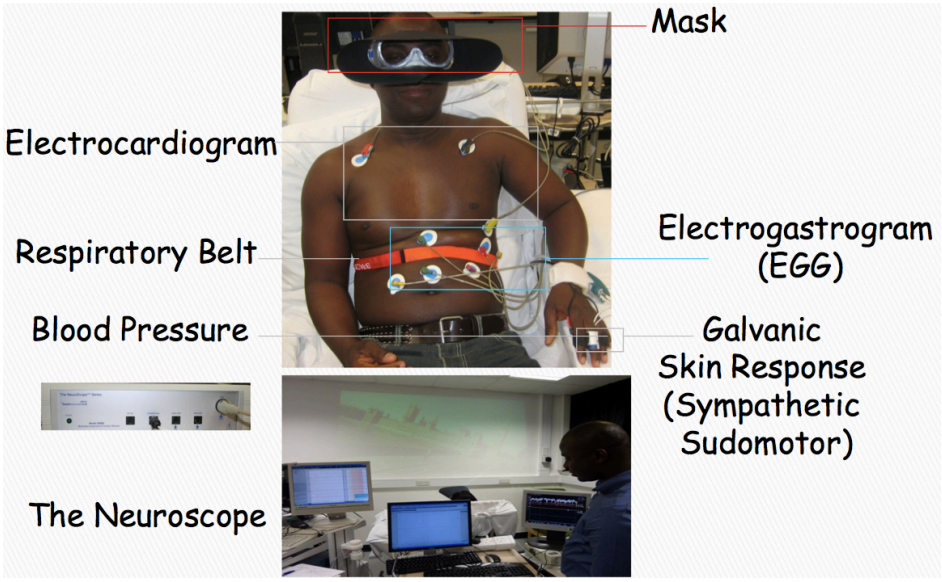

Figure 24 Subject with all the equipment on and investigator observing tracings of EGG, EDA and Neuroscope data (reproduced with subject's consent).

2.6 Baseline activity

Subjects were encouraged to relax their muscles, stay still and not talk while baseline recordings were taken over 10 minutes before the start of the stimulus. Prior to this the subjects had time to relax for up to 30 minutes.

2.7 Exposure to stimulus

Subjects watched two different videos at two different visits through a black card board designed to limit their field of view to only the screen. The videos consisted of;

- a non-nausea inducing video consisting of a stationary cityscape (control or neutral video) and
- a nausea inducing video consisting of a moving cityscape (nausea video)

The sequence of exposure was assigned randomly by the investigator. All events, including reported sensations were recorded on a data collection sheet.

2.8 Nausea markers

Subjects were questioned every minute about sensations of nausea, dizziness and anxiety which they rated on a visual analogue scale with 1 being no sensation and 4 at the other end meaning severe nausea or sensation. They also reported spontaneously any other sensations. Just before and just after the video subjects also completed the STAI-state anxiety assessment and the MSAQ to assess motion sickness sensations

including nausea. Vital signs and skin conductance responses were recorded continuously throughout the experiment.

2.9 Gastric myoelectrical monitoring with electrogastrogram

The Medtronic Polygram NET EGG system (Medtronic A/S, Denmark) was used for multichannel recordings, with four electrogastrogram (EGG) signals recorded simultaneously. Signals were sampled at ~105 Hz and then down-sampled to 1 Hz as part of the acquisition process with a low-pass and high-pass filters of 15 cpm and 0.5 cpm. Six electrodes (Ambu Blue Sensor P, Denmark) were placed on the subject's abdomen after skin preparation with an abrasive electrode paste (Nuprep, Weaver & Co, USA). The EGG system was configured to accept an electrode impedance of less than 11 kΩ after skin preparation and this was meticulously checked before the start of any recordings as the EGG is vulnerable to motion artifacts due to the nature of cutaneous measurement, The six electrodes consisted of four active recording electrodes, one reference electrode, and one ground electrode. Electrode 3 was placed halfway between the xyphoid process and the umbilicus (the conventional location for an EGG electrode (Parkman et al., 1997) while electrode 4 was placed 4 cm right horizontal to it. Meanwhile, electrodes 2 and 1 were placed 45º to the upper left of electrode 3, with an interval of 4 to 6 cm and the ground electrode was placed on the left costal margin horizontal to electrode 3. Lastly, electrode 0 (reference) was placed at the cross point of the two lines, one horizontal containing electrode 1 and one vertical containing electrode 3 (typically coincides with the xyphoid process). A motion sensor

was also attached on the abdomen above electrode 4 to aid the elimination of motion artefacts from the recording during off line analysis. EGG recordings were measured according to established guidelines (Chen and Lin, 2006).

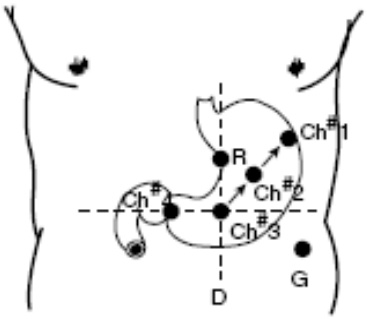

Figure 25 Positions of the EGG channel (ch1-4), reference (R) and ground (G) electrodes.

The subject sits in a reclining chair in a quiet room throughout the study and any conversations discouraged and reminded regularly to stay as still as possible to prevent motion artifacts (Lee and Hon, 1965, Hublet and Demeurisse, 1992, Eckberg, 2006).

2.10 Serum Cortisol

There are elaborate and complex sampling systems (Henley et al., 2009) to measure serum cortisol to prevent (unintentional) stress of venepuncture however the use of such complex systems in these was impractical. Thus, I inserted an intravenous cannula (21G Venflon, Beckton, UK) only once into the left antecubital fossa for all subjects at the start of the study for peripheral venous blood sampling. This is to standardise any potential effects of added stress and in most cases the patency of the

cannula was maintained without any intravenous infusion of 0.9% heparinised saline. Saline infusion may dilute serum cortisol giving falsely low measurements. A three-way extension set (Extension set, SmartSite®, Cardinal Health) with clamps on each of the three lines was used with the cannula to prevent any cross contamination between serial samples. Peripheral venous blood was collecting in silica clot-activating SST™ Vacutainers (Vacutainer, Beckton Dickinson, UK) and spun down and aliquoted to store only acellular plasma for further analysis. A portion of the plasma was also transferred to the Blood Services Department, Biochemistry Department at the Royal London Hospital for serum total cortisol assay with a competitive chemiluminescent assay (Chiron Diagnostic ACS:180 analyser, Bayer Healthcare, NY, USA). This assay was performed by the Blood Services Department, Biochemistry Department at the Royal London Hospital.

2.11 Reproducibility of the nausea study using intraclass correlation comparison (ICC)

After a minimum period of twelve months after their last visit, 20 subjects were randomly recruited to participate in a repeat of the same two initial visits. The same measures were taken and analysed in the same manner. Subsequently, the intraclass correlation (ICC) model for continuous variables were calculated between the both visits for the 20 subjects (Green et al., 2012).

2.12 Statistical Analysis of Psychophysiological Responses

Psychometric, autonomic cardiac and gastric data and cortisol data were analysed using matched-pair t-tests and Wilcoson tests to compare the means and medians. Pearson's and Spearman's correlations were used to determine the relationship between measurements. Independent-measures t-tests and Mann-Whitney tests were used to compare groups. Multi-group comparisons used a one-way ANOVA with Bonferroni correction and Kruskal-Wallis test. EGG was interpreted using the automated computer analysis package by Polygram (Medtronic, Inc., Shoreview, MN) for each channel after removing artefacts identified. Some of the ANS, EGG and cortisol data were not normally distributed and thus the data for nonparametric statistics are presented and expressed as medians and for parametric statistics presented as means and standard error of means. Reproducibility of the nausea study used intra-class correlation comparison (ICC) and agreement was measured using two-way mixed average measure ICC model for continuous variables. Confidence intervals for the ICC were calculated according to the methods of Scheffe (Green et al., 2012). ICC were interpreted according to suggestions made by Yen et al. as: - excellent (0.75-1), moderate (0.4-0.74) or poor (0-0.39) (Davis and Hallerberg, 2010). Commercially available statistics packages (SPSS, Chicago, IL, USA and GraphPad, San Diego, CA, USA) were used for the analysis. P values <0.05 were considered to be of statistical significance.

3 Results

All subjects completed and tolerated the studies well without any vomiting.

3.1 Subject characteristics

In all, 98 healthy subjects completed the study (45 females and 53 males) with a median age of 23 years (range 19-58 years) and a mean body mass index (BMI) of 22.68 kg/m^2 ± 0.38kg/m^2. Of the 98 subjects, 34 were Asian (34.69%), 14 were Orientals (14.29%), 5 were Africans (5.10%) and 45 were Europeans (45.92%).

3.2 Motion vs Control video

All subjects who completed the studies had psychometric scores consistent with healthy populations: big five inventory personality traits (extraversion 3.63, agreeableness 3.71, conscientiousness 3.67, neuroticism 2.57, openness 3.70); Spielberger trait-state anxiety inventory trait scores was 35.00; Weinberger questionnaire (Restrain 4.07; Distress 2.13; Defensiveness 2.84); Hospital Anxiety and Depression Scale (Anxiety 4.50; Depression 1.00); motion sickness susceptibility questionnaire was 68.00% (Table 6).

There were no significant differences at baseline before the control or motion video for nausea, anxiety, cortisol, cardiac or gastric autonomic markers. But when comparing the percentage change from baseline for the markers after watching either video, visual motion induced nausea was significantly higher during motion video compared to

control with associated increased anxiety, sympathetic arousal, parasympathetic withdrawal and change from normogastria to dysrhythmias. Cortisol changes were not significant with both videos showing a withdrawal (Figure 26).

There was a significant correlation between nausea VAS scores and MSSQ $p<0.05$ but with a correlation coefficient, r, of only 0.55 due to the wide variation in the MSSQ scores of the resistant subjects.

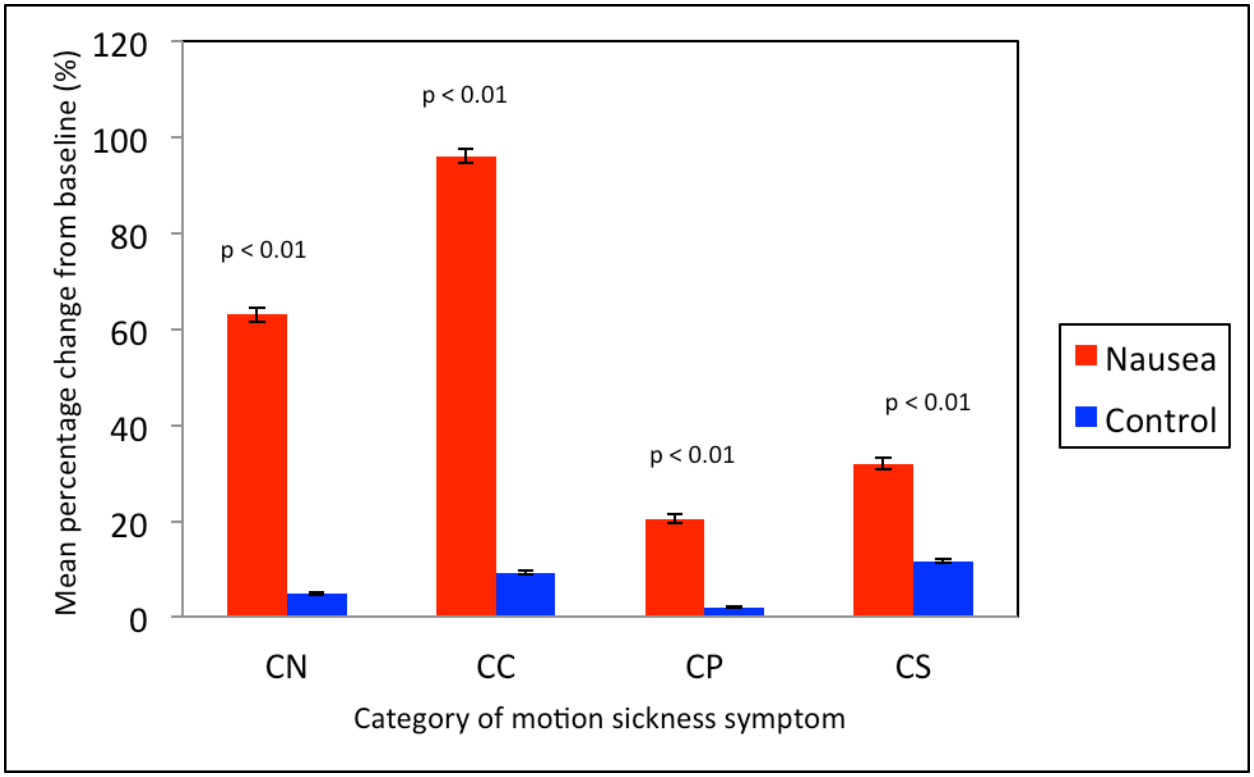

Figure 26 Mean percentage change of motion sickness sensations from baseline in motion video and control video. There were significant increases in cumulative nausea, CN, cumulative central CC, cumulative peripheral CP and cumulative sopite CS sensations reported after the motion video compared to control video.

BASELINE MEDIANS	Control Video n=98	Motion Video n=98	p value
Nausea scores (VAS)	1.00	1.00	0.61
Anxiety scores (STAI)	26.00	25.00	0.50
CVT (LVS)	10.66	9.93	0.52
CSB (∆RR/∆mmHg)	9.65	9.00	0.39
HR (bpm)	64.65	64.45	0.67
MBP (mmHg)	69.30	68.30	0.38
SBP (mmHg)	105.70	103.60	0.88
DBP (mmHg)	51.15	50.70	0.17
SCR (µS)	-0.09	-0.18	0.93
Cortisol (nMol/L)	397.00	374.50	0.61
% EGG in Normogastria (%)	73.31	75.84	0.66
CHANGE FROM BASELINE MEDIANS	Control Video n=98	Motion Video n=98	p value
Nausea scores (VAS)	+1.00	+2.00	<0.01
Anxiety scores (STAI)	+1.00	+7.00	<0.01
CVT (LVS)	-0.07	-0.85	<0.01
CSB (∆RR/∆mmHg)	-0.35	-1.95	<0.01
HR (bpm)	+0.65	+4.00	<0.01
MBP (mmHg)	+0.40	+3.55	<0.01
SBP (mmHg)	+1.35	+3.15	<0.01
DBP (mmHg)	+0.05	+2.90	<0.01
SCR (µS)	+0.49	+2.02	<0.01
Cortisol(Postvideo 15 mins nMol/L)	-71.00	-85.00	0.10
% EGG in Normogastria (%)	+2.46	-2.77	<0.01
% EGG in Dysrhythmias (%)	+0.20	+1.70	<0.03

Table 6 Associated nausea markers for all subjects recruited in the study (motion video, MV; control video, CV). Results are medians with p values shown.

3.3 Gender variability in nausea susceptibility.

Both female and male subjects who completed the studies had psychometric scores consistent with healthy populations however females report slightly more neuroticism compared to male subjects (2.86 vs 2.43 $p<0.05$) with no significant age differences between males and females.

There were no significant differences at baseline for both females and males before the motion video for nausea, cortisol, cardiac or gastric autonomic markers but females had slightly higher anxiety state compared to males (26 vs 23, $p<0.04$). There were also no significant differences when comparing the percentage change from baseline after watching motion video between females and males. Both genders still displayed increased anxiety, sympathetic arousal, parasympathetic and cortisol withdrawal and change from normogastria to dysrhythmias. (Table 7)

MEDIANS	Female MV n=45	Male MV n=53	p value
BFI – Extraversion	3.63	3.75	0.77
BFI – Agreeableness	4.00	3.80	0.22
BFI – Conscientiousness	3.89	0.08	0.19
BFI – Neuroticism	2.86	2.43	< 0.05
BFI – Openness	3.80	3.70	0.37
Age	22.00	23.00	0.30
MSSQ	68.00	68.00	0.63
STAI Trait	36.00	34.00	0.38
BASELINE MEDIANS			
Nausea scores (VAS)	2.00	2.00	0.69
Anxiety scores (STAI)	26.00	23.00	<0.04
CVT (LVS)	9.54	9.98	0.83
CSB (∆RR/∆mmHg)	9.60	8.70	0.55
HR (bpm)	8.12	8.35	0.09
MBP (mmHg)	66.60	69.10	0.29
SBP (mmHg)	101.30	105.10	0.81
DBP (mmHg)	48.70	51.10	0.09
SCR (µS)	+0.09	-0.44	0.25
Cortisol (nMol/L)	395.00	310.00	0.15
% EGG in Normogastria (%)	78.33	70.53	<0.04
CHANGE FROM BASELINE MEDIANS			
Nausea scores (VAS)	+2.00	+2.00	0.69
Anxiety scores (STAI)	+6.00	+9.00	0.16
CVT (LVS)	-0.83	-0.86	0.38
CSB (∆RR/∆mmHg)	-2.00	-1.60	0.80
HR (bpm)	+4.10	+3.70	0.89
MBP (mmHg)	+3.90	+2.60	0.13
SBP (mmHg)	+4.50	+1.60	0.11
DBP (mmHg)	+3.90	+1.90	0.22
SCR (µS)	+2.03	+2.02	0.34
Cortisol(Postvideo15mins(nMol/L))	-30.00	-44.00	0.46
% EGG in Normogastria (%)	-1.03	-3.85	0.66
% EGG in Tachygastria (%)	+1.65	+1.93	0.81

Table 7 Personality (Big Five Inventory, BFI) and Motion sickness susceptibility questionnaire (MSSQ) scores for female and male subjects recruited in the study (motion video, MV; control video, CV; nausea susceptible, NS; nausea resistant, NR). Results are medians with significant differences marked with an asterix and p values shown.

