Studying the brain mechanisms of dyspnoea with functional magnetic resonance imaging

Thesis submitted for the degree of Doctor of Philosophy

Anja Hayen

University of Oxford

Nuffield Department of Clinical Neurosciences
Balliol College
Hilary Term 2014

Für Mama und Papa

Acknowledgements

A huge thank you goes to my supervisor Kyle Pattinson for his enthusiasm, advice and support throughout the production of this thesis. Mari Herigstad was invaluable for the completion of this thesis and deserves a huge thank you. I would like to thank my co-supervisor Dr. Andrew Farmery for his encouragement and advice and my Balliol college academic advisor Jonathan Bard for his unbiased view and for his support with thinking and writing about my research.

I would additionally like to thank the members of the dyspnoea research group, as well as the various occupants of the office that provided me with company, tea and inspiration. Thank you to everyone at FMRIB, especially to the technical team Matt, Dave, Duncan, Ian and Chris and to Mark Jenkinson for his help with analyses. I would further like to thank Prof. Richard Wise and his group, as well as the FMRIB pain group for their inspiration and help. I would also like to say thanks to all my participants, who repetitively endured unpleasant respiratory stimuli and still came back for the next session.

I would like to say a special thank you to the people who helped me with the conduction and analysis of the experiments presented within this thesis, especially Kyle and Mari. For the experimental study presented in Chapter 3, I would like to thank Mari for performing the gas control for the experiment. Kyle, Martyn, Michael Kelly and Matt also deserve a thank you for helping out with the gas control. I am grateful to Imogen Davies for advise on qualitative data analysis techniques and to Bob Banzett for his helpful comments on the use of the MDP.

For the study presented in Chapter 4, I owe a huge thank you to Mari for performing all TCD measures and for providing most of the tools used for the analysis of the physiological data (including writing most of the Matlab scripts). Kevin Murphy kindly provided the scripts used for physiological noise correction of the imaging data and Michael Kelly provided help with ASL acquisition and analysis.

For the study presented in Chapter 5, I would like to thank Mari and Josie for their tireless gas control and assistance with the experimental setup in the scanner. I would also like to thank Kyle for encouraging a group of anaesthetists to help with the study. I would like to thank the team at OCMR, especially Steve Knight, for their support with this technically rather challenging study. A huge thank you goes to the anaesthetists who gave up their time to perform the drug infusions and care for our participants. Thank you Ruth Webster, Stuart Campbell, Simon Raby, Payashi Garry, Richard Rogers and Shravan Tirunagari. Further, Ellie Evans saved me many hours by meticulously computerizing the questionnaire data collected during this study, thank you for that.

I am very grateful for the support I received from Balliol College -academically, emotionally and financially. I would like to thank the Medical Research Council for funding the herein-presented research and the German National Academic Exchange Council (Deutscher Akademischer Austauschdienst, DAAD) and the NIHR Biomedical Research Centre, Oxford for their financial support.

Most importantly, I would like to thank my office mates, my family and friends, who supported me through this difficult time. A special thank you goes to Adrian for his constant support through the highs and lows of this research. Danke für eure Hilfe und euer Verständnis Mama und Kerstin. Ich hab’s geschafft!

Thesis abstract

Anja Hayen *D.Phil. Thesis, Hilary Term 2014*
Balliol College

Studying the brain mechanisms of dyspnoea with functional magnetic resonance imaging

Dyspnoea (breathlessness) is a debilitating, often poorly controlled, symptom of cardiopulmonary, neurovascular and psychological disorders. This thesis develops the necessary methodology to dissociate aspects of the acute dyspnoea experience using functional magnetic resonance imaging (FMRI) in healthy volunteers. The neuronal mechanisms underlying dyspnoea anticipation, its perceived intensity and unpleasantness and the modulation of these mechanisms by the opioid remifentanil were explored.

We investigated the subjective perception of respiratory loading, a commonly used dyspnoea stimulus, and its potential systematic confounds on FMRI due to cerebral blood flow changes. Investigation of the perception of respiratory loading at different levels of hypercapnia (increased end-tidal CO_2) showed that hypercapnia should be kept to a minimum to avoid increased baseline respiratory unpleasantness whilst maintaining the stable arterial CO_2 (isocapnia) beneficial for FMRI analysis. Investigation of the effects of respiratory loading (± 9 cmH_2O) on cerebral blood flow showed that systematic confounds of respiratory loading on perfusion-based neuroimaging data were small (~5%) and did not significantly alter neural activation in response to visual stimulation.

Isocapnic respiratory loading during a classical fear-conditioning paradigm during FMRI was used to investigate dyspnoea anticipation, and dissociate the intensity and unpleasantness of acute dyspnoea by modulating unpleasantness with remifentanil. Differential neural networks were found to be involved in perceived intensity (thalamus, insula, somatosensory cortex) and unpleasantness (hippocampus, medial prefrontal cortex). Remifentanil reduced respiratory unpleasantness without affecting the perceived intensity and differentially reduced brain activity during both dyspnoea anticipation and perception.

This thesis showed the potential of isocapnic respiratory loading for the study of dyspnoea with FMRI. This stimulus revealed, for the first time, brain activation for dyspnoea anticipation, perceived intensity and unpleasantness. The opioid-sensitive nature of the anticipation and unpleasantness of dyspnoea provides brain targets for future research and might facilitate more effective dyspnoea palliation.

Table of contents

Thesis abstract ..... **I**
Table of contents ..... **III**
Glossary ..... **VII**

Chapter 1

Dyspnoea – A complex individual experience **1**
Abstract Chapter 1 ..... **2**
1.1 Dyspnoea **3**
1.2 Perception of dyspnoea **4**
1.2.1 The impact of emotions on dyspnoea processing 5
1.2.2 Dyspnoea and cognitive processing 9
1.3 The complexity of dyspnoea: Challenges for dyspnoea management in specific sub-groups **10**
1.3.1 Aging 10
1.3.2 Obesity 10
1.3.3 Sex differences 11
1.4 Neuroimaging as a tool to understand dyspnoea **14**
1.5 Conclusion **16**

Chapter 2

The induction of dyspnoea in healthy volunteers during FMRI **17**
Abstract Chapter 2 ..... **18**
2.1 Studying the perception of dyspnoea **19**
2.2 Measuring the perception of dyspnoea **20**
2.2.1 Unidimensional scales 21
2.2.2 Sensory and affective components of dyspnoea 21
2.2.3 The multidimensionality of dyspnoea 22
2.2.4 Use of self-report measures to assess laboratory dyspnoea 25
2.3 Using functional magnetic resonance imaging to study dyspnoea in healthy volunteers **25**
2.3.1 Translation of information gained from dyspnoea models to patients 27
2.3.2 Introduction to magnetic resonance imaging 28
2.3.3 Obtaining contrasts during FMRI 29
2.4 Controlled induction of dyspnoea in the laboratory and application to FMRI **31**
2.4.1 Air hunger 32
2.4.2 Increased respiratory effort 35
2.4.3 Inducing dyspnoea during FMRI 37
2.5 Conclusion **39**
2.6 Aims of the work presented within this thesis **39**

Chapter 3

The unpleasantness of inspiratory loading is not altered by mild hypercapnia and does not habituate over time **41**
Abstract Chapter 3 ..... **42**
3.1 Introduction **43**
3.2 Materials and Methods **46**
3.2.1 Participants 46
3.2.2 Experimental paradigm 46
3.2.3 Data analysis 51
3.3 Results **53**
3.3.1 Isocapnia 53
3.3.2 Effects of hypercapnia on respiratory unpleasantness 54
3.3.4 Habituation of respiratory unpleasantness 58

3.3.5 Qualitative feedback 61
3.4 Discussion 62
3.4.1 Main findings 62
3.4.2 Benefits of isocapnia when inducing experimental dyspnoea during FMRI 63
3.4.3 First study investigating the effects of hypercapnia on unpleasantness during respiratory loading 63
3.4.4 Effects of hypercapnia on unpleasantness during unloaded breathing 65
3.4.5 Habituation of respiratory unpleasantness 65
3.5 Conclusion 68

Chapter 4

The effects of altered intrathoracic pressure on resting cerebral blood flow and its response to visual stimulation 69
Abstract Chapter 4 70
4.1 Introduction 71
4.2 Materials and Methods 74
4.2.1 Brief overview 74
4.2.2 Data acquisition 75
4.2.3 Data analysis 80
4.3 Results 82
4.3.1 Laboratory session 82
4.3.2 MRI session 82
4.4 Discussion 88
4.4.1 Summary 88
4.4.2 CBF changes with inspiratory and expiratory loading 88
4.4.3 Arterial blood pressure and CBF 89
4.4.4 Venous return 89
4.4.5 Systemic vascular resistance 90
4.4.6 Arterial CO_2 91
4.4.7 Interactions between respiratory loading and visual stimulation 92
4.4.8 Implications for the understanding of respiratory control 93
4.4.9 Clinical implications 94
4.5 Conclusion 95

Chapter 5

Investigating the neural signature of affective processing of acute and anticipated dyspnoea with the opioid remifentanil 97
Chapter Abstract 5 98
5.1 Introduction 99
5.1.1 General introduction 99
5.1.2 Dyspnoea intensity and unpleasantness 99
5.1.3 Opioids 102
5.1.4 Clinical relevance of studying the anticipatory anxiety of dyspnoea 106
5.1.5 Classical conditioning 106
5.1.6 Neuroimaging of dyspnoea anticipation 108
5.1.7 Summary and hypotheses 108
5.2 Materials and methods 110
5.2.1 Brief overview 110
5.2.2 Data acquisition 110
5.2.3 Data analysis 118
5.3 Results 124
5.3.1 Psychological and physiological data 124
5.3.2 Neuroimaging results 132
5.4 Discussion 148

5.4.1 Key findings ... 148
5.4.2 Neuronal correlates of the anticipation of dyspnoea ... 149
5.4.3 Non-subjective responses to graded inspiratory loading ... 151
5.4.4 Trial-by-trial variation within sessions ... 152
5.4.5 Neural signature of average subjective dyspnoea across session ... 156
5.4.6 Affective processing of dyspnoea ... 159
5.4.7 Limitations and methodological considerations ... 161
5.4.8 Clinical implications ... 166
5.4.9 Future research ... 167
5.5 Conclusion ... 168

Chapter 6

General discussion ... 171
Abstract Chapter 6 ... 172
6.1 Main findings of this thesis ... 173
6.2 Methodological considerations in dyspnoea research ... 174
6.2.1 The subjective perception of dyspnoea ... 174
6.2.2 Confounding factors of loading on CBF and neural responses to (visual) stimuli ... 179
6.3 Neural mechanisms of acute unpleasantness and anticipation of dyspnoea ... 180
6.4 Conclusion ... 183

Bibliography ... 185

Appendix ... 223
Appendix Ia: Older version of the MDP as used in Chapters 3 and 4 ... 225
Appendix Ib: Newer version of the MDP as used in Chapter 5 ... 227
Overview Appendix II ... 229
Appendix IIa: Understanding dyspnea as a complex individual experience ... 230
Appendix IIb: The effects of altered intrathoracic pressure on resting cerebral blood flow and its response to visual stimulation ... 236
Appendix IIc: Dyspnoea and the brain ... 246
Appendix IId: Effects of inspiratory and expiratory loading upon global and stimulus evoked CBF ... 255
Appendix IIe: Physiological noise reduction for multi-inversion time ASL ... 256
Overview Appendix III ... 257
Appendix III: Dyspnoea in COPD is associated with altered processing in the medial prefrontal cortex ... 258

Glossary

ABP	Arterial blood pressure
ACC	Anterior cingulate cortex
ASL	Arterial spin labeling
BA6	Broadmann area 6 (premotor cortex)
BDI	Baseline Dyspnea Index
BET	Brain Extraction Tool (part of FSL)
BL	Bond-Lader visual analogue scales to assess subjective mood
BOLD	Blood oxygen-level dependent
BP	Blood pressure
CBF	Cerebral blood flow
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CES-D	Center for Epidemiologic Studies Depression Scale
CV	Coefficient of variation
DLPFC	Dorsolateral prefrontal cortex
DMPFC	Dorsomedial prefrontal cortex
Dyspnoea	Breathlessness
EEG	Electroencephalography
End-tidal	At the end of the breath
EPI	Echoplanar imaging
EV	Explanatory variable
FEAT	FMRIB's Expert Analysis Tool
FILM	FMRIB's Improved Linear Model
FLAME	FMRIB's Local Analysis of Mixed Effects
FNIRT	FMRIBS's Nonlinear Image Registration Tool
FMRI	Functional magnetic resonance imaging
FMRIB	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
FSL	FMRIB's Software Library
GLM	General linear model
HR	Heart rate
Hypercapnia	Increased CO_2
Hypoxia	Decreased oxygen
Isocapnia	Stable CO_2

M1	Primary motor cortex
MCFLIRT	Motion correction using FMRIB's Linear Imaging Registration Tool
MNI	Montreal Neurological Institute
MPFC	Medial prefrontal cortex
MRC	Medical Research Council
MRI	Magnetic resonance imaging
O_2	Oxygen
$PaCO_2$	Arterial pressure of carbon dioxide
PAG	Periaqueductal grey
PCASL	Pseudo-continuous arterial spin labeling
PET	Positron emission tomography
$PETO_2$	Partial pressure of end-tidal O_2
$PETCO_2$	Partial pressure of end-tidal CO_2
PFC	Prefrontal cortex
RETROICOR	Retrospective image correction
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
TCD	Transcranial Doppler
TDI	Transitional Dyspnea Index
TE	Echo time
TI	Inversion time
TR	Repetition time
VAS	Visual analogue scale
VMPFC	Ventromedial prefrontal cortex
VLPFC	Ventrolateral prefrontal cortex

Chapter 1

Dyspnoea – A complex individual experience

Abstract Chapter 1

This chapter will introduce dyspnoea and will highlight challenges for dyspnoea management. Evidence from clinical and laboratory studies will be reviewed to show how emotions affect the perception of dyspnoea and how understanding the neural mechanisms behind these modulations can aid dyspnoea management. A particular focus is put onto the intricate link between anxiety and dyspnoea that strongly affects patients' quality of life and hence offers a valuable potential avenue for treatment. The next section highlights the complexity involved in the study of dyspnoea. The challenges of managing dyspnoea in older people, obese people and women, three populations particularly vulnerable to dyspnoea, will be introduced here. Neuroimaging of the link between dyspnoea and emotions is reviewed and neuroimaging is proposed as a way to understand the neural mechanisms involved in the perception of dyspnoea in order to better target treatment.

1.1 Dyspnoea

Dyspnoea is the highly distressing experience of breathlessness that makes simple everyday activities, like playing with the grandchildren, going to the shops or simply getting dressed in the morning, a major challenge. This chronic debilitating burden affects an increasingly large group of patients with respiratory diseases like chronic obstructive pulmonary disease (COPD) and asthma, heart failure and terminal cancer (Solano et al., 2006) and is a main symptom of panic disorder (Smoller et al., 1996). Population-based studies have shown a dyspnoea prevalence of 9% - 13% among community-dwelling adults (Bowden et al., 2011; Currow et al., 2009; Frostad et al., 2006). These figures rise to 25% - 37% in adults over 70 years of age (Ho et al., 2001). Dyspnoea is a strong predictor of mortality in patients with COPD and heart failure and amongst the most common causes for emergency department visits (Abidov et al., 2005; 1999). Due to its chronicity, costly long-term treatment of dyspnoea is necessary in many patients. For example, the annual direct treatment costs of COPD, the fifth biggest killer disease in the UK with over 30 000 annual deaths, were estimated at £820 per patient, with an additional £820 of secondary economic costs, accumulating to a total societal cost of over £2.1 billion (Britton, 2003). Similar figures are reported from the USA (Hoyert, 2012).

Dyspnoea itself can influence the course of disease and symptoms often persist after medical treatment options have been exhausted (Mahler et al., 2010). Dyspnoea can lead to early cessation of exercise and fear of dyspnoea might discourage patients from engaging in daily activities, resulting in reduced cardiovascular fitness and muscle strength, which again increase the feeling of dyspnoea, thus initiating and perpetuating a spiral of decline (American Thoracic Society, 1999). Patients and their family and friends endure great emotional suffering when facing their/their loved one's loss of independence and increasing physical distress (Gysels et al., 2007). Many patients, especially those who have smoked, are obese or

do not engage in regular exercise, feel that they have brought their symptoms upon themselves, which can bring on feelings of guilt and thus they are less likely to seek appropriate medical help (Plaufcan et al., 2012).

This chapter will introduce dyspnoea and some variations in perception and treatment success of dyspnoea that present challenges for dyspnoea management. Evidence from clinical and laboratory studies will be reviewed to show how emotions, especially anxiety, affect the perception of dyspnoea and how understanding the neural mechanisms behind these can aid dyspnoea management. The challenges of managing dyspnoea in older people, obese people and women, three populations particularly vulnerable to dyspnoea, will be highlighted and we will suggest how future research can help to understand the neural mechanisms involved in the perception of dyspnoea in order to better target treatment. Increased understanding of the neural mechanisms behind dyspnoea will allow us to individually address the most important aspects of dyspnoea suffering in each patient. While research on the neural mechanisms of dyspnoea is still in its infancy, it can reveal important directions for improvements of dyspnoea therapy and future research.

1.2 Perception of dyspnoea

Dyspnoea is not one experience, but encompasses a whole range of sensations (e.g. air hunger, feeling of increased effort, rapid breathing) that are highly subjective. Individual variation between patients in dyspnoea perception and resulting functional impairment is large and reaches beyond the underlying cause (Mahler et al., 1996). Treatment of the primary cause of dyspnoea is essential, but despite current best practice, patients often continue to suffer from dyspnoea and the associated decrease in quality of life (Mahler et al., 2010). In conjunction with disease-specific treatments, symptomatic treatments can help to manage

dyspnoea. To improve and optimally target these treatments, a better understanding of the mechanisms underlying dyspnoea is necessary because "one size does not fit all".

One model of dyspnoea perception describes a primary sensory component (intensity) and a primary affective component (unpleasantness) (Lansing et al., 2009). These are often correlated, but can vary independently (Banzett et al., 2008). This acute primary reaction is followed by a secondary, more cognitive affective component that leads to long-term emotional responses (suffering) that affect future dyspnoea-related behavior (Lansing et al., 2009) and will hence play a vital part in shaping the life of dyspnoea patients. Dyspnoea is a subjective experience and while animal models have given us insight into mechanisms behind specific pathologies, it is difficult to derive information about the subjective experience of dyspnoea from animal models. Much research on dyspnoea mechanisms has hence been performed in healthy individuals exposed to laboratory dyspnoea. Replicating the emotional component of dyspnoea in a laboratory environment is difficult, as laboratory dyspnoea does not cause the existential fears dyspnoea sufferers encounter in daily life. Hence, patient studies will be necessary in order to fully comprehend all aspects of dyspnoea.

1.2.1 The impact of emotions on dyspnoea processing

Based on the concept of total pain proposed by Dame Cicely Saunders in the 1960s, a model of total dyspnoea has highlighted the vicious circle in which dyspnoea affects and is affected by physical, psychological, spiritual and social aspects of a patient's life (Kamal et al., 2011). While an array of potential factors contributing to the perception of dyspnoea is presented in Figure 1.1, this chapter will focus on the impact of negative emotions, and the challenges that age, obesity and gender present to dyspnoea management and research.

An individual's emotional disposition, current mood, level of general anxiety and anticipation of dyspnoea, as well as the increased attention paid to one's respiration can all influence how dyspnoea is perceived. Depression and anxiety are two major comorbidities of respiratory disease, e.g. approximately 40% of patients with COPD suffer from depression (Norwood, 2007) and approximately 30% have comorbid anxiety disorders (Porzelius et al., 1992). Comorbid anxiety and depression during respiratory disease have been shown to correlate with doubled mortality rates, diminished social and physical functioning by approximately 25%, increased frequency of hospitalization by 10% and length of stay by approximately 3 days (Ng et al., 2007).

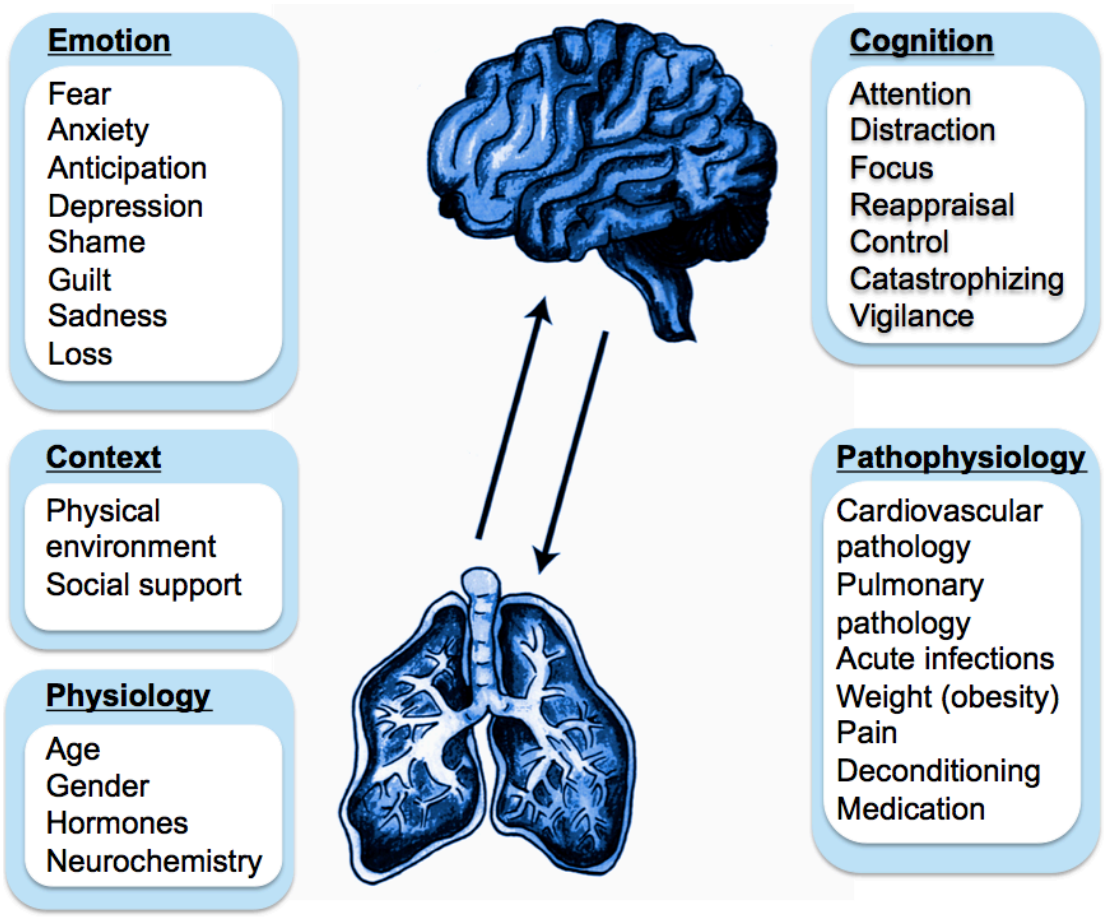


Figure 1.1: Dyspnoea is a multidimensional set of experiences that is closely linked to almost every aspect of a patient's physiological and psychological state. The brain is the main relay station integrating these complex internal and external experiences with physiological respiratory signals.

Emotions are powerful modulators of the perception of dyspnoea. This has been shown in asthmatics, where strong emotions (both positive and negative) influence pulmonary function. Positive emotions during a rollercoaster ride led to reduced dyspnoea, in spite of reduced lung function (forced expiratory volume in one second) (Rietveld and van Beest, 2007). High negative emotionality, as present in depression, was shown to increase the strength of reported dyspnoea in response to laboratory stimuli in individuals with normal lung function (Bogaerts et al., 2005) and is associated with increased symptom reporting in patients with respiratory disease (Di Marco et al., 2006), especially when sensations are ambiguous or low in intensity (1999). There is evidence that dyspnoea improvement during pulmonary rehabilitation is correlated with a decrease in experience of negative emotions (von Leupoldt et al., 2011a), but the driving force behind this interaction remains unknown.

Anxiety and dyspnoea

Despite the strong negative impact of emotions on dyspnoea perception, few studies have looked at the specific contributions of particular emotions on the perception of dyspnoea. Much research still needs to be done to fully understand the impact of emotions on the perception of dyspnoea. Section 2.1.1 will summarize knowledge about the intricate relationship between anxiety and dyspnoea. In the context of dyspnoea, we divide anxiety into general anxiety and anxiety relating directly to breathing and breathlessness (dyspnoea-specific anxiety). High general anxiety increases the perception of dyspnoea in patients (Dales et al., 1989) and is associated with decreased quality of life (Huang et al., 2013). High anxiety in turn induces maladaptive breathing that intensifies the unpleasant sensations elicited by respiratory stimuli (Alius et al., 2013), generating a positive-feedback loop. Dyspnoea-related anxiety was shown to have a mediating effect on the relationship between anxiety and dyspnoea in patients (Janssens et al., 2011) and increased perception of dyspnoea in response to respiratory stimuli in healthy volunteers, while high general anxiety did not increase

dyspnoea perception in the same experiment (Alius et al., 2013). The importance of dyspnoea-specific anxiety might explain why anxiolytics (e.g. benzodiazepines), despite having shown positive results for managing dyspnoea in individual patients, have not been successful for improving dyspnoea in clinical group trials in patients with increased general anxiety, but without measures of dyspnoea-specific anxiety (Simon et al., 2010). This indicates that patients with dyspnoea-specific anxiety might benefit more from a dyspnoea-focused treatment, like pulmonary rehabilitation, which has been shown to decrease both exercise-induced dyspnoea and dyspnoea-specific anxiety in COPD patients (Janssens et al., 2011).

Anticipatory anxiety is the anxiety felt when anticipating specific future events, so in the case of dyspnoea, it is known as acute dyspnoea-specific anxiety. Patients remember triggers of their dyspnoea, anticipate dyspnoea and alter their behaviour when encountering these situations in the future (Ley, 1999), e.g. when dyspnoea is experienced while walking up the stairs, patients will likely be wary of using the stairs and try to avoid walking upstairs in the future. An initially neutral stimulus (e.g. the stairs) begins to carry the negative associations related to dyspnoea, i.e. the patient becomes conditioned to this association. If negatively cued stimuli cannot be avoided, learnt anticipation of dyspnoea in response to a stimulus might increase the negative perception of respiratory stimuli (De Peuter et al., 2005), so patients are more likely to feel out-of breath because they know they are climbing the stairs and they expect to feel breathless. The fear of future dyspnoea and resulting avoidance of activities that might induce dyspnoea generate a positive feedback loop that drives the spiral of decline and leads to increased perception of disability at comparable pulmonary function (Yellowlees and Ruffin, 1989).

1.2.2 Dyspnoea and cognitive processing

Emotion modulates the neural processing of respiratory sensations on a higher-order level (von Leupoldt et al., 2013) and might hence interact with non-emotional mental processes, such as attention, expectation or catastrophizing, which have been found to modulate aversive experiences like pain or dyspnoea (Wiech and Tracey, 2009). Limited attentional resources are allocated according to emotional salience. Hence, if attention is used to focus on the threat of dyspnoea, lack of cognitive resources might impair performance on everyday tasks. There is evidence that high dyspnoea-specific anxiety might reduce an individual's ability to allocate attention to cognitive tasks and that this impairment is enhanced in a negative emotional context relative to a neutral emotional context (von Leupoldt et al., 2013). The consequential reduction in cognitive performance might impair a patient's quality of life. While exercise has been proven beneficial to cognitive function, it needs to be investigated whether this effect stretches to dyspnoeic patients.

The allocation of attention plays an important role in treatment. During exacerbations of their conditions, patients with lung disorders are often told to focus on their breathing. Laboratory evidence has shown that interoceptive self-awareness does decrease strength of perceived dyspnoea (Petersen and Ritz, 2011), but that this positive cognitive manipulation is susceptible to interruptions by negative emotions (Petersen and Ritz, 2011). This makes these maneuvers more challenging for dyspnoea patients with comorbid depression and/or anxiety disorders. In apparent contradiction to the positive effects of interoceptive self-awareness, external distraction has been shown to alleviate the unpleasantness of dyspnoea during exercise (von Leupoldt et al., 2007) and could therefore be used to improve exercise performance. Distraction from pain was shown to decrease brain activity in areas commonly implicated in the processing of pain intensity, whilst simultaneously increasing activity in the frontal cortex and the cingulate cortex (Bantick et al., 2002). Similar mechanisms could be

employed during distraction from dyspnoea. Understanding the important role of attention in the modulation of dyspnoea could lead to relatively straightforward and cost-effective interventions that may help relieve dyspnoea.

1.3 Challenges for dyspnoea management in specific sub-groups

1.3.1 Aging

Besides being affected by psychological state, dyspnoea perception can be shaped by an individual's particular life situation (e.g. context). A growing population of patients suffering from chronic dyspnoea are elderly, with approximately a third of elderly people (over 75) at home being affected by dyspnoea (Ho et al., 2001). Comorbid medical conditions, low fitness levels, and/or functional limitations in older adults (Nelson et al., 2007) make this population especially vulnerable to deconditioning and deterioration. Additionally, the onset of chronic dyspnoea is gradual and in older patients it is often disguised as a normal part of aging. Mistaking dyspnoea due to respiratory disease as a normal sign of aging delays diagnosis of underlying medical conditions and start of treatment. Increasing public awareness and patient education are vital to encourage elderly patients to see dyspnoea as potentially serious and to present early with dyspnoea symptoms. Understanding the neural processes leading to particular dyspnoea sensations has the potential to improve its management in elderly patients.

1.3.2 Obesity

Improving the management of dyspnoea in an aging Western population with an unhealthy diet, increasingly sedentary lifestyle and growing rates of obesity is of vital importance. In 2008, the World Health Organization estimated that 1.4 billion adults were overweight, including 200 million men and 300 million women who were obese (World Health

Organization, 2013). Dyspnoea is strongly associated with body mass index (BMI) and decreased physical activity (Gerlach et al., 2013). Obesity mechanically affects respiration by decreasing tidal volume and residual capacity (Weiss, 2005). It also leads to altered hormone levels (e.g. increased estrogen), but whether and how obesity-induced hormonal changes increase dyspnoea is not yet understood (Weiss, 2005). There is a strong association between asthma and obesity and losing weight has been shown to improve asthma (Weiss, 2005). Additional comorbidities (e.g. joint pain) makes obese dyspnoea patients especially prone to deconditioning and there is evidence indicating that obese individuals more frequently experience negative emotions (Pasco et al., 2013), which could further increase dyspnoea perception and suffering. A holistic view towards improving both dyspnoea and obesity are vital for optimum long-term patient care. Neuroimaging could be a useful tool to understand the role the burdens of obesity (e.g. deconditioning, altered hormone levels and negative emotions) play in modulating dyspnoea with a view of better targeting treatments.

1.3.3 Sex differences

Respiratory disease affects both men and women (de Marco et al., 2000; Mannino, 2002; Melgert et al., 2007; Naberan et al., 2012), but there is evidence for emotions playing a bigger role in dyspnoea perception in women. Female COPD patients are twice as likely to suffer from comorbid anxiety and depression (Laurin et al., 2007). The perception of dyspnoea in women with respiratory disease seems to be more strongly influenced by socio-emotional factors and less related to physiological measures compared to men (de Torres et al., 2007). Women with dyspnoea also perceive poorer quality of life compared to men with the same level of dyspnoea (Di Marco et al., 2006; Naberan et al., 2012). Women respond more emotionally (e.g. anger towards self) to their dyspnoea than men, which is associated with lower general wellbeing (Ketelaars et al., 1996) and might amplify the negative consequences of dyspnoea in women (Ninot et al., 2006).

It is currently not clear whether gender differences in dyspnoea perception arise from differences in physiology, hormonal make-up, social and cultural experiences and/or emotional processing strategies. Neuroimaging studies of dyspnoea have not (yet) investigated gender differences in the neural mechanisms of its perception, but there is evidence that men and women use different neural pathways when processing emotions (Weisenbach et al., 2014). Inconclusive results of behavioural and neuroimaging studies investigating responses to complex multifactorial stimuli (e.g. pain) might indicate that so-called 'gender effects' on the perception of these stimuli might be due to variations in other biopsychological factors that often vary with gender (Racine et al., 2012).

Sex hormones

The effects of sex hormones on respiration, emotion and pain perception make it likely that differences in dyspnoea perception between men and women might be related to differences in sex hormone levels. The three major sex hormones (estrogen, progesterone, and testosterone) have been shown to act at sites throughout the body, including the central nervous system (Aloisi and Bonifazi, 2006) and the cardiovascular (Traupe et al., 2007) system. Testosterone, while present at low levels in women, is predominant in men. The effects of testosterone on respiration are complex (increased ventilation response to hypoxia (Saaresranta and Polo, 2002) and worsening of obstructive sleep apnoea (Schneider et al., 1986)). Testosterone elevates mood (Douma et al., 2005), has been found to decrease during stress, including anticipatory stress, and to increase pain perception (Choi et al., 2012), but its effects on dyspnoea have not been investigated.

Progesterone and estrogen are predominant in women, with low concentrations in men. During the menstrual cycle, progesterone levels are low during the first half of the cycle, and then increase, peaking in the middle of the luteal phase (Vincent and Tracey, 2010).

Progesterone has been shown to stimulate respiration (Saaresranta and Polo, 2002) and elevate mood (Douma et al., 2005). Estrogen peaks during ovulation and is increased during the luteal phase of the cycle (Vincent and Tracey, 2010). Estrogen increases progesterone-induced respiratory stimulation (Behan et al., 2003) and elevates mood (Douma et al., 2005). These strong variations in estrogen and progesterone levels throughout a woman's cycle will likely affect her respiration, mood and perception of dyspnoea. The neural effects of these hormones on dyspnoea perception have not been shown, but evidence from pain studies suggests that increases in estrogen and progesterone seem to be associated with enhancement of the affective component of the pain experience, but the neural mechanisms are not yet understood (Vincent and Tracey, 2010).

Pregnancy and menopause

Pregnancy and menopause are two important life events in women that are often associated with dyspnoea. Over 60% of healthy pregnant women are affected by dyspnoea (Tenholder and South-Paul, 1989). During pregnancy, the body changes: the diaphragm is elevated, metabolism increases, levels of estrogen and progesterone increase and ventilation and respiratory drive increase. Mood changes, fatigue and stress associated with the pregnancy make pregnancy an emotionally challenging time for many women. When a pregnant woman complains of dyspnoea, the clinician should determine whether her symptoms are due to an underlying disease or present a normal physiologic response to pregnancy. If no pathology is found, cognitive reappraisal of dyspnoea from a life-threatening warning sign to a normal part of the physical and emotional burden of pregnancy decreases suffering. Understanding the neuronal mechanisms behind this reappraisal might inform the study of the brain mechanisms behind the emotional threat in more chronic dyspnoea.

Dyspnoea was found to have a prevalence of 20% in menopausal women in the UK (Ward et al., 2010). During menopause, estrogen and progesterone decrease, which might negatively affect mood (Douma et al., 2005) lead to increased anxiety and could in turn increase dyspnoea perception. Additionally, pharmacological treatment of hot flushes and emotional symptoms occurring during menopause might in itself induce dyspnoea. Being aware of hormonal changes, medications and their interactions and potential side-effects is vital for managing dyspnoea in menopausal women. Addressing how hormonal changes influence brain chemistry and emotional processing in the limbic system might help to better manage dyspnoea during menopause in future.

1.4 Neuroimaging as a tool to understand dyspnoea

Neuroimaging research has confirmed the important role of emotional processing in the perception of dyspnoea. There is evidence for a common emotion-related human brain network that underlies the perception of aversive bodily sensations such as dyspnoea and pain (von Leupoldt et al., 2009a).When respiratory stimuli are applied to healthy volunteers in a non-threatening laboratory context, they primarily activate somatomotor areas, but when laboratory dyspnoea is evoked, the limbic system, an array of brain structures involved in emotional processing, and paralimbic areas, like the insula (implicated in interoception, emotion and cognition), are activated (Herigstad et al., 2011a). This research hints at different neuronal networks being responsible for the different aspects of dyspnoea perception, but future research is necessary to disentangle the neuronal processes implicated in the sensory and affective processing of chronic dyspnoea.

Neuroimaging can be used to identify specific neuronal mechanisms of respiratory disease that can help us understand the processing of dyspnoea in particular conditions. Evidence

from functional magnetic resonance imaging (FMRI) suggests that emotional processing might be altered in asthma. The anterior cingulate cortex and the insula, both implicated in the regulation of the sensitivity of the response to respiratory stimuli (Rosenkranz and Davidson, 2009), might be hyperresponsive in individuals with asthma and other stress-related conditions (Rosenkranz et al., 2005), leading to a perception of increased severity of respiratory stimuli, increased use of medication (Main et al., 2003) and increased frequency of emergency hospital visits. A weak perception of dyspnoea in asthmatics, as e.g. occurs when positive emotion is experienced, can lead to a dangerous lack of necessary treatment (Rietveld and van Beest, 2007). Furthermore, dyspnoea perception might vary between patients and within the same patient at different times, which might have severe consequences. Neuroimaging might be able to provide a biological marker for the subjective perception of dyspnoea. A better understanding of the neuronal mechanisms involved in the multidimensional perception of respiratory symptoms can highlight particularly vulnerable patients and dangerous situations in order to minimize the risks associated with over- and under-treatment.

Neuroimaging could also be a useful tool to examine the link between dyspnoea and dyspnoea-specific anticipatory anxiety. Brain activation during anticipation of dyspnoea was found to correlate with brain activation during the perception of dyspnoea during blood oxygen-level dependent (BOLD) FMRI in elite athletes and healthy controls whilst performing a cognitive task (Paulus et al., 2012). Anticipation of pain has further shown that brain activation during anticipation can alter brain activity during pain perception and can amplify pain perception (Fairhurst et al., 2007). The power of anticipation to amplify the subsequent experience makes understanding the negative impact of acute dyspnoea-specific anxiety vital for introducing targeted treatments to break the spiral of decline and to increase quality of life in affected patients. FMRI could be used to provide neurobiological markers for

potential anxiolytic effects. Drug intervention studies suggest that the medial prefrontal cortex, an area central to conscious threat appraisal, might be a useful target area for dyspnoea and anxiety interactions (Evans et al., 2009; Sripada et al., 2013), but this needs to be investigated.

1.5 Conclusion

In this chapter, we have shown that dyspnoea is a complex, subjective sensation and that its successful treatment depends upon viewing dyspnoea as a composite, assessing psychological comorbidities, hormonal changes and mood changes, as well as a potential underlying respiratory cause. Future research should aim to improve our knowledge of the mechanisms involved in dyspnoea processing. The effects of dyspnoea-specific anticipation and anxiety could be of particular interest because of their detrimental contribution to patient suffering. Dyspnoea occurs in vastly different populations, e.g. elderly COPD patients with chronic dyspnoea vs. young mothers experiencing temporary dyspnoea during pregnancy. Integrating behavioural measures and carefully designed neuroimaging protocols will allow us to clearly define intricacies of the neural pathways and spatial organization of the respiratory network involved in dyspnoea perception and its modulation. Accurate diagnosis and a greater awareness and understanding of influencing factors can facilitate targeted treatments of dyspnoea and might subsequently dramatically improve clinical outcomes.

Chapter 2

The induction of dyspnoea in healthy volunteers during FMRI

Abstract Chapter 2

Dyspnoea perception relies on the complex moment-to-moment integration of physiological and psychological factors. Accurate assessment of the different emotional and sensory components of dyspnoea is essential for the study of the complex nature of dyspnoea. Dyspnoea can be induced under controlled laboratory conditions in a homogenous sample of healthy volunteers. The study of dyspnoea with FMRI requires use of a reliable dyspnoea stimulus that does not in itself induce systematic changes in FMRI signal that could mask signal of interest. This chapter will discuss some of the issues associated with studying the perception of dyspnoea in humans. It will give a brief introduction to the dimensions of dyspnoea and the complexities of measuring dyspnoea via self-report. It will then briefly discuss how FMRI could help to advance our understanding of dyspnoea. Finally, it will give an overview of laboratory stimuli that are commonly used to induce dyspnoea in healthy volunteers and will discuss their applicability to the study of dyspnoea with FMRI. The outcomes of neuroimaging studies previously using these dyspnoea stimuli are reviewed. Lastly, the need for rigorous methodological developments is discussed in order to create a valid and reliable stimulus for the study of neural mechanisms of dyspnoea with FMRI.

2.1 Studying the perception of dyspnoea

Dyspnoea is a complex symptom that arises from a multitude of physiological and psychological factors. Feedback mechanisms from chest wall and respiratory muscle mechanoreceptors, CO_2 sensitive chemoreceptors and corollary discharge from the brainstem to the sensory cortex seem to play an important role in the generation of dyspnoea (Gigliotti, 2010). These physiological signals do not correlate directly with dyspnoea perception and individuals with apparently comparable levels of lung disease report varying levels of respiratory distress (Lareau et al., 1999; O'Donnell and Webb, 1992). Dyspnoea even occurs without any form of cardiorespiratory pathology in patients suffering from idiopathic hyperventilation (Smith et al., 2009) and panic disorder (Parshall et al., 2012b). The brain might hold the key to understanding the dissociation between cardiopulmonary pathophysiology and perception of dyspnoea, as it is where peripheral, psychological and environmental signals are integrated to shape the experience of dyspnoea. Understanding these underlying neuronal mechanisms might provide the key towards improving dyspnoea treatments and is hence vitally important.

