Cerebral mechanisms of breathlessness and its relief

by

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‘***’And the Lord God formed man of the dust of the ground, and breathed into his nostrils the breath of life; and man became a living soul’’- Gen.2:7- KJV***

DEDICATION

To God be the glory

To my beloved and ever supportive Wife Sylvia Debrah for your painful sacrifices and my cheer leading daughters; Jessica Akua Debrah and Mina Yaa Debrah

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ABSTRACT

The perception of clinical breathlessness is a complex experience, modulated by physical and psychological factors. Distinguishable types of breathlessness arising from different peripheral mechanisms have been delineated, experimental models of these specific types have been developed and advances have been made in multidimensional assessments. However, current knowledge about cerebral mechanisms of breathlessness is lacking. Functional brain imaging studies have identified involvement of certain cortical structures but interpretations of these data are limited by various assumptions.

Aim: The primary purpose of this thesis was to interrogate the validity of the conclusions drawn by recent brain imaging studies and to draw further inferences about cerebral mechanisms of dyspnoea from neurological patients undergoing deep brain stimulation (DBS) for relief of other symptoms. Focusing on experimentally induced 'air hunger' (the most unpleasant form of clinical breathlessness) two primary working hypotheses were generated: (i) ***Perceptual sensitivity to experimentally induced breathlessness will be greater during MRI brain scans due to greater anxiety associated with the MRI milieu*** (ii) ***DBS of motor-thalamic nuclei generates breathlessness***

Method:(i) Experimental breathlessness was induced in healthy subjects in simulated brain scanners to measure the effects of psychological stress associated with the functional magnetic resonance imaging (fMRI) scanner. (ii) Experimental breathlessness was induced in patients with DBS of the ventral intermediate (VIM) and ventralis oralisposterior (VOP) nuclei of the thalamus when the implanted electrodes are activated(switched 'ON' to stimulate the brain tissue) and when they are not activated (switched 'OFF' to stop stimulating the brain tissue).The Dyspnoea-12 multidimensional tool was also used to assess breathlessness. (iii)Probabilistic tractography was used to estimate the white fibre

connectivity from the seed area (VIM) in the brain to brain regions of interest and to compare these in patients who responded differently in (ii).

Results: (i) Healthy volunteers with anxiety scores at or above average levels increased air hunger sensitivity in the simulated fMRI environment whereas those with low anxiety responded in the opposite direction. (ii) Contrary to the hypothesis posed, motorThalamic DBS resulted in relief of air hunger in all but one of the 6 patients studied. (iii) Tractography suggested that those patients who improved with DBS 'ON' had stronger connections to the anterior parts of the brain (orbitofrontal cortex) in contrast to the stronger connectivity to the posterior areas of the brain in the one patient who improved with DBS 'OFF'.

Conclusion :(i) Future functional brain imaging studies of breathlessness should screen healthy volunteers for anxiety in order to improve interpretation of activations detected. (ii) Neurological patients undergoing DBS provide a useful clinical model to explore cerebral mechanisms of breathlessness. While the dataare largely inconclusive due to limited numbers of patients, the orbitofrontal connections for dyspnoea relief has been flagged as being an important element of a cerebral network for dyspnoea. Potential new thalamic targets for dyspnoea relief are suggested. The novel findings of this study lay the groundwork for better understanding of cerebral mechanisms of dyspnoea and therefore potential new and more effective targets for relief of intractable dyspnoea – a goal that remains an urgent clinical need.

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ABBREVIATIONS

ACC:	Anterior cingulate cortex
AH:	Air hunger
BP:	Blood pressure
COPD:	Chronic obstructive pulmonary diseases
DLPFC:	Dorsolateral prefrontal cortex
D: 12:	Dyspnoea: 12
DBS:	Deep brain stimulation
DT:	Dystonic tremor
DTI:	Diffusion tensor imaging
ECG:	Electrocardiogram
EEG:	Electroencephalography
ET:	Essential tremor
rfMRI:	Resting functional magnetic resonance imaging
FR:	Frequency rate
FVC:	Forced Vital capacity
FEV:	Forced expiratory volume
fMRI:	Functional magnetic resonance imaging
FMRIB:	Functional magnetic resonance imaging of the brain
FSL:	FMRIB software library
GPi:	Globus pallidus interna
JHU	John Hoopkins University
LFP:	Local field potential
MDP:	Multidimensional dyspnoea profile
MEG:	Meganoencephagraph

MRI:	Magnetic resonance imaging
MBS:	Modified Borg Scale
NRS:	Numerical Rating Scale
PAG:	Periaqueductal grey
PET:	Positron emission topography
PEF:	Peak expiratory flow
PAW:	Airways pressure
PD:	Parkinson's disease
$P_{ET}CO_2$:	End-tidal carbon dioxide partial pressure
$P_{ET}O_2$:	End-tidal oxygen partial pressure
PVG:	Periventricular grey
rTMS:	Repetitive transcranial magnetic stimulation
STAI:	State: trait anxiety inventory
SMA:	Supplementary motor area
SpO_2:	Oxygen saturation
STN:	Subthalamic nucleus
TE:	Echo time
TMS:	Transcranial magnetic stimulation
VAS:	Visual analogue scale
VAT:	Volume of activation
VIM:	Ventral intermediate nucleus
VOP:	Ventralis oralis posterior
VPL:	Ventral posterolateral nucleus
VT:	Tidal volume
W/E:	Work and effort

CONTENTS

CHAPTER 1

Introduction

1.1 OVERVIEW OF BREATHLESSNESS ("DYSPNOEA")

The experience of unpleasant awareness of breathing discomfort termed clinically as breathlessness or dyspnoea can be described as unyielding and distressing (Bruera *et al*, 2000; Desbiens *et al*, 1999). The normal occurrence of breathlessness is physiologically important as a function of maintaining the homeostatic state of the essential gases such as oxygen and carbon dioxide in the body despite the constant change in respiratory mechanics. However, breathlessness as a clinical symptom may be experienced at low activity levels or even at rest. For many years, both clinicians and researchers have attempted to understand how the complex interplay of breathlessness perception is registered and interpreted in the body by using various psychophysical and clinical models, and with some success (Bakers and Tenney, 1970;Killian*et al*, 1981). However, the interactions between multiple factors known to influence the generation of the sensation and the lack of a precise single experimental model to promote the study of its mechanisms have yetto be fully explored.

1.2 DEFINITIONS AND TERMINOLOGY

Dyspnoea and breathlessness are terms that are often used differently by different research groups, service providers and user groups. Dyspnoea (meaning 'bad' or 'disordered' breathing) comes from the Greek word *dyspnoia* and has often been applied to 'strenuous' and 'difficult' breathing (Sarkar *et al*, 2006). Breathlessness, on the other hand, is the

individual feeling of laboured breathing with and without dyspnoea and/or irregular respiratory function (West and Popkess -Vawter 1994).

A consensus statement by the American Thoracic Society defined breathlessness as 'a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity' (Parshall *et al*, 2012). The salient points here are that (i) dyspnoea is by definition a subjective sensation, and (ii) clinical dyspnoea comprises distinguishable types of breathlessness such as 'air hunger' (defined as an uncomfortable urge to breathe), 'work and effort' (a sense of laboured breathing) and 'chest tightness' (usually associated with airway narrowing in asthmatics) (O'Donnell *et al*, 1997). It is notable that despite this consensus statement, many clinicians still use the term dyspnoea to denote clinical signs such as laboured and rapid, shallow breathing and grunting sounds, indicating respiratory distress even when the patient is unable to provide subjective confirmation (e.g. newborn babies). Breathlessness and dyspnoea will be used synonymously in this thesis to express difficult and laboured breathing, but it is recognised that 'shortness of breath' is the preferred term in the USA. Patients will relate to the term 'breathlessness' whereas 'dyspnoea' is exclusively used by clinicians.

References will be made to acute, refractory (or intractable) breathlessness or dyspnoea. Acute dyspnoea is defined as symptomatic sudden breathing discomfort with a known underlying cause that lasts less than one month. Refractory or intractable dyspnoea is defined as a sense of breathing discomfort which often persists for more than one month or when it is not possible to treat the underlying condition (Abernethy *et al*, 2003; Mahler *et al*, 2010). Episodic breathlessness is a newly identified form of breathlessness. It is defined as 'an increased breathlessness occurring intermittently in patients with or without underlying continuous breathlessness' (Simon, 2013a; Simon*et al,* 2013b).

1.3 PREVALENCE AND CLINICAL IMPACT OF BREATHLESSNESS

Moderate to severe clinical breathlessness is a common presenting symptom among patients with different life–limiting disease trajectories. Such conditions include cancer, heart failure, lung disease and motor neuron diseases (Solano *et al*, 2006). Despite the alarming and intimidating nature of the sensation, an effort to manage and treat symptomatic breathlessness has historically received less attention than other equally disabling and common symptoms, such as pain (Desbiens *et al,* 1997).

1.3.1 Breathlessness in the general population

Using data from a two-year survey, a community-based study by Currow *et al*, 2009, conducted in Australia over a 15 year period, reported an overall breathlessness prevalence of approximately 9% in the 5,473 sampled within the general populationat rest in normal subjects. The study looked at breathlessness prevalence in the general population and within specific age groups (<35 years, 35-49 years, 50-64 years and >65 years). Participants were asked to categorisethe severity of their breathlessness using the Medical Research Council (MRC) dyspnoea scale (The MRC scale is a clinical tool ues to categorisingthe disability caused by dyspnoea). It is graded from 1- 4, (Table 1.1). The study reported that breathlessness prevalence within each age group varies, with the highest found among those aged 65 or above (16.9%). The authors also found that quality of life was unexpectedly lower among the sampled population due to breathlessness. In addition, the kind of disability associated with the type of breathlessness sensation such as acute or chronic was not discussed in the study. Without this information, it is not possible to say for certain whether there are links between disease condition and reductions in quality of life.

In a separate study in adults by Bowden *et al*, (2011), using survey data from 5,331 participants over a two-year period, the authors reported an overallincrease of 11.1% in breathlessness of the population sampled. In addition, 3.4% of the population sampled scored between 2 and 4 on the MRC scale. The studyalso reported breathlessness prevalence of 15% among those aged 50 years and over which compromised their quality of life. (Bowden *et al*, 2011). Another study by Voll-Aanerud *et al*, (2008) of a general population sample of approximately 2,306 participants in Sweden, where breathlessness was defined as grade 2 of the MRC scale (Table 1.1), reported that 20.5% of women experienced breathlessness compared to 12% of men, providing an overall incidence rate of 16.4% (Voll-Aanerud *et al*, 2008).

Table 1.1 Medical Research Council dyspnoea scale (Adapted fromFletcher *et al*, 1960)

Grade	Degree of breathlessness related to activities
1	Not troubled by breathlessness except on strenuous exercise
2	Short of breath when hurrying or walking up a slight hill
3	Walks slower than contemporaries on level ground because of breathlessness, or has to stop for breath when walking at own pace
4	Stops for breath after walking about 100m or after a few minutes on level ground
5	Too breathless to leave the house, or breathless when dressing or undressing

An extensive epidemiological study on breathlessness conducted in the general population in the Netherlands for a 21-year period highlighted the extent of breathlessness prevalence in the general population (Figarska *et al,* 2012). The study, known as the Vlagtwedde/Vlaardingen respiratory project, involved a cohort of 7,360 people, exclusively Caucasian individuals of Dutch ancestry. The authors reported a 5% and 1% moderate and severe breathlessness perception respectively in the study sample.

Despite the present knowledge of chronic breathlessness being a common symptom in the general public, only a few large-scale community based studies on chronic breathlessness have been conducted worldwide. Although perceived breathlessness has been studied for

different categories of people within the general population, such as different age groups, the elderly, different genders and terminally ill patients, an emerging trend of higher prevalence has been found to be associated with old age. Up till now, breathlessness prevalence has been investigated in separate categories but not in one whole study. Investigation of breathlessness prevalence including all the categories such as age, gender, ethnicity, those with life limiting illness, the elderly in one study would provide an accurate reflection of the scale of the problem. This would enable us to improve current interventional strategies in both clinical and community settings.

1.3.2 Breathlessness in primary care setting

Breathlessness in the primary care setting has been widely investigated. It is acknowledged to be a common complaint on par with pain in general practice, with or without any obvious underlying pathological cause (Frese *et al*, 2011; Mu¨llerova *et al*, 2014). A prevalence study by Jolly (1978) in Canada from 1974-1975 reported an incidence and prevalence of 7.3 and 15.4 per 1,000 being susceptible per year in a sample of 121 patients.Another study conducted in the USA with a larger sample of 1000 patients over a period of 3 years (1984-1987), an incidence of 3.7% in breathlessness complaint was observed (Kroenke and Mangelsdorff, 1989). A survey by Charles *et al* (2005) in Australia from April 1998 to March 2004, using available national data from the bettering the evaluation and care of health (BEACH) program to study general practice activity, concluded that in a sample of 5,215 patients, breathlessness was the primary complaint among patients in 0.9 per 100 encounters.

1.3.3 Breathlessness among the elderly

An increase in breathlessness is generally associated with old age, and may happen without any apparent underlining clinical condition. Evidence is available from two independent studies which have randomly measured self-reported breathlessness within a specific age

group. The first study found breathlessness prevalence of 27% in adults aged between 45 and 69 years, in a random sample of 4,276 individuals taken from electoral rolls for Melbourne, Australia using the MRC scale (Abramson *et al*, 2002). Another study in the Netherlands also reported a breathlessness incidence rate of 24% in a random sample of 210 adults of 55 years and above, using the Borg dyspnoea score. It is a scale that asks an individual to rate the difficulty of their breathing. It is graded from 0-10 (Boezen *et al*, 1998). Other studies of exertional breathlessness measured among the over 65 year group in a community-based study conducted in France estimated that 60% of those sampled reported breathlessness. The percentage was found to be significantly higher among women in this study as compared to men (Barberger-Gateau *et al*, 1992).

1.3.4 Breathlessness in advanced disease conditions

Breathlessness is the predominant clinically reported and observed symptom reported by 60-88% of chronic heart failure patients (including patients with no known lung pathophysiology), 90-95% of chronic obstructive pulmonary disease (COPD) patients,10-78% of lung cancer patients, and 50-70% of all other cancer patients. It is found to be very common in advanced terminal illness such as Acquired Immune Deficiency Syndrome (AIDS) and end-stage renal disease (Bruera *et al*, 2000, Edmonds *et al*, 2001, Solano *et al*, 2006, and Thomas *et al*, 2011). Other studies conducted among seriously ill patients who are hospitalised found that half of a sample of over 1500 patients complained of breathlessness, equal to the number complaining of pain (Desbiens *et al,* 1997; Desbiens *et al,* 1999). Half of patients with advanced cancer experience moderate to severe breathlessness (Bruera *et al,* 2000), yet understanding and treatment of breathlessness in cancer patients is decades behind that of pain (Dudgeon and Rosenthal, 1996). In a study of cancer outpatients, the incidence of dyspnoea was reported to be 84% in lung cancer patients. This was followed by 54% of patients with lymphoma (Dudgeon *et al*, 2001). The

study used two breathlessness scoring scales (visual analogue scale and verbal rating scale-VAS and VRS), to assess breathlessness severity in these patients. The authors found that patients with lung cancer had the most severe breathlessness experience.

Breathlessness has been reported in some studies to be the contributing factor to initiate palliative use of drugs to reduce agitation (sedation) in end of life patients. One such study found that 25 out of 77 terminally ill patients, such as terminal cancer patients, needed sedation to control the symptom of breathlessness (Mercadante *et al*, 2009). Another multicentre observational study investigating the usefulness of sedation in palliative settings for end of life patients, found that nearly 60% of the sample population complained of unbearable breathlessness which needed sedation (Morita *et al*, 2005).

1.3.5 Clinical impact of breathlessness symptoms

In healthy subjects, moderate levels of laboratory-induced breathlessness provoke comments that emphasize its potential for emotional impact. Examples of such comments are "*just feel I can't get my breath; feel as though I am so breathless I will die; no matter how big a breath I take, it doesn't feel enough and it makes you very frightened"* (Elliott *et al*, 1991). Breathlessness is a powerful diagnostic symptom of cardiopulmonary disease that motivates patients to seek medical attention (Mahler *et al*, 1996). Unyielding breathlessness is frightening and considered as one of the best predictors of mortality (Hammond, 1964; Figarska *et al,* 2012). In a recent study, the authors reported that the number of patients who easily perceive symptomatic dyspnoea has risen and therefore it is equal to the number of patients who experience blunted perception of breathlessness (Ebihara *et al,* 2012). The more breathless the patient gets, the more likely they are to seek help. The terrifying nature of chronic breathlessness as a symptom has made a patient living with COPD redefine the condition as '*Can Only Plan Daily*' (www.nhsiq.nhs.uk/media/2486362/chris_warburton.doc). A current review of the

National Health Service (NHS) budget has indicated that COPD and asthma medications cost the organisation nearly £1bn yearly. Respiratory inhalers were among the top five medications which contribute to the high expenses (www.drugtariff.co.uk,June 2012).

1.4 NEED FOR MORE EFFECTIVE TREATMENT OPTIONS FOR BREATHLESSNESS

Currently available pharmacological agents for the relief of symptomatic breathlessness are either not fully effective or their benefit still remains unproven. Few treatment options exist for breathlessness in conditions with no discernible pathology (e.g. hyperventilation or panic–disorder syndrome).

There is some debate concerning the use of opioids for breathlessness relief with most support for the oral or parenteral routes rather than the nebulized route (Jennings *et al*, 2002). Most of the data regarding opioids for breathlessness relief have been gathered from studies in terminally ill patients such as those with COPD, cancer and interstitial lung disease. Two independent studies (Abernethy *et al*, 2003, Currow *et al*, 2011) have explored the possible use of a continued discharge of morphine and sought to define an acceptable starting dose. Ten mg of oral morphine gave a 62% improvement in breathlessness, which remained unchanged with steady dose increments (Currow *et al*, 2011). The downside of using opioids is the known respiratory depression and severe sedation side effects, but these effects were not reported in the 83 COPD patients involved in this study (Currow *et al*, 2011). Currently, there are no comprehensive studies looking at dosing of opioids for symptomatic breathlessness in patients already taking them for pain relief. Other identified symptomatic relief options are inhaled furosemide and cannabinoids, but these are unproven in the clinical setting (Jill *et al,* 1994).

The lack of confidence in pharmacological options for dyspnoea relief has led to a focus on non-pharmacological interventions to complement pharmacological ones. Most non-

pharmacological options have been studied in COPD and asthmatic patients and several reviews have been published highlighting the variability in effectiveness of the various options(Booth *et al*, 2004; Cranston *et al*, 2004; Jenkinson *et al*, 2001; Polosa *et al,* 2002;). Breathing training (e.g. pursed lips) (Garrod *et al*, 2005, Hochstetter *et al,* 2005 and Wu *et al*, 2006), counselling and support (Hermiz *et al*, 2002; McMillan and Small, 2007), and walking aids (Dalton *et al*, 1995; Gupta *et al*, 2006a and 2006b; Probst *et al*, 2004 and Solway *et al*, 2002) have demonstrated varying degrees of benefit. The evidence for non-conventional therapeutic practices, such as acupuncture, is questionable in regards to blinding of procedures and may lack adequate placebo control (Suzuki *et al,* 2008; Suzuki *et al*, 2012; Momaerts *et al,* 2012). Supplementary oxygen does not appear to be more effective in breathlessness relief than supplemental air alone (Abernethy *et al*, 2010). Hand-held fans to blow air on the face have been found to be effective in alleviating breathlessness (Baltzan *et al*, 2000; Galbraith *et al*, 2008) and may work by the stimulation of the facial nerves (Schwartzstein *etal,* 1987; Liss and Grant 1988 and Burgess *et al,* 1988).

Development of more effective pharmacological and non-pharmacological treatment options are likely to depend on a better understanding of the neurophysiological mechanisms of dyspnoea. This thesis aims to elucidate some of these mechanisms.

1.5 DEVELOPMENT OF OUR UNDERSTANDING OF BREATHLESSNESS MECHANISMS

Key milestones in the development of our understanding of the mechanisms of breathlessness perception are collected in chronological order within Table 1.2. The key theories are discussed in more detail below.

Table 1.2: Key theories leading to our understanding of breathlessness mechanisms.

Year	Author (s)	Key theory
1956-1960	Julius Comroe (1956)	'We must look for the sensory receptors, sensory pathways, and thalamic or cortical centres which are responsible for the perception of respiratory discomfort'. This was echoed in his opening remarks at the 1965 international symposium on the topic of breathlessness. This advice remains as fresh today as it was back in the 1960s.
1963	Campbell and Howell. (1963)	'Length-tension inappropriateness': This theory proposed a peripheral mechanism based on an assumption that all forms of breathlessness sensation originated from respiratory muscle feedback.
1989,1990	Simon *et al*. (1989) Elliot *et al*. (1991)	Language of breathlessness: Patients with different clinical conditions and healthy volunteers undergoing different forms of respiratory stimulation led to the realisation that clinical dyspnoea consists of several different sensations which are likely to arise from different afferent sources.
1990-1993	Banzett *et al*. (1990) Gandevia *et al*.(1993) Manning *et al*. (1992)	Complete paralysis experiments. The Campbell and Howell (1963) assertion that all dyspnoea arose from respiratory muscle afferent feedback was disproven, by a demonstration that 'air hunger' was generated by hypercapnia and relieved by raising tidal volume irrespective of respiratory muscle feedback. Further corroborated by studies in high-level quadriplegics.
1991-1996	Chen and Eldridge (1991) Eldridge and Chen (1992;1996)	Studies in cats: identified signals in the thalamus which may be the dyspnoea signal rising to the forebrain.
2000-2003	Banzett *et al*. (2000) Peiffer *et al*.(2001) Evans *et al*.(2002)	Brain imaging studies of breathlessness which identified certain brain regions which might be important in processing breathlessness perception.
2010	Yorke *et al*, (2010) Banzett *et al*, (2015)	Developed and validated dyspnoea assessment tool to score either the affective or the physical component of dyspnoea.

1.6 NEUROPHYSIOLOGICAL MECHANISM OF BREATHLESSNESS

1.6.1 The length-tension inappropriateness theory

An idea proposed by Comroe (1960) was followed and expressed in terms of 'length-tension inappropriateness' by Campbell and Howell (1963). They proposed that all dyspnoea originates from a single respiratory muscle source. Thus when the tension within

the muscle does not match the length in the muscle, this will result in respiratory discomfort. The following are some of the traditionally proposed theories, which either were developed in support of, or refine, the length-tension inappropriateness theory. These are: the efferent–reafferent dissociation by Schwartzstein *et al*, (1990) and Schwartzstein *et al*, (1989), neuroventilatory dissociation by O'Donnell *et al,* (1997), afferent mismatch by Manning *et al*, (1992), neuromechanical uncoupling by Jensen *et al*, (2009), and O'Donnell (2006, 2007 and 2009), or neuromuscular dissociation by Scano *et al*, (2010).

However, these theories are closely related and have a common limitation. This has led to constant revision to encompass the interplay amidst neurophysiological mechanisms. The limitation is that the authors focused mainly on the peripheral mechanism, which may drive and give rise to different breathlessness sensations. In addition, none of the theories was based on robust experimental design or with enough sample data. Afferent information from vagal lung receptors and mechanoreceptors of the respiratory muscles has the potential to contribute to the perception of breathlessness (Campbell and Howell, 1963 andPaintal, 1973). Several studies on the relationship between ventilatory drive and motor command have found the contribution of interneurons to be equally essential to generate the perception of breathlessness (Killian and Campbell, 1983; Gandevia, 1988).

1.6.2 Impact of language used by patients to describe respiratory discomfort

Following the work of Campbell and Howard (1963), the next two decades saw the emergence of interest in the 'language of breathlessness' in patients with different clinical conditions and healthy volunteers undergoing different breathlessness stimulation (Simon *et al*, 1989,1990; Elliott *et al*, 1991 and von Leupoldt *et al*, 2007a). This led to the realisation that clinical dyspnoea may consists of several different sensations which are

likely to arise from different afferent sources (Parshall *et al*, 2012). This led to the development of the idea of different, distinguishable types of breathlessness.

1.6.3 Perception of distinguishable types of breathlessness

Air hunger (AH) cluster:Air hunger or unsatisfied inspiration is described as an uncomfortable urge to breathe (Lansing *et al*, 2000).Relatively small increases in partial pressure of carbon dioxide (PCO_2) (<10mmHg) are sufficient to produce severe AH at constant ventilation even in healthy volunteers(Banzett *et al,*1996). Much evidence has indicated that AH is originated and amplified by imbalances between perception from respiratory motor drive from the brainstem and the feedback from the afferent stretched receptors (Moosavi *et al,* 2000 ; 2005;Parshall *et al*, 2012). A 'corollary' copy of the generated information is conveyed to the cerebral cortex. Larger tidal volumes will increase vagal afferent discharge from lung mechanoreceptors and this is believed to inhibit the 'corollary' copy as it rises through the midbrain (Eldridge *et al,* 1992). AH involves a combination of inputs reporting metabolic demand for breathing, with inputs reporting the actual prevailing ventilation. This represents a similar concept to the 'length-tension inappropriateness' that was applied to respiratory muscle feedback. Respiratory muscle activity is not involved in AH since breathlessness persists after complete muscle paralysis in healthy volunteers (Banzett *et al,* 1990) and in patients with high level spinal cord injury in whom air hunger is experienced despite sensory input from the chest wall (respiratory muscles) being absent. In a separate experiment to clearly understand the afferent pathways involve in the AH perception, subjects paralysed by polio experienced 'shortness of breath'' when CO_2 was added to the inspired air during constant mechanical ventilation. This phenomenon occurred at a considerable lower PCO_2 when ventilated at lower tidal volumes. Therefore, the authors concluded that shortness of breath resulted

from an interaction of CO_2 stimulation and inhibition produced by the lung and chest wall stretch receptors (Opie *et al*, 1959) (Figure 1.1).

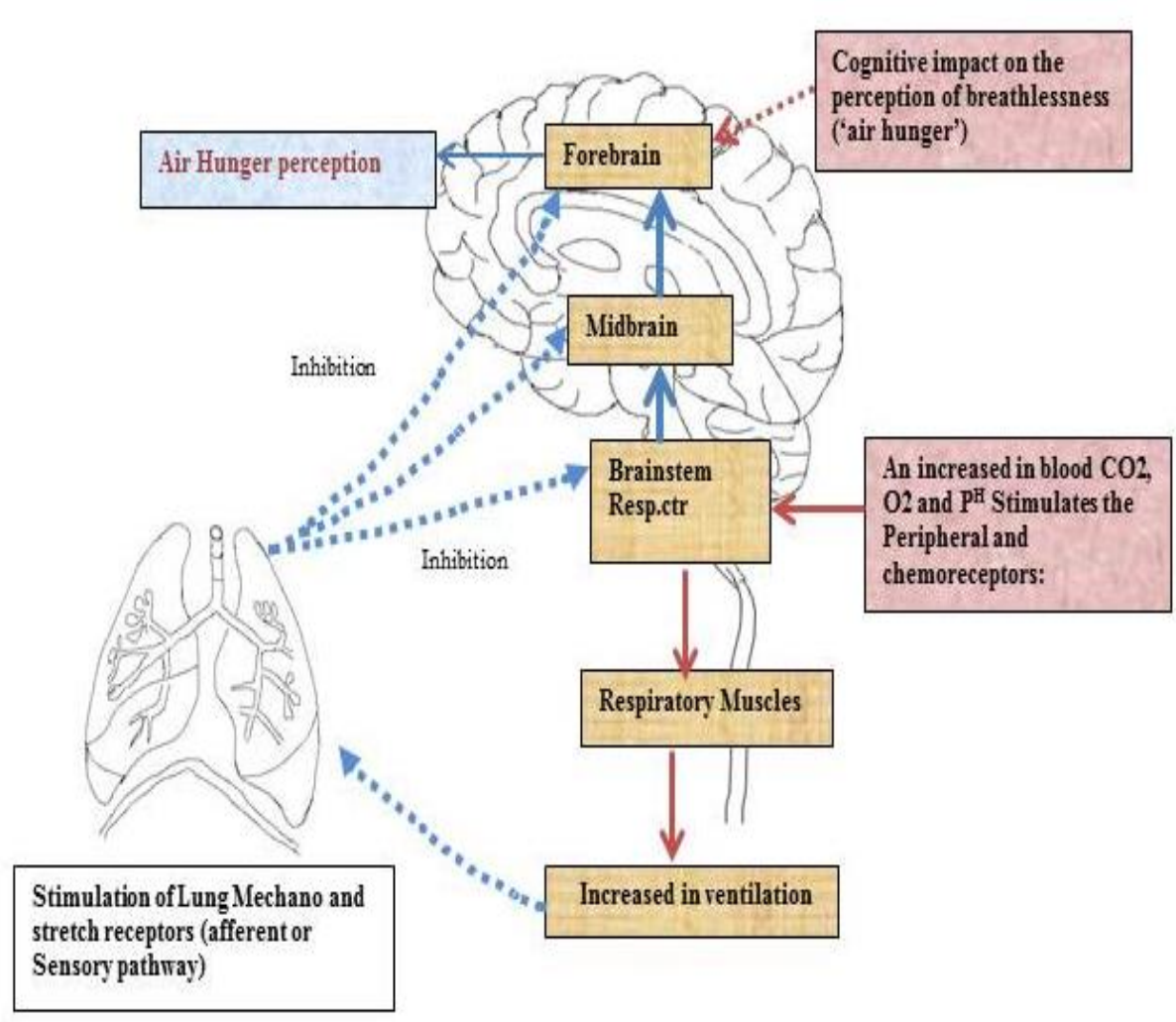


Figure 1.1: Cortical pathway underlying air hunger perception. Changes in arterial blood PCO_2, hydrogen ions and O_2 cause a demand in respiration. This signal is sent to the respiratory centers in the brainstem which are then passed on to the respiratory muscles to increase respiration. Receptors in the chest wall and the lungs respond accordingly and stretch to compensate the respiratory demands. A copy of the signal ('corollary discharge') is sent to the forebrain and to the hind brain to register air hunger. The fully stretched receptor in the lungs sends another copy of the compensatory signals back to the brainstem and the forebrain which inhibit the experience of the air hunger.(Adapted from Manning *et al*, 1992).

Effort or work/effort (W/E) cluster: Work/effort (W/E) is an uncomfortable sensation that can arise when ventilation requires more respiratory muscle activity due to exhaustion or

increased resistance to inspiration (Gandevia *et al,* 1981). W/E is often reported among patients with weak breathing muscles, with interstitial lung disease and with COPD (Simon *et al,* 1990). The sensation may arise from a combination of respiratory muscle afferent activity and 'corollary discharge' of central neural motor drives to the respiratory muscles (Moosavi *et al,* 2000 ,Gandevia *et al,* 1981). Contrary to AH, lung afferent information is not thought to have any effect in reducing W/E but direct evidence for this is currently lacking.

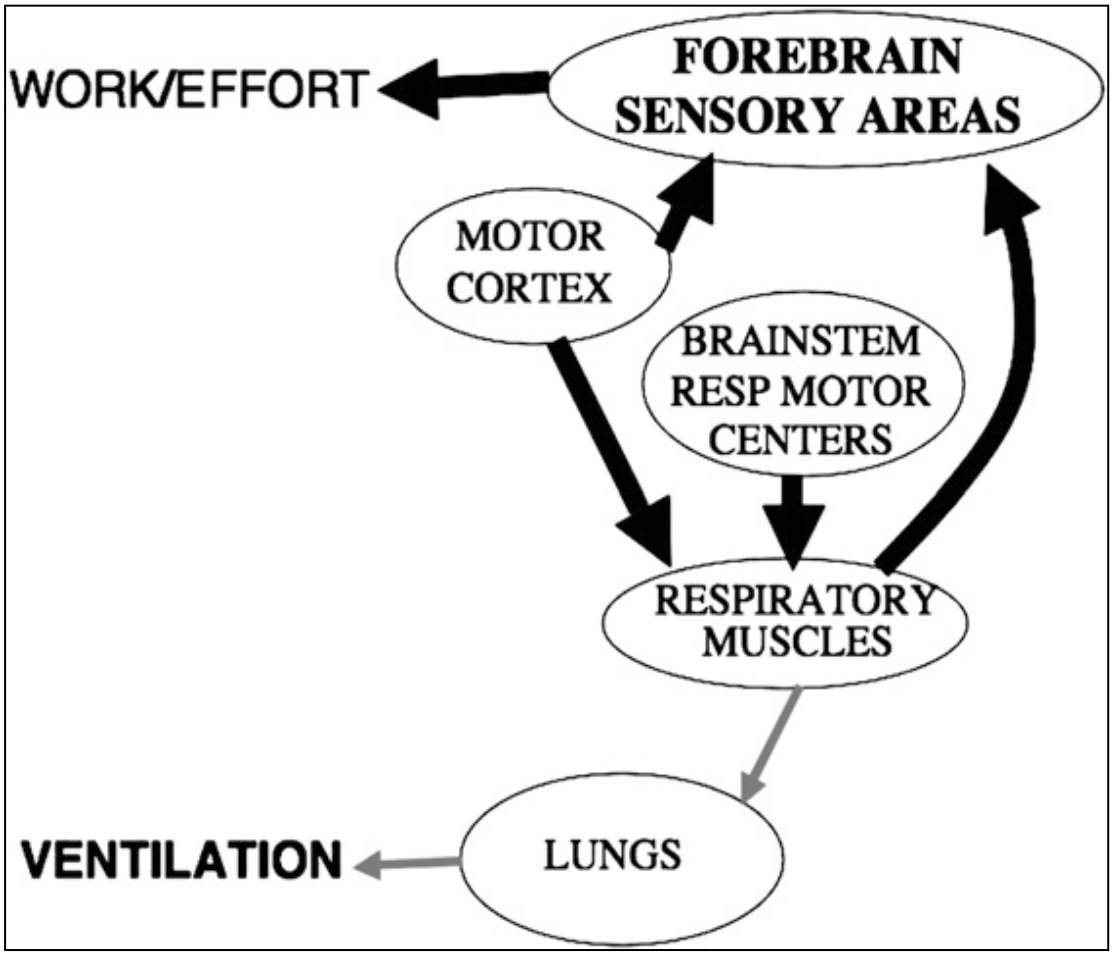


Figure 1.2: Neural mechanism underlying work and effort breathlessness type. Restriction in free breathing causes a mechanical change which sends a signal to the respiratory motor center to increase breathing. This information is passed on to the respiratory muscles and lungs where ventilation is increased. A copy of the afferent signal is sent to the forebrain and the motor cortex and work and effort sensation is perceived. (Reproduced from O'Donnell *et al*, 2007).

Chest Tightness:This type of sensation is specifically reported by asthmatics. Evidence suggests that vagal afferent information from pulmonary receptors, activated by inflammation of the airways, is the most likely cause of this form of breathlessness (Simon *et al,* 1990; Binks *et al,* 2002).

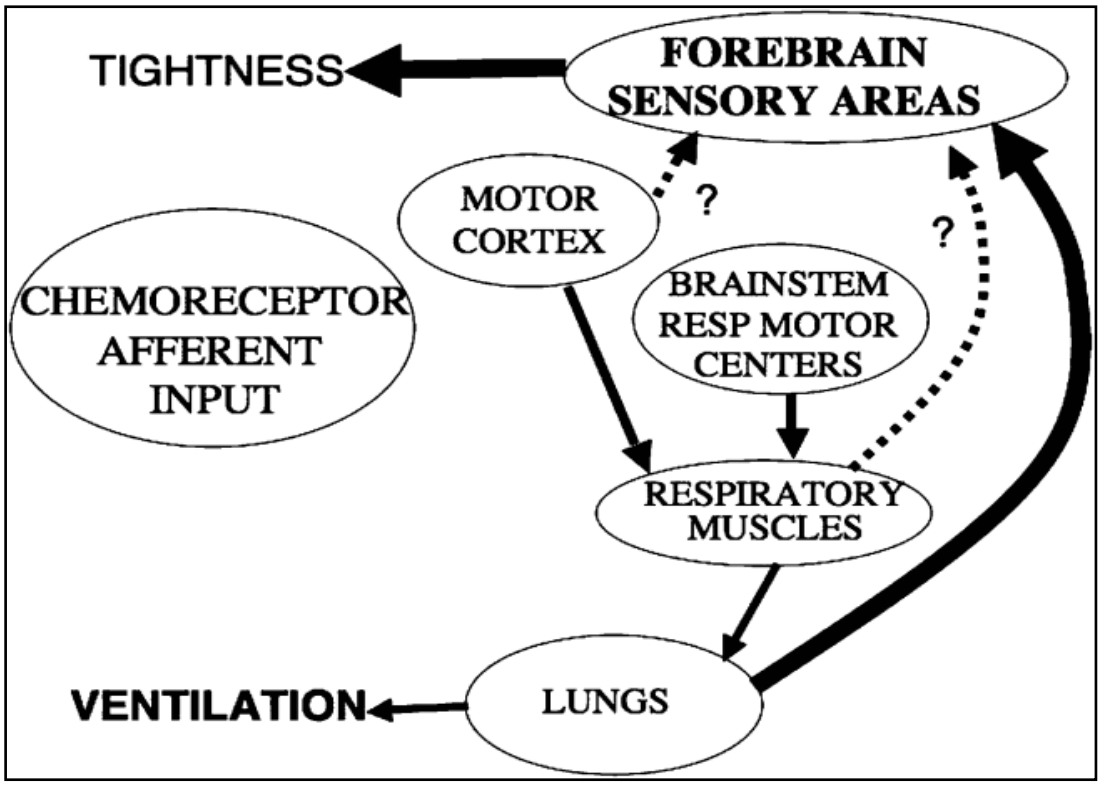


Figure 1.3: Cerebral pathway underlying chest tightness breathlessness type. Changes in ventilation due to restriction are sensed by the respiratory motor area. The signal is passed on to the respiratory muscles and the lungs to respond accordingly by increasing ventilation. Feedback information to the forebrain results in the experience of chest tightness as a type of breathlessness. This mechanism is similar to work and effort experience but thought to involve different pathways. (Reproduced from O'Donnell *et al*, 2007).

1.6.4 Respiratory muscle paralysis in humans

We can use paralysis of respiratory muscles as a tool to identify the respective roles of the brain and the body in creating dyspnoea. The cerebral mechanism of breathlessness is a complex process in both the body and the mind and not fully understood (Hyen *et al,*

2013). Early research primarily focused on the role of chemoreceptors in generating breathlessness. The peripheral process of respiratory sensory information to register the perception of breathlessness is a complex physiological mechanism involving a series of receptor interactions. In the case of AH, this involves the comparison of an afferent copy of the respiratory motor signals ascending from the brainstem to the higher brain, with the inhibitory feedback from lung mechanoreceptors. A mismatch between these signals is thought to lead to dyspnoea (Chen *et al*, 1991; O'Donnell *et al,* 2007). This mechanism has its basis of support in functional clinical observation of both normal subjects and patients under certain conditions. What is not well defined is (i) where in the brain breathlessness is actually perceived, (ii) the neural network that underlined the psychological modulations and (iii) whether the activations seen in the brain imaging studies of dyspnoea are actually part of a wider network akin to the pain perception network.

Many functional research studies have explored, and proposed the possibility of the presence and the location of a chemoreceptor such as a CO_2-sensitive cell in the brain and in the carotid bodies (Feldman *et al,* 2003; Peers and Buckler, 1995). Progressive hypercapnia and hypoxia induce sensations of breathlessness at rest and during exercise (Stark *et al*, 1981; Adams *et al*, 1985b; Lane *et al*, 1990). Therefore, it has been suggested that central and peripheral chemoreceptor independent or combined stimulation may be perceived directly as breathlessness (Adams and Guz, 1996). The role of afferent inputs originating from chemoreceptors, mechanoreceptors, chest wall and lungs stretch receptors and carotid receptors have been documented through various psychophysical experiments involving both healthy volunteers and patients with different respiratory conditions such as asthma and COPD (Campbell,*et al*, 1967; Banzett *et al*, 1987; Gandevia and Macefield, 1989).

Paralysis studies showed that there is no single afferent source. Subsequent studies using this information re-examined induced respiratory muscle paralysis and complete neuromuscular block in healthy humans (Banzett *et al,* 1990; Gandevia *et al,* 1993) and patients with high-level spinal cord injury (quadriplegics) with increased CO_2 and reduced end-tidal volume (Manning *et al*, 1992).The persistence of dyspnoea in a paralysed respiration muscle situation disproved the earlier assertion that all dyspnoea signals emanate from a single afferent source in the chest wall. Animal studies later began to unravel the various peripheral neural pathways that might be involved in the different types of dyspnoea leading to the proposal that dyspnoea arises from a mismatch of afferent–efferent signals (Eldridge and Chen, 1992). Eldridge and Chen (1992) studied the effect of changing pulmonary vagal nerve inputs in paralysed and ventilated cats. They manipulated the ventilation and the temperature under different conditions on the vagus nerve. The spinal cord was transected at C_7-T_1 where the carotid sinus nerve was cut but the vagus nerve was not. The study showed that the vagal input inhibits the respiratory afferent information to the cortex by a mechanism that is independent of its effect on respiratory drive. This is similar to the length-tension inappropriateness concept but not limited to respiratory muscle feedback. With important physiological components identified, and given that these cannot fully explain dyspnoea, we turn to the brain.

1.6.5 Brain imaging studies

The introduction of neuroimaging techniques such as functional magnetic resonance imaging (fMRI) 30 years ago has provided an important opportunity to study the neural dynamic activities of the brain in health and disease. Recent functional imaging and positron emission topography (PET) studies have reported several cortical areas of the brain, notably the insular cortex, to be more active than other areas when experimental breathlessness is perceived as shown in Figure 1.1 (Banzett *et al*, 2000; Evans *et al*, 2002).

Several lines of evidence from functional brain imaging studies of breathlessness over the last decade has provided the opportunity to begin to address the latter part of Comroe's advice –' We must look for the sensory receptors, sensory pathways, and thalamic or cortical centres which are responsible for the perception of respiratory discomfort' (Conroe, 1956). The first functional brain imaging study of breathlessness was published in 2000 (Banzett *et al,* 2000) and showed activation of the anterior insular cortex in response to induced 'air hunger'. This was quickly followed by several independent reports showing activation of this and various other cortical centres, especially within the limbic system when dyspnoea was induced in healthy volunteers (Evans *et al*, 2002; Peiffer *et al,* 2001).

Comroe's advice (1956) for a dyspnoea research strategy suggested over five decades ago has cautiously guided the translation from the involvement of peripheral sensory receptors to our current knowledge of the functional cerebral centres in dyspnoea perception.

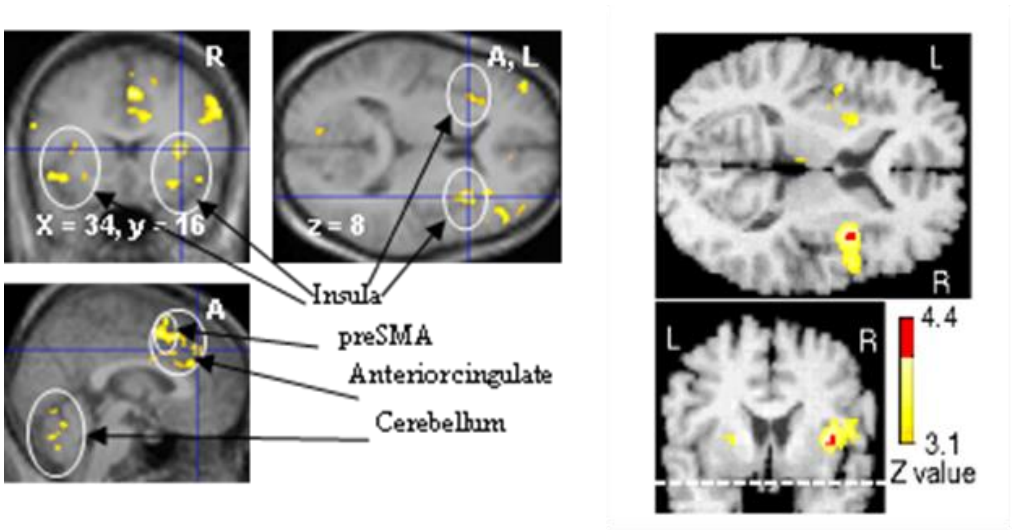


Figure 1.4: Functional brain imaging of dyspnoea. *Right:* First published brain imaging study of dyspnoea using positron emission tomography (PET) scans during experimentally induced air hunger in healthy human subjects showing predominant activation of the insular cortex (from Banzett *et al*, 2000). *Left*: The first published functional magnetic resonance imaging (fMRI) brain scan showing cerebral activation by dyspnoea in healthy volunteers undergoing experimentally induced 'air hunger' (from Evans *et al*, 2002). The higher resolution and depth of view of the fMRI technology identified additional areas beyond the insular cortex (circled structures in the top panels) such as the pre sensory motor area, (preSMA-circled structure in bottom panel).