3.4 Ethnic variability in nausea susceptibility.

The 34 Asians and 14 Chinese were grouped together as Asians. Both Asians and European subjects who completed the studies had psychometric scores within the normal range however Europeans scored slightly more for extraversion, neuroticism and openness compared to Asian subjects (Table 8).

There were no significant differences at baseline for both Europeans and Asians before the motion video for nausea, cortisol, cardiac or gastric autonomic markers. No significant differences were also seen when comparing the percentage change from baseline after watching motion video between Europeans and Asians except systolic blood pressure was increased more in Europeans. Both genders still displayed increased anxiety, sympathetic arousal, parasympathetic and cortisol withdrawal and change from normogastria to dysrhythmias. (Table 8).

MEDIANS	Asian MV n=48	European MV n=50	p value
BFI – Extraversion	3.38	3.75	< 0.05
BFI – Agreeableness	3.80	3.90	0.31
BFI – Conscientiousness	3.56	3.83	< 0.05
BFI – Neuroticism	2.57	2.64	0.66
BFI – Openness	3.70	3.80	< 0.05
Age	23.00	23.23	0.55
MSSQ	14.80	17.50	0.42
STAI Trait	36.00	32.00	< 0.01
BASELINE MEDIANS			
Nausea scores (VAS)	2.00	1.50	0.15
Anxiety scores (STAI)	26.00	25.00	0.18
CVT (LVS)	9.05	10.49	0.65
CSB (∆RR/∆mmHg)	8.90	9.25	0.44
HR (bpm)	66.50	62.45	0.14
MBP (mmHg)	68.30	68.25	0.66
SBP (mmHg)	104.70	102.10	0.56
DBP (mmHg)	50.40	50.75	0.83
SCR (µS)	-0.32	-0.06	0.98
Cortisol (nMol/L)	395.00	310.00	0.15
% EGG in Normogastria (%)	74.98	77.29	0.19
CHANGE FROM BASELINE MEDIANS			
Nausea scores (VAS)	+2.00	+1.50	0.15
Anxiety scores (STAI)	+10.00	+7.00	0.65
CVT (LVS)	-1.24	-0.68	0.48
CSB (∆RR/∆mmHg)	-2.10	-1.45	0.47
HR (bpm)	+3.15	+4.40	0.80
MBP (mmHg)	+3.55	+3.55	0.09
SBP (mmHg)	+2.15*	+3.7*	<0.05
DBP (mmHg)	+2.55	+3.30	0.28
SCR (µS)	+1.91	+2.09	0.76
Cortisol(Postvideo15mins(nMol/L))	-46.50	-35.00	0.95
% EGG in Normogastria (%)	-3.09	-2.66	0.49
% EGG in Tachygastria (%)	1.90	1.70	0.39

Table 8. Personality (Big Five Inventory, BFI) and Motion sickness susceptibility questionnaire (MSSQ) scores for subjects recruited in the study (motion video, MV; control video, CV; nausea susceptible, NS; nausea resistant, NR). Results are medians with significant differences marked with an asterix and p values shown.

3.5 Variability in nausea susceptibility.

Subjects who fell within the 1st quartile did not report any nausea when exposed to the stimulus and were grouped as nausea resistant (NR) subjects. Subjects who fell within the 4th quartile and reported at least moderate to severe nausea were grouped as nausea susceptible (NS) subjects. There were 28 who were NS, 28 who were Intermediates and 42 were NR (Figure 27). When 25 of the most nausea susceptible and 25 of the most resistant of these groups are compared, nausea susceptible subjects reported significantly higher nausea and scored significantly higher on the MSSQ compared to NR subjects. When subjects were divided into nausea susceptible and nausea resistant both CVT and CSB decreased in the susceptible subjects during the motion video with increased heart rate and blood pressures. Cortisol was significantly higher in nausea susceptible in comparison with nausea resistant subjects (Table 9). There were decreased normal gastric rhythms with dysrhythmias shown more clearly; this is illustrated in figure 7 with a single susceptible volunteer's real time EGG tracings showing a clear shift from the baseline fasting normal 3 cycles per minute (cpm) to increased dysrhythmias during the motion video which was associated with severe nausea.

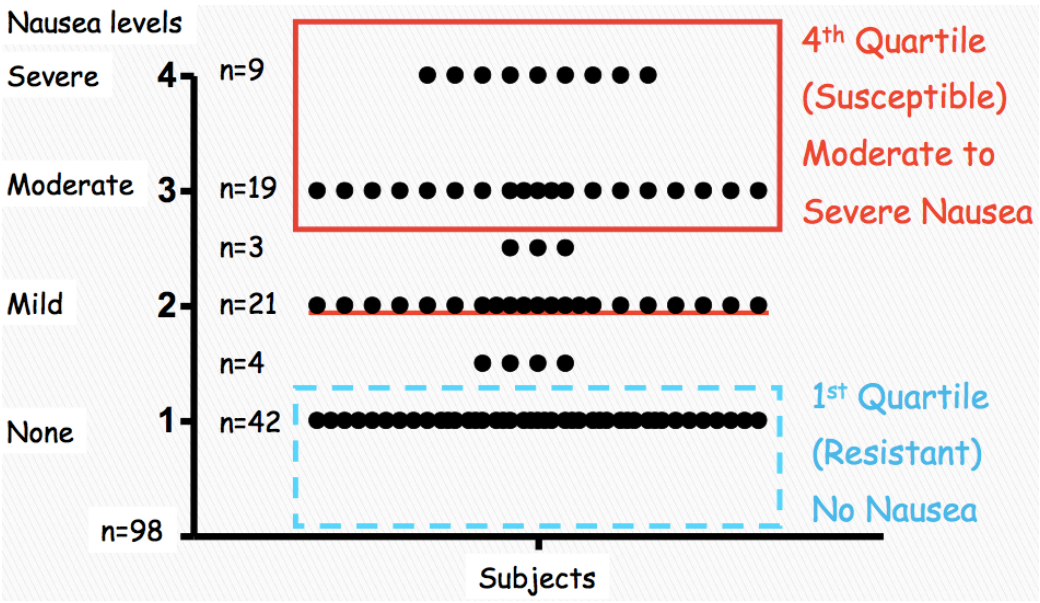

Figure 27. Nausea scores during the nausea stimuli for nausea susceptible & resistant subjects Study 1. All susceptible subjects had moderate scores and up to severe nausea during the motion video. Meanwhile, all resistant subjects had no nausea during the motion video.

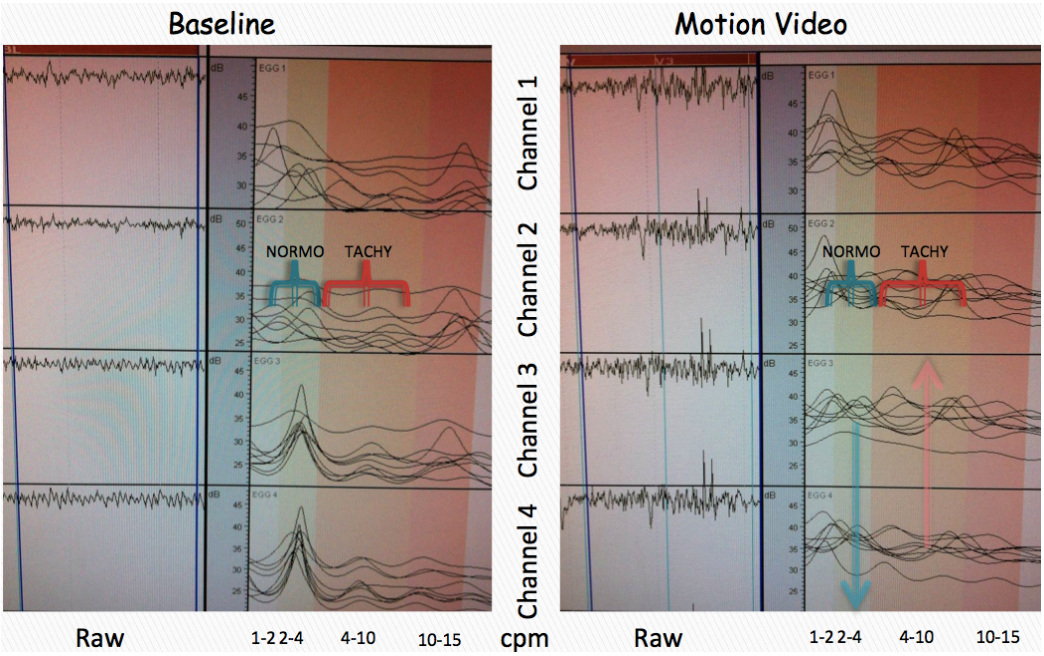

Figure 28. Gastric myoelectrical activity shown together with an example of with a single volunteer's real time EGG tracings.

MEDIANS	NR MV n=25	NS MV n=25	p value
BFI – Extraversion	66.00	68.00	0.70
BFI – Agreeableness	76.00	68.00	0.07
BFI – Conscientiousness	69.00	61.00	0.08
BFI – Neuroticism	37.00	42.00	0.37
BFI – Openness	68.00	69.00	0.70
Age	24.00	25.00	0.92
MSSQ	46.00	80.00	0.06
STAI Trait	34.00	35.00	0.23
BASELINE MEDIANS	**Control Video n=98**	**Motion Video n=98**	**p value**
Nausea scores (VAS)	1.00	1.00	0.67
Anxiety scores (STAI)	25.00	25.00	0.41
CVT (LVS)	10.83	10.60	0.55
CSB (∆RR/∆mmHg)	9.80	9.60	0.47
HR (bpm)	63.40	63.30	0.51
MBP (mmHg)	67.70	67.80	0.80
SBP (mmHg)	105.40	105.10	0.61
DBP (mmHg)	49.80	49.50	1.00
SCR (µS)	-0.01	-0.93	0.83
Cortisol (nMol/L)	505.50	298.00	<0.05
% EGG in Normogastria (%)	75.00	74.00	0.60
CHANGE FROM BASELINE MEDIANS			
Nausea scores (VAS)	+0.50*	+2.80*	< 0.01
Anxiety scores (STAI)	+3.12*	+11.34*	< 0.01
CVT (LVS)	- 5.03*	- 20.63*	< 0.01
CSB (∆RR/∆mmHg)	- 7.08*	- 27.64*	< 0.01
HR (bpm)	+4.52*	+11.05*	< 0.01
MBP (mmHg)	+2.69*	+7.73*	< 0.05
SBP (mmHg)	+1.47*	+5.67*	< 0.05
DBP (mmHg)	+4.12*	+9.94*	< 0.05
SCR (µS)	+0.51*	+2.15*	<0.01
Cortisol(Postvideo15mins(nMol/L)	- 21.93*	+17.94*	< 0.05
% EGG in Normogastria (%)	+1.60*	-2.82*	< 0.01
% EGG in Dysrhythmias (%)	+0.83*	+2.35*	< 0.05

Table 9. Personality (Big Five Inventory, BFI) and Motion sickness susceptibility questionnaire (MSSQ) scores for subjects recruited in the study (motion video, MV; control video, CV; nausea susceptible, NS; nausea resistant, NR). Results are medians with significant differences marked with an asterix and p values shown.

3.6 Reproducibility of the Nausea Study

3.6.1 Subject Characteristics

Twenty healthy subjects were recruited to the study (12 male) with a median age of 24.3 years (range 21-33 years) and a mean BMI of 21.3 kg/m2 ± 0.78 kg/m2. Of the 20 subjects, 10 were Caucasian (50%), 2 Afro-Caribbean (10%), 3 were Asian (15%), and 5 were Orientals (25%).

3.6.2 Reproducibility of Nausea Study

The similar changes are observed for the 20 subjects who repeated their studies with significantly more nausea and anxiety and the same associated markers changes (Table 10). The mean ± SEM and inter-class correlation (ICC) for each of the markers are summarised in Table 11. The baselines of all markers were consistent between study 1 and 2. The reproducibility of nausea parameters at baselines was moderate to good with ICCs between 0.52 to 0.89 indicates that there was no bias or systematic error. Participants' ratings of MSAQ, STAI, % EGG in Normogastria, HR and SCR in response to the motion video were higher in comparison to baseline in both study 1 and 2. The reproducibility was good for STAT, HR and SCR with ICCs of 0.81, 0.82 and 0.88, respectively, and moderate reproducibility for MSAQ, and % EGG in Normogastria with ICCs of 0.57 and 0.40 respectively. The nausea score, SBP, MBP and DBP levels increased moderately above the baseline after watching the motion video and had good reproducibility with ICCs of 0.81, 0.74, 0.69 and 0.53 respectively. In contrast,

participants' rating of % EGG Normogastria, CVT and CSB dropped below the baseline in both study 1 and 2 in response to the motion video and had reproducibility with ICCs of 0.55 (moderate), 0.87 and 0.73 (good), respectively.

Variable	Baseline Control: Mean (±SEM)	Baseline Nausea: Mean (±SEM)	Control Video: Mean (±SEM)	Nausea Video: Mean (±SEM)	Baseline Control vs. Baseline Nausea Video	Control vs. Nausea Video
HR (bpm)	64.95 ± 0.92	64.64 ± 0.89	65.68 ± 0.90	68.82 ± 1.00	p=0.67	p<0.01
SBP (mmHg)	104.80 ± 1.19	104.63± 1.25	106.11 ± 1.24	109.70 ± 1.47	p=0.88	p<0.01
DBP (mmHg)	52.18 ± 0.65	51.26 ± 0.63	52.49 ±0.71	54.49 ± 0.81	p=0.17	p<0.01
MBP (mmHg)	69.69 ± 0.75	69.03± 0.75	70.34 ± 0.81	72.86 ± 0.95	p=0.38	p<0.01
CVT (LVS)	11.35 ± 0.46	11.67 ± 0.57	11.18± 0.50	10.08 ± 0.49	p=0.51	p<0.01
CSB (BRS) (ΔRR/ΔmmHg)	10.16 ± 0.40	10.6 ± 0.54	9.97 ± 0.42	8.81± 0.40	p=0.39	p<0.01
SCR (μS)	-0.04 ± 0.26	-0.004 ± 0.36	1.24 ± 0.38	2.95 ± 0.49	p=0.93	p<0.01

Table 10. Mean baseline, control and motion video at baseline.

	Baseline			Motion Video		
Variable	Study 1 Mean (±SEM)	Study 2 Mean (±SEM)	ICC (95% confidence interval)	Study 1 Mean (±SEM)	Study 2 Mean (±SEM)	ICC (95% confidence interval)
Nausea Score	1.00 ± 0.23	1.00 ± 0.18	0.89	1.93 ± 0.23	1.73 ± 0.18	0.81
MSAQ Score	5.00 ± 0.24	5.00 ± 0.25	0.57	9.05 ± 1.01	8.2 ± 0.90	0.57
STAI State	26.6 ± 0.75	23.7 ± 1.32	0.85	36.08 ± 1.20	31.28 ± 3.37	0.61
% EGG in Normogastria	72.10 ± 1.51	77.23 ± 2.76	0.52	69.29 ± 3.58	68.78± 3.27	0.55
% EGG in Tachygastria	6.04 ± 1.62	5.10 ± 1.51	0.67	8.43 ± 1.74	9.78 ± 2.96	0.40
HR (bpm)	62.44 ± 1.62	61.01 ± 1.67	0.81	66.76± 1.74	64.97 ± 1.75	0.82
SBP (mmHg)	102.41± 2.83	104.74± 2.58	0.79	105.64 ± 2.90	104.87 ± 2.92	0.74
DBP (mmHg)	49.92± 1.78	49.32 ± 1.14	0.71	53.46 ± 1.35	51.92 ± 1.61	0.53
MBP (mmHg)	67.39± 1.55	67.78 ± 1.42	0.73	70.83± 1.70	68.30 ± 1.52	0.69
CVT (LVS)	10.30 ± 0.77	11.82 ± 1.18	0.73	9.18 ± 0.74	9.87 ± 0.92	0.87
CSB (BRS) (ΔRR/ΔmmHg)	8.9 ± 0.78	10.14 ± 1.01	0.73	7.51± 0.61	8.66± 0.9	0.73
SCR (μS)	1.49 ± 0.90	0.91 ± 0.87	0.84	4.22 ± 1.26	3.80 ± 1.19	0.88

Table 11. The reproducibility of nausea study parameters at baseline and following motion video.