This chapter discusses three aspects important to the study of dyspnoea. Firstly, it introduces the complexities associated with assessing dyspnoea using self-report measures. It then briefly introduces how FMRI, a non-invasive whole-brain imaging technique, allows the investigation of brain processes associated with the perception of dyspnoea. Lastly, it presents some of the most common techniques used to induce laboratory dyspnoea in healthy volunteers and discusses their applicability to the study of the perception of dyspnoea with FMRI.

2.2 Measuring the perception of dyspnoea

As a subjective experience that encompasses a wealth of highly threatening physical and emotional sensations, assessment of dyspnoea is reliant on symptom recall, which can be captured with various scales and questionnaires. Symptom recall is complex, due to limits of memory performance and variations in strategies used for recall (Broderick et al., 2006). The reliability and validity of recalled dyspnoea depend on the specificity of the instructions, the particular dimensions of dyspnoea participants are asked to rate, the time-points/periods the ratings referr to and the comparison standard, e.g. some undefined 'usual state' (Meek et al., 2001). Even recall immediately after a dyspnoea experience was shown to predominantly reflect sensations at the peak of dyspnoea intensity and at the end of dyspnoea application (Bogaerts et al., 2012), which highlights the complexity of 'accurate' dyspnoea assessment.

Unlike for the description of pain, words used to describe aspects of the sensation of dyspnoea are not part of common everyday language. Descriptors of pain are common and easy to relate to, e.g. burning, sharp or crushing. Dyspnoea on the other hand is disease specific and not generally experienced by healthy individuals except during occasional hard exercise. This makes it hard for patients to describe their experiences to healthy individuals, e.g. their family or clinician (Kjellstrom and van der Wal, 2013) and makes it very difficult to compare the sensation of dyspnoea between different individuals, especially between patients and experimental models in healthy volunteers.

Being very specific about the exact dimension to be evaluated and simultaneously assessing multiple dimensions of dyspnoea allows the meaningful measure of these difficult sensations. The increase in information gained by using multi-item scales is accompanied by a necessary 'cognitization' of the experience, in which each individual dimension needs to be separated out and evaluated independently. This makes these measures a difficult and time consuming

task, especially for dyspnoea-naive participants undergoing respiratory challenges in the laboratory.

2.2.1 Unidimensional scales

Many different unidimensional self-report scales can be used to quantify the strength of dyspnoea perceived by an individual patient at a particular point in time. Amongst the most commonly used scales are the modified Borg scale (Kendrick et al., 2000; Weingartner et al., 2013), the visual analogue scale (VAS) (Gift, 1989), other Likert-type scales (Guyatt et al., 1987) and numerical rating scales (Gift and Narsavage, 1998). In comparison with the Borg scale and the Likert scale, the VAS has been named to be the most sensitive scale to assess dyspnoea in patients during hospitalization (Kjellstrom and van der Wal, 2013) and might hence be most appropriate to capture the subtle differences evaluated during laboratory research of dyspnoea. As these unidimensional scales only provide one composite measure of dyspnoea, instructions need to be clear about which aspect(s) of dyspnoea to rate (e.g. sensory intensity of dyspnoea or distress associated with dyspnoea) and need to be consistently given, otherwise the exact measure obtained via a unidimensional scales is ambiguous (Clark et al., 2002b). Generalized unidimensional scales are hence difficult to interpret when comparing dyspnoea between different individuals and in different situations, e.g. when comparing dyspnoea induced in a laboratory setting to dyspnoea experienced by patients in the emergency room.

2.2.2 Sensory and affective components of dyspnoea

The perception of dyspnoea is often reported on two different dimensions, a sensory dimension and an affective dimension. Separating dyspnoea into sensory intensity (intensity, measuring the perceived strength of a stimulus) and affective intensity (unpleasantness, measuring how discomforting a stimulus is perceived to be) allows a more complex

assessment of dyspnoea (Lansing et al., 2009). This separation, which may be over-simplistic, might be clinically relevant as it is usually not the sensory intensity, but the accompanying affective unpleasantness that most strongly reduces quality of life for patients (von Leupoldt et al., 2010) and makes them seek treatment. Intensity and unpleasantness can vary intra-individually at a given stimulus strength in healthy volunteers (Banzett et al., 2008) and in patients (Carrieri-Kohlman et al., 2001), which makes it likely that they are produced by independent neuronal mechanisms (Davenport and Vovk, 2009). Unidimensional scales can be adapted to measure either the intensity or the unpleasantness of dyspnoea by varying the instructions given to individuals and the anchors provided on the scales. This separation of sensory and affective components allows the comparison of environments in which a composite rating of dyspnoea would capture either predominantly sensory intensity or unpleasantness according to situational influences, e.g. when comparing scores biased towards intensity of strong, but safe laboratory stimuli versus the emotional real-life threat of dyspnoea (Lansing et al., 2009).

2.2.3 The multidimensionality of dyspnoea

In addition to separating dyspnoea into affective and sensory components, qualitatively different dimensions of dyspnoea can be distinguished. A popular model of dyspnoea separates dyspnoea into three distinct sensations, based on distinct afferent sources (Lansing et al., 2009): air hunger, work/effort and chest tightness (American Thoracic Society, 1999; Binks et al., 2002; Elliott et al., 1991; Lansing et al., 2000; Simon et al., 1990). These distinct sensations have partly been linked to specific diseases. Patients with asthma for example, preferred the terms chest tightness and work/effort to describe their dyspnoea (Killian et al., 2000; Mahler et al., 1996; Simon et al., 1990), whilst patients with COPD preferred descriptors relating to respiratory work/effort (Mahler and Harver, 2000; Mahler et al., 1996; O'Donnell et al., 1997; Simon et al., 1990). Several studies provide information on the

relationship between quality of dyspnoea and the underlying physiological mechanisms producing discomfort. The sensation of air hunger seems to be associated with increased respiratory drive, particularly in the presence of hypoxia or hypercapnia, and is hence likely to be associated with the stimulation of chemoreceptors (Gandevia et al., 1993). The sensation of work/effort increases when respiratory muscle load is increased (el-Manshawi et al., 1986), when respiratory muscles are weakened by fatigue (Gandevia et al., 1981) or during an increase in lung volume (O'Donnell et al., 2000). To ensure adequate ventilation in response to increasing mechanical load on the respiratory system, the central respiratory motor command has to be increased, which could be associated with the generation of work/effort sensations of dyspnoea. The sensation of chest tightness seems to be associated with bronchoconstriction since asthmatic patients frequently describe their symptoms as a sense of chest tightness or constriction (Moy et al., 2000). The results of induced bronchoconstriction in asthmatic patients showed that the sensation of tightness does not arise from the work of breathing, suggesting that it does not depend on respiratory muscle afferents, but on stimulation of airway receptors such as rapidly adapting stretch receptors (RARs) and C-fiber receptors (Binks et al., 2002). It is likely that simultaneous stimulations of different types of receptors interact and causes qualitatively and quantitatively different sensations of dyspnoea (Nishino et al., 2007).

Currently, there exist a large number of scales to measure the severity of clinical dyspnoea, but few are commonly used in clinical practice. One of the most frequently used scales in everyday clinical practice is the Medical Research Council (MRC) breathlessness scale. The MRC breathlessness scale is a five-item questionnaire that provides a measure of respiratory disability in a few seconds (Fletcher et al., 1959). It gives a rudimentary overview of dyspnoea severity by classifying dyspnoea severity into one of five different levels. More detailed evaluation of dyspnoea can be performed with the Baseline Dyspnea Index (BDI),

which measures dyspnoea at rest, and the Transition Dyspnea Index (TDI), which evaluates changes from baseline as established with the BDI. The BDI/TDI provide interview-based or self-administered computer-based measurements of breathlessness related to activities of daily living (Mahler et al., 1984). Each scale scores functional impairment, magnitude of task and magnitude of effort on 24 items. The BDI/TDI gives a more accurate categorisation of a patients' dyspnoea, both at rest and during activities of daily living, but it is more time-consuming to administer and less widely used. Whilst these and other clinical questionnaires give a picture of the impact of dyspnoea on daily life, the assessment of dyspnoea in these questionnaires is tailored to use everyday situations in which non-symptomatic individuals would score the minimum. The resulting 'floor-effect' makes these questionnaires unsuitable for use in research studies involving healthy individuals.

Two 12-item questionnaires, that might be useful for the study of healthy volunteers, have recently been developed. The Dyspnoea-12 (Yorke et al., 2010) provides a non-disease specific global score of dyspnoea severity that incorporates sensory and affective aspects of dyspnoea. The score provides a general measure of dyspnoea 'these days'. The instructions could potentially be targeted towards the perception of laboratory dyspnoea, although this has not been validated. The second questionnaire, the Multidimensional Dyspnea Profile (MDP) (Meek et al., 2012) evaluates the multidimensionality of dyspnoea. It determines overall sensory intensity and affective intensity (unpleasantness) of dyspnoea and additionally assesses five sub-dimensions in each category, which gives an overview of the nature of the different dimensions of dyspnoea that are experienced. The MDP has been used in a clinical environment (Parshall et al., 2012a) and to distinguish different qualities of stimulus-induced laboratory dyspnoea in healthy volunteers and COPD patients (Banzett et al., 2011; O'Donnell et al., 2013). The studies presented in Chapter 3 and Chapter 4 of this thesis use a development version of the MDP, which was obtained from the developers of the scale and is

presented in Appendix Ia. This version of the MDP solely measures the six sensory dimensions of dyspnoea. The study presented in Chapter 5 uses the official version of the MDP that includes both sensory and affective components of dyspnoea ((Meek et al., 2012) see Appendix Ib).

2.2.4 Use of self-report measures to assess laboratory dyspnoea

There is a tradeoff between capturing the multidimensionality of dyspnoea in an elaborate questionnaire versus scoring a composite measure of dyspnoea immediately during or after the experience. Whilst unidimensional scales are quick and easy to administer, they rely on accurate instructions and might show poor comparability, e.g. the high intensity of a strong laboratory stimulus could give the same rating as the high unpleasantness of less intense, but more threatening exertional dyspnoea in a patient. More complex measures of dyspnoea will provide information that allows the assessment of different dyspnoea dimensions, but completion is more time consuming. When time is a limiting factor (e.g. during FMRI), unidimensional scales might be used to capture dyspnoea components of interest during or immediately after the experience of dyspnoea. These can be followed by a more thorough evaluation of the different sensory and affective components of dyspnoea after the experimental session is completed.

2.3 Using functional magnetic resonance imaging to study dyspnoea in healthy volunteers

FMRI can help to better understand the intricate relationships between different factors involved in the generation of dyspnoea. This could help to create targeted therapies and might allow the development of novel dyspnoea treatments, e.g. pharmacologic agents that attenuate

anxiety. FMRI might provide biomarkers for dyspnoea that could supplement subjective patient self-report and could be used to evaluate the efficacy of dyspnoea treatments more objectively.

Aspects of the perception of dyspnoea can be investigated in individuals with normal lung function by using experimental dyspnoea stimuli in a laboratory. This allows the researcher to test specific constructs using especially designed models that would otherwise be too complex to disentangle in real-life situations in patients. The researcher has full control over timing, context and strength of respiratory manipulations to elicit the desired sensory and emotional effects and can apply these stimuli to a homogenous population of healthy volunteers without confounds of disease, aging and medication. Healthy volunteers are readily available, tolerate FMRI and respiratory manipulations and can be selected to bear particular psychological traits of interest. Studies using healthy volunteers can be run alongside patient studies to disentangle noisy data from patients confounded by disease and comorbidities. Healthy volunteer models can also generate new hypotheses that can then be tested in patients.

While a few FMRI studies have scanned patients with asthma (Rosenkranz et al., 2005; Rosenkranz et al., 2012; von Leupoldt et al., 2009b) and idiopathic hyperventilation (Jack et al., 2010), patients with dyspnoea are a difficult population to examine with FMRI. Practical concerns like the tolerance of controlled respiratory manipulations in dyspnoea patients and the supine position necessary for MRI that often increases the sensation of dyspnoea need to be taken into account. The interpretation of FMRI data might be complicated by variations in underlying pathology, presence of comorbidities and the effects of necessary medications. As such, healthy volunteer models may remain our best bet to disentangle some of the finer details of dyspnoea processing.

2.3.1 Translation of information gained from dyspnoea models to patients

When wanting to translate findings from the laboratory to patients with dyspnoea, it is important to account for the influences of potential confounds, e.g. age, smoking history and psychological variables (e.g. anxiety and depression). Additionally, the use of volunteer models assumes that basic processing of respiratory stimuli works with the same principles in healthy participants and in patients suffering from dyspnoea and would hence be difficult to interpret if neural processing was significantly altered in patients (which is currently unknown). Care hence needs to be taken when translating findings from healthy volunteer studies into a clinical environment.

Ideally, healthy volunteer studies are run in a translational setting where patient studies can inform volunteer studies that can examine constructs in more detail and then feed back into the design of new patient studies. Each experimental model of dyspnoea is specifically designed to look at particular aspects of dyspnoea, as each laboratory stimulus has the ability to evoke distinct dyspnoea sensations. As the questions asked by neuroimaging studies become more complex, accurate replication of various sensory and emotional components of dyspnoea gain importance and future dyspnoea models will need to be targeted to answer specific questions. Modulations like hypercapnia and respiratory loading allow us to study certain aspects of dyspnoea and have been used to gain insights into the sensory aspects of dyspnoea processing. However to disentangle the mechanisms involved in processing of the affective components of dyspnoea, a more realistic dyspnoea experience is necessary. Some of the emotions of dyspnoea might only be mimicked when the sensory experience is complex, uncertain/unknown and threatening. Controlled laboratory dyspnoea seems fundamentally different from the threatening dyspnoea experienced by patients during acute exacerbations, but might be closer to replicating the dyspnoea patients tolerate on a day-to-day level when performing activities they know will evoke dyspnoea. A recent study

(O'Donnell et al., 2013) showed similar affective responses (anger, frustration and fear) to moderate and strong laboratory stimuli (hypercapnia and ventilator restriction) in patients with COPD and healthy volunteers. The strength of affective responses was similar to that experienced by patients during daily activities, which shows the ability of laboratory stimuli to induce affective components of moderate dyspnoea similar to those experienced by patients during daily life.

2.3.2 Introduction to magnetic resonance imaging

A variety of imaging techniques can be used to visualize the structure and function of the brain in health and disease. Since 1918, X-ray images in conjunction with air injections were used to visualize the ventricles and compartments for cerebrospinal fluid. The invention of X-ray computerized axial tomography (CAT) in the early 1970s was closely followed by the introduction of positron emission tomography (PET), which uses radioactive tracers in metabolically relevant molecules to visualize brain activity. The introduction of MRI in the 1980s allowed the use of magnetism to show brain structure. Soon, it was detected that this technique could also be used to look at blood-flow changes to indicate brain function. Due to its non-invasive nature, FMRI quickly gained in popularity and is now the most widely available imaging modality. Despite the benefits of being radiation-free, non-invasive and widely available, MRI is relatively expensive to run and needs installation in a specific non-magnetic suite, which makes it unsuitable for bedside investigations. The population that can safely be scanned is limited to people who are not pregnant, have no pacemakers or other ferrous or electronic objects in their body and can lie still in a supine position for the time of the scan (usually 30-60 minutes).

2.3.3 Obtaining contrasts during FMRI

The primary contrast mechanisms used in FMRI are the blood oxygenation level dependent (BOLD) contrast and the arterial spin labelling (ASL) contrast. BOLD signal is the result of a complex interaction between changes in cerebral blood flow (CBF), blood volume and oxygenation in response to neural activation. It derives its signal from a decrease in deoxyhemoglobin in response to increases in CBF ((Detre and Wang, 2002) see upper panel of Figure 2.1). During activation, increased local blood flow, in excess of neuronal oxidative requirements, results in a regionally increased ratio of oxygenated to deoxygenated hemoglobin. Oxyhemoglobin and deoxyhemoglobin have different magnetic properties that influence the magnetic susceptibility of surrounding tissues. As oxygenated blood flow increases from baseline, and thus the ratio of oxygenated to deoxygenated hemoglobin, BOLD shows a relative increase in oxygenated hemoglobin associated with regional brain activation in response to a specific task/stimulus. This change is a relative measure as it varies between different brain regions within the same participant at different times (Detre and Wang, 2002). ASL uses blood as an endogenous tracer to measure perfusion changes, both qualitatively and quantitatively (Detre and Wang, 2002). A volume of blood in the arterial tree is 'tagged' by inverting the water spins in the blood prior to their arrival in the slice of interest (Jezzard et al., 2001). The tagged blood spins then continue to the slice of interest, where they exchange with tissue water spins (Jezzard et al., 2001). By comparing the image thus obtained with one in which the arterial blood is not inverted, subtle changes in the signal intensity of the tissue image can be detected and related to blood perfusion (Jezzard et al., 2001). During ASL, signal from changes in perfusion are more uniformly distributed in the brain, whereas changes acquired with BOLD signal are tied to the veins and venules ((Duong et al., 2002) see Figure 2.1).

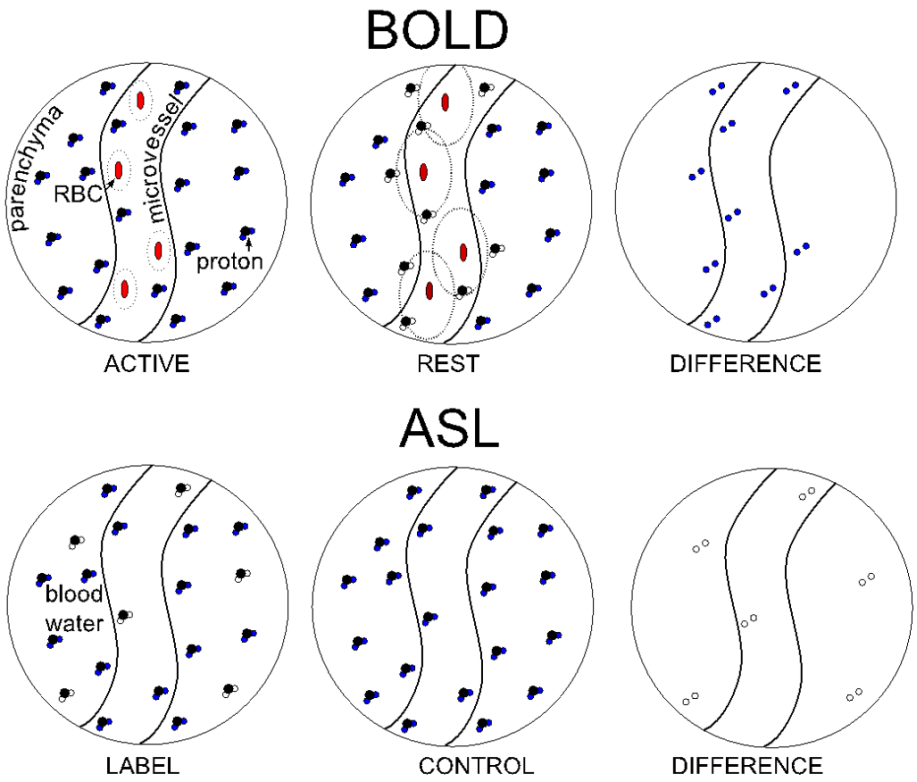


Figure 2.1: Schematic illustration of the mechanisms behind BOLD (top panel) and ASL perfusion (bottom panel) contrast. Each circular section shows brain parenchyma with a vessel. Water molecules with mobile protons are distributed in the vessel and parenchyma. Top panel: During activation, vessels contain mainly oxygenated blood. Red blood cells are highly oxygenated and have a small magnetic field around them. During rest, blood flow to a certain section of brain tissue is reduced and the blood is relatively deoxygenated, which spoils signal from nearby protons (indicated by white colour). The difference image show how a typical BOLD contrast is skewed towards the activity close to the microvessel. Bottom panel: During ASL, protons are labeled in a plane away from where the image is obtained and are allowed to perfuse into the imaging plane. These labeled protons do not contribute to the image (indicated by white colour). A second image is taken without any prior labeling. The difference image shows the even distribution of signal from diffusible water molecules throughout the vessel and surrounding tissue. RBC=red blood cell. Figure adapted from (Detre and Wang, 2002).

At present, our understanding of the brain mechanisms of dyspnoea is based largely on studies employing short respiratory stimuli during BOLD FMRI. While BOLD studies are immensely insightful towards guiding our understanding of the complexity of dyspnoea, the relation of these regions and their dynamic interactions to chronic dyspnoea is not known. The investigation of brain mechanisms relating to dyspnoea stimuli longer than a few seconds is beyond the capabilities of BOLD FMRI. This is due to the indirect nature of the BOLD

signal and the complex non-linear temporal dynamics of CBF, blood volume and oxygen consumption (Miller et al., 2001). As a direct measure of blood flow, ASL benefits from the ability to provide a quantitative measure of perfusion, lower intersubject variability and is sensitive to longer stimulus blocks than BOLD (Detre and Wang, 2002). ASL is also less prone to susceptibility artifacts, motion artifacts and baseline shifts that present major issues when analyzing BOLD contrasts (Detre and Wang, 2002). On the other hand, ASL has a lower signal to noise ratio than BOLD, which requires a higher number of stimulus repetitions and/or more participants, hence making ASL studies more expensive.

2.4 Controlled induction of dyspnoea in the laboratory and application to FMRI

Healthy individuals can experience dyspnoea in different situations, for example during breath holding, at high altitude, during heavy exercise or when experiencing strong anxiety or panic. A variety of voluntary and external challenges can been used to change chemical or mechanical respiratory characteristics resulting in the induction of aspects of the perception of dyspnoea in individuals with normal lung function. The final part of this chapter will present the laboratory stimuli that are most commonly applied to induce dyspnoea, particularly air hunger and work/effort, in participants with normal lung function. We propose three novel criteria that a physiological stimulus used to study the neural mechanisms of dyspnoea during FMRI should fulfill and we evaluate physiological stimuli according to these criteria. Firstly, we suggest that the stimulus must allow for the controlled induction of valid components of dyspnoea, as assessed with self-report measures. Secondly, we recommend that the temporal dynamics of the induced dyspnoea experience should be known in order to account for perceptual changes over time. Thirdly, we think that stimulus-induced systematic changes that

could alter FMRI signal need to be known, so that these can be accounted for during FMRI analysis.

2.4.1 Air hunger

Air hunger is thought to arise from a mismatch between ventilatory drive and ventilation. Ventilatory drive can be increased by decreasing arterial oxygen or by increasing arterial CO_2. An additional restriction of ventilation enhances sensations of air hunger. Hypoxia is a reduction of inspired oxygen that leads to a reduction in end-tidal oxygen and decreased arterial oxygenation. Hypercapnia is an increase in end-tidal CO_2 associated with increased arterial CO_2. Both hypoxia and hypercapnia increase ventilation and whilst both are potent inductors of air hunger (Moosavi et al., 2003), hypercapnia is physiologically safer than hypoxia, as it does not bear the risk of compromising the body's tissue oxygenation. Hypoxia has been used to investigate dyspnoea in the laboratory (Moosavi et al., 2004) and hypoxia has been induced during FMRI (Macey et al., 2005), but the risk associated with hypoxia and the effects of changing oxygenation during FMRI make hypoxia a model for investigation of acute dyspnoea during FMRI that needs to be approached with care.

Hypercapnia is safer than hypoxia and more commonly used to induce air hunger to study dyspnoea in the laboratory (Banzett et al., 2011; Lansing et al., 2000). Stable hypercapnia can be induced by adding CO_2 to the inspired air or by facilitating partial rebreathing of expired air higher in CO_2 from a reservoir. When additional inspiratory CO_2 is given, the amount can either be determined as a fixed percentage of the inspired gas mixture (e.g. a mild challenge of 5% CO_2 in air (Wise et al., 2007b) or a strong challenge of 35% CO_2 in air as is often used to induce panic attacks in panic disorder patients (Perna et al., 2004)) or the amount of CO_2 added to the inspired air can be individually titrated to increase partial pressure of end-tidal CO_2 ($PETCO_2$) by a certain amount from resting $PETCO_2$ (Wise et al., 2007b). Through

ongoing computer-controlled gas manipulation (dynamic end-tidal forcing (Wise et al., 2007b)) or manual breath-by-breath modulation by an experimenter (Olin et al., 2012), $PETCO_2$ can be kept relatively stable. Rebreathing of a certain amount of expired air from a reservoir can also be used to increase $PETCO_2$ (Jensen et al., 2011), which can be kept stable by varying the amount of additional fresh air available (Banzett et al., 2000).

Hypercapnia has been used in various laboratory studies to investigate dyspnoea. Air hunger induced by strong hypercapnia (+3.3kPa above baseline) for approximately 3 minutes during PET showed strong activation in the brainstem, cerebellum and in limbic and paralimbic sub-cortical and cortical areas. These include the amygdala, insula, cingulate cortex, hippocampus and parahippocampal gyrus, prefrontal cortex, middle temporal cortex, hypothalamus and thalamus (Brannan et al., 2001; Liotti et al., 2001; Parsons et al., 2001). Hypercapnia on its own has not been used to study dyspnoea during FMRI. This might be due to the strong dampening effects increased CO_2 has on the BOLD response. Using hypercapnia of sufficient strength to induce dyspnoea might hence make the associated BOLD response uninterpretable. Additionally, air hunger in response to CO_2 builds up slowly over a few breaths and is hence ill-suited for a FMRI block design in which an on-off effect is desired.

Two dyspnoea models induce air hunger through a combination of hypercapnia and restricted ventilation, which produces higher air hunger ratings than would be obtained during a given level of hypercapnia on its own. One model induces air hunger by combining hypercapnia with restricted ventilation at 0.13 liters/min/kg (Banzett et al., 2011). Ventilation is reduced by limiting airflow into a reservoir bag. Participants can breathe the air in this reservoir, but when it is exhausted, the bag collapses and no further air can be obtained. This model has been used to study aspects of air hunger, including its relief, in the laboratory (Banzett et al., 2011). The combination of ventilatory restriction during hypercapnia requires less

hypercapnia to induce a given level of air hunger than hypercapnia on its own, which would mean less signal-dampening due to CO_2 during FMRI and makes this model interesting for the study of dyspnoea during FMRI. Up until now, it has not been used during FMRI as the respiratory reservoir can collapse abruptly, which might induce significant stimulus-correlated head movement during FMRI. Hence the collapse of the reservoir might need to be softened before the model can be used during FMRI.

The second model that induces dyspnoea by reducing ventilation during hypercapnia uses passive mechanical ventilation. In this model, participants do not breathe themselves, but are externally ventilated by a machine. This technique allows respiratory parameters to be completely controlled by the experimenter. For the study of dyspnoea, mechanical ventilation has been used to reduce tidal volume in combination with hypercapnia to induce the sensation of air hunger. Reduced tidal volume from 1.47 liters to 0.75 liters at constant $PETCO_2$ at 5.5kPa induced a sensation of air hunger, whilst similar tidal volumes at a lower constant $PETCO_2$ of 4.4kPa did not invoke air hunger (Evans et al., 2002). This model has been used during FMRI and showed activity in the frontal cortex, middle temporal cortex, limbic and paralimbic areas associated with air hunger (insula, ACC, operculum, cerebellum, amygdala, thalamus, basal ganglia) (Evans et al., 2002). Whilst mechanical ventilation allows probing the neuronal mechanisms related to the perception of respiration, participants have to inhibit active breathing during mechanical ventilation, which requires extensive training and will alter neural activation related to respiratory motor commands and inhibition of respiration. Mechanical ventilation hence seems to be a useful, but relatively invasive and potentially confounded technique that can be used to study perceived air hunger at stable passive ventilation during FMRI.

2.4.2 Increased respiratory effort

Increased respiratory work refers to the awareness of effortful breathing, e.g. during hyperventilation or when breathing against respiratory resistance. The currently most widely accepted theory regarding the origin of work/effort component of dyspnoea is that it arises from a mismatch between the motor command sent by the brainstem or motor cortex to the respiratory muscles and incoming afferent information from sensory receptors (Nishino, 2011). External inspiratory loading has often been used to simulate the increased respiratory load on the inspiratory muscles and the associated work/effort that patients might feel when trying to overcome airway narrowing (Gorman et al., 1999). Respiratory resistance can be generated externally by letting participants breathe through a narrow tube (the smaller the diameter, the higher the resistance) or by introducing resistors into a respiratory circuit. The resistance can either be fixed at a certain physical strength, can be titrated to produce a specific work/effort rating or can be dynamically adjusted at a breath-by-breath level in order to produce a specific mouth pressure or sensation of work/effort. Respiratory circuits can be designed to apply mechanical respiratory loading during the inspiratory or expiratory phase of a breath or simultaneously during both phases (Nishino et al., 2007). One study has shown that the intensity of dyspnoea generated during respiratory loading applied during inspiration (inspiratory loading) and respiratory loading applied during expiration (expiratory loading) add linearly when simultaneously applied (Nishino et al., 2007). Using inspiratory and expiratory loads at 19.5cmH_2O for 100 seconds during PET, Peiffer and colleagues showed brain activation in the caudate nucleus, premotor cortex and cerebellum with dyspnoea and activation in the anterior cingulate cortex and prefrontal cortex correlating with the more emotional concept of dyspneoa relief (Peiffer et al., 2008). Using FMRI, von Leupoldt and colleagues have used inspiratory loading of 12 -13cmH2O over 24 seconds to elicit feelings of dyspnoea in healthy volunteers and asthmatics (von Leupoldt et al., 2008, 2009a; von Leupoldt et al., 2009b). These studies revealed the involvement of somatomotor areas, limbic

and paralimbic areas, the thalamus, brainstem and cerebellum during dyspnoea and highlighted the role of the insula in the perception of dyspnoea unpleasantness.

Respiratory loading seems to be the current model of choice for the investigation of dyspnoea with FMRI. Respiratory resistance can be controlled on a breath-by-breath basis and changes in resistance cause immediate changes in respiratory work/effort, which allows the clear on/off blocks or events often used during FMRI. Still, apart from its ability to induce dyspnoea, not much is known about the application of respiratory loading during FMRI. A recent study suggests that the effect of the application of respiratory loading during FMRI might be dependent on the duration of the stimulus (Raux et al., 2013), but the study was significantly underpowered (11 participants when more than 30 participants are required for effective statistical comparison), hence these effects will need to be properly investigated.

Respiratory loading is often associated with increased dead space and might hence automatically induce slight hypercapnia. The group of Takashi Nishino has previously used a combination of respiratory loading and dead space loading at different strengths (addition of 400ml -1800ml instrumental deadspace adjusted to increase $PETCO_2$ by 1.7-1.9kPa from baseline and approximately -20cmH_2O inspiratory loading and +15cmH_2O expiratory loading) to induce dyspnoea through both hypercapnia and additional respiratory work (Nishino et al., 2007; Nishino et al., 2005). Whilst the separate contributions of hypercapnia and respiratory resistance to the generation of dyspnoea with this model have not been investigated independently, inspiratory, expiratory and simultaneous inspiratory and expiratory resistance in addition to baseline hypercapnia changed the top three descriptors selected from a list from more air hunger-related descriptors towards more work/effort based descriptors (Nishino et al., 2007). Whilst this model induced moderate respiratory discomfort in the laboratory (Nishino et al., 2007), a $PETCO_2$ increase of +1.7-1.9kPa from baseline

would dampen the BOLD response in a stimulus-correlated way and would hence be detrimental to the BOLD signal of interest.

2.4.3 Inducing dyspnoea during FMRI

The induction of dyspnoea in the laboratory is complex, but additional challenges are faced when inducing dyspnoea during FMRI. To obtain brain activation relating to dyspnoea during FMRI, two main factors need to be considered. Firstly, it is important to apply a valid stimulus, i.e. a stimulus that elicits the discomfort associated with dyspnoea in the relative safety of a controlled laboratory environment. Secondly, it is important that this stimulus does not cause systematic physiological confounds of FMRI signal, e.g. by altering cerebral blood flow and hence BOLD signal in a stimulus-associated manner via CO_2 and direct pressure changes.

In order to avoid stimulus-associated CO_2 changes that would be detrimental to the analysis of FMRI data, inspiratory loading could be combined with a low level of isocapnic (stable) baseline hypercapnia. Such a model would benefit from the breath-by-breath controllability of respiratory loading and the dissociation of stimulus-induced CO_2 effects from the signal of interest during FMRI analysis. In Chapter 3 we will investigate whether such a model can be used to reliably induce moderate dyspnoea. Secondly, we will investigate whether the perception of dyspnoea remains stable over time. Stability of induced dyspnoea is important for multi-session intervention studies that allow the evaluation of the impact of experimental manipulations on subjective response and brain activation within subjects. Dyspnoea studies published to date have not used multi-session FMRI paradigms, but such paradigms could be invaluable for the investigation of the neural signature underlying treatment effects found in the laboratory (e.g. (Nishino et al., 2000)). We will therefore investigate the temporal stability

of dyspnoea induced with respiratory loading to be able to account for group-level habituation that could make between-session comparisons of dyspnoea during FMRI more difficult.

Neuroimaging of dyspnoea is still in its infancy (Herigstad et al., 2011a). Whilst previous studies have used inspiratory loading to induce dyspnoea during FMRI (e.g. (Raux et al., 2013; von Leupoldt et al., 2008)), it is currently not known whether respiratory loading might alter cerebral blood flow and might thus interfere with the generation of the BOLD signal itself. In order to validate the use of inspiratory loading for the study of dyspnoea during FMRI, it is hence necessary to investigate whether respiratory loading induces such a stimulus-correlated confound.

Neuroimaging of dyspnoea has greatly benefitted from findings from neuroimaging of pain, which is currently far better understood. Based on a common neural network for pain and dyspnoea (von Leupoldt et al., 2009a)), neuroimaging of dyspnoea has largely used small volume corrections with limited searches in order to investigate activations in brain areas that are implicated in the perception of pain (von Leupoldt et al., 2008; von Leupoldt et al., 2009b). Whilst hypothesis-driven searches based on previous findings from the pain literature have led to a significant advance in the study of dyspnoea, this approach is limited by its focus on specific brain areas from the study of pain and might hence miss activation in areas that are more strongly implicated in dyspnoea than pain.

A few studies have used a whole-brain analysis to identify areas involved in dyspnoea, but participant numbers were small and physiological noise was not incorporated into analyses (e.g. (Evans et al., 2002; Raux et al., 2013)), which might have reduced power for detection of additional effects. In order to fully appreciate the potentially complex neural network involved in the perception of dyspnoea, there is the need for a study with adequate statistical

power looking for dyspnoea-related activation in the whole brain. The inclusion of a sufficient number of participants and the full implementation state-of the art physiological noise modelling for FMRI analysis are highly important here.

2.5 Conclusion

Both hypercapnia and respiratory loading are used to investigate aspects of dyspnoea in the laboratory. Care has to be taken when applying these models to an FMRI environment. The dampening effects of hypercapnia on the BOLD response and the slow induction and relief of dyspnoea over multiple breaths suggest that hypercapnia might best be used in combination with other stimuli, like ventilatory restriction or respiratory loading. Respiratory loading seems ideally suited for the induction of dyspnoea during FMRI, but its effects on cerebral blood flow are currently unknown and might systematically confound BOLD signal. Combining mild baseline hypercapnia with respiratory loading might have advantages over using either only hypercapnia or respiratory loading during FMRI and seems to be worth investigating.

2.6 Aims of the work presented within this thesis

Dyspnoea is a complex symptom that can be studied by using a variety of laboratory stimuli. The two stimuli most commonly used to induce laboratory dyspnoea are hypercapnia and respiratory loading. Hypercapnia predominantly induces air hunger, and respiratory loading predominantly elicits an increased sensation of respiratory effort. We propose that measuring the neural mechanisms of dyspnoea through the application of dyspnoea stimuli during FMRI

necessitates a valid stimulus and that the temporal dynamics and potential FMRI confounds of this stimulus should be known. The aim of this thesis is to develop a dyspnoea stimulus that can realistically and reliably induce a sensation of dyspnoea in healthy volunteers during FMRI, to investigate potential systematic FMRI confounds of the stimulus and to apply it to elucidate the neural mechanisms involved in the anticipation and the subjective perception of dyspnoea.

The work presented in this thesis aims to use a healthy volunteer model to study the neuronal mechanisms of dyspnoea. Initially, this warrants the development of an FMRI-compatible model of dyspnoea in healthy volunteers (Chapters 3 and 4). This process necessitates the generation of a dyspnoea stimulus that induces a realistic experience of dyspnoea that can be rapidly controlled and applied over multiple MRI scanning sessions (Chapter 3) without confounding the interpretation of the blood flow dependent responses during FMRI itself (Chapter 4). Subsequently, the developed model is applied to study two important aspects of the perception of dyspnoea. Firstly, it investigates the neuronal mechanisms of anticipatory anxiety of dyspnoea, a mechanism that causes the avoidance behavior displayed by many dyspnoeic patients, decreases quality of life and contributes to rapid disease progression and increased mortality (Chapter 5). Secondly, the neuronal signature of dyspnoea unpleasantness is explored (Chapter 5). Understanding the neuropsychological bases of dyspnoea-specific anticipatory anxiety and the unpleasantness of dyspnoea will allow the development of targeted behavioural and pharmacological interventions that could increase quality of life in a large group of patients. The work presented in this thesis contributes to the study of neural mechanisms of dyspnoea and has implications for both, the study of dyspnoea with laboratory stimuli during FMRI and the understanding of the neural signature of dyspnoea and its alleviation. These contributions are discussed in Chapter 6, together with suggestions for future research required to substantiate current findings.

Chapter 3

The unpleasantness of inspiratory loading is not altered by mild hypercapnia and does not habituate over time

Abstract Chapter 3

Functional magnetic resonance imaging (FMRI) allows the study of neural mechanisms of dyspnoea. Resistive respiratory loading (to induce dyspnoea) induces changes in end-tidal CO_2 ($PETCO_2$) that confound interpretation of FMRI. Isocapnia (keeping $PETCO_2$ constant) avoids these confounds, but necessitates a continuous baseline of mild hypercapnia. We hypothesised that mild hypercapnia would have little subjective effect during unloaded breathing, but would make resistive loading more unpleasant (thus smaller resistive loads were required to achieve same dyspnoea rating). As intervention studies often require multiple sessions, we examined whether respiratory unpleasantness habituated over multiple sessions.

Ten healthy non-smokers (mean age 23 (SD±6) years, 4 females) participated in one training and four experimental sessions: poikilocapnia (freely varying $PETCO_2$), isocapnia at +0.4kPa, +0.6kPa, +0.9kPa above baseline (counterbalanced) on five consecutive days. At the beginning of each experimental session, we determined the load needed to evoke 50% respiratory unpleasantness on a visual analogue scale (VAS). This load was then applied for two 4-minute blocks with 3 minutes unloaded breathing in between (VAS ratings every 15s). Participants completed the multidimensional dyspnoea profile (MDP) after each session.

During poikilocapnia, mean $PETCO_2$ fluctuated and decreased from baseline after initial adaptation of respiratory loading. Manual gas control achieved isocapnia. Hypercapnia increased baseline unpleasantness (13%-24%VAS, p=0.010), but did not significantly decrease the load necessary to induce 50% VAS unpleasantness (p=0.486). Loads required to induce 50%VAS dyspnoea unpleasantness remained stable across all experimental sessions (no habituation). Initial application of respiratory loading in all conditions increased mouth pressure and unpleasantness during subsequent unloaded periods.

Combining respiratory loading with mild hypercapnia could benefit the investigation of dyspnoea perception during FMRI. Manually controlled continuous mild hypercapnia decreases $PETCO_2$ variability, reducing unwanted CO_2 fluctuations that affect BOLD. Baseline hypercapnia does not reduce respiratory loads necessary for the induction of moderate unpleasantness and needs to be kept low to minimize increases in unpleasantness during unloaded breathing. Respiratory unpleasantness remained stable over four sessions, which allows comparisons of stimuli between sessions, which is important for FMRI intervention studies.

3.1 Introduction

Dyspnoea causes suffering in millions of patients, yet remains poorly understood and insufficiently treated (Parshall et al., 2012b). Combining experimental dyspnoea with functional MRI allows the investigation of specific components of dyspnoea through the application of controlled respiratory challenges to a homogenous population in a standardized environment. This might help to develop reveal new therapeutic targets (Herigstad et al., 2011a). However, care must be taken to ensure that the stimulus used to induce dyspnoea mimics meaningful aspects of the sensation without interfering with FMRI signal, particularly through systematic physiological confounds.

Commonly used methods to induce dyspnoea include mechanical respiratory loading, hypercapnia and combinations of the two. Respiratory loading predominantly increases perceived respiratory work (Lansing et al., 2009). Its controllability at a breath-by-breath basis and relatively simple administration make respiratory loading a popular stimulus for the investigation of dyspnoea during FMRI (e.g. (von Leupoldt et al., 2008)). Hypercapnia, on the other hand, induces the perception of air hunger (Lansing et al., 2009), which builds up and decreases over a few breaths whilst arterial CO_2 slowly changes (Banzett et al., 1996).

Systematic physiological changes need to be considered when using respiratory stimuli to induce dyspnoea during FMRI. Blood oxygen level dependent (BOLD) FMRI signal is an indirect measure of neural activity that is dependent on a combination of metabolic oxygen consumption, changes in cerebral blood flow (CBF) and cerebral blood volume (Wise et al., 2004). A CBF increase induced by increases in partial pressure of arterial CO_2 ($PaCO_2$) increases baseline BOLD signal (Wise et al., 2004) that significantly decreases the magnitude and slows the response time of the BOLD signal induced by neural activity (Cohen et al., 2002). Respiratory loading induces respiratory changes that result in altered partial pressure

of end-tidal CO_2 ($PETCO_2$) in some, but not all individuals and is reflected in increased standard deviations and in increased group $PETCO_2$ (Gorman et al., 1999; Hornstein and Ledsome, 1984; Isono et al., 1990; McKenzie et al., 1997; Rohrbach et al., 2003; von Leupoldt et al., 2008). If not controlled for, differential CO_2 responses could dampen BOLD signal of interest by introducing stimulus-linked physiological confounds. They could also induce additional variation between individuals that would decrease power when averaging FMRI responses across participants.