1.6.6 Multidimensionality of breathlessness (sensory and affective domain)

One of the most important recent developments in our understanding of breathlessness has been the acceptance that breathlessness is multidimensional with sensory intensity, quality and affective domains, just as has been described for pain (Boring 1939; Melzack *et al*, 1968). The American Thoracic Society (ATS) consensus statement (2012), definition of dyspnoea, acknowledges the sensory and affective dimensions.

Studies have confirmed similarities between the neurophysiology of pain and of dyspnoea sensations (Gracely *et al,* 2007; von Leupoldt and Dahme 2007; Evans *et al,* 2002; von Leupoldt *et al,* 2008). This raises the possibility that the application of multidimensionality is possible in dyspnoea study, even though our current understanding of the affective dimension of dyspnoea remains less well explored (Banzett *et al,* 2000; von Leupoldt *et al,* 2006). More recent data from the study of the affective dimension of dyspnoea in both healthy subjects (Banzett *et al,* 2000; Banzett *et al,* 2008), patients with COPD (Carrieri-Kohlman *et al,* 2010) and during exercise (Meek *et al,* 2003; Carrieri-Kohlman *et al,* 1996b;) shows the coexistence of both sensory and affective dimensions in dyspnoea perception which can be measured separately. This prompted the development of breathlessness assessment tools to help in our understanding of the neurophysiological mechanisms.

1.6.7 Development of dyspnoea-12 and multi dyspnoea profile assessment scales

Two new assessment tools, dyspnoea-12 and the multidimensional dyspnoea profile (MDP), have been developed and validated to specifically score either the combined intensity/affective dimensions of dyspnoea (Yorke *et al*, 2010) or score these domains separately (Meek *et al,* 2012). The choice of final descriptor items used in dyspnoea-12 was based on a pool of language used in published literature to describe the sensation of

breathlessness. On the other hand, descriptor items used in the MDP are based on existing instruments to measure pain and dyspnoea (Parshall, 2002; Parshall *et al*, 2012;Simon *et al*, 1990).Evidence has suggested that the different clusters of respiratory descriptors are likely to arise from separate neural afferent pathways and are independent of each other (Binks *et al,* 2002; Lansing *et al,* 2000 ; Moosavi *et al,* 2000).

1.7 INSULAR CORTEX, THE LIMBIC SYSTEM AND BREATHLESSNESS

The insular cortex (Figure 1.5) has identifiable anatomical subdivisions (Augustine, 1996; Naidich *et al*, 2004) and is involved in the processing of various autonomic functions and emotional responses such as anxiety, panic and depression (Crespo-Facorro *et al*, 2000; Paulus and Stein, 2006). Damage to the insular cortex has consistently been implicated in different psychosomatic illnesses, various neurological diseases and neuropsychological disorders apart from breathlessness perception (Jones *et al*, 2010). It also forms part of a unique area in the brain termed as the eloquent brain or the limbic and paralimbic system (Figure 1.5). However, it is becoming increasingly hard to implicate the insular cortex alone in breathlessness perception (Herigstad *et al*, 2011; Hayen *et al*, 2013). Major structures of the limbic system are the amygdala, thalamus, fornix, insular cortex, corpus callosum, hypothalamus, hippocampus, mammillary body and cingulate gyrus (Rajmohan and Mohandas, 2007).

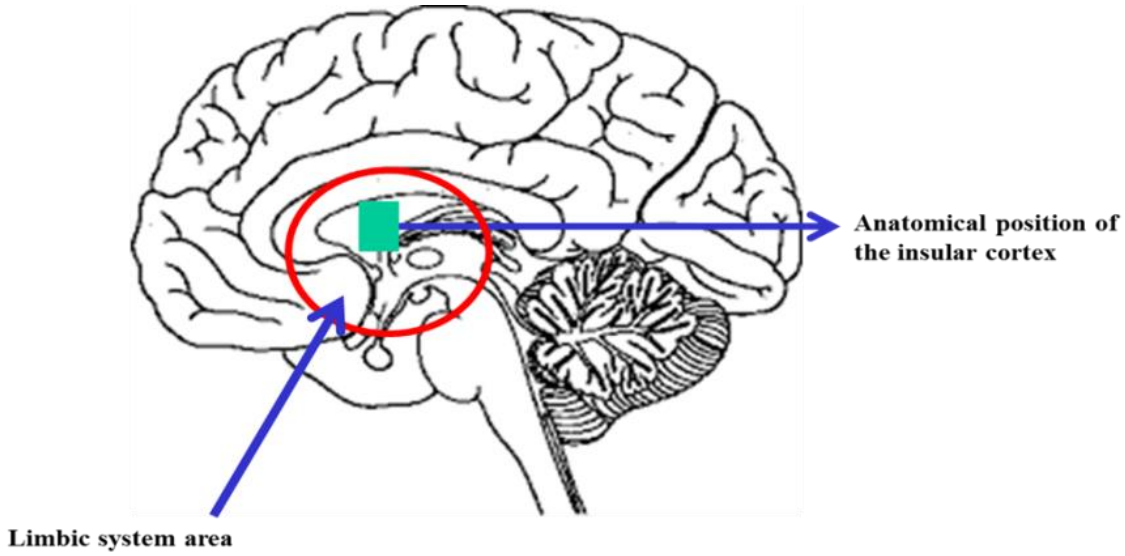


Figure 1.5: Schematic of the human brain indicating location of insular cortex. A sagittal view of the human brain, showing a schematic of the limbic system (red circled area) and the anatomical position of the insular cortex (green shaded area). (Image adapted and modified from; www.gopixpic.com).

1.7.1 Anatomy and structural organisation of the insular cortex

Anatomically, the insular cortex is considered a distinct and complex structure buried deep within the Sylvian fissure and mostly covered by the temporal lobe, opercula of the parietal and frontal lobes. Macroscopically it is divided into anterior and posterior structures by the central sulcus of the insula. The anterior part is identified by three short gyri, the anterior, middle and posterior short gyri with a supplementary accessory gyrus on the ventral side of the anterior part of the insula. The posterior part is also identified by two long gyri, an anterior and a posterior long gyrus (Türe *et al*, 1999; Nieuwenhuys 2012; Shelley *et al*, 2004; Naidich *et al*, 2004) (Figure 1.6). Microscopically, the insular is made up of six different layers. The cells and its layers within each part of the insula have been found to be morphologically distinct. The anterior part is made up of agranular pyramidal neurons arranged in different layers. The posterior part is also made up of fine granular neurons located in layers II and IV (Nieuwenhuys 2012). Although there are some individual and

species differences observed in these structures, the general pattern is supported by various studies (Morel *et al*, 2013; Kurth *et al*, 2010; Gallay *et al*, 2012). Despite the identified structural differences, there is very little experimental evidence available to support their independent functional roles in the processing of any bodily sensations. Other less functionally known neurons within the anterior region of the insular cortex called Von Economo neurons (VENs) with distinct large bipolar features have also been identified (Morel *et al*, 2013; Nieuwenhuys, 2012; Cauda *et al*, 2013). Post-mortem studies in humans have shown that the VENs are thought to be involved in interoceptive functions such as of pain perception, encoding visceral sensations and immunity by the expression of transcription factors (Stimpson *et al*, 2011; Cobos and Seeley, 2013).

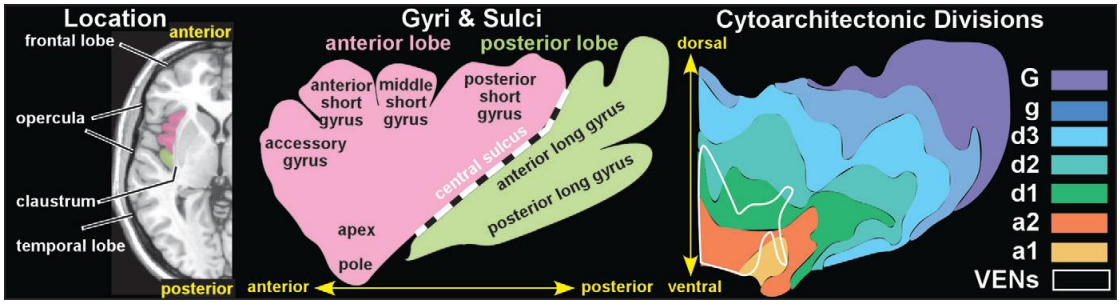


Figure 1.6: Anatomical location and extent of the insular cortex.*Left*: The insula location between the opercula and the claustrum is illustrated on an axial MRI. *Middle*: close up showing that the insula is divided by the central sulcus into anterior (pink) and posterior (green) lobes. *Right*: Cytoarchitecture map of the region identifying possible functional subdivisions. (Reproduced from Shura *et al*, 2014).

1.7.2 Structural connectivity of insular with limbic structures

The insular cortex is powerfully connected to the limbic, the para-limbic and other structures in the cerebral cortex. These areas process many unpleasant visceral sensations and modify physiological and psychological responses. Activation within these structures has been reported in a study involving pain perception and underlines its diverse roles (Augustine, 1996; Miller *et al,* 1996; Kinomura *et al,* 1994; Kettenmann *et al,* 1997;

Tataranni *et al;* 1999; Denton *et al,* 1999 ; Casey, 1999). Information from tract tracer and electrophysiological recordings in animals have suggested that the anterior and the posterior structures lie within the sensory-associated region whilst the ventral portion projects into the limbic relate areas such as the amygdala, temporal lobe and the anterior cingulate cortex (Nieuwenhuys, 2012; Stephani *et al*, 2011). Other projections, which control various stimuli such as gustatory and somatosensory information, are found in the anterior region (Figure 1.7). Stimulation studies in awake macaque monkeys have revealed multiple projections with strong connections between different insular regions and responsesto many sensations such as eating-related disgust and food refusal (Jezzini *et al* 2012). Two studies in which intraoperative electrophysiology and depth electrode stimulation were used in healthy individuals and in patients with intractable epilepsy have confirmed the functions of the posterior region in responding to sensory inputs and the sensations of pain perception and other unwanted symptoms (Almashaikhi *et al*, 2014; Stephani *et al*, 2011). Many other locations within the insular cortex have been found to evoke various symptoms through stimulation studies to confirm its involvement in the process of wider physiological sensations (Almashaikhi *et al*, 2014; Stephani *et al*, 2011). Several authors have also used the novel technique diffusion tensor imaging tractography to investigate the structural connectivity of the human insula (Cerliani *et al*, 2012; Cloutman *et al*, 2012; Jakab *et al*,2012; Dennis *et al*, 2014; Wiech *et al*, 2014). The identified subdivisions in the connectivity studies are closely linked and consistent with those found in tract-tracing studies in animals. Interestingly, more differences in connectivity patterns have been identified between the anterior and posterior regions of the insula. The progression of these patterns is similar to other microstructure mapping confirmed through a voxel-based probabilistic tractography (Cerliani *et al*, 2012) (Figure 1.8). More data are needed to confirm similar connectivity patterns in humans. The insula

is non-reciprocally connected to the orbitofrontal and parietal lobes and reciprocally to the thalamus. The posterior region is thought to receive a broad range of interoceptive afferent information from respiratory chemoreceptors, pulmonary receptors and medullary respiratory neurons (Gaytan *et al,* 1998; Hanamori *et al,* 1998a ;1998b).

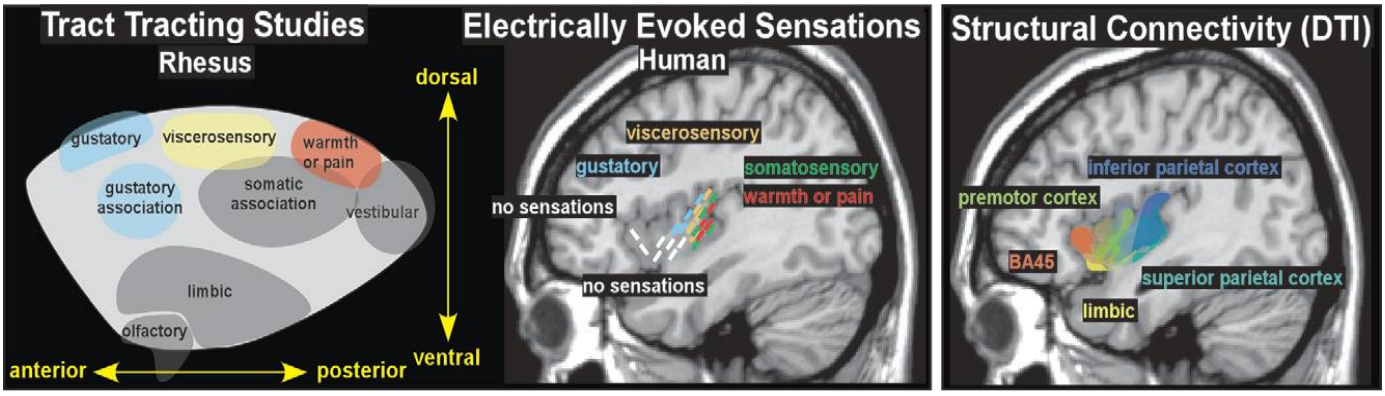


Figure 1.7: Functional and structural connections within the insular cortex. ***(Left)*** The posterior to anterior progression of functional areas identified within the dorsal area of the insula is analogous across primates. This is shown above with relative maps based on tracing of afferents in rhesus monkeys and intraoperative electrical stimulation in human subjects. Sensory functions are thought to be restrictedpredominantly to the posterior region in human butit is spread almost the whole insula in rhesus. Mostof the anterior portion of the human insula has been associated to nonsensory functions, such as cognition, emotion, and social functioning. ***(Right)*** A similar posterior to anterior development has been established using DTI tractography, which allowsan *in vivo*structural connectivity to be investigated. Results from a selected recent study (darker shades represent stronger connectivity; lighter shades represent weaker connectivity) is overlaid onto a sagittal. The authors of this study stressed that, even though the anterior and posterior regions have different connections in predictable ways, transitions were gradual rather than sudden. (Reproduced From Shura *et al*, 2014).

1.7.3 Functional connectivity of the insular cortex

Despite the anatomic benefits of structural connectivity within the insular cortex, understanding the functional connectivity is important to know which regions are co-activated or share functional responsibility based on data recorded during activities in real time. This will establish the role of the insula in the process of many local and distal threatening bodily sensations and help to either predict or uncover new roles. More

importantly, the structural-functional relationships of the insular cortex will be better understood. There are several dissimilarities in the results across single site studies where the functional connectivity of the insula has been investigated during task induction conditions or task-free (resting state functional connectivity) periods (Figure 1.9).These variations are largely due to participant selection and number, protocol, study design and data analytical methods. In-depth meta-analyses of databases of neuroimaging studies and the Functional Connectomes Project (a database containing both resting-state and functional state neuroimages pooled from a large number of healthy subjects for in-depth analysis) (Biswal *et al*, 2010) can provide a stronger technique for exploring insular functional connectivity.

In a study by Deen *et al*, (2011), two different types of fMRI (resting-state and task-activated) studies were used to investigate subdivisions within the insula. The authors identified three functional subdivisions based on shared distinct connectivity pattern. These are(i) a posterior area functionally associated with primary and secondary somatomotor areas (ii) a dorsal anterior to middle region, linked with dorsal anterior cingulate cortex, along with additional areas of a control network, previously described; and (iii) a ventral anterior region, predominantly associated with pregenual ACC. Although these three functional subdivisions have similar connectivity patterns, there exists difference in their anatomical boundaries. Two other meta-analysis studies used existing functional and structural imaging modalities to investigate subdivisions within the insula and findings consistent with the above (Kelly *et al*, 2012; Chang *et al*, 2013). The two resultant insula images were approximated from the study on a sagittal MRI scan to aid visual comparison. The imaging techniques used and the findings in the above studies indicate further brain regions that can be explored in studies of dyspnoea.

Various functionally and individually grouped tasked connectivity studies have used anatomical subdivision information generated from the resting state connectivity studies to established specific, or a wider range of, functional domains within the insula (Kelly *et al*, 2012; Kurth *et al*, 2010; Uddin *et al*, 2014). However, no one particular region has been identified to be responsive to all functional activation. This highlights the multimodal and integrative properties of the insular cortex (Uddin *et al*, 2014).

The mid-insula is thought to combine information from the higher sensory cortices and the limbic structures with interoceptive signals to register a range of emotionally related and homeostatic information (Craig 2009; 2010). This and many other proposed insular functions have made it increasingly difficult to pin point its direct and indirect roles in the process of breathlessness perception in the higher cortical region. More robust and integrative studies are needed to give a fresh perspective to its functions.

Neuroimaging measures of functional connectivity are based on using time series data to identify areas that are changing in activity at the same time and in the same way as the area of interest (seed region of interest or voxel). Two types of fMRI studies have been used to divide the insula into functional subdivisions based on shared patterns of functional connectivity: resting state and task-activated studies.

In resting state fMRI studies, considered to be measuring fundamental connectivity, the participants are simply relaxing. Although three functional subdivisions (tripartite) of the insula are the commonly reported pattern, the borders between subdivisions differ.

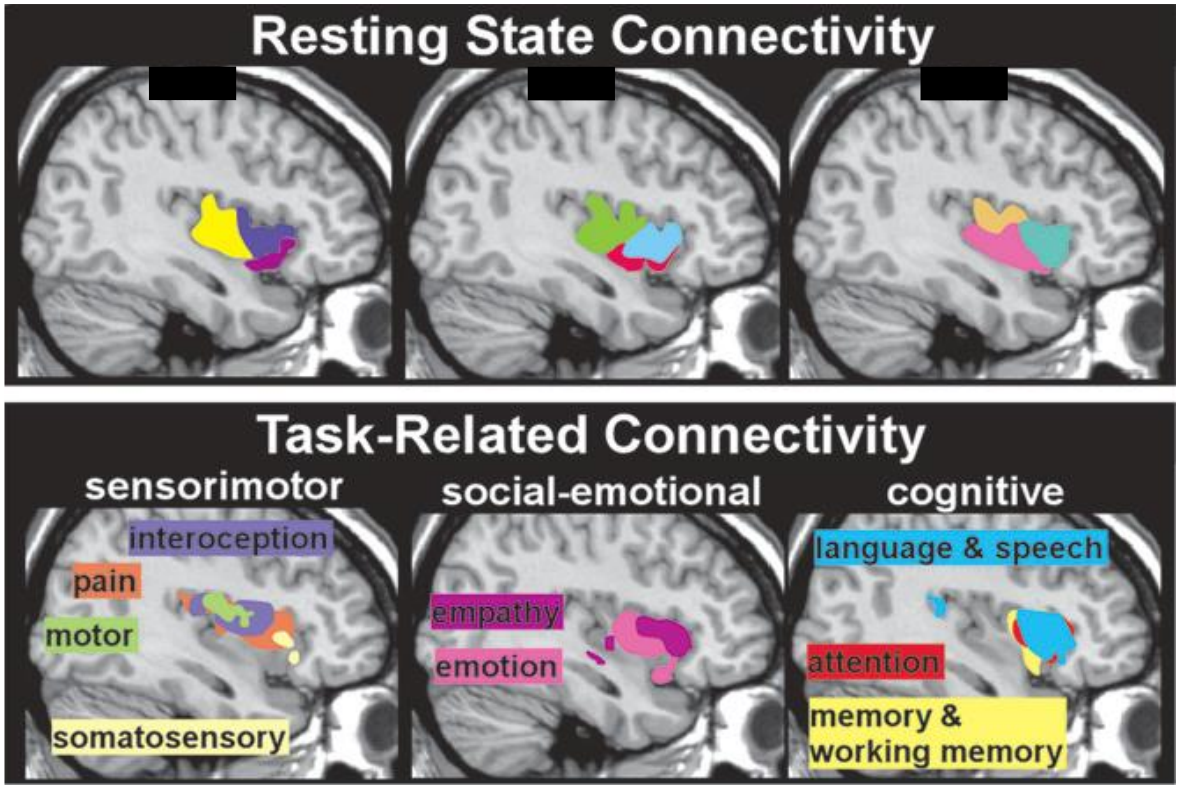


Figure 1.9: Measurement of functional connectivity within the insular cortex. (*Top row*) Intrinsic connectivity in the insular cortex measured in resting state by fMRI when the participants are in relaxing state. (*Bottom row*) Functional subdivisions of the insular cortex are identified in task stimulated fMRI studies where participants performed a specific task. These correlational functional maps are centred on a recent meta-analysis that divided the insula into subdivisions with functional specificity by organising several tasks into four functional areas. The areas activated by each task for the three areas are estimated on sagittal MRIs. Even though the three functional subdivisions are the oftenreported pattern, there are variations between borders of the subdivisions. The different regions reported in a single site studyandin two meta-analysesareestimated on sagittal MRIs to offer a rough visual assessment. (Reproduced from Shura *et al*, 2014).

1.7.4 Clinical implications of lesions restricted to the insularcortex

The insula plays an essential role in amalgamating both sensory and psychological signals to register perceptual or visceral sensations, resulting in conscious or unconscious emotional responses. The insular cortex has been implicated in many sensory, neurocognitive, motor and emotionally-related and neuropsychiatric disorders(see Jones *et al*, 2010 for extensive review). One such notable implication of the insula function was highlighted by the work of Berthier *et al*, (1988). These authors reported that the insular

cortex in a pain patient was found to be damaged resulting in changes in both the intensity and the affective component of the pain perception. Information from the above studies led to earmarking the insular cortex as a core region in the pain perception network. Various neurodegenerative disorders such as Alzheimer's disease and frontotemporal neurocognitive disorder have been associated with structural damage to the anterior insular cortex (Santillo *et al*, 2013). A number of other neuropsychiatric disorders, such as schizophrenia, have been found to substantially decrease tissue volume in the posterior part of the insula but direct evidence is needed from more studies to establish any clear clinical presentations. Also lesion restricted to the insular cortex as a result of ischaemic stroke has been found to produce different clinical presentations in a study by Lemieux *et al*, (2012),thus making the insula a complex brain area in breathlessness study.

1.7.5 Brain correlates of breathlessness

Most of our current understanding into the cortical network believed to be involved in the perception of breathlessness sensation is provided by studies involving the use of functional fMRI and PET studies in healthy volunteers. Evidence from early published PET, and blood oxygen level dependent (BOLD) fMRI studies have underlined the relevant range of several cortical areas when acute laboratory-induced breathlessness was present in a small number of healthy individuals (Corfield *et al*, 1995a, and 1995b; Liotti *et al,* 2001; Peiffer *et al,* 2001; Liotti *et al*, 2001; Banzett *et al,* 2000; Parsons *et al,* 2000; Raichle *et al,* 2001; Evans *et al,* 2002 ;von Leupoldt *et al*, 2008).

Principal activation was reported in these studies to be in the anterior insular (extending to the operculum), cerebellum, amygdala and the anterior cingulate cortex. Other activation areas included the thalamus and the supplementary motor areas (SMA). Activation of the

anterior insular cortex was consistently reported in all studies, subsequently providing an insight into the neuroanatomy of dyspnoea.

The problem of fMRI data interpretation is further compounded by the demonstration of functional differentiation within the anterior cingular cortex, the insular cortex and the periaqueductal grey (PAG) (Matthews *et al*, 2004; Kurth *et al*, 2010, Linnman *et al*, 2012) which have been implicated in breathlessness perception (Evans *et al,* 2002). The difference in functional activation in these areas raises more questions concerning their excitatory and inhibitory roles in breathlessness sensation.

Currently, the direct brain structural area(s) involved in the processing of breathlessness sensation has not clearly been defined. However, early animal studies on the role of the PAG and other structures within the brain stem in respiratory control underlined their importance in modulating breathing (Subramanian *et al*, 2008, Subramanian, 2013). Comprehensive structural neuroimaging studies of the higher brain regions involved in breathlessness perception in both healthy subjects and patient populations are limited. Evidence from a structural brain imaging study in patients with mild to moderate asthma found a significant increase in the grey matter volume in the PAG, but not in the insular cortex, relative to the disease duration (von Leupoldt *et al*, 2011). In addition, a reduction in the unpleasantness ratings recorded by induced dyspnoea correlated with the increase in grey matter volume in the PAG. In another separate imaging study using diffuse tensor imaging (DTI) technique in COPD patients with stable oxygen levels, a reduction in the integrity of the white matter microstructure was found throughout the brain and widespread disturbance in functional grey matter activation was seen compared to control subjects (Dodd *et al*, 2012). These findings, according to the authors, accounted for the observed difference in cognitive function between the patient with COPD and the control subjects.

Whilst the results from the above studies give a closer understanding of the brain mechanism underling breathlessness perception in clinical conditions, it does not fully define the specific brain structures that could be involved in the perception of breathlessness sensation. However, the potential of structural neuroimaging to further assist in defining higher brain regions that could be actively involved in processing pure breathlessness perception signals from other visceral sensations is very strong.

1.8 PSYCHOLOGICAL MODULATION OF BREATHLESSNESS PERCEPTION

Respiratory symptoms have long been known to be influenced by psychological factors such as anxiety, panic attacks, fear, depression and other emotional responses (Parshall *et al*, 2012; De Peuter *et al,* 2004). However, the extent to which it occurs is not known, and the underlying mechanism remains understood.

The insular cortex has been identified to process emotionally-related sensation and sensory sensations; therefore it is very important to distinguish pathways for which they are independently modulated. Different components of psychological factors such as anxiety, depression or anger have been found to be commonly associated with breathlessness resulting from hypoventilation, hyperventilation and patients with emphysema and other forms of COPD (Barstow 1974; Dudley *et al*, 1968). Studies conducted on healthy individuals and on asthmatic patients or COPD patients have shown that emotional sensations can substantially influence the perception of breathlessness sensation. Positive sensations reduce the level of perceived breathlessness, whereas unpleasant emotions generally heighten the perceived level of breathlessness, irrespective of the type of induced breathlessness sensation during experiments (Janssens *et al*, 2009; Livermore *et al*, 2012).The precise interactions between these factors to either heighten or

decrease respiratory sensations are complex. An in-depth review on this topic is given in Chapter 3 of this thesis.

1.8.1 Sensations of breathlessness and psychological effects

The response to the intensity and unpleasantness of breathlessness perception is known to be closely shaped by various factors such as individual subjectiveexperience,anticipation, social interactions and emotional conditions. These factors have been shown to influence the qualities of breathlessness in many different ways at a given level of respiratory challenges (Dudley *et al*, 1968; Guenard *et al*, 1995).This follows findings in the study of pain where perception is found to be heightened by previous experiences of pain (Papageorgiou *et al*, 1996; Singer *et al,* 2001) and level of anxiety (Sternbach, 1968; Grachev *et al*, 2001). The most commonly identified factor in all the studies was anxiety. For example, pain severity and affective changes have been shown to be predicted by anxiety levels in acute chronic pain patients (Kain *et al*, 2000; van den Hout *et al*, 2001). Also, the application of anxiety-reducing procedures has been reported to be successful in reducing pain related to medical procedures (Suls and Wan, 1989). A growing amount of evidence proposes that similar psychological factors, such as anxiety, panic and stress, influence the perception of respiratory symptoms such as breathlessness or breathing discomfort (Maurer *et al*, 2008; Scott *et al*, 2007). The impact of some of these factors on breathlessness have been highlighted in few studies both in healthy subjects and in certain respiratory patient groups such as those with asthma and COPD (Von Leupoldt and Dahme*,* 2007; Maurer *et al*, 2008; Van den Bergh *et al*, 2004; Gracely *et al*, 2007; Meek, 2000). The positive relationship between anxiety and reported perception of respiratory sensation has extensively been explored in one experimental study in healthy subjects (von Leupoldt *et al,* 2011). In this study, the authors concluded that anxiety is related to the increase of respiratory sensations, thus representing a neural mechanism that may underline its increase.

1.9 DO PAIN AND BREATHLESSNESS SHARE NEURAL PATHWAYS?

1.9.1 Similarities between pain and breathlessness

Although research in pain perception is more advanced than dyspnoea, emerging evidence from separate studies has reported similarity between them (Banzett and Moosavi 2001). This establishes the possibility of the existence of common neural pathways between pain and dyspnoea. Both are known to produce unyielding sensations with different sensory afferents and affective domains, which can be measured separately on a scale with a subjective outcome. This may be modulated by psychosocial factors where their extent of influence is yet to be determined. Complaint of dyspnoea is on par with pain among hospital patients, with a similar contribution to rising medical cost and health care resource use (Desbiens *et al,* 1997). In recent functional brain imaging studies using fMRI and PET techniques, areas activated by pain and those by dyspnoea have been shown to be similar, supporting the idea that these different unpleasant sensations share common neural networks. In addition, recent studies of patients with damaged insular cortex have suggested that dyspnoea sensitivity is reduced by this (Schon *et al,* 2008), as has been shown for pain (Berthier *et al,* 1988; Greenspan *et al,* 1999).

The pain multidimensionality concept has been applied to recent research on dyspnoea with some success. Indeed, the emergence of multidimensional dyspnoea tools within the last few years was based on the existing multidimensional tools already available for pain assessments.

1.9.2 Differences between pain and breathlessness

Pain and breathlessness share similar characteristics in many ways (Banzett and Moosavi, 2001). They are both unpleasant sensations and have affective and emotional components. However, differences in the cerebral mechanisms of pain and breathlessness have been noticed in few studies. A review by Gracely *et al*, (2007) has pointed out these differences.

Data from one study where pain and dyspnoea were compared in one experiment, showed slightly higher inspiratory time in dyspoeamic stimulus than in pain (von Leupoldt and Dahme, 2007). The study also reported a slight increase in end-tidal carbon dioxide ($PETCO_2$) in the dyspoeamic stimulus than painful stimulus. Furthermore, differences between pain and breathlessness could be further explored using a more powerful functional imaging equipment such as a 7 Tesla MRI and magnetoencephalography (MEG) since the resolution of currently available 1.5 Tesla MRI brain imaging tool may not be sufficient to detect differences in the cerebral network for pain and dyspnoea. Although it is now accepted that dyspnoea is as multidimensional as pain, and the newly validated multidimensional tools have the potential to improve dyspnoea assessments, there are subtle differences that must be noted. While the different types, intensity and unpleasantness domains of dyspnoea are largely analogous to features of pain, there are certain aspects of the pain experience that do not translate to dyspnoea; e.g. patients are usually able to locate their pain but this is not possible for dyspnoea.

1.9.3 Interactions between pain and breathlessness

Breathlessness and pain have clinically been observed to be co-present in patients of different disease groups. This places more emphasis on understanding the commonality of their neural pathways. Evidence from a study that investigated the effect of pain and dyspnoea on each other simultaneously, reported that an increase in pain stimulation increases dyspnoeic visual analogue scale (VAS) score, and *vice versa* (Nishino*et al,* 1999). Perhaps the most remarkable evidence was presented by Morélot-Panzini et *al* (2007), who demonstrated that endogenous analgesia can be elicited by the concomitant presence of dyspnoea.

1.10 NOVEL APPROACHES TO STUDYING BREATHLESSNESS PERCEPTION

1.10.1 Neuroimaging

Modern neuroimaging techniques have provided the opportunity for non-invasive *in vivo* measurement of local neuronalactivity of the human brain and occupy a unique position between research and clinical practice. This has helped to dissociate several cognitive processes such as movement, emotions, language, intelligence, memory and thoughts. They are however, underutilised in studies which quantify physiological sensations such as breathlessness. Currently, there are several universally accepted and safe neuroimaging methods available in use in hospitals and research facilities. Although established neuroimaging methods have expanded our knowledge on the cortical representation of breathlessness perception, recorded information from other alternative neuroimaging methods can be an added benefit to the field. Neuroimaging methods can be considered as direct, indirect, functional, structural and to lesser extent chemical (Friston, 1994, 2011).

1.10.2 Functional neuroimaging

The global application of functional neuroimaging methods to obtain quantitatively, direct or indirect physiological information from neural activities in the brain has expanded over the years. Despite the better temporal resolution and easy access to study human subjects, fMRI and PET neuroimaging of breathlessness, data are mostly limited to healthy subjects, thus making any reasonable conclusion of a particular focal cortical region being the seat of breathlessness perception very difficult. This, among other factors such as variation in method application across study and data analysis, may delay the transfer of acquired information from healthy subjects to clinical practice. To date, only a few fMRI and PET studies have investigated breathlessness in different patient population such as asthma (Rosenkranz *et al*, 2005; Rosenkranz *et al*, 2012; von Leupoldt *et al* 2009; von Leupoldt *et al*, 2009). The procedure and practical concerns such as the psychological state of the

patient in relation to respiratory stimulus manoeuvres may account for the lack of data in the patient population. Furthermore, the different disease states within and between patient conditions may modulate the distributed cortical network already perceived to be involved in breathlessness perception from healthy subjects studies in the patients, hence making data interpretation difficult.

Although the most commonly used functional neuroimaging, such as fMRI, has advanced our knowledge about the possible distributed cortical representations of acute breathlessness mechanisms, a robust study protocol favourable to study chronic dyspnoeic patients will be a step forward to fully understand the overall neurophysiologic atlas of breathlessness sensation in the human brain.

1.10.3 Structural neuroimaging

Structural imaging can be used to see if there are any differences in brain structure between patients with breathlessness and healthy controls. Several experimental studies have taken full advantage of this imaging technique to study the relationship between changes in the brain with chronic respiratory diseases versus healthy subjects and have reported some interesting findings (von Leupoldt *et al*, 2011; Esser*et al*, 2016). von Leupoldt and colleagues used an MRI to study changes in the brain white and grey matter structure with asthma disease duration. They reported a positive correlation between changes in the grey matter as the disease progresses. In another study, Esser and colleagues (2016) reported a decrease in grey matter in the brain regions essential to dyspnoea perception, especially the posterior cingulate cortex in 30 COPD patients. Dodd *et al*, (2012) used novel imaging techniques (DTI and resting state functional magnetic resonance imaging (rfMRI)) to investigate the structural changes in white matter of COPD patients and compared with healthy control subjects. The study reported a reduction in white matter integrity and widespread functional disturbance in the grey matter of COPD patients compared to the

healthy control subjects. Although, this technique has been shown to possess great potential as a tool to further enhance our knowledge about the neural mechanism underpinning breathlessness perception, it has limitations, which need to be addressed. The limitations are that, in those few studies, patients may have comorbidities that are not controlled for. Also, it is uncertain what brain structural differences really tell us without additional behavioural or functional data.

1.10.4 Tractography

Tractography is a 3D model method to visually represent, localise and estimate essential white matter fibre tracts in human brain *in vivo* using a special imaging technique known as diffusion tensor imaging (DTI). Pre and post-surgical diffusion tensor magnetic resonance imaging (DT-MRI or DTI) is currently widely used to study white matter connections (Basser *et al,* 1994; Assaf *et al*, 2008). This is able to address clinical problems since its introduction in 1994 and to quantitatively measure white matter tracts in the human brain without any enhancing agents. Generally, the concept takes advantage of the translational movement of water molecules in certain directions. It has been demonstrated that water diffusion along white matter fibres is much faster in restricted environments compared to unrestricted ones (Basser *et al,* 1994; Basser *et al*, 1996). Predominantly, tractography has become a useful clinical tool during pre-surgical planning in patients with brain tumours or lesions, to preserve other essential white matter tracts during surgical procedures and minimise any functional deficit. DTI tractography can be explored as a novel research tool to study white matter tracts in relation to the perception of breathlessness (Schonberg *et al*, 2006).

1.10.5 Lesion-deficit study

Reservations regarding the interpretation of imaging studies make them difficult to rely on in establishing brain structure-function relationships(Banzett *et al,* 2000; Evans *et al,*2002).

Determination of brain function in the early years of clinical research has been through animal studies and lesion-deficit methods. Lesion-deficit methods have been instrumental in the development of our understanding into the functional relevance of specific brain regions over the past decades. Testing dyspnoea sensitivity in ischaemic stroke patients where the damaged brain area predominantly involves the insular cortex will offer another alternative observation and may also indicate whether activation of these areas increases or decreases dyspnoea. Although the lesion deficit method remains very popular among researchers, its limitations - such as the possibility of brain re-generation and precise lesion location and volume - have led to the recommendation that both functional imaging and the lesion deficit techniques are used to complement each other for better results (Price and Friston, 2002).

1.10.6 Deep brain stimulation

Deep brain stimulation (DBS) surgery in specific areas of the brain such as the periaqueductal grey (PAG), sensory thalamus (ST), the subthalamic nucleus (STN), globulus pallidiun (GPi) and the anterior cingulate cortex (ACC) have recently been shown to relievetreatment-resistant pain, tremor in Parkinson disease, dystonia and epileptic seizures (Owen *et al,* 2007). Another novel way to investigate breathlessness perception would to study the changes in currents generated from the electrodes in patients with DBS. In deep brain stimulation patients, local field potential (LFP) can be recorded from the implanted electrode whilst AH is induced by increasing CO_2 with fixed breathing. The role of AH in LFP changes can be determined with the appropriate control in the future. This will provide the opportunity to investigate the role of these areas in dyspnoea perception directly from the brain. Similarities have been noted between pain and dyspnoea perception (Banzett and Moosavi, 2001) raising the possibility that these two unpleasant feelings may share a distributed cerebral network.

1.10.7 Surgical resection – low grade insular gliomas

Surgical resection is the removal of a tissue from the body aimed to improve both physical and clinical function. One example of such practice is tumor in the brain especially the insular cortex. Assessing the effect of insular gliomas on breathlessness perception using an established AH breathlessness model before and after surgical removal of insular gliomas (tumours affecting the insular cortex in the brain) may increase our knowledge of the damaged site in the brain perceived to be essential for breathlessness perception. Although the effect of surgery on other brain functions such as motor movement and cognitive responses has been investigated (Wu *et al,* 2011; Sanai *et al,* 2010), no study has yet investigated breathlessness perception in these patients. Insular glioma patients are perfect candidates to better our understanding of the insular involvement in breathlessness perception from the structural point of view, as the insular cortex has been shown to be activated in breathlessness in several recently published functional imaging studies (Banzett *et al*, 2000, Evans *et al,* 2002).

1.11 STUDY RATIONALE/HYPOTHESIS AND AIMS

Recent functional brain imaging studies have identified several sites activated by breathlessness in healthy subjects when dyspnoea was present, predominantly the anterior insular (extending to operculum), cerebellum and anterior cingulate. However, activation when dyspnoea is present could be incidental rather than essential for the perception of dyspnoea. One of the potential confounding factors with functional brain imaging, particularly the fMRI technique, is that the procedure generates considerable psychological stress such as claustrophobia. Thus the question remains to what extent the activations detected by fMRI are linked specifically to dyspnoea perception and which might be related non-specifically to anxiety brought on by the fMRI environment. This led to the

hypothesis that ***perceptual sensitivity to experimentally induced breathlessness will be greater during MRI brain scans due to greater anxiety associated with the MRI milieu***.

Neurological patients undergoing DBS of various brain nuclei with surgically implanted electrodes (for treatment of a variety of clinical symptoms including pain, tremor epilepsy) form a hitherto untapped clinical model to explore cerebral mechanisms of dyspnoea. Having an alternative approach to functional brain imaging will allow interrogation of the interpretation of brain imaging studies of breathlessness.

In the current study, guided by anecdotal evidence from neurosurgeons,a second hypothesis was generated in this thesis which postulated that ***patients with DBS of motor-thalamic (VIM and VOP) will experience heightened breathlessness when the electrodes are stimulated (switched 'ON').***

The specific aims to address these hypotheses are stated in the individual experimental chapters.

1.11.1 CLINICAL SIGNIFICANCE OF THE STUDY

Chronic obstructive pulmonary disease (COPD) is a fast growing chronic condition in the world. Breathlessness is a cardinal feature of COPD and nearly all other heart and lung conditions including asthma, lung cancer and chronic heart failure. Unlike for pain, there are no effective treatment options (that do not carry dangerous side-effects) to reduce breathlessness and improve quality of life when the underlying condition is incurable. We now know that clinical dyspnoea comprises several unpleasant qualities which probably arise from different underlying mechanisms depending on the pathophysiology. This complexity provides a diagnostic challenge to clinicians and makes appropriate treatment choice very difficult. While it is accepted that the breathlessness mechanisms can be considerably modulated by psychosocial, physiological and environmental factors such as

anxiety, hostility, depression, stress of inadequate social support, threat of job loss and exposure to disasters (e.g. wars), the extent to which this is true is yet to be determined. Understanding the neural mechanisms of breathlessness may help to distinguish brain areas that process breathlessness sensation and those related to general psychosocial symptoms such as anxiety, stress, panic and depression and thereby improve the interpretation of brain imaging studies of breathlessness. This will in turn, improve diagnosis and ensure tailored treatment or management option for patients when the underlying condition cannot be treated.

1.12 ORGANISATION OF THESIS

The thesis is organised as follows:

Chapter 1 provides the reader with the general introduction into the thesis. This comprises the relevant literature review and background of all the studies undertaken in this thesis.

Chapter 2 captures the general methodology emphasising the multidisciplinary nature of the study merging physiological, psychological and neurologically techniques.

Chapter 3 presents a study which explores the effect of psychological modulations on breathlessness perception using a mock brain imaging scanner.

Chapter 4 describes the use of DBS as an investigative tool to explore the neural mechanism of breathlessness perception (air hunger component). This is the first study to have used DBS as a clinical model to investigate breathlessness mechanism.

Chapter 5 presents a study where the DTI tractography technique was used as a novel way to better understand the anatomical connectivity of white matter tracts in the brain in relation to air hunger perception and to specifically compare the patients in Chapter 4 who found air hunger relief with DBS ‘ON’ with those who got relief with DBS ‘OFF’ .

Chapter 6 summarises the important findings in the thesis, draws conclusions which offer new insights into the cerebral mechanisms of breathlessness and provides guidance on future studies.

CHAPTER 2

GENERAL METHODOLOGY

2.1 INTRODUCTION

Discussion of methodology employed in this thesis is divided into 3 major sections:

(1) Physiological methods, with particular attention to the experimental model of air hunger which is the most unpleasant component of clinical dyspnoea. This model involves the systematic induction of graded levels of air hunger in healthy volunteers using hypercapnia and constrained ventilation. This is now a much used, well established and validated technique.

(2) Psychological and psychophysical techniques. The experimental model of air hunger includes psychophysical methods, established in their own right and used extensively in other fields such as pain perception, and now proving to be just as valuable in studying breathlessness. Given that the respiratory pump is subjected to control by both an automatic controller in the brainstem and by behavioural control from higher brain regions, this makes the system particularly prone to modulation by psychological factors.

(3) Neurological and brain imaging techniques. The novelty of this thesis rides on its multidisciplinary nature. This is particularly evident in connection to the use of new techniques in neuroscience for stimulating and recording from cortical and sub-cortical sites for both therapeutic and experimental interventions.

2.2 PHYSIOLOGICAL METHODS

2.2.1 The need for a robust experimental model of clinical dyspnoea

Breathlessness consists of different types of sensation known to arise through different neurophysiological mechanisms, thus making its assessment in both clinical and research settings challenging. Conducting hypothesis driven or intervention studies in breathless patients is fraught with difficulties:

(1) Breathlessness affects patients with varied clinical underlying conditions which presents a challenge in pin-pointing the source of the sensation.

(2) Clinical dyspnoea is known to comprise multiple sensations including air hunger, chest tightness and sense of breathing effort (Parshall *et al*, 2012; Simon *et al*, 1989; Elliot *et al*. 1991). Since different components of dyspnoea are likely to arise from different afferent sources, this makes interpretation of clinical studies highly problematic.

(3) Disease trajectory may vary considerably (Booth *et al*, 2008). Exacerbations of the disease can be associated with changes in the quality and the intensity of dyspnoea. For example an asthma attack starts predominantly with a sense of chest tightness but this is overtaken by the sense of breathing effort as the patients recruit accessory muscles to overcome the airway collapse finally leading to intense air hunger if treatment with bronchodilator is delayed or ineffective (Moy *et al*, 1998)

(4) Hypothesis driven experiments to test new interventions often require a stable level of breathlessness in order to ascertain the specific effect of the intervention.

(5) Studies of dyspnoea mechanisms in patients with clinical dyspnoea are often confounded by co-morbidities and effects of medication.

To circumvent many of the issues identified above, reliable, safe, reproducible experimental models of the different components of clinical dyspnoea induced in healthy volunteers were established. Thus chest tightness can be induced in healthy volunteers

through methacholine or histamine challenges (Binks *et al*, 2002), sense of breathing effort can be specifically induced by targeted hyperventilation with an inspiratory resistive load (Banzett *et al*,2008) and air hunger can be generated systematically with hypercapnia while ventilation is constrained to the resting level (e.g. Moosavi *et al*, 2007). While these methods have been used successfully to advance our knowledge of dyspnoea mechanisms, how equivalent the induced sensations are to components of clinical dyspnoea is still open to question. A recent study by O'Donnell *et al* (2013) has suggested that experimentally induced dyspnoea in healthy volunteers is quantifiably less than that in COPD patients but both lie on a continuum. The current thesis focussed on the experimental model of air hunger as this component of dyspnoea has been shown to be the most unpleasant (Banzett *et al*, 2008).