4 DISCUSSION

4.1 Motion versus control video

The results show that nausea video is able to provoke nausea in more than half (57%) of all the subjects with a range of responses, and more importantly nausea sensations receded quickly within five minutes post-stimulus. The nausea video also produced classical changes in markers associated with nausea for all subjects, like the withdrawal of parasympathetic activity and cortisol with sympathetic arousal. The percentage of time with EGG in normal gastric rhythm was also reduced during motion versus control video with an increase in dysrhythmias (e.g., increase in tachygastrias and arrhythmias). The presence of increased anxiety during the control video may be due to the anticipation of a nauseogenic stimulus (Jacobsen et al., 1988). While there was an increase of state anxiety during nausea video, this study is unable to conclude whether the elevated anxiety level is the indirect result or cause of nausea experienced by the subjects. Considering previous reports on the correlation between elevated trait and state anxiety and anticipatory nausea and vomiting in cancer patients receiving chemotherapy (Andrykowski, 1990), it is likely that anxiety plays an important role in nausea development.

In general, the results from my study does not show that Asians or Chinese were more susceptible to nausea compared to other ethnicities (Stern et al., 1993) nor were there any gender or personality subclass differences (Turner and Griffin, 1999). The females

in this study did score slightly more for neuroticism personality trait as well as higher baseline anxiety compared to the males.

The reproducibility of the markers at study visits one year apart for visual motion induced nausea appear to be stable in a representative cohort of 20 out of the original 98 subjects studied. There is good reproducibility of the nausea VAS scores with moderate ICC for the more detailed MSAQ scores with scores mainly differing in the three other dimensions of motion sickness rather than nausea complaints (Golding 2006). This is also consistent with a postoperative nausea and vomiting study where the PONV Intensity Scale showed 0.99 ICC. The reproducibility of EGG was poor to moderate and this probably reflects the sensitivity of the EGG to noise although a study measuring dominant power and frequency daily while fasting over three days showed there was no significant difference with analysis of variance.

4.2 Selecting nausea susceptible versus resistant subjects

In the comparison between susceptible and resistant subjects, nausea scores were positively correlated with the higher mean percentage motion sickness susceptibility of NS subjects, while the median MSSQ scores for NS vs NR subjects showed a trend towards higher scores in those who were nausea susceptible. The MSSQ may potentially predict a moderate to severe nausea reactivity to the motion video if the subjects score highly that is similar with previous findings (Golding, 1998).

Susceptible subjects withdrew parasympathetic activity and cortisol with sympathetic arousal. The percentage of time with EGG in normal gastric rhythm was also reduced during motion versus control video with an increase in dysrhythmias (e.g., increase in tachygastrias and arrhythmias).

Cardiac sympathetic arousal during nausea are similar to responses in 'fight or flight' situations and are consistent with findings from previous studies of vection (Himi et al., 2004, Cowings et al., 1986). The cardiac parasympathetic withdrawal (CVT, CSB) during the stimulus was also in-line with previous reports (Himi et al., 2004, Cowings et al., 1986). Contrary to previous findings (Parker and Wilsoncroft (Parker and Wilsoncroft, 1978b, Himi et al., 2004), baseline sympathetic and parasympathetic responses do not seem to influence the manifestation of nausea in this group of subjects as this study found no significant differences in those parameters between susceptible and resistant subjects.

Susceptible subjects had a lower baseline cortisol compared to resistant subjects that is consistent with previous findings although the expected rise in cortisol was not seen in this study (Koch KL, 1985, Otto et al., 2006). This might be due to the fact that this is a milder stimulus compared to actual motion and thus less stressful. The parasympathetic activity could be a form of innate protective mechanism however another study has shown interesting observations of high baseline parasympathetic tone leading to increased nausea susceptibility (Rawat et al., 2002).

The EGG results above are consistent with previous studies (Imai et al., 2006) however the dominant frequency and dominant power were not significantly increased possibly due to the multiple assessments done simultaneously that would increase subject movement and as EGG is a very weak signal and sensitive to noise, this would make it difficult to get accurate readings (Stern et al., 1985, Muth et al., 1995).

4.3 Limitations and future work

In summary, the study showed that the virtual reality video human model of nausea is a good and safe stimulus for studying nausea comprehensively without vomiting with multiple modalities of recordings. The stimulus was chosen for its safety profile and may not be generalised for other conditions although there some evidence that susceptibility to nausea from motion sickness may potentially predict susceptibility to nausea in chemotherapy patients and physically-induced motion sickness (Golding, 2006). As there are no comprehensive studies of nausea especially one that is adaptable for brain imaging, this is the most suitable stimulus currently available and the subjects identified as susceptible and resistant in this study are potentially good candidates for a functional brain imaging study (Stern et al., 2011).

Visual motion induced nausea has the weakness of other collinear presentations of nausea with the previous observation of several dimensions of sensations closely linked to it (Muth et al., 1996, Gianaros et al., 2001). The three observed before are central, peripheral, sopite sensations (Gianaros et al., 2001). It is also closely linked with anxiety

as it causes general discomfort and is stressful (Burish and Carey, 1986, Haug et al., 2002). However, in this study the effects of the stimulus may be due to nausea because of no correlations between the nausea scores and other related motion sickness sensations, low BFI neuroticism score and low STAI state and trait scores in all subjects.

Another limitation to consider is that all the investigations are indirect assessments of the systems involved and may not be truly representative of the cortical processing of nausea. This is where there is a need for functional brain imaging studies to be able to decipher better the associated nausea markers that sometimes provides conflicting results (Stern et al., 2011).

CHAPTER 4

FUNCTIONAL MAGNETIC RESONANCE IMAGING BRAIN PROCESSING OF VISUAL MOTION INDUCED NAUSEA IN SUSCEPTIBLE VS RESISTANT SUBJECTS

1 Introduction

The human studies specifically studying nausea genesis in the brain include a 1996 magnetic source imaging study whereby one subject underwent yaw-axis rotations with side-to-side head movements and ipecac ingestion showing inferior frontal gyrus activation (Miller et al., 1996). This activation was reversed when the same subject was administered the anti-emetic drug ondansetron, a 5-hydroxytryptamine$_3$ receptor antagonist. These results supported an older 1993 electro-encephalography study which demonstrated increased activity in the temporo-frontal region during motion sickness (Chelen et al., 1993). The inferior frontal gyrus was also activated by galvanic vestibular stimulation (Bense et al., 2001, Stephan et al., 2005) caloric vestibular stimulation (Fasold et al., 2002) without nausea in human fMRI studies. Galvanic stimulation also activated the basal ganglia, inferior and middle frontal gyrus, parahippocampal gyrus and hippocampus (limbic), cerebellum (crus I, vermal lobule IV), anterior and posterior insula and retroinsular regions (interoception and visceral autonomic response), superior temporal gyrus, temporoparietal cortex, precentral gyrus, thalamus, anterior cingulate gyrus, and the supplementary motor area (Bense et al., 2001, Stephan et al., 2005). More recently, an fMRI study of visual motion induced nausea on 28 women discovered that there were also activation of the dorsolateral prefrontal cortices bilaterally and in addition a broader network involving the interoceptive, limbic, somatosensory brain regions were also stimulated (Napadow et al., 2012b). Activation of the insula and cingulate cortices have also been shown to play an important role in animal and other related human studies (Stern et al., 2011). There

is now a need for a larger functional magnetic resonance imaging (fMRI) study of a similar design to Miller et al., (1996) with a balanced recruitment of both men and women (Napadow et al., 2012b) using preselected subjects who are susceptible with resistant subjects for comparison (Stern et al., 2011), with a safe stimulus (Kowalski et al., 2006) that allows for repeat volunteer visits.

1.1 Functional Magnetic Resonance Imaging

The fMRI has excellent spatiotemporal resolutions, can exhibit whole brain networks while subjects are stimulated, and subjects are not exposed to harmful materials (Aziz and Thompson, 1998). Thus it is preferred over magnetoencephalography (MEG), computed tomography (CT) and positron emitting tomography (PET). The investigation of the cortical pathways involved in nausea genesis may potentially uncover new targets as well as form the basis for quantitative pharmacological studies of nausea (Borsook et al., 2006a). This is important for the clinical management of nausea especially those seen in postchemotherapy patients as it is currently the target of oncological societies to control of nausea, the greatest remaining emetogenic challenge (Roila et al., 2010).

This neuroimaging technique measures changes in the blood oxygenation levels in microcirculation that provides an indirect measure of neural activity. Neural activity increases blood supply to the surrounding capillary beds overcompensating for neural oxygen consumption causing an increase in oxyhaemoglobin and decrease in deoxyhaemoglobin concentration. Oxyhaemoglobin is less paramagnetic than

deoxyhaemoglobin causing the magnetic resonance (MR) signal intensities to change i.e. increased oxyhaemoglobin concentrations leads to higher MR signals. A net increase in MR signal intensity (usually about 0.5–5% in magnitude) is thus detected and is dependent on the amount increased blood flow evoked that correlates with increased neural activity (Logothetis, 2008, Raichle and Mintun, 2006).

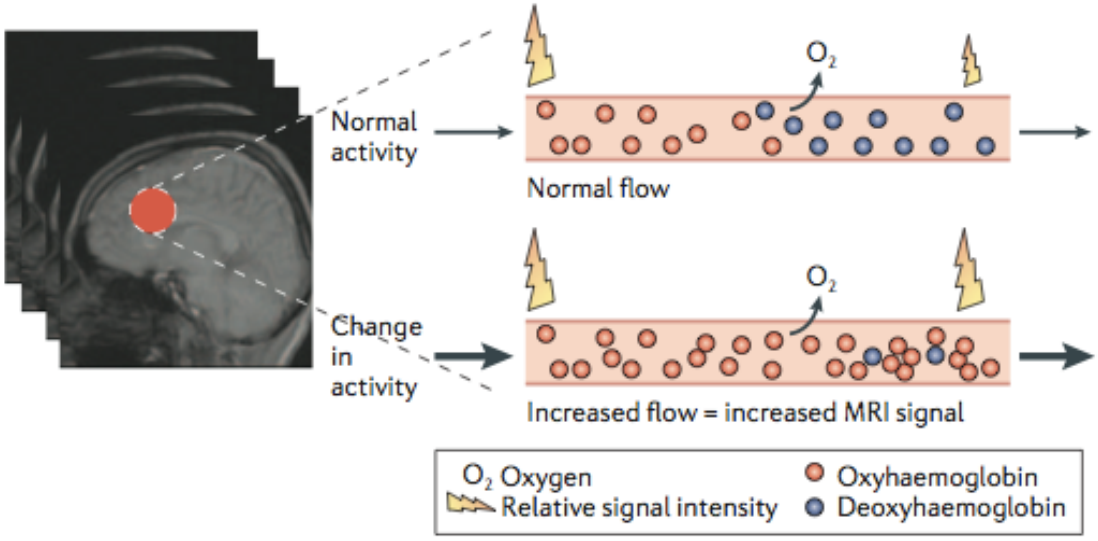

Figure 29. BOLD fMRI measures changes in the blood oxygenation levels in the microcirculation providing an indirect measure of neural activity. Brain activity increases blood flow to the surrounding capillary beds overcompensating for neural oxygen consumption causing an increase in oxyhaemoglobin and decrease in deoxyhaemoglobin concentration. As oxyhaemoglobin is less paramagnetic than deoxyhaemoglobin, the magnetic resonance (MR) signal intensities change i.e. increased oxyhaemoglobin concentrations leads to higher MR signals. A net increase in MR signal intensity (usually about 0.5–5% in magnitude) is thus detected and is dependent on the amount increased blood flow evoked that correlates with increased neural activity (Logothetis, 2008, Raichle and Mintun, 2006).

1.2 Knowledge gaps

Whilst there have been numerous studies of nausea using multiple animal models, this is somewhat inadequate in the evaluation of a subjective, descriptive experience of nausea, especially when comparing animals with such differing neuroanatomy (Hermer and Spelke, 1994, Hermer and Spelke, 1996). Progress in the understanding of the neurophysiological mechanisms of nausea, is hindered by a scarcity of human brain imaging studies to evaluate the brain processing of nausea (Stern et al., 2011). Thus, there is a need to delineate the specific areas of the brain generating nausea better in humans.

1.3 Research aims and hypothesis

The aim of the present study was to use the previously validated methods now to study the brain processing of nausea and compare brain activity in susceptible and resistant subjects. I hypothesise that subjects preselected by previous exposure to the stimulus as either susceptible or resistant will show differences in the brain processing of nausea.

2 Method

2.1 *Study design and setting*

This was a crossover study i.e. the same subject is exposed to both a control and experimental condition. It was carried out at the Institute of Psychiatry, King's College London (KCL).

2.2 Ethical approval

The King's College London Research Ethics Committee (PNM/09/09-04) approved these studies.

2.3 Subjects

30 healthy right handed volunteers from the Chapter 3 study were invited for this study: 17 nausea susceptible (8 males and 9 females) median age 24 years, range 19 - 34 years, and 11 nausea resistant (6 males and 5 females) median age 22 years, range 20 - 33 years were preselected based upon previous exposure to the stimulus. All subjects gave written informed consent. Volunteers were recruited to meet the following criteria: (i) normal body mass index, (ii) no abnormality on clinical examination, including a history or presence of cardiac, ophthalmologic, gastro-intestinal, hepatic, or renal disease, or other condition known to alter their response to visually induced motion

sickness nausea e.g. vestibular disease, (iii) no abnormality on electrocardiogram examination at screening (iv) no abuse of alcohol (defined as an average intake >21 units per week or 3 units per day); and (v) no history or presence of neurological or psychiatric conditions (e.g. stroke, traumatic brain injury, epilepsy, space-occupying lesions, multiple sclerosis, Parkinson's disease, vascular dementia, transient ischaemic attack, schizophrenia, major depression, etc). Subjects with any of the following were excluded: (i) received prescribed medication within 14 days prior to the first visit, which might interfere with the study procedures or compromise safety, (ii) received over-the-counter medicine within 48h of the study, (iii) participated in a trial with any drug within 3 months before the first visit, (iv) had a caffeinated drink within 24 h of visit.

2.4 Materials and Protocol

After the same preparation as the protocol for Chapter 3 study, with the addition of MRI safety measures, the subjects were brought into the MRI room. Subjects were provided with a pair of goggles with questionnaires administered assessing nausea (VAS and MSAQ) and anxiety (STAI-state). After starting motion or control video, minute-to-minute nausea reporting using a four button box with first button for no nausea and then mild, moderate and last button for severe nausea was collected during the fMRI scans. Blood samples were not taken during this study.

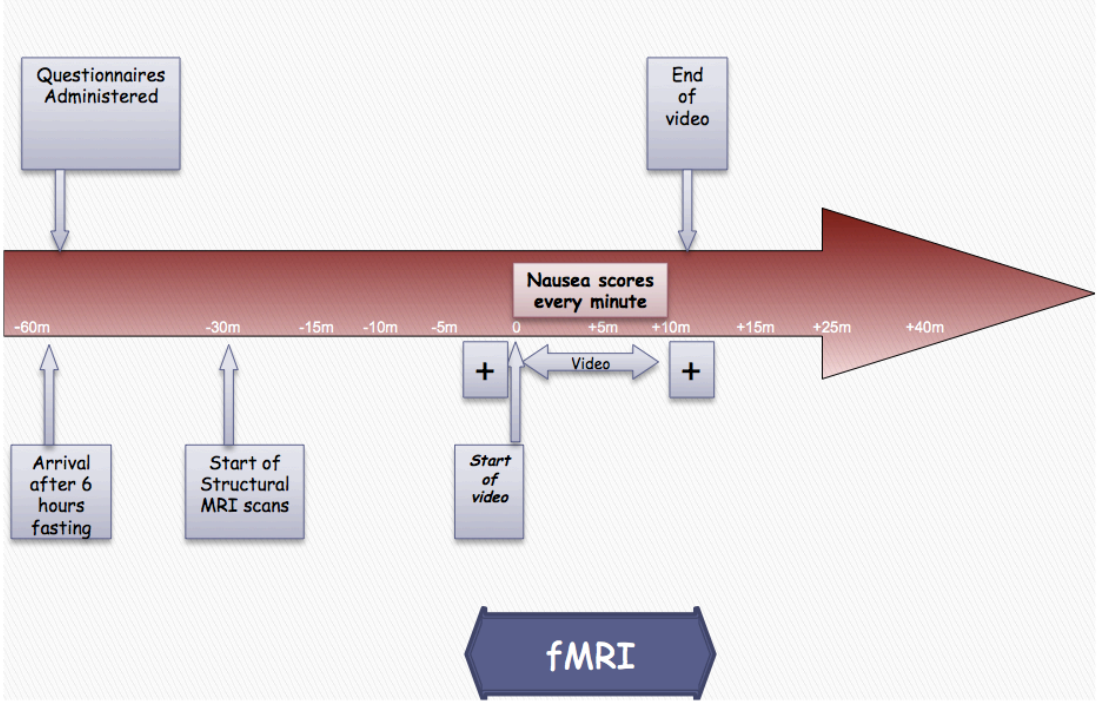

Figure 30. Schematic summary of chapter 4 experimental protocol: After 6 hours of fasting subjects arrived for chapter 4 answering questionnaires which e.g. assess motion sickness symptoms and questionnaires which e.g. assess motion sickness symptoms and, reassessed just before starting motion or control video after which minute to minute nausea reporting was determined and another MSAQ and STAI-S questionnaire were adminstered at the end of the video. This is essentially similar with study 1 protocol with the exception that no bloods being taken.