Isocapnia (constant CO_2) can be used to counteract stimulus-induced CO_2 effects (Wise et al., 2007b) and can additionally reduce CBF variability, a major source of noise during BOLD imaging (Kruger and Glover, 2001). Fluctuations in $PETCO_2$ can be counteracted through external breath-by breath adjustments of inspired CO_2 (Wise et al., 2007b). This necessitates mild hypercapnia in order to be able to adjust inspired CO_2 upwards and downwards in order to correct for increases as well as decreases in $PETCO_2$.

Here we investigate whether the addition of continuous mild hypercapnia to respiratory loading could be advantageous for the induction of laboratory dyspnoea during FMRI. During FMRI, hypercapnic isocapnia will allow the dissociation of stimulus-induced CO_2 changes from neuronal BOLD activation of interest, which would increase sensitivity of detecting neuronal activation related to dyspnoea during FMRI. Additionally, continuous mild hypercapnia might amplify the subjective response to respiratory loading, which would allow a decrease in the intensity of applied respiratory loads and their associated physiological confounds on the BOLD response.

Perceptual habituation to respiratory stimuli may be a problem when stimuli are applied over long or multiple sessions. FMRI paradigms often compare brain activation acquired during

different conditions (e.g. drug/placebo) in multiple sessions. Perceptual and neural decreases in response magnitude over time (habituation) might therefore cause problems when interpreting results. Whilst the strongly enhanced emotional effects of laboratory respiratory stimuli to naïve participants are well known and often avoided by the use of training sessions to familiarize participants with respiratory stimuli (e.g. (Nishino et al., 2007; O'Donnell et al., 2013)), the effect of repeated administration of respiratory stimulation on perceived respiratory sensations beyond this initial application remains unknown. Studies looking at respiratory sensations show evidence for both habituation of subjective perception of hypercapnia (Li et al., 2006; Wan et al., 2009) and for stable shortness of breath (no habituation) during exercise in patients with obstructive pulmonary disease, in healthy participants during breath hold and in ventilated patients who showed stable stimulus-response curves to CO_2 challenges during chronically increased CO_2 (Bloch-Salisbury et al., 1996; Carrieri-Kohlman et al., 2001; Nishino et al., 1996).

In this study we applied respiratory loading superimposed upon different levels of mild hypercapnia in order to investigate the effects of isocapnic application of respiratory loading that could benefit FMRI analysis on three different perceptual aspects. Firstly, we investigated the effects of hypercapnia on the unpleasantness perceived during unloaded breathing. We hypothesized that mild baseline hypercapnia would not increase respiratory unpleasantness significantly compared to unloaded breathing. Secondly, we investigated whether hypercapnia and respiratory loading had an additive effect on respiratory unpleasantness. We hypothesized that hypercapnia would decrease the amount of respiratory resistance necessary to induce the same level of respiratory unpleasantness. Thirdly, we explored whether the evoked subjective perceptions remained stable over time within each session and across four sessions.

3.2 Materials and Methods

3.2.1 Participants

Ten volunteers (aged 23 ± 6 years, 4 females) participated in this study after giving written informed consent in accordance with the Oxfordshire Research Ethics Committee. Participants were healthy right-handed non-smokers with no history of significant psychological, neurological, pulmonary or cardiovascular disease and free from acute illness (e.g. acute upper respiratory tract infection). Participants abstained from heavy meals and physical activity (cycling, exercise) for at least one hour before each session. The study consisted of five sessions performed on consecutive days. Participants were tested at the same time of day in order to avoid circadian changes in hormone level and cognitive performance throughout the day (Carrier and Monk, 2000). To minimize the biasing effects of initial first-time adaptation to a respiratory apparatus, the first session was a training session similar in length and stimulus strength to the experimental sessions. This was followed by four experimental sessions.

3.2.2 Experimental paradigm

Participants were seated comfortably and were breathing through through a custom-built respiratory circuit connected to a facemask (Hans Rudolf; Figure 3.1). To this circuit, a mixture of compressed medical air (humidified) was added in which inspired CO_2 was adjusted by mixing in a CO_2-rich gas (10% CO_2, 21% O_2, balance nitrogen). To reduce auditory distractions, participants wore standard foam earplugs and headphones (Sony MV300, Sony), through which a constant sound (‘pink noise’) was played at a volume sufficient to mask sounds of the respiratory system (Banzett et al., 1996). The experimental paradigm shown in Figure 3.2 was run for all experimental sessions.

Hypercapnia

After a five-minute period in which participants familiarized themselves with breathing on the respiratory circuit, their $PETCO_2$ baseline was determined over one minute. Over the next 5 minutes, an investigator manually adjusted the inspired CO_2 to raise $PETCO_2$ to the desired amount above baseline. Each participant underwent a poikilocapnia ($PETCO_2$ allowed to fluctuate naturally) session and three sessions in which $PETCO_2$ was experimentally raised by different amounts from the participant's natural breathing baseline. In the first hypercapnia condition, $PETCO_2$ was increased and kept stable at approximately +0.4kPa above baseline breathing. In the second hypercapnia condition $PETCO_2$ was increased by approximately +0.6kPa above baseline and in the third hypercapnia condition by approximately +0.9kPa above baseline breathing. The levels of continuous baseline hypercapnia were chosen to be low (+0.4kPa, +0.6kPa and +0.9kPa) to be able to translate the findings to a hypercapnia-sensitive BOLD environment.

Subjective ratings of respiratory unpleasantness

Participants did not rate their respiratory unpleasantness during the first 11 minutes of the paradigm (the time during which familiarization with the breathing system took place and $PETCO_2$ was adjusted to experimental level). After the familiarization period, participants rated perceived respiratory unpleasantness four times a minute (i.e. 12 ratings were given during a 3-minute block of normal breathing). Intervals between the ratings were semi-randomized to values between 10-20 seconds. Participants rated their breathing unpleasantness on a horizontal visual analog scale (VAS) stating the question 'How unpleasant did your breathing feel?' with the anchors 'not unpleasant' on the left and 'very unpleasant' on the right. Behavioural responses were made via a custom-made button box. Standardized instructions relayed the concepts of respiratory intensity and unpleasantness before the training session. An experimenter verbally confirmed participants' understanding

of the concepts of respiratory intensity and unpleasantness before the training session and asked whether participants felt able to distinguish both concepts after the training session. Participants were not exposed to a maximally-tolerable stimulus, but were instructed to use the scale so that a maximum rating would correlate with the degree of unpleasantness that would make them immediately stop the experiment.

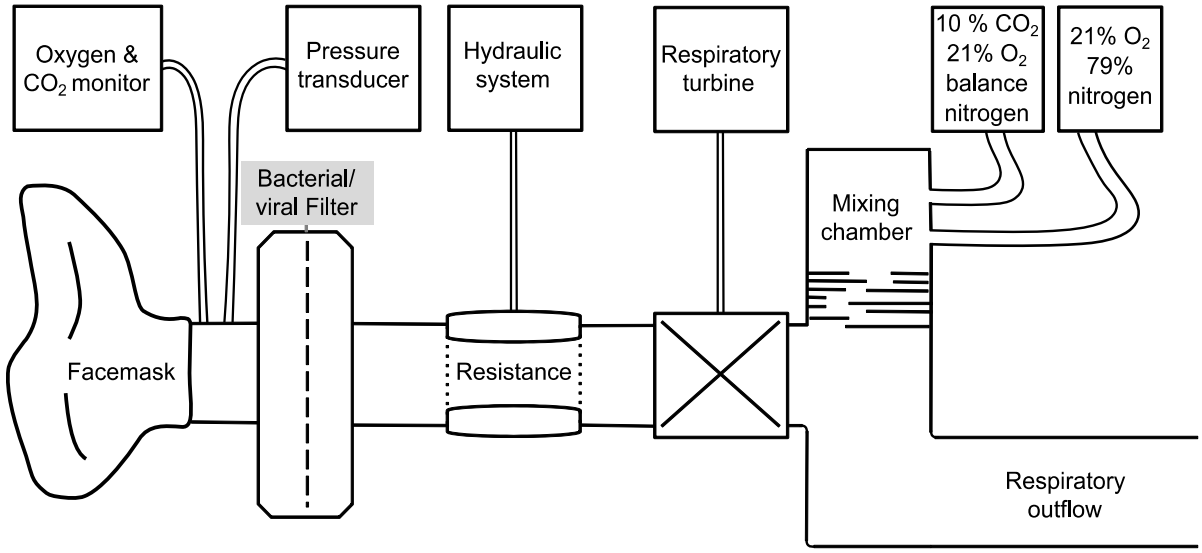


Figure 3.1: Schematic illustration of custom-built respiratory circuit. A facemask (7450 SeriesV2™ Mask, Hans Rudolph, USA) was connected to two sampling lines measuring respiratory gases and respiratory pressure via polyethylene extension tubing (Vygon SA, Ecouen, France). One sampling line led to a pressure transducer (MP 45, +/-50cmH2O, Validyne Corp., Northridge, CA, USA) connected to an amplifier (Pressure transducer indicator, PK Morgan Ltd, Kent, UK) and provided readings of current respiratory pressure at the mouth. Tidal CO_2 and tidal oxygen were continuously sampled and analyzed using an infrared analyzer with side-stream sampling (Capnomac Ultima, Datex Ohmeda, Helsinki, Finland). The diameter of a 5cm long section of respiratory tubing is lined with a rubber party balloon connected via non-distensible plastic tubing to a 10ml syringe filled with water and can be altered remotely to increase respiratory resistance. A turbine (VMM-400, Interface Associates, Aliso Viejo, CA, USA) was used to record inspiratory and expiratory volumes. Medical gases were fed from cylinders and entered a mixing chamber that allowed thorough mixing before inspiration.

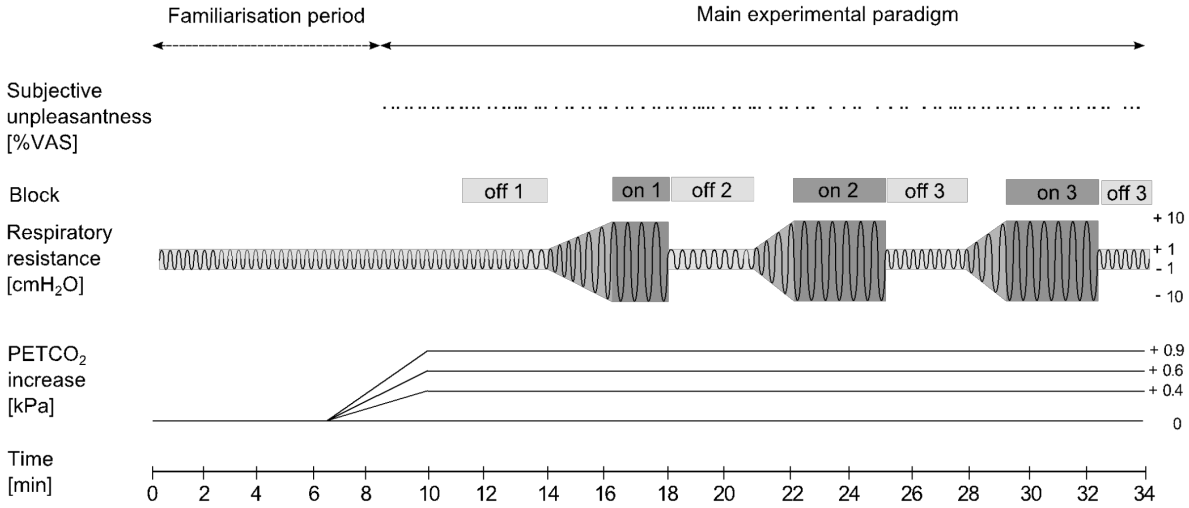


Figure 3.2: Timeline of experimental session. Each experimental session lasted 34 minutes. After an adjustment period of 5 minutes, baseline $PETCO_2$ was determined. In the isocapnia conditions, $PETCO_2$ was then increased according to condition (+0.4kPa, +0.6kPa, +0.9kPa) and was kept stable at the target $PETCO_2$ level. $PETCO_2$ was allowed to freely vary during the poikilocapnia condition. Participants rated perceived respiratory unpleasantness at semi-random intervals four times a minute. The strength of respiratory loading applied during the session was determined during the first two minutes of the first respiratory loading block. Three blocks of respiratory loading of 4 minutes length were administered at 14, 21 and 28 minutes.

Respiratory resistance

Simultaneous inspiratory and expiratory loading was added to the respiratory circuit. A hydraulic system was used to decrease the diameter of a 5cm section of respiratory tubing that was lined with a party balloon. Inflating the balloon decreased the diameter of the tube. The increase in respiratory pressure was measured at the mouth (mouth pressure). Mouth pressure amplitude was used as a physiological measure of respiratory intensity, indicating the successful application of the respiratory manipulation. The change in mouth pressure during respiratory loading was titrated to elicit 50% unpleasantness during each individual session in each individual participant (loads were slowly increased over two minutes until subjective ratings indicated 50% VAS for two successive ratings and this mouth pressure was noted as

the target level for the rest of the session). A dedicated experimenter maintained mouth pressure constant through breath-by-breath adjustment of inspiratory resistance (Figure 3.1). During the two successive blocks of respiratory loading during that session, loading was increased over 30 seconds till the target mouth pressure was reached. Mouth pressures were then kept stable throughout the loading block, representing the manipulation (independent variable) and not a dependent variable.

Physiological monitoring

Heart rate was measured with a pulse oximeter (Capnomac Ultima, Datex Ohmeda, Helsinki, Finland). All physiological data were sampled at 100Hz and were logged via a Power1401 using Spike2.7 (CED, Cambridge, UK).

Questionnaires

Participants answered a short pre-study questionnaire immediately before being connected to the respiratory circuit. Participants answered the following four questions on a horizontal VAS with 'not at all' as the left anchor and 'very' as the right anchor: 'How tense are you feeling? How nervous are you about this session? How bad do you expect this session to be? How relaxed are you feeling right now?'. Immediately after participants were released from the respiratory circuit, they filled in an early version of the Multidimensional Dyspnoea Profile (MDP) that measured six sensory qualities of dyspnoea as well as overall shortness of breath and respiratory unpleasantness (Banzett et al., 2008). A version of the questionnaire used in this study can be found in Appendix Ia. After completing the questionnaires, participants were asked 'How did your breathing feel during the experiment?'. The experimenter recorded the answers participants gave and prompted for additional information once participants had completed their assessment. Participants were fully debriefed after completion of the experiment.

3.2.3 Data analysis

Respiratory data (mouth pressure and $PETCO_2$), heart rate and unpleasantness ratings were exported to MatLab and analyzed on a breath-by-breath basis using custom written scripts (MathWorks Inc., Natick, MA, USA). Data were averaged over resistance and non-resistance blocks (excluding the first 120 seconds of the first block and the first 30 seconds of the remaining two resistance blocks, whilst pressure was adjusted to target level). All statistical analyses were performed using the SPSS statistical package version 21 (SPSS Inc., Chicago, IL). Prior to analysis, descriptive statistics were calculated for all variables to ensure the correct choice of test. For all analyses, two-tailed testing at $p< 0.05$ was used to determine statistical significance.

Isocapnia

Fluctuations in $PETCO_2$ and their reduction by our isocapnia manipulation were assessed by computing the temporal coefficient of variation (CV) by taking the temporal standard deviation (SD) for each breath, dividing it by the mean and multiplying it by 100 to receive a result in %. A 2 (resistance) x 4 (CO_2 condition) repeated measures ANOVA was performed to test for differences in the coefficient of variation between CO_2 conditions. Post-hoc tests were based on estimated marginal means and were corrected for multiple comparisons with Bonferroni corrections.

Effects of hypercapnia on unpleasantness

In this study, mouth pressure amplitude provided a measure of physical stimulus intensity. A repeated measures ANOVA was performed on the block averages of mouth pressure amplitude during respiratory loading to check for successful application of respiratory loading (mouth pressure amplitude as independent variable). A second repeated measures ANOVA was performed on the block averages of mouth pressure amplitude during respiratory loading

to investigate differences relating to application of hypercapnia (mouth pressure as dependent variable). Because unpleasantness ratings during non-resistance blocks and MDP data were not normally distributed, significant differences in unpleasantness were assessed with two related-samples Friedman's two-way analyses of variance by ranks, one for effects of CO_2 condition on respiratory loading blocks, one for effects of CO_2 on blocks without respiratory loading. One related-samples Friedman's two-way analysis of variance by ranks was performed for each dimension of the MDP comparing all four experimental CO_2 conditions (df=3).

Habituation

Another set of repeated measures ANOVAs was performed on the block averages of mouth pressure amplitude to investigate adaptation of participants' perceptions within- and between-sessions, separate for blocks with and without respiratory resistance. Bonferroni corrections were used to correct for multiple comparisons. Average values are expressed as mean and standard deviation (SD) in the text.

Two Friedman's two-way analyses of variance by ranks were performed on unpleasantness ratings for effects of chronological session (on blocks with and blocks without respiratory loading separately).

Qualitative data

Qualitative data were entered into NVivo qualitative data analysis software (QSR International Pty Ltd. Version 10, 2012). Each statement was coded into a node according to its meaning. Interesting themes emerged from the data and are presented to supplement quantitative data.

3.3 Results

All ten participants tolerated CO_2 loading and the addition of respiratory loading and completed the experimental protocol. Two participants only performed a training session and three experimental sessions (Table 3.1).

3.3.1 Isocapnia

$PETCO_2$ fluctuated between blocks of loaded/unloaded breathing during poikilocapnia (Figure 3.3). Manual gas control in the three hypercapnia conditions achieved the desired effect of keeping $PETCO_2$ stable in most participants (Figure 3.3) and reduced interindividual differences in CO_2 level between participants. A 2 (resistance) x 4 (CO_2 condition) repeated measures ANOVA on the coefficient of variability of $PETCO_2$ showed a main effect of CO_2 condition, $F=(3,5)=12.99$ $p<0.0001$. Post-hoc tests determined that the $PETCO_2$ coefficient of variability decreased with increased hypercapnia (poikilocapnia and 0.4kPa hypercapnia ($p=0.075$), poikilocapnia and 0.6kPa hypercapnia ($p=0.019$), poikilocapnia and 0.9kPa hypercapnia ($p=0.015$)). The ANOVA showed no mean effect for resistance, $F(1,7)=5.09$ $p=0.059$. An interaction effect of resistance and CO_2 condition showed a stronger decrease in coefficient of $PETCO_2$ variability during resistance periods compared to non-resistance periods with increased hypercapnia, $F(3,5)=5.06$ $p=0.009$ (Figure 3.3). There is no significant difference in the coefficient of variability of $PETCO_2$ between poikilocapnia and 0.4kPa hypercapnia (mean difference 2.8%, $p=0.131$), but a difference between poikilocapnia and 0.6kPa and 0.9kPa hypercapnia (poikilocapnia vs. 0.6kPa: mean difference 4.5% $p=0.033$, poikilocapnia vs. 0.9kPa: mean difference 4.3% $p=0.029$). These results suggest 0.6kPa hypercapnia to be the lowest CO_2 increase at which manual gas control achieved the desired effect of significantly reducing $PETCO_2$ variability compared to poikilocapnia (Table 3.2).

3.3.2 Effects of hypercapnia on respiratory unpleasantness

Comparing unpleasantness during all four $PETCO_2$ conditions (poikilocapnia, hypercapnia at +0.4kPa, +0.6kPa and +0.9kPa) showed a significant increase in unpleasantness during pure hypercapnia (no respiratory loading) compared to completely unloaded periods ($p=0.036$; Table 3.2, Figure 3.4), but no effect of $PETCO_2$ condition during application of respiratory loading ($p=0.682$, Figure 3.4). Comparing mouth pressure amplitude during all four $PETCO_2$ conditions showed a significant increase of mouth pressure amplitude when hypercapnia was applied during unloaded periods ($F=10.39$, $df=3$, $p<0.0001$; Table 3.2, Figure 3.4), but no effect of $PETCO_2$ condition during application of respiratory loading ($F=.014$, $df=3$, $p=0.998$, Figure 3.4). A sample trace of a poikilocapnia session is shown in Figure 3.5.

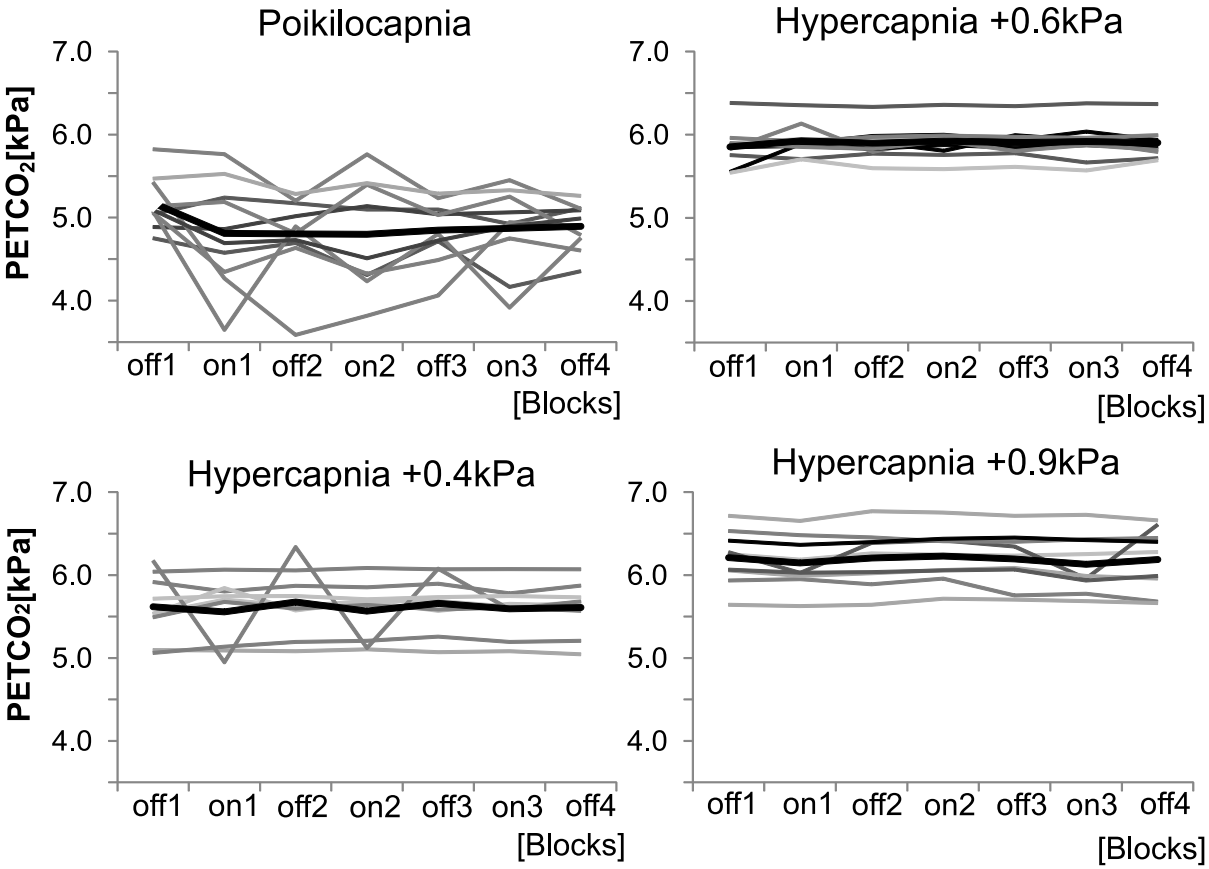


Figure 3.3: Plot of mean $PETCO_2$ averaged over resistance blocks and plotted according to $PETCO_2$ session. The black line represents the average of all participants. Individual traces for all participants are plotted in grey shades. N=10.

3.3.3 Characterisation of dyspnoea stimuli

Hypercapnia compared to poikilocapnia increased concentration from 37% to 56%VAS (p=0.017) and chest tightness from 10% to 37%VAS (p=0.028) during application of respiratory loading. Hypercapnia increased the sensation of breathing deeply from 14% to 21%VAS (p=0.007) compared to poikilocapnia (Figure 3.6).

Table 3.1: Participant characteristics.

Participant number	Age [years]	Sex	Height [cm]	Weight [kg]	Fitness level	Previous respiratory experience	Worst previous breathlessness
1*	18	F	167	66	casual sports	none	after rowing race
2	26	M	190	86	very sporty	mild allergy to dust mites	during intense exercise in hot weather
3	23	F	158	50	casual sports	diving	after a long treadmill session
4	22	F	186	77	very sporty	plays wind instrument	after a cross-country race
5	22	M	183	70	not sporty	none	during the 1500m run at school
6	21	M	162	48	not sporty	none	none
7#	38	M	183	83	not sporty	childhood asthma, diving, respiratory apparatus	childhood asthma
8	22	F	149	50	casual sports	none	none
9	18	M	170	65	casual sports	diving	after an intense period of running
10	23	M	183	79	casual sports	none	after a long treadmill session
Median	22		177	68			

* Participant did not complete +0.4kPa hypercapnia condition. # Participant did not complete +0.9kPa hypercapnia condition. F=female, M=male.

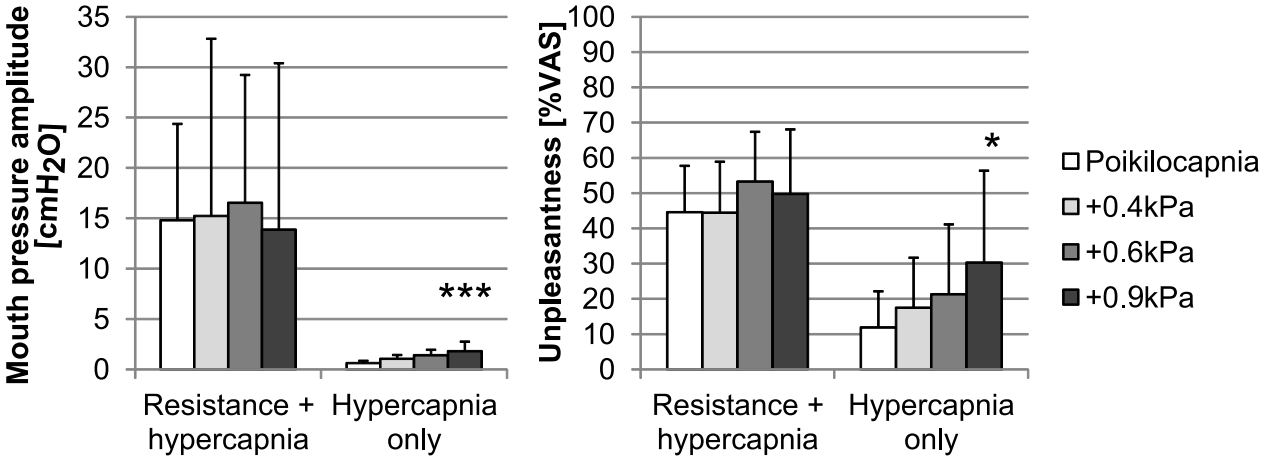


Figure 3.4: Mouth pressure amplitude and unpleasantness averaged over all participants by experimental condition (error bars depict SD). *p<0.05, ***p<0.001. N=10.

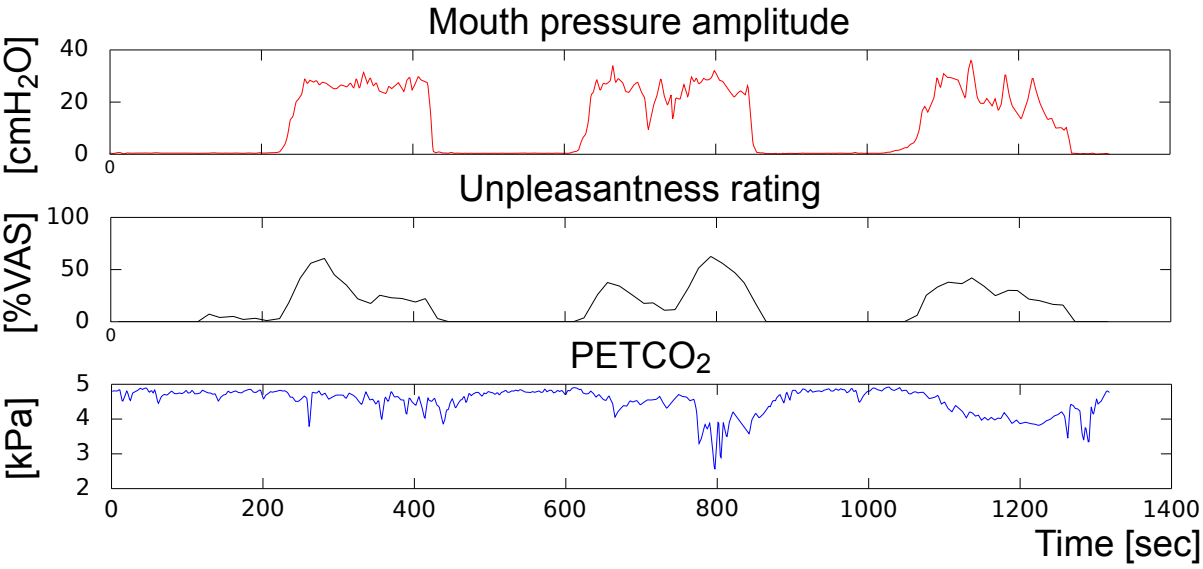


Figure 3.5: Representative timecourse of mouth pressure, unpleasantness rating and $PETCO_2$ during main part of experiment for one participant during the poikilocapnia session.

Table 3.2: Mouth pressure amplitude, unpleasantness, $PETCO_2$, end-tidal oxygen ($PETO_2$) and heart rate averaged over individual experimental blocks for all 10 participants. Blocks are labeled chronologically (first unloaded block = off1, first block with respiratory resistance = On 1) within sessions and are presented for poikilocapnia and the three hypercapnia conditions (+0.4kPa, +0.6kPa and +0.9kPa). P = poikilocapnia. CV = coefficient of variation. All values are mean (SD) for 10 participants.
* different from remaining off blocks throughout condition

Variable	CO_2	Off 1	On 1	Off 2	On 2	Off 3	On 3	Off 4	All off	All on
Mouth pressure amplitude [cmH_2O]	P	0.63 (0.24)	15.87 (10.11)	0.67 (0.33)	14.11 (8.94)	0.62 (0.22)	14.43 (10.11)	0.57 (0.23)	0.62 (0.23)	14.80 (9.57)
	+0.4kPa	0.91 (0.32)	15.13 (16.66)	1.11 (0.38)	15.68 (18.03)	1.11 (0.49)	14.89 (18.18)	1.07 (0.59)	1.05 (0.38)	15.23 (17.59)
	+0.6kPa	1.06* (0.39)	16.51 (12.46)	1.35 (0.47)	16.15 (12.38)	1.56 (0.67)	16.99 (13.35)	1.60 (0.79)	1.39 (0.56)	16.55 (12.68)
	+0.9kPa	1.55* (0.81)	13.32 (16.42)	1.81 (0.89)	15.45 (18.69)	1.97 (1.00)	12.83 (14.56)	1.91 (1.15)	1.81 (0.94)	13.87 (16.53)
Unplea-santness [%VAS]	P	9.33 (9.47)	44.06 (13.37)	13.01 (10.28)	46.97 (16.83)	11.81 (10.86)	42.81 (14.45)	13.44 (13.79)	11.90 (10.24)	44.61 (13.14)
	+0.4kPa	14.33 (14.15)	47.54 (16.93)	20.15 (19.25)	45.03 (17.74)	18.02 (17.98)	40.85 (13.68)	17.50 (15.30)	17.50 (14.19)	44.48 (14.44)
	+0.6kPa	14.20 (15.18)	53.29 (12.16)	22.16 (22.67)	55.74 (14.75)	23.55 (20.50)	50.87 (17.95)	25.38 (24.17)	21.32 (19.81)	53.30 (14.09)
	+0.9kPa	29.68 (31.60)	51.73 (18.48)	33.06 (27.59)	50.51 (19.52)	29.59 (20.16)	47.10 (19.98)	28.99 (27.35)	30.33 (26.04)	49.78 (18.29)
$PETCO_2$ [kPa]	P	5.18 (0.31)	4.81 (0.64)	4.80 (0.48)	4.80 (0.64)	4.85 (0.37)	4.87 (0.49)	4.90 (0.27)	4.93 (0.28)	4.83 (0.55)
	+0.4kPa	5.62 (0.39)	5.56 (0.39)	5.68 (0.39)	5.57 (0.34)	5.66 (0.34)	5.59 (0.30)	5.61 (0.31)	5.64 (0.35)	5.57 (0.33)
	+0.6kPa	5.85 (0.23)	5.93 (0.19)	5.90 (0.19)	5.92 (0.20)	5.90 (0.19)	5.92 (0.22)	5.90 (0.19)	5.89 (0.19)	5.92 (0.20)
	+0.9kPa	6.21 (0.33)	6.15 (0.31)	6.21 (0.34)	6.23 (0.31)	6.2 (0.33)	6.13 (0.35)	6.19 (0.38)	6.20 (0.34)	6.17 (0.32)
CV $PETCO_2$ [%]	P	3.51 (2.34)	5.86 (4.09)	3.89 (1.94)	6.99 (3.65)	4.84 (1.92)	6.89 (3.73)	4.72 (3.40)	4.24 (1.69)	6.58 (3.24)
	+0.4kPa	2.35 (0.95)	3.27 (2.63)	2.68 (1.76)	3.87 (3.51)	2.01 (0.79)	3.47 (2.97)	2.37 (1.68)	2.35 (1.05)	3.54 (2.37)
	+0.6kPa	1.98 (1.69)	2.08 (1.00)	2.01 (1.96)	1.93 (1.02)	2.14 (0.98)	2.21 (1.51)	1.25 (0.86)	1.85 (1.17)	2.07 (0.93)
	+0.9kPa	1.55 (0.75)	2.08 (1.36)	1.75 (1.13)	2.11 (1.04)	1.84 (1.11)	2.61 (2.28)	1.93 (0.92)	1.77 (0.75)	2.27 (1.23)
$PETO_2$ [kPa]	P	15.07 (0.49)	15.69 (1.04)	15.53 (0.88)	15.51 (0.99)	15.36 (0.42)	15.10 (0.89)	15.17 (0.62)	15.28 (0.33)	15.44 (0.71)
	+0.4kPa	17.17 (0.55)	16.62 (1.14)	17.58 (0.40)	17.01 (0.97)	17.67 (0.56)	17.01 (0.94)	17.44 (0.97)	17.47 (0.57)	16.88 (0.98)
	+0.6kPa	17.70 (0.64)	17.59 (0.72)	18.31 (0.53)	18.06 (0.72)	18.40 (0.49)	18.12 (0.70)	18.41 (0.54)	18.20 (0.50)	17.92 (0.67)
	+0.9kPa	18.10 (0.69)	17.92 (0.86)	18.42 (0.46)	18.12 (0.88)	18.49 (0.55)	18.12 (0.81)	18.43 (0.55)	18.36 (0.53)	18.06 (0.84)
Heart rate [bpm]	P	77.57 (11.42)	79.67 (9.77)	79.90 (9.95)	79.86 (10.56)	80.33 (10.92)	82.01 (10.26)	79.60 (11.10)	79.35 (10.32)	80.51 (9.95)
	+0.4kPa	75.82 (8.58)	79.75 (10.02)	80.65 (10.60)	78.76 (8.44)	78.74 (9.10)	78.14 (8.83)	79.15 (8.85)	78.59 (8.61)	78.89 (8.54)
	+0.6kPa	75.49 (10.20)	75.83 (9.68)	77.68 (8.38)	79.55 (10.46)	80.40 (10.57)	78.93 (11.04)	80.46 (11.12)	78.51 (9.63)	78.10 (10.17)
	+0.9kPa	81.92 (12.75)	80.12 (14.13)	82.62 (12.31)	83.22 (13.75)	82.62 (14.08)	84.34 (13.82)	83.76 (13.38)	82.73 (12.84)	82.56 (13.59)

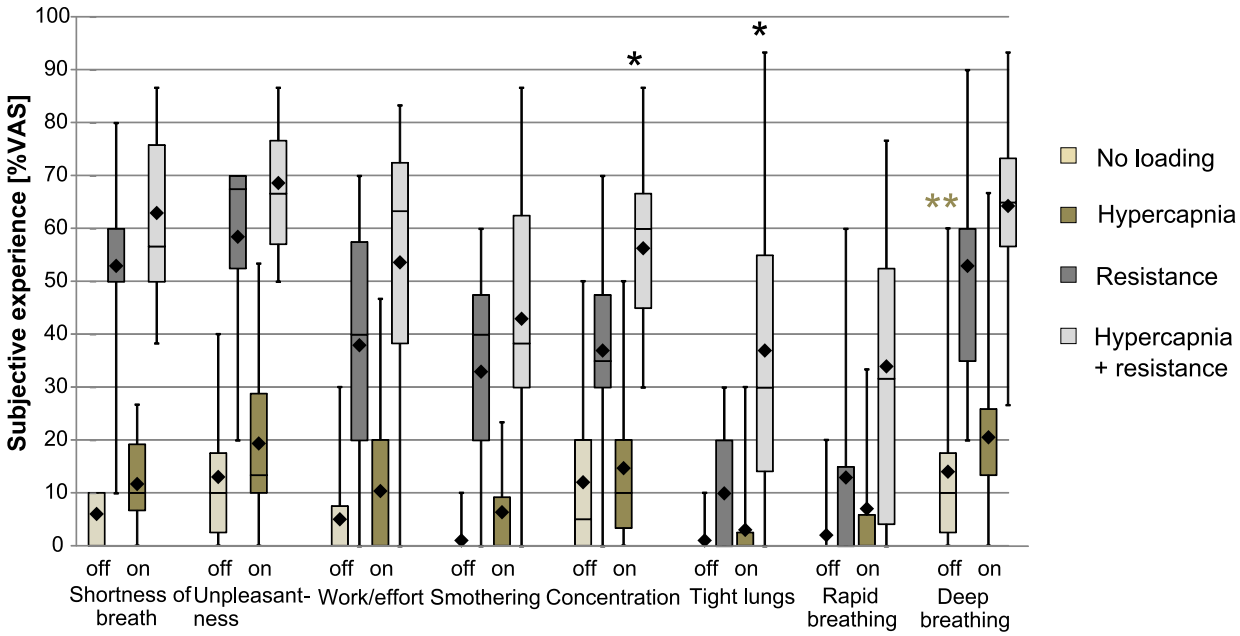


Figure 3.6: Sensory components of respiratory sensations during unloaded breathing (light beige), hypercapnia (average of +0.4kPa, +0.6kPa and +0.9kPa hypercapnia, brown colour), respiratory loading (dark grey) and respiratory loading and hypercapnia (average of +0.4kPa, +0.6kPa and +0.9kPa hypercapnia, light grey). Data presented as box-plots with interquartile ranges. Horizontal lines depict the median. Diamond shapes depict the mean. N=10.
* $p<0.05$, ** $p<0.01$ (uncorrected).

3.3.4 Habituation of respiratory unpleasantness

Figure 3.7 presents average unpleasantness and mouth pressure amplitude for each experimental block of each session. Average values for mouth pressure amplitude, unpleasantness, $PETCO_2$, $PETO_2$ and heart rate are displayed in Table 3.3.

Within-session habituation

During unloaded periods, perceived unpleasantness increased from 17% in the first block to approximately 22% during the remaining blocks ($F=3.74$, $df=3$, $p=0.027$) and mouth pressure amplitude increased from 1.0cmH_2O in the first block to approximately 1.2cmH_2O after the first block within each session ($F=3.73$, $df=3$, $p=0.027$). Across all respiratory loading blocks within each session, perceived unpleasantness ($F=2.19$, $df=2$, $p=0.149$) and mouth pressure amplitude ($F=2.29$, $df=2$, $p=0.546$) did not change.