2.2.2 Experimental model of air hunger

Hypercapnia with constrained ventilation: Subjects breathed a gas mixture that was mostly air but with some added oxygen and carbon dioxide (CO_2) while their breathing was restricted to their normal resting level. Breathing with restricted tidal volume and added CO_2 will give the subject varying amounts of breathlessness. This method of inducing breathlessness is well established in several labs around the world (Moosavi *et al*, 2004). The amounts of CO_2 added to the inspired air at any time did not exceed the amount being expired so that subjects did not detect ('taste') the CO_2. The total amount of CO_2 added was limited to an amount that would not raise end-tidal PCO_2 (a non-invasive estimate of arterial $PaCO_2$; Asmussen and Nielsen, 1956; Whipp and Wasserman, 1969; Robbins *et al*, 1990) by more than 20mmHg from the normal resting level of 40mmHg. Most subjects reach the top of the visual analogue scale of breathlessness (i.e. intolerable level) by the time end-tidal $PaCO_2$ rises by only half this amount. Thus only small changes in inspired

fraction of CO_2 are enough to generate a stimulus-response relationship between CO_2 and the level of air hunger that is rated by the subjects.

The validity of using end-tidal PCO_2 measurements as an indicator of arterial $PaCO_2$ depends on certain criteria; (i) there must be a good plateau on the continuous $PaCO_2$ waveform measured at the mouth. If this condition is satisfied, the plateau level of $PaCO_2$ can be taken to be a good measure of alveolar $PaCO_2$. (ii) There should not be any diffusion defects or other clinical factors affecting gas exchange in the lungs. Thus, the assumption that end-tidal PCO_2 is equivalent to arterial $PaCO_2$ must be treated with caution if studying patients with lung disease. In Chapter 3 only healthy volunteers were studied. In Chapter 4, one of the DBS patients studied had co-existing COPD but was not subjected to breathing tests (involving end-tidal $PaCO_2$ measures). It is also well known that raised inspired CO_2 can lead to autonomic side effects. To check this, previous data collected within this laboratory for other projects (not related to the studies included in this thesis) which involved 13 healthy volunteers was examined: this data indicates that on average the end-tidal $PaCO_2$ has to be raised above 50 mmHg before any rise in blood pressure is detected (Figure 2.1).

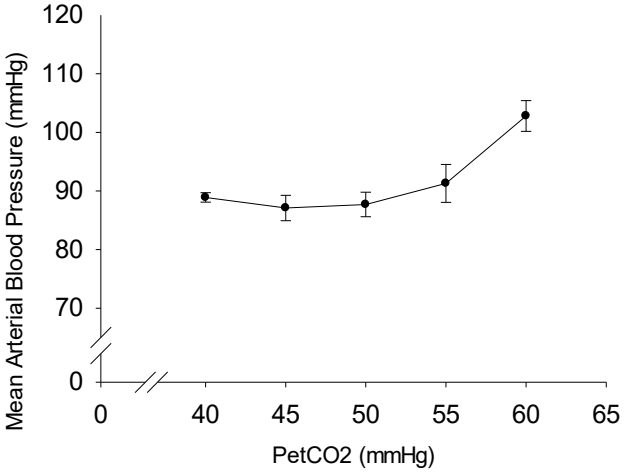


Figure 2.1: Blood pressure changes in response to hypercapnia. Data collected from three previous studies of air hunger in this laboratory (not related to the studies included in this thesis). Total number of healthy young adult male subjects was 13 with mean±sd age 24±3.6years. Data show that mean arterial blood pressure increased only after $PaCO_2$ rose beyond 50mmHg.

The composition of the inspired air was modified by mixing (Sechrist air-oxygen mixer, USA) the gas from a cylinder of medical air and a second cylinder containing 10% CO_2, 21% O_2, balanced N_2 (BOC, England). This gas was heated and humidified (Fisher and Paykel HCL150) prior to feeding a reservoir of inspired air in a 3 litre anaesthetic bag. Subject's respiratory rate (f_R) was fixed and kept constant by breathing to a metronome set at 12 breaths per minute. Subjects breathed from a 3 litre anaesthetic bag that served as a reservoir of heated humidified fresh gas (Figure 2.2). A flowmeter was used to set initial flow into the reservoir at a level matching the subject's resting alveolar ventilation (i.e. bag just collapsed with each breath). One-way breathing valves (Hans Rudolph**,** model No.5710, USA) were used to separate inspiration from expiration.

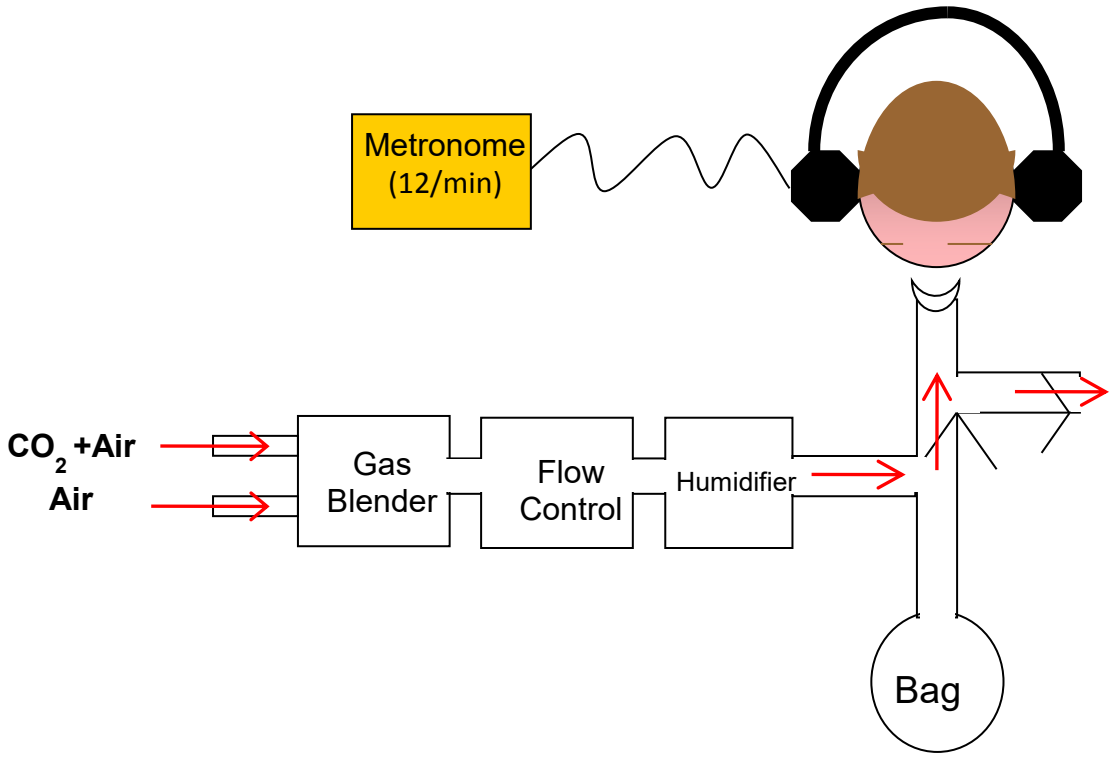


Figure 2.2: Inducing experimental air hunger set up. A gas blender was used to produce the required CO2 – medical air mix. A flow-meter regulated the rate at which this air mixture filled the anaesthetic rubber bag from which the subjects had to breathe. Subjects expired out through a one way valve. Subjects had to breathe in time with a metronome which determined their respiratory frequency. Ventilation was limited by the bag collapsing when the subjects reached the required VT ('bag limited' method).

2.2.3 Physiological measurements and recording

Ventilatory measurements: Tidal volume was derived by electronic integration of the airflow signal from a pneumotachometer (Fleisch type #2) attached to the mouth piece. PCO2 was measured continuously using a fast responding infra-red capnometer (BCI, capnometer, Smith Medical –see Chapter 4; ADInstruments gas analyser, Oxford-see Chapter 3) via a small bore sampling tube inserted in the mouthpiece. This was derived from expired PCO_2 recorded continuously by fast-responding infra-red sensor (Applied Electrochemistry P-61B) through a fine bore catheter inserted into the mouth piece. Changes in airway pressure ("Pmouth'') were measured by a pressure transducer (Validyne DP45-30) through a second fine-bore catheter inserted into the mouth piece. This signal was used as a measure of how much subjects were pulling on the collapsed inspiratory reservoir bag during constrained ventilation. If they over exerted themselves in an attempt to derive more air from the collapsed bag this could lead to sensations of respiratory effort so subjects were instructed to avoid this. All analogue signals were sampled at 20Hz, by an A-D converter (CED Micro 1401, Cambridge, UK) and recorded on a computer for off-line analysis (Spike 2 version 6.13 software, CED, Cambridge, UK).

Safety monitoring: To ensure the safety of all subjects and patients, the following important physiological parameters were monitored, measured and recorded during the experiment.Blood pressure (BP) was automatically and non-invasively recorded every two minutes via an inflatable cuff on the upper arm (Datex-Ohmeda, F-CM1-04 in chapter 3) or a beat by beat continuous BP device via a finger probe (Portapres - Finapres Medical Systems-UK, for the study reported in Chapter 4). Electrocardiogram (ECG) traces were derived from a 3-lead ECG electrode attached to the chest (HME Lifetrak). Arterial oxygen saturation (SpO_2) was monitored continuously using a capnometer and pulse oximeter (Nellcor) through a finger probe with an inbuilt infra-red sensor. Experiments were

stopped if there were any cases where SpO2 fell below 95%, BP rose above 150mmHg, and heart rate rose above 150 beats per min or frequent ectopics developed on the ECG trace.

2.2.4 Quality control procedures

Calibrations: All essential recorded respiratory and physiological parameters were calibrated at the beginning of each experiment using an inbuilt script command in spike 2 version 6.13 to ensure accuracy and precision in data collection.

Sterilisation and disinfection: To prevent cross infection and minimise the risk of contamination between subjects, the breathing circuit was dismantled after each experiment and soaked in an antiseptic solution for at least 10 minutes. Thecircuit was then thoroughly rinsed and left to dry.

Checking for leaks: Prior to each experiment, the circuit was checked for leaks by using a 3 litre syringe to try to 'inspire' from the mouthpiece while the inspiratory gas supply was closed off.

2.3 PSYCHOLOGY AND PSYCHOPHYSICAL METHODS

2.3.1 Rating scales

Visual Analogue Scale (VAS): The different sensations involved in breathlessness perception has required the use of a comprehensive and reliable measuring scale, yet current available validated rating scales fall short in many ways. However, few recently published systematic reviews on the existing measuring scales have acknowledged the need to use them to complement each other since none are considered most favourable (Dorman *et al,* 2007; Bausewein *et al,* 2008). This is due to an observed overlap between rating interpretations and the underlying pathology of patients with different clinical conditions such as COPD and cancer (Wilcock *et al,* 2002). The most recognised devices

are the Visual Analogue Scale (VAS) which has proved consistent within the same subjects on various occasions, the Numerical Rating Scale (NRS), which is preferred in pain assessment than VAS and the Modified Borg Scale (MBS) which has the advantage of being more reproducible than VAS. Their respective rating scores inform the severity, unpleasantness and the state of quality of daily life of the dyspnoeic patient. These tests are more widely used in pain assessment compared to dyspnoea. Even though they are thought to be convenient and user friendly, they present different strengths and limitations under different operational conditions, hence the need for constant evaluation.

All subjects and patients were cued to rate their breathlessness sensations every twenty seconds by Light Emitting Diode (LED) light indicator. Ratings were made on a 100mm vertical VAS, controlled by a 100mm linear potentiometer. The limits of the scale were labelled "none'' (indicating no sensation) and "extreme'' (indicating their tolerable limit). Word labels of "slight'', "moderate'' and "severe'' were also added to the scale at locations selected by the subjects –this is thought to help subjects remember how much of the scale means how much sensation from one occasion to the next (Lansing *et al*, 2003). Extreme ratings activated an alarm, and the stimulus was immediately reduced to a tolerable level if this occurred.

2.3.2 Questionnaires

Several disease-specific questionnaires are currently used in dyspnoea measurement to complement the use of the rating scales. These questionnaires are divided into activity-based and multidimensional. The most frequently used activity-based questionnaire in clinical setting in the United Kingdom is the Medical Research Council Scale (MRC), which assesses the impact of dyspnoea on the activity in daily life (Fletcher *et al*, 1959; Bestall*et al*, 1999. Other questionnaires considered to be multidimensional in its application are, the Cancer Dyspnoea Scale (CDS), Chronic Respiratory Disease

Questionnaire (CQR), the SF36, St. George's Respiratory Questionnaire and recently developed Dyspnoea-12 questionnaire (Yorke *et al*, 2010). Integrating these questionnaires is necessary to give dyspnoea research a more focused direction.

Multidimensional dyspnoea questionnaire: In this thesis the Dyspnoea-12 assessment tool has been used as it is simple and quick to use and provides a total score for dyspnoea that includes the physical and affective domains. While it was not designed to measure physical and affective components separately, it was possible to make these measurements to see if any changes were predominantly related to one or other dimension.

Structured interviews and debriefs: Volunteered comments from individuals who experienced either clinical or experimental breathlessness plays a significant role in understanding the various mechanisms which underlie the breathlessness sensation. A standard post-debrief form was used to collect volunteered comments from subjects about their experience of breathing sensation in the test just completed. This was followed by presenting a pre-set list of respiratory descriptors (including air hunger and work/effort clusters) for subjects to choose the most relevant descriptors. These descriptors were used to identify and establish the quality of the breathlessness experienced, to guide participants' ratings for subsequent test runs, and to ensure that the stimulus was comparable between test runs. The debriefs also queried whether subjects experienced any other non-respiratory symptoms (e.g. headache, flushed or warm etc).

State-Trait Anxiety Inventory (STAI): This is a well-established psychological instrument that produces a validated score of both the tendency to be anxious generally (Trait anxiety; TAI) and the level of anxiety at any particular moment in time (STAI). The STAI and TAI inventories have been proven to be reliable in a large population of healthy subjects with different age groups (Crawford *et al*, 2011). This measure provided a useful stratification

of the data in Chapter 3 which generated an important new observation regarding the sensitivity to breathlessness inside a mock MRI scanner.

2.3.3 Consistency, repeatability and need for practice tests

Ensuring that subjects specifically rated air hunger and used VAS correctly: Healthy individuals do not normally experience intense breathlessness. Since breathlessness consists of different components arising from different mechanisms, it was necessary to ensure that subjects recognised the sensation they were required to rate and used the full VAS reliably. To achieve this an initial 'RAMP' air hunger test was always performed first in which hypercapnia was increased every two minutes until subjects had reached the tolerable limit (top of VAS) or came off the mouthpiece. The instruction for this first test was always to rate 'any uncomfortable breathing sensations' on the scale. At the end of this test, the debrief determined that they had in fact rated the required air hunger cluster of sensations. The healthy volunteers studied in Chapter 3 were given a practice session to familiarise themselves with the equipment and procedures. An example of a RAMP test in one individual is shown in Figure 2.3.

Steady state AH tests: Steady state tests involved 4 random steps of CO_2 determined for each individual from their initial ramp tests. Each step lasted 4 minutes. Four minutes steps are required to ensure the stimulus and perceptual ratings reach a steady state (Banzett, 1996). An example of a typical air hunger step produced from using this method is shown in Figure 2.4. It was important that two different practice sessions are run in order to obtain consistency in data collection. Below are the two types of practice sessions which were run.

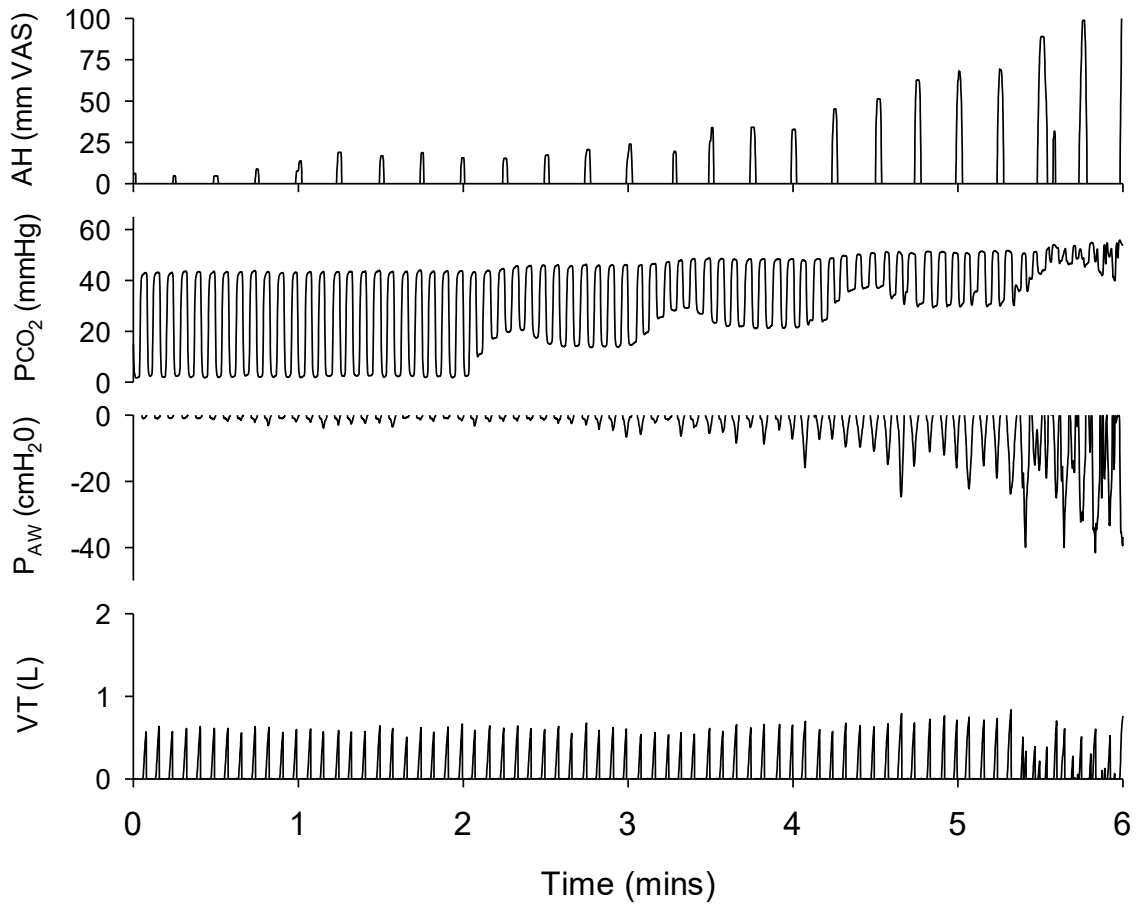


Figure 2.3: An example of an air hunger "ramp" test in one subject. This involved a minute-by-minute increase in inspired CO_2. The top panel shows the subject's AH rating (mmVAS) provided every 15 seconds on a 100 mm visual analogue scale. The second panel shows continuous P_{CO2} measured from the mouthpiece. The third panel shows continuous breath-by-breath airway pressure (P_{AW}). Bottom panel shows breath-by-breath tidal volume (L). The breathing circuit fixed tidal volume, while inspired CO_2 was increased to raise end-tidal $P_{CO_2}$to achieve a desired AH stimulus.

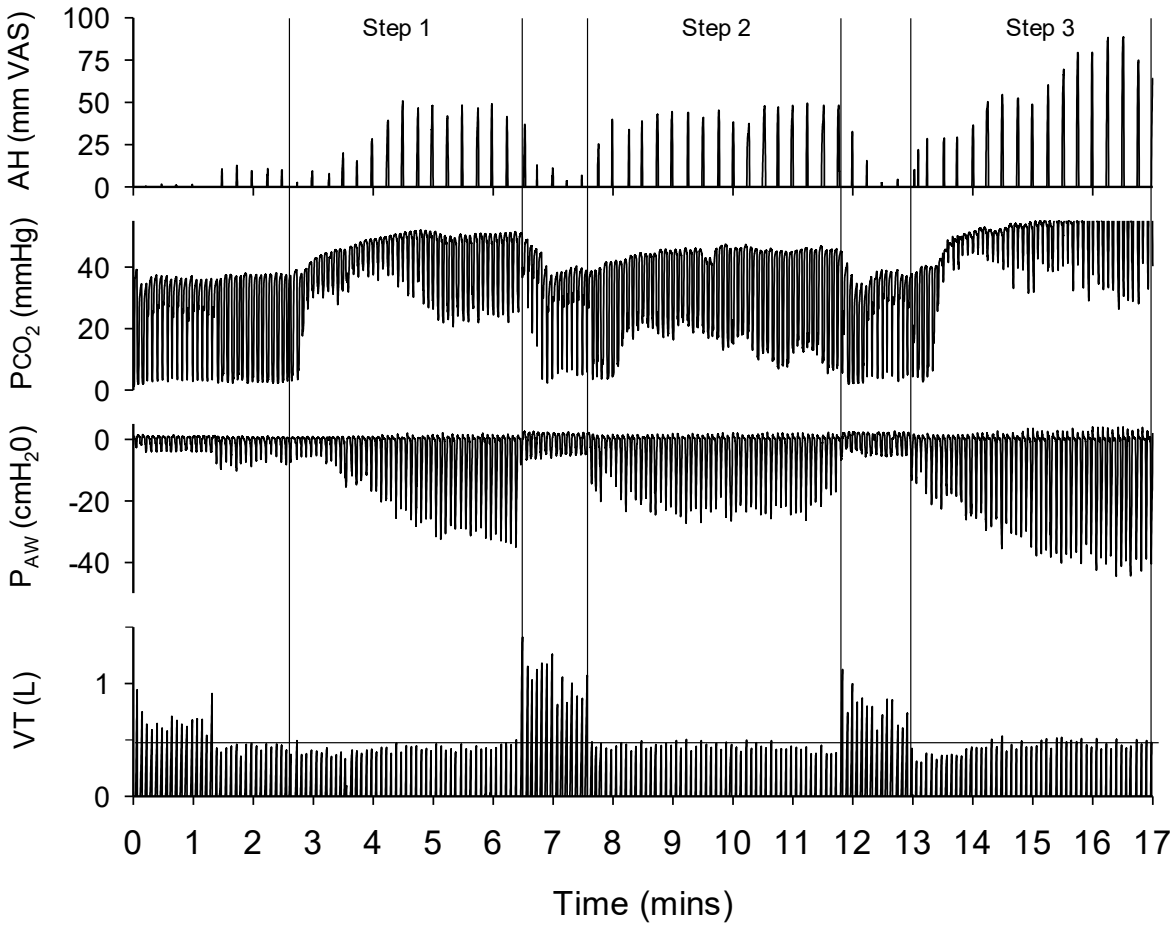
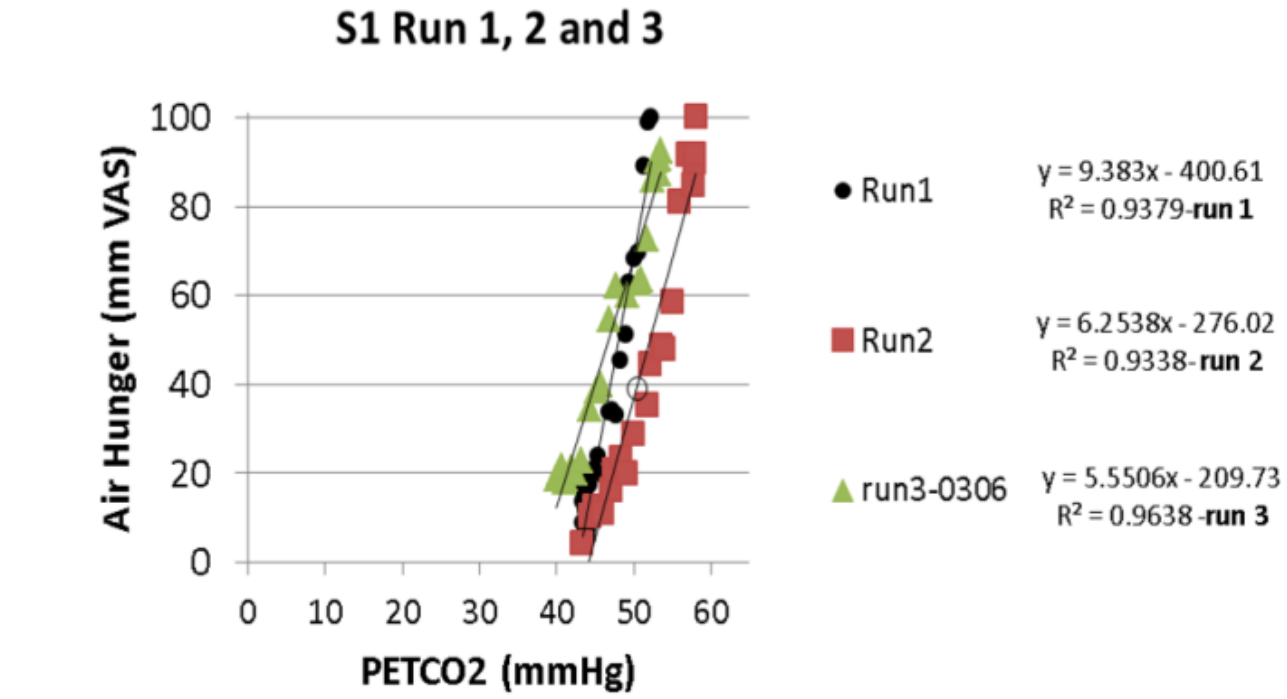


Figure 2.4: An example of a typical air hunger 'steady state test'. There are three 4min steps (step 1, step 2, step 3) with approximately one minute periods of free breathing between each step. The top panel shows ratings of air hunger (AH) provided by subjects on a Visual Analogue Scale (VAS) every 20s. The second panel shows continuous measurements of tidal P_{CO2} from which end-tidal $P_{CO2}$was derived. The third panel shows continuous tidal airway pressure (PAW) which reflects the efforts made to breathe against the ventilator constraint. The bottom panel shows the subject'sbreathe-by-breath tidal volume (VT) in Litres (L).

Consistency and repeatability data: The accurate measurement of the AH-sensitivity stimulus response curve depends on the precise use of the breathing circuit and robust data collection techniques. Although the breathing method used to generate experimental breathlessness is well established, a consistency test was conducted in two subjects over 3 different days and times to check that the assembled circuit was working properly and to establish (i) the need for practice sessions and (ii) need to perform tests at the same time of

day. Results indicated (i) good reproducibility from day to day, (ii) consistency of ratings by subjects using a VAS after only 1 practice session and (iii) a higher threshold PCO_2 and lower slope of AH stimulus-response if performed in the afternoon compared to morning (Figure 2.5).



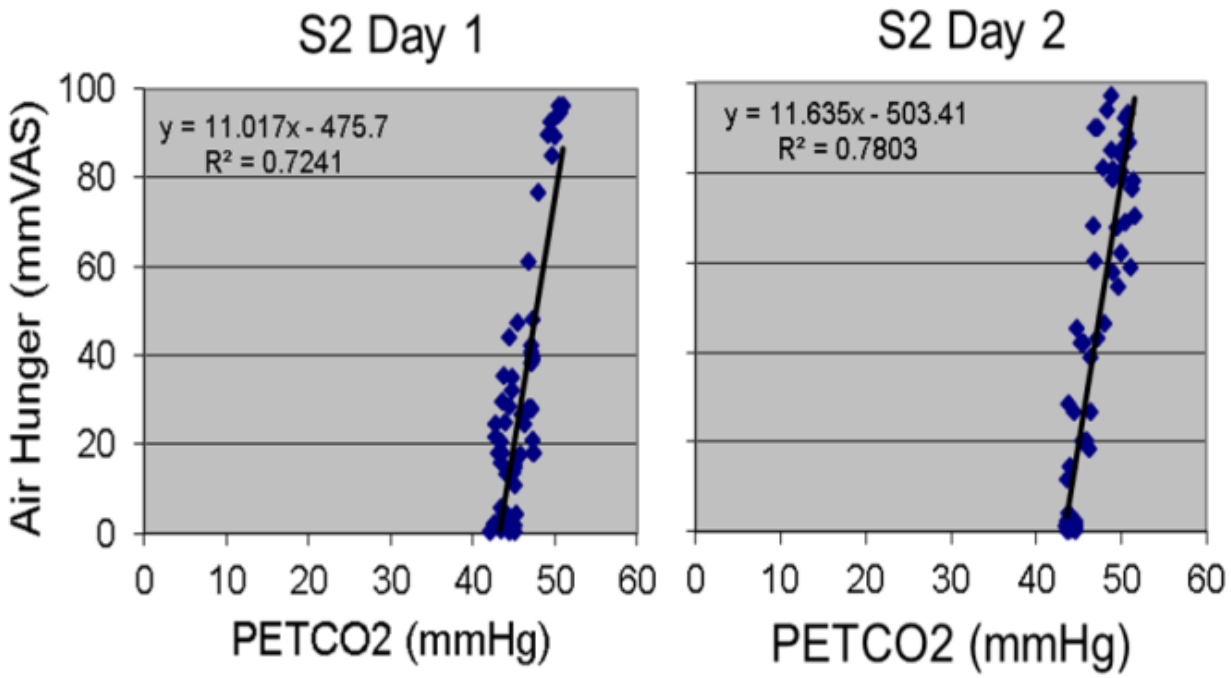


Figure 2.5: Repeatability and consistency of AH stimulus response curves.*Left panel*: Stimulus response curves obtained from the same test performed three times in the same subject (S1). Run 1 on Day 1 (am), Run 2 on Day 1 (pm) and Run 3 on Day 2 (am) illustrates the importance of conducting tests at the same time of day if they are repeated on separate days. *Right panel*: Test repeated on a second subject at the same time of day on two separate days shows good reproducibility (AH sensitivity = 11.0 and 11.7 mmVAS/mmHg PCO_2).

2.3.4 Development of mock brain scanners

Mock Magnetic Resonance Imaging Scanner (mMRI): A standard custom-made mock MRI scanner was purpose built for the study reported in Chapter 3 replicating the 1.5 Tesla MRI system(Siemen, Erlangen, Germany). A similar mock scanner has been used in other laboratories (Wood and McGlynn, 2000). The construction of the mock scanner is illustrated in Figure 2.6. The 60cm bore size (matching the 1.5 Tesla scanner) was cut from plywood and lined into a cylindrical shape with a flexible plastic Perspex sheet. It was painted with a white acrylic paint to reduce transparency in the tunnel and also reproduce a clinical setting. A 6ft MDF board coated with foam was used to construct a wheeled platform to slide the supine subject into the scanner. A head coil was constructed from a plastic cylindrical bucket. Audio speakers were inserted into the body of the mock scanner and connected to a laptop which played acoustic sounds of various scan sequences.

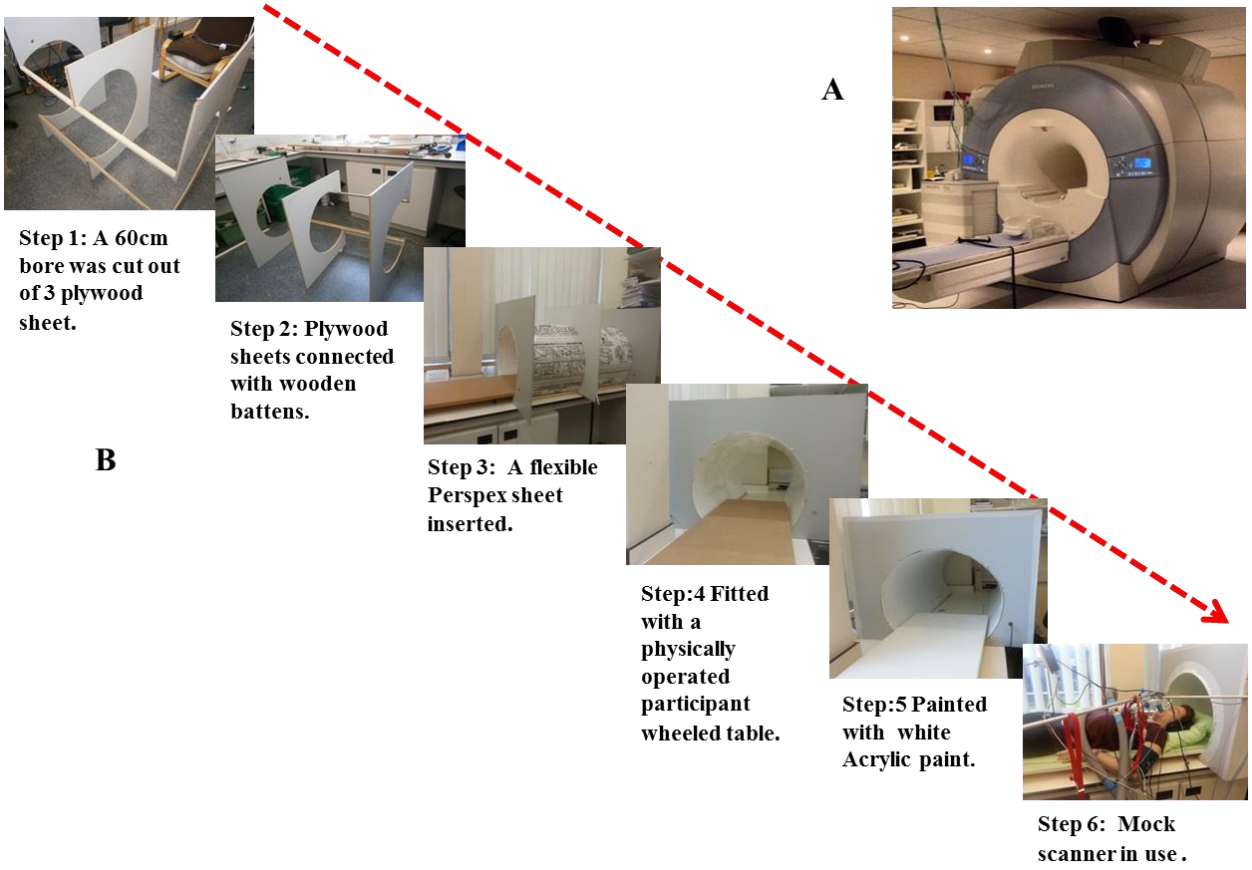


Figure 2.6: A set up of mock MRI scanner. (A) An actual Siemens TIMTRIO 3T fMRI scanner (B) A step by step standard custom built mock fMRI scanner. It was fitted with speakers to produce acoustic sounds of various scan sequences that are used in the actual fMRI. (Picture credit for MRI scanner (A) by Dr Mari Herigstad).

Mock magnetoencephalography brain scanner (mMEG):An adjustable floor standing salon style 'hair dryer' was used to mimic a MEG scanner. This was considered to be an adequate replication of the actual MEG brain imaging scanner shown in Figure 2.7A. Standard electroencephalography (EEG hydrocele Geodesic Sensor Net of medium size 54-56 cm; HCGSN Electronical Geodesic Inc., Oregon, USA) was worn by the subject while seated upright with their head in the mock MEG scanner (Figure 2.7B).

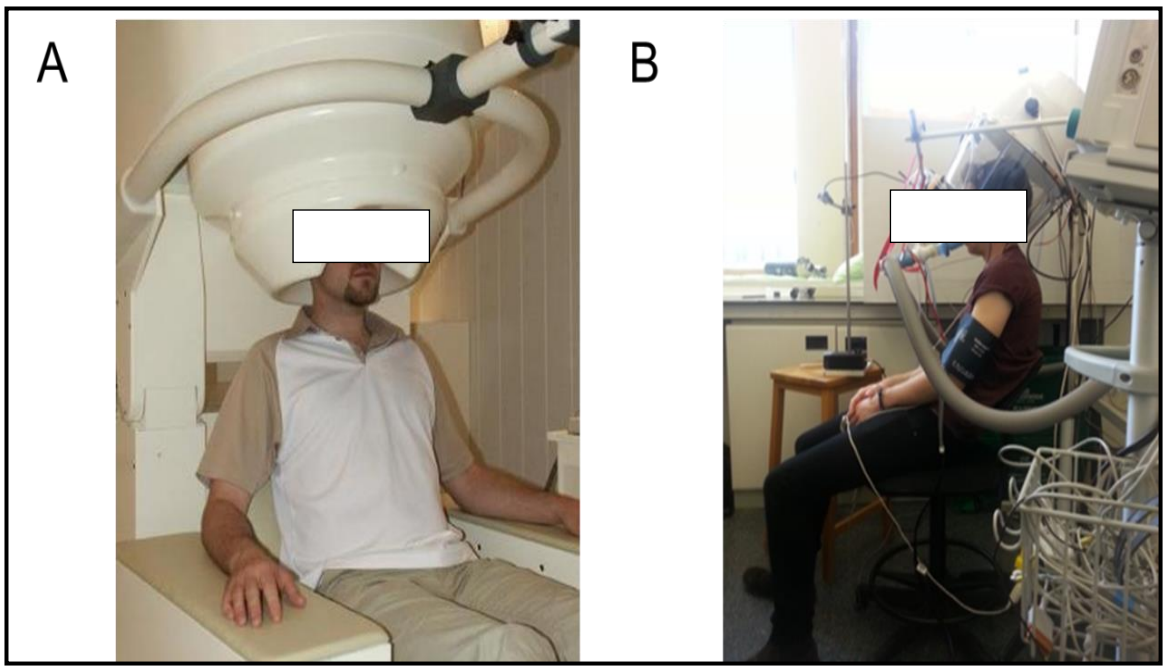


Figure 2.7: An MEG imaging scanner. (A) An actual MEG scanner (Image credit; mcgovern.mit.edu). (B) A purchased 900W adjustable floor standing 'hair dryer' as a mock MEG scanner (Hong Kong Aosen International Group Co, Limited). **Note**: the mock scanner does not have a huge overheard tube like the actual one, although the effect of having something over the head during scanning is the same.

2.4 NEUROLOGICAL AND BRAIN IMAGING TECHNIQUES

2.4.1 Brain Neuroimaging

Brain neuroimaging techniques such as functional and structural scans used in this study have made measurement of the brain electrical activities possible to researchers in clinical studies compared to the last 10 years. It can provide detailed information on normal anatomic structures of the brain and the measurements of different contrast characteristics.

Structural Magnetic Resonance Imaging (MRI): Structural MRI is a resonance imaging technique which provides quantitative information about the size, shape and details of both grey and white matter structures in the brain. It is a useful clinical and research tool to

investigate structure of brain structures in health and disease. Although it is currently an established neuroimaging technique, it has some limitationsassociated with it. Examples of such limitations are that, the data collection sequence is highly sensitive to movement artefacts, thus requiring patient's or individual subject's to lie motionless for a longer period of time during data collection.It also has the potential to induce claustrophobia in individuals with known or unknown history of anxiety and panic attack. It is expansive and involves loud noise during processing.Finally, structural MRI cannot differentiatebetween inhibitory and excitatory activations, thus mathematical assumptions are made during data interpretation.A high-resolution CT, T1 and T2 weighted functional and structural MRI scanning was performed in all patients where necessary on a 3 Tesla Magnetom-TRIO MR scanner (Siemens Medical Solutions, Erlangen, Germany) with a standard head coil to obtain the required neuroimages.

For each patient a pre functional MRI and post structural CT T1 and T2 weighted scans were acquired to be anatomically compared with other images obtained by different means. Details of image acquisition, processing and analysis will be provided in the subsequent chapters where applicable. Furthermore the target selection, location and evaluation of implanted DBS electrodes will also be provided in the appropriate chapters.

2.4.2 DBS surgical procedure

The precise methods used for DBS surgery differ amongst neurosurgical teams. In this study, the methods adopted in Professor Tipu Aziz' Functional Neurosurgical Centre in Oxford (OFN) are presented and focus on surgical procedures used for DBS for symptomatic relief of tremor, dystonia, neuropathic pain and Parkinson disease tremor. Target regions were the ventral intermediate nucleus of the thalamus (VIM), the ventral oralis posterior (VOP), the Globus Pallidius (GPi), the periventricular and periaqueductal grey (PVG/PAG) and the ventral posterior lateral nucleus (VPL). Details of electrode

position can be found in Chapter 4. Details of the full procedure can be found in Appendix 1.

2.4.3 Tractography technique

Tractography is a non-invasive neuroimaging technique used to visually identify and represent the neural connections in the white matter of the brain. It uses data collected by diffusion magnetic resonance imaging (DTI) MRI. MRI is a detailed anatomical image of the body using a non-damaging radiation. While the DTI image provides detailed cellular structures by measuring the diffusion of water molecules in the brain, tractography provides quantitative information about the neural fibre tracts in the living human brain in health and disease conditions and used as a clinical and research tool. There are two types tractography; (i) deterministic which uses a single orientation from the origin of interest to measure connectivity and (ii) probabilistic which uses a distribution of orientation to predict fibre connection in the brain. The latter was used in this study as it has the advantage of estimating wider fibre connection distributions than the former.

Tractography is able to indirectly measure the connectivity of white mater across the entire brain. The principle of tractography is based on the directionality of water. Water molecules are assumed to diffuse longitudinal along an axon bundle but arerestricted in the perpendicular axis. Tractography analysis was conducted using a 3D modelling technique to reconstruct the neural tracts (white matter) using data obtained by diffusion-tensor imaging (DTI MRI). The technique has been validated and tested for reproducibility and accepted as a useful analytical tool (Dyrby *et al*, 2007). Probabilistictractography FMRIB Software Library (FSL) analysis software developed by Functional Magnetic Resonance Imaging of the brain (FMRIB) (Oxford) was used for all analysis. Detailed explanation of the step- by- step and type of tractography with generated algorithms involved are

provided in Chapter 5 of the thesis. Here, a brief overview of analysis principles was provided.

An established automated connectivity mapping pipeline to construct white matter fibre networks from pre surgical DTI imaging data was employed. T1-weighted, T-2 weighted and DTI imaging data were used in the analysisemployed. Linear and nonlinear methods to process image registration were used as appropriate. Connectivity strength and pathways were also measured.

2.5 DATA ANALYSIS

The primary outcome measures in this thesis were VAS ratings in mm and total D12 scores. For the study in Chapter 3, it was possible to determine a sample size estimate based on previous studies that provided a standard deviation for the mean change in VAS for unit change in end-tidal $PaCO_2$ (mmVAS/mmHg PCO_2). The details of this estimation are provided within Chapter 3 itself. While the correct number of participants was recruited for this study, the dropout rate was excessive reducing the power of the study. For Chapter 4, which was a prospective study, no a priori sample size estimation was possible. The number of patients depended on clinical recruitment rates. Within the time available for this study, the number of patients recruited was not adequate for conclusive statistical tests and definitive outcomes. Nonetheless, even with limited numbers some substantial and significant trends were observed indicating that properly powered studies would likely detecthighly significant findings.

Thus the data presented in this thesis did not warrant elaborate statistical analyses and the data were presented descriptively with only occasional paired t-tests to detect trends. The data are at best preliminary and pilot but nonetheless raises important new information to the field.

Boxcar analysis of P_{ETCO_2}: A 60sec box-car average was run through the breath-by-breath CO_2 data during RAMP air hunger tests. This was necessary because a RAMP test is a non-steady state test - it is not obvious in this case which level of PCO_2 corresponds to which subjective rating. Banzett *et al*(1996) determined that a boxcar average of 60 seconds through the PCO_2 data would empirically align the data taking account of circulation delays from the lungs to the chemoreceptors, and the delayed response in air hunger ratings when a square-wave change in PCO_2 is presented.

CHAPTER 3

EFFECT OF SIMULATED BRAIN SCANNING ON BREATHLESSNESS SENSITIVITY

3.1 INTRODUCTION

This chapter investigates the influence of simulated brain scanning procedures and environments (which are known to generate psychological distress such as anxiety) on the perception of experimentally induced breathlessness (hypercapnic stimulus with ventilatory constraint). The general aim of this study is to quantify the impact of psychological factors on breathlessness perception. There are several studies of breathlessness which explore the psychological consequences of being breathless but few studies which have looked at the impact of psychological factors on breathlessness sensitivity. Examples of the latter include studies reporting the effect of 'negative affect' (e.g. sad images) on breathlessness in patients with COPD (von Leupoldt *et al*, 2007; von Leupoldt *et al*, 2010; von Leupoldt and Dahme, 2007). Other studies have reported strong positive correlation between the levels of anxiety among asthmatics and the severity of their asthma (DePeuter*et al*, 2008; Janseen *et al*, 2009), however a causative link can only be assumed from these data.