2.5 Baseline activity

Subjects were encouraged to relax and focus on a target presented in the goggles for two and a half minutes before the video is started. This forms the baseline recordings.

2.6 Assessment of motion sickness susceptibility and anxiety levels

Subjects used a button box (four button box with first button for none and then mild, moderate and last button for severe) on their right hands to self-report nausea and anxiety scores before and at the end of each video as well as nausea scores every minute during the video. The ratings were reported using the same visual analogue scale used during study 1 explained above. Both MSAQ and STAI-S questionnaires were also administered just before and at the end of the videos. A two-way, random effects, average measures intra-class correlation (ICC) model for continuous variables were calculated between the chapter 3 and 4 visits for the 28 subjects (Green et al., 2012) to assess reproducibility of their nausea induction. Please refer Chapter 2 section 2.4.6 for more details.

2.7 Exposure to stimulus

Subjects watched two different videos consecutively through a pair of MRI compatible goggles provided as standard equipment by the MRI manufacturer. The goggles are positioned with rubber eyepieces to cover their eyes to limit their field of view to only the screen and delivered the stimulus using two LCD screens in front of each eye to create an illusion of a large screen in front of them:

. The videos consisted of;

- a non-nausea inducing video consisting of a stationary cityscape (control or neutral video) and
- a nausea inducing video consisting of a moving cityscape (nausea video)

The first and second videos were separated by a washout period of 10 minutes during which subjects continued to be questioned every minute for symptoms of nausea, anxiety or dizziness until no symptoms are reported. This was to avoid a carry-over effect of symptoms from one video onto another. A red target was put in the video at regular intervals to assess the subject's attention on the video, as shown in Figure 3.

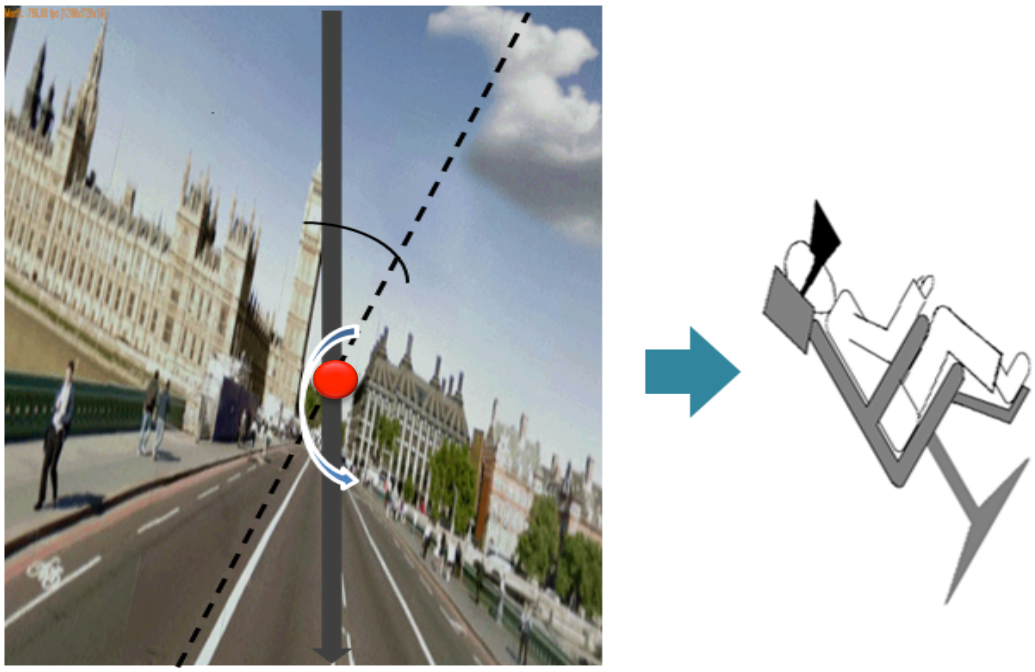

Figure 31. The novel stimulus - projected in front of a subject with goggles to limit their peripheral vision to the stimulus. The off-vertical tilt helps create an illusion that the subject was actually spinning, at an angle which is found to hasten the onset of MSIN (Bijveld et al., 2008b)

2.8 Subjective monitoring

A visual-analogue-scale symptoms (VAS) questionnaire and motion sickness assessment questionnaire (MSAQ) described in chapter 2 section 2.4.7 documented any symptoms reported by the subjects. A visual scale from 1 to 4 was used with 1 being, without symptom, and 2 being mild symptom, 3 being moderate symptoms and 4 being maximum level of tolerated symptom. State anxiety status was assessed using the state version of the STAI.

2.9 Objective monitoring

The SCR was recorded on the subject's left hand continuously throughout the experiment. Cardiac pulse and respiratory effort data were monitored using a pulse oximeter (InVivo) and a respiratory effort transducer (BIOPAC), respectively. The pulse oximeter was placed on the subject's left index finger. The respiratory effort belt was placed around the subject's abdomen. The vital signs monitoring was performed similar to those described in chapter 2. Video was delivered through fMRI compatible goggles with an eye-tracker video to monitor subject's attention to the stimulus and pupil location (NordicNeuroLab GmbH VisualSystem, Norway).

The fMRI data (T2*-weighted images) was collected on a General Electric Signa Excite II 1.5 T HD scanner based at the Centre for Neuroimaging Sciences, Institute of Psychiatry, King's College London. Head movement was restricted using foam padding

within the head coil and an eye movement's tracker was mounted onto the head coil together with the in-built MRI compatible goggles delivering the stimulus. Prior to the start of the fMRI experiment, a high-resolution gradient echo structural scan (43x3mm slices, 0.3 interslice gap, TE 40ms, TR 3000ms, flip angle 90˚, matrix 128^2, in plane voxel dimensions 1.875x1.875) was acquired in each volunteer to be used for Talairach normalisation. During fMRI, a total of 300 T2* weighted images per slice (40x3mm slices, 0.3 interslice gap, TE 25ms, TR 3500ms, flip angle 90˚, matrix 64^2), depicting blood oxygen level dependant (BOLD) contrast were collected as subjects viewed the control and motion video.

2.10 Statistical analysis

XBAM version 4.1 (http://brainmap.co.uk/ referenced on the 8th of August 2012), a package developed at the Institute of Psychiatry, King's College London, was used to analyse fMRI data. It implements permutation-based nonparametric methods to minimise the number of assumptions used in making statistical inference (Brammer et al., 1997). After acquisition, fMRI data pre-processing, smoothing and individual brain activation mapping was performed (Coen et al., 2009). Analysis of covariance was performed on the effect size maps in Talairach and Tournoux's standard space (Talairach and Tournoux, 1988) with each voxel statistic corrected for the actual number of participants contributing to the calculation (Thirion et al., 2007). An analysis of covariance (ANCOVA) examining the main effect of several different groups on whole-

brain neural activity was done and a clusterwise p value of 0.01 (corrected for whole brain volume using permutation testing). Correlation analysis for brain activity to the level of nausea reported were analysed during the nausea video. Comparisons were also made between activity in the whole brain for all subjects between control versus motion video and between resistant versus susceptible subjects during the motion video.

Psychometric, nausea questionnaires, and autonomic data were analysed using matched-pair t-tests and Wilcoxon tests to compare the means and medians. Pearson's and Spearman's correlations were used to determine the relationship between measurements. Independent-measures t-tests and Mann-Whitney tests were used to compare groups. Reproducibility of the studies used intra-class correlation comparison (ICC) and agreement was measured using two-way mixed average measure ICC model for continuous variables. Confidence intervals for the ICC were calculated according to the methods of Scheffe (Green et al., 2012). ICC were interpreted according to suggestions made by Yen et al (2002) as: - excellent (0.75-1), moderate (0.4-0.74) or poor (0-0.39) (Davis and Hallerberg, 2010). Commercially available statistics packages (SPSS, Chicago, IL, USA and GraphPad, San Diego, CA, USA) were used for the analysis. P values <0.05 were considered to be of statistical significance.

3 RESULTS

All 30 subjects completed and tolerated the studies well without any vomiting or retching. 2 subjects' (1 nausea susceptible and 1 nausea resistant) data were excluded from analysis due to excessive motion artefact (>3mm translation in any axis or spiking >1.5mm) or scanner anxiety.

3.1 Response to videos

The 17 nausea susceptible subjects reported significantly higher nausea compared to the 11 resistant subjects during the motion video (Figure 32). The susceptible subjects reporting more nausea also reported more anxiety on the STAI compared to the resistant subjects who did not report much nausea (+7 vs +1, $p<0.05$). The 17 susceptible subjects' nausea responses during the motion video when compared to the initial visit responses had an ICC of 0.539 with a slight reduction of reported nausea percentage change compared to baseline during the motion video (-5.3% ± 1.1, $p>0.05$).

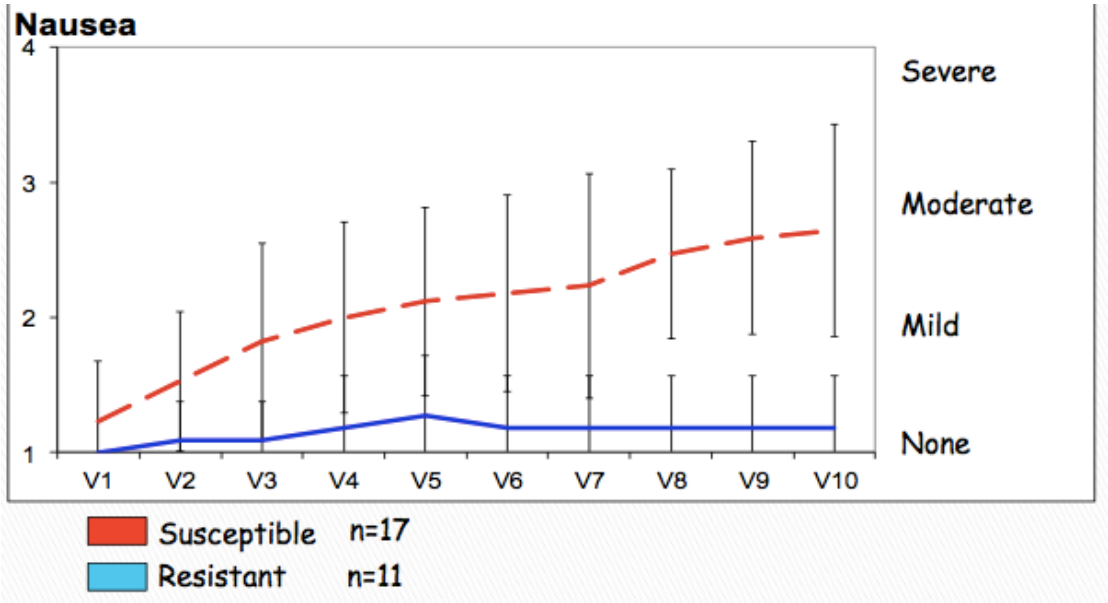

Figure 32. Nausea scores during the fMRI motion video for 17 nausea susceptible and 11 nausea resistant subjects. All 17 nausea susceptible subjects experienced a gradual increase in nausea scores during the motion video with statistically significant peaking at the end of the video ($p<0.05$). Meanwhile the 11 nausea resistant subjects did not have any nausea or had mild nausea during the motion video.

3.2 Control versus motion video for all subjects

All 28 subjects demonstrated an increase in activity in the right middle temporal gyrus and the left occipital lobe cuneus (Table 12); and a decrease in activity in the left parahippocampal gyrus and the right cerebellar tonsil while watching the motion video compared to watching the control video (Table 13).

Cerebral Region	3D Cluster size	Peak Talairach Coordinates (x, y, z)	Probability
Right Middle Temporal Gyrus	63	43.33, -59.26, -3.30	0.0039
Left Occipital Lobe Cuneus	921	-18.06, -74.07, 9.90	0.0001

Table 12. Brain activity in all subjects during control vs motion video. There was increased activity in these brain areas during motion video compared to control video in all 28 subjects.

Cerebral Region	3D Cluster size	Peak Talairach Coordinates (x, y, z)	Probability
Left Parahippocampal Gyrus	54	-21.67, -25.93, -13.20	0.0034
Right Cerebellar Tonsil	107	3.61, -55.56, -39.60	0.0006

Table 13. Brain activity in all subjects during control vs motion video. There was decreased activity in these brain areas during motion video compared to control video in all 28 subjects.

3.3 Nausea susceptible versus nausea resistant subjects

Nausea susceptible subjects demonstrated an increased in activity in right substantia nigra (Peak Talairach Coordinates (x, y, z) 3.61, -18.52, 16.50; <p = 0.01) compared to resistant subjects during the nausea stimulus (Figure 33) and decrease in activity in left cerebellar declive and right parahippocampal gyrus (Table 14).

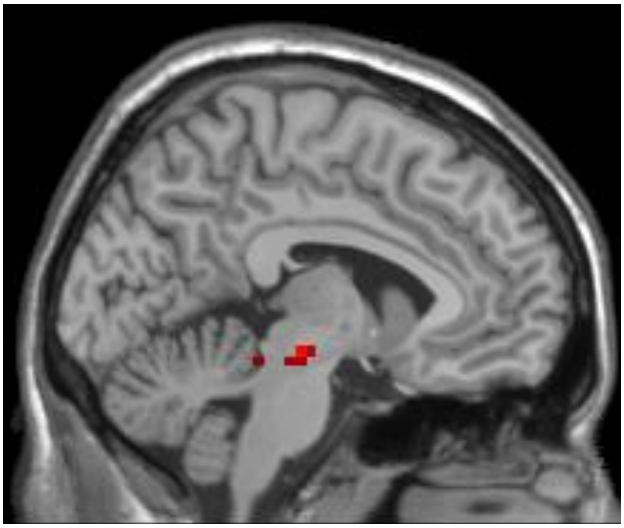

Figure 33. Brain activity in susceptible subjects vs resistant subjects during nausea video. Red spots marks the increased activity in the substantia nigra in 17 nausea susceptible subjects compared to 11 resistant subjects during the nausea video.

Cerebral Region	3D Cluster size	Peak Talairach Coordinates (x, y, z)	Probability
Left Cerebellar Declive	197	-10.83, -66.67, -13.20	0.0012
Right Parahippocampal Gyrus	66	21.67, -44.44, -6.60	0.0034

Table 14. Brain activity in susceptible subjects vs resistant subjects during nausea video. There is decreased activity these brain areas in 17 nausea susceptible subjects compared to 11 resistant subjects during the nausea video.

3.4 Correlations between nausea scores and brain activity:

Nausea scores also positively correlated with left inferior frontal gyrus (Peak Talairach Coordinates (x, y, z) -54.17, 25.93, -6.60; <p = 0.01) activity (

Figure 34 and Figure 35) where activity increased with increasing scores of nausea. Nausea scores negatively correlate with right occipital cuneus, left anterior cerebellar culmen, left occipital lingual gyrus, right parahippocampus and left posterior cerebellar declive activity for susceptible subjects (Table 15).

Cerebral Region	3D Cluster size	Peak Talairach Coordinates (x, y, z)	Probability
Right Occipital Cuneus	79	7.22, -70.37, 13.20	0.0005
Left Anterior Cerebellar Culmen	38	-7.22, -51.85, -3.30	0.0016
Left Occipital Lingual Gyrus	151	-3.61, -70.37, -3.30	0.0001
Right Parahippocampus	70	21.67, -25.93, -13.20	0.0006
Left Posterior Cerebellar Declive	64	-32.50, -51.85, -13.20	0.0005

Table 15. Brain activity correlated with nausea scores. There was decreased activity in these brain areas that is correlated with decreased reporting of nausea scores in 17 nausea susceptible subjects during the nausea video.

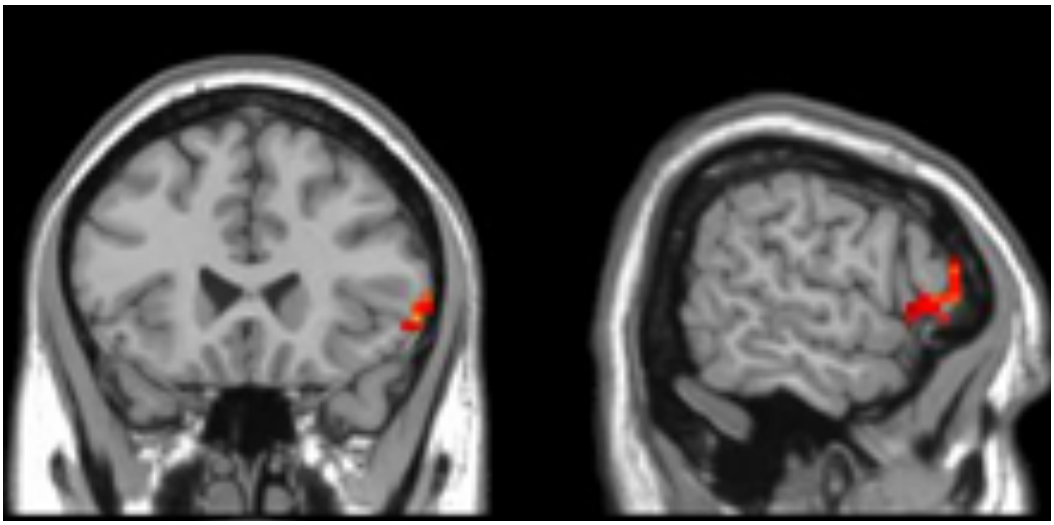

Figure 34. Brain activity correlated with nausea scores. Red spots mark the increased activity in the inferior frontal gyrus that is correlated with increased reporting of nausea scores in 17 nausea susceptible subjects during the nausea video.