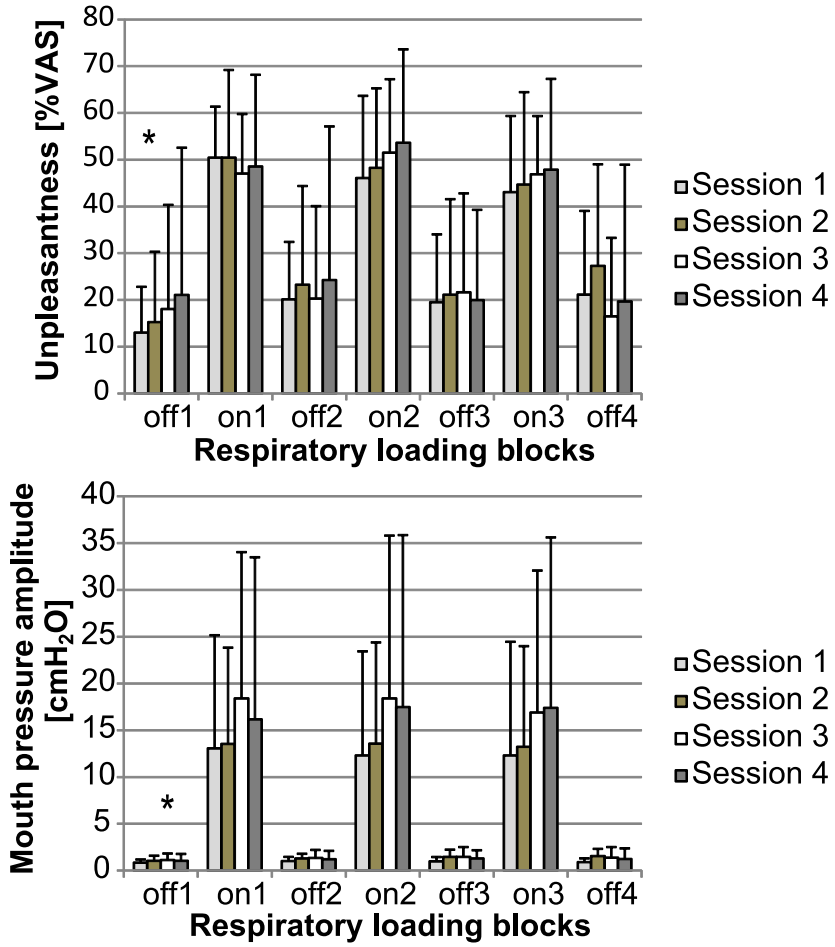


Figure 3.7: Adaptation within and between sessions. Chronological plot of unpleasantness ratings and mouth pressure amplitudes averaged over all participants (mean +/- standard error) during the four no resistance and the three resistance blocks within each session (off1= first unloaded block, on1=first respiratory resistance block). Data presented averaged over all poikilocapnia and hypercapnia conditions (counterbalanced) sorted according to session (Session 1 = first experimental session after training). N=10.
* mean of first non-resistance block is lower than subsequent unloaded blocks at p=0.05.

Mouth pressure during the unloaded periods did not change throughout the poikilocapnia ($F=1.23$, $df=3$, $p=0.317$) and the +0.4kPa ($F=0.98$, $df=3$, $p=0.416$) hypercapnia session. Mouth pressure significantly increased after application of the first respiratory load during the +0.6kPa ($F= 8.69$, $df=3$, $p<0.001$) and the +0.9kPa ($F=4.20$, $df=3$, $p=0.016$) hypercapnia sessions and remained elevated throughout the remaining unloaded blocks (Table 3.2). $PETCO_2$, $PETO_2$ and heart rate remained stable throughout the session for all CO_2 conditions.

Table 3.3: Basic physiology table. Means for 10 participants over all blocks according to chronological session (1-4). CV = coefficient of variation. All values are mean (SD) for 10 participants.

Variable	Session	Off 1	On 1	Off 2	On 2	Off 3	On 3	Off 4	All off	All on
Mouth pressure amplitude [cmH_2O]	1	0.86 (0.33)	13.08 (12.08)	1.03 (0.44)	12.31 (11.13)	0.99 (0.47)	12.31 (12.15)	0.93 (0.38)	0.95 (0.38)	12.57 (11.75)
	2	1.05 (0.54)	13.56 (10.27)	1.30 (0.51)	13.58 10.82)	1.48 (0.77)	13.24 (10.75)	1.55 (0.78)	1.34 (0.63)	13.46 (10.50)
	3	1.14 (0.69)	18.40 (15.64)	1.35 (0.87)	18.41 (17.41)	1.47 (1.05)	16.89 (15.18)	1.39 (1.13)	1.34 (0.92)	17.90 (15.93)
	4	1.05 (0.73)	16.17 (17.32)	1.23 (0.89)	17.47 (18.39)	1.29 (0.89)	17.41 (18.21)	1.23 (1.14)	1.20 (0.88)	17.02 (17.93)
Unpleasant-ness [%VAS]	1	12.99 (9.82)	50.42 (10.93)	20.12 (12.29)	46.06 (17.59)	19.53 (14.49)	43.06 (16.28)	21.12 (17.93)	18.44 (12.18)	46.51 (12.71)
	2	15.27 (15.02)	50.45 (18.73)	23.24 (21.12)	48.24 (17.02)	21.13 (20.42)	44.65 (19.79)	27.28 (21.73)	21.73 (18.31)	47.78 (17.67)
	3	18.04 (22.29)	47.02 (12.75)	20.29 (19.76)	51.48 (15.73)	21.61 (21.18)	46.85 (12.47)	16.51 (16.77)	19.11 (19.35)	48.45 (12.34)
	4	21.06 (31.51)	48.53 (19.63)	24.25 (32.86)	53.65 (19.95)	19.94 (19.33)	47.84 (19.45)	19.69 (29.24)	21.23 (27.79)	50.01 (19.01)
$PETCO_2$ [kPa]	1	5.59 (0.55)	5.44 (0.70)	5.35 (0.88)	5.43 (0.83)	5.44 (0.76)	5.53 (0.57)	5.50 (0.60)	5.47 (0.67)	5.47 (0.69)
	2	5.86 (0.35)	5.71 (0.74)	5.90 (0.40)	5.84 (0.60)	5.89 (0.42)	5.76 (0.67)	5.90 (0.48)	5.89 (0.41)	5.77 (0.66)
	3	5.73 (0.46)	5.75 (0.50)	5.63 (0.48)	5.69 (0.56)	5.63 (0.47)	5.61 (0.59)	5.57 (0.56)	5.64 (0.48)	5.68 (0.55)
	4	5.62 (0.62)	5.45 (0.73)	5.63 (0.67)	5.47 (0.71)	5.58 (0.69)	5.55 (0.62)	5.56 (0.62)	5.60 (0.64)	5.49 (0.67)
CV $PETCO_2$ [%]	1	2.52 (1.75)	3.52 (2.69)	2.19 (1.34)	3.34 (3.16)	2.93 (2.30)	3.66 (2.61)	3.13 (3.03)	2.69 (1.85)	3.50 (2.21)
	2	2.15 (1.37)	3.38 (3.18)	2.42 (1.66)	3.40 (3.60)	2.42 (1.31)	4.22 (4.64)	1.50 (1.28)	2.12 (0.94)	3.67 (3.67)
	3	1.95 (1.30)	2.46 (1.83)	2.68 (2.04)	4.19 (3.63)	2.68 (1.41)	3.77 (2.80)	3.05 (2.79)	2.59 (1.57)	3.47 (2.42)
	4	2.89 (2.36)	4.15 (4.08)	3.16 (2.49)	4.13 (3.18)	2.94 (2.11)	3.72 (3.29)	2.55 (1.79)	2.89 (1.86)	4.00 (3.19)
$PETO_2$ [kPa]	1	16.85 (1.14)	17.08 (0.85)	17.40 (1.04)	17.14 (1.14)	17.16 (1.32)	16.58 (1.67)	16.99 (1.59)	17.10 (1.20)	16.93 (1.10)
	2	17.36 (1.26)	17.68 (0.80)	17.98 (1.13)	18.00 (1.00)	18.09 (1.11)	18.04 (0.98)	18.26 (0.95)	17.92 (1.09)	17.91 (0.91)
	3	17.07 (1.55)	16.81 (1.62)	17.42 (1.53)	16.96 (1.62)	17.36 (1.59)	17.10 (1.48)	17.26 (1.60)	17.28 (1.55)	16.96 (1.56)
	4	16.54 (1.46)	15.99 (1.25)	16.81 (1.55)	16.35 (1.42)	17.13 (1.52)	16.40 (1.46)	16.69 (1.62)	16.79 (1.51)	16.25 (1.31)
Heart rate [bpm]	1	76.86 (10.53)	81.02 (9.92)	81.89 (11.45)	79.54 (11.32)	80.39 (11.94)	81.52 (11.89)	80.54 (11.91)	79.92 (10.97)	80.69 (10.61)
	2	76.50 (12.97)	77.17 (11.46)	78.64 (9.50)	79.69 (11.51)	79.53 (12.52)	79.93 (12.28)	80.49 (12.60)	78.79 (11.56)	78.93 (11.57)
	3	80.98 (9.11)	78.20 (12.12)	81.65 (11.02)	80.39 (10.29)	82.07 (10.44)	80.69 (10.99)	81.47 (9.32)	81.54 (9.59)	79.76 (10.58)
	4	75.85 (10.82)	78.74 (10.38)	77.93 (8.87)	81.97 (10.99)	79.96 (9.89)	81.30 (9.93)	80.23 (11.37)	78.49 (9.63)	80.67 (10.33)

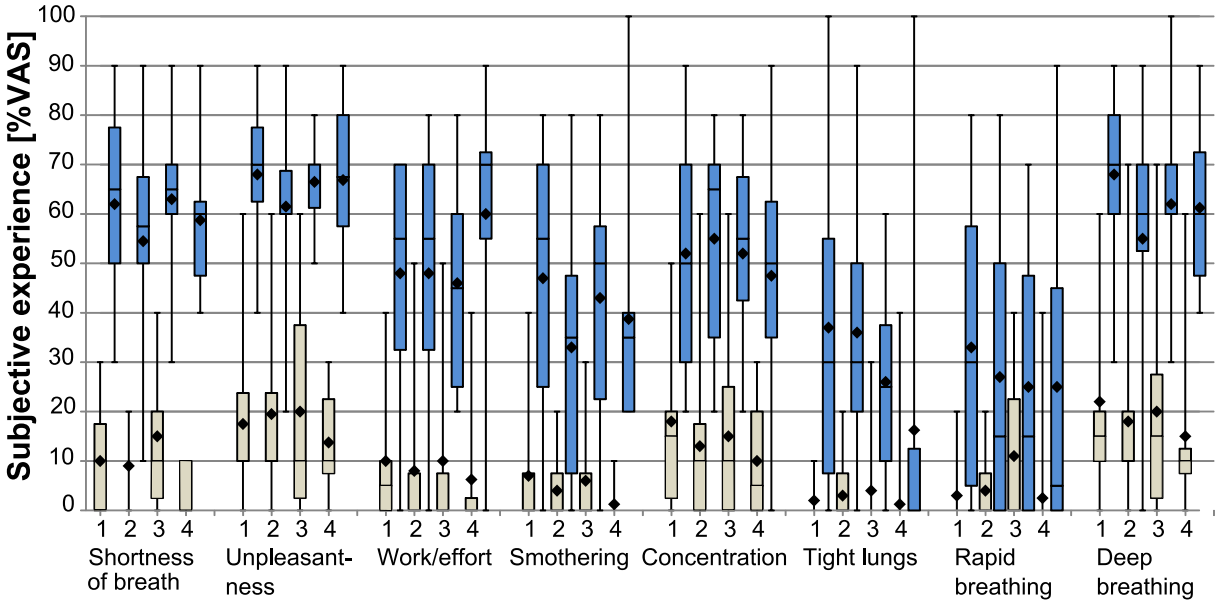


Figure 3.8: Subjective perception of dyspnoea measured by the MDP and presented by study visit (visits 1 to 4 after training session). Beige colour: data for unloaded periods. Blue colour: data for respiratory loading periods. Data presented as box-plots with interquartile ranges. Horizontal lines depict the median. Diamond shapes depict the mean. N=10.

Habituation between sessions

Mouth pressure necessary to induce 50%VAS unpleasantness did not change between sessions (F=.632, df=3, p=0.546) and the achieved unpleasantness did not vary (p=0.256). During unloaded periods, unpleasantness (p=0.199) and mouth pressure amplitude (F=.514, df=3, p=0.677) did not change between the four sessions. The subjective perception of dyspnoea measured with the MDP after each visit remained stable over all four study visits (Figure 3.8).

3.3.5 Qualitative feedback

The continuous mild hypercapnia used in this study was applied before ratings of respiratory unpleasantness were started, so that it would not be perceived as a change from baseline. During debrief after all sessions, all participants stated that they were not aware that the received continuous hypercapnia during some of the sessions. In a verbal interview, some participants also mentioned that they found it very difficult to evaluate the unpleasantness of

mild respiratory sensations in the lower third of the spectrum. A few participants further mentioned that they were not sure whether the change they noticed was real when it was mild or whether they fabricated a change because they were asked about their breathing and were trying to perceive a change.

The amount of CO_2-related physiological effects increased with increasing strength of hypercapnia (two participants felt uncomfortably hot and one participant felt claustrophobic during +0.9kPa hypercapnia, one participant felt uncomfortably hot during +0.6kPa hypercapnia).

3.4 Discussion

3.4.1 Main findings

In this study, we perception-locked the amount of respiratory loading (physical stimulus) to elicit a comparable level of respiratory unpleasantness (50%VAS) between participants at the beginning of each experimental session and then let respiratory unpleasantness vary whilst we measured respiratory unpleasantness to this respiratory load over the remainder of the session in order to investigate cchanges in perception. The three main findings of this study are: Firstly, mild hypercapnia (without respiratory loading) of between +0.4 and +0.9kPa significantly increased respiratory unpleasantness compared to completely unloaded breathing (no hypercapnia and no respiratory loading; Figure 3.4, hypercapnia). Secondly, the same amount of respiratory loading was necessary to elicit 50%VAS unpleasantness with and without hypercapnia, hence hypercapnia did not amplify unpleasantness in response to respiratory loading (Figure 3.4, hypercapnia and respiratory loading). Thirdly, unpleasantness

of respiratory loading did not habituate as a result of repeated application within and between sessions (Figure 3.7).

3.4.2 Benefits of isocapnia when inducing experimental dyspnoea during FMRI

Manual gas control during unloaded breathing and during the application of respiratory loading minimized intra- and interindividual variation of $PETCO_2$ (Figure 3.3). This has two potential benefits for using respiratory loading to investigate dyspnoea during FMRI. Firstly, isocapnia reduced $PETCO_2$ fluctuations within individual sessions compared to poikilocapnia. Controlling $PETCO_2$ in this way has the benefit of reducing intraindividual variations in BOLD responsiveness (Harris et al., 2006) and allows the dissociation of the signal of interest from BOLD activity caused by CO_2 fluctuations. Secondly, isocapnia reduced $PETCO_2$ fluctuations between participants. Previous literature shows hypercapnia or unaltered $PETCO_2$ in response to respiratory loading at a group level (Gorman et al., 1999; Hornstein and Ledsome, 1984; Isono et al., 1990; McKenzie et al., 1997; Rohrbach et al., 2003; von Leupoldt et al., 2008), which indicates between-subject variability in respiratory response to respiratory loads. In this study, application of a respiratory load that elicited 50%VAS unpleasantness increased variation in $PETCO_2$ within a group of ten participants during poikilocapnia (increase in CO_2 coefficient of variability, see Figure 3.2). During mild hypercapnia of +0.6kPa and +0.9kPa in the current study, application of such a load did not increase CO_2 variability (no change in CO_2 coefficient of variability, see Figure 3.2). Reducing this variability in $PETCO_2$ variability is beneficial for group FMRI studies.

3.4.3 First study investigating the effects of hypercapnia on unpleasantness during respiratory loading

To our knowledge, this is the first study investigating the effects of hypercapnia on the perception of respiratory loading. Here, we initially perception-locked the amount of

respiratory loading applied to elicit 50%VAS in order to generate a consistent stimulus-perception. We have shown that hypercapnia did not decrease the amount of respiratory loading necessary to induce 50%VAS unpleasantness and hence did not linearly increase respiratory unpleasantness. Therefore, adding mild continuous hypercapnia to respiratory loading at the levels used in this study does not provide the additional benefit of reducing the stimulus strength necessary for the induction of moderate dyspnoea unpleasantness that could reduce physiological confounds of respiratory loading during FMRI.

This study was not designed to investigate the mechanisms behind potential interactions of hypercapnia and respiratory loading, but our results in combination with the finding that inspiratory and expiratory loading are additive (Nishino et al., 2007) suggests three potential hypotheses for investigation. Firstly, it is possible that intensity and unpleasantness of dyspnoea behave differently with the intensity of dyspnoea increasing more linearly than the unpleasantness. Secondly, differences in linearity might be due to the different underlying physiological mechanisms by which respiratory loading and hypercapnia cause dyspnoea. It has previously been suggested that the dyspnoea caused by mechanical respiratory loading is mainly caused by activation of lung receptors, whilst hypercapnia mainly causes dyspnoea due to chemoreceptor activation (Lansing et al., 2009). It might be possible that dyspnoea evoked through the same physiological pathway influences sensations in an additive way, whilst dyspnoea that is processed by different afferent pathways is as strong as the most severe dyspnoea perceived. Thirdly, differences in top-down categorization of respiratory sensations have been shown to override differences in sensory input (Petersen et al., 2014). Hence differential interoceptive categorization of inspiratory loading as a dyspnoea stimulus and hypercapnia as ‘normal breathing’ might account for perceptual stability during respiratory loading. Future research studies are required to carefully investigate the

mechanistic underpinnings of these results, which could be a major milestone in understanding dyspnoea perception.

3.4.4 Effects of hypercapnia on unpleasantness during unloaded breathing

As hypercapnia is well known to induce air hunger (Lansing et al., 2009) and is one of the main stimuli used to investigate dyspnoea in the laboratory, it is not surprising that mild hypercapnia increases perceived unpleasantness compared to the unloaded condition. Increased unpleasantness during unloaded breathing is not ideal for the study of dyspnoea with BOLD as BOLD relies on the comparison of different conditions (e.g. unloaded breathing and respiratory loading). Increasing unpleasantness during unloaded breathing might decrease the change between the unloaded condition and respiratory loading on a perceptual and neural level. Perceptual ratings during unloaded respiratory periods are therefore advisable in order to correctly interpret BOLD differences between conditions.

3.4.5 Habituation of respiratory unpleasantness

Within-session habituation

Perceived unpleasantness remained stable over three consecutive 4-minute administrations of respiratory loading within one session. This is good news for the investigation of experimental dyspnoea during FMRI, where neural changes are usually obtained by averaging over multiple stimuli. A relatively stable stimulus is therefore necessary to obtain comparable neural responses at the beginning and at the end of the scan and we have shown that respiratory loading fulfills this criterion over the time course of a normal FMRI study.

Contrary to our expectations, our study has shown that unpleasantness during unloaded periods increased after the initial application of respiratory loading in each session. This effect remained present throughout four consecutive sessions that followed an initial exposure

session. During poikilocapnia, unpleasantness, mouth pressure and $PETCO_2$ were increased after the first respiratory load. Whilst isocapnia eliminated effects of respiratory loading on mouth pressure and $PETCO_2$, the difference in unpleasantness remained. This consistent increase in respiratory unpleasantness during unloaded breathing following the initial application of respiratory loading highlights the difficulty of assessing 'normal breathing' on a respiratory circuit. Unloaded breathing was rated at 10%VAS unpleasantness, which varied throughout the experiment. During the post-study interview, participants stated difficulties determining 'normal breathing' while they were on the respiratory circuit and many used a strategy in which they adjusted their rating given for the immediately previous experience by the currently perceived difference in unpleasantness. It hence seems possible that application of an unpleasant respiratory load alters the way in which unloaded breathing is evaluated. The variability in unpleasantness scores obtained during unloaded breathing and the qualitative interview both highlight the benefits of measuring subjective perception of unloaded breathing throughout the session to aid interpretation of results.

Habituation across sessions

There was no adaptation of respiratory unpleasantness across sessions during loaded or unloaded breathing. This makes respiratory loading a valuable tool for comparing dyspnoea over multiple sessions and allows its use during multi-session FMRI studies. Such a stable manipulation is important for FMRI intervention studies, e.g. pharmacological studies that involve administration of drug and control in separate sessions.

Perception of respiratory unpleasantness was stable in response to repeated respiratory loading. Previous studies have shown varying degrees of perceptual habituation to different respiratory stimuli. No habituation of dyspnoea perception in response to hypercapnia was shown in a study by Bloch-Salisbury and colleagues, who gave hypercapnic challenges to

four mechanically ventilated patients with experimentally induced continuous hypercapnia over two weeks (Bloch-Salisbury et al., 1996). Patients retained their original stimulus-response to the load. Whilst perceptual stability was also found during repeated breath holds within one laboratory session (Nishino et al., 1996) and during repeated exercise over 12 sessions in patients with chronic obstructive pulmonary disease (Carrieri-Kohlman et al., 2001), there is evidence of perceptual habituation in response to repeated hypercapnic rebreathing challenges in healthy individuals (Li et al., 2006; Wan et al., 2009). It seems like habituation of dyspnoea perception occurs during short laboratory challenges of hypercapnia, whilst repeated administration of hypercapnia in ventilated patients, respiratory loading, breath holding and exercise show higher perceptual stability. This would need to be investigated within the same study in order to disentangle differences that might originate from differences in study context and exact sensations rated.

Understanding the mechanisms underlying the habituation of sensory and affective responses to respiratory sensations could explain in which contexts and to which stimuli experiential habituation occurs. To our knowledge, potential neuronal mechanisms of habituation to respiratory unpleasantness in response to experimental dyspnoea stimuli have not been investigated, but one study has shown perceptual and neuronal habituation to short inspiratory occlusions (12 blocks) during late compared to early experimental periods used electroencephalography (EEG) (von Leupoldt et al., 2011b). This suggests the habituation of neural processing of respiratory sensations as a potential mechanism for reduced respiratory perceptions. Additional clues might be found when investigating the complex interplay between dyspnoea and other cognitive and emotional states. Trait anxiety has been shown to influence the rate of perceptual habituation to dyspnoea stimuli, with higher anxiety resulting in less experiential habituation (Li et al., 2006). A striking additional finding in our study was the large interindividual variation in respiratory unpleasantness. The interindividual

differences in perception of dyspnoea in the current study largely outweighed the more subtle differences between hypercapnia conditions and might mask subtle habituation effects of particular groups, which would need to be investigated in future studies.

3.5 Conclusion

This study set out to test the applicability of isocapnic respiratory loading to the investigation of dyspnoea during FMRI. We showed that mild hypercapnia can be used to generate isocapnia that reduces $PETCO_2$ variability within sessions and between participants, which has advantages for the analysis of experiments using respiratory loading to investigate neural mechanisms of dyspnoea using BOLD. There was no advantage of hypercapnia on perception of respiratory loading, whilst the increased unpleasantness during unloaded breathing could be deleterious to FMRI if not accounted for. The unpleasantness associated with repeated induction of dyspnoea with respiratory loading remained stable over four sessions, indicating the usability of this model for multi-session FMRI paradigms. Whilst no one model is presently ideal for the study of breathlessness, we conclude that a carefully titrated combination of continuous mild hypercapnia and respiratory loading (stimulus to induce dyspnoea) will allow the study of the perception of dyspnoea in healthy volunteers during multi-session FMRI studies. Future technical neuroimaging studies will be needed in order to optimize the benefits and disadvantages of hypercapnic isocapnia for the investigation of dyspnoea.

Chapter 4

The effects of altered intrathoracic pressure on resting cerebral blood flow and its response to visual stimulation

Abstract Chapter 4

Investigating how intrathoracic pressure changes affect cerebral blood flow (CBF) is important for a clear interpretation of neuroimaging data in patients with altered respiratory physiology (due to disease or mechanical ventilation) and in research paradigms that might affect intrathoracic pressure. Here, we investigated the effect of experimentally increased and decreased intrathoracic pressures on grey matter CBF and the stimulus-evoked hemodynamic response to visual stimulation.

Twenty healthy volunteers received intermittent inspiratory and expiratory loads (plus or minus 9cmH_2O for 270 seconds) and viewed an intermittent 2Hz flashing checkerboard, while maintaining stable end-tidal CO_2. CBF was recorded with transcranial Doppler sonography (TCD) and whole-brain pseudo-continuous arterial spin labeling magnetic resonance imaging (PCASL MRI).

Application of inspiratory loading showed an increase in TCD-measured CBF of 4% and a PCASL-measured increase in grey matter CBF of 5% at stable mean arterial pressure (MAP). Expiratory loading did not alter CBF, while MAP increased by 3%. Neither loading condition altered the perfusion response to visual stimulation in the primary visual cortex. Localized relative CBF increases were shown in the somatomotor cortices and the cerebellum.

Altered intrathoracic pressures, whether induced experimentally, therapeutically or through a disease process, have possible significant effects on CBF and should be considered as a potential systematic confound in the interpretation of perfusion-based neuroimaging data.

4.1 Introduction

The brain regulates its blood supply in order to ensure adequate delivery of oxygen and nutrients in the face of changing systemic blood pressure and regional changes in metabolic demand. Cerebral autoregulation is the hemodynamic process that keeps cerebral blood flow (CBF) within tight physiological limits during changes in perfusion pressure (mean arterial pressure minus intracranial pressure) via adaptations in cerebrovascular resistance (van Beek et al., 2008), while neurovascular coupling matches local CBF to changes in underlying neuronal activity (Nielsen and Lauritzen, 2001).

Global CBF can be affected by physiological modulations or pharmacological agents and in turn alters the CBF-derived blood oxygen level dependent (BOLD) response (Brown et al., 2003; Cohen et al., 2002). Altered intrathoracic pressures could potentially affect CBF and could therefore be a potentially significant confound of CBF-derived imaging methods via direct localized pressure effects on the great arteries of the chest, as well as through systemic changes in arterial blood pressure and venous return from the brain (Kolbitsch et al., 2000). It is therefore important to examine how these potential effects might confound the interpretation of CBF-derived functional neuroimaging methods, such as arterial spin labeling (ASL) or BOLD contrast. Studies using transcranial Doppler (TCD) sonography as an indirect measure of CBF during experimentally decreased inspiratory pressures (and hence reduced intrathoracic pressures) have shown increases in CBF of up to 18% depending on the strength of the respiratory maneuver (Reinhard et al., 2000), while increasing expiratory pressure to +40cmH_2O has been shown to reduce CBF measured with TCD by ~35% (Tiecks et al., 1996).

Changes in intrathoracic pressure beyond the normal range of 0 to -5cmH_2O are relevant to a variety of clinical situations. For example, intermittent positive pressure ventilation (a technique in which air is blown into the lungs in a normal inspiratory rhythm, causing increased intrathoracic pressure during inspiration) is used to ventilate unconscious patients during surgery and critically ill intensive care patients at pressures of +15 to +30cmH_2O. Positive end expiratory pressure (PEEP) of up to +10cmH_2O is often added to prevent lung collapse. Continuous positive airway pressure (CPAP) breathing, during which expiratory pressures of +5 to +10cmH_2O are applied continuously throughout the respiratory cycle, is used as a treatment of sleep apnea. Additionally, CBF regulation is important in a wide range of medical disorders during neuro-intensive care, where both the CBF effects of the underlying condition (e.g. ischemic stroke) and the ventilator-induced intrathoracic pressure changes contribute to CBF.

Furthermore, chronically altered intrathoracic pressures occur in several specific disease states, such as in chronic asthma, where inspiratory pressures during an attack might reach up to -30cmH_2O, and in chronic obstructive pulmonary disease (COPD), where the lungs are chronically hyperinflated and total ventilation is increased. Inspiratory pressure at rest measured in 25 patients with severe COPD reached -13cmH_2O, with expiratory pressures of approximately +3cmH_2O (Montes de Oca et al., 1996). Investigating the effects of altered chronic respiratory pressures on CBF might improve our understanding of the specific mechanisms of respiratory disorders and their implications for cerebral and systemic circulation.

Intrathoracic pressure may be altered by breathing against a respiratory load (inspiratory, expiratory or both). Respiratory loading is an integral part of laboratory models studying dyspnoea (breathlessness), a major symptom of respiratory conditions. This method has been applied to patients (COPD (Sturdy et al., 2004) and asthma (von Leupoldt et al., 2009b)), as well as to create a sensation of heightened respiratory work/effort in healthy volunteers (Banzett et al., 2008). These healthy volunteer models can be used to gain an understanding of the neuronal mechanisms involved in the perception of breathlessness with FMRI (Evans et al., 2002). As such, it is important to understand the effects of such interventions for scientific investigations and for common clinical scenarios.

Classical physiology teaching states that CBF is affected by systemic factors (e.g. systemic vascular resistance, arterial blood pressure, venous return, arterial carbon dioxide and oxygen tensions) and by local factors within the brain (e.g. autoregulation). In order to use FMRI to examine brain activity of patients with altered intrathoracic pressure or of healthy volunteers undergoing respiratory loading, we need to establish the effects of respiratory loading, both on baseline CBF and on CBF changes during neuronal activation. In this study we aimed to show the effects of inspiratory and expiratory loading on CBF measured in two ways; with TCD and with arterial spin labeling (ASL) FMRI, to determine whether or not respiratory loading induces CBF changes that might alter the perfusion response to visual stimulation. Firstly, informed by previous studies using TCD during inspiratory loading (decreased inspiratory pressure), we hypothesize that inspiratory loading will lead to increased CBF and that expiratory loading (increased expiratory pressure) will lead to a decrease in CBF. If the induced CBF changes are relatively large, these could potentially alter the CBF response to visual stimulation, similar to the way in which increased CO_2 and the subsequent increase in CBF decrease the BOLD response to visual activation (Cohen et al., 2002). We furthermore

expect inspiratory and expiratory loading to show locally increased perfusion in brain areas that are engaged in the sensorimotor control of breathing.

4.2 Materials and Methods

4.2.1 Brief overview

Our study consisted of three sessions: a training session, a laboratory session in which CBF was measured indirectly (with TCD) and continuous non-invasive mean arterial blood pressure (MAP) measurements were taken and a third session in the MRI scanner during which CBF was measured in the whole brain with PCASL FMRI. TCD enables the beat-by-beat investigation of local blood velocity changes in the middle cerebral artery velocity (MCAV), which reliably correlate with changes in CBF (Bishop et al., 1986) and hence can be used as an indirect measure of CBF as long as a stable diameter of the insolated vessel and constant blood vessel resistance and permeability throughout the brain can be assumed.

Arterial spin labeling (ASL) allows serial measurement of relative CBF to be taken over several minutes from the whole brain (Dai et al., 2008). During ASL, water molecules in flowing blood are magnetically labeled at the level of the neck. An image is acquired in the brain once this labeled blood water has exchanged from the vasculature into the brain tissue. Tissue perfusion, or CBF, can then be assessed by subtracting this labeled image from a control image in which the inflowing blood water is not magnetically labeled. We used a recently developed variant of ASL, PCASL (Dai et al., 2008), which provides enhanced labeling efficiency and a resultant increase in the signal-to-noise ratio of the CBF measurements.

4.2.2 Data acquisition

Participants

Twenty volunteers (mean age 27 (SD±6) years, 6 female) participated in this study after giving written informed consent in accordance with the Oxfordshire Clinical Research Ethics Committee. Participants were healthy right-handed non-smokers with no history of significant neurological, pulmonary or cardiovascular disease, who were free from acute illness (e.g. acute upper respiratory tract infection). Participants abstained from excessive consumption of alcohol (24 hours), caffeine (six hours) and heavy meals (two hours) before the experiment and had sufficient sleep the preceding night. Female participants either used hormonal contraception or were at the same stage of their menstrual cycle during both parts of the experiment.

The 20 volunteers were drawn from a total of 27 participants, seven of which were excluded for the following reasons: one did not tolerate the visual stimulus, four did not comply with the study protocol, and two displayed excessive head motion during FMRI. These complete datasets were excluded from all analyses.

Respiratory loading

The same experimental paradigm was performed in both experimental sessions (Figure 4.1). In a supine position, participants wore a nose clip (Air Safety Ltd, Morecambe, UK) and standard foam earplugs while breathing room air through a mouthpiece connected to a custom-built respiratory circuit (shown in Figure 4.2). Participants were exposed to intermittent inspiratory or expiratory loads of either minus or plus 9cmH_2O, which was induced by partially occluding either the inspiratory or the expiratory limb of the breathing circuit with a balloon that was remotely controlled by a hydraulic system. Intrathoracic

pressure can be inferred through non-invasive measurements of mouth pressure, which is approximately equivalent to measurements of esophageal pressure (Baydur et al., 1982).

Respiratory control

As a strong cerebrovascular vasodilator, CO_2 has been shown to alter CBF and to induce changes in the BOLD FMRI signal (Cohen et al., 2002). To avoid confounding influences of CO_2, the partial pressure of end-tidal CO_2 ($PETCO_2$) was kept stable throughout this study. A $PETCO_2$ baseline value was determined for each individual at the beginning of each experimental session by monitoring $PETCO_2$ over two minutes after an 8-minute habituation period. The $PETCO_2$ target value was set 0.75mmHg (0.1kPa) lower than the baseline. During the training session, participants learnt to keep their end-tidal CO_2 stable. At the beginning of each session, participants were instructed to keep their $PETCO_2$ stable within 0.75mmHg of their target value throughout the paradigm via numerical visual feedback that was back-projected to participants onto the centre of a screen behind them, visible via a mirror (similar set-up to Pattinson et al. (2009)).

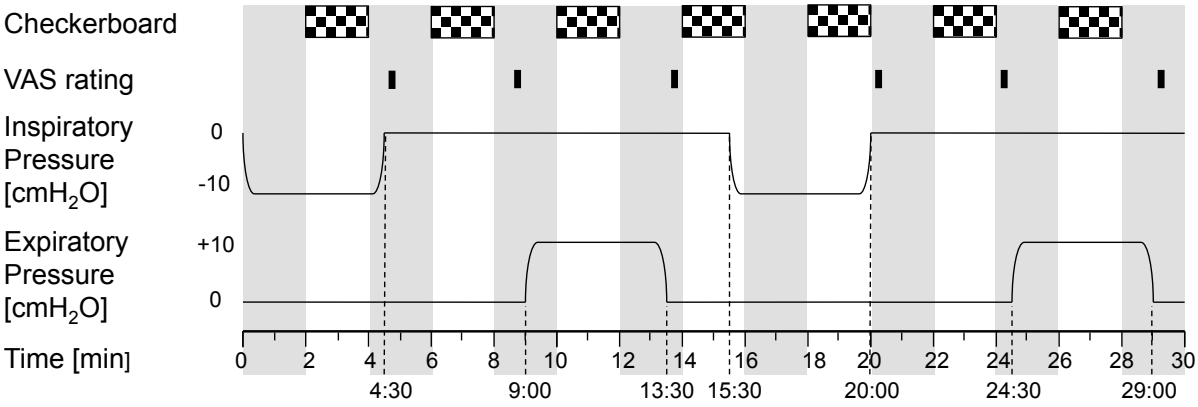


Figure 4.1: Timeline of experimental paradigm, consisting of a 4 ½-minute block of inspiratory resistance of -9cmH_2O, 4 ½ min of normal breathing and 4 1/2min of expiratory resistance of +9cmH_2O, a normal breathing period of 2 minutes and a second cycle of inspiratory, normal and expiratory resistances (4 1/2min each). After each block, participants rated their average breathlessness over that block. A 2Hz black and white flashing checkerboard was presented for 120 seconds every 240 seconds. $PETCO_2$ values remained visible throughout baseline and visual stimulation.

All physiological data were sampled at 50Hz and were logged via a Power1401 (CED, Cambridge, UK) using Spike2.7 (CED, Cambridge, UK). $PETCO_2$ was calculated on a breath-by-breath basis through an online script in Spike2.7 and was fed back to the participant via a stimulus presentation program (Presentation, Neurobehavioral Systems, Albany, USA) on a second computer.

Behavioral responses were made using a custom-made button box and were represented on a horizontal visual analog scale (VAS). This scale allowed participants to answer the question "How strong was your breathlessness averaged over the last 4 ½ min block?" with the anchors 'none' on the left and 'unbearable' on the right. Participants were told to interpret the bar as a scale from 0 to 10, where 9 would indicate severe breathlessness and the wish to terminate the experiment.

Every four minutes, participants were shown a 2Hz flashing checkerboard for two minutes (see Figure 4.1). A 2Hz flashing checkerboard induces robust activations of the visual cortex (Fox and Raichle, 1985), hence allowing us to detect potential intrathoracic pressure related changes in the perfusion increase to visual activation with high sensitivity.

Immediately after each session, participants completed an early version of the Multidimensional Dyspnoea Profile (MDP). This questionnaire was used to evaluate the mean unpleasantness of breathing during the three respiratory conditions (unloaded, inspiratory and expiratory loading) on a scale from zero to 10, as well as the occurrence and strength of six sensory dimensions of dyspnoea (work/effort, smothering, concentration, tight lungs, rapid breathing and deep breathing) on a scale from one to 10. A version of the questionnaire as used in this study can be found in Appendix Ia.

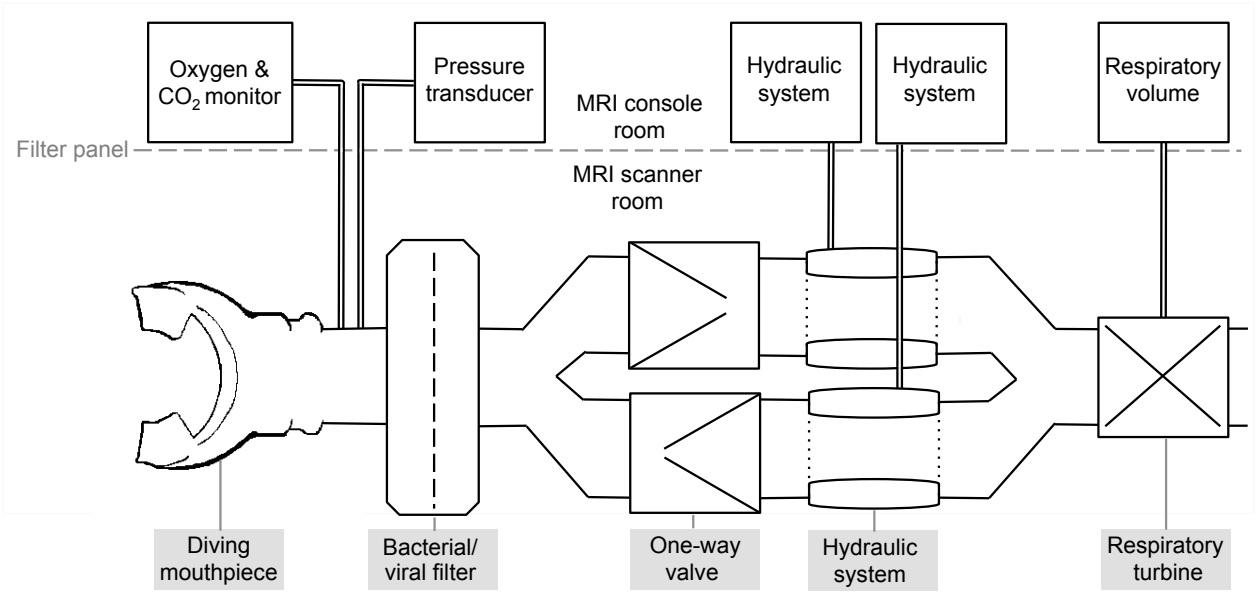


Figure 4.2: Schematic diagram of respiratory circuit. A diving mouthpiece (Scubapro UK Ltd, Mitcham, UK) connects to a bacterial and viral filter (Vitalograph, Buckingham, UK) from which respiratory gases and respiratory pressure are sampled via polyethylene extension tubing (Vygon SA, Ecouen, France). One sampling line leads to a pressure transducer (MP 45, +/-50cmH_2O, Validyne Corp., Northridge, CA, USA) connected to an amplifier (Pressure transducer indicator, PK Morgan Ltd, Kent, UK). During the physiology session, the second sampling line connects to a gas analyzer that samples oxygen and CO_2 (Capnomac Ultima, Datex Ohmeda, Helsinki, Finland), while during the scanning session tidal CO_2 was measured with a rapidly responding gas analyzer (CD-3A and S-3A; AEI Technologies, Pittsburgh, PA, USA). The filter connects to a Y-piece, creating an inspiratory and an expiratory limb through one-way valves (Hans Rudolf, Kansas City, MO, USA). The diameter of a 5cm long section of the inspiratory and the expiratory channel can be altered remotely via a hydraulic system. Each modifiable section of tubing is lined with a rubber party balloon connected via non-distensible plastic tubing to a 20ml syringe filled with water and used for inflation and deflation of the balloon. The inspiratory and expiratory tubes recombine through a Y-piece and an attached turbine (VMM-400, Interface Associates, Aliso Viejo, CA, USA) records inspiratory (V_i) and expiratory (V_e) volumes.

Recordings during laboratory session

A 2.0 MHz pulsed wave Doppler transducer (DWL Elektronische Systeme GMbH, Sipplingen, Germany), held over the right temporal bone window, provided continuous MCAV. Standard vessel identification criteria were used and the signal was sampled at the individual's optimum depth between 50–55 mm. Continuous non-invasive blood pressure was measured with a Finapres blood pressure monitor (Ohmeda 2300, BOC Health Care, USA). The cuff was applied to the midphalanx of the middle or ring finger of the non-dominant hand

and placed at heart level. Beat-to-beat systolic and diastolic pressures, as well as MAP, were computed. MAP was obtained as the integral of pressure over one beat divided by the corresponding beat interval. Pulse pressure (PP) was calculated as the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP). Cerebrovascular resistance (CVR) was calculated with the formula CVR = MAP/ MCAV. Blood pressure was recorded during the laboratory session only, due to the lack of availability of an MRI compatible device. As both experimental sessions were otherwise performed in an identical manner, and as the physiology measurements and CBF changes in both sessions were comparable, we assume that similar blood pressure changes occurred during the MRI scanning session. Heart rate was measured with a pulse oximeter (Capnomac Ultima, Datex Ohmeda, Helsinki, Finland). Respiratory measures were obtained as explained in Figure 4.2.