The use of MRI simulators to investigate fear response, behavioural patterns and claustrophobia in healthy participants has increased substantially over the years. The nature and extent of behavioural response has been reported to vary greatly among 64 college students undergoing mock scanning assessments (McGlynn *et al,* 2003; McGlynn *et al*,

2007). These responses were categorised as fear of suffocation, fear of restriction and anxiety sensitivity. Respiratory muscles have dual control mechanisms, automatic reflex control from the brainstem on the one hand and behavioural control from higher brain areas that can modulate or override this automatic control (Moosavi *et al*, 2005). On the otherhand, it is very likely that the outcome measures of respiratory studies involving brain scanning procedures will be especially prone to modulation by the psychological valence of the scanning procedures themselves. The 'mouthpiece effect' on breathing is a well-known example of how the measured response is affected by measurement procedure itself usually producing an increase in tidal volume and ventilation (Askanazi *et al*, 1980). Experiments involving breathing measurements must incorporate sufficient practice and familiarisation sessions to minimize the confounding effects of the measurement procedures or environment on the outcome measures.

How much the confounding effects of the measurement procedures can be minimised will also depend on which neuroimaging technique is employed. In breathlessness research, positron emmision tomography (PET), blood oxygen level dependent magnetic resonance imaging (BOLD fMRI) and magnetoencephalography (MEG) techniques have all been used (Banzett *et al,* 2000, Evans *et al,* 2002, Johnson *et al,* 2010). MEG directly measures magnetic fields produced by electrical currents in active nerve cells through the scalp. It has a poor spatial resolution but high temporal resolution compared to fMRI and PET and detects wide spread of electrical currents in the brain instead of differences in oxygen content or perfusion dynamics of the blood. MEG is considered to be a less stressful procedure because it does not encroach on an individual's 'personal space' as extensively as the MRI or PET environment. In addition, PET studies involve infusion of radioactive agents and MRI procedures involve lengthy periods of movement restriction accompanied

by loud auditory noises. Thus PET and MRI studies are likely to invoke greater claustrophobia and anxiety.

In addition to differences in psychological valence between different neuroimaging techniques, there are also physiological differences that need to be taken into consideration. MEG is usually performed sitting upright while PET and MRI brain scans are almost invariably conducted in the supine posture. Posture is known to influence breathing mechanics and control (Frederiksen 1996; Gordon 1987; Snell 1988; Steenstrup 2004;Prince*et al*, 2014; Sundberg *et al*, 1991). Gravity will act in different directions when supine compared to upright (i.e. base-apex of lungs to ventral-dorsal). When healthy individuals move from an upright to supine posture, a significant reduction in FVC and FEV1 has been reported (Hojat and Mahdi 2011; Blair and Hickam, 1955). It is also likely that the degree of ventilation-perfusion mismatching may change as a result of the change in posture.

3.1.1 Rational for study

The experimental breathlessness sensation experienced by healthy subjects and the symptomatic breathlessness experienced by patients has been shown to be influenced by environmental, physiological and psychological factors such as anxiety, fear, emotional distress and panic (De Peuter *et al*, 2004). The claustrophobic environment associated with fMRI scanner can generate or augment these factors.Currently, the importance of these factors on breathlessness has not yet been fully investigated and little attention has been paid to the influence induced by test procedures such as fMRI on the interpretation of the imaging results in breathlessness research. This study therefore proposes to measure the extent to which psychological factors (e.g. anxiety or claustrophobia) and physiological factors (e.g., posture) associated with brain imaging scans impact on breathlessness

sensitivity in healthy individuals. To do this, breathlessness sensitivity was measured using a standard test of 'air hunger' (AH) in a mock MRI scanner (a more 'stressful' environment) and a mock MEG scanner (a less 'stressful' environment).

Specific aims:

A. To compare the AH measured on a visual analogue scale (VAS) for a given stimulus (hypercapnia with constrained ventilation) in the same subjects tested in four different conditions; sitting upright (baseline), sitting upright in mock MEG scanner (mMEG), lying supine (SUP) and lying supine in mock MRI scanner (mMRI).

B. To determine to what extent differences in breathlessness sensitivity measured in the different conditions (as above) are due to differences in psychological factors (low versus high trait anxiety) or in physiological factors (postural differences).

C. To measure the 'apprehension' (state anxiety) experienced by participants just prior to being tested for breathlessness sensitivity in each of the 4 conditions.

Working hypothesis is that: ***The perceptual sensitivity to experimentally induced breathlessness ('air hunger') will be greater during MRI brain scans due to the greater anxiety associated with the MRI scanner.*** Understanding the level of influence of the environment will generate important information that has implications for how brain imaging studies involving MRI scanning should be interpreted and inform future clinical studies. Greater breathlessness for a given stimulus when lying in the mock MRI scanner, which is not accountable to physiological factors (postural differences), would be consistent with a powerful psychological modulation of breathlessness due to a more stressful environment.

3.2 METHODS AND MEASUREMENTS

3.2.1 Study participants

Twenty three healthy volunteers were recruited (mean age 27 years, range 20-31 years, 12 males). Fourteen completed participation (8 males and 6 females). All participants were students and staff from Oxford Brookes University (OBU). Of the subjects who completed participation, two were current smokers and five past smokers (Table 3.1). Exclusion criteria included any indication or history of cardiorespiratory, neurological or psychological conditions including anxiety disorders. Subjects were also excluded if they had upper respiratory tract infection or were on prescription or herbal medications within the 2 weeks prior to participation. The study was carried out at the Respiratory Physiology Laboratory, Faculty of Health and Life Sciences, OBU. All participants gave written informed consent and completed their participation over a mean±SD period of 14±9 days. The study was approved by the University Research Ethics Committee (URECNo: 140861).

3.2.2. Sample size

A previous study estimated the slope of the AH ratings measured on a VAS in response to increasing P_{ETCO_2} in healthy volunteers, and found the effect size to be 6.7±2.4 mmVAS per mmHg P_{ETCO_2} (Banzett *et al*, 1996). Based on this, it was determined that a sample size of 16 participants would be needed to be able to detect a mean difference in slope of AH-P_{ETCO_2} relationship of 20% with 80% power for significance at the $p<0.05$ level of probability. With greater numbers the study would have greater power to detect smaller changes that are significant but would not have the same clinical importance.

Table 3.1 Subject demographics: Gender, age, height, weight, smoking history and absolute Trait Anxiety Inventory (TAI, Spelberger, 1975, 1983 and 1985) score in all subjects recruited for the study including those who did not complete, n=23. Reasons for withdrawal included; "found experiment to be too difficult", "too distressful" or "felt sick on the breathing circuit". (n=23)

Subject (ID)	Gender (M/F)	Age (yrs)	Height (cm)	Weight (kg)	Smoking history	TAI (/80)	Status	Reason for withdrawal
S1	M	20	165	68	Past	41	Completed	
S2	M	21	189	104	Never	28	Completed	
S3	M	20	189	104	Never	36	Completed	
S4	M	23	182	73	Current	21	Completed	
S5	F	20	158	63	Never	25	Completed	
S6	F	20	152	55	Never	24	Completed	
S7	M	29	186	96	Never	20	Completed	
S8	M	24	177	78	Current	32	Completed	
S9	F	20	165	50	Never	31	Completed	
S10	M	25	179	90	Past	39	Completed	
S11	F	19	177	64	Past	35	Withdrew	Procedure was stressful
S12	F	21	162	47	Never	31	Withdrew	Procedure was stressful
S13	F	54	168	58	Never	40	Withdrew	Could not cope with procedure
S14	F	31	175	60	Never	25	Completed	
S15	M	26	178	68	Past	23	Completed	
S16	M	51	193	102	Past	37	Withdrew	Found it difficult to cope
S17	M	31	178	78	Never	44	Withdrew	Procedure was stressful
S18	M	41	153	85	Never	30	Withdrew	Found it difficult to cope
S19	F	26	165	62	Never	40	Completed	
S20	M	22	181	86	Never	39	Withdrew	No reason given
S21	F	27	155	70	Never	40	Completed	
S22	M	25	190	126	Never	39	Withdrew	No reason given
S23	F	26	167	51	Never	34	Withdrew	Procedure was stressful
Mean		**27**	**173**	**76**		**32.8**		
SD		**9.5**	**12.4**	**21**		**7.2**		

3.2.3. Different testing environments or conditions

Baseline: Subjects sat semi-reclined in a comfortable chair (IKEA. Poang chair) while breathing through a mouthpiece connected to a breathing circuit.

mMEG: The only differences between this condition and the baseline condition were that (i) a floor standing salon hair drier (model No-110052., Logistics Vibes, UK) was lowered over the head of the subjects' down to the eyebrows to mimic an MEG scan, and (ii) an EEG electrode cap was placed over the subjects head.

Lying supine: All subjects lay supine on a platform made of wood (3/4 inch MDF), which was padded with a yoga mat for comfort. The orientation of the breathing circuit was adjusted to enable the mouthpiece to be placed in the subjects' mouth while they remained comfortably supine. The length of breathing tubes and deadspace within the circuit was identical in all conditions.

mMRI: Subjects lay supine on the platform (see above) which was then wheeled into the purpose-built mock MRI scanner. The orientation of the breathing circuit was again adjusted to enable the mouthpiece to be situated within the mock scanner positioned such that the subject was able to place it in their mouth while remaining comfortably supine. The mock scanner was fitted with speakers to playback the sounds of a typical MRI scan sequence. A mirror was attached to the inner ceiling of the mock scanner, positioned to enable subject to view the VAS placed at the opening of the scanner (head end). The subject was able to indicate their AH on this VAS using the same linear potentiometer placed by their right hand. Mock scanner construction is shown in section 2.5.

3.2.4. Standard test for air hunger

Gas from a cylinder containing 21% oxygen and 79% nitrogen and another gas from a cylinder containing 21% oxygen, 10% CO_2, 69% nitrogen were mixed using a gas blender

(Sechrist, model No 3500) to enable the fraction of CO_2 to be varied between 0 and 10% while maintaining near normal inspired fraction of oxygen. The gas output from the blender was heated and humidified (Fischer & Paykel, model No HC 325-New Zealand) before supplying an inspiratory reservoir (3 litre anaesthetic bag) at a flow rate that matched the subject's resting alveolar ventilation. Subjects breathed from the inspiratory reservoir via a mouthpiece connected by wide bore corrugated breathing tubing to a one way valve (Hans Rudolph model No.5710 -USA). Subjects breathed out into the room via a second one way valve (Hans Rudolph model No.5710 -USA). Air hunger was generated by increasing the inspired fraction of CO_2 in the inspiratory reservoir while ventilation was maintained at the resting level. The respiratory rate (f_R) was fixed as subjects breathed in time with the metronome set at 12 beeps per minute. Since ventilation was also fixed by the flow of fresh gas into the inspiratory reservoir, the tidal volume was also therefore fixed. Breathing with restricted tidal volume and added CO_2 gave the subject varying amounts of air hunger. The standard test of AH during the test session comprised 4 levels of end-tidal PCO_2 ($PETCO_2$) each held for 4mins and separated by 1min of free breathing. The level used for each condition was the same but order of steps was randomised.

3.2.5. Physiological and Psychophysical Measurements

Spirometry: Subjects were asked to blow as hard and as fast as they could into a spirometer, which computed values for Forced Expiratory Volume (FEV1), Forced Vital Capacity (FVC) and Peak Expiratory Flow (PEF). The test was done on day 1 in both sitting upright and supine positions.

Flow measurement: Airflow was measured and recorded by a pneumotach (AD instruments, Oxford, UK) connected to a pressure transducer (Validyne mp45-1, ±2 cmH_2O). Tidal volume (V_T) and (f_R) were calculated from the airflow signal. PCO_2 was monitored and recorded continuously (AD Instruments gas analyser ML 206, Oxford) via a

small diameter tube inserted into the mouthpiece. For the safety of the subject a number of physiological variables were monitored: a 3 lead ECG was monitored (HME Lifepulse LP10) from which heart rate was derived. Blood pressure was measured and recorded continuously every two minutes (Datex-Ohmeda F-CM1-04) via a cuff placed around the upper arm. S_PO_2 was continuously monitored (Datex-Ohmeda F-CM1-04) via a pulse oximeter finger probe. All analogue signals were amplified and then digitised (sample rate of 20Hz) using Spike2 v6 software (Cambridge Electronic Design) for later analysis.

Subjective ratings of air hunger (Visual Analogue Scale): Subjects rated air hunger using a 100 mm visual analogue scale, similar to that used in previous studies (Moosavi *et al*, 2007). The ends of the scale were labelled 'none' -defined as no sensation, and 'extreme'- defined as an intolerable level. The subjects were informed that if they reached extreme the stimulus would immediately be reduced or stopped. The subjects were also instructed to place three additional labels on the scale ('slight', 'moderate' and 'severe') at locations of their choice beside the scale; this was to ensure that they used the scale consistently between tests (Lansing *et al*, 2003). Subjects were cued to rate by a light that flashed once every 20 seconds.During practice sessions subjects were instructed to rate 'any uncomfortable breathing discomfort' while undergoing an initial 'ramp' test in which inspired CO_2 was raised in 1 minute increments. Subjects filled out a questionnaire after this first ramp test in which they selected respiratory descriptors from list to describe their experience. Subjects who selected phrases connected to air hunger were instructed to continue rating those sensations. Subjects who described other sensations predominantly (e.g. breathing felt harder) were asked not to include them in the ratings and to report them after each test.

3.2.6. Study protocol and experimental design

All participants attended the laboratory on 3 separate days for 1-2 hours on each day; each subject was tested at the same time of day at each visit (Figure 3.1).

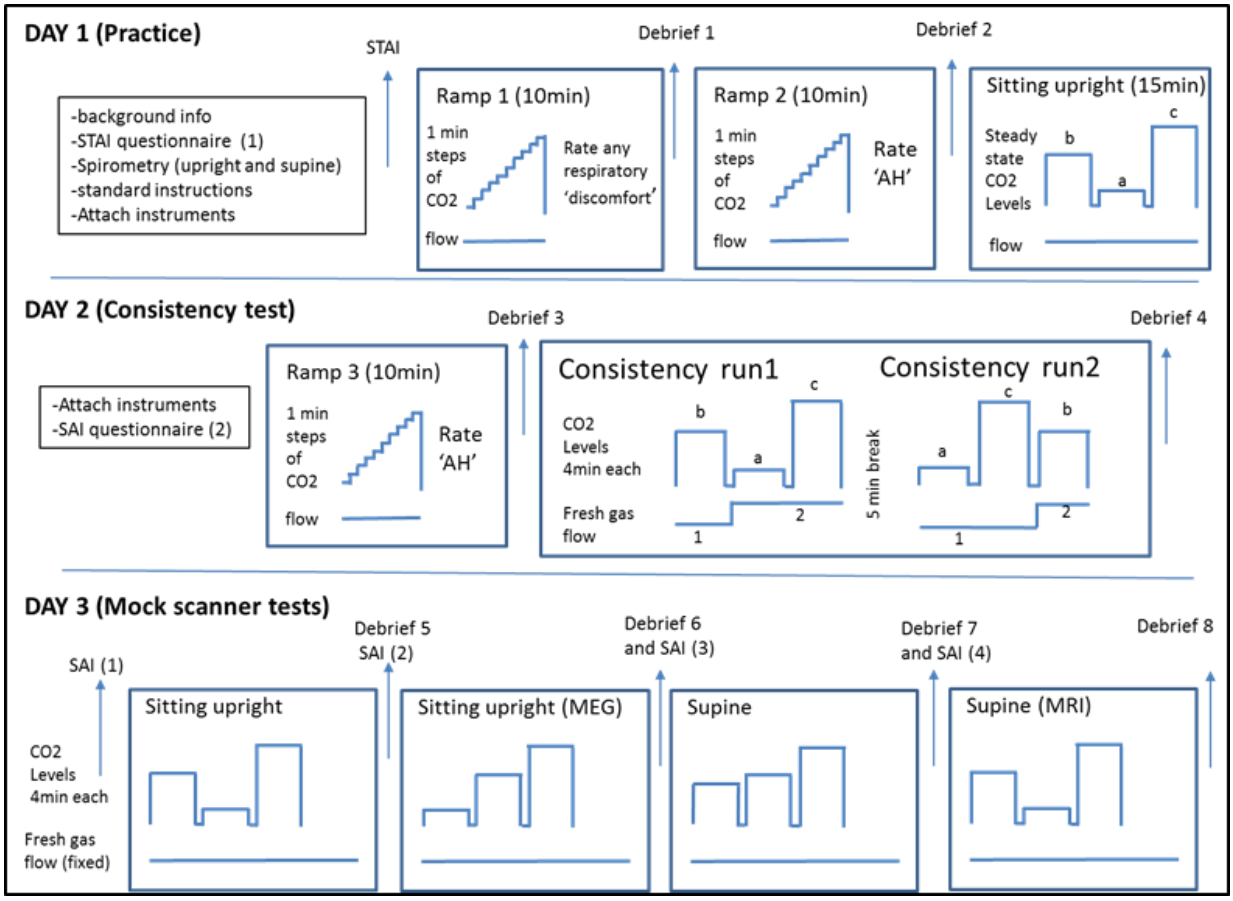


Figure 3.1: Study design. Day 1: (Practice): All subjects performed spirometry and completed the state and trait anxiety inventories (STAI and SAI). Two 'ramp' tests were performed in which the stimulus (added inspired CO_2) was increased every minute until subjects indicated 'intolerable' on the VAS scale. Subjects were initially instructed to rate any uncomfortable breathing sensations. The standard debrief following Ramp 1 included a list of respiratory descriptors for them to select the ones which best described their experience; their choice was checked to confirm that the stimulus specifically generated AH. This was followed by a steady state test targeting 3 levels of CO_2 to determine the targeted test levels of end-tidal $PaCO_2$ for use in subsequent test days. Day 2 (Consistency test): A third ramp test was followed by two steady-state tests at the target PCO_2 levels from Day 1, at a high and low ventilatory constraint (high and low flow). Day 3 (Test day): Three target levels of end tidal $PaCO_2$ were imposed for 4mins each separated by 1min of free breathing, in each of the four conditions (sitting upright baseline, MEG, supine, supine in MRI scanner). The order of conditions and the order of CO_2 levels within conditions were randomised. Prior to each condition, subjects were asked to complete the SAI for how anxious they felt just prior to the next test that was pointed to them. Standard debriefs were completed immediately following each condition.

Practice session (Day 1): This was to familiarise participants to the breathing circuit and breathlessness rating system and to enable them to distinguish the AH component of breathlessness from other uncomfortable sensations. Subjects were instructed to rate any uncomfortable breathing sensation during the first ramp test and were debriefed afterwards to determine which respiratory descriptors they selected to describe their experience. Subjects were instructed to focus on the air hunger descriptors they chose following the first ramp, and not include any other forms of breathlessness or discomfort they may have identified from the first ramp, in subsequent tests. The levels of steady state CO_2 to be used during day 3 test sessions (see below) were determined from the data generated in this practice session. (Figure 3.1, top panels).

Consistency tests (Day 2): This session provided further practice and familiarisation and included a test of consistency to gauge whether rating reliability had been established – these consistency tests involved three steady state levels of P_{ETCO_2} presented twice each, once with ventilation constrained to resting alveolar ventilation and once at a higher level (see Figure 3.1, middle panels).

Test sessions (Day 3): This consisted of three 4 minute steady state CO_2 tests, each done under different conditions (baseline sitting upright, mock MEG, supine, mock MRI).

Each subject completed a debrief questionnaire containing descriptors which indicate the type of sensation experienced, immediately after each test session. Just before each test condition, subjects were instructed to indicate how they felt about the condition which they are about to be tested by completing a state anxiety inventory questionnaire. This was to assess their emotional state at that particular moment as compared to the general anxiety trait questionnairethey completed on day of the study (see Figure 3.1, bottom panels).

3.2.7. Data processing and analysis

AH ratings and breath-by-breath P_{ETCO_2} levels in the last minute of each 4 minute step of targeted P_{ETCO_2} were measured and averaged (mean±sd) -P_{ETCO_2} levels which were found to be noisy were not used. This was done for each condition in each subject. For each individual, a test level of P_{ETCO_2} was selected which targeted a level of air hunger during the baseline condition that approximated the midpoint of the VAS and was well-matched between conditions within each subject (deviating by no more than 1mmHg). A paired Student's t-test was performed between changes from supine condition to mMRI condition. Trait and state anxiety scores were analysed using the scoring key provided by Spielberger *et al* (1975). The total score was calculated for each subject and the subjects were grouped into those with 'low' (≤30) and 'high' trait anxiety scores (>30). The threshold of 30 TAI was chosen because subject's with TAI score above 30 had high air hunger ratings compared to those with TAI score lower than 30 at the a given level of PCO_2.Mean±sd changes in spirometry measurements (FVC, FEV1 and FVC/FEV1 ratio) were determined and compared between supine and sitting conditions.

3.3 RESULTS

Fourteen of the 23 participants initially recruited went on to complete the study. All participants completed day 1 (training session). The 9 who dropped out of the study made comments such as "I find the experiment very distressful", "I do not want to experience such discomfort again". Those participants who dropped out were found to have a significantly higher trait anxiety score than those who completed the study (mean±sd 37±5 versus 30±8; unpaired t-test, p=0.04). Although 14 subjects completed the study, only 12 datasets were considered acceptable for inclusion in data analysis. The reasons for this were due to inconsistency in ratings or poor targeting of P_{ETCO_2} levels.

3.3.1 Group mean AH for a given steady state PCO2 between conditions.

A typical raw data set in one individual is shown in Figure 3.2 for the last minute of each target level of P_{ETCO_2} for each experimental condition. For the target P_{ETCO_2} level of 47-48 mmHg (indicated in Figure3.2), this subject rated the highest AH levels for the mMEG and mMRI conditions.

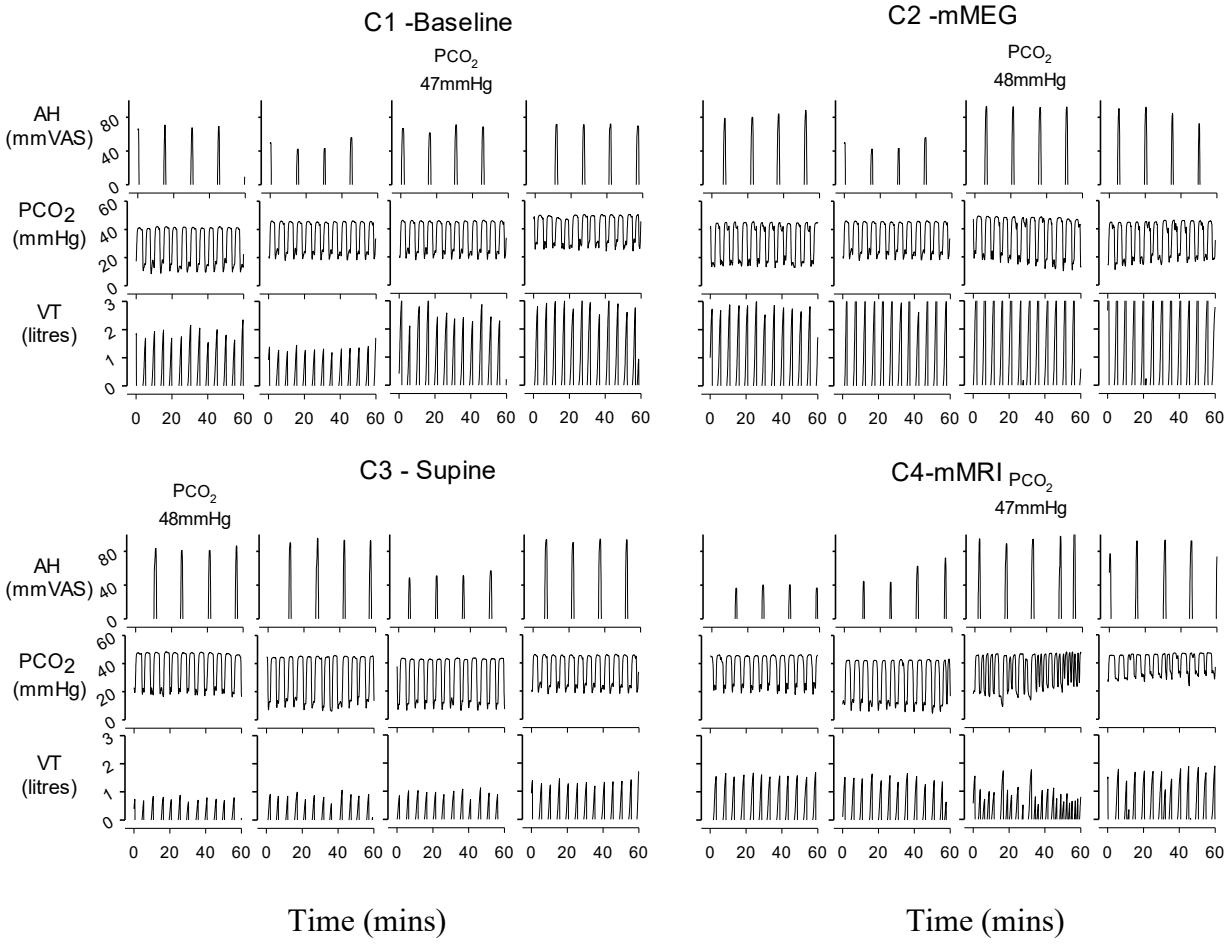


Figure 3.2: Typical raw steady state or step session air hunger sensitivity data set tested under four different conditions in one subject (n=1) (C1, C2, C3 and C4). C1=Sitting upright, C2=mMEG, C3=Lying supine and C4=mMRI scanner. C=Condition, mMRI= mock magnetic resonance imaging, mMEG= mock magnetoencephalography. There are four minute steps. The top panel shows the subjects' air hunger rating (mmVAS), the second panel shows continuous P_{ETCO_2} and the bottom panelshows breath-by-breath tidal volume (L). The modified circuit fixes tidal volume, while P_{ETCO_2} is varied. The subjects' perception of air hunger correlates with P_{ETCO_2} as reflected in the VAS ratings.

However on average, sitting upright in a mock MEG scanner (mMEG), lying supine (SUP) and lying supine in a mock MRI scanner (mMRI) did not appreciably alter the level of AH associated with a given steady state P_{CO_2} stimulus at baseline: The mean±sd target P_{ETCO_2} levels of 48.6±4, 48.4±4, 49.0±4, 48.5±4 mmHg were associated with mean±sd AH levels of 59±15, 60±21, 58±18 and 61±26 mmVAS for the baseline, mMEG, SUP and mMRI and mean conditions respectively. Thus group average data provided no evidence of a condition effect (Figure 3.3).

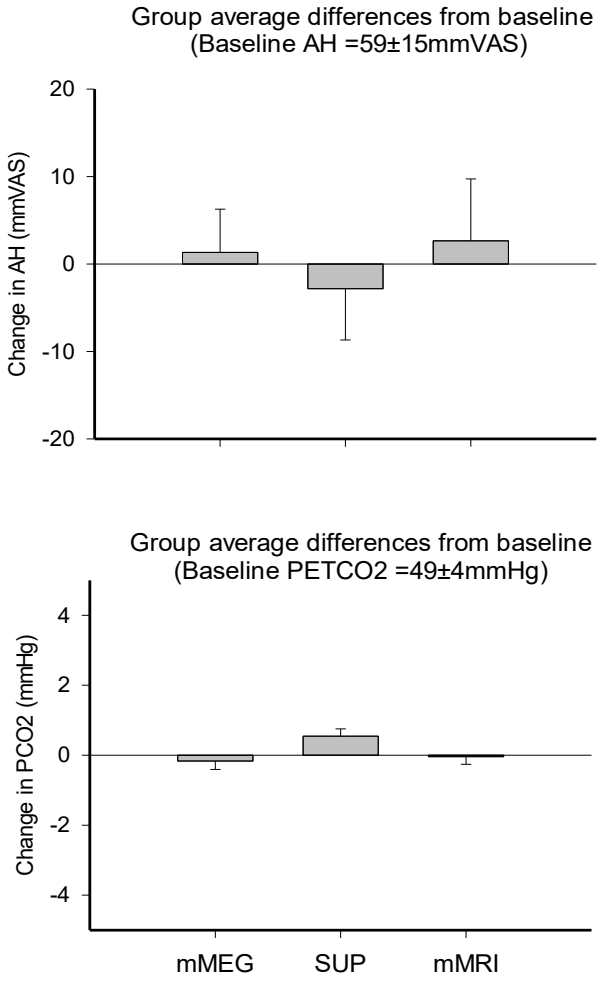


Figure 3.3 No significant group mean condition effectsfor change in AH from baseline for a given CO2 stimulus.*Top panel:* Group mean±SEM changes in air hunger (AH) ratings for a given target level of P_{ETCO_2} for each of the test conditions (mMEG, SUP and mMRI) relative to the baseline condition. *Bottom panel*: Corresponding group mean±SEM differences in target level of P_{ETCO_2} for each of the test conditions (mMEG, SUP and mMRI) relative to the baseline condition. The absolute mean target P_{ETCO_2} level did not vary by more than ±1mmHg between conditions.

3.3.2 Individual variability in condition effects for a given steady state PCO_2.

Although the average data shown in Figure 3.3 indicated no appreciable changes in AH sensitivity, there was a high degree of underlying variability among individual responses (Figure 3.4). For the mMRI condition, 6 individuals produced a drop in AH and 6 produced a rise in AH for the same stimulus compared to the baseline condition (Figure 3.3, top panel). For the other conditions (SUP and mMEG) the variability between individuals was just as great but did not synchronise with the variability for the mMRI condition (i.e. if AH fell relative to baseline for the mMRI condition this did not necessarily mean that AH fell relative to baseline for the other conditions as well). The mean±sd trait anxiety score was 26±6.7 for the subjects who rated less AH and 31±6.6 for those who rated higher AH, during the mMRI condition relative to the baseline condition for the same stimulus (Figure 3.4, top panel). This difference in TAI was statistically significant (unpaired t test; $p=0.04$).

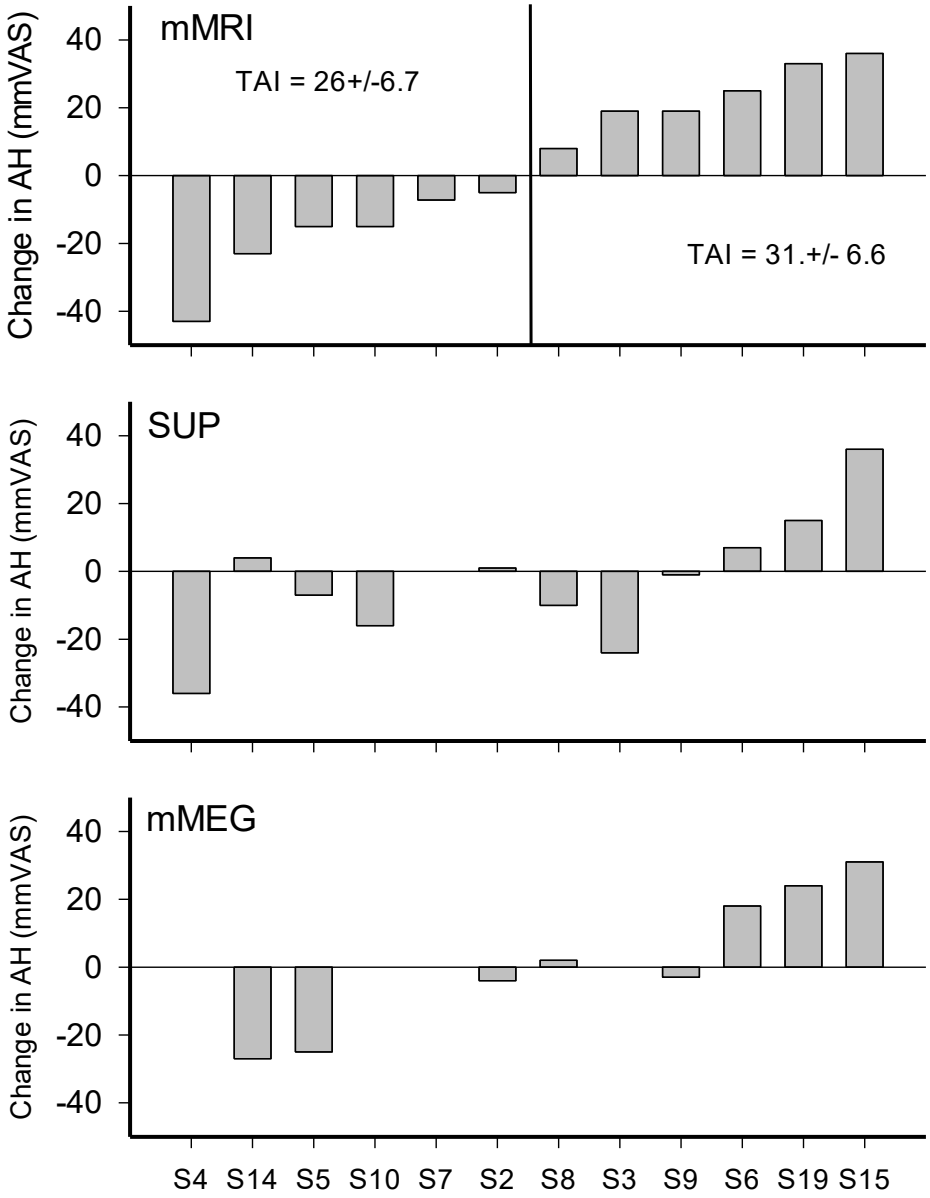


Figure 3.4 Individual changes in AH relative to baseline for each condition. *Top panel*: The individual data are plotted in order from the biggest to the smallest fall in AH rating for a given stimulus during the mMRI condition relative to baseline. Those individuals, who showed an increase in AH during the mMRI condition relative to baseline, had a higher average trait anxiety (TAI) score. *Middle panel*: Corresponding changes in AH ratings relative to baseline for the supine (SUP) condition. *Bottom panel*: Corresponding changes in AH ratings relative to baseline for the mMEG condition.

3.3.3 Is there a correlation between TAI scores and condition effects on AH?

When the individual changes in AH ratings from the baseline condition for a given stimulus were plotted as a function of individual trait anxiety (TAI) scores, there was a

tendency (p=0.24) towards a positive correlation (Figure 3.5, r^2=0.12). In contrast, the corresponding plots for the supine condition relative to baseline and the mMEG condition relative to baseline did not reveal appreciable tendencies to vary with TAI scores with p-values 0.50 and 0.60 respectively(Figure 3.5, middle and bottom panels).

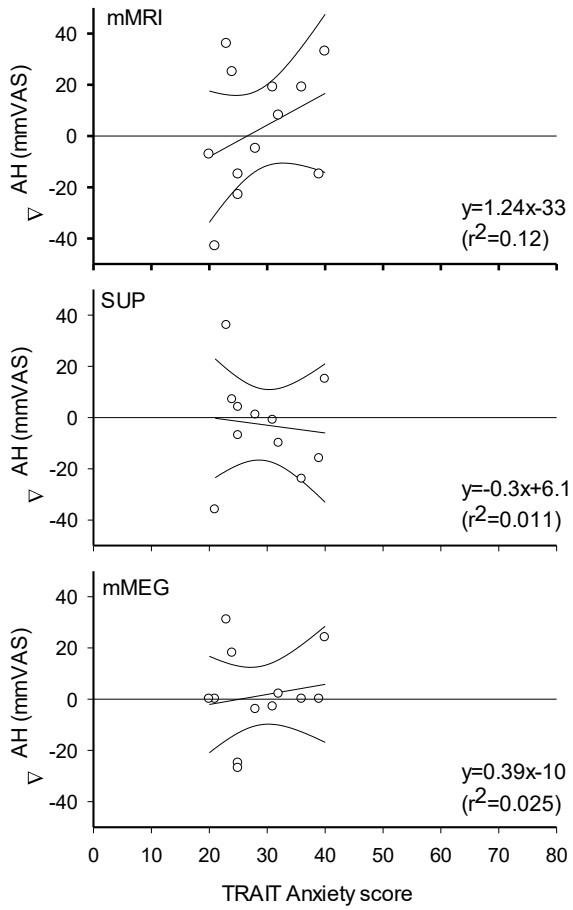


Figure 3.5: Changes in AH sensitivity as a function of individual trait anxiety scores
Top panel: Individual changes in AH rating in the mMRI environment relative to baseline for a given stimulus, plotted as a function of individual trait anxiety scores. The line of linear regression through the data is shown with 95% confidence intervals. The r^2 value of 0.12 and p-value of 0.24 indicates a tendency for bigger increases in AH ratings in the mMRI scanner relative to baseline with increasing Trait anxiety scores. *Middle panel*: The corresponding plot for the changes associated with the supine condition relative to baseline (p=0.50). *Bottom panel*: The corresponding changes associated with the mMEG condition relative to the baseline condition (p=0.60).

A stronger correlation emerged between differences in AH sensitivity and individual trait anxiety scores when the mMRI condition was compared to the SUP condition (Figure 3.6, r^2=0.29). The mean±sem change in AH relative to baseline for a given stimulus during

SUP compared to during mMRI was -5±15 mmVAS for those individuals who had a trait anxiety score less than 30 (TAI=24±2.3) and +20 mmVAS for those individuals who had a trait anxiety score greater than 30 (TAI=36±4.0). This difference was statistically significant (unpaired t-test; p=0.02).

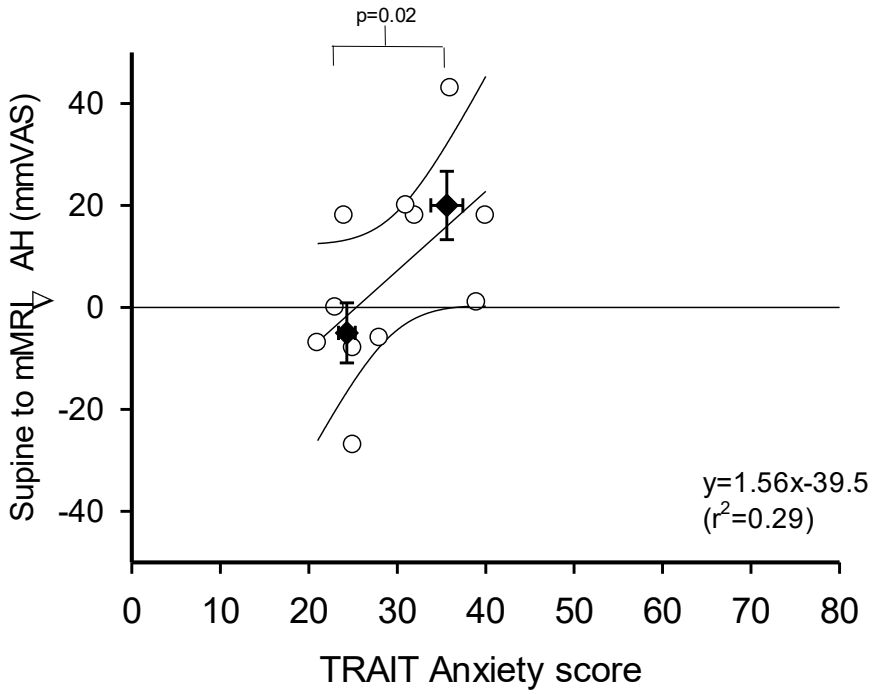


Figure 3.6: Differences in AH sensitivity relative to baseline between SUP and mMRI as a function of trait anxiety. The change in air hunger relative to baseline for a given stimulus when going from the supine condition to the mock MRI condition (ΔAH) tended to become more positive with increasing trait anxiety scores (r^2=0.29). Solid diamonds indicate the mean±semΔAH and mean±sem trait anxiety scores for subjects with trait anxiety scores less than 30 and for those with trait anxiety scores greater than 30. Unpaired t-test indicated a significant difference in ΔAH between these subgroups (p=0.02).The threshold of 30 TAI was chosen because subject's with TAI score above 30 had high air hunger ratings compared to those with TAI score lower than 30 at the a given level of PCO_2.

3.3.4 Changes in state anxiety scores in anticipation of conditions

Figure 3.7 indicates that subjects who had a trait anxiety score greater than 30 (TAI=37±4) consistently scored higher state anxiety scores prior to each AH test than those subjects who had a trait anxiety score less than 30 (TAI=24±3). High TAI subjects were inclined to have the lowest state anxiety prior to commencing the SUP condition and the highest prior to commencing the mMRI condition (Figure 3.7, right) – this pattern was not evident in the low TAI subjects (Figure 3.7, left).

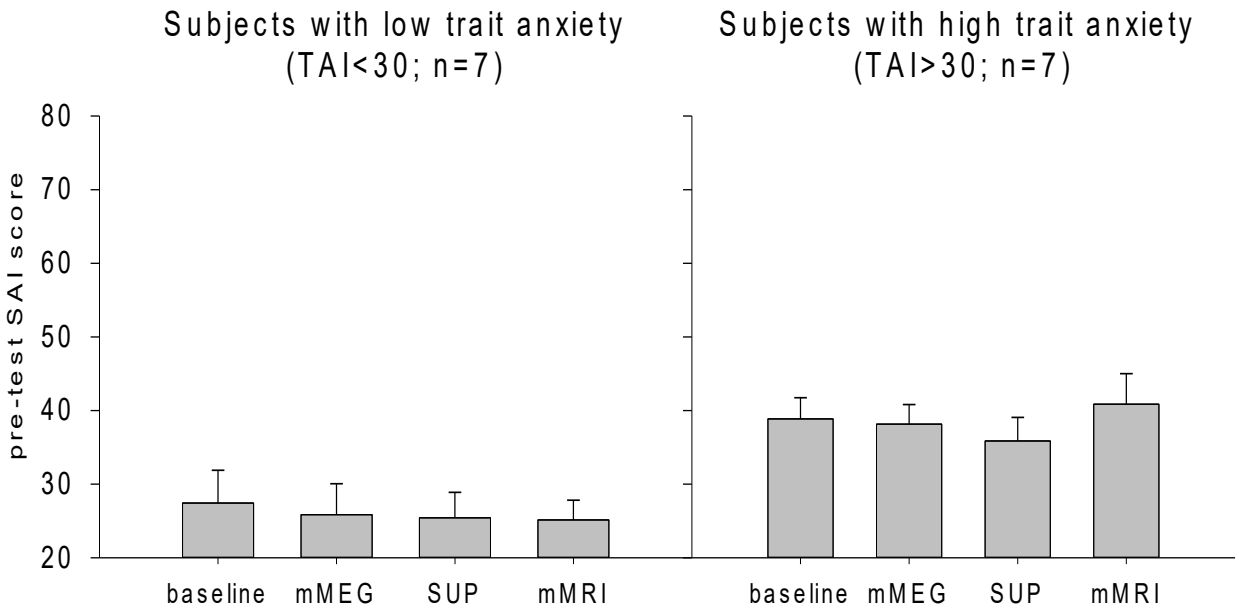


Figure 3.7: State anxiety scores immediately prior to AH test in different conditions. *Left panel*: State anxiety (pre-test SAI scores) associated with knowing under which condition the AH test was about to be performed; sitting upright (baseline), sitting upright under the mock MEG scanner (mMEG), lying supine (SUP) and lying supine in the mock MRI scanner (mMRI). Data are the mean±sem absolute scores in the subgroup of subjects are(n=7) who had a trait anxiety score less than 30 (mean±sem TAI=24±3). *Right panel*: The corresponding data plotted for the subgroup of subjects (n=7) who had a trait anxiety score higher than 30 (mean±sem TAI=37±4).

The difference between the subject subgroups (low TAI versus high TAI) in terms of differences in pre-test SAI scores can be seen more readily when plotted for the mMEG, Sup and mMRI conditions relative to baseline pre-test SAI scores (Figure 3.8). For the

mMRI condition, the high TAI subgroup showed a mean±sd change in pre-test SAI score (relative to baseline condition) of 2±10.3 while for the low TAI subgroup the corresponding change was -2.3±7.8. While the direction of change is opposite for the low and high TAI groups for this condition (see mMRI bars in Figure 3.8), this difference did not achieve significance (unpaired t-test, p=0.265).