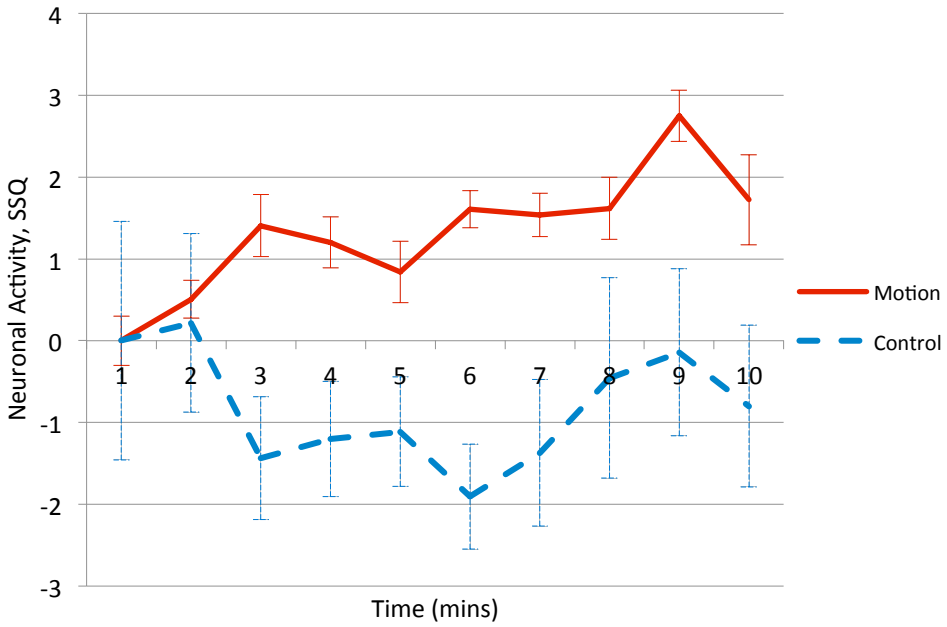

Figure 35. Time course Of Brain Activity (Raw diagram of minute by minute average brain activity in the inferior frontal gyrus in 17 nausea susceptible subjects that progressively increases while watching the motion video for 10 minutes reaching a peak in the last third of the video that corresponds with the nausea scores reported by the nausea susceptible subjects. In contrast, there is no specific trend seen during the control video).

4 DISCUSSION

4.1 Adaptation of a novel visually induced motion sickness nausea model to study the brain processing of nausea

Nausea and anxiety levels was higher in the susceptible compared to resistant subjects with the ICC of moderate to good reproducibility between chapter 3 and chapter 4 studies although less with the MRI goggles video compared to the projected video in Chapter 3 possibly due to a larger field of view in the projected video (Bos et al., 2010). As these subjects have had their subjective reports of nausea validated by objective cardiac sympathetic arousal, parasympathetic withdrawal, shift of normal to dysrhythmic gastric myoelectrical activity, increased cortisol and increase state anxiety (refer Chapter 3), we are making the assumption that they are experiencing the same psychophysiological changes as described in chapter 3 and thus similar brain activity was likely when they were reporting nausea inside the MRI scanner.

All 28 subjects increased activity in the middle temporal gyrus and occipital lobe; and decreased activity in cerebellum and parahippocampal gyrus while watching the motion video compared to control. As previously demonstrated the increased activity in the occipital lobe (Brandt et al., 1998) (Napadow et al., 2012a) and the middle temporal gyrus (Napadow et al., 2012a) is likely due to the effect of the motion video on the visual cortices compared to the control.

Of possibly greater importance, decreased cerebellar and parahippocampal activity may be associated with the sensory mismatch interactions proposed to be the mechanism behind the development of vection and eventually motion sickness. It is likely that the conflict in the visual stimuli informing the brain that the subject is in motion with the information from the vestibular system suggesting that the subject is stationary led to the vestibular system being inhibited (Brandt et al., 1998). The reverse has also been shown to be true with vestibular system activations inhibiting the parieto-occipital visual pathways in fMRI studies using a mild vection stimulus without achieving nausea (Brandt et al., 1998, Brandt et al., 2002, Wenzel et al., 1996).

I have demonstrated that the virtual reality stimulus can be adapted to the fMRI investigative environment with the pre-existing infrastructure and not needing complicated or expensive modifications to the MRI infrastructure (Napadow et al., 2012a, Kowalski et al., 2006). To our knowledge, this is the first easily adaptable virtual reality stimulus based on real world scenery that has been successfully utilised for the fMRI study of nausea specifically.

4.2 Brain processing of visual motion induced nausea

When comparing the nausea susceptible versus resistant subjects during the motion video there was increased substantia nigra activity in susceptibles. As substantia nigra is part of the basal ganglia pathway that is involved in maintaining posture (Henderson et al., 2005, Su et al., 2002), its activation may be due to increased motion related brain

processing in the susceptible individuals that are experiencing significant motion effects from the stimulus. Substantia nigra is involved in motor control (Hodge and Butcher, 1980) and typical postural responses to motion stimuli which are altered when experiencing motion sickness (Shepard et al., 1990).

Further analysis in the nausea susceptible subjects revealed that increasing inferior frontal gyrus activation was positively correlated with increasing levels of nausea reported. This is consistent with an earlier preliminary study (Miller et al., 1996) using Magnetic Source Imaging with head yaw-axis rotation and ingested syrup of ipecac as the stimuli to induce nausea that was reversed with a 5-hydroxytryptamine$_3$ receptor antagonist. This was also supported by a recent study showing bilateral prefrontal cortical activation with simulated optokinetic drum inducing nausea (Napadow et al., 2012b). The same area was also activated by galvanic and caloric vestibular stimulation evoking feelings of motion or nystagmus but not up to the point of nausea in fMRI (Bense et al., 2001, Fasold et al., 2002). Furthermore, it was observed in PET studies in migraine patients that onset of headaches associated with nausea activated the inferior frontal gyrus (Denuelle et al., 2007). Taking these results together the inferior frontal gyrus is either involved in (conscious) perception of discordant information of body motion or of nausea, with the latter hypothesis supported by the (Miller et al., 1996) report of activation by a nauseogenic stimulus i.e ingested ipecac which likely acted via abdominal visceral afferent pathways.

Other than regions of the brain that was activated in these studies, there were consistent inhibitions seen in all the aforementioned analysis in the parahippocampus. When comparing between the motion versus control video for all subjects the parahippocampus was inhibited during motion video. Furthermore, the parahippocampus activity was also decreased when comparing nausea susceptible and resistant subjects during the motion video. On top of that, parahippocampus activity decreased as nausea levels increased in susceptible subjects. Conversely, the parahippocampus was activated in resistant subjects as a group during the motion video (right parahippocampus, cluster size 798, peak talairach coordinates (x=25.28, y=-18.52, z=-9.90), $p<0.0002$).

It is possible to speculate that the parahippocampus is a pivotal area involved in the processing of the visual motion induced nausea in our subjects such that its inhibitions signifies the disorientation induced by the stimulus leading to the sensory conflict (Reason and Brand, 1975) postulated to generate nausea. Interestingly the nausea resistant individuals as a group increased left parahippocampal activity that may confer some protection against disorientation that may be involved in the generation of nausea.

It was postulated before that motion sickness develops specifically when postural control is threatened by misinterpretation of the environment (Riccio and Stoffregen, 1991), consistent with the evidence that spatial orientation loss inevitably produces loss of balance in addition to provoking nausea (Takahashi et al., 1995). Behavioural studies

have demonstrated that human infants spatially disorientated by being spun around with their eyes closed and left facing a random wall in a room (Hermer and Spelke, 1994, Hermer and Spelke, 1996) re-orientate themselves solely on the basis of the geometry of the local visual scenery like the horizon or the corner of a room. The same was seen with rats during spatial disorientation also (Cheng, 1986, Margules and Gallistel, 1988). It is proposed that this is evidence for a phylogenetically and developmentally primitive component involved in spatial orientation. In addition, clinical lesions in humans (Habib and Sirigu, 1987, Hublet and Demeurisse, 1992) in the parahippocampus presents with spatial disorientation to their surroundings with reports of a patient who selectively lost the ability to orient himself in the environment after a stroke involving the right parahippocampal gyrus (Luzzi et al., 2000). As disorientation is common with vection caused by visual motion induced nausea (Kennedy et al., 2010), it is likely that the parahippocampus plays an important role.

Observations from fMRI human studies using topographic recall and learning of a virtual maze revealed that the parahippocampus plays a pivotal role in spatial awareness and navigation especially in studies using cityscapes like the ones used in this study's motion video stimulus (Aguirre et al., 1996, Epstein and Kanwisher, 1998, Ishai et al., 1999).

In this study, the hippocampus (left hippocampus, cluster size 3484, peak talairach coordinates (x=-28.89, y=-18.52, z=-9.90), $p<0.0012$) was also inhibited in all subjects during the motion video. There is a clinical case of a young woman with chronic

topographical disorientation after a haemorrhagic lesion of the right temporo-occipital region involving the hippocampus (Rusconi et al., 2008). Primates research in aged female rhesus macaques revealed the hippocampal M1 muscarinic receptor function was associated with spatial learning and memory (Haley et al., 2011). Older studies showed the rat hippocampus has neurons with receptive fields for current position in the environment (spatial awareness), with lesions there disrupting place learning suggested that the hippocampus is involved in processing large-scale environmental space (O'Keefe and Dostrovsky, 1971). However, further research showed that the rat hippocampus plays an important role in spatial representation and learning with lesions there leading to disorientation (Morris et al., 1982). Thus the inhibition of the hippocampus in all our subjects during the motion video that is not seen during the control video might be related to some form of disordered processing of the visual stimuli as part of the sensory conflict theory hypothesised to play a role in the generation of nausea (Reason and Brand, 1975).

Thus evidence from both human and animal studies suggests that the parahippocampus and the hippocampus respectively are critical for orientation and navigation; and clinical lesions of the hippocampus and parahippocampus present with disorientation. Visual motion induced nausea possibly starts from vection generating disorientation that develops into nausea and both worsen progressively with increasing exposure to the stimulus. This may possibly be the first objective evidence of the pathway involved in the universally acknowledged and accepted sensory conflict theory initially proposed by (Reason and Brand, 1975). What is equally as important if not more

is the fact that the parahippocampus appears to have a protective role against the development of visual motion induced nausea. This now needs specific studies targeting the limbic structures

4.3 Limitations and future studies

More detailed investigation using non-visual/vestibular stimuli to evoke nausea will be needed to determine if the brain processing of nausea is similar with different input pathways or if there are important differences that may potentially be important when thinking about treating the widely varying causes of nausea for the clinician. Investigating disease states associated with nausea may be a potential option such as cyclic vomiting syndrome (Olden and Chepyala, 2008) and migraine (Cuomo-Granston and Drummond, 2010).

In this study, areas of the brainstem like the brainstem nuclei or the NTS that are postulated to play a key role in nausea pathways were not seen, however these medullary nuclei are at the limit of fMRI spatial resolution and are also susceptible to cardiorespiratory artefacts. In future fMRI studies specifically targeting the brainstem using 3 Tesla MRIs for better spatial resolution or possibly complementary studies like high resolution research tomography (HRRT) that was recently shown to be able to quantify the serotonin transporter availability in the brainstem may be helpful in developing a better understanding of brain stem processing of nausea (Schain et al., 2012).

This stimulus evoked specific/characteristic pattern of changes in brain activity that will now permit studies of pharmacological interventions aimed at normalising these changes with the intention of treating nausea. It is hoped that with the identification of the possible brain pathways involved in processing nausea that the pharmacological studies will be able to be further refined.

It would now be useful to know if the nausea brain response stimulated by the motion video can be modulated by drugs treating motion sickness (Miller et al., 1996) to ensure the areas of the brain highlighted above are reversed (suggesting that it plays a key role in nausea processing) when individuals are able reduce their nauseous response to the same stimulus.

CHAPTER 5

SCOPOLAMINE MODULATION OF THE BRAIN PROCESSING OF VISUAL MOTION INDUCED NAUSEA

1 Introduction

The prefrontal and limbic cortexes as well as the substantia nigra appear to play a role in nausea generation in nausea susceptible individuals. It would now be useful to know if these brain regions are specific for nausea with pharmacological studies. There are many on-going debates for the best treatment of nausea and vomiting depending on its cause and the jury is still out (Green et al., 2012, Davis and Hallerberg, 2010). From the available literature scopolamine appears to be the first line recommendation for its efficacy and low incidence of side effects in comparison with other agents (Spinks et al., 2004).

Hyoscine bromide is an antimuscarinic compound and a derivative of scopolamine. It has a diverse role in medicine, such as the prevention of motion sickness, pelvic magnetic resonance imaging (MRI) by suppression of intestinal and uterine smooth muscle contractions (Fujimoto et al., 2010, Dosda et al., 2003, Winkler and Hricak, 1986, Nakai et al., 2008). Hyoscine has high affinity for muscarinic receptors (Elrod and Buccafusco, 1988) [little selectivity for receptor subtypes M1–M5; (Renner et al., 2005)] and competitively antagonizes acetylcholine on postsynaptic muscarinic receptor sites (Deutsch, 1971). Scopolamine has negligible affinity for histaminergic and dopaminergic receptors (Peroutka and Snyder, 1982). The central nervous system (CNS) effects consist of drowsiness, reduced attention and memory impairment, and a range of other CNS effects including changes in several EEG frequency bands (Ebert et al., 2001, Ebert and Kirch, 1998, Ebert et al., 1998). The peripheral effects of scopolamine include

typical antimuscarinic effects like a dry mouth, skin and throat, decreased blood pressure, decreased heart rate, difficulty urinating, constipation, pupil dilatation and impaired eye focusing (mydriasis and cycloplegia).

It is interesting to note that muscarinic acetylcholine receptors were shown to be present in high concentrations in the prefrontal cortex and the hippocampus (Nathanson, 2008) and to have a role in spatial learning and memory in rodents, nonhuman primates, and humans (Wisman et al., 2008, Gage et al., 1988, Fredrickson et al., 2008, Thomas et al., 2008). Scopolamine impairs cognitive task performance in rats (Biggan et al., 1996), dogs (Araujo et al., 2005), rhesus monkeys (Savage et al., 1996, Taffe et al., 1999), and humans (Rosier et al., 1998) and possibly reducing the conscious sensation of nausea. The regions critical for cognitive function like the prefrontal cortices and the hippocampus express type 1 (M1) subtype muscarinic receptors mostly (Gage et al., 1988, Fredrickson et al., 2008, Thomas et al., 2008, Flynn et al., 1995a, Flynn et al., 1995b, Tamminga, 2006, Wisman et al., 2008) and the muscarinic type 2 (M2) after (Jagoda et al., 2003, Rouse et al., 2000). The M2 may also contribute to cognitive function (Gautam et al., 2006). With the brain regions of interest that were discovered in chapter 4 known to have expressions of M1 and M2 muscarinic receptors, scopolamine is a likely candidate to reverse the prefrontal and limbic cortical activation due to visual motion induced nausea.

There hasn't been any study designed specifically to look at the effects of scopolamine modulation on the fMRI brain activity with nausea to my knowledge. Thus what we know

regarding scopolamine's effects on the brain are mostly from previous studies using scopolamine as a pharmacological model substance based on the "cholinergic hypothesis" of memory loss in senile dementia of the Alzheimer type. In the human brain by fMRI, it has been found to modulate the hippocampus/parahippocampal gyrus and disrupt spatial memory (Antonova et al., 2011b).

1.1 Hypothesis

Therefore my hypothesis was that the prefrontal and limbic involved in the genesis of visual motion induced nausea would be reversed by scopolamine intervention.

2 Method

2.1 Study design and setting

This was a crossover study i.e. the same subject is exposed to both scopolamine and placebo administration during motion video. It was carried out at the Institute of Psychiatry, King's College London (KCL).

2.2 Ethical approval

The NRES Committee South Central - Portsmouth (12/SC/0117) approved these studies.