Recordings during MRI session

Participants were scanned with a 3 tesla Siemens Verio MRI scanner (Siemens, Erlangen, Germany) with a 32-channel radiofrequency receive coil, using foam inserts to minimize head motion. A whole-brain PCASL sequence was utilized with a gradient-echo echo-planar (EPI) readout (PCASL module flip angle 20°, labeling duration=1.4 s, post labeling delay = 1 s, EPI readout flip angle 90°, TR= 4.0 s, TE= 14ms, 6/8 k-space). We acquired twenty-two axial slices in ascending order (450 volumes, 3.8 x 3.8 x 5.0 mm voxels, interslice gap=1.75 mm,). Pre-saturation of the imaging region was performed just prior to labeling to suppress static tissue signals, reducing the sensitivity to subject motion and physiological noise. A time-of-flight angiography scan was acquired in the neck to allow visualization of the main feeding arteries. The PCASL labeling plane was positioned approximately halfway between the upper and lower contortions of the vertebral arteries, so that the plane was perpendicular to both the vertebral and carotid arteries, thereby optimizing the labeling efficiency. For each subject an MPRAGE structural image was acquired to enable registration of functional data. Continuous

heart rate was recorded with a pulse oximeter (MR multigas monitor, MR Equipment, model 9500) and respiratory measures were obtained as described in Figure 4.2.

4.2.3 Data analysis

Physiological recordings

Data were exported to MatLab (MathWorks Inc., Natick, MA, USA) and analyzed using custom-written scripts. Respiratory parameters (CO_2 partial pressures and respiratory pressures), circulatory parameters (heart rate and MAP) and MCAV were averaged within subject across each of the six respiratory resistance periods (unloaded breathing, inspiratory loading and expiratory loading [x2]). No difference between the first and the second repeat of a condition were found and the results for both repeats were combined.

PCASL

Analysis of the PCASL FMRI images to identify regions exhibiting significant perfusion changes was carried out using tools from the FMRIB Software Library (FSL, www.fmrib.ox.ac.uk/fsl). Full perfusion signal modeling was performed in FEAT (FMRI Expert Analysis Tool in FSL), with the ASL tag-control difference and the stimulus time courses modeled as separate explanatory variables (EV) and perfusion change obtained from their interaction. Non-brain structures (i.e. skull and surrounding tissues) were removed using BET (Smith, 2002) and 4D images were motion corrected using MCFLIRT (Jenkinson et al., 2002b). Spatial smoothing using a Gaussian kernel of full-width-half-maximum 5mm and high pass temporal filtering were performed (Gaussian-weighted least-squares straight line fitting with a high-pass-filter cutoff of 12 seconds). After preprocessing, the functional scans were co-registered to the individual's structural scan (linear registration) and then to the MNI152 standard space (average T1 brain image constructed from 152 normal subjects at the Montreal Neurological institute, Montreal, QC, Canada) using nonlinear registration. ASL

images were corrected for physiological noise using RETROICOR (Glover et al., 2000; Harvey et al., 2008). RETROICOR involves fitting low-order Fourier series to the EPI data based on the time of each image acquisition relative to the phase of the cardiac (3 terms) and respiratory cycles (4 terms) and their interaction (1 term).

FEAT (http://www.fmrib.ox.ac.uk/fsl/feat5/index.html) was used to compare the relative CBF change in response to inspiratory and expiratory resistance to CBF while breathing was unloaded. The general linear model (GLM) included the following EVs: mouth pressure during inspiration (P_{min}) that indicated the presence of inspiratory loading, mouth pressure during expiration (P_{max}) that indicated the presence of expiratory loading, $PETCO_2$, visual stimulation, the act of rating, breathlessness score and six motion parameters. Interactions between inspiratory loading and visual activation and expiratory loading and visual activation were modeled. Voxel-wise statistical analysis was extended to a second (group) level in a mixed effects analysis using a cluster threshold of $Z>2.3$ and a (corrected) cluster significance threshold of $P=0.05$.

A region-of-interest approach was used to calculate mean fractional perfusion changes in response to inspiratory and expiratory loading in the whole brain grey matter (grey matter masks determined individually by segmenting participants' structural scans with FAST (http://www.fmrib.ox.ac.uk/analysis/research/fast/)), and in the individual brain areas with significantly increased perfusion demonstrated on voxel-wise analysis. It is possible that localized CBF increases (e.g. activation due to the work of breathing against load etc.) might elevate the general global CBF change. Therefore, we performed a region of interest analysis specifically excluding localized CBF increases (i.e. sensorimotor cortices and cerebellum) that were assumed to be task specific.

An anatomical mask of the primary visual cortex (V1) was created using the Juelich histological atlas within FSLView (http://www.fmrib.ox.ac.uk/fsl/fslview/index.html) with a probability threshold applied at 0.35. This mask was used to determine perfusion changes in the visual cortex in response to visual stimulation and the interactions between visual stimulation and respiratory loading.

4.3 Results

4.3.1 Laboratory session

Physiological results of the laboratory session are reported in the first part of Table 4.1. There was no significant difference between the first and second block of each stimulation condition, hence values are presented as averages over both blocks. Mean arterial pressure increased from 90.7±15.2mmHg to 93.8±15.7mmHg, during expiratory loading, while $PETCO_2$ and HR remained stable for both loading conditions. The time course of MCAV, $PETCO_2$ and minimum and maximum mouth pressure values throughout the first half of the laboratory session are illustrated in Figure 4.3.

4.3.2 MRI session

The mean change in relative CBF (±SD) in the grey matter and the primary visual cortex (V1) are reported in Table 4.2. Areas in which perfusion increased during inspiratory loading are shown in the top panel of Figure 4.4. Areas in which perfusion increased during expiratory loading are shown in the bottom panel of Figure 4.4. Locations of peak perfusion increase, Z scores of peak voxels (Z_{max}) and % perfusion increase during inspiratory and expiratory loading within the respective activity masks are provided in Table 4.3. No relative CBF decreases were seen in any of these regions of interest during the different loading conditions.

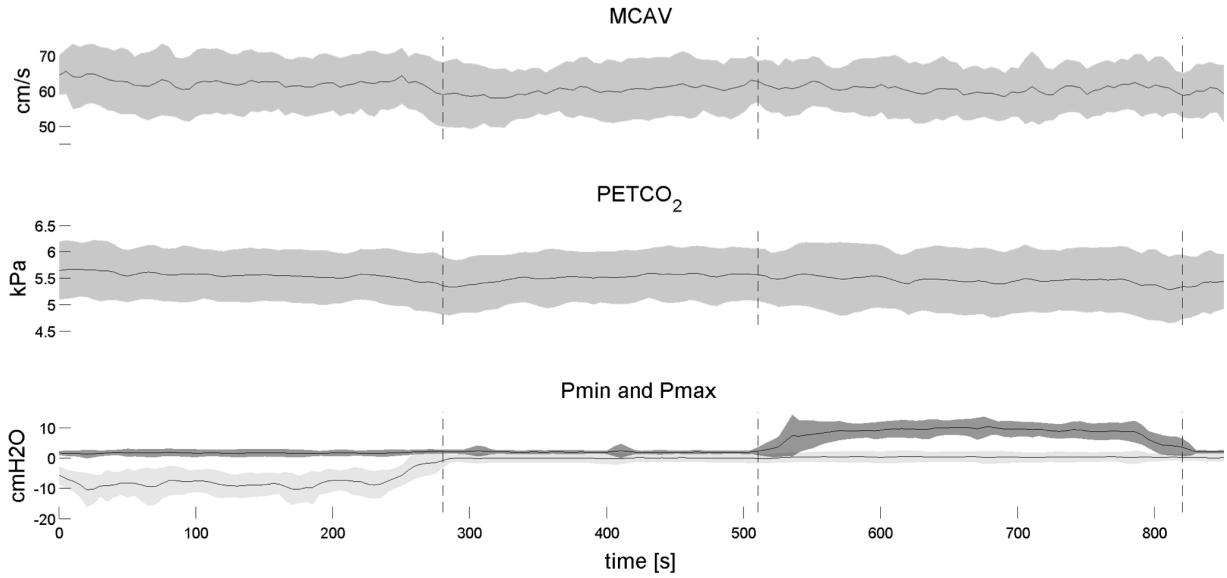


Figure 4.3: MCAV, $PETCO_2$, Pmin (light grey SD) and Pmax (dark grey SD) averaged across 20 participants and plotted over the first half of the physiological recording session (mean ± SD).

In response to inspiratory loading, significant regional increases in perfusion were found in the right primary and secondary somatosensory cortices, the right premotor and primary motor cortices and in cerebellar lobe VI. Significant perfusion increases in the somatomotor cluster extended over the primary and secondary somatosensory and the primary motor cortex and the premotor cortex. The locations and Z scores (Z_{max}) of local peak voxels are shown in Table 4.3. Additional areas commonly activated in respiratory studies using BOLD showed increased perfusion in our data only when no cluster threshold was applied. Table 4.4 shows the location of peak voxels in clusters that showed a significant Z score of above 2.3, but that did not survive the application of the cluster threshold.

Table 4.1: The first part of the table shows physiological measures during the laboratory session during unloaded breathing, inspiratory loading and expiratory loading. The lower part of the table shows physiological measures collected during the scanning session.

	Unloaded breathing	**Inspiratory loading**	**Expiratory loading**
Physiology session			
MCAV [cm/s]	60.6 ± 6.2	62.6 ± 7.0*	60.7 ± 6.1
MAP [mmHg]	90.7 ± 15.2	89.8 ± 15.4	93.8 ± 15.7**
SBP [mmHg]	134.8 ± 19.1	134.1 ± 18.6	138.4 ± 17.9**
DBP [mmHg]	68.7 ± 14.6	67.8 ± 14.6	71.4 ± 16.0*
Pulse pressure [mmHg]	66.1 ± 11.6	66.2 ± 9.8	67.0 ± 11.8
CVR [mmHg/cm/s]	1.5 ± .30	1.4 ± .30**	1.53 ± .31
HR [bpm]	59.0 ± 7.3	59.3 ± 6.6	59.0 ± 6.7
$PETCO_2$ [mmHg]	41.3 ± 3.8	41.3 ± 3.8	41.3 ± 4.5
$PETO_2$ [mmHg]	110.3 ± 6.0	109.5 ± 6.0	110.3 ± 8.3
Minute ventilation [l/min]	6.3 ± 2.0	5.9 ± 2.8	6.3 ± 2.0
Resp. rate [breaths/min]	18.2 ± 11.5	16.3 ± 11.3	15.9 ± 10.0
P_{min} [cmH_2O]	-0.1 ± 1.5	-8.8 ± 2.6***	0.2 ± 1.7
P_{max} [cmH_2O]	2.0 ± 0.7	1.6 ± 1.1*	9.3 ± 1.9***
Breathlessness [VAS 0-10]	0.9 ± 1.2	4.1 ± 1.6***	3.6 ± 2.0***
MRI session			
$HR_{(scan)}$ [bpm]	55.9 ± 7.8	56.7 ± 7.3	56.6 ± 7.3
$PETCO_{2\,(scan)}$ [kPa]	5.5 ± 0.7	5.5 ± 0.7	5.5 ± 0.8
$P_{min\,(scan)}$ [cmH_2O]	-0.8 ± 0.4	-9.8 ± 1.7***	0.2 ± 0.9**
$P_{max\,(scan)}$ [cmH_2O]	1.0 ± 0.5	0.3 ± 0.9***	9.1 ± 2.0***
$Breathlessness_{(scan)}$ [VAS 0-10]	0.7 ± 1.0	4.1 ± 1.9***	3.9 ± 2.1***

Stars indicate statistically significant differences between respective loading condition and unloaded breathing. * $p<0.05$, ** $p<0.01$, ***$p<0.001$. Mean ±SD MCAV (middle cerebral artery velocity), MAP (mean arterial pressure), SBP (systolic blood pressure), DBP (diastolic blood pressure), pulse pressure, CVR (cerebrovascular resistance), HR (heart rate), $PETCO_2$ (partial pressure of end-tidal CO_2), $PETO_2$ (partial pressure of end-tidal oxygen), minute ventilation, resp. rate (respiratory rate), P_{min} (minimum pressure at mouth), P_{max} (maximum pressure at mouth), breathlessness ratings averaged over both 4 ½min blocks for each respiratory loading condition and during unloaded breathing, unpleasantness as indicated on the questionnaire after the session. The subscript $_{(scan)}$ indicates that data was collected during the MRI session.

Even when the localized brain activity related to increased breathing effort was excluded from analysis, global CBF remained increased during inspiratory loading, which indicates a small, but significant increase in baseline CBF (+0.5% per cmH_2O, p=0.026, Table 4.2).

Table 4.2: Mean ± SD relative CBF changes measured with arterial spin labeling as percentage change from baseline in the global grey matter (total GM), the mean grey matter CBF excluding areas specifically activated in response to the loading task (non-task GM) and in an anatomical mask of the primary visual cortex (V1) in response to inspiratory and expiratory loading, CO_2, visual stimulation and the interaction between visual stimulation and inspiratory or expiratory loading.

Condition	Brain region	CBF change
Inspiratory load	Total GM	+0.5 ± 0.8* [%/cmH_2O]
	Non-task GM-V1	+0.5 ± 0.8* [%/cmH_2O]
Expiratory load	Total GM	+0.3 ± 0.8 [%/cmH_2O]
	Non-task GM-V1	+0.2 ± 0.8 [%/cmH_2O]
CO_2	Total GM	+24.0 ± 11.3*** [%/kPa]
Visual stimulation	V1	+14.7 ± 5.8*** [%]
Interaction: visual and inspiratory loading	V1	+0.1 ± 1.9 [%]
Interaction: visual and expiratory loading	V1	+0.6 ± 1.9 [%]

Stars indicate statistically significant differences between respective loading condition and unloaded breathing. * p<0.05, ** p<0.01, ***p<0.001.

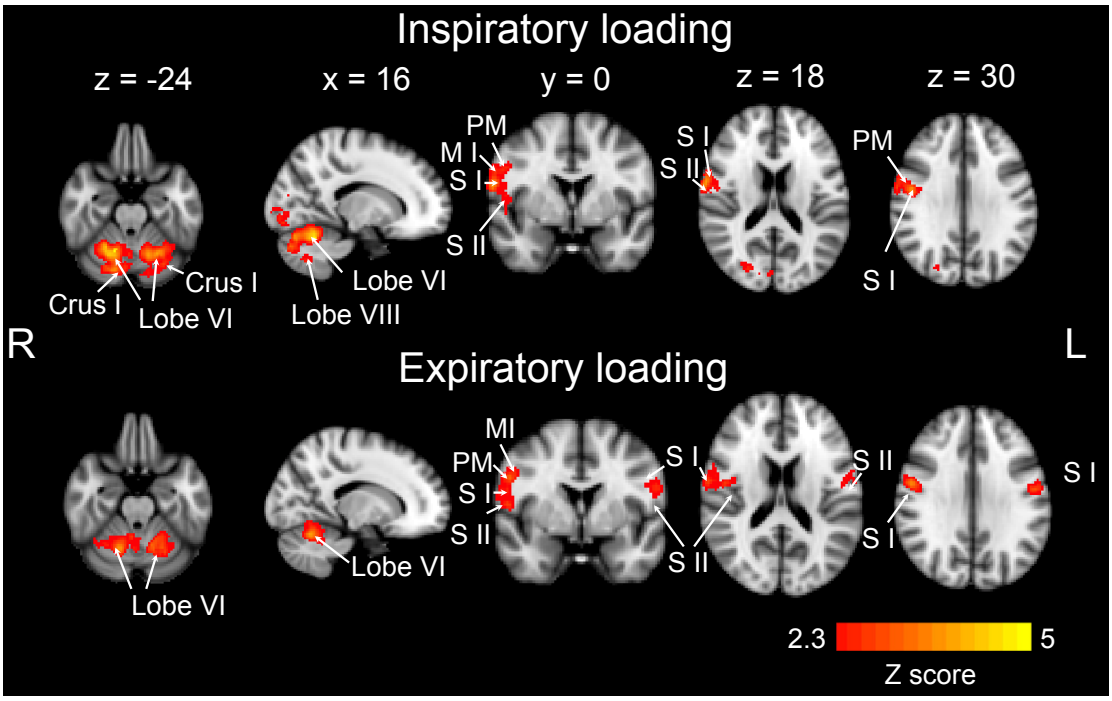


Figure 4.4: Significant CBF increases (ASL) during inspiratory and expiratory loading compared to unloaded breathing (N=20). PM = premotor area, M1 =motor cortex, S I = primary somatosensory cortex, S II = secondary somatosensory cortex.

Table 4.3: Peak voxel locations, peak voxel Z scores (Z_{max}) and % perfusion increase (averaged over cluster) in activation cluster during inspiratory and expiratory loading.

Condition	Region	Peak voxel location	Z_{max}	Perfusion increase [%/cmH_2O]
Inspiratory loading	Right somatosensory cortex	X=62, Y=0, Z=18 X=48, Y=-4, Z=30 X=62, Y=-4, Z=30 X=52, Y=-2, Z=10	4.13 3.96 3.26 3.13	1.3 ± 0.9***
	Cerebellum	X=16, Y=-58, Z=-24	5.09	1.5 ± 1.0***
Expiratory loading	Primary motor cortex	Right: X=54, Y=-4, Z=32 X=50, Y=-10, Z=38 X=50, Y=-8, Z=34 Left: X=-50, Y=-8, Z=40 X=-52, Y=-8, Z=40 X=-44, Y=-12, Z=38	Right: 4.01 3.95 3.86 Left: 3.78 3.63 3.41	1.0 ± 0.9***
	Somatosensory cortex	Right: X=62, X=-6, X=16 X=44, X=-8, X=22 X=56, X=0, X=10 Left: X=-56, Y=-8, Z=26 X=-58, Y=-4, Z=24 X=-52, Y=-6, Z=20	Right: 3.35 3.25 3.23 Left: 3.53 3.49 3.12	
	Cerebellum	X=18, Y=-60, Z=-22	4.23	1.4 ± 1.3***

Stars indicate statistically significant differences between respective loading condition and unloaded breathing. ***p<0.001.

During application of expiratory loading, relative CBF increased in the bilateral somatosensory and motor cortices and in the cerebellar lobe VI. Six local maxima were found in the left and right somatomotor clusters. Their locations and Z scores are shown in Table 4.3.

The voxel-wise analysis demonstrated a robust bilateral increase in the PCASL difference signal in the visual cortex in response to visual stimulation (Z_{max}= 7.11, not shown). This corresponds to a 14.7% increase in relative in CBF response to visual stimulation compared to baseline in a standard anatomic mask of the primary visual cortex (V1). No significant interactions were observed between the relative CBF response to visual stimulation and inspiratory or expiratory loading (Table 4.2).

Table 4.4 Z scores (Z_{max}) of peak voxels and coordinates of clusters of relative CBF increase in regions previously implicated in respiratory studies that did not survive the application of our cluster threshold during inspiratory and expiratory loading.

Condition	Region	Peak voxel location	Z_{max}
Inspiratory loading	Left somatomotor cortex	X=-58, Y=-6, Z=36	3.78
	Amygdala	Right: X=30, Y=2, Z=-16 Left: X=-14, Y=-4, Z=-22	Right: 2.3 Left: 2.2
	Right anterior cingulate gyrus	X=0, Y=-12, Z=42	2.2
	Left posterior cingulate gyrus	X= -4,Y=-4, Z=4	2.4
	Right anterior insula	X=40, Y=80, Z=-2	2.5
	Right posterior insula	X=40, Y=-6, Z=2	2.4
Expiratory loading	Right posterior insula	X=38, Y=-14, Z=6	2.7
	Amygdala	Right: X=24, Y=-12, Z=-12 Left: X=-14, Y=-2, Z=-18	Right: 2.6 Left: 2.3
	Right anterior cingulate gyrus	X=12, Y=40, Z=2	2.2
	Posterior cingulate gyrus	Right: X=6, Y=-14, Z=46 Left: X=-2, Y=-48, Z=2	Right: 2.3 Left: 2.7

Results of the physiological recordings during the scanning session are reported in the lower part of Table 4.1 and show stable $PETCO_2$ and HR for all respiratory conditions. As during the laboratory session, there were no differences between the first and second block of each condition, hence values are presented as averages over both blocks.

In order to estimate the power of our study, we used the data from our region of interest analysis of the ASL data to retrospectively examine the effect sizes of changes in relative CBF detectable with our study design. A confidence interval of 80% detection rate was chosen as sufficient as it is commonly used, e.g. in a previous study of our group (Pattinson et al., 2007). Based on our sample size and standard deviations, our study can detect a change in relative global grey matter CBF of 0.5% with 80% confidence and a change of 1.3% of the visual-stimulus induced change in perfusion with 80% confidence for the interaction between visual stimulation and inspiratory or expiratory resistance in V1.

4.4 Discussion

4.4.1 Summary

The key findings of this study are:

1) Inspiratory loading caused an increase in relative CBF of approximately 0.5% per cmH_2O, but did not have any effect upon arterial blood pressure. This global increase in relative CBF remained evident even when localized CBF increases in brain areas thought to be responsible for performing the breathing tasks were excluded in a subsequent region of interest analysis.

2) Expiratory loading had no effect on global CBF (either including or excluding task-specific regions of interest), but was associated with an increase in mean arterial pressure from 90.7±15.2mmHg to 93.8±15.7mmHg (approximately 0.3% per cmH_2O, $p<0.01$).

3) There was no generalized modulation of the CBF response to visual stimulation in the primary visual cortex by either inspiratory or expiratory resistive loading.

4) Localized perfusion increases were observed in the sensorimotor cortices and the cerebellum during inspiratory and expiratory loading.

4.4.2 CBF changes with inspiratory and expiratory loading

This study was motivated by the need to understand how the well-described effects of respiratory loading on systemic arterial and venous systems (Scharf, 1991) might affect CBF. Other studies of respiratory loading using a variety of different techniques have shown varying effects on CBF (Cooke et al., 2006; Droste et al., 1999; Kolbitsch et al., 2000; Lorenz et al., 2001; Reinhard et al., 2000). The conflicting results of previous studies may possibly depend on the specific method used to generate respiratory loads, the differing techniques

used to measure CBF and the fact that studies tended to only apply either inspiratory or expiratory loading and the effects might therefore not be directly comparable. With ASL we have additionally been able to examine the effect of regional changes in CBF across the whole brain and to test how the CBF response to neural activation might be affected by respiratory loading.

4.4.3 Arterial blood pressure and CBF

Inspiratory loading elicited no changes in blood pressure (although CBF increased), while expiratory loading caused a small increase in blood pressure (but was not associated with altered CBF). In terms of understanding CBF regulation, our data therefore suggest that small changes in blood pressure are directly caused by expiratory loading, but that a different mechanism is responsible for the increase in CBF observed during expiratory loading. We speculate that the increase in blood pressure during expiratory loading is most likely due to a primary localized direct pressure effect of increased intrathoracic pressure on the great arteries of the chest, e.g. the aorta, as other cardiovascular parameters (heart rate, pulse pressure) were unchanged, making it unlikely that the increased blood pressure was secondary to systemic factors, such as increased sympathetic activation or increased systemic vascular resistance.

4.4.4 Venous return

Venous return from the brain to the heart is important for the regulation of the cerebral circulation. In an upright position, gravity maintains a slightly negative pressure in the jugular veins, resulting in unimpeded venous drainage from the brain. Venous drainage is further assisted by the "thoracic pump", the slight negative intrathoracic pressure generated during normal inspiration that is transmitted to the great veins in the thorax (e.g. the superior vena cava). In a supine position like in this study, gravity no longer has such a profound effect and

jugular venous pressure (JVP) increases to approximately 8mmHg, potentially impeding venous outflow from the brain. Decreased intrathoracic pressure caused by inspiratory loading in a supine position decreases JVP through direct pressure effects on the great veins in the thorax that are in direct continuity with the jugular veins. Inspiratory loading therefore may act to accentuate the normal thoracic pump mechanism and will therefore increase the arterio-venous pressure gradient across the brain, leading to increased CBF.

Increased intrathoracic pressure during expiratory loading has the opposite effect on the central venous circulation and will tend to elevate the pressure in the jugular vein, decreasing the arterio-venous pressure gradient across the brain. Without an effective compensatory mechanism, this would decrease CBF. We therefore speculate that this reduction in venous return would be compensated by the increased MAP during expiratory loading, leading to unchanged CBF. Jugular venous pressure was not measured during this study (due to the invasive nature of this measurement), but the effects of intrathoracic pressure on JVP have been well documented (Hall and Guyton, 2011).

4.4.5 Systemic vascular resistance

Another possibility is that alterations in atrial transmural pressure induced by respiratory loading stimulate the release of atrial natriuretic peptide, a powerful vasodilator that decreases systemic vascular resistance. The difference between the systolic and diastolic blood pressures (i.e. pulse pressure) is proportional to systemic vascular resistance. Although we observed an increase in mean arterial pressure during expiratory loading, this was associated with equivalent increases in diastolic and systolic pressures, while pulse pressure remained constant. Therefore, the increase in CBF during inspiratory loading is unlikely to originate from altered systemic arterial tone and we therefore remain confident in our hypothesis that alterations in venous return may explain the increased CBF during inspiratory loading.

4.4.6 Arterial CO_2

Additionally, arterial CO_2 tension has a strong impact on the regulation of CBF. For each 1mmHg change in partial pressure of arterial CO_2 ($PaCO_2$), there is a corresponding CBF change in the same direction of approximately 2.5% in hypocapnia, and 4.5% in hypercapnia (Ide et al., 2003) and there are correspondingly profound effects on the BOLD response, which is decreased in hypercapnia and increased in hypocapnia (Cohen et al., 2002). As changes in respiration potentially affect $PaCO_2$ (and therefore CBF), we have paid meticulous attention to the maintenance of constant CO_2 levels. We trained our research participants to maintain their $PETCO_2$ constant through a visual feedback system. Without this training, respiratory loading, equivalent to that used here during spontaneous breathing, is well documented to cause hypercapnia due to decreases in ventilation (Davies et al., 1919), of between 1 and 3 mmHg, therefore further increasing CBF by approximately 4.5% to 13.5%. Such a CBF increase would confound the interpretation of our results. As we have maintained constant $PETCO_2$, we can be confident that the CBF increase during inspiratory loading is a true primary physiological effect, that taken in isolation is small, but could contribute to a more profound systematic confound due to secondary effects of loading on ventilation.

Such careful control is important not only in respiratory studies, but also in any other paradigm that may alter respiration. One example of the importance of respiratory confounds is the caffeine-induced decrease of CBF (Chen and Parrish, 2009), which confounds the BOLD response. Caffeine stimulates both the respiratory system (causing hypocapnia (Bell et al., 1999)) and the cardiovascular system (increasing blood pressure (Robertson et al., 1978)), both important contributors to CBF regulation. We are not aware of any current reports of caffeine's effect on CBF having obtained what we consider essential measures of systemic physiology and its direct effects on CBF are therefore not clear.

4.4.7 Interactions between respiratory loading and visual stimulation

Inspiratory and expiratory loading did not modulate the stimulus-evoked perfusion increase in V1. Therefore, the theoretical confounding effects of arterial blood pressure (ABP) and of baseline CBF on stimulus-evoked CBF responses as suggested by Payne (2006) were not observed. This finding is in agreement with a recent paper by Gauthier et al (2011), demonstrating a profound effect of carbogen on baseline CBF and the visually-evoked BOLD response in the visual cortex during simultaneous carbogen inhalation and visual stimulation, which were evident at the lowest level of carbogen inhalation (2.5% CO2, 38.25% O2), while relative CBF increases in the visual cortex induced by visual stimulation remained unaffected during carbogen inhalation.

The interpretation of the BOLD signal, which is a composite signal comprising CBF, cerebral blood volume and the cerebral metabolic rate of oxygen (Kim et al., 1999), is highly susceptible to changes in blood deoxyhemoglobin level and to the size of the perfusion response to activation (Gauthier et al., 2011). A small CBF increase, as seen during inspiratory loading in our study, could hence lead to a lower fraction of deoxyhemoglobin, an elevated BOLD baseline and a reduced BOLD response to functional tasks. Pooling of venous blood due to increased venous pressure might lead to a further decrease in the BOLD response (Lorenz et al., 2001). As such, CBF effects might confound the comparison of BOLD activations across different levels of inspiratory loading, e.g. in von Leupoldt et al (von Leupoldt et al., 2009b), where the CO_2 effects of loading will affect CBF in addition to the direct effects of negative intrathoracic pressure. Similarly, Evans et al. compared positive pressure ventilation with spontaneous breathing without controlling for CO_2, hence their results might be confounded due to opposing effects on CBF and venous drainage.

As we only used ASL during this study we can only comment on the CBF component of the BOLD signal. One approach to control for potential CBF influences on the BOLD response is to acquire a measure of baseline CBF, which can be used as a regressor to aid the differentiation of local metabolic effects from purely vascular effects when interpreting BOLD signal change (Pattinson et al., 2009). Intrathoracic pressure changes might additionally affect other components of the BOLD signal, which could be investigated in future studies.

4.4.8 Implications for the understanding of respiratory control

Activation in somatomotor and cerebellar areas has previously been found to be implicated in neuroimaging studies of modified respiration (reviewed in Herigstad et al (2011b)). Increased perfusion in the premotor, motor and supplementary motor cortices is likely to indicate stronger mechanical breathing effort during loading periods, as these areas are implicated in conscious breathing (Pattinson et al., 2009; Pattinson et al., 2007), while the increased perfusion in the somatosensory cortices in this study might indicate an increased sensation of breathing. Using positron emission tomography (PET) in 6 healthy volunteers, Isaev et al (2002) did not demonstrate activity in the primary sensory or motor cortices in response to 2 minutes of inspiratory loading, but considered that their study design was not sensitive enough to detect such changes. Our data confirms that the sensory and motor cortices are in fact implicated in breathing against a load, and therefore support Isaev's hypothesis that the respiratory response to loading is not solely explained on the basis of respiratory reflexes activated from the chest wall, diaphragm and airways.

We have also shown increased perfusion in cerebellar lobe VI, which has previously been shown during resistive loading (Isaev et al., 2002a), but its role in respiratory control is unclear. Based on increased BOLD signal in cerebellar lobe VI found during other

sensorimotor tasks and during the application of aversive stimuli in other studies (Moulton et al., 2011), we speculate that this activity might be due to the sensorimotor control of breathing (sensorimotor cerebellum) and might also represent the negative psychological affect experienced during respiratory loading.

CBF increases were also identified in the insula, amygdala and cingulate gyrus in response to both inspiratory and expiratory loading, but did not survive cluster thresholding. These brain areas have consistently been identified in neuroimaging studies using resistive loading to induce breathlessness (reviewed in Herigstad et al. 2011). We suspect that the poorer SNR of ASL explains why these CBF increases have not reached our statistical thresholds. Furthermore, in a study by von Leupoldt data analysis necessitated small volume correction to identify respiratory-related activity in these structures. We have chosen not to follow this analysis approach, as identifying brain structures activated by respiratory loading was not a primary aim of this study.

4.4.9 Clinical implications

The duration of the manipulations used here, with each condition lasting 4 ½ minutes, allows us to investigate static changes in CBF after the dynamic autoregulatory response of the first few seconds has been completed. This makes the results relevant when considering ventilated patients or patients with medical conditions inducing altered intrathoracic pressure. Patients requiring ventilation during intensive care (inducing a positive intrathoracic pressure of up to +30cmH_2O) might undergo FMRI or other perfusion-based measurements, in which the ventilation could potentially alter their CBF. Our results have shown that the increased intrathoracic pressure does not significantly confound these measures.

Decreased intrathoracic pressures are encountered in patients suffering from exacerbations of COPD, or obstructive sleep apnea. While our results indicate that the increase in CBF induced by negative intrathoracic pressure is unlikely to be a major confound in the interpretation of ASL, at least at the levels employed in this study, it could potentially confound BOLD responses in patients with respiratory diseases, thus care needs to be taken when comparing their BOLD responses with those of healthy controls. However, ASL seems less prone to these confounds than BOLD in this respect (Brown et al., 2003).

Research involving altered respiration can also benefit from using ASL in combination with careful $PETCO_2$ control. The advantage of using ASL to detect respiratory-related CBF responses is that ASL gives higher temporal signal stability during longer stimulus blocks than BOLD FMRI (Wang et al., 2003), while not requiring the radioactive isotopes necessary for PET. The advantage of longer stimulus blocks is that longer periods of modulated breathing are more realistic with respect to patient experiences, while experimental respiratory sensations take some time to build-up (Evans et al., 2002). Furthermore, ASL allows the investigation of a single physiological parameter (i.e. CBF) as opposed to BOLD, which is a compounded signal encompassing different underlying physiological components, and which can therefore be influenced by physiological changes beyond CBF.

4.5 Conclusion

Inspiratory loading at 9cmH_2O increases CBF by ~5% (0.5% CBF increase per 1 cmH_2O inspiratory loading). We have demonstrated that this does not affect the ASL perfusion contrast, but we cannot exclude a systemic vascular effect that could potentially dampen the BOLD response in patients with clinical conditions involving decreased intrathoracic

pressures. It appears that expiratory loading is better compensated for, as it does not change CBF. Taking into account direct effects of intrathoracic pressure, especially during inspiratory loading, will allow the comparison of patients with altered respiratory physiology to healthy controls. Understanding how physiological factors may affect CBF in diseases such as COPD will level the path towards understanding disease mechanisms and drug action in the brains of patients with altered cerebrovascular and respiratory physiology.

Chapter 5

Investigating the neural signature of affective processing of acute and anticipated dyspnoea with the opioid remifentanil

Chapter Abstract 5

Acute dyspnoea is severely distressing. Additionally, when dyspnoea becomes chronic it is accompanied by increased dyspnoea-specific anticipatory anxiety that further reduces quality of life. Understanding the neuropsychological basis of the acute and anticipatory emotional components of dyspnoea will allow the development of selectively targeted interventions to maximise quality of life in dyspnoeic patients. Here, we study perceived unpleasantness and anticipation of acute dyspnoea and their change with the opioid remifentanil in a classical conditioning paradigm.

19 healthy right-handed non-smokers (aged 24 (+-7SD) years, 10 females) were trained through an aversive delay-conditioning paradigm to associate visual cues with -3cmH_2O and -11cmH_2O inspiratory loading. After initial conditioning, participants performed two identical scanning sessions with intravenous infusion of remifentanil (0.7ng/ml effect site concentration) or saline (placebo, counterbalanced) whilst performing the same conditioning paradigm.

Processing of dyspnoea intensity and unpleasantness rely on two separate, but interlinking neural systems. The anterior insula and the hippocampus were identified as key structures processing dyspnoea. The hippocampus plays a role in re-experiencing dyspnoea unpleasantness in a fear conditioning context and might hence be a promising target for behavioural treatments to alleviate dyspnoea. Also, remifentanil revealed scaling of the change in trial-by-trial evaluation of dyspnoea intensity in the hippocampus and amygdala. Remifentanil revealed that the prefrontal cortex modulates subjective dyspnoea perception and might be a prominent target for behavioural dyspnoea therapies.

Anticipatory anxiety and subjective perception of dyspnoea are strongly linked in the anterior insula. During remifentanil, the insula shows lower activation during anticipation, but not perception of actual stimuli following remifentanil administration. This prominent effect on dyspnoea-specific anticipatory anxiety could explain the high potency of opioids in treating dyspnoea. Unaltered encoding during stimulus perception with remifentanil highlights the differential role of the insula in dyspnoea. We conclude that the subjective evaluation of dyspnoea intensity, immediate and secondary unpleasantness relies on spatially distinct but interlinked systems for cognitive and emotional evaluation.

5.1 Introduction

5.1.1 General introduction

Refractory dyspnoea is the distressing experience of breathing difficulty that persists at rest or with minimal activity despite optimal therapy of the underlying condition (Mahler et al., 2010). It is a prominent and disabling symptom of lung and heart disease accompanied by immense acute suffering, and anxiety and fear of the next dyspnoea attack. Whilst the behavioural effects are strong, little is known about the neural processes behind these sensations. In this study we set out to dissociate the neural mechanisms processing perceived intensity, immediate unpleasantness and anticipatory anxiety of laboratory dyspnoea in healthy volunteers. To dissociate these mechanisms, we modulate respiratory unpleasantness with the opioid remifentanil in a classical fear-conditioning paradigm.

5.1.2 Dyspnoea intensity and unpleasantness

As we have seen in Chapter 1, it is not the mere intensity of dyspnoea (sensory dimension), but the associated suffering (affective dimension) that warrants palliation. Therefore, exploring the different domains and qualities of dyspnoea is relevant to understanding how specific therapies, such as opioids, relieve refractory dyspnoea (Mahler, 2013). It has been hypothesized that dyspnoea might consist of distinct sensory and affective components. Two systems have been proposed to process and integrate acute respiratory sensations in the brain (Lansing et al., 2009). The first proposed system discriminates the intensity of dyspnoea. It could encompass the conduction of afferent information from the respiratory muscles through the brainstem medulla, then via the ventroposterior thalamus to the primary and secondary somatosensory cortices (Davenport and Vovk, 2009; Mahler, 2013; von Leupoldt and Dahme, 2005). The second suggested system processes information related to the affective perception of dyspnoea and might comprise vagal conduction of signals from the airway/lung receptors through the medulla to the amygdala and thalamus, from where they get forwarded to the

insula and anterior cingulate cortex (ACC; (Davenport and Vovk, 2009; Mahler, 2013; von Leupoldt and Dahme, 2005)). Currently, it is unknown whether these suggested neural systems are independent or overlapping.

Through independent manipulation of negative emotion through affectively charged pictures during the perception of dyspnoea, von Leupoldt and colleagues (2008) showed that dyspnoea unpleasantness was associated with activation in the right anterior insula and the right amygdala. This finding was obtained by using small volume corrections to perform directed searches in areas of the limbic system in 14 participants. Whilst small volume corrections reduce the number of statistical comparisons by providing smaller search regions and are more sensitive to activation within the search areas, this approach might miss important activations outside specific search areas. Peiffer and colleagues (2001) identified activation in the right posterior cingulate cortex in association with perceived intensity of dyspnoea during simultaneous inspiratory and expiratory loading during positron emission tomography (PET) in eight participants. This study did not dissociate intensity and unpleasantness of dyspnoea as participants gave one rating of perceived dyspnoea intensity, which per instructions included the affective component and the sensory experience. A more recent study has found correlations between the ACC/medial prefrontal cortex (MPFC) and insula/inferior frontal gyrus and ratings of unpleasantness of inspiratory loads during FMRI (Paulus et al., 2012). Whilst ratings of respiratory unpleasantness were obtained, this study predominantly compared performance during inspiratory loading between a group of elite athletes and control participants. Participants performed a cognitive task during loading, which might have distracted from the unpleasantness of the task and might have altered unpleasantness ratings. Additionally, unpleasantness during unloaded baseline periods was relatively high during this study (~30%VAS), with only a small increase in unpleasantness during inspiratory loading (~15%VAS increase). On top of that, the study did not correct for potential changes in

$PETCO_2$ during inspiratory loading of 40cmH_2O, which could affect regional CBF and hence BOLD response (see Chapter 4). It hence remains questionable whether activation supposedly associated with unpleasantness reflects true unpleasantness. Results will hence need to be interpreted with caution.

The anterior insula and ACC have long been known to facilitate complex integrations between sensory-motor and affective/cognitive information in the processing of unpleasant sensory experiences like pain (Peyron et al., 2000; Treede et al., 1999). In the study of pain, the thalamus, the insula, the midcingulate cortex (MCC) and the primary somatosensory cortex have consistently been found to scale with intensity of nociceptive inputs and resultant pain perception (Apkarian et al., 2005; Tracey and Mantyh, 2007). The dorsolateral prefrontal cortex (DLPFC) might be involved in coordinating pain modulation with goal-directed behaviour as evidence suggests that it modulates pain perception (Lorenz et al., 2003) in line with its activation during anticipation of pain (Yaguez et al., 2005) and the localization and encoding of attention to a noxious stimulus (Peyron et al., 1999).

Whilst the neuroimaging studies of dyspnoea described above have looked at the response to a specific stimulus by averaging over multiple applications of the same stimulus, laboratory experiments are starting to show that the perception of respiratory sensations varies not only by stimulus condition, but fluctuates strongly over time within a given context. Pattinson and colleagues have shown variations in the 'urge-to-breathe' with repeated performance of 15-second breath-holds during the same session (Pattinson et al., 2009). In Chapter 3, we have also shown that whilst the average respiratory unpleasantness did not change between sessions, subjective perception of dyspnoea did not directly follow stimulus strength, but fluctuated within and between blocks (see Figure 3.7, Table 3.3 and Figure 3.5).

5.1.3 Opioids

There is a large need for palliation of dyspnoea. Whilst opioids, a class of potent painkillers, may be effective for this purpose (Abernethy et al., 2003; Booth et al., 2008; Simon et al., 2010), they are also associated with serious side effects. Opioids decrease central respiratory drive with a hypothesised associated decrease in corollary discharge (the efferent copy of the motor command to somatosensory regions that provides the perception of expended effort; (Mahler, 2013)). This effect is regulated by the brainstem respiratory centers (Weil et al., 1975) and is directly proportional to the administered dose (Eckenhoff and Oech, 1960). Whilst respiratory depression is a major concern for treatment of patients with respiratory disease, evidence is accumulating for severe side effects including immunodepression (Vallejo et al., 2004) and endocrine impairment (Katz and Mazer, 2009) with long-term opioid therapy. Opioid-induced hyperalgesia (increased pain sensitivity) is also a major concern, added by the potential for addiction (Mehendale et al., 2013) and rapidly increasing tolerance to treatment effects (Williams et al., 2013). Balancing these serious side effects with the potential for dyspnoea relief make opioids a controversial treatment for dyspnoea.