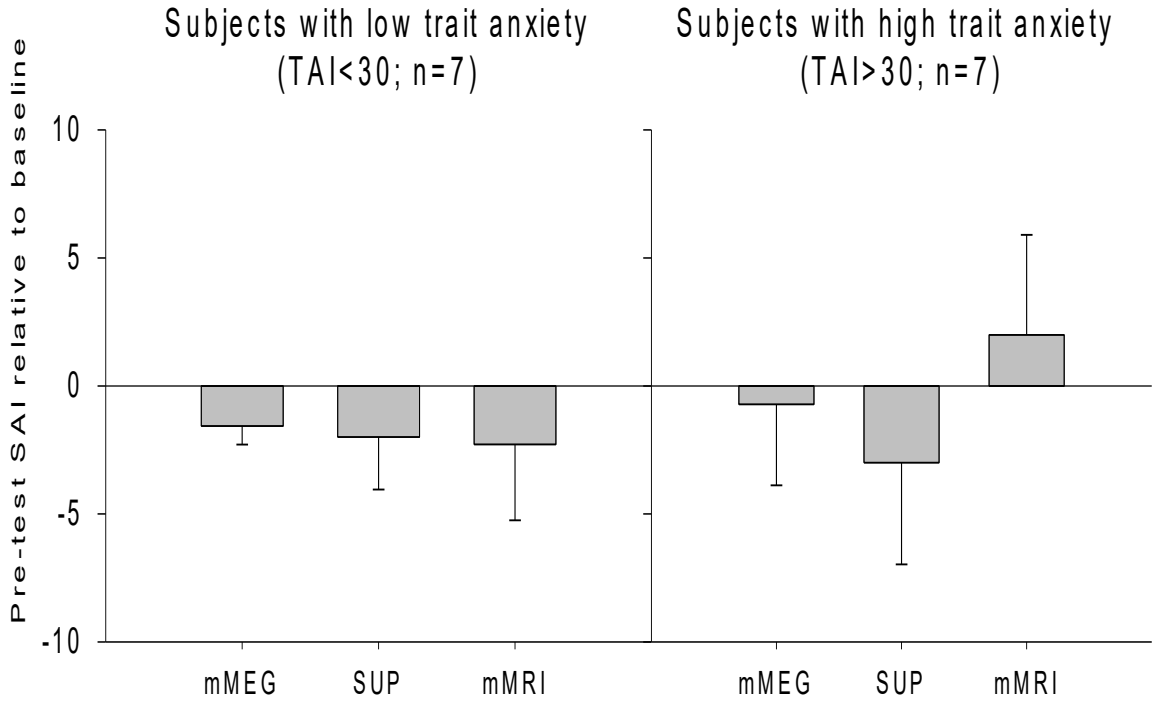


Figure 3.8:Pre-test state anxiety scores relative to baseline for low versus high TAI. *Left panel*: The low TAI subgroup (mean±sem TAI=24±3; n=7) show a tendency for reduced pre-test state anxiety (SAI) for each condition relative to baseline associated with knowing under which condition the AH test was about to be performed; sitting upright under the mock MEG scanner (mMEG), lying supine (SUP) and lying supine in the mock MRI scanner (mMRI). Data are the mean±sem change in pre-test SAI scores relative to the pre-test SAI for the baseline condition. *Right panel*: The corresponding data plotted for the subgroup of subjects are(n=7) who had a trait anxiety score higher than 30 (mean±sem TAI=37±4) indicates a tendency for state anxiety to rise relative to the pre-test SAI scores for the baseline condition. This tendency did not achieve statistical significance (p=0.27).

3.3.5 Effect of posture on spirometry

All subjects had a lower FEV1 and lower FVC in the supine position as opposed to the sitting upright position (Table 3.2). Although the differences were small (mean±sd changes of -0.34±0.3 and -0.36±0.4 liters respectively) they did achieve statistical significance (paired t-test; p=0.0005 and p=0.0033 respectively). However, because both FEV1 and FVC change by a similar amount when adopting the supine position the resultant change in FEV1% was not significant;0.2±2.4 (p=0.64, Table 3.2).

Table 3.2: Individual spirometry and Body Mass Index data
Body Mass Index (BMI) and spirometry data are shown for each subject. The spirometry includes Forced Vital Capacity (FVC), Forced expired Volume in 1 (FEV1) and the ratio of FEV1 to FVC (FEV1%) for supine and sitting upright conditions. The effect on the spirometry values due to change in posture from sitting to supine is also indicated. * = P<0.05; **=P<0.01; ***=P<0.001: paired student's t test.

ID	BMI kg/m^2	FVC litres sitting	FVC litres supine	FVC litres change	FEV1 litres sitting	FEV1 litres supine	FEV1 Litres change	FEV1 % sitting	FEV1 % supine	FEV1 % change
S2	29.1	6.13	5.04	-1.09	5.55	4.87	-0.68	90.5	96.6	6.1
S3	29.1	5.87	5.72	-0.15	5.29	5.04	-0.25	90.1	88.1	-2.0
S4	21.5	4.59	4.19	-0.4	4.57	4.19	-0.38	99.6	100.0	0.4
S5	25.2	3.25	3.17	-0.08	3.13	3.02	-0.11	96.3	95.3	-1.0
S6	20.8	2.28	2.21	-0.07	2.28	2.21	-0.07	100.0	100.0	0.0
S7	27.6	5.7	5.63	-0.07	4.96	4.67	-0.29	87.0	82.9	-4.1
S8	24.9	3.31	3.24	-0.07	3.27	3.17	-0.1	98.8	97.8	-1.0
S9	18.4	3.33	3.29	-0.04	3.28	3.23	-0.05	98.5	98.2	-0.3
S10	28.1	4.40	3.84	-0.56	4.12	3.59	-0.53	93.6	93.5	-0.1
S14	19.6	3.03	2.86	-0.17	2.93	2.8	-0.13	96.7	97.9	1.2
S15	21.5	4.43	4.06	-0.37	4.32	3.98	-0.34	97.5	98.0	0.5
S19	22.8	3.54	2.87	-0.67	3.54	2.87	-0.67	100.0	100.0	0.0
S21	29.1	3.83	2.89	-0.94	3.74	2.9	-0.84	97.7	100.0	2.3
Ave	**24.4**	**4.1**	**4.7**	**-0.36****	**3.9**	**3.6**	**-0.34*****	**95.9**	**96.0**	**0.2**
SD	**3.9**	**1.2**	**4**	**0.4**	**1.0**	**0.9**	**0.3**	**4.2**	**5.2**	**2.4**

The mean±sdBMI was within the normal range (24.4±3.9 kg/m^2) although 3 individuals were borderline overweight with BMI values close to 30 kg/m^2 (Table 3.2). Individuals

with higher BMI tended to have greater reductions in both FVC and FEV1 with change in posture from sitting to supine (Figure 3.9).

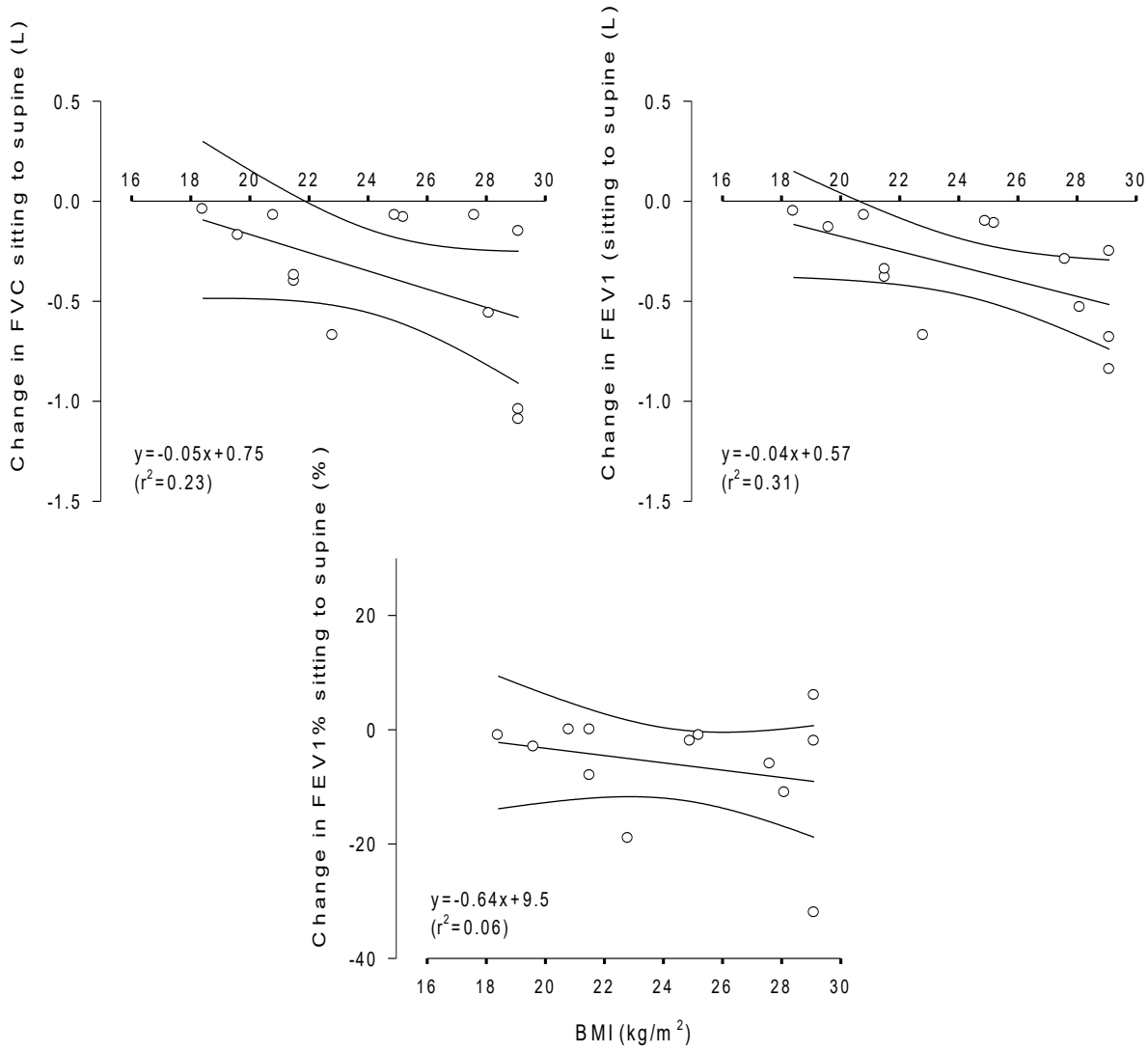


Figure 3.9: Change in dynamic lung volumes with posture as a function of BMI.
Top Left: Individual changes in Forced Vital Capacity (FVC, top left), Forced Expired Volume in 1 second (FEV1, top right) and the ratio of FEV1 to FVC (FEV1%, bottom panel) each plotted against individual Body Mass Index values (BMI). Solid lines indicate the linear regressions along with 95% confidence intervals for each of the scatter plots. The equations of the lines of linear regression through the scatter and the r2 values are indicated for each plot.

3.3.6 Effect of posture on oxygen saturation

All subjects had a slight increase in arterial oxygen saturation when in the lying supine posture compared to the sitting upright posture. Thus the mean±sd SpO2% was 97.1±0.8 as an average of the two sitting upright conditions (baseline and mMEG) and 98.6±0.6 as an

average of the two lying supine conditions (supine and supine in mMRI). This small difference was highly significant statistically as it was seen in all subjects (paired t-test, $p<0.000001$).

Table 3.2 Arterial oxygen saturation during sitting upright and supine posture (n=14). Oxygen saturation (SpO_2%) measured prior to AH testing at the start of each of the different conditions (sitting upright baseline, sitting upright in mMEG, lying supine and lying supine in mMRI) for each individual subject who completed the study. The average of the two sitting upright conditions and the average of the two lying supine conditions are also provided. Mean±sd of SpO_2 for the condition one (sitting upright) was found to be 96.9±1.0, sitting upright (mMEG) was 97.4±0.6, lying supine outside mMRI was 98.6±0.8 and supine in (mMRI) was 98.5±0.7.

Subject	SpO_2 (%) SITTING UPRIGHT			SpO_2 (%) LYING SUPINE		
	Baseline	mMEG	Average	Supine	mMRI	Average
S1	97	97	97.0	99	99	99.0
S2	96	97	96.5	98	98	98.0
S3	95	97	96.0	98	97	97.5
S4	98	98	98.0	99	99	99.0
S5	98	98	98.0	98	98	98.0
S6	97	98	97.5	100	99	99.5
S7	97	97	97.0	99	99	99.0
S8	95	96	95.5	97	98	97.5
S9	97	97	97.0	99	99	99.0
S10	98	98	98.0	100	99	99.5
S14	96	97	96.5	99	98	98.5
S15	97	98	97.5	98	99	98.5
S19	97	97	97.0	99	98	98.5
S21	98	98	98.0	98	99	98.5
Mean	96.9	97.4	97.1	98.6	98.5	98.6
SD	1.0	0.6	0.8	0.8	0.65	0.6

3.4 DISCUSSION

3.4.1 Main findings

(1) On average there was no significant change in air hunger sensitivity in the different environments compared to baseline. However this masked a considerable degree of individual variability. When the data were stratified according to trait anxiety scores, a

powerful trend emerged despite the small number of subjects in each group. In particular, those individuals with 'high' anxiety either dropped out of the study or increased their AH sensitivity for a given stimulus within the mock MRI scanner. In contrast those with 'low' anxiety showed on average a reduced AH sensitivity in the mock MRI scanner.

(2) There was a general tendency for reduced state anxiety associated with subjects knowing that they were about to do the supine condition –but the mean fall in scores was not significantly different for high or low TAI subgroups. A more interesting observation was that the change in SAI (for baseline versus mMRI conditions) was in opposite directions for the 'high' trait anxiety and low trait anxiety subgroups –again this difference was not significant but may warrant further investigation with greater power.

(3) Expected physiological changes were observed when adopting a supine posture, including a small but highly significant improvement in oxygen saturation. In addition therewere small but significant reductions in FEV1 and FVC of similar amounts such that the FEV1/FVC ratio was unaffected.

3.4.2 Adopting the supine posture

Gravitational effects on lung mechanics: The role of gravitational forces on pulmonary function in healthy subjects relative to different postures has been well documented by many authors over the years (Hojat and Mahdi, 2011; Makhsous *et al*, 2004; Townsend, 1984). Those studies which measured the relationship between intrapulmonary gas mixture and dynamic lung volumes in both sitting and supine positions in healthy subjects and patients all reported lower values for FVC and FEV1 in the supine position (consistent with findings in this study). However, different magnitudes of change were reported (Blair and Hickman, 1955; Clauss *et al*, 1968; Barach, 1974; Navajas *et al*, 1988; Liu *et al*, 1991; Xie *et al*, 1991; Vilke *et al*, 2000; Melam *et al*, 2014; Patel and Thakar, 2015). The

decreased dynamic lung volumes in the supine posture aregenerally attributed to adverse lung mechanics (e.g. through the increased pressure of the abdominal contents on the diaphragm) which might be expected to increase breathlessness through a need for greater respiratory muscle effort. With this in mind, the observed reduction in AH sensitivity when supine was unexpected. However, since supine dynamic lung volumes do not fall outside the 'normal' predicted values and do not significantly alter FEV1/FVC ratio the adverse effects on lung mechanics may be well within the capacity of the inspiratory muscle strength of healthy individuals.

There was a tendency for air hunger to be reduced for a given PCO_2 when going from upright to supine posture. While this does not tally with reduced lung mechanics, it is recognised that changes in FVC and FEV1 do not correlate well with measures of breathlessness. Another explanation for the reduced AH sensitivity when supine is that there is an improvement in ventilation-perfusion matching (consistent with a rise in SpO2 from 97 to 99%) and this could reduce the response of peripheral chemoreceptors to raised PCO_2 and therefore reduce reflex increase in drive to breathe and therefore change the need to breathe-how much you are breathingmismatch in favour of less air hunger.

Gravitational effects on ventilation and perfusion distribution: Intrapleural pressures at the base of the lungs are normally more negative because inspiratory muscle contraction leads to bigger changes in intrapleural pressures at the base; a feature of how the lungs are suspended in the upright position. Thus, in the upright position, ventilation tends to be greater at the base. The effects of gravity also cause the blood pressure to be lower at the apex of the lungs as this is higher than the level of the heart thus causing less perfusion at the apex. The relative magnitude of ventilation and perfusion changes from base to apex in the upright lungs normally leads to a net decrease in ventilation (V)/perfusion (Q) ratio from base to the apex of the lungs. When adopting a supine posture, the V and Q become

uniform between apex and base but a mismatch now appears in the posterior vs anterior lung direction; it is conceivable that in healthy individuals, this may lead to a reduction in the small V/Q mismatch that is normally present (Riley and Cournand, 1949; Riley and Cournand, 1951; Fowler *et al,* 1952). Consistent with this possibility in the present experiment, arterial oxygen saturation (SpO_2%) tended to be closer to 100% in the supine posture than when seated.

An increase in SpO_2% in the supine posture could also be a consequence of improved signal quality in the pulse oximeter as a result of the finger probe being at heart level. Whether this small increase in oxygenation could account for a decreased AH sensitivity is debatable. There do not appear to be any studies that have investigated changes in SPO_2 when lying supine against other positions and its applications.

Psychological benefit of adopting supine posture:A lower state anxiety associated with the supine posture would suggest that any reduced AH sensitivity in the supine posture may at least in part be due to changes in psychological factors rather than entirely due to physiological changes especially if the AH sensitivity returns when supine in the MRI scanner. Although the underlying mechanism by which this might occur is unclear, it is possible that supine position promotes intrinsic relaxation and good feelings, but that this is to some extent reversed inside an enclosed environment. The finding that this is more apparent in those with a higher general anxiety level suggests that these individuals benefit more from adopting the supine posture. While interesting this may only apply to healthy individuals as pathophysiological effects of adopting a supine posture in patients with lung disease could outweigh any psychological benefit.

3.4.3Effect of entering the MRI scanner

The physically enclosed design of an fMRI scanner can be intimidating to patients and research participants. The thought of undergoing the process can be very unsettling and undeniably produces subjective distress (Dantendorfer *et al*, 1997; Flaherty and Hoskinson, 1989; Kilborn and Labbe, 1990) and episodes of panic attacks in individuals with no history of psychotic illness (Spiegelhalder *et al*., 2009). In a study by Maddock *et al*, (2003), the authors reported that four out of six patients who underwent MRI scan reported a sense of anxiousness. In a separate study by Bystritsky *et al* (2001), panic symptoms in patients were found to be much higher compared to controls. Despite the better temporal and spatial resolution of the fMRI compared to other neuroimaging techniques, the incidence of psychological disorders such as anxiety, panic attacks, claustrophobia, fear and emotional distress has long been associated with the fMRI scanning procedure (Quirk *et al,* 1989; Melendez and McCrank, 1993; Dewey *et al*, 2007). Claustrophobia has been defined by several authors as the expression of anxiety, panic or fear in conditions which promote restriction of movement, suffocation and invasion of personal space (Harris *et al,* 1999; Bigley *et al,* 2010). This situation has been reported to be worse in psychiatric patients (Quirk *et al,* 1989) and in children undergoing the MRI procedure (Raschle *et al*, 2012).

The identified contributing factors which heighten claustrophobic reactions such as anxiety and panic in an MRI scanner are: the small bore size of modern scanners (60cm for the 3Tesla scanner) generating a confined environment, the average scanning time of approximately 20-90 minutes for functional scans, and the loud acoustic noise from the scanner (MacIsaac *et al*, 1998). A number of studies have tried to reduce the problem by using open scanners and by giving participants lots of practice to familiarise themselves with the environment. Although open scanners have limitations such as poor image quality,

poor magnetic strength and longer examination times, they are now widely used in both clinical and research settings. Despite the improvement in imaging techniques, the number of premature terminations of procedures and the production of unreliable imaging data remains high (Enders *et al*, 2011b; Entsar *et al*, 2013). It has been acknowledged that the training and the practice session is time consuming as well.

3.4.4 Significance of study for fMRI and MEG studies of breathlessness

When supine individuals were wheeled into the MRI scanner, this study found that only those with high trait anxiety increased their AH sensitivity in the scanner. These subjects had trait anxiety score greater than 30 but lower than the score of 33 reported for the general population aged 22-78 years (Crawford *et al*, 2011). Furthermore, the subjects who dropped out of the study also had trait anxiety higher than 30 compared to those who completedthe study. Together these findings suggest thatsubjects recruited for fMRI studies of breathlessness are mostly those with trait anxiety below 30 and therefore not representative of the general population. This calls into question the general assumption of the activations reported in brain imaging studies of breathlessness particularly when the fMRI technique is employed.

While it is widely acknowledged that the breathlessness mechanisms can be considerably modulated by psychological factors such as anxiety, panic attack and emotional distress (De Peuter *et al*, 2004; von Leupoldt *et al*, 2008), the extent to which this is relevant for neuroimaging studies on breathlessness is yet to be determined. Understanding the level of the psychological impact on breathlessness may help to distinguish brain areas that process breathlessness sensation and those related to general psychological symptoms and thereby improve the interpretation of brain imaging studies of breathlessness. This will, in turn, improve diagnosis and ensure tailored treatment or management option for thepatient. This

study also indicates that participants who undertake functional brain imaging studies for research purposes should complete a state-trait anxiety inventory which is likely to aid interpretation of findings.

Although it is beyond the scope of this present study, if preliminary findings are confirmed, this would underscore a need for actual fMRI studies alongside actual MEG studies of breathlessness in the same subjects. This could help distinguish activations related to anxiety and those to breathlessness per se.

3.4.5 Limitations of thestudy

Number of subjects: Due to the drop-out rate in this study, the total number of subjects included in the analysis (n=13) fell below the number stipulated by the original sample size estimation. However, even with less subjects and with splitting the subjects into high and low trait anxiety, strong tendencies were detected some of which achieved significance (Figure 3.6). This suggests that these are powerful effects.

Order effect: The order of conditions was initially not randomised for the first 6 subjects, but was randomised for the rest of the subjects, thus differences in AH sensitivity and in state anxiety between conditions may reflect an order effect to acertain extent. Both the state anxiety and the AH data suggest that there may be an order effect such that the first condition completed will always be the one with the highest sensitivity. The protocol design was revised for the remaining subjects such that the order of conditions was randomised.

Administration of state trait anxiety inventory questionnaire: The first 6 subjects were asked to complete the state anxiety inventory with reference to how they felt during the condition just completed. The inventory was not designed to be used in this way, but rather to get subjects to complete the form for how they were feeling at that time. This was

corrected for the remaining subjects who were told which condition they will be tested in next and just before starting were instructed to complete the state anxiety form for how they were feeling at that moment. This is likely to generate a more direct assessment of how much anxiety is generated by each condition and may therefore provide a more powerful demonstration of the affective valence of the MRI environment.

Testing equipment: The purpose built mock MRI scanner along with the makeshift head coil successfully generated considerable psychological stress in the subjects. This suggests that the mock scanner was a good simulation of actual fMRI for the purposes of this study.

3.4.6 Conclusion

This study demonstrates that the air hunger sensitivity is affected by the MRI environment *if* the subject has high trait anxiety. This leads to two considerations: (i) that brain imaging studies trying to find the source of AH perception in the brain using MRI technology may be basing their conclusions on a biased population (those with low trait anxiety) and(ii) thatbrain imaging studies used for researchingperception of any unpleasant sensations should take account of individual trait anxiety scores as this may well influence the pattern of activations detected. Finally, this is the first study to quantify the impact of a stressful environment (e.g. mock MRI) on air hunger sensitivity. Furthermore, the novel aspect of this work is that, this increase in AH sensitivity depends on an individual's trait anxiety levelsand it should be considered before any imaging procedure for either clinical or research purpose.

CHAPTER 4

EFFECT OF DEEP BRAIN STIMULATION OF THALAMIC NUCLEI ON DYSPNOEA

4.1. INTRODUCTION

Deep brain stimulation (DBS) refers to the delivery of an electric current directly to a specific subcortical structure of the brain via surgically implanted electrodes. The stimulation parameters (frequency, amplitude and pulse width) are titrated on individual patient basis for optimal therapeutic relief.

4.1.1. Overview of deep brain stimulation

Deep brain stimulation has rapidly emerged as a choice of intervention to treat patients with a wide spectrum of neurological symptoms, when conventional treatments (e.g. pharmacological) are ineffective and the patient's symptoms become intolerable. More than 100,000 patients worldwide have received DBS, predominantly for movement disorders, chronic neuropathic pain and epileptic seizures (Shen, 2014; Fisher *et al,* 2010; Benabid *et al*, 1987, 1996). This number is only likely to increase exponentially in the near future with improvements in surgical techniques and technical specifications of the electrodes (Udupa and Chen, 2015), and as the benefits of the treatment become clearer. DBS procedures have evolved significantly since its use was first reported in the early 1980's (Benabid *et al*, 1987). Recent explorative studies have identified further neurological and psychiatric conditions that can potentially be treated by DBS (Lyons 2011; Hariz *et al*, 2013).

DBS procedures consist of two key stages: (i) surgical implantation of a single or multiple intra-cerebral microelectrodes into targeted brain nuclei and, (ii) implantation of programmable pulse generator (IPG) under the skin of the chest where electrical current is generated and delivered to the electrode via leads tunnelled under the skin. In between these two key steps, externalised leads from the implanted electrodes are connected to an external programming device to determine the individual stimulus parameters to achieve the desired therapeutic effect. Although DBS is invasive and involves major surgery, it is reversible and personalised based on symptom severity and overall intended outcome to suit the treatment need of each patient (Bain *et al*, 2009).

The therapeutic benefit of DBS in chronic neuropathic pain and movement disorder conditions such as Parkinson's disease has been considered remarkable with high success in functional improvements for most patients. Long term therapeutic benefit is illustrated by a growing number of patients who continue to benefit even after 10 years post-surgery (Bittar *et al*, 2005; Franzini *et al*; 2010, Boccard *et al*, 2013). Neurologists, neurosurgeons and a selected group of patients have expressed confidence in theDBS procedures (Green *et al*, 2006; Hyam *et al*, 2012).

4.1.2 Utility of DBS in clinical and research settings

The deep cortical structures in the human brain are inaccessible under normal circumstances, however, DBS has made the subcortical brain structures easily accessible for clinical research and has led to a better understanding of pathophysiology of neurological diseases through opportunistic recordings of local field potentials (LFP) (Green *et al*, 2012; Brown and Williams 2005; Sverrisdottir *et al,* 2014) –i.e. the electrodes can be used to record from rather than stimulate the local neural tissue. Other psychosomatic illnesses such as severe depression or obsessive compulsive disorder which

have pharmacologically become refractory to medication have also been reported to achieve sufficient relief from the procedure (Mayberg *et al*, 2005).

4.1.3 DBS, breathing and breathlessness

Since there appears to be considerable overlap between pain perception mechanisms and dyspnoea perception mechanisms (Banzett and Moosavi, 2001;Gracely *et al*, 2007), it is reasonable to speculate that relief of chronic neurological pain by DBS of the anterior cingulate cortex (Boccard *et al,* 2013) would also relieve the perception of experimentally induced dyspnoea. This idea has not been previously explored.

First hand experimental evidence about the effect of DBS on ventilation is scarce. Recent data from a neuro-functional study have shown that DBS of the subthalamic nucleus (STN) and the globus pallidus (GPi) improves the ratio of forced expiratory volume in 1 second to forced vital capacity (FEV1/FVC) in patients with chronic neurological pain, indicating a reduction in bronchoconstriction (Hyam *et al*, 2012). Thus DBS of the STN for relief of Parkinsonian tremor appears to also have the potential to reduce autonomic dysfunction (e.g. relieve bronchoconstriction). It has also been reported in a prospective study that the experience of dyspnoea can be a side effect of STN DBS (Chalif *et al*, 2014) –this raises the potential for this technique to be used as a tool to investigate cerebral mechanisms of dyspnoea.

4.1.4. Working hypothesis and specific aim of study

Functional brain imaging studies of experimentally induced breathlessness in healthy individuals has identified various brain structures that may be involved in the cerebral mechanisms of dyspnoea (Banzett *et al*, 2000; Evans *et al*, 2002). It has been noted that there is considerable overlap between these areas and those identified for pain perception (e.g. ACC and insular cortex), although some differences are also evident (e.g. pain

perception appears to be more linked to posterior insular activations while dyspnoea perception more anterior insula). Recent experimental evidence has indicated a remarkable relief for neuropathic pain from DBS of the ACC.

Recently, anecdotal evidence has emerged from patients receiving DBS in the ventrolateral-intermediate nuclei (VIM) of the thalamus for the clinical purpose of relieving essential or dystonic tremor. Neurosurgeons in the Oxford Functional Neurosurgery Centre have noted that a small subgroup of these patients report dyspnoea as an undesirable side effect when the stimulus is 'on' (personal communication; Green 2016). This has led to a working hypothesis for the current chapter: ***DBS of motor-thalamic nuclei generates breathlessness***. If sensitivity to experimentally induced air hunger was increased when electrodes were activated, this would be consistent with this hypothesis.

Patients recruited for this study included one who had co-existing chronic obstructive pulmonary disease (COPD). In this case the patient was already breathless at rest as a result of the co-existing COPD and therefore did not require experimentally induced air hunger. This patient provided the opportunity to explore the effect of DBS on their existing perception of clinical dyspnoea. Since this patient had electrodes implanted in multiple nuclei, this also provided the additional advantage of comparing stimulation of different nuclei in the same patient.

4. 2. METHODS

4.2.1. Patients

Recruitment and sample size: All patients with DBS electrodes implanted in motor-thalamic nuclei or in nuclei ventral to the thalamus were recruited prospectively over a 1 year period from the Oxford Functional Neurosurgery Centre based at theJohn Radcliffe Hospital. Sixteen patients (14 males, mean±SD age at surgery 66±8.8 years) were recruited for the study over this period (Table 4.1). A previous study estimated the slope of the AH ratings measured on a visual analogue scale (VAS) in response to increasing $PETCO_2$ in healthy volunteers, and found the effect size to be 6.7±2.4 mmVAS per mmHg $PETCO_2$ (Banzett *et al*, 1996). Based on this, it was determined that a sample size of 16 participants would enable detection of a mean difference in slope of AH-$PETCO_2$ relationship of 20% with 80% power for significance at the $p<0.05$ level of probability.

Predominant clinical symptoms and target nuclei: Eight patients were treated for either essential or dystonic tremor predominantly affecting the right limb, with either bilateral ventral intermediate nucleus (VIM), bilateral globus pallidus internus (GPi) or bilateral ventro-oralis posterior (VOP) DBS stimulation. Five patients were treated for Parkinsonian tremor with either bilateral subthalamic nucleus (STN) or bilateral GPi DBS stimulation. Two patients were treated for chronic neuropathic pain with either ACC or peri-aquaductal grey (PAG) DBS stimulation.

Co-existing lung disease (patient 9 in Table 4.1): One patient was a 65 year old right-handed male with pre-existing clinically diagnosed COPD. There was documented video evidence of pursed-lips breathing during walking assessments. This patient was treated for a combination of post stroke pain, essential tremor and dystonic tremor in the left arm. Electrodes were implanted in four different brain nuclei unilaterally. Target nuclei in this

patient were the GPi for relief of dystonia, PVG for relief of post-stroke pain, and two thalamic nuclei for the relief of essential tremor and pain; the VIM and ventral posteriolateral nucleus (VPL) (Table 4.1).

Table 4.1: Physical demography of all patient cohorts recruited in this study.
The laterality of the implanted electrode was defined as either unilateral (Unil) or bilateral (Bil). The target nuclei for essential or dystonic tremor (ET or DT) relief were ventral intermediate nucleus (VIM), ventro-oralis-posterior (VOP) or globus pallidus internus (GPi). Target nucleus for Parkinsonian tremor (PT) was the subthalamic nucleus (STN). Target nuclei for chronic or post-stroke pain (CP or PSP) were anterior cingulate cortex (ACC), peri-aquaductal grey (PAG), peri-ventricular grey (PVG) or ventro-postero-lateral (VPL). (*=patients who did not complete the study and excluded from any analysis; †=patient with co-existing chronic obstructive pulmonary disease). Number of patients (n=16)

ID	Sex (M/F)	Age (yrs)	Wt (kg)	Ht (cm)	DBS target	Laterality	Treated symptom
P1*	M	72	85	155	STN	Bil	PT
P2*	M	50	60	152	ACC	Bil	CP
P3*	M	57	75	160	PAG	Bil	CP
P4	M	69	72	183	STN	Bil	PT
P5*	M	65	78	164	GPi	Bil	PT
P6	M	73	75	164	VIM	Bil	ET
P7*	M	50	94	155	GPi	Bil	DT
P8*	M	62	78	152	STN	Bil	PT
P9†	M	67	80	165	PVG,VIM,VPL,Gpi	Unil	ET, PSP
P10	M	69	94	170	VIM	Bil	ET
P11	M	76	85	171	VOP	Bil	ET
P12	F	66	89	166	VOP	Bil	DT
P13	M	78	83	179	VIM	Bil	ET
P14	M	67	78	178	VIM	Bil	ET
P15	M	68	73	180	STN	Bil	PT
P16	F	71	75	152	VIM	Bil	DT
Mean		66	79.6	165			
SD		8.82	8.7	10.7			

Medications on the day of testing: Patient 9 (with co-existing COPD) was the only one who had taken any medications on the day of testing, in this case inhaled bronchodilator 3 hours prior to testing.

Exclusion criteria: Patients were excluded if their neuropsychological profiles included any indication of psychiatric symptoms or disorders including neuroses, compulsive habits, or any indication of compromised cognitive functioning. Patients were also excluded if they had any speech impairment because this has been shown to be associated with lack of reliability in subjective ratings (Saint-Cyr *et al*, 2000; Shulman *et al*, 1982).

All patients provided written informed consent. The study was added as amendment 5 to an existing study ("Non-Invasive Cerebral Blood Flow Monitoring in Patients with Deep Brain and Occipital Nerve Stimulators"; PI Mr. Alex Green) and approved by the Oxford South Central C research ethics committee (Ref: 11/SC/0229).

4.2.2. Air hunger tests

Non steady-state ramp tests*:* Patients breathed via a mouthpiece and a non-rebreathing one way valve (Hans Rudolph, 5700-USA) from a 3 litre anaesthetic bag into which heated, humidified air was continually fed at a flow rate that matched the individual patient's resting baseline ventilation. Breathing frequency was fixed at 12 breaths per minute by instructing patients to breathe in time with an audible beep delivered via headphones. An air-oxygen blender (Sechrist-350, USA) supplied by a cylinder of medical air (input 1) and by a cylinder containing 10% CO_2, 21% O_2 balance N_2 (input 2) was used to add increasing amounts of CO_2 to the inspiratory reservoir (anaesthetic bag) while maintaining a normal fraction of inspired oxygen. Thus hypercapnia was 'ramped' up by raising the inspired fraction of CO_2 by an amount that resulted in 2-3mmHg increments in end-tidal PCO_2 every minute.

Steady state air hunger tests: Steady state air hunger tests involved increasing the inspired level of CO_2 to a level that generated air hunger ratings approximating 50% of the VAS (determined from the ramp test data). The hypercapnic stimulus was maintained for 5 minutes assuming that a steady state will have been established between the stimulus (hypercapnia with ventilation constrained to baseline level) and the response (VAS ratings of air hunger) (Banzett *et al*, 1996). Detailed discussion of standard ramp and steady-state air hunger tests, including their validity and reliability is provided in Chapter 2 of this thesis.

4.2.3 Physiological and psychophysical measurements

Respiratory flow measurement: Airflow was measured using a pneumotach (AD instruments, Oxford UK) connected to a pressure transducer (Validyne mp45-1, ± 2cm water). Tidal volume (V_t) and respiratory frequency were derived from airflow. P_{CO_2} was monitored and recorded continuously (AD instruments gas analyser ML 206) via a small diameter tube inserted into the mouthpiece.

Blood pressure, ECG, heart rate and arterial blood oxygen saturation: A 3 lead ECG was monitored (HME Lifepulse LP10) from which heart rate was derived. SpO_2 was monitored continuously by pulsed oximetry using a finger probe (BCI, Capnocheck plus, Wisconson USA). Changes in blood pressure were monitored using a non-invasive automated beat-by-beat monitor using a second finger probe (Portapres –Finapres Medical Systems UK). All analogue signals were digitalised using an A-D converter (1401, Cambridge Electronic Design, Cambridge UK) at a sample rate of 20Hz (except ECG which was digitised at a sample rate of 100Hz) and recorded on a computer using Spike 2 software (version 6.13 Cambridge Electronic Design, Cambridge, UK) for later off-line analysis.

Subjective ratings of air hunger (visual analogue scale): Patients rated air hunger using a 100 mm visual analogue scale similar to that used byMoosavi *et al*, (2007).The ends of the scale were defined as “no sensation” and an “extreme” level, the latter was described as an ‘intolerable’ level. Patients were informed that if they reached “extreme” the stimulus would immediately be reduced or stopped. The patients were also instructed to place three additional written labels on the scale (‘slight’, ‘moderate’ and ‘severe’) at locations of their choice beside the scale; this was to ensure that they used the scale consistently between tests (Lansing *et al*, 2003). Patients were cued to rate their AH perceptionby a light that flashed once every fifteen seconds.They filled out a questionnaire after the first ramp on day one in which they described their sensations. Patients who described phrases connected to air hunger were instructed to continue rating those sensations. Those patients who described other sensations rather than air hunger (e.g. breathing felt harder) were asked not to include them in the ratings and to report them after each test.

Dyspnoea-12 questionnaire (D-12): All patients completed a dyspnoea-12 questionnaire containing 7 physical and 5 affective descriptors to obtain perceptual information from patients after each experiment. Patients indicated on a Likert scale(none, mild, moderate or severe) what extent each item on the questionnaire applied to feelings experienced during the test just completed. D-12 data were obtained for each DBS settings when the electrodes were not activated (switched ‘OFF’), when the electrodes were activated (switched ‘ON’), and also when the electrodes were set at 25% and 75%of the overall therapeutic threshold). In situations where patients were not able to score due to mild to severe tremor, an accompanying family member was allowed to tick a score on their behalf after the patient had indicated their choice. A trained technician or clinician from the neurosurgery team was present during testing to ensure patient safety and manipulate the DBS frequency and/or amplitude settings.

Standard debrief questionnaire:A standard debrief questionnaire was administered following each test to gather any volunteered comments and to collect patients' selection of respiratory descriptive phrases (from a standard list) that best described their experience of breathing during the test just completed.

4.2.4 Protocol

All patients attended the "Gait laboratory" situated within the Neuroscience department, level 3, West Wing of the John Radcliffe Hospital for no longer than 2 hours.

Protocol for air hunger testing:An initial ramp test was performed in which patients were instructed to rate "any uncomfortable breathing sensations". The standard debrief that immediately followed this initial ramp test was used to ensure that AH was the predominant sensation generated and that patients used the VAS reliably. For subsequent ramp tests, patients were instructed to specifically rate "air hunger". The second and third ramp tests were done with the DBS set 'ON' and 'OFF' in random order (Figure 4.2A).

Steady state AH tests: For each patient a test level of inspired CO_2 was determined from the ramp tests. This was a level that generated air hunger at approximately 50% of the VAS. Up to Four 5min steady state tests were then performed with inspired CO_2 raised to the individual patient's test level. DBS was 'ON' for one, 'OFF' for another, at 25% of the therapeutic threshold level for a third and at 75% of the therapeutic threshold for the fourth test. The order of tests was randomised (Figure 4.2B).

Protocol for patient with co-existing COPD: This patient (P9 in Table 4.1), who had electrodes implanted in four locations, was not subjected to experimentally induced air hunger since they were already breathless at rest. This patient underwent a different protocol involving repeated administration of the D12 questionnaire on two separate visits. On the first visit day, the D12 assessments were carried out at baseline (all electrodes OFF)

and at 20 minutes after each of the GPi, VIM and PVG electrodes were individually switched 'ON' (with all other electrodes 'OFF') in that order (Figure 4.3). Stimulation of the VPL electrode was not tested on the first day due to time constraints brought about by other clinical tests scheduled for this patient. The protocol was repeated on a second visit 11 weeks later when the patient returned for their routine follow-up clinic appointment. All four electrodes were tested, including the ones located in the VPL, on this occasion. Although stimulating parameters of the electrodes were different during each assessment (Visit 1 and 2), the stimulating parameters of each pair (GPi/VIM and PVG/VPL) had the same stimulating values during each assessment (Table 4.2).

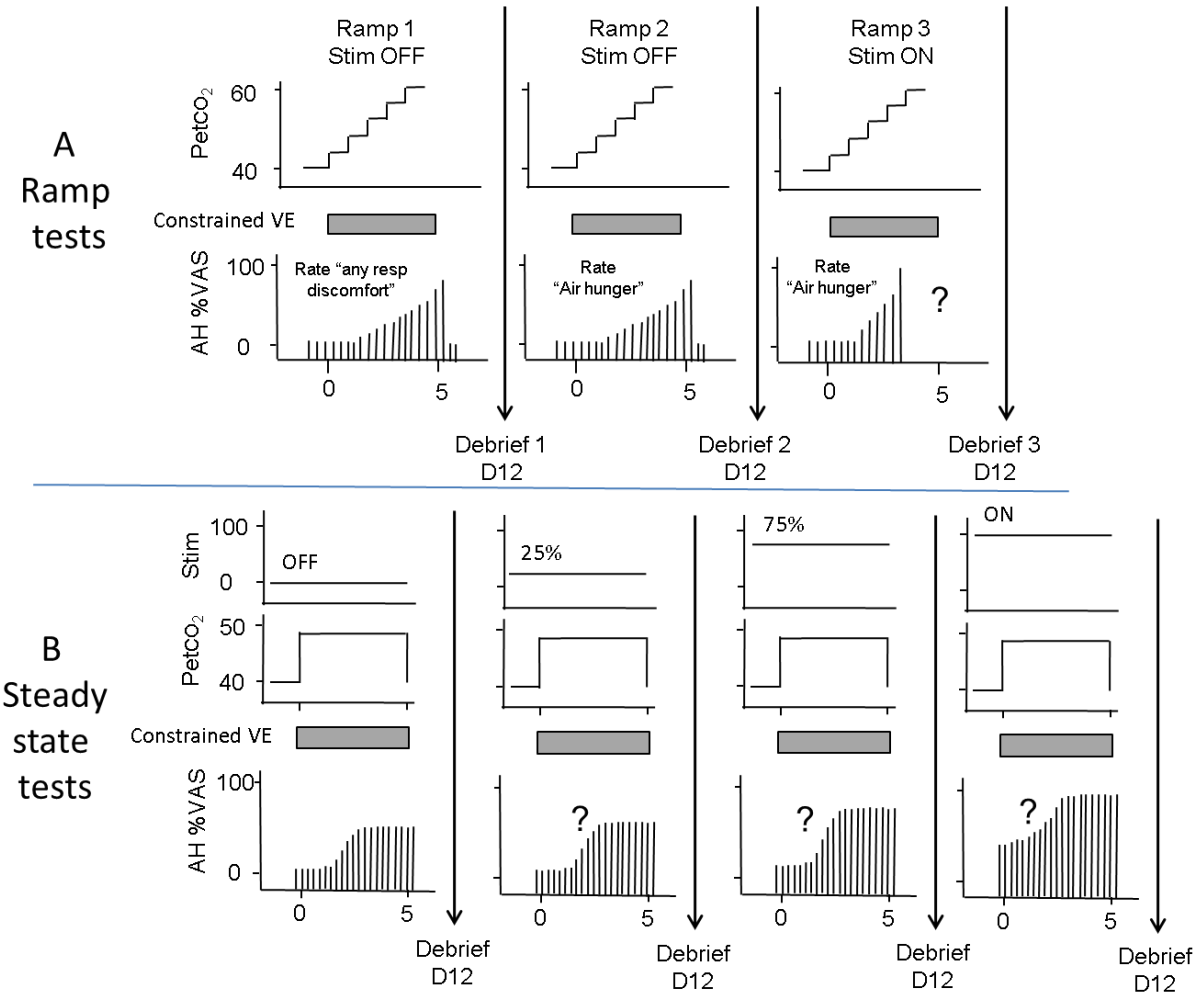


Figure 4.1: Schematics of the protocol for DBS patients who underwent AH test.

A: The initial Ramp test involved 1min increments in inspired CO_2 during constrained breathing and stimulator switched 'OFF' and 'ON'. ***B***: Four 5min tests were performed with inspired CO_2 raised to a test level that generated AH ratings at approximately half way up the scale.