2.3 Subjects

Sixteen (8 males, 8 females) healthy right handed nausea susceptible volunteers median age 24 years, range 19 - 36 years were recruited with 5 subjects who had participated in studies described in Chapters 3 and 4. Subjects were preselected based upon moderate to severe nauseous response to previous exposure of the stimulus. All subjects gave written informed consent. Volunteers were recruited to meet the following criteria: (i) normal body mass index, (ii) no abnormality on clinical examination, including a history or presence of cardiac, ophthalmologic, gastro-intestinal, hepatic, or renal disease, or other condition known to alter their response to visually induced motion

sickness nausea e.g. vestibular disease, (iii) no abnormality on electrocardiogram examination at screening (iv) no abuse of alcohol (defined as an average intake >21 units per week or 3 units per day); and (v) no history or presence of neurological or psychiatric conditions (e.g. stroke, traumatic brain injury, epilepsy, space-occupying lesions, multiple sclerosis, Parkinson's disease, vascular dementia, transient ischaemic attack, schizophrenia, major depression, etc). Subjects with any of the following were excluded: (i) received prescribed medication within 14 days prior to the first visit, which might interfere with the study procedures or compromise safety, (ii) received over-the-counter medicine within 48h of the study, (iii) participated in a trial with any drug within 3 months before the first visit, (iv) had a caffeinated drink within 24 h of visit.

2.4 Materials and Protocol

Subjects arrived 60 minutes before their experiment to allow for the accurate timing of the administration of the drug or placebo capsule 30 minutes before the experiment. Before the administration of the capsule, subjects heart rate were assessed as well as the 'n-back' task and questionnaires monitoring motion sickness symptoms and anxiety state that were repeated at the end of the experiment. Heart rate was also assessed before the capsule was given and at the end of the experiment 2 hours later. After similar preparation as the protocol for Chapter 4, the subject was brought into the MRI room. Subjects were provided with a pair of goggles that displayed the stimulus and questionnaires in front of the subject's eyes. An emergency buzzer was also put into

their left hand to allow the subjects to end the experiment at any point in time if they become too uncomfortable due to the video or drug side effects. Close monitoring of their vital signs with pulse oximetry, skin conductance response (SCR) and respiratory belt was started once they are inside the scanner room with microphones picking up their voices as well as a video overseeing the subject in the scanner in the monitoring panel for safety reasons. After starting the stimulus, minute-to-minute nausea reporting using a visual analogue scale was collected during the fMRI scans using a button box on their right hand.

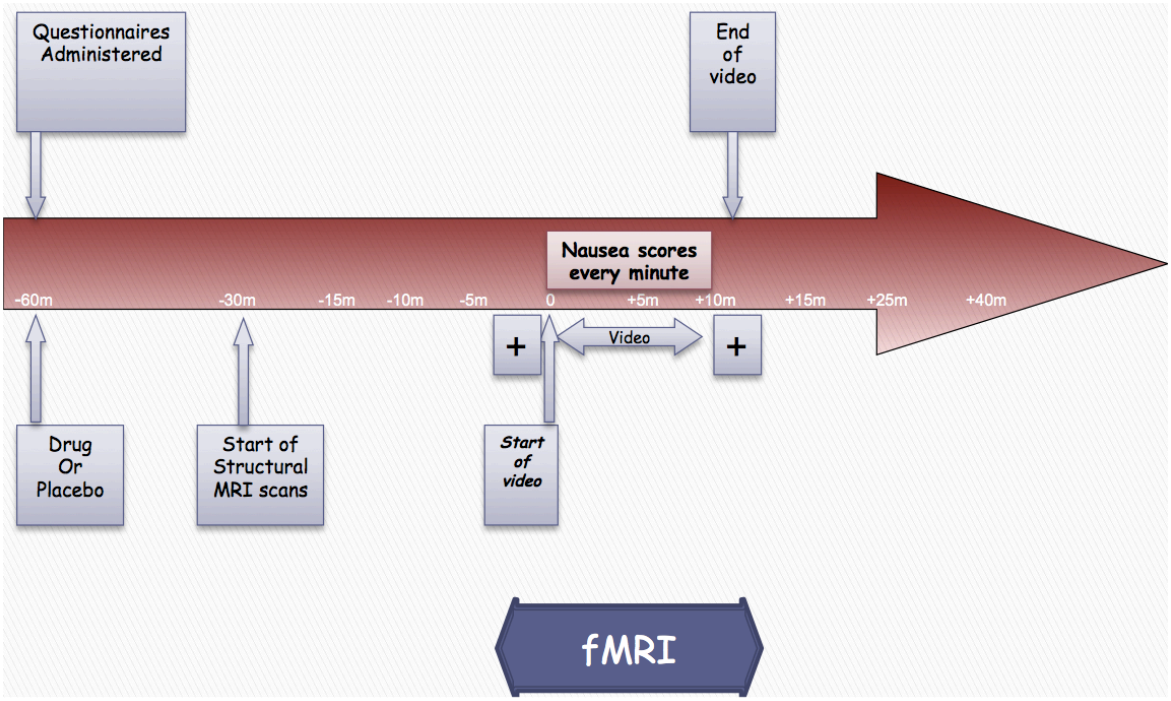

Figure 36. Schematic summary of the experimental protocol: After 6 hours of fasting subjects arrived for either drug or placebo administration and questionnaires which e.g. assess motion sickness symptoms and anxiety, reassessed just before starting the video after which minute to minute nausea reporting was determined and another MSAQ and STAI-S questionnaire done at the end of the video. This is essentially similar with chapter 4 protocol with the exception that drug or placebo was administered.

2.5 Distraction task (n-back)

As scopolamine causes significant drowsiness as a side effect (Spinks et al., 2004), a distraction task (letter version of *n*-back; (Ragland et al., 2002)) was used to assess subjects level of attention. The *n*-back has been used for experimental research in working memory [reviewed by (Jaeggi et al., 2010)]. A sequential presentation of letters putting a constant demand on attentional resources by requiring constant update and retrieval of information was administered to the subjects with three levels of difficulty: 1-back condition requires a response to any letter identical to the one before (i.e. one letter back); 2 and 3-back needs a response to any letter that is identical to the letter presented 2 or 3 letters back respectively (see

Figure 37). The response required is pressing a button connected to a PC that stores the response times and accuracy for post-hoc analysis.

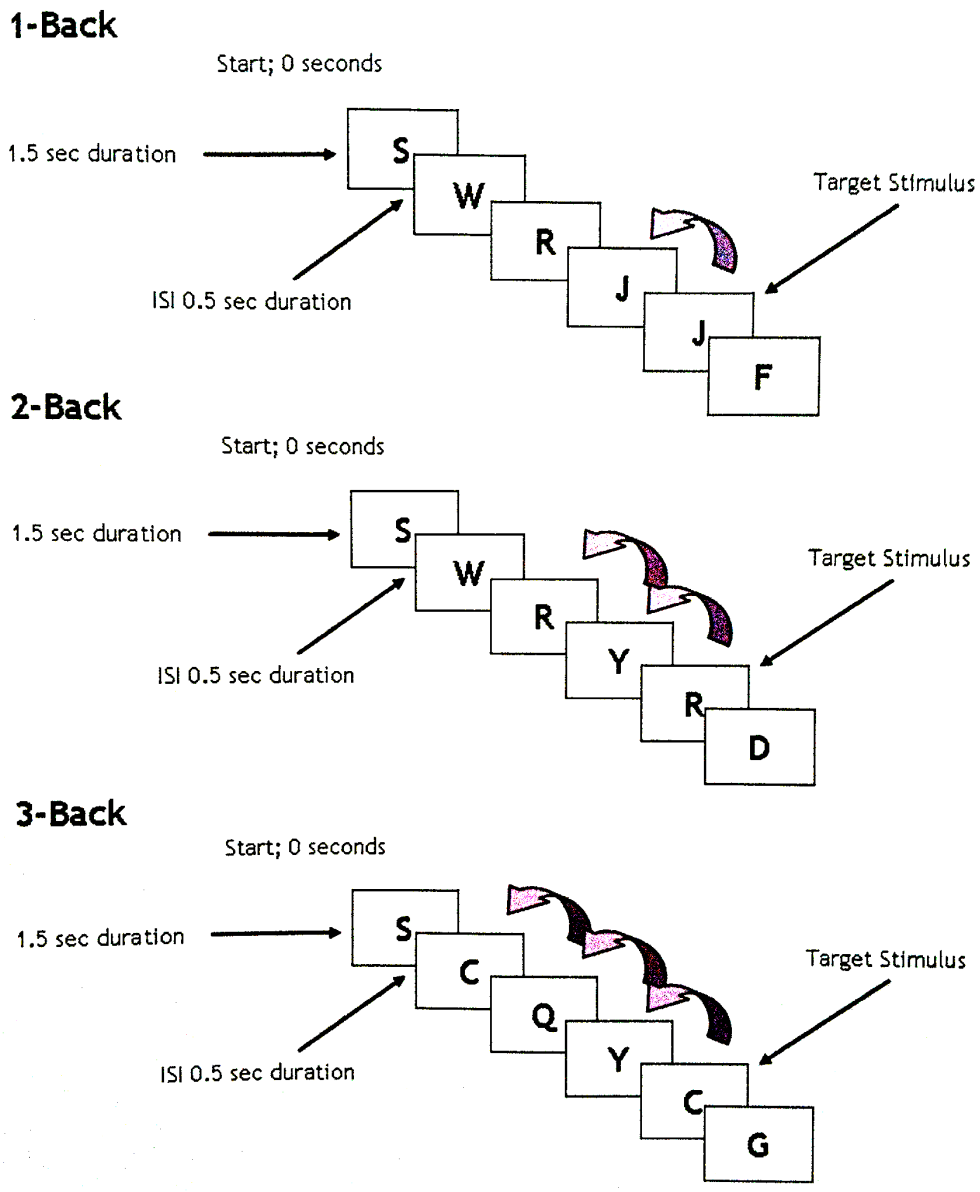

Figure 37: Pictorial representation of 1,2 and 3-back of the *n*-back tasks. Subjects are presented a sequence of letters and respond by pressing a button when they see a letter identical to 1, 2 or 3 letters before it respectively. (Adapted from (Coen et al., 2007).

2.6 Baseline activity

Subjects were encouraged to relax and focus on a target presented in the goggles for two and a half minutes before the video is started. This forms the baseline recordings.

2.7 Assessment of motion sickness susceptibility and anxiety levels

Subjects used a button box (four button box with first button for none and then mild, moderate and last button for severe) on their right hands to self-report nausea and anxiety scores before and at the end of each video as well as nausea scores every minute during the video. The ratings were reported using the same visual analogue scale used during study 1 explained above. Both MSAQ and STAI-S questionnaires were also administered just before and at the end of the videos. A two-way, random effects, average measures intra-class correlation (ICC) model for continuous variables were calculated between the chapter 3 and 4 visits for the 28 subjects (Green et al., 2012) to assess reproducibility of their nausea induction. Please refer Chapter 2 section 2.4.6 for more details.

2.8 Exposure to motion video stimulus

Subjects watched the motion video through a pair of goggles as in chapter 4 section 2.7. The goggles are positioned with rubber eyepieces to cover their eyes to limit their field of view to only the screen and delivered the stimulus using two LCD screens in front of each eye to create an illusion of a large screen in front of them.

2.9 Hyoscine hydrobromide administration for the prevention of motion sickness

The prevention and control of motion sickness symptoms includes pharmacological interventions, behavioural therapy and complementary medicine with varying success. This initial study of the pharmacological modulation of nausea pathways in the brain was approached with the safety and comfort of the subjects first and foremost as they are healthy human volunteers. Hyoscine bromide was chosen as it is licensed and widely used for oral prophylaxis of motion sickness and has low incidences of side effects compared with other agents with the pharmacokinetics and pharmacodynamics of scopolamine known and is currently the first line recommendation (Spinks et al., 2004). Thus Kwells Hyoscine Hydrobromide 300 microgram orally was administered 30 minutes before the start of the experiment (Liem-Moolenaar et al., 2011) in one visit with another visit a placebo was given in a double-blinded manner. Both were manufactured and the order of the drug and placebo randomised by the Royal Victoria Infirmary, Newcastle upon Tyne Hospitals NHS Foundation Trust.

2.10 Subjective monitoring

A visual-analogue-scale symptoms (VAS) questionnaire and motion sickness assessment questionnaire (MSAQ) described in chapter 2 section 2.4.7 documented any symptoms reported by the subjects. A visual scale from 1 to 4 was used with 1 being, without symptom, and 2 being mild symptom, 3 being moderate symptoms and 4 being maximum level of tolerated symptom. State anxiety status was assessed using the state version of the STAI.

2.11 Objective monitoring

The SCR was recorded on the subject's left hand continuously throughout the experiment. Cardiac pulse and respiratory effort data were monitored using a pulse oximeter (InVivo) and a respiratory effort transducer (BIOPAC), respectively. The pulse oximeter was placed on the subject's left index finger. The respiratory effort belt was placed around the subject's abdomen. The vital signs monitoring was performed similar to those described in chapter 2. Video was delivered through fMRI compatible goggles with an eye-tracker video to monitor subject's attention to the stimulus and pupil location (NordicNeuroLab GmbH VisualSystem, Norway).

The fMRI data (T2*-weighted images) was collected on a General Electric Signa Excite II 1.5 T HD scanner based at the Centre for Neuroimaging Sciences, Institute of

Psychiatry, King's College London. Head movement was restricted using foam padding within the head coil and an eye movement's tracker was mounted onto the head coil together with the in-built MRI compatible goggles delivering the stimulus. Prior to the start of the fMRI experiment, a high-resolution gradient echo structural scan (43x3mm slices, 0.3 interslice gap, TE 40ms, TR 3000ms, flip angle 90˚, matrix 128^2, in plane voxel dimensions 1.875x1.875) was acquired in each volunteer to be used for Talairach normalisation. During fMRI, a total of 300 T2* weighted images per slice (40x3mm slices, 0.3 interslice gap, TE 25ms, TR 3500ms, flip angle 90˚, matrix 64^2), depicting blood oxygen level dependant (BOLD) contrast were collected as subjects viewed the control and motion video.

2.14 Statistical analysis

XBAM version 4.1 (http://brainmap.co.uk/ referenced on the 8th of August 2012), a package developed at the Institute of Psychiatry, King's College London, was used to analyse fMRI data. It implements permutation-based nonparametric methods to minimise the number of assumptions used in making statistical inference (Brammer et al., 1997). After acquisition, fMRI data pre-processing, smoothing and individual brain activation mapping was performed (Coen et al., 2009). Analysis of covariance was performed on the effect size maps in Talairach and Tournoux's standard space (Talairach and Tournoux, 1988) with each voxel statistic corrected for the actual number of participants contributing to the calculation (Thirion et al., 2007). An analysis of covariance (ANCOVA) examining the main effect of several different groups on whole-

brain neural activity was done and a clusterwise p value of 0.01 (corrected for whole brain volume using permutation testing). Correlation analysis for brain activity to the level of nausea reported were analysed during the nausea video. Comparisons were also made between activity in the whole brain for all subjects between placebo versus scopolamine and during the motion video.

Psychometric, nausea questionnaires, and autonomic data were analysed using matched-pair t-tests and Wilcoxon tests to compare the means and medians. Pearson's and Spearman's correlations were used to determine the relationship between measurements. Independent-measures t-tests and Mann-Whitney tests were used to compare groups. Reproducibility of the studies used intra-class correlation comparison (ICC) and agreement was measured using two-way mixed average measure ICC model for continuous variables. Confidence intervals for the ICC were calculated according to the methods of Scheffe (Green et al., 2012). ICC were interpreted according to suggestions made by Yen et al (2002) as: - excellent (0.75-1), moderate (0.4-0.74) or poor (0-0.39) (Davis and Hallerberg, 2010). Commercially available statistics packages (SPSS, Chicago, IL, USA and GraphPad, San Diego, CA, USA) were used for the analysis. P values <0.05 were considered to be of statistical significance.

3 RESULTS

3.1 Response to videos during the fMRI study

All 16 subjects completed and tolerated the studies well. There was significantly more nausea after the video during the placebo visits. There were no other significant changes. The overall results are summarised in Table 16.

Five of the subjects responded to treatment with scopolamine (5 scopolamine modulated) and had less nausea during the motion video compared to after placebo. There was also a slight but not statistically significant lower heart rate and a deterioration of the 1-back task performed with higher response time (ms) and lower accuracy (% correct).

Six of the subjects appear to have developed more nausea during the drug visit compared to placebo visit. Meanwhile, 5 of the subjects did not have any response to the motion video. There was also a slight but not statistically significant lower heart rate and a deterioration of the 1-back task performed with higher response time (ms) and lower accuracy (% correct).

Variable	Baseline Median	Placebo	Hyoscine	Baseline vs. Placebo	Baseline vs. Hyoscine	Placebo vs. Hyoscine
Nausea scores (VAS)	1.21	1.93	1.35	p<0.05	p=0.35	p=0.16
Anxiety scores (STAI)	25.00	30.00	28.00	p=0.09	p=0.29	p=0.57
HR (bpm)	68.32	67.11	67.93	p=0.13	p=0.36	p=0.72
1-back response time (ms)	535.98	554.18	562.37	p=0.25	p=0.16	p=0.62
1-back accuracy (%)	93.28	92.21	91.89	p=0.19	p=0.08	p=0.33

Table 16 Medians of baseline, placebo and hyoscine visits and the statistical comparisons between them (Wilcoxon test).

3.2 Brain activity in placebo versus scopolamine for all subjects

All subjects demonstrated an increase in activity in the left occipital lobe and lingual gyrus after placebo compared to scopolamine (Table 17).