Opioids bind to peripheral and central opioid receptors. These are G-protein coupled receptors that can be activated by brain-internal peptides (endogenous opioids) and by a variety of externally administered natural opiates (e.g. morphine) as well as processed, partly synthetic or synthetic opiates. Their main effect is a decrease in neuronal excitability (Benarroch, 2012). Mu opioid receptors are the primary binding site for morphine and other analgesics, as well as for the endogenous opioid beta-endorphin (Hughes and Kosterlitz, 1983). They are prominent in a number of brain regions involved in the processing of emotional information, stress responses and reward (Mansour et al., 1995), especially in the thalamus, basal ganglia insula and operculum, the ACC, MCC and DLPFC, amygdala and periaqueductal gray (PAG) (Baumgärtner et al., 2006; McNally et al., 2004b; Vogt, 2005).

Behaviourally, opioids have shown potential in dissociating the affective and sensory component of unpleasant stimuli. Low doses of opioids reduce the affective component of pain more strongly than the sensory component (Price et al., 1985). Hence, it has been proposed that opioids might be more effective in relieving the affective dimension of dyspnoea compared to its intensity (Horton and Rocker, 2010), but evidence is scarce. Using naloxone to block opioid receptor signalling, investigations have shown that endogenous opioids (i.e. beta-endorphin) affect the perception of dyspnoea in patients with chronic obstructive pulmonary disease (COPD) during exercise (Mahler et al., 2009). One study has looked at the effect of endogenous opioids on the intensity and unpleasantness of dyspnoea in response to inspiratory loading (Gifford et al., 2011). The study used a small sample size (N=14) and a relatively high bolus of naloxone (10mg/25ml) to block endogenous opioid activity that was chosen to be effective for reduction of dyspnoea based on previous studies. It was not adjusted with the aim of dissociating dyspnoea intensity and unpleasantness and was hence not designed to find such effects.

Administration of external opioids might give higher control over effective compound concentrations, which might be crucial for dissociation of affective and sensory encoding of dyspnoea. A reduction of perceived dyspnoea intensity with exogenous opioids has been shown in patients (Abernethy et al., 2003; Jennings et al., 2002), but to our knowledge there is only one study that investigated the effects of opioids on dyspnoea unpleasantness. In the laboratory, Banzett and colleagues (Banzett et al., 2011) showed that intravenous morphine reduced air hunger and anxiety ratings during hypercapnia and ventilatory restriction. Whilst this study indicates that exogenous opioids can impact the affective component of dyspnoea, it did not investigate effects on subjective respiratory intensity. In the present study, we use a low dose of the fast-acting opioid remifentanil to dissociate the sensory from the affective dimension of dyspnoea with the aim of examining their underlying neural substrates.

Our understanding of how opioids reduce dyspnoea is limited. Neuroimaging suggests that the mu opioid receptor agonist remifentanil may modulate the central processing of dyspnoea. Pattinson et al. (2009) have shown that 1ng/ml of the opioid remifentanil reduced activation in the insula, ACC, DLPFC, PAG and cerebellum in healthy volunteers performing breath holds. These areas have previously been associated with air hunger (Herigstad et al., 2011a) that may be relevant to dyspnoea. In contrast, the 'urge to breathe', their measure of the subjective component of a 15-second breath hold, scaled with activation in the bilateral insula and operculum and the secondary somatosensory cortex, but was not altered by administration of 1ng/ml of the opioid remifentanil. The absence of an effect of remifentanil on the emotional component of a breath-hold might be due to the relatively low 'urge to breathe' ratings during the challenges (28/100 reduced to 14/100 with remifenanil), which might not be truly unpleasant. Additionally, a breath-hold is not a realistic model of dyspnoea as experienced in clinical conditions. An externally controlled dyspnoea challenge, as used in this study, is likely to be more realistic as it captures brain activation related with the anxiety of being out of control.

Dissociating dyspnoea intensity and unpleasantness during FMRI is necessary in order to reveal brain activation involved in differentially processing the sensation and its associated affect, as well as brain areas that are involved in processing of both dyspnoea intensity and unpleasantness. Structures linking dyspnoea intensity and unpleasantness might represent key components of the neural network processing dyspnoea and could be vital for dyspnoea treatment. Dyspnoea treatment is predominantly concerned with alleviating dyspnoea unpleasantness in order to increase quality of life, hence understanding how the sensation of unpleasantness is generated could help to explain how current treatments alleviate dyspnoea and might help to provide targets for novel therapies.

Opioids have also shown profound effects on associative learning. Associative learning is an important component of the secondary affective response to dyspnoea, i.e. patients learn associations between walking upstairs and feeling breathless, which will foster anticipation of a breathlessness episode when patients walk upstairs. The endogenous opioid system is involved in fear learning in rodents with opioid agonists attenuating (Westbrook et al., 1991) and opioid antagonists facilitating (McNally et al., 2004a) the acquisition of conditioned fear, potentially by affecting a conditioning-induced amygdala/brainstem-dependent inhibitory signal that acts on ascending processing of the unconditioned stimulus (Fanselow, 1998). Additionally, mu-opioid receptors regulate the consolidation of emotional memory through interactions with noraderenergic terminals in the amygdala (Quirarte et al., 1998). An inhibitory role for opioids in human acquisition of fear was shown to implicate the amygdala (Eippert et al., 2008) and opioids are involved in regulating prediction errors (a difference between expected and actual outcome) during aversive learning. This mechanism might be located in the midbrain and linked with the dopamine system (Bourdy and Barrot, 2012) and could be crucial for understanding dyspnoea-related anxiety.

Remifentanil is a short-acting mu-selective opioid analgesic that is clinically used in anaesthesia. We chose remifentanil because it is a good model for opioids that are in clinical use (e.g. morphine), but it is more convenient to use in an experimental setting. It has a short half-life of 3 to 4 minutes, which allows rapid adjustment of plasma levels during a target-controlled infusion (infusion through a computer-controlled pump pre-programmed with a pharmacokinetic model of the drug). The short half-life means that it can be administered by infusion and plasma levels can be changed rapidly. In terms of volunteer safety, this means it is possible to achieve a relatively strong opioid effect with remifentanil, but the infusion can be terminated and effects reversed within minutes.

5.1.4 Clinical relevance of studying the anticipatory anxiety of dyspnoea

Dyspnoea amplification by anxiety is an integral part of dyspnoea and anticipatory dyspnoea-related anxiety plays a large role in the downward spiral of decline that decreases quality of life and increases mortality in patients with lung and heart diseases (Heim et al., 1972; Huang et al., 2013; Nici et al., 2006; Smoller et al., 1996). Patients form associations between previously neutral stimuli (e.g. walking upstairs) and the aversive outcome of dyspnoea (De Peuter et al., 2004). Anticipation of future dyspnoea leads patients to alter their behaviour when encountering these situations in the future (Ley, 1999), e.g. avoidance of stairs. If negatively cued stimuli cannot be avoided, learnt anticipation of dyspnoea in response to a stimulus might increase the negative perception of respiratory stimuli (De Peuter et al., 2005), so patients are more likely to feel out-of breath because they know they are climbing the stairs and they expect to feel breathless. Alteration of fear responses is a component of pulmonary rehabilitation, an exercise-based treatment with educational elements that has been shown to decrease dyspnoea-related anxiety and to increase quality of life in patients with COPD (Lacasse et al., 2007). Whist some patients strongly improve, treatment benefits are not universal and understanding the intricate relationship between anxiety and dyspnoea could help to target treatment more efficiently.

5.1.5 Classical conditioning

Anticipatory anxiety of dyspnoea represents associative fear learning with patients learning to associate the performance of a strenuous task, e.g. walking upstairs, with dyspnoea and hence anticipate dyspnoea when approaching a staircase. Breathing is under both voluntary and involuntary control and can thus be conditioned directly by classical conditioning (Ley, 2001). Classical conditioning is a simple form of associative learning that has been studied extensively in animals and humans (Clark et al., 2002a). It is a procedure in which a biologically significant stimulus, e.g. an unpleasant respiratory stimulus, (the unconditioned

stimulus) is paired with an initially neutral stimulus, e.g. a staircase (the conditioned stimulus). If the conditioned stimulus is repeatedly presented just before the unconditioned stimulus, participants learn that the conditioned stimulus predicts the unconditioned stimulus. This process is called acquisition and is relevant for dyspnoea patients because exertional dyspnoea occurs after performance of strenuous activities, e.g. walking upstairs, hence the idea of performing this activity elicits expectations of dyspnoea. The conditioned stimulus thus becomes predictive of the unconditioned stimulus and presentation of the conditioned stimulus in itself elicits a response, the conditioned response (Clark et al., 2002a). After repeated pairing of the conditioned and the unconditioned stimulus, the conditioned stimulus elicits a response that is appropriate to the anticipated unconditioned stimulus, e.g. change in heart rate, skin conductance and anxiety level during aversive conditioning (Carter et al., 2006). Conditioning can be unconscious or can involve conscious awareness of the predictive contingency between the conditioned and the unconditioned stimulus, which is called contingency awareness (Carter et al., 2006).

The simplest form of conditioning is called delay conditioning. In delay conditioning, the conditioned and the unconditioned stimulus overlap in time. Because no memory components are required to acquire contingency (compared to trace conditioning, in which the conditioned and the unconditioned stimulus are temporally separate), explicit knowledge of the contingency is easily acquired (Sehlmeyer et al., 2009). The cerebellum and brainstem have been shown to be sufficient for retention of eyeblink conditioning in rabbits (Mauk and Thompson, 1987), but a core network comprising the amygdala, insula and the ACC is activated in most human neuroimaging protocols using conditioning (Sehlmeyer et al., 2009).

Classical conditioning has been extensively used to investigate the neural structures and functions that underlie learning and memory, and over time conditioning protocols have

become more intricate in order to investigate more complex memory processes. (Sehlmeyer et al., 2009).

5.1.6 Neuroimaging of dyspnoea anticipation

A recent imaging study has investigated the anticipation of respiratory stimuli with FMRI, showing activation in the anterior insula/orbitofrontal cortex and dorsal ACC/dorsomedial prefrontal cortex (DMPFC) was shown during anticipation of hyperventilation (Holtz et al., 2012). Participants with increased fear of somatic symptoms showed increased activation in these areas, which links increased anticipatory activation with increased anxiety (Holtz et al., 2012).

A ground-breaking study by Ploghaus et al. (Ploghaus et al., 1999) showed that similar, but more anterior regions of the medial frontal lobe, insular cortex, and cerebellum were activated during the anticipation of painful stimuli compared to their perception (Ploghaus et al., 1999). We further know from the study of pain that the brain state preceding painful stimulation predicts responses to experimental (Ploner et al., 2010) and clinical (Loggia et al., 2013) pain. The anterior insula and ACC seem to be especially important for subsequent pain perception with changes in connectivity between the anterior insula and the periaqueductal grey (PAG) during the anticipation of pain relating to its subjective perception (Fairhurst et al., 2007; Ploner et al., 2010). Some of the same regions may fulfil a similar function in dyspnoea.

5.1.7 Summary and hypotheses

In this study, we adopted a whole-brain approach to localize the neuronal network involved in the subjective perception of dyspnoea induced by inspiratory loads during isocapnia (stable CO_2, induced by mild hypercapnia). We aimed to dissociate acute dyspnoea intensity and unpleasantness and dyspnoea-specific anticipatory anxiety.

Dissociating the intensity and unpleasantness of dyspnoea is important for understanding their differential neural processing and to identify key brain structures that might provide treatment targets. Three previous studies consistently show activation of the anterior insula that scales with dyspnoea unpleasantness. Additional activations in the amygdala, ACC and MPFC was not consistent, hence a sufficiently powered whole-brain study is necessary to investigate dyspnoea unpleasantness. Behaviourally, low-dose opioids have been shown to decrease pain unpleasantness at stable intensity, but have not been used to differentially affect dyspnoea intensity and unpleasantness. We hypothesise that a low dose of the opioid remifentanil would reduce respiratory unpleasantness without affecting respiratory intensity. These changes would correlate with neural changes in brain areas involved in processing dyspnoea unpleasantness, potentially including the anterior insula, ACC and MPFC.

Opioids have been shown to alter processing of respiratory stimuli and might also affect neural processing of dyspnoea, but such an effect has not been shown. In the laboratory, opioids induce a generally more relaxed and less unpleasant psychological state during processing of dyspnoea stimuli (Banzett et al., 2011). Additionally, trial-by-trial variations in stimulus perception within the same context have been shown (Pattinson et al., 2009). Assessment of such variations might hence allow investigation of encoding of dyspnoea intensity and unpleasantness in different psychological contexts. We hypothesise that trial-by-trial variations in dyspnoea perception in the remifentanil session would change in line with altered psychological state during remifentanil.

During an associative fear learning process, situations and actions that trigger dyspnoea become ‘dyspnoea-cues’ for patients. These cues elicit dyspnoea-specific anticipatory anxiety. Classical conditioning paradigms can be used to investigate associative fear learning in an experimental context to investigate anticipatory anxiety. The anticipation of respiratory

stimuli causing dyspnoea has not been investigated. One study has shown activation in the anterior insula and the ACC/DMPFC during anticipation of hyperventilation and anticipation of pain has shown activation in limbic areas including the insula and ACC and the brainstem. We investigate the anticipation of dyspnoea in response to inspiratory loading. We hypothesise that BOLD activation will occur in limbic regions including the anterior insula during anticipation of dyspnoea.

5.2 Materials and methods

5.2.1 Brief overview

The study consisted of three sessions, a conditioning session and two double-blinded FMRI sessions, all between 24 and 48 hours apart. An aversive delay-conditioning paradigm was used to investigate the anticipation and perception of mild and strong dyspnoea, induced by -3cmH_2O (mild) and -11cmH_2O (strong) inspiratory loading respectively.

5.2.2 Data acquisition

Participants

Twenty-nine healthy right-handed non-smokers (16 females, mean age 24 (+/-6 SD) years) participated in this study after giving written informed consent in accordance with the Oxfordshire Research Ethics Committee. Participants were generally healthy, had no history of neurological, pulmonary or cardiovascular disease, were free from acute respiratory infections and were currently not on any medication. Participants abstained from excessive consumption of alcohol 24 hours before and during the study and fasted solids for 6 hours and liquids for 2 hours before every session.

Of the 29 volunteers, 21 completed the study. Eight participants did not complete the study for the following reasons: Two participants suffered from vasovagal reactions when the infusion line was inserted, one participant did not comply with study instructions, four participants were not aware of the association between visual cues and respiratory stimuli, one participant was not able to complete the study due to technical difficulties with the MRI scanner. Two data sets were excluded because of technical difficulties (insufficient image quality and computer error). Data sets from nineteen participants (10 females, age 24 (±7 SD) years) were included in the analysis.

Training session

The initial behavioural session was used to determine participant suitability, to stabilize anticipatory anxiety and to control for rating strategy and learning effects. The session included a medical assessment confirming suitability for remifentanil administration by a consultant anaesthetist. Participants then performed the aversive conditioning paradigm (Figure 5.1) during which they learned associations between three visual cues (conditioned stimuli, CS) and three different respiratory sensations (unconditioned stimuli, US). The three breathing conditions consisted of normal breathing (baseline), mild inspiratory loading of -3cmH_2O (control stimulus) and strong inspiratory loading of -11cmH_2O (stimulus). The three visual cues were abstract shapes (a star, a triangle and a square) in white outline on a black background. Each visual stimulus (CS) was consistently paired with one breathing condition (US) for each participant (counterbalanced between participants). Participants who did not form these associations were excluded from the study. Participants received a 20-minute test-dose of remifentanil to ensure that they reacted well to the drug and to characterize physiological changes with remifentanil.

Experimental sessions

After successful conditioning during the first session, we confirmed conditioning to the three shapes at the beginning of each session. The two experimental sessions were performed identically (Figure 5.1), but in a double-blinded design, participants received an infusion of remifentanil or saline (placebo) in a counterbalanced order. Participants and the experimenters running the study were blinded to the treatment condition. Before the MRI scan, an intravenous cannula was placed on the back of the participant's left hand. Once the participant was supine in the MRI scanner, the infusion was connected to the cannula. Galvanic skin conductance, pulse rate (PR), blood oxygen saturation (SpO_2), respiratory rate (RR), ventilation and end-tidal carbon dioxide partial pressure ($PETCO_2$) and end-tidal oxygen partial pressure ($PETO_2$) and ventilation were monitored.

Drug infusion

A target-controlled infusion (TCI) pump (Graseby 3500 TCI incorporating Diprisor, SIMS Graseby Ltd, Watford, UK) delivered remifentanil intravenously into the left hand. The TCI pump was pre-programmed with the three-compartment pharmacokinetic model of remifentanil (Minto et al., 1997a; Minto et al., 1997b). This model controls the infusion rate of remifentanil to achieve and maintain a desired effect site concentration based on the participant's gender, age, weight and height. We used a steady-state effect site concentration of 0.7ng /ml. The total duration of the infusion was 45 minutes, which allowed for a 10-minute ramp-up period to reach effect site concentration. Volunteers were supervised for 1h after termination of the infusion.

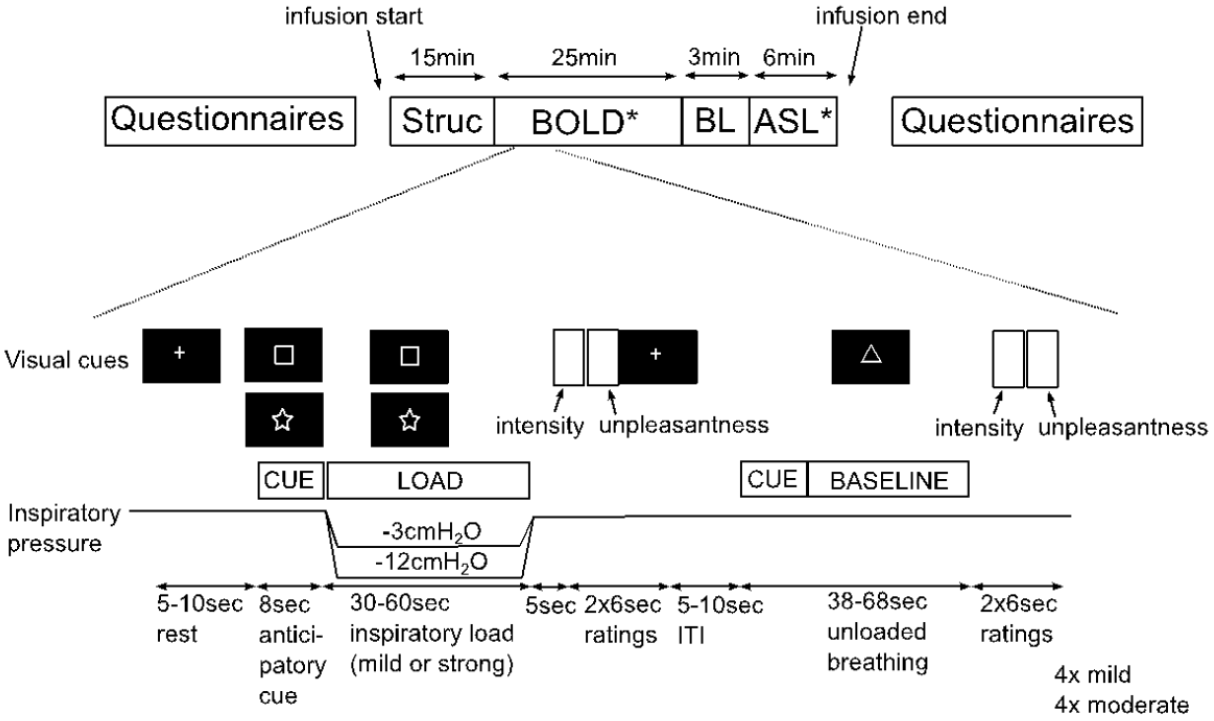


Figure 5.1: Schematic illustration of experimental session and aversive conditioning paradigm. Prior to the application of each inspiratory load, the fixation cross on the screen changed into a visual cue (CS) for eight seconds. This anticipation period signaled the imminent application of a respiratory stimulus (US; mild inspiratory load, strong inspiratory load). The visual cue (CS) remained on the screen during application of the respiratory stimulus (US; delay conditioning). Respiratory stimuli lasted for 30-60seconds. After the stimulation period, the visual cue disappeared and a fixation cross was displayed for 5seconds. This was followed by ratings of respiratory intensity and unpleasantness on visual analogue scales (VAS). Each application of a mild or strong inspiratory load was followed by a baseline period without a respiratory manipulation, indicated by a visual cue that stayed on throughout the duration of this resting period (30-60seconds). After VAS ratings of respiratory intensity and unpleasantness a variable intertrial interval (ITI) was inserted. Participants received four mild and four strong inspiratory loads followed by eight baseline-breathing periods. The use of relatively long dyspnoea stimuli was chosen to maximize the emotional responses associated with anticipation of dyspnoea. Visual stimuli were generated and presented in white on a black background using the Cogent toolbox (www.vislab.ucl.ac.uk/Cogent/) for MATLAB (Mathworks Inc., Natick, MA, USA). Struc=structural and other technical scans; BL=Bond Lader VAS scales.

Respiratory circuit

An in-house developed breathing circuit was used to deliver inspiratory gases and respiratory resistances (Figure 5.2). Physiological data was recorded at 1000 Hz in LabChart (ADInstruments Ltd, Oxford, UK). After participants adjusted to the breathing system for at least 5 minutes, $PETCO_2$ was increased by +0.3kPa from baseline breathing. $PETO_2$ was increased to 20kPa to ensure medical safety during both the remifentanil and placebo scans. A dedicated experimenter kept end-tidal gases stable throughout the sessions with the methods developed in Chapter 3.

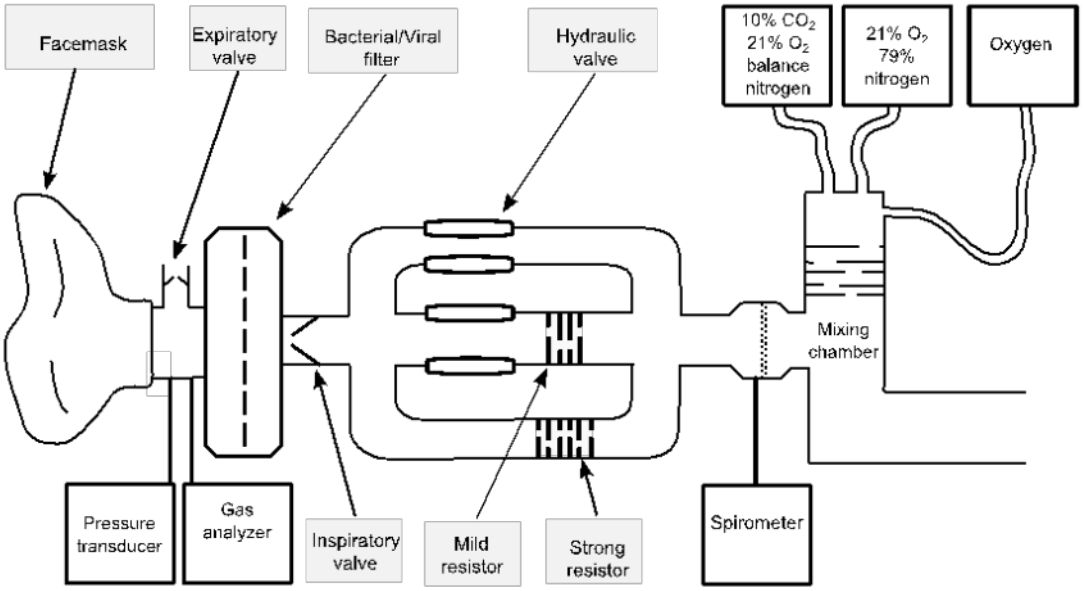


Figure 5.2: Schematic diagram of respiratory circuit. A facemask (Hans Rudolph, Kansas City, MO, USA) connects to a bacterial and viral filter (Vitalograph, Buckingham, UK) from which respiratory gases and respiratory pressure are sampled via polyethylene extension tubing (Vygon SA, Ecouen, France). One sampling line leads to a pressure transducer (MP 45, ± 50 cmH_2O, Validyne Corp., Northridge, CA, USA) connected to an amplifier (Pressure transducer indicator, PK Morgan Ltd, Kent, UK). The second sampling line connects to a gas analyser that samples oxygen and CO_2 (ADInstrument Ltd, Oxford, UK). A one-way valve allows expired air to escape close to the mouth in order to minimize rebreathing (Hans Rudolf, Kansas City, MO, USA). The breathing system contains three arms. Participants usually breathe through the first, unobstructed arm. This arm can be closed off by inflating a balloon (embolectomy balloon, Microtek Medical B.V., Zutphen, Netherlands) via a hydraulic system. Closure of the arm forces participants to breathe through the second arm, which contains a paediatric respiratory filter (mild resistor, Intersurgical, Wokingham, UK) and a second balloon valve. Closure of both valves forces breathing through a third arm, which contains a porous glass disk (strong resistor). The three arms recombine into a spirometry module that records respiratory flow (ADInstruments Ltd, Oxford, UK) connected to a custom-made mixing chamber in which medical air, oxygen and 10% CO_2 in air are combined.

Behavioural ratings

Immediately after each inspiratory load, participants rated the intensity and unpleasantness of their breathing on a horizontal visual analogue scale (VAS) with the anchors 'no breathlessness' on the left and 'severe breathlessness' on the right for intensity ratings and 'not unpleasant' on the right and 'extremely unpleasant' on the left for unpleasantness ratings. The 16-item Bond-Lader VAS (Bond et al. 1974) was used to track mood changes induced by remifentanil 15 minutes before, during and 15 minutes after each scan. These items were analysed to assess the following three dimensions of mood: sociability, tranquillity and sedation (Bond et al. 1974). Additionally, we assessed state anxiety with the state component of the Spielberger State-Trait Anxiety Inventory (STAI-S, (Spielberger, 2010)) 15 minutes before and 15 minutes after each scan. VAS scales were scored as VAS% from 0-100.

Approximately 10 minutes after application of all loads, participants completed the Multidimensional Dyspnea Profile (MDP) (Meek et al., 2012). The version of the questionnaire used in this study can be found in Appendix Ib.

Personality questionnaires

At the beginning of the training session, participants completed these eight psychological self-report questionnaires:

1. The Center for Epidemiologic Studies Depression Scale (CES-D; (Radloff, 1977)). This common depression-screening tool was used to assess whether participants were clinically depressed, with a score of 16 being indicative of clinical depression.

2. The Behavioral Inhibition System and Behavioral Activation System Scale (BIS/BAS; (Carver and White, 1994)). This questionnaire was employed to investigate the strengths of

motivational systems responding to aversive and rewarding stimuli, which have previously been linked to endogenous opioids (Schreckenberger et al., 2008).

3. The Levenson Multidimensional Locus of Control Inventory (Levenson, 1973). This scale was used to assess whether participants feel that they themselves are in control of their life (internal locus of control) or whether they feel that control over their life lies with external forces (external locus of control; 2 scales: Powerful Others and Chance). An internal locus of control has been found to be advantageous for coping with chronic pain (Crisson and Keefe, 1988) and locus of control might have similar effects on coping with dyspnoea.

4. The Thought Control Questionnaire (TCQ; (Reynolds and Wells, 1999)). This questionnaire was employed in order to indicate how individuals control unpleasant and unwanted thoughts, a process that might be applicable to perceived respiratory unpleasantness during loading.

5. The Defence Style Questionnaire (DSQ-40; (Muris and Merckelbach, 1996)) scores individuals on different unconscious dimensions of coping. This questionnaire was used in order to assess differences between the healthy volunteers in this study and patients with COPD, who have been found to have a relatively immature and neurotic defensive profile (Albuquerque et al., 2011).

6. Positive Affect Negative Affect Schedule (PANAS-X; (Watson and Clark, 1994)). The PANAS provides a measure of positive and negative affective state. Negative affect was shown to contribute to health-related quality of life in COPD patients (Hu and Meek, 2005) and might hence be an important concept for the holistic understanding of dyspnoea. We want to record the affective profile of our healthy participants for comparison.

7. Spielberger State-Trait Anxiety Inventory (STAI; (Spielberger, 2010)). Trait anxiety was measured in order to characterize how anxious participants are in general (trait). State differences in anxiety levels were examined on top of that baseline.

8. Anxiety Sensitivity Index (ASI; (Reiss et al., 1986)). Because we were particularly interested in anxiety of physiological sensations, we used the ASI as a measure of fear of anxiety induced by bodily sensations.

FMRI data acquisition

MRI data were acquired on a 3 tesla-Siemens whole-body magnetic resonance scanner. A head-only gradient coil was used with a 32-channel radiofrequency coil for pulse transmission and signal reception. A T1-weighted structural (MPRAGE sequence, time repetition (TR)=1720ms, time echo (TE)=4.68ms, flip angle 8°, voxel size1.0mm^3) image was acquired for image registration. A whole-brain gradient echo, echo-planar-imaging (EPI) sequence (TR=3000ms, TE=30ms, field of view: 192x192mm, voxel size 3.0mm^3, 45 slices, 380 volumes) was used for functional scans. Fieldmaps were obtained using a symmetric-asymmetric spin-echo sequence before the baseline functional scans (30ms echo time, 0.5494 ms dwell time, field of view and matrix identical to EPI) and were used to correct for magnetic field inhomogeneities. A multi-inversion time pseudo-continuous arterial spin labelling sequence (multi-TI PCASL; TIs= 1.65, 1.9, 2.15, 2.4, 2.65, 2.9sec, TI cycles=6, tag duration – 1.4sec, TR=4000ms, TE=13ms, voxel size 3.8x3.8x5.0mm, 24 slices, 72 volumes) was performed to measure changes in CBF induced by remifentanil infusion (MacIntosh et al., 2008). After running a calibration gradient and spin echo (GRASE) scan to allow CBF quantification, a phase contrast scan was performed in order to allow measurement of blood flow changes in the neck. Values obtained from this scan allowed correction of reduced blood

flow velocities with remifentanil compared to saline when quantifying CBF and hence increased accuracy of CBF estimates.

5.2.3 Data analysis

Behavioural ratings

Behavioural data were analysed in SPSS (Version 21, IBM Corp., Armonk, NY). Because data from online ratings were not normally distributed, Friedman's 2-way ANOVA by ranks was performed to compare ratings for unloaded, mild and strong respiratory loads during saline and remifentanil (3x2) intensity and unpleasantness separately.

Separate Wilcoxon signed rank tests were performed to examine differences in the three dimensions of the Bond-Lader questionnaire between saline and remifentanil: sedation, tension and contentment at three time points (15 minutes before infusion, 10-20 minutes after effect site concentration was reached during infusion and 15 minutes after infusion). Differences between the saline and remifentanil sessions in subjective scores of intensity and unpleasantness were correlated with differences in sedation, discontentment and tension in SPSS.

Separate Wilcoxon signed rank tests were performed for each pair of remifentanil and saline ratings at each drug level for each qualitative dimension of the MDP (uncorrected).

Physiological data

Physiological recordings were exported to MatLab (MathWorks Inc., Natick, MA, USA) and analysed using custom-written scripts.

FMRI analysis

Image pre-processing

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The cluster Z threshold was set to 2.3 and a corrected cluster significance threshold of p=0.05 was applied.

Pre-processing of the data was performed with MCFLIRT motion correction (Jenkinson et al., 2002a), non-brain removal using BET (Smith, 2002), spatial smoothing using a full-width-half-maximum Gaussian kernel of 5mm, high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=75.0s) with 150 seconds cut-off. Registration to high resolution structural was carried out using boundary-based-registration (BBR; FSL software library), registration from high resolution structural to standard space was then further refined using nonlinear registration (FNIRT; FSL software library).

BOLD images were corrected for physiological noise with physiological noise modelling integrated in FEAT (Brooks et al., 2008) using two respiratory, two cardiac terms and their first-level interaction EVs. Additional motion correction was performed by using FSL Motion Outliers (FSL software library) to exclude time points in the dataset that are subjected to large, rapid motion artefacts. These data points were excluded from analysis using a confound matrix. Standard and additional motion parameters were calculated by FEAT and used to minimize motion artefacts.

First level analysis

Linear models were designed to describe the data. First level analysis used a general linear modelling (GLM) approach with multiple explanatory variables (EVs). Activation was convolved with a gamma function and a 6-second hemodynamic delay was assumed.

Temporal derivatives were incorporated into the GLM. Individual subject contrasts were generated for unloaded, mild and strong anticipation periods and mild and strong stimulus periods. Periods when participants rated their preceding sensations and the 5-second periods after each respiratory manipulation (fixation cross; presented to give participants a chance to recover before giving their subjective ratings) and the variable fixation period between trials (intertrial interval, ITI) were modelled as regressors of no interest. Setting the model up in this way used the periods of normal breathing starting from 8 seconds into presentation of the visual cue associated with normal breathing, till the end of cue presentation as a functional baseline. $PETCO_2$ was entered as a separate EV in order to account for fluctuations in CO_2 that could affect BOLD signal as discussed in Chapter 3. An additional EV contained the cerebrospinal fluid (CSF) time course throughout the experiment. This EV was prepared by calculating the mean time series of activation within a CSF mask (obtained by running FMRIB's Automated Segmentation Tool (FAST; FSL software library) on each individual structural scan). Lastly, we were interested in dissociating brain activation that correlated with the intensity from that correlating with the unpleasantness of strong inspiratory loads (the main stimulus). Intensity and unpleasantness ratings for normal breathing periods (baseline) and for mild respiratory loads (control stimulus) were very low and not of primary interest and were hence not modelled. Subjective ratings of respiratory intensity and respiratory unpleasantness during strong inspiratory loading were demeaned. They were then entered into the model and orthogonalised to the EV modeling strong inspiratory loads as on/off blocks. They represent the trial-by-trial variability of the subjective response to strong dyspnoea within session. These intensity and unpleasantness regressors were available for 17 participants. Analysis of the remaining two participants was performed as above, but without the added intensity and unpleasantness regressors.

Higher level analyses

Individual effects of remifentanil on the anticipation and perception of respiratory sensations and on the application of the respiratory stimulus were determined by subtracting BOLD activation during the remifentanil session of a given condition from BOLD activation during the saline session of a given condition in a fixed effects analysis (e.g. mild $stimulus_{saline}$ – mild $stimulus_{remi}$) for each participant.

The resulting contrasts were fed into two third-level mixed effects analyses. The primary third-level analysis averaged over the 17 participants with complete subjective ratings on a trial-by-trial basis. This analysis was used to show brain areas related to trial-by-trial variations in perceived intensity and perceived unpleasantness.

The second third-level analysis averaged over 19 participants and was used to determine main effects of stimulus anticipation and stimulus application. Anticipation of strong inspiratory loading was contrasted with anticipation of mild inspiratory loading (contrast $anticipation_{strong}$ - $anticipation_{mild}$) in order to account for potential effects of inspiratory loading on attention. Anticipation of strong respiratory loading was also contrasted to the anticipation of normal breathing (the fist 8 seconds of the safety cue; contrast $anticipation_{strong}$ – $anticipation_{norm}$). During the resting condition, attention was free-floating, whilst it would be focused on bodily sensations in both the mild and strong loading conditions.

Because we were also interested in brain areas that relate to the perception of unpleasantness at a between-subject level, we entered average session intensity and unpleasantness as a covariate. These averages (available for all 19 participants) were added as covariates and their main effects on strong respiratory loading (cleared of trial-by-trial variation) were determined. Brain activation that was not unique to respiratory unpleasantness, but that could reflect

respiratory intensity or unpleasantness, due to their high correlation, was determined by an F-test.

At the third level, FMRIB's Local Analysis of Mixed Effects (FLAME 1+2 (Woolrich et al., 2004)) was used to process images with automatic outlier de-weighing. The difference between resting CBF values during saline and remifentanil sessions was inserted as a voxel-wise regressor in order to account for remifentanil-induced perfusion changes (Pattinson et al., 2009). A cluster threshold of >2.3 and a corrected cluster significance threshold of p=0.05 were used.

Correlation analyses

Additional regression analyses were performed between subjective ratings of strong dyspnoea and neural activation during strong dyspnoea in order to link neural activation to subjective perception. In order to account for potential changes in sedation, sedation was entered as an additional covariate into third level analyses, but no significant correlations were identified. Sedation was hence not further included in the model.

To investigate whether the anticipation of dyspnoea correlates with subjective perception of dyspnoea, parameter estimates were extracted from a 3mm sphere around the peak voxel of difference (X=40, Y=12, Z=2) from the contrast saline ($anticipation_{strong} > anticipation_{mild}$) > remi ($anticipation_{strong} > anticipation_{mild}$). Parameter estimates during mild and strong inspiratory loading were extracted for the saline and remifentanil conditions using Featquery (http://www.fmrib.ox.ac.uk/fsl/feat5/featquery.html).

ASL analysis

Generation of blood-perfusion maps

Blood flow velocities for the left and right carotid and the left and right vertebral arteries were extracted from the phase contrast scan with a custom-written Matlab script. As they showed a 20% perfusion increase from 31 l/sec to 36 l/sec in the left and right carotid arteries ($p=0.005$) during remifentanil compared to saline, this was used to adjust the CBF calculation in order correct for differences in flow when calculating CBF.

Multi-TI ASL data was corrected for physiological noise using RETROICOR (Glover et al., 2000) with a custom-written script that corrected multi-TI data by separating the time points by TI and by tag and control condition, before combining them back together (see Murphy et al. in Appendix). A quantitative perfusion map was generated for each session with a custom-written Matlab script that ran registration with FLIRT (FSL software library) and estimation of CBF with BASIL (Bayesian Inference for Arterial Spin Labeling MRI; part FSL software library). The resulting voxel-wise CBF averages for each session were incorporated into higher-level FMRI analyses to help account for the confounding effects of global CBF changes with remifentanil on the BOLD signal (Pattinson et al., 2009).

Visualisation of perfusion changes with remifentanil

Differences in CBF during remifentanil compared to saline infusion were calculated by subtracting CBF during saline infusion from CBF during remifentanil for each participant. Significant voxels were determined across the group by permutation testing using threshold-free cluster enhancement (TFCE) thresholded at $p<0.005$ using randomise (part of FSL).

5.3 Results

5.3.1 Psychological and physiological data

Respiratory intensity and unpleasantness

Subjective ratings of perceived intensity and unpleasantness of respiration confirmed the successful induction of mild and strong dyspnoea. During the saline condition, participants perceived respiratory intensity of 12%VAS (±17%SD) and respiratory unpleasantness of 10%VAS (±18%SD) during normal breathing. Mild inspiratory loading (-3cmH_2O) was associated with respiratory intensity of 29%VAS (±16%SD) and respiratory unpleasantness of 21%VAS (±21.5%SD) and strong inspiratory loading (-12cmH_2O) with a respiratory intensity of 71%VAS (±20%SD) and respiratory unpleasantness of 61%VAS (±31%SD) (Figure 5.3). Remifentanil did not significantly change perceived respiratory intensity (p=0.275), but significantly reduced respiratory unpleasantness (p=0.036; Figure 5.3).

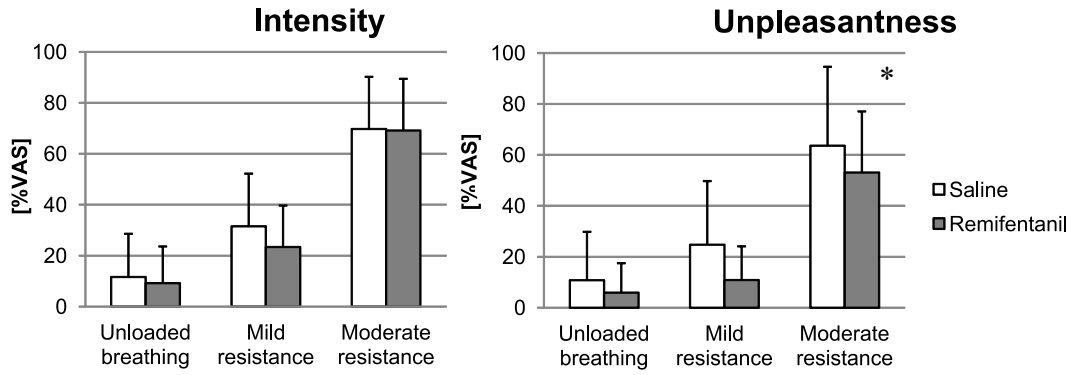


Figure 5.3: Mean respiratory intensity and unpleasantness induced by slightly CO_2-loaded breathing (unloaded breathing), additional mild inspiratory loading (mild resistance) and additional moderate inspiratory loading (moderate resistance) as rated during saline and remifentanil infusions. Data are mean ± SD for 19 participants. *=significant main effect at p=0.05.

Evaluation of the three different stimulus levels (unloaded, mild inspiratory load and strong inspiratory load) on the six sensory and the six affective dimensions of the MDP after each saline and remifentanil session are shown in Figure 5.4.

Effects of remifentanil on mood and alertness

Self-reported sedation was significantly increased during remifentanil infusion (26%-40%VAS, $p<0.0001$) and 15 minutes after remifentanil infusion (22%-40%, $p<0.0001$, Figure 5.5). Self-reported contentment and tension were decreased during the infusion of remifentanil compared to saline infusion (contentment: 32%-23%VAS, p=0. 002; tension: 40% to 21%VAS, p=0.002, Figure 5.5). State anxiety scores were low for all participants and did not significantly differ 15 minutes before and 15 minutes after the infusions (Figure 5.5).

The reduction in dyspnoea unpleasantness did not correlate with increased sedation during remifentanil compared to saline (R=.149; p=0.544, not shown). Decreased tension during remifentanil did not correlate with reductions in unpleasantness ratings (R=.292, p=0.225, not shown). The reduction in dyspnoea unpleasantness did negatively correlate with increased contentment during remifentanil compared to saline (R=-.476, p=0.039, not shown).

Psychological characterisation of participants

Participants scored relatively low on psychological dimensions relating to negative affect and depression, anxiety and catastrophizing. Results of the psychological questionnaires used to characterize participants in the study are presented in Table 5.1.