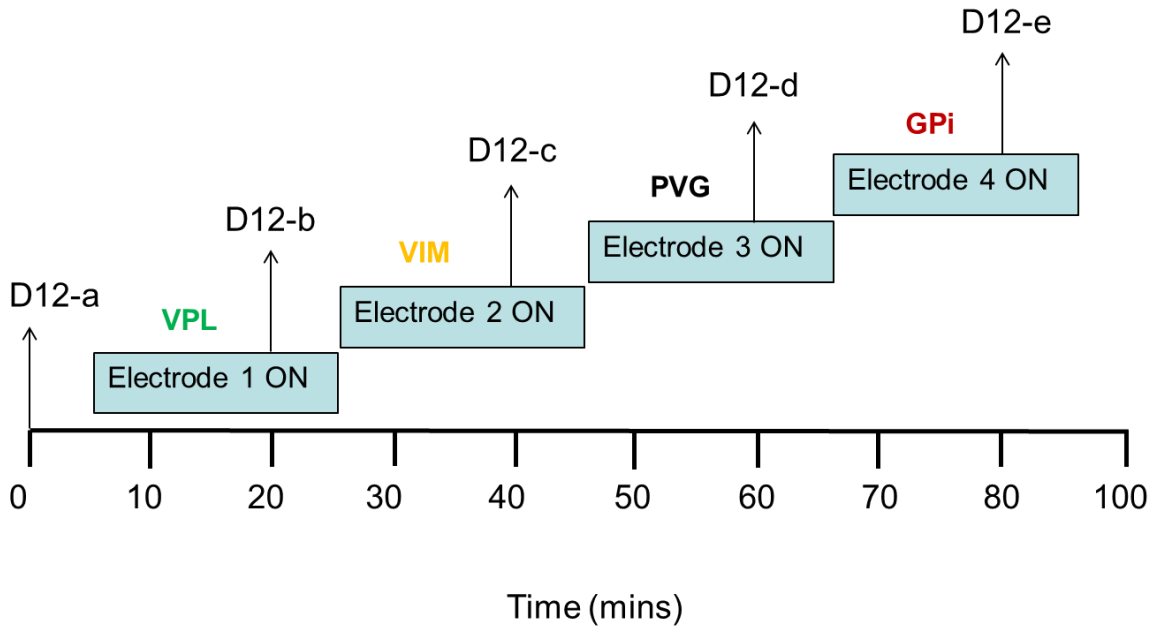


Figure 4.2: Protocol for patient 9 who had co-existing COPD.
Breathlessness was assessed using the Dyspnoea-12 questionnaire repeatedly: at baseline with all electrodes 'OFF' (D12-a) and then 15min after each of the electrodes (located in VPL, VIM, PVG and GPi respectively) were turned "ON" in turn with all other electrodes 'OFF' (D12-b, D12-c, D12-d, D12-e). This protocol was repeated 11 weeks later when patient 9 returned for his routine follow-up clinic appointment. VPL electrode was not tested on visit 1 due to time constraints.

Table 4.2 Change in stimulating parameters of electrodes between visits for P9.
On visit 1 each stimulation parameter was still under trial programming, thus they either remained the same or changed on the second visit for optimal therapeutic relief.

	Visit 1		Visit 2	
Electrode locations	GPi + VIM	PVG+VPL	GPi + VIM	PVG+VPL
Clinical indication	Dystonia/Tremor	Pain	Dystonia/Tremor	Pain
Amplitude (V)	3.7	5.5	7	6
Pulse Width (μs)	110	450	110	230
Frequency (Hz)	130	37	139	40

4.2.5 Neuropsychological profiling and tremor scores

All patients who participated in this study had cognitive function assessments as part of their clinical management. A battery of pre and post-operative neuropsychological test results were available for pre and postoperative motor symptom activity evaluations in each patient to help establish the level of motor impairment before and after surgery. Other quality of life assessment tools were also used. These assessments had been performed with all patients on medications and with stimulation 'on' during post-operative testing.

4.2.6 Data processing and analysis

Ramp AH test:A 60 second boxcar average was run through the breath-by-breath end-tidal PCO_2 data throughout the RAMP AH tests in order align the AH ratings to the CO_2 stimulus at the chemoreceptor sites (see section 2.5 in Chapter 2). The AH ratings were then plotted against the corresponding end-tidal PCO_2 from the corrected breath-by-breath data. From these individual plots, the slope and thresholds were determined by applying a linear regression through the data-points and computing a coefficient of variation (r-squared) which were then compared for the RAMP tests with DBS 'ON' and OFF'. Since only 3 patients produced analysable data, the slopes were compared descriptively from the graphs with no formal statistical analysis.

Steady state AH tests: The AH ratings and breath-by-breath end-tidal PCO_2 values in the last minute of the 4 minute period of the targeted PCO_2 were averaged for each 4 minute test under the different DBS settings for each patient. Only 3 patients provided analysable data so the averaged AH ratings were plotted against DBS settings for each patient individually.

D12 assessments following AH tests: The total, physical and emotion domain scores for the D12 were expressed as a % of the maximum possible scores. This was done for D12

questionnaires administered following RAMP AH tests with DBS 'OFF' and 'ON', and following steady state AH tests at 25% and 75% DBS settings in all motor-thalamic patients (with VIM or VOP electrodes) who completed the tests. A paired t-test was used to compare the mean D-12-total scores between the electrodes 'ON' and 'OFF' ramp tests.

D12 assessments for different target locations in the same patient: The D12 total, physical and emotion scores were determined for the baseline condition (resting dyspnoea; when all electrodes were not activated (switched 'OFF') and then when each electrode was activated (switched 'ON') for 20 minutes in turn (with all other electrodes switched 'OFF'). The absolute percentageof D-12 scores and the change in percentageD-12 scores from baseline (when all the electrodes were switched 'OFF') were compared graphically between each electrode in this single subject.

4.3 RESULTS

A total of 10 patients participated in this study (mean±sd age at surgery 70±4years, height 171±10cm, weight 80±7kg; 8 males). Eight of these patients (patients 6, 9, 10, 11, 12, 13, 14 and 16) had electrodes implanted in motor-thalamic nuclei (5 in VIM bilaterally, 1 in VIM unilaterally and 2 in VOP bilaterally),Figure 4.4. These eight patients were treated as a group for analysis purposes; the mean±sd disease duration was 23±8 years, and mean±sd time since surgery was 16±16 months in this group (Table 4.3).

Table 4.3 <u>The 'motorthalamic nuclei' group.</u>
Clinical information of patients with essential or dystonic tremor who had DBS electrodes implanted in motor-thalamic nuclei (VIM or VOP). NA= data not available, RH=Right hand, LH=Left hand.

ID	Sex (M/F)	Worse affected limb	Disease duration (years)	Pre-op tremor score	Post-op tremor score	Time since electrode implant (months)	Stimulating parameters		
							Amp (V)	Pulse width(μs)	Freq (Hz)
P6	M	RH	15	15	3	9	3.1	80	130
P9	M	LH	20	35	NA	2	2.5	110	130
P10	M	RH	16	25	NA	1	2.1	90	130
P11	M	RH	30	NA	NA	48	2.6	90	150
P12	F	LH	35	NA	NA	12	2.8	90	150
P13	M	RH	25	36	15	26	4.2	80	130
P14	M	RH	30	28	12	24	2.8	130	130
P16	F	RH	12	65	NA	2	1.5	380	130

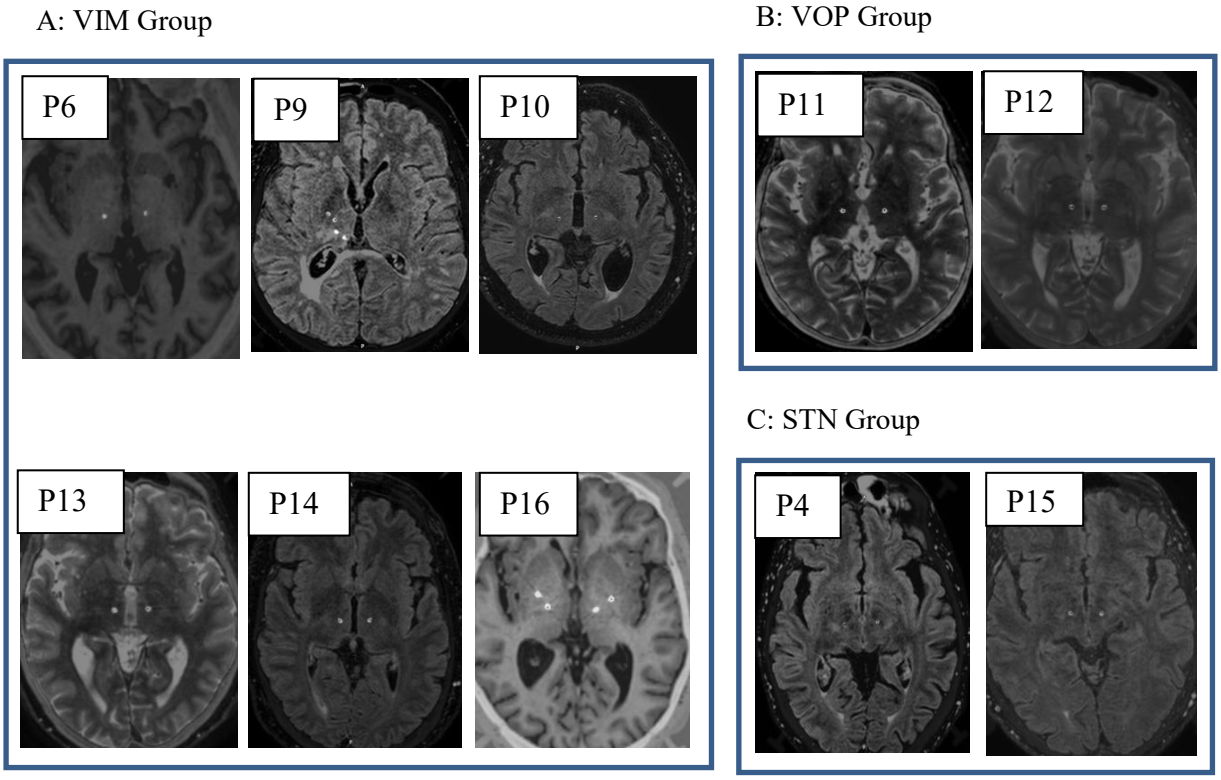


Figure 4.3: Location of electrodes in DBS patients who completed the study
Axial T1-weighted MRI images with different contrast of patients with DBS electrodes implanted in VIM (A), VOP (B) and STN (C) nuclei. Groups A and B together formed the 'motor-thalamic' patient group. The MRI images confirmed that all patients except P9 had electrodes implanted bilaterally. The electrodes are shown as a '**white spots**' in the images. P9 had electrodes placed unilateral in four different targets on the right side (VIM, VPL, GPi, and VPG). P16 had electrodes placed bilaterally in two different targets (GPi and VIM). During testing in P16, the GPi was not switched on (not stimulated as external programming had not yet established therapeutic threshold to relieve symptoms for which it was intended).

One unilateral VIM patient (patient 9) only provided D12 questionnaire data because they had co-existing COPD. One bilateral VIM patient (P13) and one bilateral VOP patient (P12) also only provided D12 questionnaire data because they could not cope with the AH breathing tests. In addition, one of the bilateral VIM patients (P16) rated zero AH despite end-tidal PCO_2 being made to rise above 60mmHg and so was excluded from the formal analysis and their results are discussed separately. The remaining two patients not included in the motor-thalamic nuclei group (patients 4 and 15) had bilateral STN electrodes -the results for these two patients are commented on separately.

4.3.1 Dyspnoea assessments in the motor-thalamic nuclei group

AH ramp data: A raw dataset in one patient (P10) is shown in Figure 4.5. It can clearly be seen that when the bilateral VIM stimulation is 'ON' AH climbs at a much slower rate as end-tidal PCO_2 is ramped up, than when the bilateral VIM stimulation is 'OFF'.

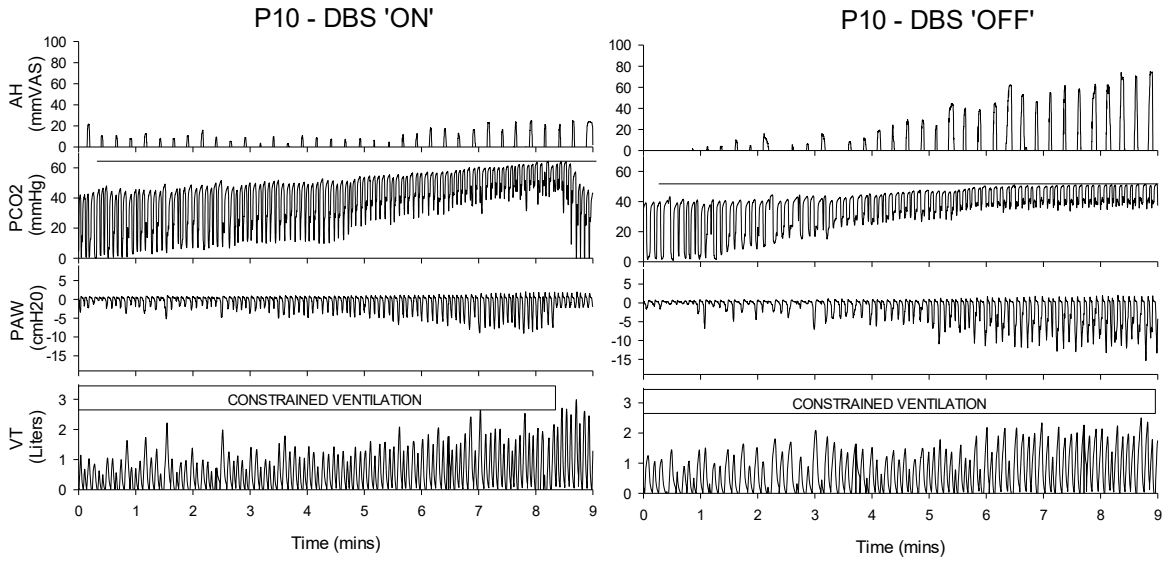


Figure 4.4: Ramp AH tests in one patient (P10) with and without bilateral VIM DBS Recordings of breath-by-breath ventilatory data (middle channel; PCO_2, bottom channels; tidal volume VT), and discrete ratings of AH every 15s (top channels) during RAMP AH tests with DBS 'OFF' (right panel) and with DBS 'ON' (left panel).

The effect of turning the DBS 'ON' produced changes in AH stimulus-response relationships in the 4 'motor-thalamic' patients who completed the breathing tests. These changes varied among patients: P6 (bilateral VIM) had a rightward shift in the AH threshold but slope of response was also increased, P10 (bilateral VIM) had a markedly reduced slope but with an unchanged threshold, P11 had a lower baseline AH but with no discernible slope with either DBS 'ON' or 'OFF' and P14 had a steeper slope with little change in threshold (Figure 4.6).

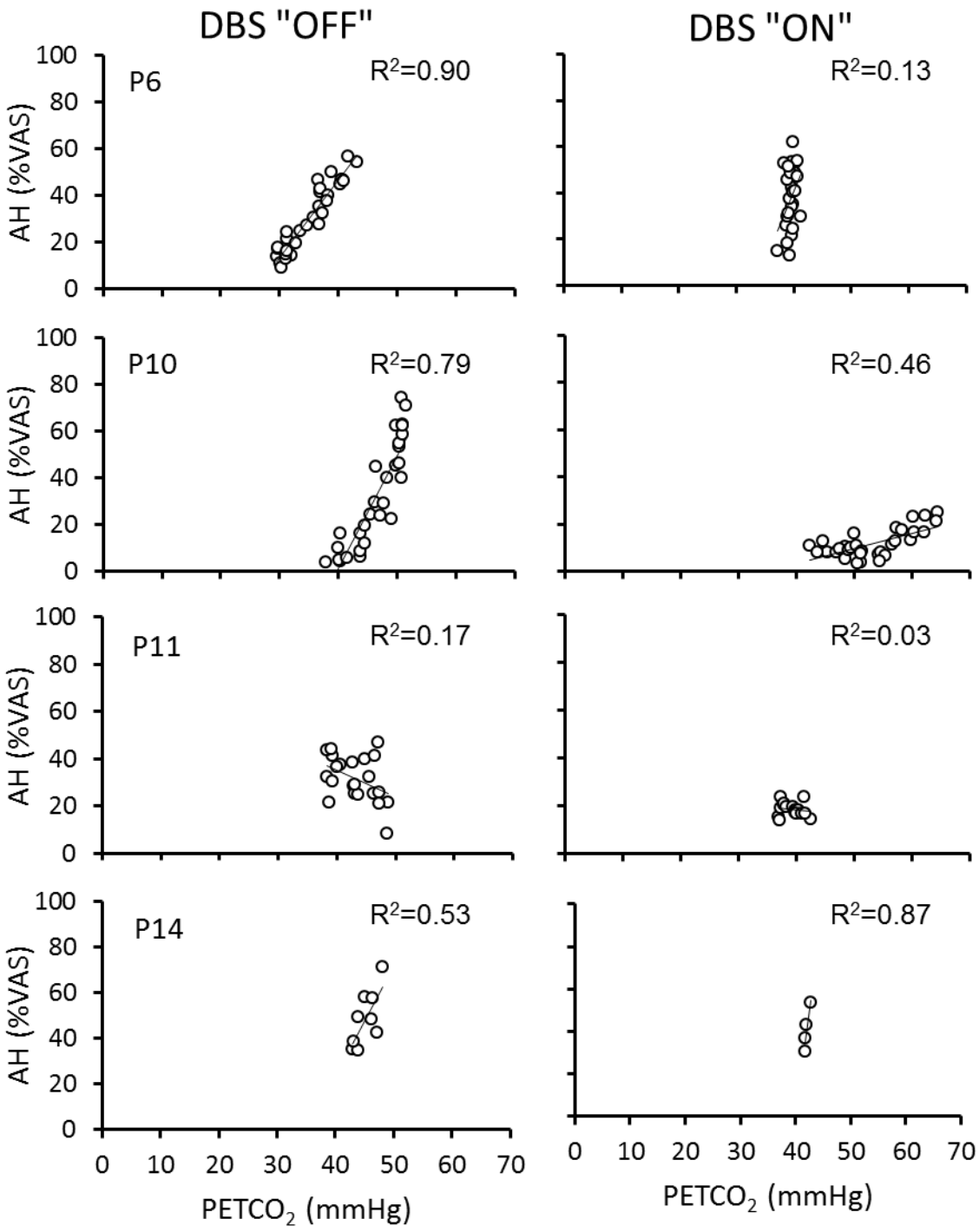


Fig 4.5: Stimulus response slopes for RAMP AH tests in motor-thalamic patients
Change in VAS ratings of air hunger (AH %VAS) with increasing end-tidal PCO_2 in 4 patients with electrodes implanted in motor thalamic nuclei.

Steady state AH tests at different %threshold DBS: Steady state AH measured in the third minute of a 4 min step of fixed hypercapnia (at a level that generated AH at approximately half way on the VAS scale during the Ramp tests) verified the changes seen when DBS was turned 'ON' in the RAMP tests (Figure 4.7).

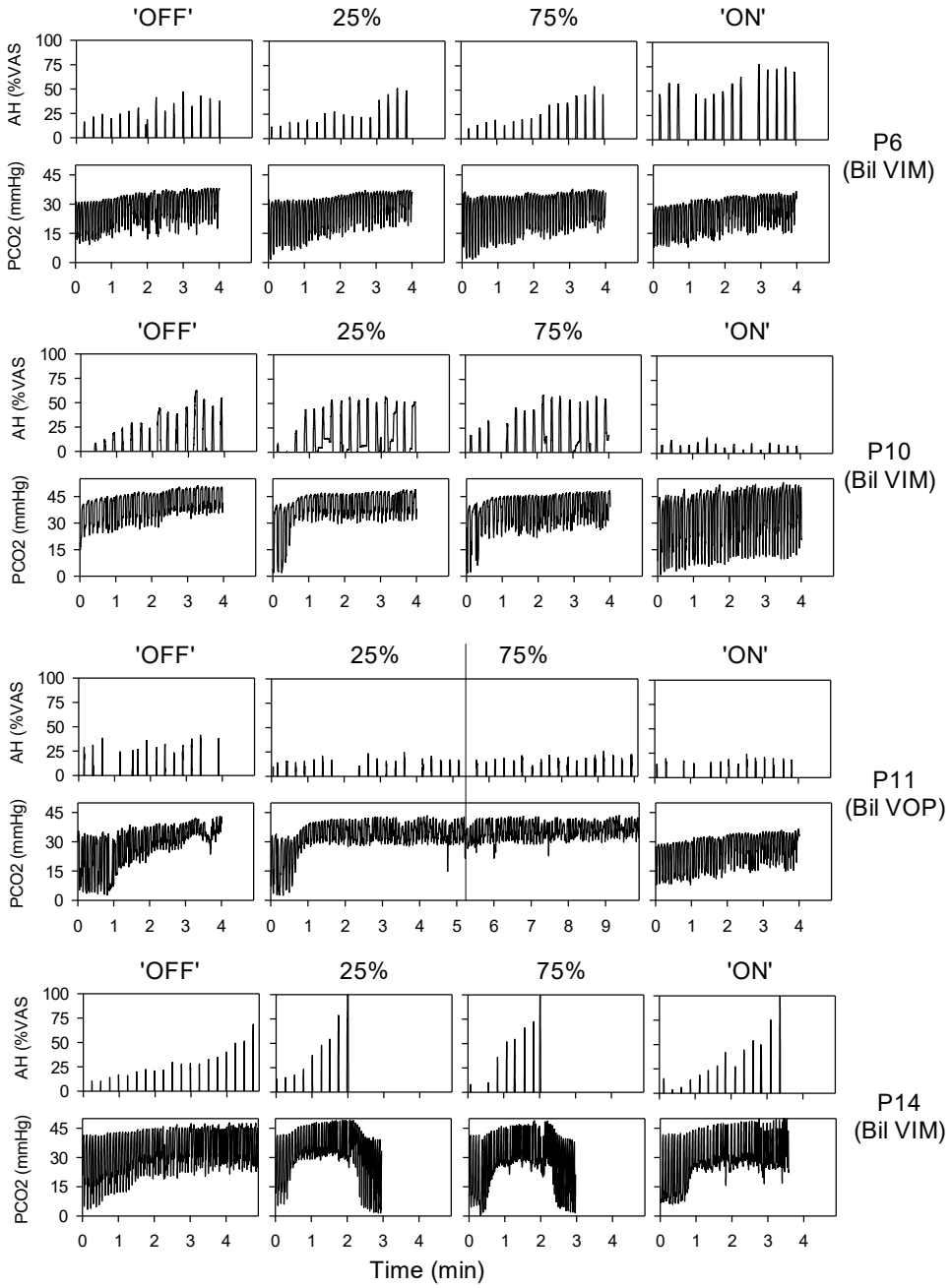


Figure 4.6: Individual AH steady state tests. Discrete ratings of air hunger (AH % VAS) taken every 15s during a 4 minute step of raised end-tidal PCO_2 (PCO_2 mmHg) repeated with 4 DBS states ('OFF', 25%, 75% and 100% threshold referred to as 'ON') in 4 motor-thalamic DBS patients. In P11, the 25% was switched to 75% during the same breathing test.

A clear and substantial increase in steady state AH was recorded for the target PCO_2 when the DBS of bilateral VIM was 'ON' compared to when DBS was 'OFF (or at 25% or 75% of the therapeutic threshold) in patient P6 (Figure 4.7, top panels). Despite the steady state PCO_2 being a little below the target level during the 'ON' state compared to the other states in this patient, the steady state AH ratings are still clearly higher for this state. However in patients P10 (bilateral VIM) and P11 (bilateral VOP) a substantial reduction was recorded for the 'ON' state (Figure 4.7, middle panels). In patient P14, the target PCO_2 was set too high such that a steady state was not achieved –this patient's data were therefore inconclusive (Figure 4.7; bottom panels). The average steady state AH in minute 3-4 of the raised PCO_2 step is shown for P6, P10 and P11 in Figure 4.8 for each level of DBS. There is a suggestion that P11 (bilateral VOP) had a 'dose-dependent' decrease in steady state AH with regard to DBS settings, however P6 and P10 data suggested that in these patients the DBS setting needed to be fully 'ON' before any appreciable changes in steady state AH were evident (Figure 4.8).

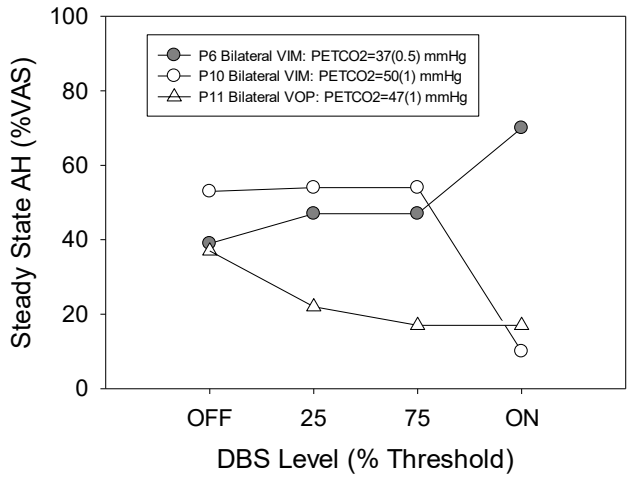


Figure 4.7: Effect of DBS state on steady-state AH. The average of 4 AH ratings in minute 3 to 4 of the 4 minute periods of raised end-tidal PCO_2 with four different DBS states ('OFF', 25%, 75% and 100% of therapeutic threshold referred to as 'ON'). Two patients (open and closed circles) showed an 'all or none' response (in opposite directions) whereas P11 appeared to have a 'dose-dependent' change with DBS state (open triangles). The mean (sd) end-tidal PCO_2 across DBS level is shown for each patient.

D-12 assessments of dyspnoea**:** The mean±sd total D-12 score associated with the Ramp AH test performedwith DBS 'OFF' was 37±26%. The corresponding score associated with the AH test performed with DBS 'ON' was 23±25% (Figure 4.9). This difference approached significance (paired t-test; P=0.06). This difference was primarily due to a change in the 'physical' domain of the D-12 instrument 'OFF' versus 'ON' mean ±sd; 48±33% and 33±34%. Five of the 6 patients included in this analysis scored lower on the D-12 following the 'ON' ramp AH test compared to the 'OFF' ramp AH test (Table 4.4). The change in D-12 total score for P6 indicated that they felt worse when the DBS was 'ON'. When P6 was excluded from the analysis, the paired t-test produced a significant fall in D-12 total score between 'OFF' and 'ON' ramp AH tests (P=0.03).

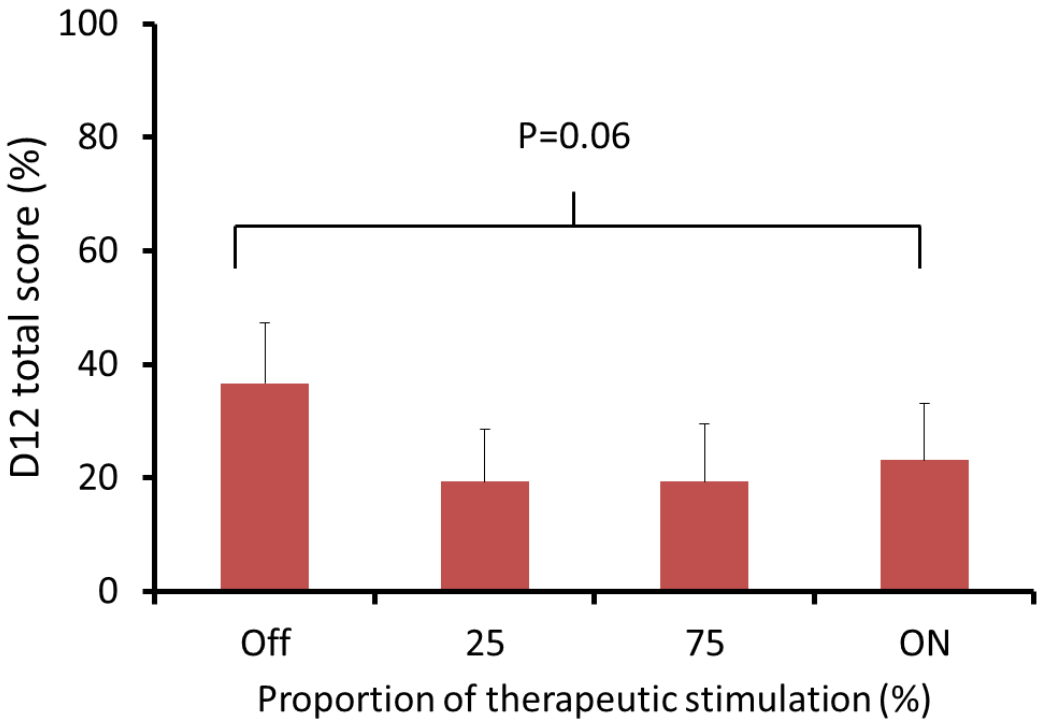


Figure 4.8: Average Dyspnoea 12 total scores for different DBS levels. During 'OFF' state, all motor-thalamic patients (P10, P11, P13, P14 and P16) reported an increase in air hunger compared to when stimulation was switched 'ON'. The 25% and 75% scores were taken after steady state AH tests while the 'OFF and 'ON' scores were taken after Ramp AH tests-thus comparison between the 25 or 75% scores and the 'on'/'off' scores should be treated with caution.

Table 4.4 <u>The 'motorThalamic nuclei' group: D12 scores following AH tests</u>

Dyspnoea 12 questionnaire (D12) scores (T=total, P=physical domain, E=emotion domain) obtained immediately after Ramp tests with DBS 'OFF' and 'ON', and after steady state AH tests at 25% and 75% DBS. The instruction given was that they should score according to what they experienced when rating high on the scale in the test just completed. The patients included in this table are those who had motor-thalamic electrodes (bilateral VOP in P11 and bilateral VIM in the others). The 25% and 75% scores for P11 were obtained from the steady state test in which the DBS level was changed while the patient continued to breathe on the circuit (see Figure 4.7) in this case, P11 was asked to score twice, once for the first half of test and once for the second half of test.

	OFF			25%			75%			ON		
	D12 T (%)	D12 P (%)	D12 E (%)	D12 T (%)	D12 P (%)	D12 E (%)	D12 T (%)	D12 P (%)	D12 E (%)	D12 T (%)	D12 P (%)	D12 E (%)
P6	50	57	40	39	52	20	53	67	33	53	67	33
P10	22	33	7	8	14	0	6	10	0	3	5	0
P11	17	29	0	6	10	0	6	10	0	6	10	0
P13	67	100	13	No steady state test			No steady state test			53	81	13
P14	61	67	53	44	62	20	33	57	0	25	38	7
P16	3	5	0	0	0	0	0	0	0	0	0	0
Ave	**37**	48	19	**19**	28	8	**19**	29	7	**23**	33	9
sd	**26**	33	22	**20**	28	11	**23**	31	15	**25**	34	13

4.3.2 D12 assessments with different sites of DBS in the same patient

Electrode locations in P9 are shown in Figure 4.10. During visit 1, the biggest fall in this patient's pre-existing dyspnoea (baseline D-12a with all electrodes 'OFF' in Figure 4.3) was produced by turning DBS 'ON' for the VIM target while all other electrodes were 'OFF'. The results showed that, baseline D-12 scores remained unchanged in visit 2. However, during visit 2, all electrodes produced this level of reduction in D-12 total scores from baseline (Figure 4.11). These changes were due to changes in the physical domain scores of the D12 instrument with little change in the emotion domain scores.

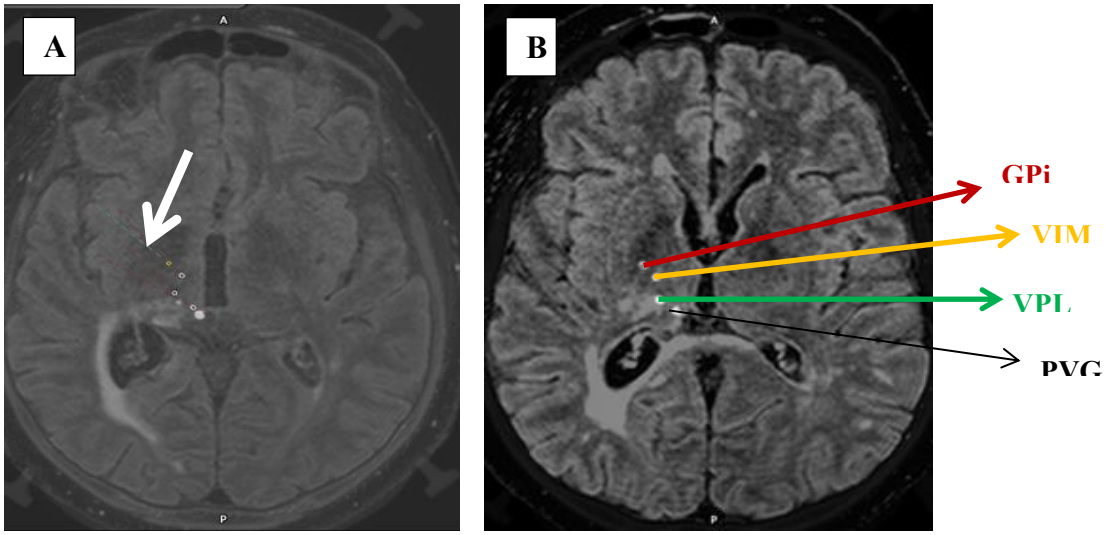


Figure 4.9: A coronal T1-weighted MRI image obtained for patient with COPD. (A) Pre-op MRI planning of electrode target in four different subcortical targets and (B) Post-op confirmation of electrode location. Each dotted line in (A) represent the electrode trajectory through which the electrode passes to the defined target in reference to the anterior commissure-posterior commissure (AC-PC) axis. The 4 DBS targets are confirmed by 'white' dots and labelled in B. Dotted lines are indicated by the 'white arrow in (A).

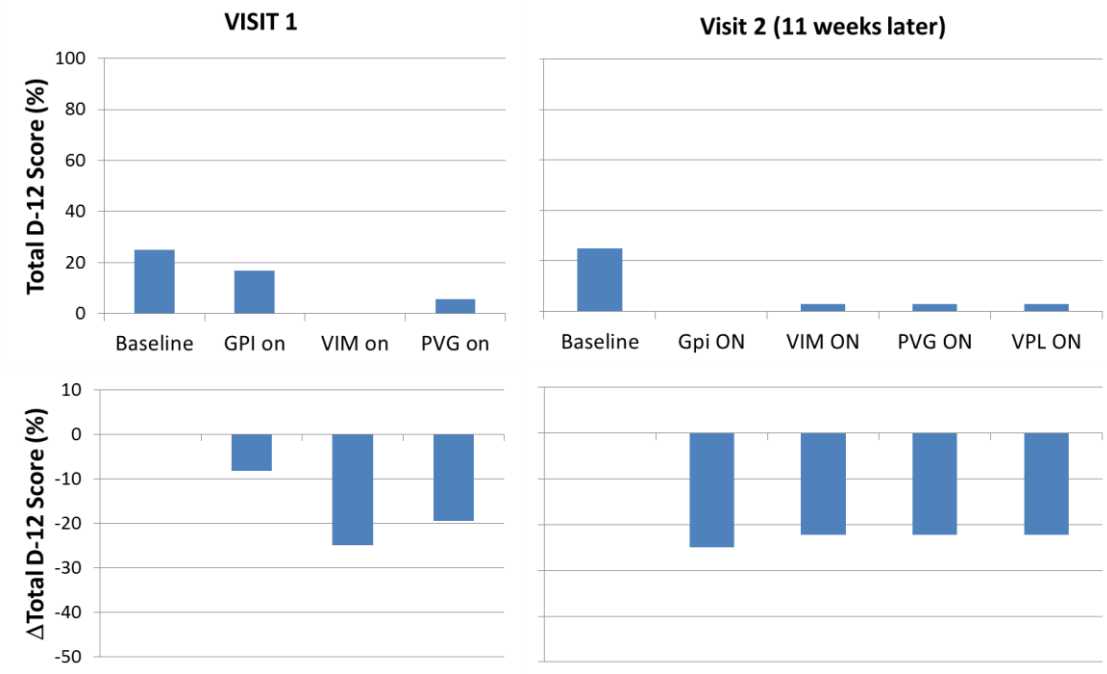


Figure 4.10: Effect of stimulating different DBS targets on D-12 in a patient with COPD. (n=1). The total D12 score (top panels) and the change in total D12 scores from baseline (bottom panels) in patient P9 on two visits; within 1 week after surgery (visit 1; left panels) and 11 weeks later (visit 2; right panels). On each visit, the total scores and change in total scores from baseline are shown for DBS of each target in turn while all other electrodes are 'OFF'. VPL was not studied in visit 1 due to time constraints of the patient.

4.4 DISCUSSION

4.4.1 Main findings

The working hypothesis of this study was that when electrodes implanted in motor-thalamic nuclei are turned 'ON' patients will experience greater dyspnoea as a side effect.

- One patient with bilateral VIM electrodes experienced increased dyspnoea both in terms of air hunger associated with a given PCO_2 stimulus and in terms of D12 scores of their experience during the tests.
- Two other motor-thalamic DBS patients who generated analysable air hunger data experienced the opposite with substantial reductions in dyspnoea sensitivity when electrodes were 'ON'. This again tallied with their D12 scores.
- The net result among 6 patients was that the D12 scores associated with air hunger tests fell substantially when electrodes were 'ON', a finding which approached significance. If the one patient who concurred with the original hypothesis was removed from this analysis, the change in D12 scores was highly significant. The changes in total D12 scores were principally due to changes in the physical domain not the emotion domain.
- The patient with pre-existing clinical dyspnoea as a result of COPD also showed a marked reduction in D12 scores of dyspnoea when the VIM electrode was 'ON' and this change from baseline was greatest for this target nucleus. Eleven weeks later the other 'ON' state for the other electrode targets in this patient (GPi, VPL and PVG) also produced equivalent relief of dyspnoea as the VIM.

Thus this study suggests that *in general* motor-thalamic DBS results in reduced dyspnoea sensitivity contrary to the working hypothesis. The working hypothesis was generated on the basis of anecdotal evidence from neurosurgeons who noted that some patients with DBS electrode implants in motor-thalamic nuclei complained of dyspnoea following surgery. The current study only found 1 out 7 patients who fell in this category but unexpectedly found several who responded the other way. It is not surprising that this bigger subgroup would not be flagged up by the neurosurgeons since they do not experience any dyspnoea to start with. It was only the experimental induction of air hunger that enabled these patients with dyspnoea relief to be identified.

4.4.2 The functional role of the motor thalamic nuclei (VIM) in tremor control

The motor thalamic nucleus (VIM) has long been identified as an important target to relieve symptomatic tremor in various neurological conditions through several treatment procedures such as ablation of parts of the thalamus (Hassler and Riechert, 1954; Zirh *et al*, 1999) through DBS (Klein *et al*; 2012; Papavassiliou *et al*, 2008) including use of fixated ultrasound for this purpose (Elias *et al*, 2013). These treatments have yielded remarkable therapeutic outcomes in patients suffering from various types of tremor but theoverall mechanism underlying tremor pathogenesis and the neural network is not completely understood (Elias and Shah, 2014; Louis, 2014). The success of these results together with other neurophysiological recordings during thalamic lesioning surgery has functionally implicated the VIM as the seat for tremor generating cells, therefore forming the rationale for targeting the VIM through stimulation (Benabid *et al*, 1996).

4.4.3 Neural connectivity of the VIM to other subcortical regions

The thalamus is considered as a relay region with afferent and efferent projections to other subcortical areas predominantly, the motor area and the cerebello-thalamo-cortical tracts (CTC) of the brain. It is actively involved in processing both sensory and motor sensations

to affect movement and other unpleasant sensations such as pain. The results from this study suggest the, breathlessness signal from the mid-thalamic and brainstem region can also be targeted for relief just like pain. Other invasive track-tracing studies in primates have also demonstrated the connectivity of the VIM to the motor cortex and the cerebellum, thus forming the VIM-motor cortex (MC)-cerebellum (CELM) neural circuit (Asanuma *et al*, 1983a; Yamamoto *et al*, 1983). Evidence from similar studies in humans using in vivo non-invasive DTI method have identified identical existing anatomical connectivity between the VIM and the cerebellum (Behrens et al, 2003; Johansen-Berg *et al*, 2005; Kincses *et al*, 2012; Anderson *et al*, 2011). Since many areas are involved in forming the tremor network and the VIM has connections with much of this network, this makes it likely that the VIM has a role in tremor mechanisms. Evidence from MRI imaging studies and advance colour coded maps technique has shown the location of the VIM lying laterally to the pyramidal tract and medially to the sensory target (VPL) of the thalamus (Yamada *et al*, 2010). More importantly, since the thalamus receives signal sensationsfrom two different areas; afferentsignals from the cerebella area and efferent signals from the pallidial area, it has made the complete understanding of the mechanism of VIM DBS in the reduction of tremor more complex.

4.4.4 Mechanism of tremor reduction by VIM-DBS stimulation

Despite the success of VIM DBS in controlling tremor, consensus on the precise underlying neurophysiological mechanism remains fragmentary. However, several prevailing theoretical and few experimental studies agree that thalamic stimulation disrupts the functional connectivity in the VIM-MC-CELM circuit (Fang *et al*, 2015). Tremor cells have extensively been studied and are thought to display a distinct irregular pathological bursting discharge activity during electrophysiological recordings (Lenz *et al*, 1994; 1988). It is therefore believed that DBS functions by either blocking (inhibition) the

abnormal oscillation signal produced by tremor cells when the VIM is stimulated at frequency greater than 100 Hz or pathologically, overrides (activates) the irregular tremor signal and turns it into more regular firing with a constant high frequency. This theoretical idea is supported by empirical evidence from early intraoperative chronic stimulation studies which showed that frequency greater than 100 Hz was able to arrest tremor cells in the thalamus (Hassler *et al*, 1960; Ohye *et al*, 1964).

4.4.5 Mechanism of dyspnoea modulation by VIM-DBS stimulation

The possible mechanisms by which the VIM stimulation modulates dyspnoea sensitivity are as follows:

Proximity of the breathlessness signal pathway to VIM: It could be speculated that dyspnoea modulation occurs in the same way as tremor suppression. As indicated earlier about the location of the VIM nucleus in relation to other sensory nucleus such as the ventroposterior lateral nucleus (VPL), it has been proposed that, when an electrode is activated, any tissue found within 2 to 3mm of the stimulated electrode pathway is more likely to be activated as well (Boraud *et al*, 1996). Therefore activation of nearby nuclei responsible for processing sensory information by VIM stimulation can be speculated to either inhibit or cross-activate nearby sensory tissues responsible for processing sensory information. This mechanism could affect sensory changes leading to functional changes in respiratory sensations.

Relief of potential tremor related mechanical feedback from respiratory muscles: It is unknown whether tremor or dystonia affects respiratory muscles themselves. If this is the case then relief of tremor by VIM DBS could remove mechanosensory feedback from respiratory muscles that contributes to the dyspnoea signal. However, this pre-supposes that the patient's relief would be more linked to relief of the sense of breathing effort rather

than the sense of air hunger. The feedback from respiratory muscles is not thought to be involved in generation of air hunger (Banzett *et al* 1990, Gandevia *et al,* 1992). In this study the patients were only available for testing on one occasion for 1-2 hours only. This limited the time available to ensure that they were in fact rating air hunger and not breathing effort during air hunger tests.

Overlap with pain modulation mechanisms: The unexpected finding of AH reduction during VIM stimulation is consistent with similar findings in pain modulation studies when the VPL of the thalamus was stimulated. The ascending spinothalamic tract has been shown to terminate in the VPL of the thalamus and receives, integrates and processes sensory information. They are therefore propagating to the motor cortex and affect a response through the descending tracts. Neurons which respond to both visceral and noxious mechanical stimuli are reported to be present in the VPL by electrophysiological studies carried out in primates (Guilbaud *et al*, 1980). Banzett and Moosavi (2000) have reported an interaction in terms of experience between pain and breathlessness perception, thus suggesting that they both share a classical pathway. Unlike pain, the physical and affective pathway mechanisms could not be distinguished in this current study, therefore, the reduction in AH could be as a combination the activation or descending inhibition to regulate pain reduction or motor pathways.

Could the VIM have sensory properties as well as motor? Although not clear, it is possible that tremor cells and networks may exhibit sensory properties which influence sensations discretely reserved to be sensory when stimulated at high or low frequency.

4.4.6 Clinical application of current study

Up until now, the only way in which cerebral mechanisms of breathlessness have been investigated is through brain imaging of mostly healthy volunteers with experimentally

induced breathlessness or through indirect manipulation of psychological factors (see Chapter 3). The current study represents a novel approach to exploring cerebral mechanisms of dyspnoea. The study contributes new information that will need further evaluation before any clinical application can be proposed. The new findings in this study have two major clinical applications.

Firstly, VIM stimulation could potentially offer a new target for clinical management of intractable breathlessness through surgical intervention. The baseline clinical dyspnoea in the patient with co-existing COPD was completely abolished by DBS of the right VIM. Furthermore it appears from this case study that unilateral VIM DBS is adequate to abolish substantial clinical dyspnoea. The case study in the COPD patient also provided valuable information about potential longitudinal effects suggesting that the effect is nonspecific but varying in duration for full manifestation depending on the site of DBS. Thus the amelioration of dyspnoea extended to DBS of other target nuclei 11 weeks after initial testing. However, the caveat to this is that the stimulation parameters for the therapeutic threshold had changed which could account for the more non-specific targets for the relief on the re-visit. Also, the extent of differences in steady state AH for a given PCO2 between the DBS 'OFF' and 'ON' states in the 3 patients who showed relief in this study was substantially greater than the minimal clinically relevant change in VAS rating of dyspnoea that has been reported in the literature (Ries, 2005).