Cerebral Region	3D Cluster size	Peak Talairach Coordinates (x, y, z)	Probability
Left Occipital Lingual	192	-14.44 -77.78 -6.60	0.0007

Table 17. Brain Activity in Placebo versus Scopolamine during Motion Video for All Subjects There was increased activity in these brain areas during motion video after placebo versus scopolamine in all 16 subjects.

3.3 Brain activity in placebo versus scopolamine for the five scopolamine modulated subjects

All subjects demonstrated an increase in activity in the left occipital lobe, cuneus (Table 19); and a decrease in activity in the left occipital lobe, middle occipital gyrus while watching the motion video (Table 19)

Cerebral Region	3D Cluster size	Peak Talairach Coordinates (x, y, z)	Probability
Left Occipital Cuneus	41	-10.83 -81.48 3.30	0.0035

Table 18. Brain Activity for Scopolamine Modulated Subjects during the Motion Video in Placebo versus Scopolamine Administration. There was increased activity in these brain areas during motion video after placebo versus scopolamine in all 5 subjects.

Cerebral Region	3D Cluster size	Peak Talairach Coordinates (x, y, z)	Probability
Left Middle Occipital Gyrus	85	-43.33 -85.19 9.90	0.0022

Table 19. Brain Activity for Scopolamine Modulated Subjects during the Motion Video in Placebo versus Scopolamine Administration. There was decreased activity in these brain areas during motion video after placebo versus scopolamine in all 5 subjects.

4 DISCUSSION

4.1 Varying responses from 16 healthy volunteers to scopolamine

Five of the sixteen subjects responded to treatment with scopolamine (5 scopolamine modulated) and had less nausea during the motion video compared to after placebo. The rest of the subjects did not. This is consistent with the variable scopolamine efficacy. For instance (Spinks et al., 2004) showed meta-analysis of transdermal scopolamine studies having a relative risk to develop nausea of 0.48 (95% confidence interval (CI) 0.32 to 0.73) however oral scopolamine studies were not meta-analysed due to too widely varying differences between studies (e.g. different sample sizes, different dose of oral scopolamine).

Six subjects developed more nausea during the drug visit compared to placebo visit that may be due to factors like scanner anxiety (2 subjects reported scanner anxiety during their drug visit and that may be potentially avoided in the future with an additional mock scan (Lueken et al., 2012). Another 2 subjects had excessive drowsiness due to lack of sleep night before. One of the subjects scored the Weinberger test as possibly lying and thus it would be best to exclude that person. A possible explanation is that these subjects were not sensitive to scopolamine as all subjects did not report any classical scopolamine side effects e.g. dry mouth, increase in heart rate after drug, or deterioration in n-Back task and this is consistent with previous studies (Spinks et al., 2004). In terms of adequate dosing of scopolamine, previous studies have shown with

doses of 300 and 600 micrograms that both doses were adequate for prevention of motion sickness with more side effects seen in 600 and increasing side effects noted at 900 micrograms (Renner et al., 2005, Ebert et al., 2000). Thus the recommended 300 microgram was aimed for with the stimulus given when scopolamine should peak about an hour after dose (ibid). The dose chosen was both for safety as well as to prevent confounding factors of side effects and subject discomfort and yet have adequate treatment.

Meanwhile, 5 of the subjects had mostly mild and up to moderate response to the motion video. This may be due to habituation as they were involved in the previous studies as well (Dai et al., 2011, Bos et al., 2010). All possible precaution was taken to prevent this with the initial visits taking place 6 months before the 1st MRI visits and then in between visits another week. Unfortunately in this case all the subjects who had been exposed to the stimulus before appear to have habituated to the stimulus in the MRI even though they still reported moderate to severe nausea during the screening study 2 weeks before the MRI visit with the video projected upon a large screen.

This was a pilot study and the results herein discussed must be viewed with the knowledge that when analysing all the subjects together they are a heterogenous group and also it is likely to be underpowered when looking at the scopolamine-modulated subjects' sample size of five. But this is the first visually induced motion sickness nausea study with scopolamine designed specifically to modulate the cortical pathways involved in the brain processing of visual motion induced nausea and it may provide us

with a preliminary glimpse into looking at the nausea pathways modulated by scopolamine.

4.2 Developing a safe and reliable stimulus for studying nausea

All the subjects completed all studies and did not have any retching or vomiting. Furthermore, none of the subjects reported any side effects after scopolamine and thus we have likely erred on the side of caution here. Thus it was a safe study.

However, there was no clear scopolamine modulation of the stimulus due to the wide individual variations to scopolamine response. It is possible that an additional visit to determine if these volunteers can show scopolamine modulation of their responses to the video might have proved useful.

Nausea was still induced in 69% of the subjects however subjects reported poor ICC to nausea and anxiety scores (intra-class correlation coefficiency of 0.23). This is likely due to the smaller field of view (Bos et al., 2010) and the possibility of reduced anticipation of nausea as they are being told they might or might not get a drug to reduce their nausea before the study (Morrow et al., 2002b).

In terms of the fMRI data, when comparing the scopolamine versus placebo in all 16 subjects there was decreased occipital lingual activity with no regions of significantly increased activity. However when only the 5 scopolamine modulated subjects were analysed together comparing scopolamine versus placebo, there was significant increase in the occipital cuneus and a decrease in the middle occipital gyrus activity.

These visual pathway regions are likely involved in processing the visual stimuli. This is consistent with previous studies of apparent self-motion using translating (Napadow et al., 2012b, Brandt et al., 1998) and stationary (Riedel et al., 2005) visual stimuli suggesting that vection is mediated by medial temporal gyrus and parieto-occipital areas that are part of the visual pathway.

Future studies will need to preselect subjects who are susceptible to the nausea stimulus and then study if these subjects are scopolamine sensitive before proceeding to the fMRI study.

4.3 Prefrontal cortices modulation by scopolamine

Chapter 4 studies showed that the inferior frontal gyrus was positively correlated with increasing nausea. The same correlation was not seen and neither was there a negative correlation after placebo for all subjects. Thus the chapter 5 subjects may not be comparable with the previous study, as the same activation was not seen during placebo visit. The placebo visit in chapter 5 should arguably be similar to the motion video stimulation in chapter 4 studies except that their anticipation may be altered due to taking the drug or placebo. Thus it was to no surprise that there were no significant correlations seen for all subjects during the scopolamine visit.

When looking at the single group level activity, all subjects after placebo showed significant prefrontal cortex activation in the superior frontal gyrus. This is consistent with the chapter 4 findings of increased prefrontal cortex activity with motion video. When all the subjects were investigated after scopolamine, this activation had been reversed with significant inhibition of bilateral inferior frontal gyri and left middle frontal gyrus. Thus, although the between groups of placebo versus scopolamine comparisons did not show a significant relationship, the more basic group level activations may possibly be moving along the right direction as was expected. This is taken with the naïve and likely wrong assumption that the prefrontal cortices can be grouped together.

Further analysis however did show possible supporting evidence for the inferior frontal gyrus in the genesis of nausea. After scopolamine administration all the subjects as a group had significantly reduced bilateral inferior frontal gyrus activity. When placebo was compared with scopolamine brain activity, there was also a trend towards the bilateral inferior frontal gyrus becoming less active (post-hoc reanalysis of 5 subjects modulated by scopolamine with inferior frontal gyrus significantly inhibited bilaterally after lowering Bonferonni correction with cluster p value at $p=0.05$; Left and right cluster size 43 & 37; peak talairach coordinates x,y,z are -36.11, 25.93, -9.90 & 25.28, 14.81, -9.90; $p<0.02$ & $p<0.03$).

4.3 Limbic cortex

It was also observed in the chapter 4 studies that the limbic parahippocampus and hippocampus was inhibited with increasing nausea. The same correlation was not seen and neither was there a positive correlation after placebo for all subjects. And again there were no significant correlations seen for all subjects during the scopolamine visit. There were also no significant changes when comparing placebo versus scopolamine.

It was also only with further post-hoc analysis that we were able to observe that there was a trend of increased left parahippocampus activity after scopolamine administration versus placebo in the 5 scopolamine modulated subjects with a trend of decreased right parahippocampus activity.

4.4 Limitation and future studies

It is a limitation of this study that the mild to moderate visual motion induced nausea stimulus was habituated to by about a third of our subjects. And it is also arguable that the mild to moderate nausea induced in these subjects may not have been strong enough to show a reversal with an intervention. Thus it is possibly necessary at this juncture to discuss what are the other options for a nausea stimulus we may consider as the other stimuli may also be considered for future studies in fMRI to ascertain if the visual motion induced nausea pathway is generalizable to other pathway(s) of nausea.

Ideally a stimulus induces nausea alone is used to investigate the central nervous system pathways of nausea.

When considering an ingested or an injected agent, its appropriate dose selection is critical. This relies on the availability of detailed dose-response information in humans. A fast acting agent like apomorphine where onset of nausea and vomiting may be within minutes it may be difficult to separate the pathways involved in the two events. Other alternatives include ingested agents like syrup of ipecac (Minton et al., 1993), (-) tryptophan (Greenwood et al., 1975), L-DOPA (Davis et al., 1986), and the partial 5-HT_{1A} receptor agonist buspirone, that potentiates morphine induced nausea (Oertel et al., 2007).

Another possibility would be avoiding the agonist drugs and consider antagonists. They may need concurrent administration of an emetic stimulus since some work by reducing the threshold for vomiting although some can induce nausea when given alone. An example would be the opioid antagonist naloxone (Kobrinsky et al., 1988) and the CB_1 receptor antagonist Rimonabant (Pi-Sunyer et al., 2006) that induce nausea as a dose-related side effect.

A major factor to consider is the risk of vomiting. There is a real danger of aspiration if the subject is supine in a scanner with head restrains as it may not be possible to remove the subject rapidly other than vomiting being a potentially confounding factor (Ladabaum et al., 2001). The scanner may also be contaminated by aerosolized vomit containing infectious agents. These issues may be resolved by technical developments in scanner design with vertical more open designs however there is no known timeline when they may come out of development.

Alternatively a well studied, easily controlled, discrete experimental stimulus for the induction of nausea and its associated gastrointestinal motor changes would be caloric or galvanic vestibular stimulation (Brandt and Strupp, 2005, Wolf, 1965). It would still be necessary to compare these results with other stimuli activating the area postrema and/or the abdominal vagal afferents.

Last but not least, there is evidence that a number of observations in crude early electrical stimulation studies may be worth pursuing with more sophisticated techniques presently available. Brief looks at these studies that must be interpreted with caution, are for example stimulation of the extreme lateral portion of the primary somatosensory cortex (S1) provokes nausea and a sick-feeling (Penfield and Rasmussen, 1950). Meanwhile, anterior cingulate cortex stimulation induced nausea, vomiting, and epigastric awareness (Devinsky et al., 1995). In addition, stimulation of the frontal lobe in either cerebral hemisphere (Sem-Jacobsen, 1968) evoked “Nausea I” — where the subject reported nausea that was followed by sudden vomiting and immediate recovery; and “Nausea II” — where the subject reported more intense nausea with perspiration and increased breathing rate and depth. Thus, more developed techniques such as deep brain stimulation or the non-invasive transcranial electromagnetic stimulation could potentially confirm the involvement in nausea of sites identified by imaging studies.

As discussed above, although other modalities of studying nausea are available, each of them have their own difficulties none currently are able to produce a state of sustained nausea with a minimal risk of vomiting.

CHAPTER 6

CONCLUDING DISCUSSION

1 The human model of nausea

There is a need for using functional brain imaging in humans to obtain an insight into brain processing of nausea. This understanding will in future help to develop quantitative means for assessing anti-emetic efficacy. With few functional brain imaging studies focused on nausea genesis, our knowledge is mainly based on animal studies that come primarily from the rat (lacking an ability to vomit), cat, dog, ferret and nonhuman primates but there are substantial differences in cerebral cortical anatomy between species e.g. the primates, cetaceans, and other mammals (Craig, 2009b, Craig, 2009a, Craig, 2002, Dunbar and Shultz, 2007, Marino, 2007, Butler et al., 1996). There is some indirect information for nausea genesis from human studies where nausea was an associated or incidental finding however these studies are difficult to interpret as other symptoms such as acute pain act as confounding factors.

The human studies specifically studying nausea genesis in the brain include a 1996 magnetic source imaging study whereby one subject underwent yaw-axis rotations with side-to-side head movements and ipecac ingestion which led to the inferior frontal gyrus being activated (Miller et al., 1996). This activation was reversed when the same subject was administered the anti-emetic drug ondansetron, which is a 5-HT3 receptor antagonist. These results supported an older 1993 electro-encephalography study which demonstrated increased activity in the temporo-frontal region during motion sickness (Chelen et al., 1993). More recently, an fMRI study of visual motion induced nausea on 28 women discovered that there were also activation of the dorsolateral

prefrontal cortices bilaterally and in addition a broader network involving the interoceptive, limbic, somatosensory brain regions were also stimulated (Napadow et al., 2012b). Activation of the insula and cingulate cortices have also been shown to play an important role in animal and other related human studies (Stern et al., 2011).

Previous studies have also shown there are important associated psychophysiological changes (e.g. anxiety), cardiac autonomic, gastric and hormonal activity during visual motion induced nausea and it is important to preselect the subjects who demonstrate these changes to ensure they are actually experiencing nausea as self-reporting is prone to bias (Stern et al., 2011).

In short, as nausea increases in an individual they will demonstrate increasing levels of anxiety; sympathetic arousal and parasympathetic withdrawal; shift from normal gastric activity to abnormal activity that is predominantly tachygastric; and also an increase in vasopressin and cortisol (Figure 38).

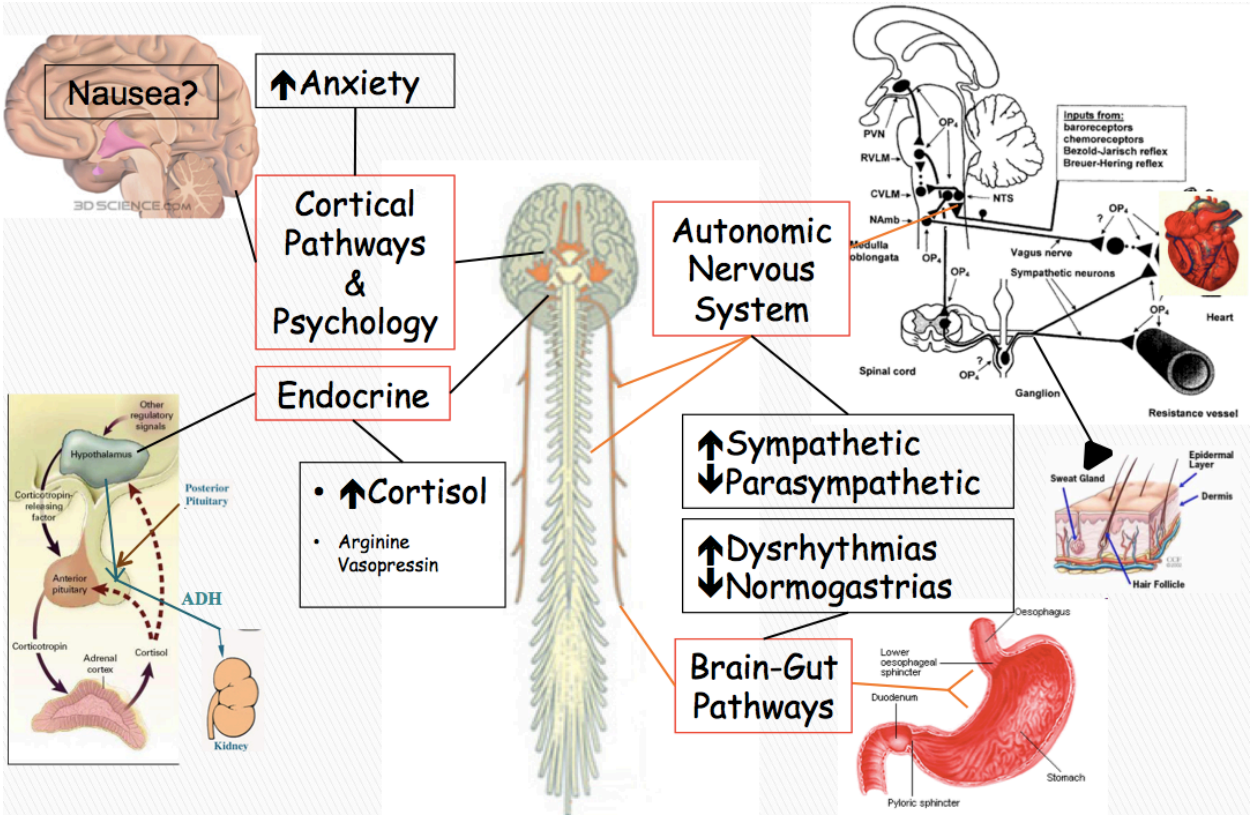

Figure 38 Changes associated with visual motion induced nausea. There is increased anxiety; sympathetic arousal and parasympathetic withdrawal; shift from normal gastric activity to abnormal gastric activity that is predominantly tachygastric; and an increase in vasopressin and cortisol.