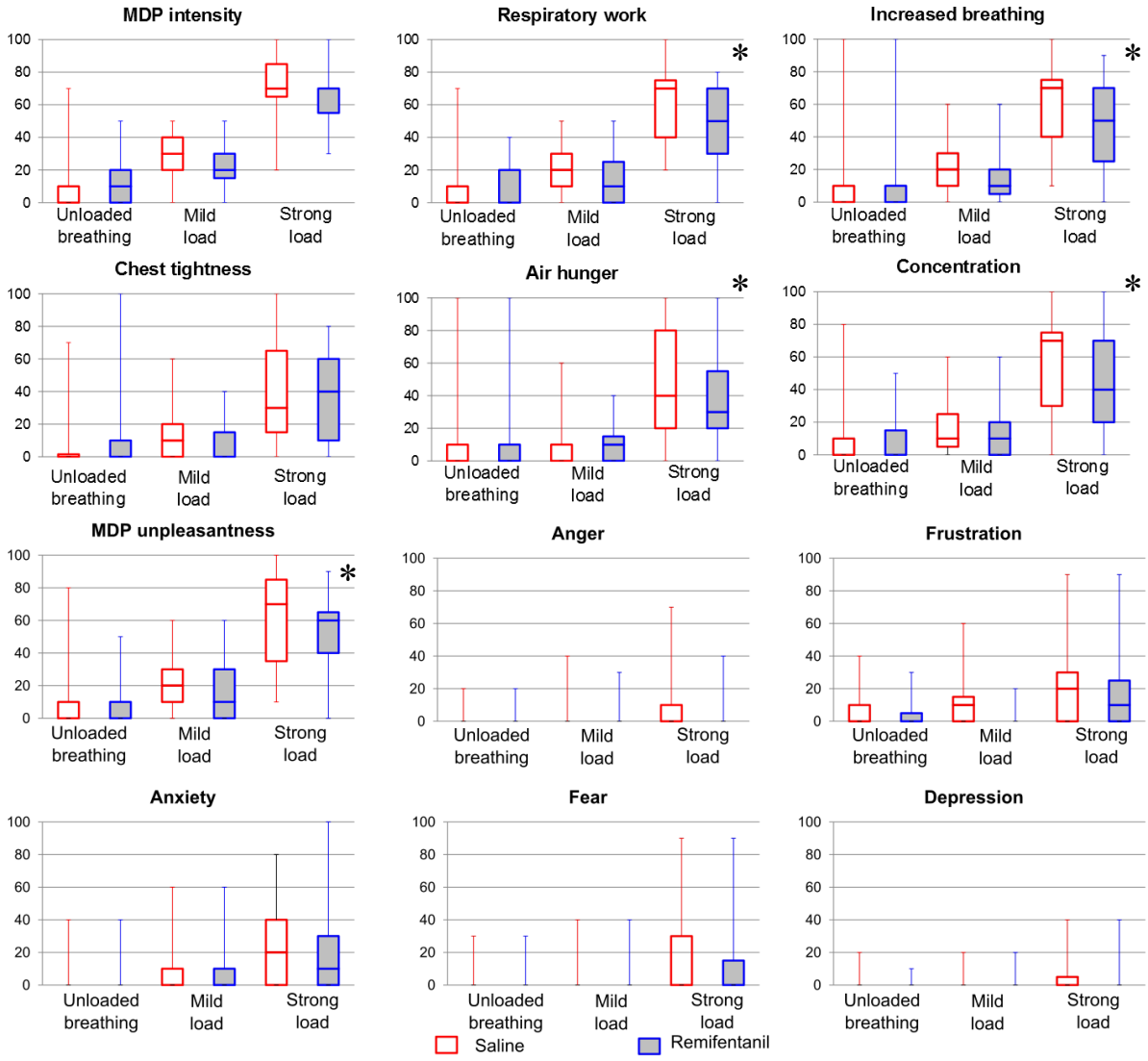


Figure 5.4: Sensory and affective dimensions of dyspnoea during saline (red) and remifentanil (blue). Mean ± SD. N=19. Data presented as box plots. Significance determined with individual Wilcoxon signed rank tests for each pair of each dimension.
*significant difference between saline and remifentanil condition at $p<0.05$ (uncorrected).

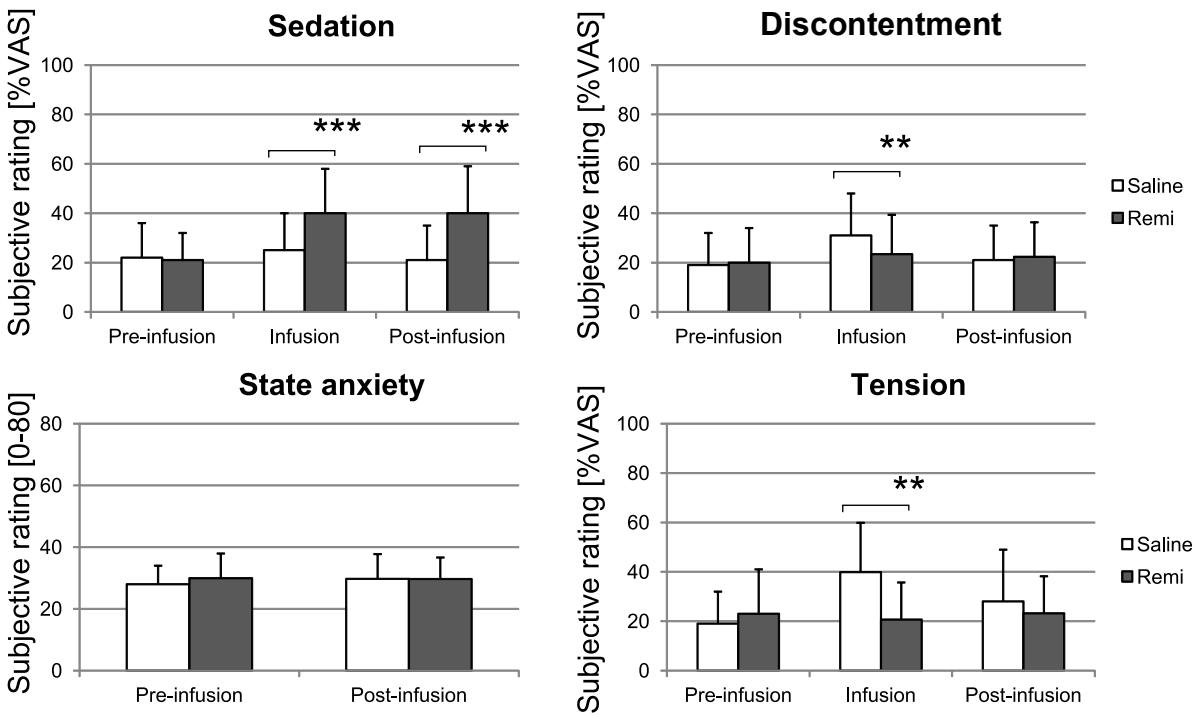


Figure 5.5: Sedation, tension and contentment (3 dimensions of the Bond-Lader questionnaire) 15 minutes before the infusion (pre-infusion), halfway through the infusion (infusion) and 15 minutes after the infusion (post-infusion) for saline and remifentanil. State anxiety levels 15 minutes before and 15 minutes after infusion for saline and remifentanil. Data are mean ± standard deviation for 19 participants. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 5.1: Means and standard deviations for questionnaire scores for all 19 participants. Possible ranges of scores are reported in parentheses behind each questionnaire abbreviation.

Questionnaire	Subscale	Possible range	Range in sample	Mean	SD
PANAS-X	Negative affect	(10-50)	10-26	14	5
	Positive affect	(10-50)	16-48	32	8
	Total affect*	(-40-40)	-5-37	17	10
BIS/BAS	BAS drive	(4-16)	9-16	12	2
	BAS fun seeking	(4-16)	6-16	12	3
	BAS reward	(5-20)	12-20	17	2
	Total BAS**	(13-52)	28-47	41	5
	BIS	(7-28)	10-26	20	4
LOC	Internal	(0-48)	19-42	32	6
	Powerful others	(0-48)	0-30	15	9
	Chance	(0-48)	4-33	17	7
DSQ-40	Mature	(2-18)	9-15	12	2
	Neurotic	(2-18)	7-14	9	2
	Immature	(2-18)	3-10	7	2
ASI	ASI	(0-64)	5-31	15	7
STAI-Trait	TAI	(20-80)	23-57	34	9
TCQ	TCQ total	(30-120)	47-78	63	10
	Distraction	(6-24)	13-20	17	2
	Punishment	(6-24)	6-14	9	2
	Reappraisal	(6-24)	11-19	15	2
	Worry	(6-24)	6-12	8	2
	Social control	(6-24)	6-24	14	5
CES-D	CES-D	(0-60)	0-23	7	6
Catastrophizing		(0-52)	0-35	8	10

N=19

* positive affect minus negative affect

** BAS drive + BAS fun seeking + BAS reward

Effects of remifentanil on blood flow and physiology

Effects of remifentanil on physiological variables for all participants during BOLD are shown in Table 5.2. Remifentanil decreased minimum, maximum and amplitude of mouth pressure and increased $PETCO_2$ compared to saline, but did not alter $PETO_2$ and heart rate. Remifentanil increased cerebral blood flow by approximately 10% compared to saline (Figure 5.6, Table 5.3). Physiological data collected during ASL are shown in Table 5.3.

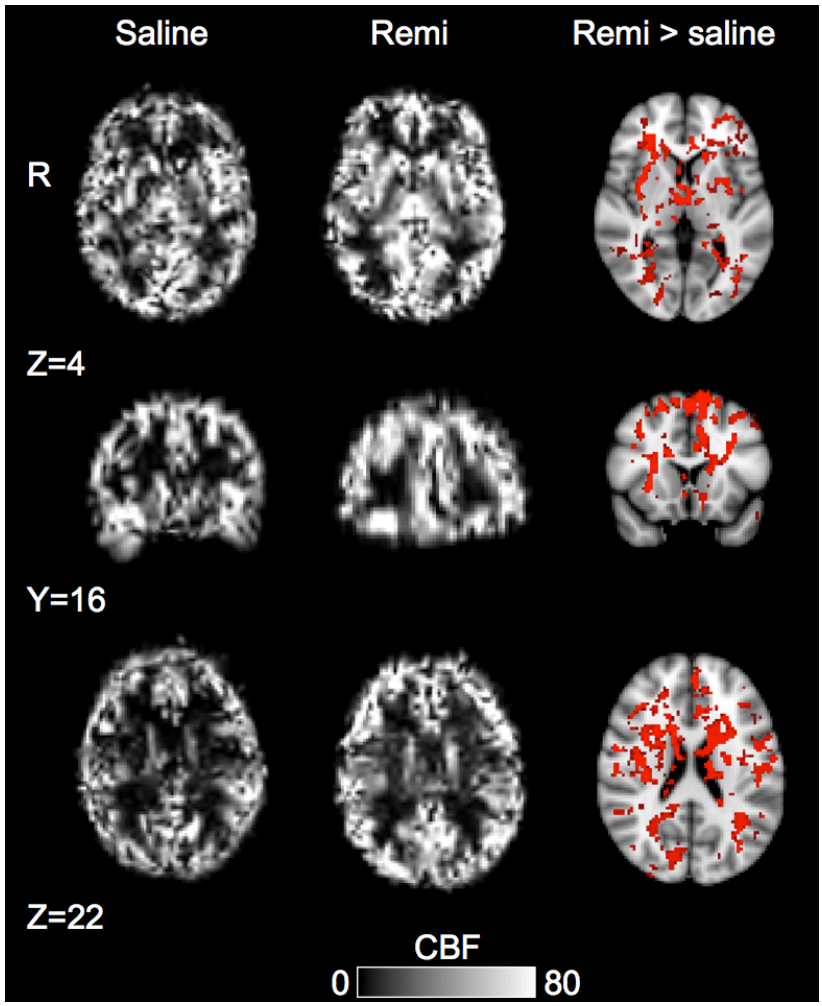


Figure 5.6: Average CBF for saline (left) and remifentanil sessions (middle). Voxel-wise comparison of CBF between saline and remifentanil infusion (right) is shown on the right. Voxels significantly increased during remifentanil compared to saline across the group (N=19) were determined by permutation testing ($P<0.05$, TFCE) and are indicated in red.

Table 5.2: Effects of mild and moderate respiratory loading on respiratory, cardiovascular and subjective parameters (N=19). Values are presented as mean (SD). * =significantly different from unloaded period at p=0.05.

	Saline			**Remifentanil**			
Variable	**Unloaded breathing**	**Mild loading**	**Strong loading**	**Unloaded breathing**	**Mild loading**	**Strong loading**	**Remi-saline comparison (p value)**
Minimum mouth pressure [cmH_2O]	-1.6 (0.4)	-3.1 (0.7)*	-12.0 (4.0)*	-1.3 (0.4)	-2.5 (0.6)*	-10.4 (3.4)*	.035
Maximun mouth pressure [cmH_2O]	0.8 (0.2)	0.9 (0.2)	0.5 (0.8)	0.6 (0.2)	0.7 (0.2)	0.4 (1.0)	.0001
Mouth pressure amplitude [cmH_2O]	2.4 (0.5)	4.0 (0.8)*	12.7 (4.1)*	2.0 (0.4)	3.2 (0.6) *	10.9 (3.4)*	.018
$PETCO_2$ [kPa]	5.6 (0.6)	5.5 (0.5)	5.5 (0.6)	6.0 (0.6)	6.0 (0.6)	6.0 (0.6)	>.0001
$PETO_2$ [kPa]	19.6 (1.3)	19.9 (1.5)	19.9 (1.2)	19.9 (1.1)	20.2 (1.2)	20.3 (1.3)	.371
Heart rate (N=14) [bpm]	65.9 (12.3)	66.0 (14.0)	67.4 (12.8)	68.6 (14.4)	66.3 (10.0)	66.3 (10.0)	.849
Intensity rating [%VAS]	11.6 (17.0)	31.5 (20.7)*	69.7 (20.5)*	9.2 (14.4)	23.4 (16.3)*	69.1 (20.3)*	.275
Unpleasantness rating [%VAS]	10.8 (19.0)	24.8 (25.0)*	63.6 (31.0)*	5.9 (11.5)	10.8 (13.3)*	53.1 (24.0)*	.036

Table 5.3: Effects of remifentanil on respiratory and cardiac parameters during ASL (N=19). Values are presented as mean (SD).

Variable	Saline	Remifentanil	Remi-saline comparison (p value)
Right carotid artery flow	31.1 (6.6)	36.3 (8.4)	.005
Left carotid artery flow [l/s]	30.9 (7.4)	35.5 (10.7)	.005
GM CBF [ml/100g/min]	40.7 (4.4)	44.8 (4.2)	.003
Minimum mouth pressure [cmH_2O]	-1.3 (0.4)	-1.1 (0.33)	.035
Maximun mouth pressure [cmH_2O]	0.7 (0.2)	0.7 (0.2)	.270
Mouth pressure amplitude [cmH_2O]	2.0 (0.4)	1.7 (0.3)	.054
$PETCO_2$ [kPa]	5.5 (0.6)	6.0 (0.6)	<.0001
$PETO_2$ [kPa]	19.5 (1.4)	19.5 (1.4)	.973
Heart rate [bpm] (N=18)	66.2 (11.0)	64.2 (8.5)	.246

5.3.2 Neuroimaging results

Anticipation

The contrast $anticipation_{strong}$-$anticipation_{mild}$ showed activation in the anterior insula/operculum, premotor cortex and the precentral gyrus (Figure 5.7, Table 5.4).

Anticipation of strong dyspnoea (contrast $anticipation_{strong}$ – $anticiaption_{normal}$) elicited BOLD activation in the bilateral anterior insula/operculum, premotor cortex, the ACC/ paracingulate, right DLPFC, left precentral gyrus, left putamen and cerebellar crus I (Table 5.4).

Anticipation of mild dyspnoea elicited changes in visual cortices (not shown).

The contrast $anticipation_{strong}$-$anticipation_{mild}$ showed lower activation in the areas activated during the saline condition (i.e. anterior insula/operculum, paracingulate cortex and left precental gyrus) with remifentanil compared to saline (Figure 5.7, Table 5.4).

During anticipation of strong dyspnoea, remifentanil reduced brain activation in the ACC and right DLPFC, areas that were activated during strong anticipation in the saline condition (Table 5.4). Additionally, remifentanil decreased activation in the left dorsomedial prefrontal cortex (Table 5.4).

The BOLD response to the anticipation of a mild respiratory load was not altered by remifentanil.

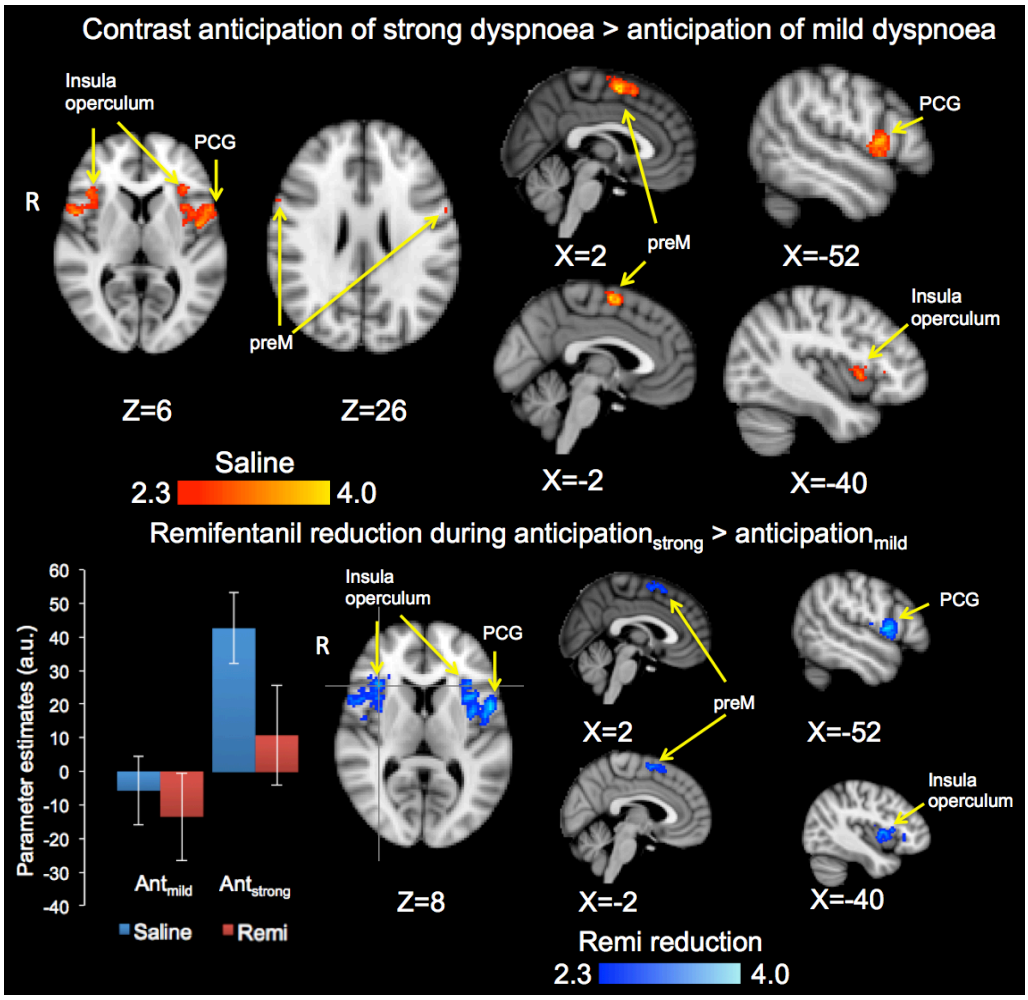


Figure 5.7: BOLD activation in the contrast anticipation$_{strong}$ > anticipation$_{mild}$ and significant reductions in BOLD activation during remifentanil compared to saline in the contrast anticipation$_{strong}$ > anticipation$_{mild}$. Parameter estimates were derived from 3mm sphere around peak voxel of contrast saline (anticipation$_{strong}$ > anticipation$_{mild}$) > remi (anticipation$_{strong}$ > anticipation$_{mild}$). Bar graph represents Mean + SEM of 3mm sphere around voxel X=38, Y=24, Z=8. preM=premotor cortex. N=19. PCG=precentral gyrus.

Table 5.4: Locations of significant activation peaks of BOLD activation during the contrast $anticipation_{strong}$ > $anticipation_{mild}$ and the reduction in BOLD with remifentanil (contrast saline ($anticipation_{strong}$ > $anticipation_{mild}$)> remi ($anticipation_{strong}$ > $anticipation_{mild}$)). Locations of significant activation peaks of BOLD activation in contrast $anticipation_{strong}$ > $anticipation_{no\ change}$ and reduction in BOLD activation during $anticipation_{strong}$ with remifentanil compared to saline (contrast saline ($anticipation_{strong}$ > $anticipation_{no\ change}$)> remi ($anticipation_{strong}$ > $anticipation_{no\ change}$)). Coordinates are derived from cluster-based analyses. The most significant maximum is listed for each anatomic location. Co-ordinates are in mm standard space of MNI. L=left, R=right. $_{norm}$=first eight seconds of normal breathing. $^{\#}$=no reduction in activation with remifentanil. FOC=frontal opercular cortex. ACC=anterior cingulate cortex. DLPFC=dorsolateral prefrontal cortex, DMPFC=dorsomedial prefrontal cortex, S2=secondary somatosensory cortex.

Condition	**Region**	**Hemisphere**	**max Z score**	**X**	**Y**	**Z**
Saline $Anticipation_{strong>mild}$	Precentral gyrus	L	3.62	-52	8	10
	FOC	R	3.67	48	10	2
	S2	L	3.45	-46	-2	6
	Anterior insula	L	3.23	-38	10	0
	Anterior insula/operculum	R	3.39	38	24	8
	Premotor cortex	L	3.97	-2	-4	62
	Premotor cortex	R	3.94	2	0	58
Saline > remi $Anticipation_{strong>mild}$	Precentral gyrus	L	3.86	-52	4	8
	FOC	R	3.92	50	12	2
	S2	L	3.87	-46	-2	6
	Anterior insula/operculum	L	3.69	-38	2	6
	Anterior insula/operculum	R	3.60	36	22	8
	Premotor cortex	L	3.48	-4	0	62
	Premotor cortex	R	3.20	4	4	60
Saline $Anticipation_{strong>normal}$	Anterior insula/operculum$^{\#}$	L	3.70	-40	16	0
	Anterior insula/operculum$^{\#}$	R	3.84	36	18	8
	ACC	L	3.78	-10	8	38
	ACC	R	3.36	4	16	38
	DLPFC	R	3.86	32	48	32
	Precentral gyrus$^{\#}$	L	4.08	-58	8	14
	FOC$^{\#}$	R	3.67	48	10	2
	Premotor cortex$^{\#}$	R	4.36	2	2	60
	Putamen$^{\#}$	L	3.21	-22	8	10
	Cerebellum crus I$^{\#}$	L	3.82	-40	-60	-28
Saline > remi $anticipation_{strong>normal}$	DLPFC	L	3.52	-26	54	28
	DMPFC	L	3.81	-14	52	36
	Paracingulate	L	3.72	4	48	26
	Paracingulate/ACC	R	3.71	6	36	30

BOLD response to inspiratory loading

BOLD activation in response to mild inspiratory loading elicited bilateral BOLD activation in S1, S2, M1, premotor cortex and activation in the left DLPFC and frontal opercular cortex (FOC) (Figure 5.8, Table 5.5).

BOLD activation in response to strong inspiratory loading elicited bilateral BOLD activation in the right posterior insula, bilateral primary and secondary somatosensory cortices, premotor and motor cortices, the precentral gyrus, temporal poles, MTG, frontal orbital cortex, fusiform cortex and cerebellum (Figure 5.8, Table 5.6).

The contrast inspiratory $loading_{strong} > loading_{mild}$ reveals activation associated with higher inspiratory loading in the bilateral premotor, motor, primary and secondary somatosensory cortices, the right temporal pole and cerebellum (Figure 5.9, Table 5.7).

BOLD activation in the neural network activated during mild inspiratory loading did not change with remifentanil, but remifentanil reduced BOLD activation in V1 (not shown, Table 5.5).

During strong inspiratory loading, remifentanil reduced activation in areas activated during strong inspiratory loading (i.e. the primary and secondary somatosensory cortices, premotor and primary motor cortices, right anterior insula and right temporal pole; Figure 5.8, Table 5.6). Additionally, remifentanil reduced activation in the left posterior insula, right frontal opercular cortex, right ventromedial prefrontal cortex and temporal pole (Figure 5.8, Table 5.6). Activation in the precentral gyrus, left temporal pole, middle temporal gyrus, left frontal opercular cortex, TOFC and cerebellum did not change with remifentanil (Table 5.6).

In the contrast $load_{strong}$ > $load_{mild,}$ remifentanil reduced activation in areas activated during this contrast during saline (i.e. primary and secondary somatosensory cortices, premotor and primary motor cortices, and cerebellum; Figure 5.9, Table 5.7). Additionally remifentanil decreased activation in the posterior cingulate cortex, fusiform cortex, precentral gyrus and thalamus (Figure 5.9, Table 5.7). BOLD activation in the medial temporal gyrus, temporal pole and cerebellum were not altered by remifentanil (Table 5.7).

Table 5.5: Locations of significant activation peaks of BOLD activation during $load_{mild}$, and reduction in BOLD activation with remifentanil $load_{mild}$. Coordinates are derived from cluster-based analyses. The most significant maximum is listed for each anatomic location. Co-ordinates are in mm standard space of MNI. L=left, R=right. S1=primary somatosensory cortex, S2=secondary somatosensory cortex, M1=primary motor cortex, BA6=premotor cortex, BA44=Broadman area 44, DLPFC=dorsolateral prefrontal cortex, V3= third visual complex, V1=primary visual cortex.

Condition	**Region**	**Hemisphere**	**max Z score**	**X**	**Y**	**Z**
Saline $load_{mild}$	S1	L	4.6	-60	-12	42
	S1	R	4.72	60	-12	44
	S2	L	4.45	-66	-14	24
	S2	R	4.96	60	-12	12
	M1	L	3.59	-52	-8	40
	M1	R	3.84	48	-8	40
	BA6	L	4.53	-50	-4	52
	BA6	R	5.35	56	-4	44
	BA44	L	4.84	-60	10	8
	BA44	R	4.92	60	14	2
	Anterior insula	L	3.98	-38	-2	12
	Anterior insula	R	3.67	38	4	6
	DLPFC	L	3.49	-30	60	20
	V3	R	5.71	36	-88	-10
Saline > remi $load_{mild}$	V1	R	4.32	18	-100	6

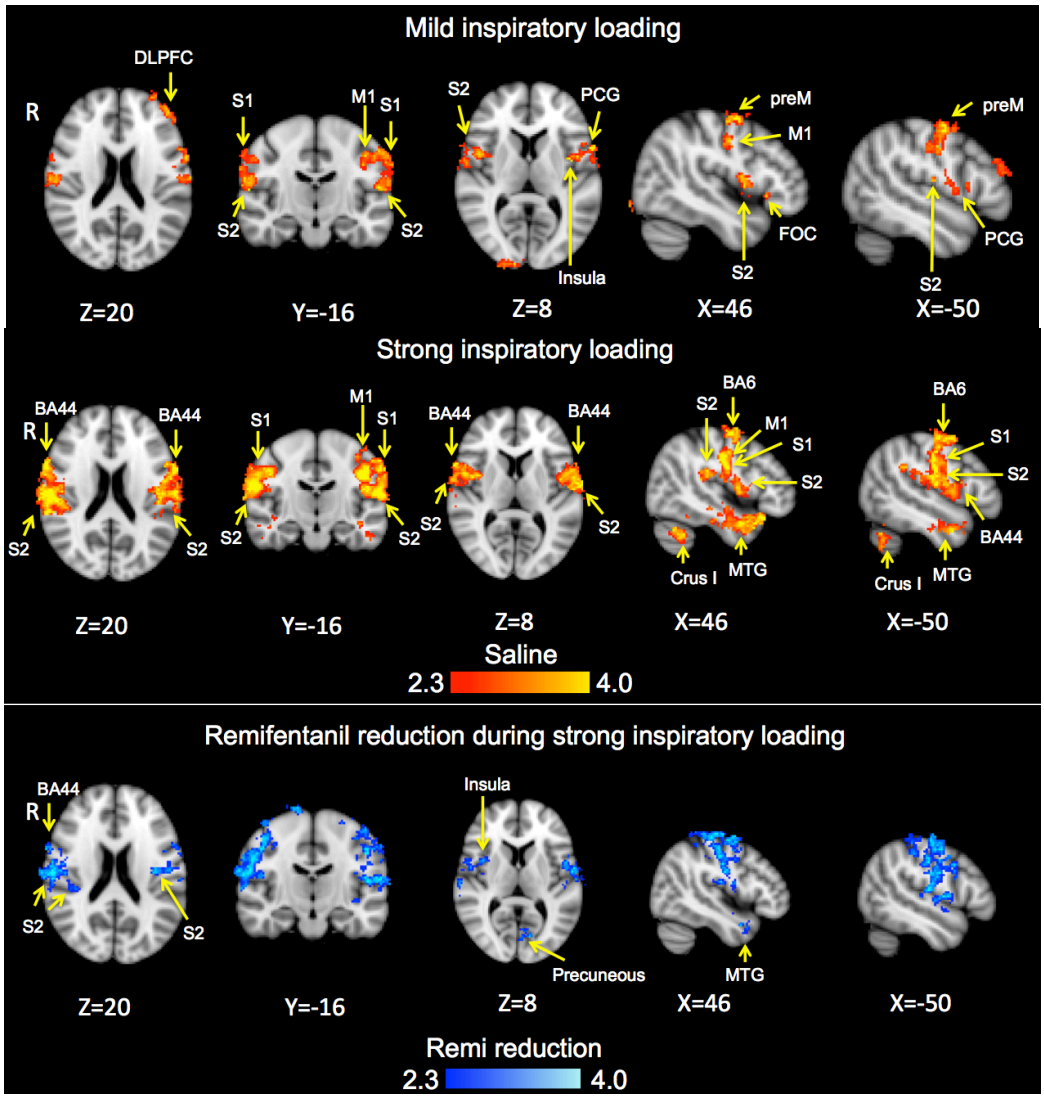


Figure 5.8: BOLD activation during mild inspiratory loading and strong inspiratory loading in 19 participants after having accounted for CO_2-induced vasodilation. Activation during saline is indicated in red-yellow. Reduction in activation during remifentanil infusion is depicted in blue-lightblue. Image consists of a colour-rendered statistical map superimposed on a standard (MNI) brain. Significant regions are displayed with a threshold of $Z<2.3$ with a cluster probability threshold of $p<0.05$ (corrected for multiple comparisons). R=right. S1=primary somatosensory cortex, S2=secondary somatosensory cortex, M1=primary motor cortex, BA6=premotor cortex, MTG=middle temporal gyrus, BA44=Broadman area 44, DLPFC=dorsolateral prefrontal cortex. FOC=frontal opercular cortex.

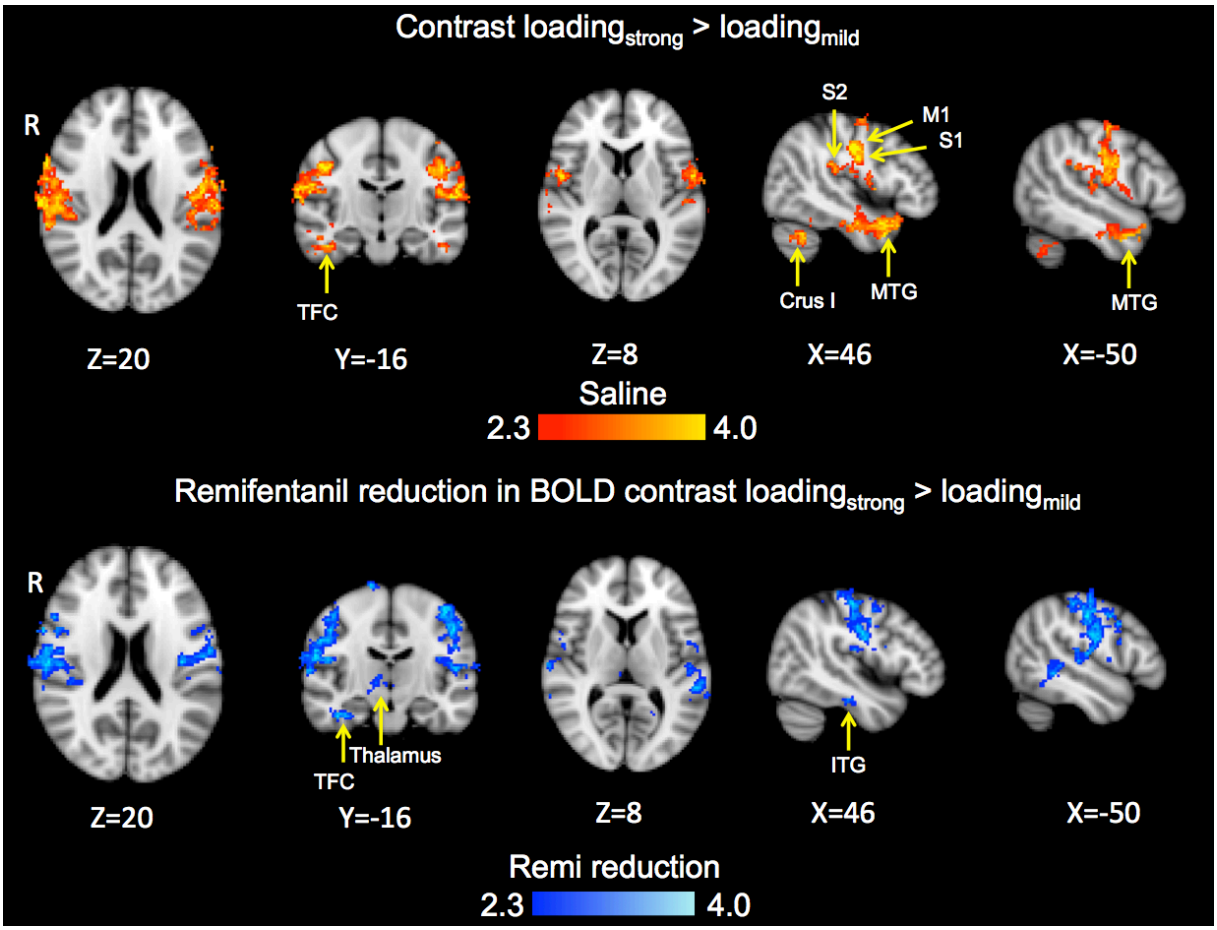


Figure 5.9: BOLD activation during the contrast loading$_{strong}$ > loading$_{mild}$ in 19 participants after having accounted for CO_2-induced vasodilation. Image consists of a colour-rendered statistical map superimpose on a standard (MNI) brain. Significant regions are displayed with a threshold of Z<2.3 with a cluster probability threshold of p<0.05 (corrected for multiple comparisons). Enhanced activity for load$_{strong}$ > load$_{mild}$ during the saline condition compared to the remifentanil session is shown in blue. R=right. TFC=temporal fusiform cortex, S1=primary somatosensory cortex, S2=secondary somatosensory cortex, M1=primary motor cortex, MTG=middle temporal gyrus, ITG=inferior temporal gyrus.

Table 5.6: Locations of significant activation peaks of BOLD activation during $load_{strong}$ and reduction in BOLD activation with remifentanil $load_{strong}$. Coordinates are derived from cluster-based analyses. The most significant maximum is listed for each anatomic location. Co-ordinates are in mm standard space of MNI. L=left, R=right. #=no reduction in activation with remifentanil.

Condition	Region	Hemisphere	max Z score	X	Y	Z
Saline $load_{strong}$	S1	L	6.43	-58	-18	40
	S1	R	7.27	48	-10	28
	S2	L	6.45	-64	-18	30
	S2	R	8	56	-6	12
	M1	L	5.89	-52	-10	44
	M1	R	6.06	44	-12	36
	Premotor cortex	L	4.22	-2	2	56
	Premotor cortex	R	6.48	2	0	58
	Precentral gyrus#	L	4.43	-56	6	12
	Precentral gyrus	R	4.08	60	8	4
	Anterior insula	R	3.89	38	2	8
	Temporal pole#	L	4.81	-46	14	-26
	Temporal pole	R	4.88	42	18	-26
	Middle temporal gyrus#	L	4.47	-46	-2	-26
	Middle temporal gyrus#	R	4.68	54	-4	-22
	Frontal orbital cortex#	L	4.25	-34	22	-26
	Temporal occipital fusiform cortex#	L	3.86	-38	-48	-20
	Temporal occipital fusiform cortex#	R	3.4	40	-44	-20
	Cerebellum VI#	R	5.39	20	-58	-22
	Cerebellum Crus I#	L	4.56	-52	-62	-30
	Cerebellum Crus I#	R	4.55	42	-52	-32
	Cerebellum Crus II#	L	4.59	-40	-52	-48
	Cerebellum VIIIa#	R	4.1	32	-54	-48
	Cerebellum VIIIb#	L	5.53	-36	-52	-48
Saline > remi $load_{strong}$	S1	L	5.7	-54	-10	40
	S1	R	4.71	48	-8	28
	S2	L	5.05	-60	-16	14
	S2	R	5.46	60	-14	20
	M1	L	3.83	-48	-6	40
	M1	R	4.31	48	-12	44
	Premotor cortex	L	4.4	-48	-4	48
	Premotor cortex	R	4.78	42	-22	56
	Posterior insula	L	3.24	-40	-12	-4
	Anterior insula	R	4.01	38	6	6
	Frontal opercular cortex	R	4.79	24	30	-14
	Ventromedial PFC	R	3.41	16	42	-18
	Temporal pole	R	4.03	44	6	-26
	Planum polare	R	3.98	42	-4	-16
	Lateral occipital cortex	R	4.06	12	-70	62
	Lingual gyrus	L	3.87	-22	-48	-4
	V1	L	3.85	-12	-74	10

Table 5.7: Locations of significant activation peaks of BOLD activation during contrast $load_{strong}$ > $load_{mild}$ and reduction in BOLD activation with remifentanil in contrast $load_{strong}$ > $load_{mild}$. Coordinates are derived from cluster-based analyses. The most significant maximum is listed for each anatomic location. Co-ordinates are in mm standard space of MNI. L=left, R=right. #=no reduction in activation with remifentanil. S1=primary somatosensory cortex, S2=secondary somatosensory cortex, M1=primary motor cortex, BA6=premotor cortex, MTG=middle temporal gyrus, PCC=posterior cingulate cortex, TOFC= temporal occipital fusiform cortex, TFC= temporal fusiform cortex.

Condition	Region	Hemisphere	max Z score	X	Y	Z
Saline $load_{strong>mild}$	S1	L	4.75	-58	-10	38
	S1	R	6.08	62	0	24
	S2	L	5.54	-64	-18	18
	S2	R	5.88	56	-2	14
	M1	L	4.14	-42	-12	36
	M1	R	5.4	50	-6	34
	BA6	L	4.17	-44	-12	60
	BA6	R	5.54	58	-8	34
	Temporal pole#	L	5.11	-48	4	-26
	Temporal pole#	R	5.26	52	8	-26
	MTG#	L	4.39	-58	-6	-24
	MTG#	R	5.04	54	4	-30
	Cerebellum VI#	L	4.28	-14	-64	-20
	Cerebellum VI#	R	4.31	14	-68	-18
	Cerebellum crus I	L	3.73	-44	-56	-30
	Cerebellum crus I#	R	4.05	44	-52	-36
	Cerebellum right VIIIb#	R	3.90	40	-54	-48
Saline > remi $load_{strong>mild}$	S1	L	4.18	-48	-18	44
	S1	R	4.3	48	-10	28
	S2	L	3.6	-50	-16	12
	S2	R	3.99	60	-14	22
	M1	L	3.8	-36	-22	46
	M1	R	3.77	44	-18	48
	BA6	L	4.22	-42	-18	58
	BA6	R	3.98	32	-4	48
	PCC	R	3.79	14	-38	-2
	TOFC	L	3.27	-34	-44	-16
	TOFC	R	3.72	30	-40	-20
	TFC	R	3.73	42	-16	-26
	Thalamus	L	3.5	-2	-22	0
	Thalamus	R	3.53	6	-22	-2
	Superior parietal lobule	R	3.97	16	-70	62
	Lingual gyrus	L	3.8	-20	-48	-6
	Precentral gyrus (BA44)	L	3.86	-54	10	36
	Precentral gyrus (BA44)	R	3.74	62	8	20
	Cerebellum crus I	L	2.55	-40	-54	-28

Trial-by-trial variations in dyspnoea perception

Trial-by-trial variation in perception of dyspnoea intensity during strong inspiratory loading was correlated with BOLD activation in the left anterior and posterior insula, left thalamus, left precentral gyrus, left premotor cortex and left secondary somatosensory cortex (Figure 5.10, Table 5.8).

Trial-by-trial variation in the perception of dyspnoea unpleasantness during strong inspiratory loading was correlated with BOLD activation in the right dorsal prefrontal cortex, ventromedial prefrontal cortex, anterior cingulate cortex and cuneal cortex (Figure 5.10, Table 5.9).

Remifentanil reduced activation correlated with dyspnoea intensity in structures activated during the saline condition (i.e. left thalamus, left precentral gyrus; Figure 5.10, Table 5.8). Additionally remifentanil revealed reduced activation in the right thalamus, right hippocampus, right amygdala and right periaqueductal grey (PAG; Figure 5.10, Table 5.8).