Secondly, in some patients this study could provide extra information to assist the planning of stereotactic functional surgery to avoid pathways in the VIM which may induce undesirable respiratory discomfort such as breathlessness.

Thirdly, this study raises a concern about neurological patients who receive DBS for other symptoms leading to a side effect of inability to perceive dyspnoea. Thus they could be put at risk of medical complications as a result of reduced dyspnoea sensitivity. Neurologists would need to be made aware of these findings so that such patients can be monitored for changes in dyspnoea sensitivity much like the routine assessment of pain perception by neurologists at the bedside.

4.4.7 Critique of the study

This study had several limitations:

Sample size of patient and recruitment: The most prominent of all was the recruitment of suitable DBS patients. Most patients receiving DBS treatment were over 50 years of age and frail as a result their respective neurological condition. Recruitment for suitable patients was very challenging as most have coexisting clinical symptoms which prevented them from taking part in the study. However, the importance of the findings of this study are amplified by the fact that even such small numbers of patients has revealed effects that are greater than minimum clinically relevant changes (D-12 scores) and approach statistical significance (air hunger VAS ratings).

Disease duration before DBS surgery: Differences in disease duration and pathological changes prior to surgery may contribute to difference in AH response and influence the results (e.g., the severity of a patient's tremor at the affected limb made it difficult to accurately move the slider on the rating scale to reflect the actual AH experience when prompted to do so).

Control group: There was no control groups included in the study. The reason was that patients served as their own control.

Effect of prescribed medication: Pre-surgery, symptoms were treated unsuccessfully by prescribed medications. Following surgery, medication intake was substantially reduced in

most patients and completely stopped in some indicating successful outcome for DBS as evidenced by substantially reduced tremor scores. The only patient to take any medication on the day of testing for the current study was the patient with co-existing COPD-he had taken inhaled bronchodilator three hours prior to testing. Since testing was completed on the same day any residual effects of medication are unlikely to account for differences between DBS 'ON' and DBS 'OFF'.

Variation in electrode location and type of implant device: All patients who completed the study had electrodes implanted in the VIM nucleus according to the atlas-based coordinates used during surgical planning. However, the precise anatomical location of each electrode may differ slightly when examined in detail due to structural differences between patient. The implanted device type varied among patients (Medtronic and Boston, USA, hardware and accessory), therefore design specifications and functional configurations may influence the outcome. No published studies were found which compared the two devices in DBS for any target nucleus.

Air hunger practice session: Due to time constraints, patients were not given enough time to familiarise themselves with the breathing equipment and also practise their rating of AH. This has the potential to introduce confounding factors in the results as available data from a technical study have showed that, test subjects need at least 3 practice sessions in order to produce reproducible AH ratings (Reed and Feisal-Subhan, 1995).

4.4.8 Future studies

1) The current study provides new and promising data but more DBS patients need to be studied along these lines to establish the extent of the different subgroups of responders and for conclusions to be definitive.

2) The focus of the current study, based on the working hypothesis, was the motor-thalamic nuclei. The VIM was targeted for particular attention because of the observation

in the patient with co-existing COPD. However both the patient with COPD and multiple DBS targets (including GPi, PVG and VPL) and the limited numbers of patients with VOP and STN stimulation studied indicate that there may be subtle differences in response relating to these different targets. For example, the one VOP patient who performed reliable AH testing suggested that a dose-dependent fall in steady state AH occurs with different DBS settings whereas with bilateral VIM the patients exhibit an all or none response.

3) With regard to STN target, the two patients studied were not included in the analysis as the focus was on motor-thalamic nuclei. One of these patients performed AH tests but there was no obvious change in AH sensitivity. This may or may not be consistent with the published study on DBS of STN targets (Chalif*et al*, 2014). In view of the importance of potential increases in dyspnoea sensitivity in Parkinson's patients treated with DBS of STN it will be necessary to confirm the previous findings.

4) Patients with ACC DBS need to be studied as was originally proposed as a hypothesis for this project. The ACC has been identified to form part of the major pain pathway, thus modulate pain relief. It has also been identified to be activated in abreathlessness imaging study (Banzett *et al*, 2000; Evans *et al*, 2002). This has made the ACC an attractive candidate to be studied in breathlessness perception. More importantly, pain and breathlessness has been shown to share certain similarities and overlapping cortical pathways, therefore information from proposed mechanism which relieve pain through DBS will be useful and shed more light on the cerebral pathways or network that may modulate breathlessness sensitivity.

5) In future, spirometry measurement prior to and after DBS surgery would add useful information to better understand the breathlessness pathways since the expression of tremor or dystonia on respiratory muscle activity has not been explored. Furthermore, it

would be pertinent to study the effort component of clinical dyspnoea if it is found that tremor and dystonia areexpressed in respiratory muscle activity.

4.4.9 Concluding statement

This is the first report of the use of DBS patients to explore cerebral mechanisms of breathlessness. The VIM of the thalamus has unexpectedly shown to offer a potential new target area for relief of intractable breathlessness when directly stimulated in neurological patients receiving DBS treatment to control tremor. As exciting as these findings are and will add to our current knowledge of the cerebral mechanism of breathlessness perception, the sample size does not permit a forgone conclusion to be made. Therefore, caution must be applied to interpreting the current results. However, the prospect of motor-thalamic areas as a target for dyspnoea relief will depend on determining the cause of opposite responses in some patients with DBS of the same motor-thalamic targets. Fortunately the technique of tractography was available to explore potential differences between the patients included in this study. Thus the next chapter examined (i) whether there were any differences in the white fibre tract connections or projections of the VIM to other areas and (ii) whether there were any differences in the volume of activation, in the patient who showed increased dyspnoea sensitivity and the patients who improved when DBS was 'OFF'.

CHAPTER 5

TRACTOGRAPHY OF THE MOTOR THALAMIC VENTRAL INTERMEDIATE NUCLEUS (VIM) AND THE PERCEPTION OF AIR HUNGER

5.1. INTRODUCTION

The human brain consists of approximately 86 billion neurons. The white matter fibres of the brain (WMF) are made up of these neurons (Azevedo *et al*, 2009). The neurons are interconnected to form structurally similar neuralnetworksin the brain, but these networks perform different functions and can be implicated in both health and disease. These white matter networks process, modulate and control how information is distributed across the wider brain regions.

Diffusion tensor magnetic resonance imaging (DTI) tractography is a neuroimaging method which uses proton MRI to measure water diffusion in specific volumes (voxels) of brain tissues (Basser *et al*, 1994). The principle and method of DTI data collection takes advantage of the movement of water molecules within the brain tissues. Diffusion of water molecules in white matter fibres in the brain is constrained directionally along axon bundles rather than across the same bundles by the surrounding myelin sheath. Therefore, taking measurements from multiple different angles (thus tensor), the orientation of white matter fibre tracts can be measured. This phenomenon allows an estimation and differentiation of individual white matter connections in the brain to be associated with specific tasks and functions as well as dysfunctions. Tractography algorithms utilise local

modelling of water distribution to offer segmental approximations of white matter tracts by following the course of least restriction to water movement (Behrens *et al*, 2009). Currently, the application of tractography to quantify WMF tracts to gain understanding of psychiatric and neurological conditions is well established both in humans (Margaret*et al*, 2003; Wang*et al*, 2008; 2009; Derek *et al*, 2000) and animals (Xue *et al*, 1999). Although the estimated connections may not be a true quantification of axon numbers within the fibre bundle, they are understood to be influenced by their connectivity strength (Jbabdi *et al*, 2011; Jones *et al*, 2013).

As a research tool, tractography has been explored to reconstruct key fibre connections within the motor thalamus nuclei (Kincses*et al*, 2012; Hyam *et al*, 2012). Recently, Boccard *et al*, (2015) demonstrated that tractography could be used in concert with DBS of the anterior cingulate cortex (ACC) to explore the pathways involved in mediating neuropathic pain. The study showed that all the ACC painpatients in the study had the same connectivity for all brain areas except for the precuneus. Patients who did not benefit from the procedure showed stronger white matter connectivityto the precuneus. The findings of this study thus have the potential to help optimise DBS presurgical planning for pain patients by avoiding the fibre tracts to the precuneus.

This chapter outlines how DBS-induced changes in air hunger perception correlate with white matter fibre tracts stimulation. In this study, probabilistic tractography was used to estimate the connectivity strength of WMF tracts between the volume of activation (VAT)and brain regions previously identified as essential to air hunger perception (Banzett *et al*, 2000; Evans *et al*, 2002). The VIM was chosen as a target of interest based on the findings presented in Chapter 4.

This is the first tractography study tracking how electrode placement in patients receiving motor thalamic VIM-DBS treatment for tremor symptoms affects experimentally induced air hunger. A better understanding of how the white matter fibre pathways interact structurally, their response to neurological injuries and how physiological sensations such as breathlessness are processed may help to better define the breathlessness neural network.

5.2. METHODS AND MEASUREMENTS

5.2.1 Patients

Six patients (5 males, aged 71+/-4.2 years) assigned motor thalamic (VIM) DBS for symptomatic relief of essential or dystonic tremors were recruited and five of them were included in the data analysis. One patient (number 10) was recruited but excluded from data analysis due to distorted CT and MRI scans. Pre-surgical and post-surgical tremor scores and cognitive evaluations were performed in all patients. Patients included in this study are a sub group of those recruited for the work described in Chapter 4, and some of the data from Chapter 4 were used to conduct the analysis presented in this chapter. Patients were selected based on electrode placement, with only those having at least one DBS electrode located in the VIM included in the present study. Ethical approval and recruitment details are given in Chapter 4. Details of the protocol can also be found in Chapter 4, although the salient points will be repeated in the present chapter.

Mean preoperative and postoperative tremor scores for the patients were 34±17.0 (range 15-65) and 10±6.2 (range 3-15), respectively. Patient 16 did not experience any tremor relief during the trial week programming. Although patient number 6 experienced tremor relief from the surgery as an expected outcome, he complained of breathlessness as a side effect when the electrodes were activated(switched ‘ON’). Therefore, patient 6 was

classified as having improved in AH perception when the electrodes are not activated (switched 'OFF'). Patients 4, 10, 9 and 13 were classified as having improved in AH perception when the electrodes were activated (switched 'ON'). Patient 16 showed unreliableair hunger (AH) ratings, but the D-12 results tended towards improvement in AH when the stimulation was 'ON' (see table 4.4 in Chapter 4). This individual was therefore included in the 'ON' category alongside patients 4, 9, 10 and 13. Clinical and demographic information is for all six patients are provided in Table 5.1.

Table 5.1 Physical demography of patients in the study. Demographic characteristics of patients selected from Chapter 4 are presented in the table. Mean age was 71±4.2years, height was 168 ±10.1 cm and weight was 81±7.1kg. (n=6)

Patient number	Gender (M/F)	Age (yrs)	Height (cm)	Weight (kg)	DBS target	Laterality	Diagnosis
6	M	73	164	75	VIM	Bil	ET
9	M	67	165	80	VIM	Bil	ET,PSP
10	M	69	170	94	VIM	Bil	ET
13	M	78	179	83	VIM	Bil	DT
14	M	67	178	78	VIM	Bil	ET
16	F	71	152	75	VIM	Bil	DT
Mean		**71**	**168**	**81**			
SD		**4.2**	**10.1**	**7.1**			

5.2.2 Study Protocol

Details of the experimental breathlessness (functional AH test) protocol used in this study can be found in Chapter 4, section 4.2.2. All patients recruited into this study had MRI and DTI scans within 1 month before surgery, and this study utilised these already existing MRI, DTI and CT data for each patient, obtained before they underwent the functional AH test.

On the basis of the results presented in Chapter 4, patients were separated into two categories (patients in whom dyspnoea decreased when the electrodes were activated (switched 'ON') and one patient in whom dyspnoea increased when the electrodes was activated (switched 'ON'). Tractography (connectivity mapping analysis) was performed in these two patient categories to determine the connectivity strength between VAT and the underlying fibre tracks region of interest (ROI). A conceptual experimental protocol was developed (Figure 5.1).

A postoperative CT scan was used to confirm the position and coordinates of the electrode. This was registered to DTI space via a MRI T1 (or T2, if the T1 registration was unsuccessful (both acquired before the surgery)). It is considered unsafe toperform an MRI after the electrode has been implanted as there is metal in the electrode, hence a CT was used. The sections below will outline data acquisition and analysis steps in detail.

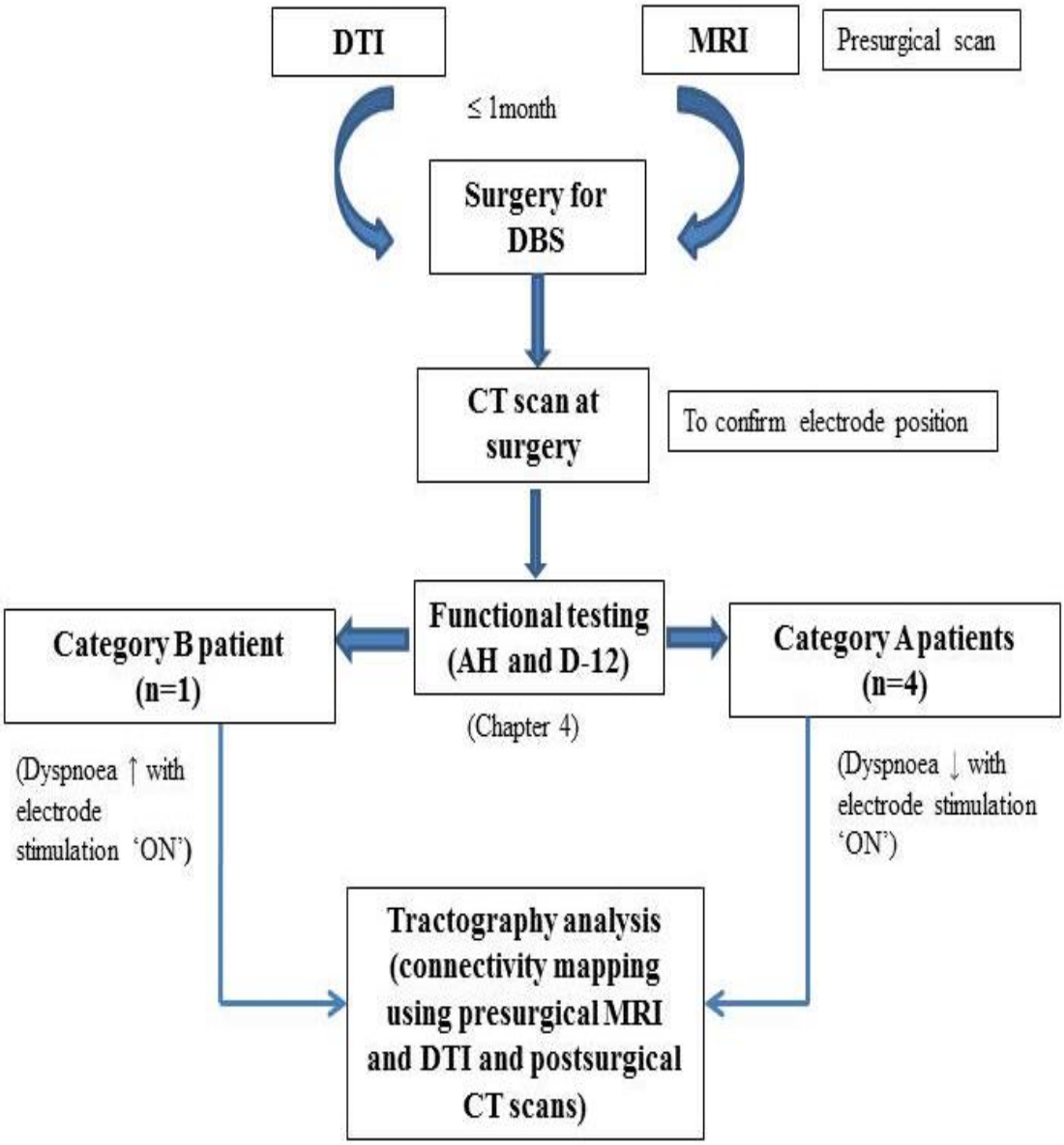


Figure 5.1: Conceptual overview of study protocol. Three types of imaging data were used for this study (Pre-surgical MRI, DTI and post-surgical CT scans). AH= Air hunger, CT= Computerised tomography scan, MRI= Magnetic resonance image; DTI= Diffusion tensor imaging; D-12= Dyspnoea-12 scale. (n=5).

5.3 DATA ACQUISITION

5.3.1 Magnetic resonance imaging parameters

As part of the preoperative assessment, high resolution T1 and T2-weighted structural MRI images were obtained for all patients by using a Philips Achieva 1.5 Tesla magnet. Diffusion weighted data were acquired using a single-shot echo planar sequence. The following are the scanning parameters: echo time (TE), 65 ms, repetition time (TR), 9390 ms, 176 x 176 reconstructed matrix, voxel size of 1.8 x 1.8 x 2 mm, and slice thickness of 2 mm. DTI data were acquired with 33 optimal nonlinear diffusion gradient directions (b ¼ = 1200 s/mm2) and one non diffusion-weighted volume (b ¼ = 0) (Boccard *et al*, 2015).

5.3.2 Pre-processing of diffusion images

Pre-processing of diffusion images was carried out in FSL (FMRIB Software Library, www.fmrib.ox.ac.uk/fsl) (Jenkinson *et al*, 2012). Distorted scans due to head motion were first corrected for artefacts using eddy current correction (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/EDDY). The BET algorithm was used to remove extracranial tissues from post-surgery CT scans and presurgery MRI scans.

5.3.3 Registration of diffusion images

FMRIB Linear Image Registration Tool (FLIRT) was used to register each patient's brain into standard Montreal Neurological Institute space(MNI). A nonlinear registration procedure using FMRIB nonlinear image registration tool (FNIRT) was tested on one patient (patient number 10) whose CT and MRI scans could not be satisfactorily registered due to large ventricles. However, FNIRT did not adequately register this patient's brain, leading to the exclusion of this participant. FLIRT was used successfully for all other registrations in this study. In each instance the transform image was computed from CT scan into MRI scans and finally into standard MNI space to be registered.

5.3.4Simulation of the volume of activated tissue (VAT)

The tissues stimulated around each electrode in the VIM DBS were estimated as in Boccard *et al*(2015). This was achieved by employing an empirical model for estimating the volume of activated tissue (VAT) which was developed to assess monopolar implanted electrode (electrodes with one active contact) stimulation and validated for movement disorders, such as refractory Parkinson's disease (Madler and Coenen, 2012). The prevailing thought is that the electric field which wasproduced and distributed by the bipolar electrode contacts(electrodes with two active contacts) stimulation between non-neighbouring electrodecontacts produces a continuous VAT between the contacts, with reduced radial distribution of activated tissues(Montgomery, 2010). This was followed by the linking together of the resulting individual masks (Madler and Coenen, 2012). The electric field distribution between the electrode contacts varies between each patient and was estimated by simulating the VAT for each of the active electrode contacts.

5.3.5 Anatomic division of targets

As the thalamic VIM was the main DBS target, this region was initially the area under study. The subcortical regions such as the ACC, the insula, brainstem, cerebellum, pre-central cortex, somatosensory motor area (SMA), amygdala and the thalamus (entire structure) were also considered as regions of interest. This was because these regions have been identified through functional imaging studies to play a prominent role in processing air hunger perception (Banzett *et al*, 2000; Evans *et al*, 2002). Specific subdivisions of the orbitofrontal cortex involved in the breathlessness network were also investigated (Herigstad *et al*, 2015). The thalamus and its subdivisions were identified using the Harvard-Oxford atlas (provided by the Harvard Centre for Morphometric Analysis-FSL). The other regions and subdivisions of the frontal cortex including the dorsolateral prefrontal cortex (DLPFC), the frontal orbital and ventromedial prefrontal cortex (vmPFC),

insula, brainstem, cerebellum, precentral gyrus (Brodmann area 4), SMA and the ACC were identified using the automated anatomic labelling (AAL) template. All regions of interest are presented in Figure 5.2.

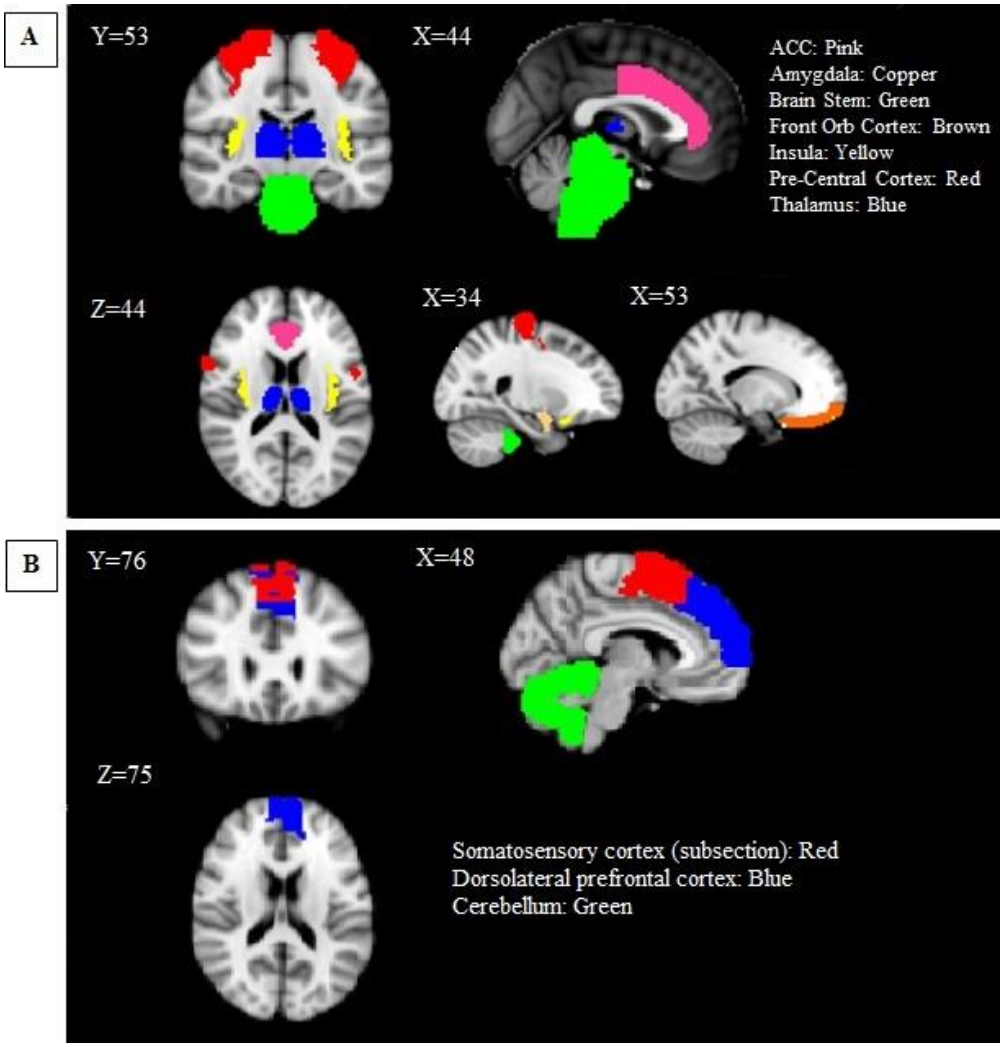


Figure 5.2: Anatomic Parcellation. The regions of interest are identified by colour coding. (A) ACC (anterior cingulate cortex, pink), thalamus (blue), amygdala (copper), insula (yellow), precentral cortex (red), brainstem (green), front orb cortex (frontal orbital cortex, brown) produced from the Harvard-Oxford atlas. (B) Subsection of the somatosensory cortex (red), dorsolateral prefrontal cortex (blue) and cerebellum (green) from the automated anatomic labeling (AAL) atlas. All parcellations are displayed on the standard MNI standard brain.

5.3.6 Probabilistic tractography

Probabilistic tractography is a method used to identify white matter pathways in the human brain. The process consists of several steps which involved the collection of pregurgical DTI and MRI data and postsurgical CT scans of each patient. The raw CT and DTI scans were registered into the MRI scan and processed to locate the electrodes in the brain area of interest.The newly created data set was then registered intoa standard MNIspace. A seed location in the brain area of interest was chosen as a starting point and waypoints for the white fibre orientation at every point of the brain to be investigated was determined. The probable preferred direction of the interested WFT was followed until a pathway was constructed and visualised in a 3-dimensional form. For each patient, probabilistic tractography was performed using the DBS electrode VAT in the (VIM) as the seed area and the regions of interest (ROIs) included in the anatomical division template as target areas. Five thousand sample streamlines were generated and seeded from each of the voxels within the VAT seed region. A "connection probability map" was then generated. This was a spatial histogram outlining the probability of streamline location. It was constructed by logging the number of streamlines going through each voxel of the brain. In order to measure the connectivity from the VAT to the selected regions of interest, the mean intensity of non-zero voxels within the masks was computed (Rozanski *et al*, 2014).

5.4 RESULTS

5.4.1 Spatial localisation of electrode contacts

Electrodes were successfully implanted bilaterally in the VIM of the thalamus in all patientsthrough surgery.Thedesired position of the electrodes was confirmed by postsurgical CT scan and the most effective electrode stimulation setting for each patient was determined. The locationof the deepest contact (C0) was different for all participants

and for both hemispheres. Standard MNI coordinates for all patients excepting patient 10 (excluded) are presented in Table 5.2.

5.4.2 Number of connections measured in each individual patient for region of interest.

The connectivity to each remote target was quantitatively estimated to represent the number of fibre tracts in that region from the seed region (VIM) (Table 5.2). The results showed a variation in fibre tract numbers to each region for each patient. The strongest connectivity across the sample was to the thalamus (Figure 5.3). Patient 6 and 16 had a greater number of connections to the ACC, brainstem and the SMA compared to patients 9, 13, 14 (Figure 5.3). However, it should be noted that the AH ratings of patient 16 was deemed unreliable (see Chapter 4) and excluded from category comparisons analysis. Patients 14 had the highest connectivity with the amygdala and patients 16 and 13 had the highest connectivity with the pre-central cortex. The individual variation in fibre connections was high for the amygdala, insula, precentral cortex and DLPFC (Table 5.2).

Table 5.2 The number of fibre connections to each region. ACC= anterior cingulate cortex, SMA= somatosensory cortex, DLPFC= dorsolateral prefrontal cortex, CER= cerebellum, Thal=thalamus, Front Orb=frontal orbital cortex, Precentral=precentral cortex, CER=cerebellum, Amyg= amygdala. ✱= unreliable air hunger ratings patient. Ave*= average and standard deviation for 9, 13 and 14. (n=5)

ID	ACC	Amyg	Brain stem	Front Orb	Insula	Pre-Central	Thal	SMA	DLPFC	CER
6	43	3	86	6	7	7	837	43	0	2
9	1	0	7	7	1	1	726	0	0	0
13	2	1	37	10	2	71	592	10	3	0
14	0	21	38	10	19	2	644	1	0	0
16✱	149	11	201	9	58	123	568	173	1	8
Ave *	**1**	**7.3**	**27.3**	**9**	**7.3**	**24.7**	**654**	**3.66**	**1**	**0**
sd	**1**	**11.8**	**17.6**	**1.7**	**10.11**	**40.1**	**67.6**	**5.5**	**1.7**	**0**

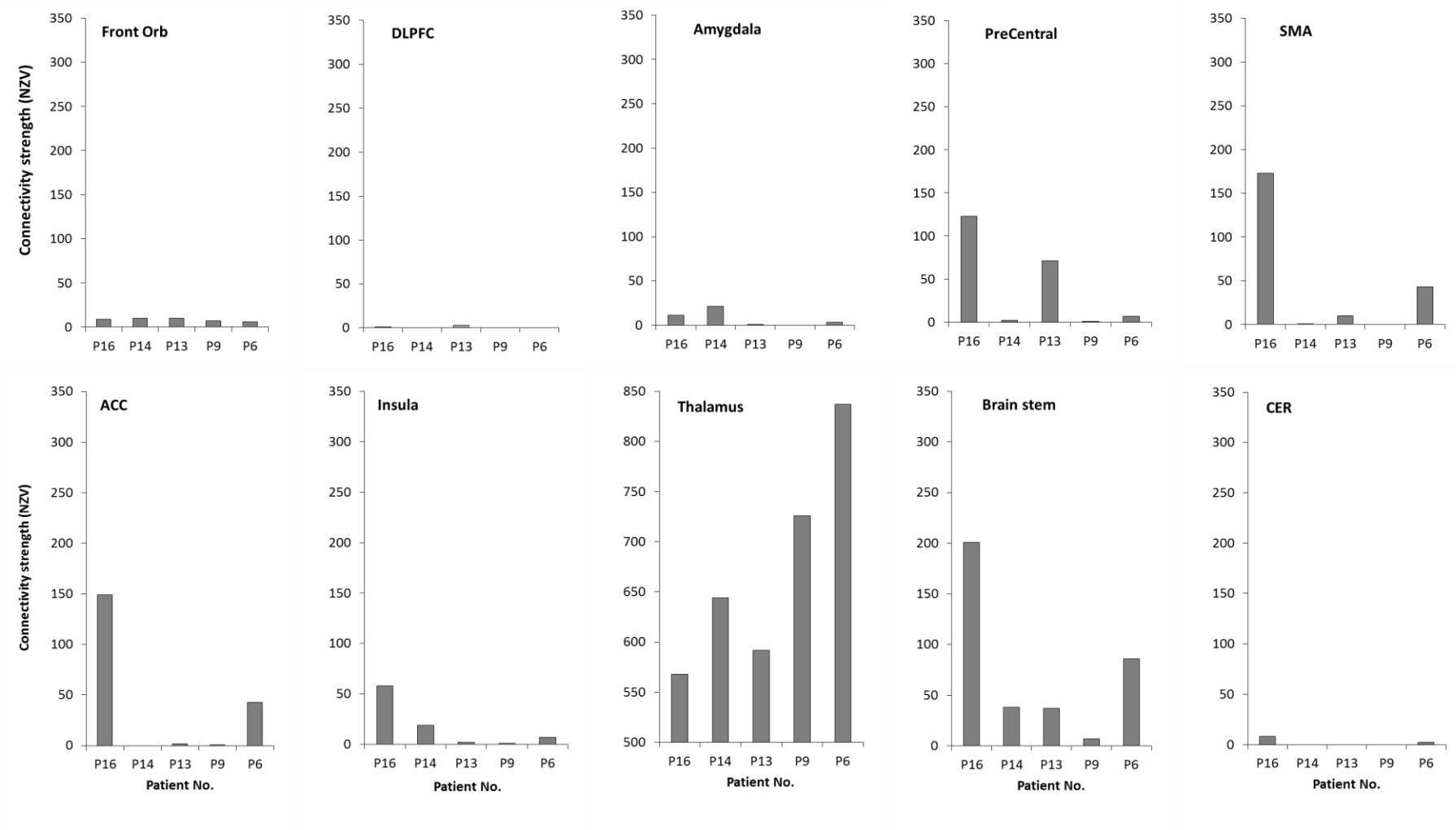


Figure 5.3: Individual connectivity strength to regions of interest. Connectivity to region of interest for each patient fromis shown. ACC= anterior cingulate cortex, SMA= somatosensory cortex, DLPFC= dorsolateral prefrontal cortex, CER= cerebellum, Front orb= frontal orbital cortex.

5.4.3. Connectivity strength to regions of interest for the two patient categories

A strong connectivity between the VIM and the thalamus was observed in all patients. The weakest connectivity of the VIM for all patients was with the front orbital cortex, DLPFC, amygdala and cerebellum. Compared to the group who experienced less AH for the same stimulus when electrodes were 'ON', the patient who improved in AH with electrodes 'OFF' showed a relatively greater connectivity between the VIM, thalamus, precentral and brainstem regions. This was most evident between the VIM and the Thalamus(Figure 5.4 and Table 5.3 for details on connectivity strengths). No significance testing of connectivity strength between the two categories was conducted due to small sample size.

Table 5.3 Connectivity strengths (number of connections) for the two VIM patient categories.ACC= anterior cingulate cortex, SMA= somatosensory cortex, DLPFC= dorsolateral prefrontal cortex, CER= cerebellum, Thal= thalamus, Amyg= amygdala, Front orb= frontal orbital cortex.

Patient category	ACC	Amyg	Brain Stem	Front Orb	Insula	Pre-central	Thal	SMA	DLPFC	CER
Improved 'ON'	38	8	71	9	20	49	633	184	4	9
Improved 'OFF'	43	3	86	6	7	93	837	43	0	2
Average	81	11	157	15	27	142	1470	227	4	11

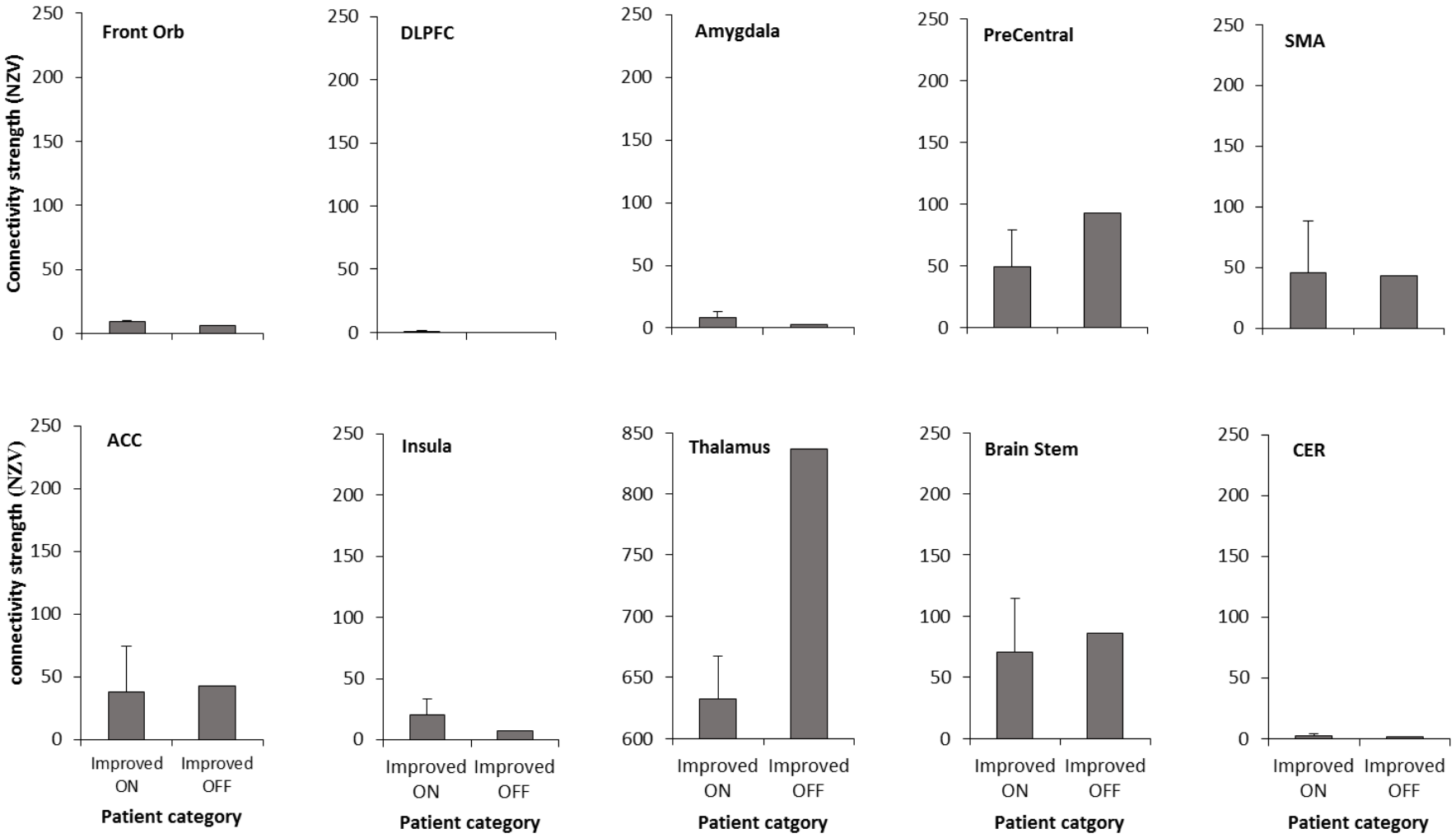


Figure 5.4: Patient category connectivity strength to regions of interest. Connectivity for improved patient with stimulation 'OFF' is presented in (blue) and the mean for the improved with stimulation 'ON' in (green). DLPFC= dorsolateral prefrontal cortex, SMA= Somatosensory cortex, ACC= anterior cingulate cortex, CER= cerebellum, Front orb= frontal orbital cortex.

5.4.4 Connectivity profiles

Figure 5.5 superimposes the connectivity tracts for the VIM on a standard MNI brain, when AH is improved with electrodes 'ON' (shown in green) and when AH is improved with electrodes 'OFF' (show in red) .

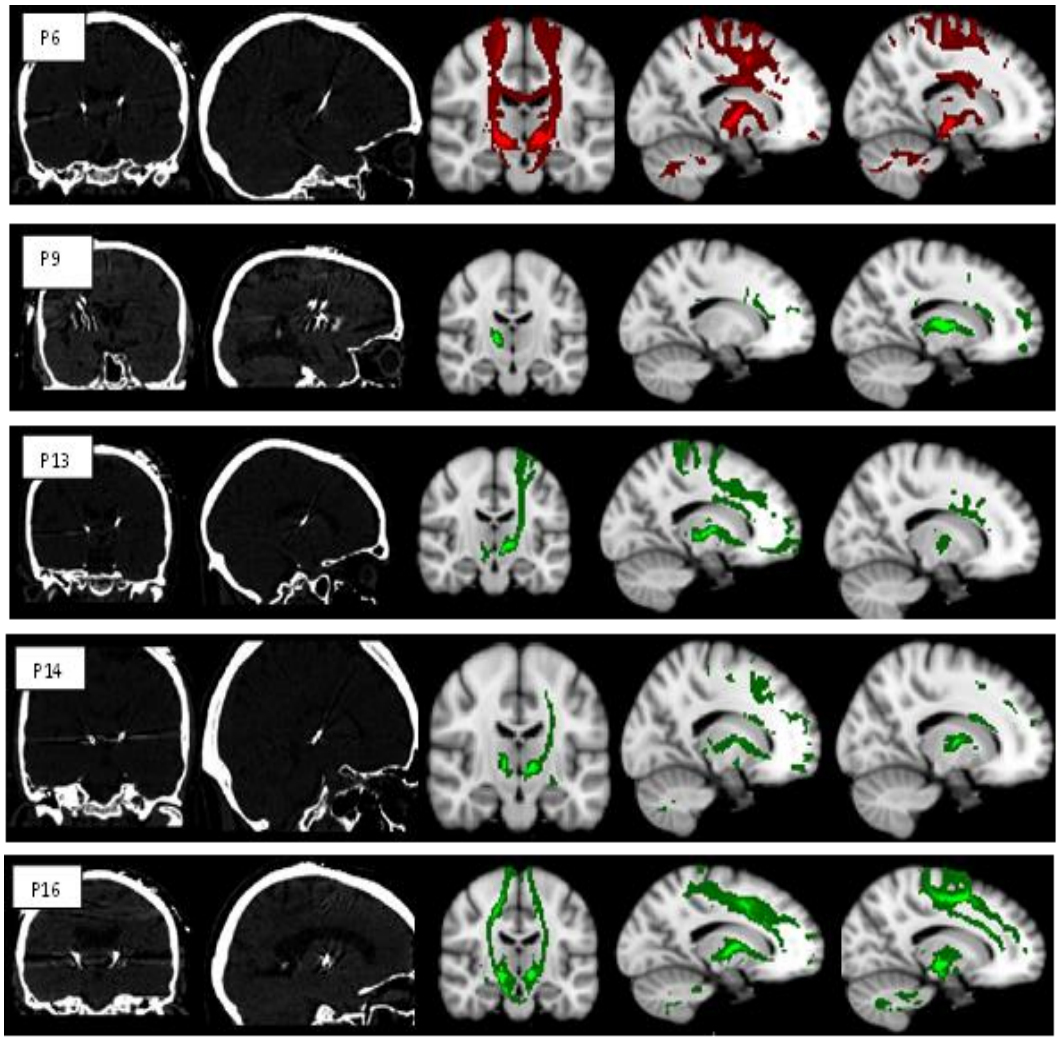


Figure 5.5: Connectivity profiles for air hunger at the average VAT coordinates for each patient in the 2-mm MNI standard brain. Electrode locations are presented for each patient. Improved patients when electrodes were activated (switched 'ON') (9, 13, 14 and 16) are showed in green, and unimproved patient when electrodes were activated (switched 'ON') (6) is shown in red. Coronal view (Y = 12), right sagittal view (X = 8), and left sagittal view (X = -8). Electrode location is shown on coronal and sagittal views of the postoperative CT scan (***left side***). The tractography was thresholded at probability levels 10/5000. (n=6).

5.4.5 Subtracted connectivity maps for the two patient categories

Connectivity maps for the two patient categories were overlapped and the difference between these calculated. In patients who improved with stimulation 'ON', connectivity was projected towards more anterior regions of the brain. However, the patient who improved with stimulation ‘OFF’ showed a marked connectivity profile towards more posterior areas of the brain (Figure 5.6). This is consistent with the data presented in Figures 5.3, 5.4 and 5.5.

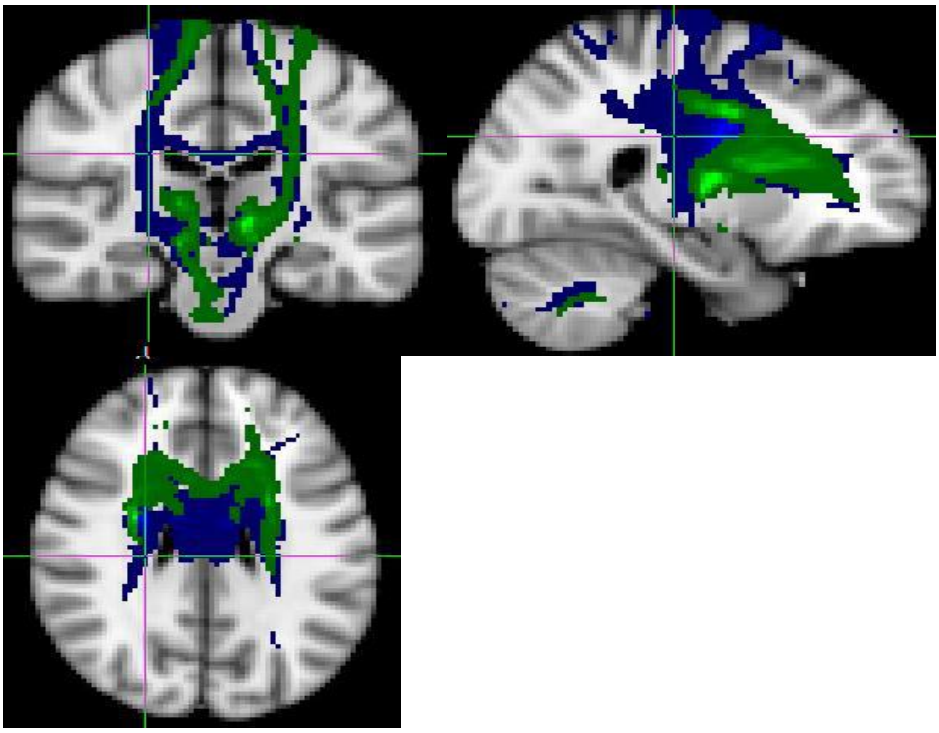


Figure 5.6: Subtracted connectivity maps for the different patient categories (VIM target). For each voxel, when connectivity is stronger for ‘ON’ than for ‘OFF’, the voxel is presented in green. When connectivity is stronger for ‘off’ than for “on” the voxel is presented in blue. The tractography was thresholded at probability levels 10/5000.

5.5 DISCUSSION

5.5.1 Summary of main findings

In order to understand the neural mechanisms of breathlessness (air hunger) through the use of DBS, probabilistic tractography was compared between two categories of patients: those who improved in AH when the VIM stimulation was 'ON' and those in whom AH worsened when the VIM stimulation was 'ON'. The electrode locations were similar in all patients, but the connection profiles of the patients differed. The results presented here show that connectivity strength from the VIM in the thalamus to anterior brain regions is stronger in patients who exhibited improved AH when stimulation was 'ON'. Conversely, the patient who improved in AH when the VIM stimulation was turned 'OFF' (i.e. worsened AH with VIM stimulation) exhibited a more posterior connectivity pattern which included stronger connectivity to the thalamus, ACC, brainstem and the precentral cortex. The weakest connectivity with the VIM was found to be with the pre-frontal lobe, amygdala and cerebellum regardless of patient category.This is the first study to report differential cortical fibre connectivity associated with DBS to add to our current understanding of the cerebral mechanism of breathlessness.