It is thus recommended that a human model of nausea investigated by functional magnetic resonance imaging after carefully selecting a safe stimulus and the right subjects may shed light upon the brain processing of the poorly understood “personal experience” of nausea (Kowalski et al., 2006).

2 Developing a human model of nausea

2.1 Developing a safe and reliable stimulus for studying nausea

The first and most important criterion for a suitable stimulus would be safety as we are conducting non-therapeutic clinical research on human subjects according to the Declaration of Helsinki (Williams, 2008). This criterion was met in our studies with the stimulus inducing nausea in approximately half of the subjects and with the majority of them only reporting mild to moderate levels of nausea that resolved within 5 minutes of stopping the stimulus. In addition, even those subjects who experienced severe nausea felt safe enough and in control of the situation that they were able to complete all the studies as they were able to close their eyes whenever the stimulus proved to be too much. This prevented any retching or vomiting in our subjects for all the studies. Furthermore, none of the subjects reported any lasting effects after the studies when questioned up to a year after the studies. In fact, we were able to re-recruit 5 of the subjects for all three of the main phases of the study and 30 subjects for two main phases of the study.

The visual motion induced nausea model in humans appears to be an effective and reproducible model with the caveat that habituation to the stimulus will occur at some point if the same subjects are repeatedly exposed to the stimulus (in our study it happened after the stimulus was presented for the 5^{th} time) as reported in the literature (Bos et al., 2010). This model is also able to provoke the classical changes associated with nausea in the subjects who are susceptible more than the resistant subjects that

will help with subject selection for further fMRI studies but there are other considerations we should be mindful of that are discussed below.

Visual motion induced nausea has the weakness (Muth et al., 1996, Gianaros et al., 2001) that multiple sensation can be evoked that are described as central, peripheral, and sopite sensations (Gianaros et al., 2001). The sensation of nausea in our study was also moderately correlated with Spielberger state anxiety inventory ($r=0.63$, $p<0.05$) but this has been reported previously and expected as nausea causes general discomfort and is stressful (Burish and Carey, 1986, Haug et al., 2002). However, the psychophysiological responses to the nausea stimulus seen in our study are likely to be specific to the nausea induced because of only weak to moderate correlations were seen between the nausea scores and headache or STAI state anxiety scores. There were poor correlations between the nausea induced in our study with the STAI trait scores, other related motion sickness sensations, and neuroticism score.

The motion video stimulus was also chosen for its safety profile and may not be generalizable for other nausea inducing stimuli although there is some evidence to suggest that susceptibility to nausea from motion sickness may potentially predict susceptibility to nausea in chemotherapy patients and physically-induced motion sickness (Golding, 2006). In terms of identifying a nausea stimulus that is adaptable for brain imaging, the visual motion induced nausea described in this studies is one of the most suitable stimulus currently available and it allows identification of subjects that are either susceptible and resistant (Stern et al., 2011). It would be advantageous for future

studies to explore the other possible stimuli for nausea induction and also to study nausea in patients with pre-existing medical condition or various drugs, however this may be fraught with difficulties as such stimuli would be difficult to predict and control and therefore may be unsafe in the brain imaging environment.

2.2 Subjects selection for a fMRI human model of nausea

Nausea is a “personal experience” with large variations between individuals (Stern et al., 2011). Thus, to ascertain that an individual is actually feeling nauseous it is necessary to be able to associate the nausea reports with objective psychophysiological changes.

As the experience of nausea may be different in each individual, it would be ideal to remove these possible biases by using the same individual as their own controls. Furthermore, the individual variations to the responses of visual motion induced nausea can help us identify those who are more susceptible and those who are more resistant and the comparisons between the two may uncover important differences in how they process the stimulus that may explain the reasons for the differences observed.

As seen in chapter 3, there is a widely spread variation in responses to the stimulus used in these studies but it was possible to identify 28 subjects who were susceptible (reporting moderate to severe nausea), 42 subjects who were resistant (with no nausea reported) and 28 subjects who were intermediate. The susceptible subjects showed the

classical changes associated with nausea with regards to the cardiac autonomic and gastric activity while the resistant subjects did not. These changes were not seen in the susceptible subjects during control video. Thus, it was possible to show that this model can be used to preselect suitable individuals before the study to allow for the best possible outcome during the fMRI studies.

It is important to bear in mind that these volunteers may be self-selecting either because they know that they are relatively immune to the stimulus, or conversely because they are curious about the fact that they may be susceptible to the stimulus, or even because they consider it a sign of machismo (Stern et al., 2011). On top of that, the extent to which the decision to participate in the study is influenced by financial or other reward is not known. However, these issues are similar to those involved in recruiting humans for studies of pain (Langley et al., 2008).

2.3 Adapting the stimulus for a fMRI human model of nausea

I have demonstrated that the virtual reality stimulus can be adapted to the fMRI investigative environment with the pre-existing infrastructure without the need for complicated or expensive modifications to the MRI infrastructure (Napadow et al., 2012a, Kowalski et al., 2006). Nausea is still effectively induced and the susceptible and resistant subjects still reported similar levels of nausea and anxiety (intra-class correlation coefficiency of moderate to good reproducibility) although less with MRI goggles video compared to the projected video in Chapter 3 due to a larger field of view

in projected videos (Bos et al., 2010). As these subjects have had their subjective reports of nausea validated by objective cardiac sympathetic arousal, parasympathetic withdrawal, shift of normal to dysrhythmic gastric myoelectrical activity, increased cortisol and increase state anxiety (refer Chapter 3), we are making the assumption that they are experiencing the same psychophysiological changes as described in chapter 3.

It is also important in developing fMRI models that we design a control task (Iannetti and Wise, 2007) and the static cityscape without any motion used in our study appears to be a good control as this task still requires the subjects to perform all the usual visual tasks but without the development of nausea. The activation of visual areas of the brain in both motion and control video groups confirms that the two tasks were well matched for activation of the visual pathways.

More detailed investigation using non-visual/vestibular stimuli to evoke nausea will be needed to determine if the brain processing of nausea is similar with different input pathways or if there are important differences that may potentially be important when thinking about treating the widely varying causes of nausea for the clinician. Investigating disease states associated with nausea may be a potential option such as cyclic vomiting syndrome (Olden and Chepyala, 2008) and migraine (Cuomo-Granston and Drummond, 2010).

In this study, areas of the brainstem like the brainstem nuclei or the NTS that are postulated to play a key role in nausea pathways were not seen, however these medullary nuclei are at the limit of fMRI spatial resolution and are also susceptible to

cardiorespiratory artifacts. In future fMRI studies specifically targeting the brainstem using 3 Tesla MRIs for better spatial resolution or possibly complementary studies like high resolution research tomography (HRRT) that was recently shown to be able to quantify the serotonin transporter availability in the brainstem may be helpful in developing a better understanding of brain stem processing of nausea (Schain et al., 2012).

My study in chapter 5 demonstrated that the central effects of scopolamine may be investigated using the model of visual motion induced nausea. Unfortunately, the study also shows that subject preselection is likely necessary with the wide individual variation in the responses to scopolamine (Spinks et al., 2004). Thus future studies will need to preselect subjects who are susceptible to the nausea stimulus and subsequently preselect those who show scopolamine induced modulation of nausea and associated psychophysiological responses before studying them in the fMRI environment.

In summary, these studies have advanced the development of a fMRI human model of nausea in several aspects, particularly with respects to a more versatile and easily adaptable stimulus and better subject preselection.

3 Mechanisms involved in visual motion induced nausea

3.1 Prefrontal cortices

These studies show that the inferior frontal gyrus was positively correlated with nausea and this activation may potentially be reversed with scopolamine. After scopolamine administration all the subjects as a group had significantly reduced bilateral inferior frontal gyrus activity. When placebo was compared with scopolamine brain activity, there was also a trend towards the bilateral inferior frontal gyrus becoming less active (post-hoc reanalysis of 5 subjects modulated by scopolamine with inferior frontal gyrus significantly inhibited bilaterally after lowering Bonferonni correction with cluster p value at p=0.05; Left and right cluster size 43 & 37; peak talairach coordinates x,y,z are -36.11, 25.93, -9.90 & 25.28, 14.81, -9.90; $p<0.02$ & $p<0.03$). This appears to suggest that the inferior frontal gyrus plays an important role in the nausea pathway consistent with the previous studies (Napadow et al., 2012b, Bense et al., 2001, Fasold et al., 2002, Denuelle et al., 2007, Miller et al., 1996). The prefrontal cortices may also play a role in spatial processing that may be important in visual motion induced nausea as a human study using a virtual reality version of the Morris Water Maze (well established spatial test in animals) showed there was medial and middle frontal gyrus activation during scopolamine with no inferior frontal gyrus activation seen (Antonova et al., 2011a). This warrants further studies with more advanced technology for example the electro-encephalography with 3 Tesla or higher fMRI that has better temporal and spatial resolution (Iannetti and Wise, 2007).

3.2 Parahippocampus

When comparing the brain activity between the motion video and control video for all subjects the left parahippocampus was inhibited in the motion video. Furthermore, the right parahippocampus activity was also decreased when comparing nausea susceptible and resistant subjects during the motion video. In addition, right parahippocampus activity decreased as nausea levels increased in susceptible subjects. Conversely, the left parahippocampus was activated in resistant subjects as a group during the motion video. There was also a trend of increased left parahippocampus activity after scopolamine administration versus after placebo in the 5 scopolamine modulated subjects with a trend of decreased right parahippocampus activity. Thus it appears that the parahippocampus may play a role in the nausea pathway with scopolamine appearing to possibly modulate it however further work is needed to confirm this.

It is possible to speculate that the parahippocampus is a pivotal area involved in the processing of the visual motion induced nausea in our subjects such that its inhibition signifies the disorientation induced by the stimulus leading to the sensory conflict (Reason and Brand, 1975) postulated to generate nausea. Interestingly the nausea resistant individuals as a group increased left parahippocampal activity that may confer some protection against disorientation that may be involved in the generation of nausea.

In this study, the hippocampus (left hippocampus, cluster size 3484, peak talairach coordinates (x=-28.89, y=-18.52, z=-9.90), $p<0.0012$) was also inhibited in all subjects during the motion video.

There is evidence from both human (Aguirre et al., 1996, Epstein and Kanwisher, 1998, Ishai et al., 1999) and animal (Haley et al., 2011) studies suggesting that the parahippocampus and hippocampus play a role in orientation and navigation; and clinical lesions of the hippocampus and parahippocampus present with disorientation (Habib and Sirigu, 1987, Hublet and Demeurisse, 1992). There is also evidence that muscarinic receptors are present in high concentrations in the prefrontal cortex (PFC) and the hippocampus (Nathanson, 2008) and have an integral role in spatial learning and memory in rodents, nonhuman primates, and humans (Wisman et al., 2008, Gage et al., 1988, Fredrickson et al., 2008, Thomas et al., 2008). And scopolamine has been shown to modulate the hippocampus/parahippocampal gyrus and disrupt spatial orientation memory in the human brain by fMRI (Antonova et al., 2011b).

Visual motion induced nausea starts from vection generating disorientation that develops into nausea and both worsen progressively with increasing exposure to the stimulus. My studies may possibly provide the first objective evidence of the brain pathways involved in the universally acknowledged and accepted sensory conflict theory initially proposed by (Reason and Brand, 1975) with the parahippocampus appearing to have a protective role against the development of visual motion induced nausea.

I have adapted a diagram with the proposed pathways of nausea generation to include the additional pathways in this study on the right with the possible interactions in red arrows (Figure 39).

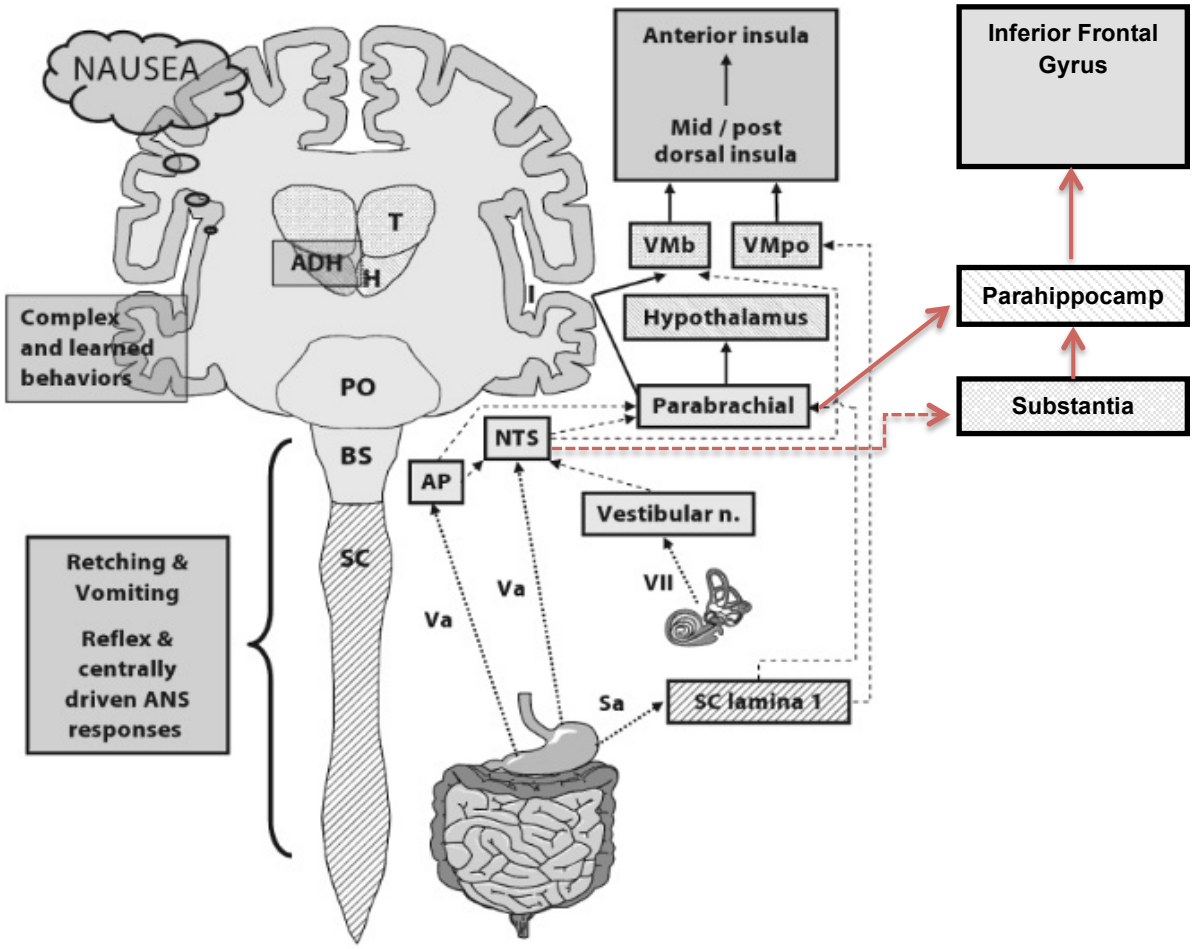

Figure 39. Adapted diagram (Stern et al., 2011) summarizing major pathways involved in the sensation of nausea. The pathways shown combine Craig's (2002) primate pathways involved in the processing of abdominal vagal afferent information; and projections of the area postrema and vestibular system (Loewy and Spyer, 1990, Yates et al., 1998, Saper, 2002) thus providing a pathway by which nausea could be induced by their activation. It also highlights the hierarchical information processing by shading brain structures with specific structures indicated with a dotted line (...). Second order projections with a dashed line (---) and higher order projections with a solid (__) line. The boxes on the right side indicate the additional pathways suggested by this study with the possible interactions in red arrows. Abbreviations: ANS-Autonomic Nervous System; AP-Area Postrema; BS-Brain Stem; H-Hypothalamus (particularly Posterior hypothalamus, supraoptic and paraventricular nuclei); I-Insular region of the Cerebral Cortex; NTS-Nucleus Tractus Solitarius; PO- Pons; Sa – Greater Splanchnic Nerve Afferent Fibres; SC-Spinal Cord; T-Thalamus; Va-Abdominal Vagal Afferent Nerves; Vestibular n.-Vestibular Nerve Nucleus; VII-Vestibular Nerve; VMb-The basal region of the ventromedial thalamic nucleus; Vmpo-The posterior region of the ventromedial nucleus of the thalamus.

In summary, further studies with other types of nauseogenic stimuli and other pharmacological agents are now warranted to explore further the role of the prefrontal cortices and the parahippocampus in the genesis of nausea. New technologies with better temporal resolution like electro-encephalography with fMRI may possibly provide more answers.

4 Conclusion

Understanding the pathways involved in the genesis of visual motion induced nausea is clearly relevant to the identification of new treatments for patients suffering from motion induced nausea and it may also provide an important tool for quantitative pharmacological studies in the future. There are also more general implications as motion induced nausea susceptibility have been shown to predict susceptibility to post-chemotherapy nausea. Thus, a better understanding of all pathways involved in visual motion induced nausea may provide a basis to recognise the mechanisms and treat successfully conditions in which nausea is induced through unknown or incompletely understood mechanisms.

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