5.5.2 Breathlessness assessment and classification

Dyspnoea is complex and may be unpredictable. It is prone to subjective and environmental factors, is multidimensional and can be hard to assess both for the experimenter and the patient (e.g. patient number 16). It may be that two categories or groups are too simplistic and that patients fall instead into a spectrum. Nevertheless, for the purposes of this study and given the small sample size, a binary model was deemed appropriate. Also, the AH method used in this study is robust and sensitive (Moosavi *et al,* 2004), which aids categorisation even in such small numbers.

5.5.3 AREAS POTENTIALLY INCREASING THE AH PERCEPTION

The ACC

The ACC is a large structure located in the medial cortex. It is immediately connected to the prefrontal cortex, amygdala, the thalamus, and the motor areas and remotely to the brainstem. Several functional brain imaging studies have linked activation of the ACC to air hunger perception (Banzett *et al*, 2000; Evans *et al*, 2002; Peiffer *et al*, 2001; Corfield *et al*, 1995; Liotti *et a*l, 2001). In this study, the patient who experienced breathlessness when the stimulation was turned 'ON' exhibited relatively strong connectivity between the VIM (VAT) to the ACC. This connectivity was not shared by the group of patients who reported lower breathlessness when the stimulation was turned 'ON'. Although the connectivity was also stronger in patient 16 who improved in air hunger when stimulation 'ON', this patient's rating of AH was not reliable. Indeed, patient 16 only reported improvements in AH after the experimental test rather than during the test. A recent study in malignant mesothelioma patients reported an improvement in pain and dyspnoea through the surgical removal of the ACC (Pereira *et al*, 2014). The ACC is also known to process other emotionally related sensations such as motivation and empathy which could influence the outcome of either the affective or the emotional aspect of air hunger (Boccard *et al*, 2015). The findings reported here are in line with the literature on AH and provide further support for the role of the ACC in breathlessness processing.

Brainstem and SMA

The individual connectivity to the brainstem and SMA was stronger in patients 16 and 6 but less in patients 9, 13 and 14. As discussed above, it is possible that patient 16 does not fit the binary division of this study, and this patient was indeed excluded from group analyses due to inconsistent AH ratings. It is thus possible that patient 16 is more appropriately grouped with patient 6, since AH perception was improved with stimulation

'OFF'. Nevertheless, it is interesting to note that this patient who exhibited conflicting AH results also showed a connectivity pattern similar to the 'OFF' patient. A second possible interpretation is that these areas could play different roles in modulating AH perception.

Several studies on respiratory perception which used inspiratory and expiratory loading and hypercapnia stimuli have reported activation in the brainstem (Brannan *et al*, 2001; Harper *et al*, 2005; Peiffer *et al*, 2001). This is because the brainstem has been demonstrated in various experiments to house the respiratory centres and responds to respiratory sensations (Lumsden, 1923; Pattinson, 2008). However, the precise role of the brainstem in breathlessness perception (beyond respiratory control) is not yet established.

The SMA is known to be activated and involved in fine motor processing during respiratory loading experiments (von Leupoldt *et al*, 2008 and 2009). The SMA forms part of the cerebral cortex and is anatomically located anterior to the primary motor cortex. Neurons located in the SMA are known to project directly to the spinal cord. It is thought to be involved in motor coordination such as posture on both sides of the body. Anatomic connectivity studies in non-humanprimates have shown that the SMA has subregions which may be functionally different from each other (Luppino *et al*, 1993; He *et al*, 1995; Bates and Goldman, 1993). These findings make the actual role of the SMA in breathlessness perception hard to define. In this study, remote stimulation of this region was observed to be associated with elevated breathlessness in patient 6, whereas patients with lower connectivity to this region showed lowered breathlessness during DBS. This is in line with the literature and supports the role of the SMA in breathlessness.

Cerebellum

Different regions within the cerebellum are thought to be involved in sensorimotor and cognitive processing of various unpleasant sensations (Catz and Thier, 2007; Parsons *et al*,

2000) and low ventilation (Evans *et al*, 2002). Several imaging studies have shown cerebellar activation when hypercapnic stimuli were applied (Corfield *et al*, 1995a,b; Parsons *et al*, 2001; Harper *et al*, 2005). Here, little connectivity between the VIM and the cerebellum was observed, and it is therefore not possible to draw any further conclusions on the potential importance of the cerebellum in AH on the basis of this study.

Amygdala and the insular cortex

The investigation into the connectivity to the amygdala and the insular cortex reported here was primarily based on their central role in the experience of air hunger (Banzett *et al*, 2000; Evans *et al*, 2002). They are part of the limbic system, which also processes other emotional and cognitive sensations (Evans *et al*, 2002; Liotti *et al*, 2001). The anterior insula has been demonstrated to be involved in the integration of sensory information such as pain and breathlessness together with other emotional sensations such as empathy (Critchley *et al*, 2004; Craig, 2009; Gu *et al*, 2012). Although weak, the connectivity to both areas appears to favour the 'improved when stimulation ON' category. This may be driven in part by patients 14 and 16. Given the small sample size as well as the inter-individual variability; it is not possible to arrive at any conclusions as to the precise involvement of the insula and amygdala in AH processingin this study.

Prefrontal cortex

Connectivity to the prefrontal cortex was investigated because it has been shown to be involved in processing both inspiratory and expiratory respiratory sensations (Macey *et al*, 2004, 2005, 2006), as well as breathlessness processing using an emotional task in healthy controls (DLPFC, Herigstad *et al,*2015; Evans *et al*, 2002) and COPD patients (medial PFC; Herigstad *et al*, 2015).

The pre-frontal cortex has previously been associated with the integration and interpreting of higher cognitive functions with feedbacks from the limbic system (Miller and Cohen, 2001). Although the PFC has been associated with the perception of respiratory sensations from functional imaging studies, its role in breathlessness remains unclear, in particular the mechanism behind its involvement. The importance of this region in breathlessness processing has been demonstrated in COPD patients who are breathless at rest (Herigstad *et al,* 2015). No differences between patient categories were observed in the current study with respect to connectivity between the VIM and the prefrontal cortex –both categories had very weak connectivity. This may reflect the fact that this study involved experimentally induced dyspnoea under controlled conditions in patients who did not normally experience air hunger (as opposed to the COPD patients reported in Herigstad et al 2015).

It is also worthnoting that overlapping the average connectivity pattern of the 'OFF' patient with anatomical atlases, the tract connecting the VIM to the SMA was indirectly connected to the prefrontal cortex through the corpus callosum (using the JHU DTI-based white-matter atlases in FSL) and the medio-dorsal nucleus of the thalamus (the Oxford Thalamic Connectivity Atlas, Behrens *et al*, 2003). The patient who had improved AH with VIM stimulation 'OFF' had a greater connectivity to the SMA compared to the other category but did not express greater connectivity to the pre-frontal cortex. One might have expected that a greater SMA connectivity would be linked to greater connectivity to the pre-frontal cortex in this patient. This might suggest that the indirect connectivity between VIM and prefrontal cortex (via SMA) may not be related to AH processing.

Thalamus (VIM)

Quantitative connectivity to the thalamus was strong in all patients and both categories. Given the proximity to the VIM, the strong connection with the thalamus is not surprising. Neural activity has been recorded in the thalamus in respiratory loading studies (Chen *et al*, 1991) as well as several breathlessness studies, thus making it an important area in air hunger perception. Connections from the thalamus to the insula, amygdala and the ACC cortical areas and respiratory associated afferents form a special cortical network that may process both the intensity and the affective components of air hunger and other somatosensory stimuli (von Leupoldt *et al*, 2007). The connectivity results reported here confirmed the importance of the thalamus in modulation of breathlessness perception, suggesting that its upregulation is part of increased perception of breathlessness.However, an interesting observation was that the connectivity was substantially stronger in the 'improved when stimulation was 'OFF' patient and less in the improved in 'ON' patients. This contributes to the more posterior connectivity profile of this category. Given that electrode placement was the same (as was the VAT) between categories, this would suggest that the patient who felt more AH with VIM stmulation had stronger connections with elements of the thalamus that relate to AH generation than the other category of patient.

5.5.4 CLINICAL IMPLICATIONS AND SIGNIFICANCE

The present study shows that DBS and tractography are fruitful techniques for breathlessness research. This approach may aid our understanding of breathlessness perception. If the findings reported here are confirmed in larger studies, the cortical areas identified in this study can be targeted through DBS stimulation to reduce chronic air hunger perception in patients. This may improve quality of life when the underlying

pathology cannot be cured. It can also help stereotactic surgical planning in patients undergoing DBS surgery to either directly or remotely avoid the networks or fibre tracts that are likely to cause an increase in air hunger perception when stimulated.

5.5.5 STUDY LIMITATIONS

There are limitations on what may be drawn from the findings in this current study as it is a pilot study. To the best of the author's knowledge this is the first study investigating dissimilarities in white fibre tracts in patients who improved in AH when DBS stimulation was 'ON' and improved in AH when it was 'OFF' using tractography.

Number of patients: The first limitation is the small sample size. Therefore, these findings must be interpreted with care. More data will be required to confirm these findings.

Regions of interest: The regions of interest identified in this study were based on functional imaging studies on breathlessness. Remote connectivity to more areas should be investigated in future studies to reveal more possible cortical neural network subserving air hunger perception.

Technique: The technique used in this study is based on probability analysis, and it is not possible to be certain that the model has accurately captured the exact number of preferred fibre tracts. It is also not possible to determine the direction of the connections. Also, the technique did not specifically determine the termination points of pathways from the VIM to the remote areas investigated. However, probabilistic tractography technique has beenshown to be successfulin guiding the implantation of electrodes in DBS surgery, andalso information from the technique has been used to successfully delineate pain pathways during DBS surgery. Therefore, this information gives confidence that the results are representative of the actual white matter pathways.

Distinction between intensity and affective fibre tracts of air hunger: Air hunger is a multidimensional sensation consisting of both physical (intensity) and affective (emotional) components. This study did not distinguish between these two components which may have different neural pathways. Future studies should endeavour to distinguish between affective and physical components.

Reliability of air hunger ratings: Classification of patients into improved when 'ON' and 'OFF' were based on results presented in Chapter 4 of this thesis. Patient 16's air hunger ratings were not fully reliable and this patient's classification is therefore in some doubt. Studies have indicated that healthy volunteers require several practice sessions to become reliable and reproducible raters of experimentally induced breathlessness using visual analogue scales (Reed and Subhan, 1995). Inthe current study of DBS patients it was only possible to have access to the patients for upto two hours on one occasion only. Thus the reliability of their AH ratings are not guaranteed. This is important as there are several distinguishable components of dyspnoea that may arise from different neural pathways and could therefore call into question the binary model used in this study.

5.5.6 FUTURE STUDIES

In order for the current results to be conclusive, tractography data from more patients who improved when DBS stimulation is 'ON' or 'OFF' will need to be studied. Connectivity to more remote targets has to be investigated with the aim of forming a cortical network or map for air hunger perception. A more advanced tractography algorithm will be needed to measure afferent and efferent pathways separately. This may help to distinguish between the intensity and the affective pathways underlying air hunger perception.

5.5.7 CONCLUDING REMARKS

This is the first pilot study to use VIM DBS stimulation and DTI probabilistic tractography to investigate the neural network underlying the modulation of air hunger. Although results from this study are not conclusive, observations made have yielded important information to enhance our current understanding of the cortical and subcortical pathways of breathlessness. The results provide an insight into the possible groups of remote areas which may be essential to either enhance or reduce air hunger perception.

CHAPTER 6

DISCUSSION

6.1 INTRODUCTION

Many hypothesis driven experimental approaches to understanding the mechanisms of perceived breathlessness have been attempted over the past decade. Breathlessness research has made significant progress by slowly moving from implicating respiratory receptors as the single source of generating the sensation to identifying higher brain regions thought to be essential in processing the breathlessness signal through functional imaging studies. However, we cannot infer functional-structural relationships from these findings alone, and other approaches to the question must be found in order to interrogate the findings of brain imaging based studies. The overall breathlessness cortical network remains unclear. This chapter provides an overview of the major findings in this thesis (section 6.2), attempts to integrate the findings (section 6.3), positions its novel contribution within the field (section 6.4) and suggests future trajectories for the current approach to unravelling the cerebral network for breathlessness perception (section 6.5).

6.2 OVERVIEW OF MAJOR FINDINGS

6.2.1 Psychological modulation - what do we know now?

The influence of psychological factors such as anxiety, emotions and stress generated by environments on the experience of respiratory sensations such as breathlessness perception in both healthy subjects and the patient population has been extensively reviewed

(Depeuter *et al*, 2004; von Leupoldt *et al*, 2008; von Leupoldt and Dahme, 2007; von Leupoldt *et al*, 2011; Thomas *et al*, 2011; Scano *et al*, 2013).

However, most brain imaging studies of breathlessness have failed to account for this influence in the interpretation of brain regions activated. Cortical substrates considered to modulate breathlessness perception are also known to process other emotional, psychological and unpleasant sensations (Peiffer *et al*, 2001; Brannan *et al, 2001;* Parsons*et al*, 2001; Liottiet *al*, 2001). Thus by identifying distinct cerebral regions and proposing that they might serve purely to process breathlessness perception, the brain imaging studies have inadvertently strayed away from pursuing a cerebral network of regions that incorporates those involved in other psychosomatic processing. In Chapter 3, it was demonstrated that the direction in which the sensitivity to breathlessness changes in a claustrophic environment will depend on an individual's trait anxiety level with a threshold close to the population average. This has implications for the interpretation of fMRI studies of breathlessness; reported activations may only apply to a limited portion of the general population (low trait anxiety individuals) or may be associated more generally with anxiety rather than to breathlessness per se.

Taking account of individual trait and state anxiety levels in future fMRI studies of breathlessness will reposition regions of interest, and refocus the goal to one of identifying a network for breathlessness perception rather than a seat of perception.There are also implications for clinical MRI proceduresThis new information will address many setbacks such as the cost, poor data quality and early termination of scanning procedures (Enders *et al*, 2011; Eshed *et al*, 2007; Sarji *et al*, 1998). Individuals who are found to be highly anxious during MRI scanning procedures should be evaluated initially to assess the degree of their anxiety. Although there is a consensus among most studies that highlights the

problem of claustrophobia among certain groups of patients or healthy individuals (Szameitat *et al*, 2009; Mclsaac *et al*, 1998; Dewey *et al*, 2007), the level to which the environment induces this undesirable effect was not known. Thestudy in Chapter 3 has added to our current knowledge about the threat posed by these environments and its detrimental effect on the interpretation of brain imaging studies in breathlessness.

6.2.2 Functional cerebral network for breathlessness sensitivity?

Development of deep brain stmulation for relief of a variety of neurological symptoms (tremor, pain, epileptic seizures, dystonia) has offered a new way to identify brain areas that might be involved in breathlessness perception or its modulation.Direct access to the thalamic motor nucleus (VIM) via stimulation in the study described in Chapter 4 has raised the possibility that the motor thalamus may be a potential target for dyspnoea relief.

How it is that stimulation of a motorthalamic nucleus could relieve breathlessness sensation? There are several possibilities. (i) The VIM is known to have extensive connections with the motor cortex and stimulation of the motor cortex has been identified as an effective means of relieving facial neuropathic pain in several reviews (Monsalve, 2012; Nguyen *et al*, 1999; Brown and Pillitsis, 2005) and original experimental studies (Tsubokawa *et al*, 1991, 1993; Fagundes-Pereyra *et al*, 2010; Velasco *et al*, 2008).The relief of breathlessness in patients undergoing VIM stimulation (Chapter 4) may share this pathway involving the connections between the VIM and the motor cortex. (ii) It is also possible that stimulation of the VIM results in blocking or modulation of the breathlessness signal as it ascends through the thalamus. This idea is supported by the observation in the COPD patient with multiple implanted-electrodes, reported in Chapter 4. On the first visit the only electrode to completely ameliorate the breathlessness was the VIM. On return at 11 weeks all electrodes produced complete relief. Since the volume of activation (VAT) by

DBS increases with changes in the amplitude of stimulation (Xiao and Johnson, 2015), and the amplitude setting was indeed higher for all electrodes on the second visit, this might explain this observation.The study in Chapter 4 identifies new potential targets for dyspnoea relief, fuels discussion of the cerebral mechanisms of dyspnoea and in addition flags up that a reduction in breathlessness sensitivity after surgery needs clinical monitoring by the neurologist as this could lead to adverse medical complications if patients are unable to detect breathlessness.

6.2.3Structural mapping of white matter fibre tracking

A novel approach to investigating potential components of a putative cerebral network for breathlessness perception has arisen from the use of DTI probabilistic tractography.

The data from the tractography study described in Chapter 5 suggests that white fibre tract connectivity is directed to relatively more anterior parts of the brain in patients who experiencedrelief of experimentally induced AH when the electrodes were activated (switched 'ON'). The strongest connectivity in the patient in whom breathlessness increased as a side effect of switching ON the motorthalamic nucleus was within the thalamus, braintem and the pre-central gyrus. This is inconsistent with the findings of the brain imaging studies because it suggests that generation of breathlessness is associated with connectivity within the brainstem, thalamus and pre-central gyrus rather than more anterior aspects such as the ACC, Insula, SMA and amygdala (Evans *et al*, 2000; Banzett *et al*, 2002; Pieffer *et al*, 2001). Since only one patient has been found with increased breathlessness with electrode 'ON', this interpretation must be treated with caution. The findings of the study reported in Chapter 5, although very preliminary, do suggest that it may be possible in future to identify potential new therapeutic targets for positive intervention or treatment for chronic breathlessness in individual patients.

6.3. INTEGRATED SUMMARY OF FINDINGS IN THIS STUDY

Mechanistic understanding of central neural breathlessness mechanisms has predominantly been driven by a few early functional PET and fMRI imaging studies involving healthy subjects (Corfield *et al,* 1995; Banzett *et al*, 2000; Evans*et al*, 2002). Current information from imaging studies permits the general understanding that breathlessness is processed in various brain regions in response to different respiratory stimuli (von Leupoldt *et al*, 2008; Peiffer *et al*, 2001).The problem with this approach (functional brain imaging) is that (i) there is no way of knowing whether activations are associated with relief or with generation of breathlessness, (ii) it is not possible to determine how the activations might be connected as part of a cerebral network for breathlessness perception and (iii) have focussed almost entirely on healthy individuals with no 'clinical anxiety' and little 'trait' anxiety.

The current thesis offers three new approaches to address the short-comings of the brain imaging approach listed above:(i) The use of the DBS model to identify specific areas of the brain which when stimulated will generate or relive breathlessness, (ii) use of DTI probabilistic tractography to explore white fibre tract connectivity between regions of interest to begin to build a conceptual network for breathlessness perception and (iii) to refine the interpretatbility of the functional brain imaging studies to account for psychological factors.Table 6.1 compares areas of the brain and their putative roles in breathlessness perception between the brain imaging studies and the new approaches (stimulation and connectivity) adopted in this thesis. It should be emphasised that the outcomes of the current thesis are more to do with feasibility of the novel approaches rather than to the findings themselves since the number of patients studied is limited.

Table 6.1: Comparison of findings from studies using different methodological approaches to investigate cerebral mechanisms of breathlessness.
CER=cerebellum, AMYG=amygdala, ACC= anterior cingulate cortex, DLPFC= dorsolateral prefrontal cortex, vmPFC= ventromedial prefrontal cortex, SMA= somatosensory area, STN=Subthalamic nucleus, VIM=Ventral-intermediate nucleus

Methodological approach:	**Brain areas: Breathlessness generation?**	**Brain areas: Breathlessnessrelief?**
Imaging studies* Banzett *et al*, 2000. Evans *et al*, 2002. Herigstad *et al*, 2013. Peiffer *et al*, 2001.	ACC, Thalamus, Brainstem, Insula, DLPFC, vmPFC, PAG, Cer, SMA, Amyg	
Stimulation studies Ψ Chapter 4. ϕ Chalif *et al,* 2015.	ϕ STN	Ψ VIM
Connectivity studyChapter 5.	€ Posterior aspect VIM to Brainstem, Thalamus and Pre-Central.	€ Anterior aspect VIM to Frontal Orbital, DLPFC, Insula, Amygdala.

*Imaging studies make the assumption that activations relate to 'generation' of breathlessness perception.
€Connectivity areas based on differences in relative connectivity strengths between patients who improved with VIM DBS 'ON' and patients with VIM DBS 'OFF' shown by the height of the bars in Figure 5.4(Note : absolute connectivity strength from VIM to DLFPC, Frontal Orbital and Insula is weak).

6.4 THE NOVEL CONTRIBUTIONS MADE BY THE WORK DESCRIBED IN THIS THESIS

The following are novel contributions made by this study:

A possible new brain target for breathlessness relief: This study has found new evidence that air hunger can be both reduced and enhanced by motor-thalamic 'stimulation' (VIM and VOP). The mechanism of this interaction needs to be further investigated but it raises the possibility that thalamic DBS in general may be a target for treatment of intractable breathlessness. The nuerosurgeons employing DBS are only flagging up those patients in whom breathlessness is appearing as a side effect of the DBS whereas the current study has

found that most have reduced sensitivity to breathlessness stimuli when DBS motorthalamic DBS is 'ON'. This raises concerns because an inability to perceive breathlessness appropriately could lead to neglect of medical issues (such as aspiration pneumonias) suggesting that these patients should be monitored by the neurosurgeons as well as those who experience breathlessness as a side effect.

The need to evaluate patients and research participant's anxiety status before an fMRI scan: The study in Chapter 4 has demonstrated that the effect of psychological factors, such as anxiety and panic induced by closed environments, on breathlessness can be quantified. In doing so, the study has identified a threshold trait anxiety score about which individuals are likely to produce very different brain activations. Averaging these activations would be misleading and therefore previous brain imaging studies should be re-interpreted in light of this. Furthermore the anxiety status of either patients or healthy subjects should be evaluated before MRI scanning procedures in future imaging studies.

The use of probabilistic tractography technique to delineate white matter tracts in breathlessness mechanisms: This study has demonstrated that, probabilistic white matter connections to the ***anterior*** region of the brain could be associated with reduced air hunger perception when the implanted DBS electrodes are activated (switched 'ON') to stimulate the surrounding tissues. Connectivity to the ***posterior*** part of the brain wasassociated with improvement in AH when stimulation was 'OFF'. This represents the first foray into developing a framework for a dyspnoea perception network much like that which has been developed for pain perception. In particular, this method enables distinguishing between parts of the brain associated with generating breathlessness with those parts associated with relief of breathlessness-not easily achieved with brain imaging studies alone.

Novel use of the experimentally-induced air hunger test.: This is the first use of experimentally induced breathlessness (air hunger model) in DBS patients. In past years, the use of experimentally induced breathlessness models such as 'air hunger' has mostly been limited to healthy subjects and certain patient groups such as COPD and asthmatics. Study of the patient groups is often confined to use of breathlessness questionnaires. Since the air hunger breathlessness model is described as a very unpleasant form of breathlessness and DBS patients studied here were vulnerable in terms of motor coordination and medical complications, the feasibility of using this experimental model reliably was questionable. The limited amount of time to study each patient did not permit long practice sessions on the breathing circuit –this is known to be required to establish reliability. The issue was circumvented by giving patients breathing practice on a metronome and use of VAS while they were on the ward as part of the recruitment process before they came to the lab. This process undoubtedly improved patient recruitment and substantially reduced the number of patients who dropped out of the study because they could not cope with the procedure.

6.5 FUTURE DIRECTIONS

6.5.1 Possibility of using MEG, EEG and rTMS to study breathlessness

In recent years, the possibility of using alternative functional brain neuroimaging methods as investigative tools to study the mechanism of breathlessness perception has gathered interest. Two such methods which are in direct competition and have been identified to possess a great potential to fill this niche are electroencephalography (EEG) and magnetoencephalograhy (MEG). This is because both EEG and MEG do not induce claustrophobia compared to fMRI scanning. This is very important to ensure that recorded

signals are from the brain areas which process mainly physiological signals with very little emotional interference as in the case of fMRI. They both reflect electrical activity from the brain with milliseconds temporal resolution and are non-invasive techniques.

A recent feasibility study, where MEG was used to measure neural activities related to changes in physically induced breathlessness in patients with chronic symptoms, concluded that MEG is an acceptable and useful investigative tool to study the perception of breathlessness (Johnson *et al*, 2015). Despite the advantages and strength of MEG as an investigative tool compared to EEG (due to superior localisation, better spatial resolution, the ability of the magnetic field to pass through the skull and various tissues undistorted) (Proudfoot*et al*, 2014), the authors agreed the need for more robust experiments with a wider range of patient groups and age-matched healthy subjects for the results to be conclusive. Unlike MEG, in terms of expense, EEG is cheaper and an attractive option to study the neural mechanisms of breathlessness. It measures tiny electric fields tangentially with millisecond temporal resolution despite its shortcomings in terms of being prone to structure distortion.

The advantage of MEG and other functional neuroimaging modalities becoming investigative tools in breathlessness perception is that they would help to dissociate pure psychological signals from physiological ones where certain brain areas found to process breathlessness perception also process emotionally-related signals. Finally, other less used imaging tools such as transcranial magnetic stimulation (TMS) and repeated transcranial magnetic stimulation (rTMS) (Rollnik *et al*, 2000, 2002; Pleger *et al*, 2004) should also be evaluated for potential usefulness in studying breathlessness perception. Based on findings of Chapter 3 in this thesis, the lack of significant changes in AH sensitivity during mock MEG compared to baseline adds further support for future MEG based studies of

breathlessness. On the other hand, using both techniques together may in future enable the activations associated with air hunger per se and those associated with general anxiety to be separated out.

6.5.2 Lesion-deficit study

Uncertainty in the interpretation of imaging studies make them difficult to rely on in establishing brain structure-function relationships (Banzett *et al,* 2000; Evans *et al,* 2000). Lesion-deficit methods have been instrumental in the development of our understanding of the functional relevance of specific brain regions over the past decades. They are still widely used in the investigation of language pathology in cognitive neuroscience to understand possible brain areas which might be involved in speech disorders (Dronkers 1996; Dronkers and Lundy, 1998). Testing dyspnoea sensitivity in ischaemic stroke patients where the damaged brain area predominantly involves the insular cortex offers an alternative observation and may also indicate whether activation of these areas increases or decreases dyspnoea. Although this method remains very popular among researchers, its limitations - such as the possibility of brain re-generation and precise lesion location and volume - have led to the recent introduction of functional neuroimaging techniques. These two techniques have been recommended to be used to complement each other for better results (Price and Friston, 2002).

6.5.3. Surgical resection –low grade insular gliomas

The few available data from early studies on the effect of anterior cingulotomy (surgical removal of the anterior cingulate in the brain) to relieve chronic pain and other psychological disorders have reported a positive autonomic outcome in approximately 60% of patients (Sharma 1973; Corkin *et al,* 1980). However, various cognitive functions were observed in more recent studies to be significantly compromised in some patients and in certain cases resulted in mortality after some time (Cohen *et al,* 1994). Despite these

challenges, testing breathlessness perception in anterior cingulotomy patients using an AH model will provide a novel insight into our current knowledge on the cerebral mechanism underpinning breathlessness since it may share common cerebral pathways with pain.

The effect of surgery on other brain functions such as motor movement and cognitive responses has been investigated in insular glioma patients (Wu *et al,* 2011; Sanai *et al,* 2010). Evaluating breathlessness perception before and after surgical removal of low grade insular gliomas in this patient group would increase our knowledge of the damaged site in the brain perceived to be essential for breathlessness perception. A change in breathlessness sensitivity has not been studied and reported in any available literature that assesses the autonomic outcomes in patients before and after surgery. Low grade insular glioma patients (who tend to be younger and have few if any co-morbidities) are perfect candidates to better our understanding of the insular involvement in breathlessness perception from the structural point of view, as the insular has been shown to be activated in several published breathlessness studies using functional imaging (Banzett *et al*, 2000, Evans *et al*, 2000, 2002). Blunted perception after surgery, if noted, presents a novel opportunity to use a diffuse tensor imaging technique (DTI) to examine the state of the destroyed cortical fibres by the tumour in relation to reduced perception. However, an increase in sudden death has been noted in these patients.Possible causeshave been investigated (Sanai *et al*, 2010) but it is not known to what extent a blunted perception of breathlessness might be involved.

6.6 CONCLUDING REMARKS

The knowledge about the central neural processing of chronic or acute breathlessness remains incomplete. A number of studies have proposed distinct brain areas to modulate either the unpleasantness or the affective component of breathlessness perception through functional brain imaging studies (Banzett *et al*, 2000; Evans *et al*, 2002; Peiffer *et al*, 2001; von Leupoldt and Dahme, 2005; von Leupoldt *et al*, 2008).This thesis has introduced novel methods to improve the interpretability of these previous approaches and thereby improve our understanding of cerebral mechanisms of breathlessness. While the findings are very preliminary, they are also very tantalising (e.g. proposing potential new DBS targets for complete relief of COPD breathlessness). In addition, the steps taken to apply the novel techniques make them more feasible. Clearly the years ahead hold much promise for great advances in both understanding of cerebral mechanisms of dyspnoea but also in its management.

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APPENDIX 1

DEEP BRAIN STIMULATION SURGICAL PROCEDURE

All patients underwent surgery in two stages, including nine steps in total (Figure below). Stage one was the implantation of a microelectrode in the selected subcortical target under local or general anaesthesia. After a week of continuous external programming to find the best stimulating levels to relieve the patient's symptoms, an implantable impulse generator is placed under the skin and controlled by an external programmer. This second stage was performed under general anaesthesia. Accuracy in stereotactic surgery is essential and this is achieved by utilising a fusion between preoperative high resolution multi-slice fine cut MRI and intra-operative CT scan with a Cosman-Roberts-Wells (CRW) head frame. The steps of the procedure will be described in detail below.

Stage 1:

Step 1: On the day of surgery, stereotactic CRW frame with metal rod localiser was fixed to the patient's head under local anaesthesia.

Step 2: These localiser rods or 'fiducials' were detected on CT scan as a hyper intense ring forming fixed anatomical landmarks outside the skull and provide the basis of the relationship of anterior- posterior midline (A-P) of the brain to the skull.

Step 3: The patients CT image was then fussed volumetrically with their preoperative MRI, using commercial software (Neuroinspire, Renishaw, Gloucestershire, United Kingdom, Figure 7.2), for pre-operative planning. A

triangulation method is used to calculate three-dimensional Cartesian coordinates of the target nuclei. In the case of GPi and STN, a visual approach is used to locate the target because they are visible when the two scans are fused together. The coordinates of the target nuclei are transferred onto a phantom frame before it is placed on the stereotactic CRW frame. Subthalamic nuclei and Globus Pallidius internus can be identified based on the distinct radiological anatomy whilst the periventricular gray and ventral posterior lateral nucleus of thalamus is based on anatomical landmarks as described above. During the planning stage, the best position of entry and a safe electrode trajectory tract is selected. This is done to avoid the ventricles and major cerebral vessels.

Step 4: After routine scalp preparation, local anaesthesia is applied to the target entry point. An adequate curvilinear incision is made on the scalp and haemostasis is secured. A 2.7 mm craniotomy is performed using a twist drill. The stereotactic frame module is then affixed to the CRW frame. A straight metal guide (recording electrode) is introduced through a special holder fixed on the stereotactic frame within the line of the calculated trajectory into the target nuclei. This holder has a security stopper which holds the guide so it will not go any deeper than the calculated coordinate. The dura is opened and a TM electrode (Radionics, Burlington MA) is introduced using the planned trajectory to reach the target nuclei.

Step 5: Series of intraoperative microrecordings are made by adjusting the frequency, amplitude and pulse width of the stimulating electrode to establish an acceptable neurophysiological outcome and minimise any adverse motor, cognitive and verbal effect.

Step 6: The DBS electrode (Medtronic (3387 or 3389 model) or Boston scientific) is then passed and permanently placed into the target. Once the final position of the electrode tip is satisfactory placed, the electrode was secured to the skull using a titanium bioplate. The distal end of the electrode is connected to a temporary extension lead and tunnelled under the scalp to a safe distance from the wound as part of infection control practice.

Step 7: An intraoperative marcrostimulation is performed to confirm the therapeutic benefit recorded and achieved during step 5.

Step 8: Post-operative CT scan is performed after surgery to confirm final electrode location.

Stage 2:

Following a one-week period where the best stimulating parameters out of a wider range were tested on continuous occasions to tailor the treatment for each patient's clinical need.

Step 9: An impulse generator (battery) is implanted, normally in a pouch below the collar bone under the skin. Extensions lead connecting the electrodes in the brain. Any provisional extension leads for test programming are disconnected. A series of electrical tests was performed on the system to ensure that all the components and connections are secure and working well. A simple schematic of the DBS procedure is shown in figure 7.1 and the stages of procedure are shown in the figure below.

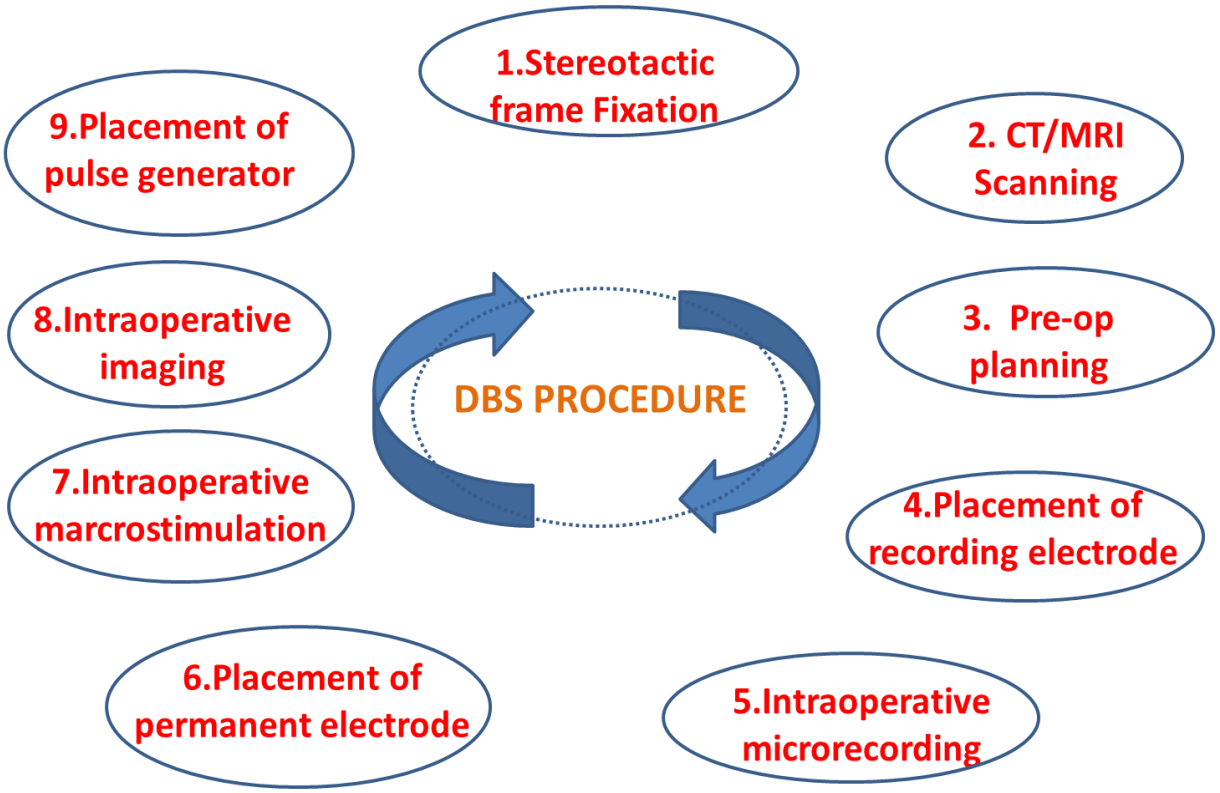

Figure 7.1: Simple schematic of the steps involved in DBS surgery adopted by the Oxford functional neurosurgery team. Step 4 in the procedure could also be done by recording the local field potential (LFPs) of the cells within the targeted nuclei to aid in its confirmation. CT= computerised tomography, MRI= magnetic resonance imaging, DBS= deep brain stimulation.

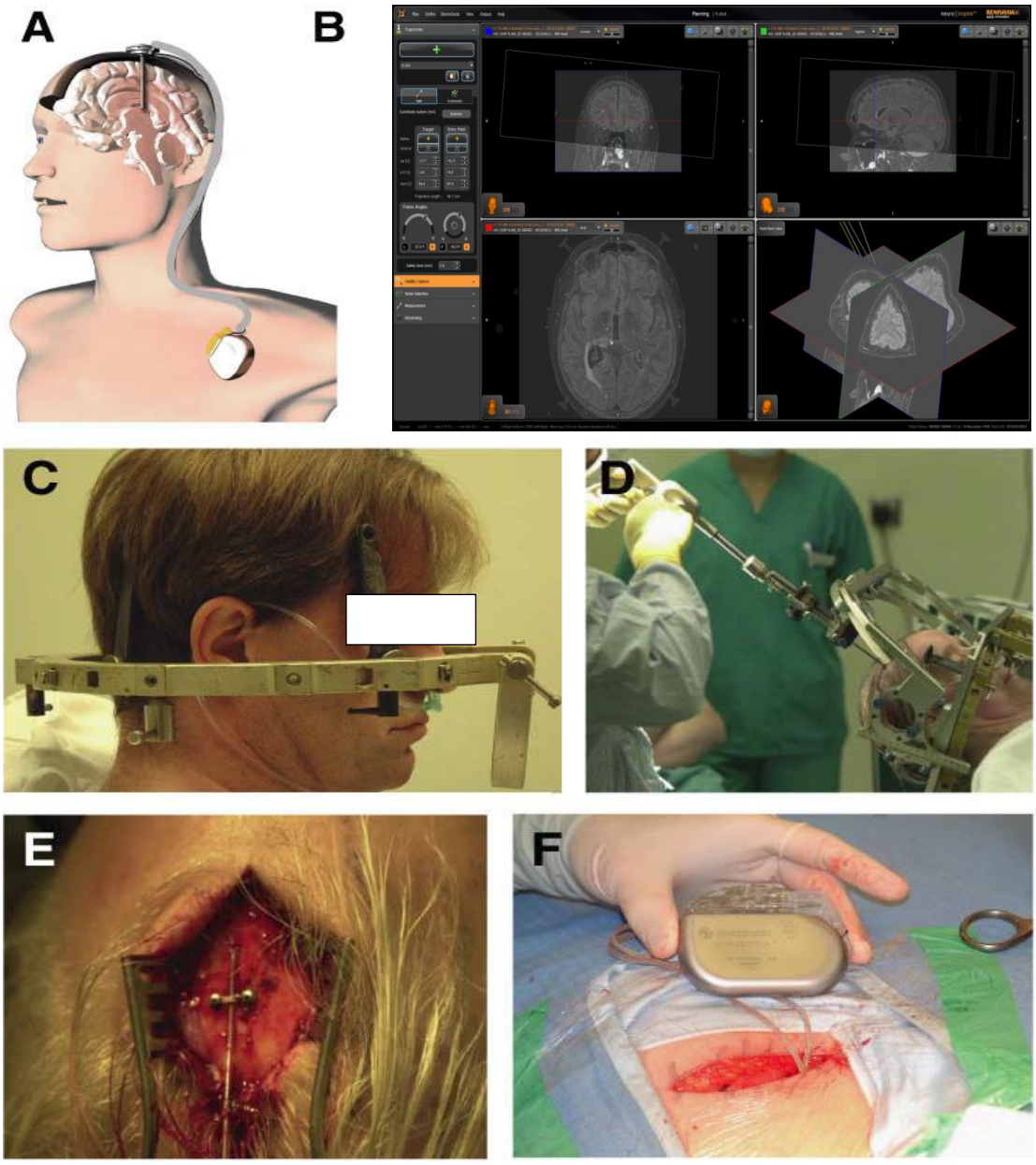

Figure 7.2: The neurosurgical procedures involved in DBS in OFN centre. **A)** Schematic of DBS mechanical principles. **B**) Illustration of the process of the neurosurgical pre-planning. C) Application of the Cosman-Roberts-Wells stereotactic head frame on the patient. The base ring is set parallel to the orbitomeatal line. D) The precise positioning of both the recording and the permanent electrode is by making a hole through the calvarium with a twist drill. E) Securing the quadriploar microelectrode to the skull with a titanium mini bioplate and screws. F) Placement of the implantable pulse generator in a subcutaneous pouch under the collar bone. (Adapted from Kringelbach *et al*, 2009). OFN= oxford functional neurosurgery, DBS= deep brain stimulation.

APPENDIX 2 (A)

ETHICAL APPROVAL FOR STUDY IN CHAPTER 3

Dr Shakeeb Moosavi
Director of Studies
Department of Biological and Medical Sciences
Faculty of Health and Life Sciences
Oxford Brookes University
Gipsy Lane
Headington

20 October 2014

Dear Dr Moosavi

UREC Registration No: 140861
Breathlessness sensitivity measured during mock brain imaging scans

Thank you for submitting the application to the University Research Ethics Committee on behalf of your research student Emmanuel Debrah. The Committee reviewed the application at its meeting on 9 October 2014, and have agreed approval subject to meeting the following conditions:

1. Please could you clarify whether the mock scanners simulate the noise of an MRI / MEG scanner? If so, please explain this in the participant information sheet.

2. Please include all exclusion criteria on the poster e.g. a history of panic attacks or anxiety disorder, so only those eligible to participate express an interest in the study.

3. It is not clear whether the copyright permission should be included on the Trait Anxiety Questionnaire – please would you check this and add if necessary.

4. The Committee thought it may be preferable to reword the title of the study as 'Breathlessness sensitivity measured during *simulated* brain imaging scans' on all correspondence with participants.

5. Some amendments are required to the participant information sheet, as follows:

    a. The wording is rather dense and could be somewhat overwhelming for potential participants. Please simplify this so that the text is more accessible to the intended audience.
    b. It may be useful to include a picture of the mock scanner.
    c. Points 8 and 9 are not relevant to this study and should therefore be removed.

d. The study has been reviewed by the University Research Ethics Committee, rather than the Faculty REC and this should be amended.
e. There are some typographic errors – please could you ensure careful proof reading prior to circulation.

6. Please remove the note on the consent form requiring a copy of this to be filed in the patient's notes. This NRES requirement does not apply in this study.

Could you please confirm in writing, within the next three weeks that you will meet these conditions? A covering letter should be included explaining how the conditions have been met along with copies of any revised documentation? When this has been received and agreed, I will send another letter indicating full approval.

Yours sincerely

Hazel Abbott
Chair of the University Research Ethics Committee

cc Mari Herigstad, Second Supervisor
Emmanuel Debrah, Research Student
Dido Green, Research Ethics Officer
Jill Organ, Research Degrees Team
Louise Wood, UREC Administrator

UNIVERSITY RESEARCH ETHICS COMMITTEE, FACULTY OF HEALTH AND LIFE SCIENCES

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APPENDIX 2 (B)

ETHICAL APPROVAL FOR STUDY IN CHAPTER 4 AND 5

NHS
Health Research Authority

NRES Committee South Central - Oxford B
Bristol Research Ethics Committee Centre
Whitefriars
Level 3, Block B
Lewin's Mead
Bristol
BS1 2NT

Tel: 0117 342 1333
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03 June 2013

Mr Alexander L Green
Consultant Neurosurgeon and Spalding Senior Lecturer
University of Oxford
Department of Neurosurgery
John Radcliffe Hospital,
Oxford
OX3 9DU

Dear Mr Green

Study title:	**Near Infra Red Spectroscopy (NIRS) and Transcranial Doppler (TCD) monitoring of cerebral blood flow, regional cortical oxygen saturation and extraction index in patients with deep brain and occipital nerve stimulators.**
REC reference:	**11/SC/0229**
Amendment number:	
Amendment date:	**15 May 2013**
IRAS project ID:	**73534**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The Committee found there to be no items of ethical concern.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
CV - Emmanuel Debrah		
CV - Syed Shakeeb Hassan Moosavi		

Questionnaire: Dyspnea Score Chart	1	
Participant Consent Form	5	01 May 2013
Participant Information Sheet	5	01 May 2013
Protocol	5	01 May 2013
Notice of Substantial Amendment (non-CTIMPs)		15 May 2013
Covering Letter		16 May 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

11/SC/0229:	**Please quote this number on all correspondence**

Yours sincerely

Mr Robert King
Vice Chair

E-mail: NRESCommittee.SouthCentral-Oxfordb@nhs.net

Enclosures: List of names and professions of members who took part in the review

Copy to: Mrs Heather House
Karl.shepherd@admin.ox.ac.uk