Remifentanil reduced activation correlated with dyspnoea unpleasantness in the anterior cingulate cortex and right dorsomedial prefrontal cortex, both previously active in the saline condition (Figure 5.10, Table 5.9).

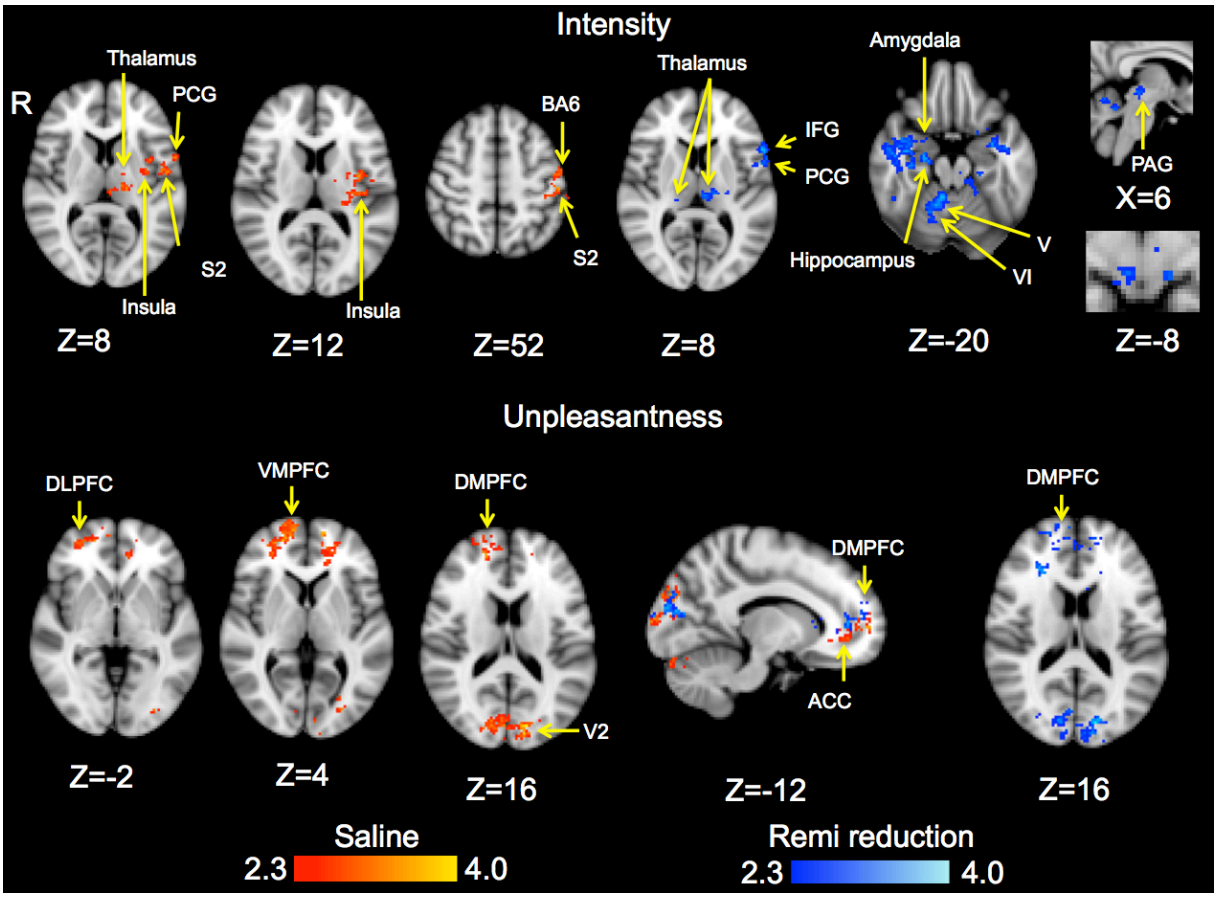


Figure 5.10: Trial-by-trial variations in subjective perception of dyspnoea intensity and unpleasantness in 17 participants. Activation during saline is indicated in hot colours. Reduction in activation during remifentanil infusion is depicted in cold colours. Image consists of a colour-rendered statistical map superimposed on a standard (MNI) brain. Significant regions are displayed with a threshold of $Z<2.3$ with a cluster probability threshold of $p<0.05$ (corrected for multiple comparisons). R=right. S2=secondary somatosensory cortex, PCG=precentral gyrus, DLPFC=dorsolateral prefrontal cortex, VMPFC=ventromedial prefrontal cortex, DMPFC=dorsomedial prefrontal cortex, ACC=anterior cingulate cortex, V2=secondary visual cortex, V= Cerebellar lobe V, VI=Cerebellar lobe VI.

Table 5.8: Bold activation related to trial-by-trial variation in perceived dyspnoea intensity during strong inspiratory loading. Coordinates are derived from cluster-based analyses. The most significant maximum is listed for each anatomic location. Co-ordinates are in mm standard space of MNI. L=left, R=right. S1=primary somatosensory cortex, S2=secondary somatosensory cortex, BA6=premotor cortex, PAG=periaqueductal gray.

Condition	**Brain region**	**Side**	**max Z score**	**X**	**Y**	**Z**
Intensity saline	Anterior insula	L	3.35	-36	-4	12
	Posterior insula	L	3.05	-40	-18	12
	Thalamus	L	3.51	-16	-20	4
	Thalamus	L	3.3	-10	-14	2
	BA6	L	3.79	-38	-18	68
	Precentral gyrus	L	3.48	-56	4	6
	S1	L	3.55	-48	-24	56
	S2	L	3.48	-50	-6	10
	Superior temporal gyrus	L	3.49	-56	-2	-8
	Temporal pole	L	3.12	-58	6	-4
Intensity saline > remi	Posterior insula	L	3.46	-38	-4	-16
	Posterior insula	R	3.75	40	0	-18
	Thalamus	L	3.57	-16	-26	0
	Thalamus	R	3.33	14	-24	-2
	Amygdala	L	3.37	-32	-2	-20
	Amygdala	R	3.14	30	0	-18
	Hippocampus	L	3.33	-24	-12	-32
	Hippocampus	R	3.84	34	-20	-24
	Precentral gyrus	L	3.71	-56	6	6
	Temporal pole	L	3.26	-50	6	-6
	Temporal pole	R	4.14	54	10	-10
	Inferior temporal gyrus	L	3.6	44	-52	-10
	Cerebellum V	L	3.2	-18	-42	-24
	Cerebellum V	R	3.96	12	-50	-24
	Cerebellum VI	R	3.71	24	-58	-30
	PAG	R	3.10	6	-26	-8

Table 5.9: Bold activation related to trial-by-trial variation in perceived dyspnoea unpleasantness during strong inspiratory loading. Coordinates are derived from cluster-based analyses. The most significant maximum is listed for each anatomic location. Co-ordinates are in mm standard space of MNI. L=left, R=right. STG=superior temporal gyrus, DLPFC=dorsolateral prefrontal cortex, VMPFC=ventromedial prefrontal cortex, ACC=anterior cingulate cortex, V2=secondary visual cortex.

Condition	Brain region	Side	max Z score	X	Y	Z
Unpleasantness saline	DLPFC	R	3.87	28	54	6
	VMPFC	R	3.69	20	56	-4
	ACC/paracingulate	L	3.15	-10	42	-4
	Superior occipital cortex	R	3.66	28	-86	38
	Cuneal cortex	L	4.09	-8	-88	26
	V2	R	3.76	6	-82	24
	Cerebellar crus I	L	3.68	-24	-88	-26
Unpleasantness saline > remi	ACC/paracingulate	L	3.4	-12	44	6
	DMPFC	R	2.99	6	62	16
	Planum temporale	R	3.28	62	-18	4
	STG	R	3.24	62	-22	-4
	Cuneal cortex	L	3.45	-10	-88	20
	Primary auditory cortex	R	3.21	56	-6	0

Table 5.10: BOLD correlations with subjective dyspnoea perception during strong inspiratory loading during saline and saline > remifentanil. Coordinates are derived from cluster-based analyses. The most significant maximum is listed for each anatomic location. Co-ordinates are in mm standard space of MNI. L=left, R=right. FOC=frontal orbital cortex, DLPFC=dorsolateral prefrontal cortex, VLPFC=ventrolateral prefrontal cortex, V1=primary visual cortex, V2=secondary visual cortex, V3=third visual cormplex.

Condition	Brain region	Side	max Z score	X	Y	Z
Saline perception	Anterior insula	R	3.89	36	22	0
	FOC	R	3.48	42	18	0
	DLPFC	R	3.7	36	46	8
	VLPFC	R	3.32	44	48	-6
	V1	L	3.78	-8	-98	6
	V2	L	4.12	-10	-94	-8
	V3	L	3.23	-14	-88	-2
Saline > remi perception	Cerebellar crus I	L	4.12	-18	-82	-26
	Vermis VI	L	3.34	-2	-68	-26

Between-session differences in subjective perception of dyspnoea

Subjective ratings of intensity and unpleasantness of strong inspiratory loading were strongly correlated during saline ($R=.88$, $p<0.0001$).

During the saline condition, participants with higher perceived unpleasantness showed higher BOLD activation in the right hippocampus, right temporal pole and the right secondary somatosensory cortex that correlated specifically with unpleasantness of strong dyspnoea(Figure 5.11, Table 5.12).

To ensure that we did not miss activation that did not uniquely correlate with unpleasantness due to its high correlation with intensity, we performed an F-test for average respiratory intensity and average respiratory unpleasantness of strong respiratory loading. This test showed activation in the right anterior insula and right lateral prefrontal cortex correlated with subjective perception of dyspnoea (Figure 5.11, Table 5.10). These results show that the right anterior insula and right lateral prefrontal cortex are involved in the perception of respiratory sensations, but that their activation is not unique to either respiratory unpleasantness or intensity.

Whilst we showed a significant reduction of perceived respiratory unpleasantness at stable intensity, reductions in average intensity and unpleasantness ratings were strongly correlated ($R=.74$, $p<0.0001$).

Participants with higher average unpleasantness ratings showed higher activation of the dorsal prefrontal cortexA reduction in BOLD activation during remifentanil compared to saline during strong inspiratory loading occurred in the dorsal prefrontal cortex, an area not activated in this contrast during saline (Figure 5.11, Table 5.12).

A reduction of BOLD activation in the cerebellum (not activated during saline) with remifentanil was correlated to subjective perception of dyspnoea (Figure 5.11, Table 5.10).

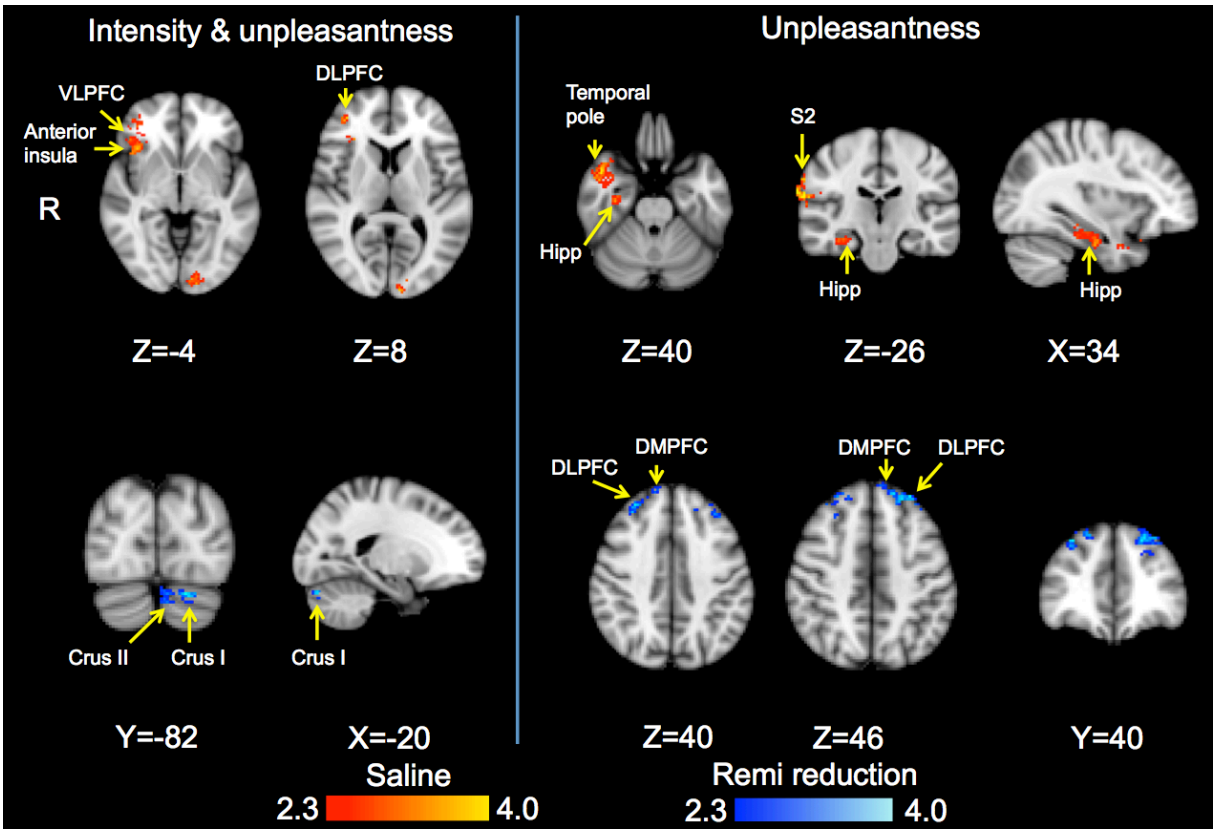


Figure 5.11: BOLD activations during strong dyspnoea that correlate with both intensity and unpleasantness per session (activation that could not be individually assigned to intensity or unpleasantness by the GLM, but correlated with both. Image from F-test using the intensity and unpleasantness contrasts) and average unpleasantness per session during strong inspiratory loading in 19 participants. Activation during saline is indicated in red-yellow. Reduction in activation during remifentanil infusion is depicted in blue-lightblue. Image consists of a colour-rendered statistical map superimposed on a standard (MNI) brain. Significant regions are displayed with a threshold of $Z<2.3$ with a cluster probability threshold of $p<0.05$ (corrected for multiple comparisons). R=right. S2=secondary somatosensory cortex, DLPFC=dorsolateral prefrontal cortex, DMPFC=dorsomedial prefrontal cortex, V2=secondary visual cortex. Hipp=hippocampus, VLPFC=ventrolateral prefrontal cortex.

Table 5.11: Between-session variation in perceived dyspnoea unpleasantness during strong inspiratory loading during saline and saline > remifentanil. Coordinates are derived from cluster-based analyses. The most significant maximum is listed for each anatomic location. Co-ordinates are in mm standard space of MNI. L=left, R=right. SMG=supramargingal gyrus, V1=primary visual cortex, V2=secondary visual cortex, DLPFC=dorsolateral prefrontal cortex, VLPFC=ventrolateral prefrontal cortex, S1= primary somatosensory cortex, BA6=premotor cortex, MTG=middle temporal gyrus, ITG=inferior temporal gyrus, TFC=temporal fusiform cortex.

Condition	**Brain region**	**Side**	**max Z score**	**X**	**Y**	**Z**
Saline intensity	SMG	L	4.31	-40	-46	40
	SMG	R	4.42	42	-46	44
	Angular gyrus	R	4.68	36	-56	46
	Inferior parietal lobule	R	4.87	40	-62	48
	V1	L	6.08	-6	-100	0
	V1	R	6.06	18	-96	0
	V2	L	6.5	-12	-94	-4
	V2	R	6.45	10	-96	20
	Cerebellar crus I	L	5.94	-8	-76	-26
	Cerebellar crus I	R	5.18	34	-76	-26
	Cerebellar crus II	L	4.8	-10	-78	-32
	Cerebellar vermis VI	L	5.75	-4	-74	-28
Saline > remi intensity	Hippocampus	L	4.38	-30	-22	-14
	Hippocampus	R	3.26	28	-38	-4
	DLPFC	R	5.3	40	48	0
	VLPFC	R	4.27	46	40	-4
	S1	L	4.66	-58	-8	34
	S1	R	5.13	50	-16	42
	M1	L	4.17	-52	-2	28
	M1	R	5.13	52	-10	46
	BA6	L	3.71	-60	4	30
	BA6	R	3.53	40	-8	54
	TFC	R	5.59	42	-14	-28
	MTG	L	4.7	-62	-54	0
	MTG	R	5.41	56	-8	-28
	ITG	L	4.76	-56	-60	-14
	ITG	R	4.97	50	-12	-28
	Cerebellum VI	L	4.56	-20	-62	-26
	Cerebellum VI	R	3.14	24	-56	-34
	Cerebellar vermis VI	L	4.79	-4	-66	-28
	Cerebellum crus I	R	4.54	24	-64	-36
	Cerebellum crus II	R	3.53	32	-56	-42
	Cerebellum IIIa	R	2.95	34	-56	-52
	Cerebellum IIIb	R	2.98	34	-56	-48

Table 5.12: Between-session variation in perceived dyspnoea unpleasantness during strong inspiratory loading during saline and saline > remifentanil. Coordinates are derived from cluster-based analyses. The most significant maximum is listed for each anatomic location. Co-ordinates are in mm standard space of MNI. L=left, R=right. S2=secondary somatosensory cortex, DLPFC=dorsolateral prefrontal cortex, DMPFC=dorsomedial prefrontal cortex.

Condition	Brain region	Side	max Z score	X	Y	Z
Saline unpleasantness	Hippocampus	R	3.72	22	-30	-12
	S2	R	4.93	66	-22	20
	Temporal pole	R	4.27	46	10	-22
Saline > remi unpleasantness	DLPFC	L	4	-28	42	46
	DLPFC	R	4.47	28	38	50
	DMPFC	L	4.02	-20	40	44
	DMPFC	R	4.38	20	42	48

5.4 Discussion

5.4.1 Key findings

This study set out to dissociate three aspects of dyspnoea perception, dyspnoea-specific anticipatory anxiety and the intensity and unpleasantness of acute dyspnoea. We used the opioid remifentanil to differentially modulate dyspnoea intensity and unpleasantness and to evaluate the effects of remifentanil on behavioural ratings and neural processing of these dimensions.

This study has two main findings:

1) The neural correlates of dyspnoea-specific anticipatory anxiety were found to be located in the anterior insula, midinsula/precentral gyrus and premotor cortex. Remifentanil infusion led to decreases in brain activity in these areas (Figure 5.7).

2) During the saline condition, trial-by-trial changes in perceived intensity were encoded by the thalamus, posterior insula and secondary somatosensory cortex. Trial-by-trial variations in perceived dyspnoea unpleasantness covaried with changes in brain activation in the medial prefrontal cortex and anterior cingulate cortex. Remifentanil decreased dyspnoea unpleasantness whilst subjective intensity remained constant (Figure 5.3). During remifentanil administration, activation in the PAG, hippocampus and amygdala that covaried with perceived intensity was dampened compared to saline, whilst brain activation related to perceived unpleasantness was dampened in the medial prefrontal cortex and the ACC (Figure 5.10).

5.4.2 Neuronal correlates of the anticipation of dyspnoea

During the anticipation of dyspnoea BOLD activation increased in the anterior insula, midinsula/precentral gyrus and premotor cortex. This is the first study investigating neural activation during the anticipation and perception of acute dyspnoea in the same paradigm. The only other study looking at anticipation to a respiratory stimulus in the scanner showed increased activation in the anterior insula and dorsal anterior cingulate/dorsomedial prefrontal cortex when contrasting anticipation of hyperventilation to anticipation of a safe condition (Holtz et al., 2012). However this study did not actually apply the subsequent hyperventilation task in the scanner. Holtz et al (2012) could thus not determine brain activation during hyperventilation in relation to its anticipation.

The anterior insula is an area functionally associated with emotional processing (Casey et al., 1995) and self-awareness (Craig, 2009). It has consistently been activated in neuroimaging studies looking at the anticipation of pain (e.g. (MacIntosh et al., 2008; Ploner et al., 2010)) and also by Holzt et al (2012) during anticipation of hypercapnia. Both the insula and the

precentral gyrus are part of the interoceptive cortex (Craig, 2003), and their activation during anticipation may be interoceptive in nature.

It is worth noting that activation in the anterior insula during the anticipation of inspiratory loading was reduced with remifentanil, whilst activation that scaled with acute dyspnoea perception did not change with remifentanil. This differential insula activation is in line with the insula as a parsimonious structure (Gu et al., 2013; Menon and Uddin, 2010) and might indicate that the perceptive saliency of the emotionally loaded anticipatory cue is reduced with remifentanil administration, whilst the role of the anterior insula in encoding of the subjective response does not appear to be altered with remifentanil.

The premotor cortex is involved in the planning of complex, coordinated movements, which are prepared in response to the visual cue in expectation of the necessity to overcome strong inspiratory loading. This area is not generally activated in anticipation of painful stimuli, but shows a unique role in eliciting the respiratory manipulations necessary to overcome dyspnoea.

If strong anticipation is compared to anticipation of a normal breathing period as opposed to mild respiratory loading (see previous paragraphs), the anterior insula/operculum, PCG and premotor cortex are again activated and additional activation is seen in the ACC, DLPFC and the cerebellum. This could indicate that their activation during strong dyspnoea might not be unique to the anticipation of dyspnoea, but that these areas could be more generally involved in the anticipation of physiological sensations (such as mild inspiratory loading), e.g. in response to changes in interoceptive attention or saliency. Indeed, Holtz et al (2012) showed activation in the ACC and DLPFC with their single-level paradigm. It may therefore be prudent for future studies to 'clean up' their paradigms using a contrast rather than one set

stimulus. This may help to tease apart expectations of general physiological sensation and the specific sensation of interest.

Remifentanil has a clear, non-differential effect on dyspnoea anticipation. Remifentanil reduced neural activation in all areas activated during the saline session (anterior insula, premotor cortices, left precentral gyrus, left secondary somatosensory cortex, right frontal operculum). Reduced brain activations in the anterior insula/opercular and somatosensory areas would be in line with a reduction of anticipatory anxiety, whilst reduced activations in the premotor cortices might be related to reduced preparations for motor activity.

5.4.3 Non-subjective responses to graded inspiratory loading

Investigation of the trial-by-trial invariant component of loading revealed a graded response to mild and strong inspiratory loading (Figure 5.8). These trial-invariant brain activations represent core activation in response to inspiratory loading and are not linked to subjective stimulus perception.

Mild and strong inspiratory loading largely activated the same somatosensory areas, with strong inspiratory loading leading to increased extent and magnitude of activations. This graded neural response has not previously been shown with inspiratory loading. Both mild and strong levels of loading recruited the anterior insula, and strong loading additionally activated the cerebellum and temporal lobes. Peak BOLD activations in primary and secondary somatosensory areas, the primary motor cortex and cerebellum during strong inspiratory loading (saline) were in very similar locations to peak perfusion changes in the ASL study performed in Chapter 4 (Table 4.3), and specific regions overlap well with those identified in studies on breath holds (primary motor cortex, (Pattinson et al., 2009)), laryngeal motor control (primary motor cortex, (Brown et al., 2008)) and respiratory loading (cerebellar

lobe VI, (Isaev et al., 2002b)). The neural response to strong compared to mild inspiratory loading was largely opioid-sensitive. Similar reduced activation patterns have been observed in the pain matrix with remifentanil during heat pain (Bingel et al., 2011), and research in rats (Kalliomaki et al., 1998) indicate that the effect might occur early on in the processing pathway.

The right anterior insula was activated during both mild and strong inspiratory loading and did not show a graded response. The persisting insular activation thus represents a stimulus-dependent mechanism that is not altered with a low dose opioid. The insula has been shown to have an important role in the salience network (Wiech et al., 2010), which may explain the insula's continued activation in non-subjective dyspnoea, as both inspiratory loads, even during remifentanil, were strong enough to elicit a response (salient stimuli). Similarly, the cerebellum is traditionally implicated in motor responses (Manto et al., 2012) and the motor response to changes in loading would not be significantly altered by remifentanil. This is in contrast to the decrease in cerebellar activation with remifentanil that is related to subjective intensity. We therefore suggest a dual role of the cerebellum, in line with the literature (Manto et al., 2012; Stoodley and Schmahmann, 2009) in the present study.

5.4.4 Trial-by-trial variation within sessions

To tease apart the fluctuations associated with either unpleasantness or intensity of dyspnoea perception, we used the online ratings of either dimension. Although correlated, these were sufficiently different to allow for this particular analysis approach.

Unpleasantness

Subjective dyspnoea unpleasantness fluctuated between trials within each session and these variations correlated with opioid-sensitive BOLD signal in the pregenual ACC and right dorsolateral medial prefrontal cortex.

The pregenual ACC is a highly opioid-sensitive area. Within the ACC, it shows the highest level of opioid receptor binding (Vogt et al., 1995). It is also a site of the opioid placebo effect (Petrovic et al., 2002) and the region most active during attention to the unpleasantness of nociceptive cutaneous stimulation (Kulkarni et al., 2005). The ACC is activated during dyspnoea relief (Peiffer et al., 2008), in line with its role in processing both pain and emotion (Vogt, 2005), and it may be an integrative structure between sensory and affective processing of pain (Price, 2000). Our study shows that the ACC is also important for the emotional processing of dyspnoea and that its activation scales with unpleasantness reduction with remifentanil.

Prefrontal cortex activation has been shown in response to hypercapnia, resistive loading and breath-holds (Evans et al., 2002; Pattinson et al., 2009; Peiffer et al., 2008). We show here that activation in the prefrontal cortex plays a specific role in evaluating dyspnoea unpleasantness and that it is reduced in line with reduced unpleasantness during remifentanil.

This indicates that the ACC and PFC work in concert to evaluate unpleasantness in dyspnoea, and that this activation may be modulated using opioids. This is of particular interest given a recent study in chronic obstructive pulmonary disease (COPD) patients which, using a trial-by-trial word paradigm, showed exaggerated activation in these particular regions in dyspnoeic patients compared to controls (Herigstad et al. in preparation, see Appendix). As

we have shown that this activation is opioid-sensitive, this opens up a valuable venue for research into therapeutic effects of opioids in COPD.

Intensity

Subjective dyspnoea intensity varied between trials within each session and these variations correlated with neural changes in the right thalamus, right mid/posterior insula, right premotor cortex and right secondary somatosensory cortex. The thalamus, posterior insula and secondary somatosensory cortex are part of the cortico-limbic pathway that encodes the subjective intensity of pain (Craig, 2003; Price, 2002). In this pathway, neurons in the spinal chord send activation in response to noxious signal to different thalamic nuclei that differentially project to the posterior insula, primary and somatosensory cortices and the ACC (Treede et al., 1999). Its activation during encoding of intensity in our study suggests similarities in processing of pain and dyspnoea.

We also found activation in the premotor cortex, which may be a consequence of the continuous need for additional respiration to overcome a respiratory load, an aspect, which would apply to dyspnoea but not pain. This may function as a reminder that neural processing of pain and dyspnoea, although sharing some similarities, is not identical.

Remifentanil did not change activation in the insula, but reduced BOLD activation in the thalamus and precentral gyrus, as well as in the inferior frontal gyrus, PAG, amygdala and hippocampus. The precentral gyrus and inferior frontal gyrus are areas closely associated to motor processing and could hence indicate the higher effort expended to overcome inspiratory loading that scales with its subjective perception. The amygdala is involved in encoding dyspnoea unpleasantness (von Leupoldt et al., 2008) and has been activated during prolonged unpleasant hypercapnia (Evans et al., 2002; Liotti et al., 2001). Hippocampal and amygdala

activations are traditionally more associated with affective dimensions, for example in pain (Lee et al., 2013; Ploghaus et al., 2001; Price, 2000).

The PAG receives inputs from the frontal cortex (Beitz, 1982), insula (Hardy and Leichnetz, 1981) and amygdala (Gray and Magnuson, 1992) and could hence mediate central modulation of ascending sensory responses. The PAG is predictive of changes in perceived pain intensity and may be a critical centre for the control of nociceptive perception through descending activity from higher cortical regions (Tracey et al., 2002).

This is the first study investigating both the subjective intensity and unpleasantness of dyspnoea in the same paradigm. During remifentanil administration, baseline levels of tension and discomfort are reduced compared to saline. Hence baseline emotional state differs between the two sessions. This might be important for interpreting reductions in PAG, amygdala and hippocampus that scale with variations in intensity during remifentanil. These perceptual fluctuations occur within the same drug state and highlight that despite no changes in average intensity, the encoding of individual stimuli is accompanied by neural dampening in brain areas involved in emotional modulation of pain.

Overall, the trial-by-trial activation linked to both intensity (posterior insula, somatosensory and motor regions) and unpleasantness (prefrontal regions) is closely associated with findings in earlier studies of dyspnoea. However, probing these activation patterns with remifentanil yields novel results, suggests a role for the hippocampus, amygdala and PAG in processing of intensity as well as unpleasantness of dyspnoea, and offers potential targets for therapy in dyspnoeic patients.

5.4.5 Neural signature of average subjective dyspnoea across session

Unpleasantness

Because we were particularly interested in the unpleasantness of dyspnoea, we performed a secondary analysis in which we looked at brain activation correlated with average unpleasantness over a given session. Activation in the right hippocampus scaled with average unpleasantness of the trial-by-trial invariant component of dyspnoea (response to strong loading cleared of within-participant variation) over each session, suggesting that it is generally important during repeated exposures to dyspnoea. Hippocampal activation was shown in two studies using PET during experimental hypercapnia. Both studies applied hypercapnia over multiple minutes (6 minutes of hypercapnia (Corfield et al., 1995) and 3 minutes of hypercapnia (Brannan et al., 2001; Liotti et al., 2001)) and reported high respiratory unpleasantness (air hunger). These studies did not specifically investigate the unpleasantness of dyspnoea and did not correlate neural activations with subjective ratings. The hippocampus is, however, not commonly reported in studies using short respiratory loads (<30 seconds) during FMRI.

Electrical stimulation of the hippocampus in anaesthetised rats has been shown to slow breathing (Ruit and Neafsey, 1988) and other animal studies indicate a potential role of the hippocampus in modulating inspiratory drive (Harper et al., 1998; Poe et al., 1996), but more research and especially evidence in humans is needed to confirm this potential hippocampal function.

The hippocampus is known to form episodic representations of the emotional significance and interpretation of events (Phelps, 2004). Hippocampal activation has also been shown to scale with pain unpleasantness, especially in response to changes in anxiety level (Ploghaus et al., 2001; Price, 2000). In our conditioning paradigm, participants had a very clear idea about the

strength and significance of the stimulus they encountered and might have been continuously evaluating stimulus unpleasantness in order to rate the stimulus after each trial.

Unpleasantness also correlated with activation in the temporal pole. The function of the temporal pole is not well understood, but it might play a role in binding complex, highly processed perceptual inputs to visceral emotional responses (Olson et al., 2007).

These activations (hippocampus, temporal lobe) did not change with remifentanil, but remifentanil reduced activation in the dorsal prefrontal cortex. This suggests the DLPFC is associated with dyspnoea, perhaps as a modulator of perceived unpleasantness. Such an interpretation is supported by studies in pain, showing that the DLPFC is engaged during modulation of nociception (Lorenz et al., 2002) and is in line with our behavioural findings of reduced tension with remifentanil compared to saline.

BOLD activation that is potentially, but not uniquely related to unpleasantness

In order to capture any brain activation that might be related to respiratory unpleasantness, but could not uniquely be distinguished from respiratory intensity, we investigated the shared activation between perceived unpleasantness and intensity (F-test). Activation of the right anterior insula and the right lateral prefrontal cortex was not mathematically dissociable between respiratory unpleasantness and respiratory intensity (F-test). This non-specificity leaves room for two interpretations. Firstly, the right anterior insula and prefrontal cortex might be involved in the processing of both dyspnoea intensity and unpleasantness and might be part of a network providing a conglomerate dyspnoea perception. Similarly, these areas might scale with general salience and awareness of the stimulus (Craig, 2009), which would potentially correlate with, but might only be a part of overt subjective dyspnoea perception. These activations were not reduced with remifentanil, but remifentanil reduced activation in

the cerebellar crus I and II. The insula has been involved in most studies on dyspnoea to date (Herigstad et al., 2011a). For example, activation in the anterior insula was previously shown to relate to perceived unpleasantness in response to inspiratory loading (Paulus et al., 2012; von Leupoldt et al., 2008). Von Leupoldt and colleagues (2008) used a hypothesis-driven approach employing small volume corrections to show that activation in the insula and amygdala scaled with perceived unpleasantness during a psychological manipulation. Dyspnoea intensity was measured and did not change between conditions. It was then not further considered during imaging analysis. We have shown that anterior insula activation is robust enough to survive a stringent cluster threshold at a whole-brain level in line with the results of Paulus and colleagues (2012) who paired inspiratory loading with a (potentially distractive) cognitive task and again correlated brain activity with unpleasantness, but not intensity ratings.

At present, few studies have demonstrated prefrontal involvement in dyspnoea (Evans et al., 2002; Paulus et al., 2012). Lateral prefrontal activation is associated with attention and action (Badre, 2008), as well as cognitive control of motor behaviour (Cieslik et al., 2013). It is the endpoint of the that establishes how to interact with a stimulus and comprises the highest cortical area involved in motor planning and organization (Abe and Hanakawa, 2009). At the other end of the hedonic spectrum, the DLPFC initiates reward-seeking behaviour by modulating midbrain dopaminergic areas and consequentially initiating motivated behaviour (Ballard et al., 2011). Hence its activation during anticipation of pain and dyspnoea might indicate preparation for a goal directed avoidance response.

In addition to its classic role in motor processing (Manto et al., 2012), a growing body of literature supports a cognitive and emotional role for the cerebellum. A meta-analysis concluded that the primary area of activation in crus I may be implicated in processing of

emotion and executive functions (Stoodley and Schmahmann, 2009) and cerebellum might play a role in processing pain and unpleasant pictures as well as other aversive stimuli in the context of associative learning (Moulton et al., 2011). Strong functional connectivity between non-modality specific processing areas in the cerebellar crus I and II with the lateral prefrontal cortex was established from resting state data (O'Reilly et al., 2010).

Overall, we demonstrate that dyspnoea perception might be associated with emotional and executive brain regions, including the anterior insula, PFC, hippocampus (unpleasantness specific) and temoporal pole (unpleasantness specific). Remifentanil lowers unpleasantness, and this modulation may rely on activation in the DLPFC. Our findings support previous studies in dyspnoea, and further establish the central role of the anterior insula in dyspnoea perception. However, it is becoming clear that the insula is only one part in a greater network of regions, albeit an important part. This highlights the importance of a whole-brain approach.

5.4.6 Affective processing of dyspnoea

This study reveals a widespread cortical and subcortical network associated with subjective processing of acute dyspnoea intensity, unpleasantness and dyspnoea-specific anticipation. The unpleasantness of dyspnoea is associated with activation in the hippocampus and temporal pole. Fluctuations in responses are associated with neural changes in the PFC and ACC, and the unpleasantness-related activation in the PFC is dampened by remifentanil along with ratings of tension, unpleasantness and discontent. Together, this suggests a prefrontal involvement in the top-down modulation of unpleasant respiratory sensations. This may be the mechanism by which some behavioural treatments reduce dyspnoea through anxiety alleviation (i.e. pulmonary rehabilitation).

The neural network that processes fluctuations in dyspnoea unpleasantness is distinct from that processing fluctuations in dyspnoea intensity (thalamus, posterior insula, somatosensory cortex, PFC). Remifentanil dampens this intensity-related activation in the thalamus, PAG, amygdala, hippocampus and cerebellum, areas involved in emotion-processing with regard to aversive stimuli. While we observe an opioid-dependent reduction in tension and discontent, there is no significant change in intensity ratings. This suggests that dampening of this neural network for emotion processing is distinct from evaluation of subjective intensity perception.

The insula probably plays a role in both the general perception of dyspnoea, fluctuations in dyspnoea intensity (mid/posterior regions) as well as the anticipation of a dyspnoeic stimulus. However, only the activation pertaining to anticipation is opioid-modulated. This suggests that the anterior insula plays a differential role in both conditions. We suggest that its activation during anticipation of dyspnoea might reflect a role in processing stimulus saliency that scales with underlying psychological variations (e.g. change in tension and discomfort), whilst the role of the anterior insula in processing acute dyspnoea might be strongly driven by bottom-up changes in stimulus-characteristics, hence its activity scales with the stimulus despite general psychological changes.

Overall, we observe that the hippocampus is central to dyspnoea unpleasantness, possibly through learnt emotional associations, while somatosensory, motor and subcortical regions are involved in the intensity dimension of dyspnoea and less subject to modulation with remifentanil. Opioids may, however, modulate unpleasantness through the PFC (acute unpleasantness) and/or the anterior insula (anticipation of dyspnoea).

5.4.7 Limitations and methodological considerations

Effects of remifentanil

In addition to their analgesic properties, opioids (including remifentanil) depress breathing (Pattinson et al., 2007; Wagner et al., 2001). Respiratory depression causes an increased partial pressure of arterial CO_2 ($PaCO_2$), which then increases the baseline BOLD signal throughout the brain. Unless accounted for, this constitutes a significant confounding factor in the interpretation of FMRI studies (Cohen et al., 2002). In a study by (MacIntosh et al., 2008), administration of 1ng/ml of remifentanil produced a 21% increase in end-tidal CO_2 and a 35% increase in CBF. In the current study, we only gave 0.7ng/ml remifentanil, which therefore had a much smaller effect on CBF (~10% increase). We chose this low dose because we hypothesised that that is where we would see the biggest differentiation between intensity and unpleasantness. This has the additional advantage of keeping remifentanil effects on BOLD to a minimum.

Great care was taken to reduce the effects of remifentanil upon the BOLD response by minimizing the dose of remifentanil that was given. Remifentanil increased baseline CBF (Figure 5.6). A voxel-wise perfusion regressor was therefore included in the analysis to dissociate the global perfusion effect of remifentanil from the specific neuronal effects of the drug. This has been shown to explain changes in baseline CBF related to perfusion effects (Pattinson et al., 2009), but it can only approximate the effects of remifentanil and is unlikely to completely account for all remifentanil-induced CBF changes.

A differential effect of a low dose of opioids (0.04 and 0.06mg/kg intravenous morphine, or 2.8mg and 4.2mg for a 70kg man) on sensory and affective dimensions has been shown in pain (Price et al., 1985), but has not previously been applied to the study of dyspnoea. One of the two previous laboratory studies of dyspnoea using opioids has shown no treatment effect

of 90mg of oral codeine (approximately equivalent to 13.5mg oral morphine) on perceived effort, respiratory discomfort or time to exhaustion during respiratory loading (Supinski et al., 1990). The authors themselves argue that a larger dose might have been necessary for viable effects on respiratory sensations, especially because participants were instructed to manually control their ventilation, which might have distracted from a full evaluation of respiratory sensations. The second study has shown equal reduction of unpleasantness and intensity of dyspnoea during intravenous infusion of a clinically effective dose of morphine (infusion of 0.07mg/kg morphine sulphate over five minutes or 4.5mg for a 70kg man; a dose in the range of effective doses when morphine is given for chronic pain (Banzett et al., 2011)). This dose was significantly higher than the low experimental dose of remifentanil infused during the present study (0.07ng/ml of remifentanil effect size concentration). This suggests a dose-dependent effect of opioids on the intensity and unpleasantness of dyspnoea and shows that a moderate effect size concentration of 0.07ng/ml remifentanil can differentially modulate respiratory intensity and unpleasantness.

Respiratory gases

We maintained $PETO_2$ constant at very mild hyperoxia ($PETO_2$: 20kPa), which did not influence BOLD (Wise et al., 2007a). $PETCO_2$ was increased by +0.3kPa from baseline to allow isocapnia in the scanner. A dedicated experimenter maintained isocapnia in response to breathing changes. Small remaining variations in $PETCO_2$ were accounted for in the statistical model.

Participants were presented with the same physical stimulus during the saline and remifentanil sessions. Due to the respiratory suppressive effects of remifentanil, participant's mouth pressure was decreased during the remifentanil session. The slightly reduced magnitude of breaths taken during the remifentanil compared to the saline session needs to be considered

when interpreting the results. As these changes are very small, it is unlikely that they account for the reduction in respiratory-load related BOLD activation during the remifentanil session, especially in view of the differential reduction of BOLD activation by remifentanil in the somatomotor and premotor areas, but not the cerebellum or temporal lobe.

Ratings of intensity and unpleasantness

The difference in average ratings of intensity and unpleasantness between saline and remifentanil sessions remained correlated and we were therefore unable to completely dissociate the highly correlated ratings for dyspnoea intensity and unpleasantness in the general linear model during our imaging analysis. Our interpretation of our neuroimaging results has therefore been guided by the differential behavioural change in unpleasantness.

Disentangling intensity and unpleasantness of dyspnoea, or indeed other aversive stimuli, is highly challenging, and it may be prudent to choose one over the other in a given experimental paradigm. This should be considered when designing future studies on dyspnoea perception.

The long durations of respiratory stimuli necessary to induce unpleasantness make it more difficult to apply conditioning paradigms to respiratory stimuli than to shorter aversive stimuli like electric shocks or thermal pain stimuli. Our finding that hippocampal activation scales directly with dyspnoea unpleasantness in response to inspiratory loads of 30-60 seconds, taken together with the fact that it is not found in response to application of shorter respiratory loads questions whether such short respiratory stimuli elicit a truly unpleasant experience. This should be considered during future studies using inspiratory loading to study dyspnoea unpleasantness.

Emotional state

The FMRI scan was perceived as a mildly aversive experience that caused tension and discontentment. These effects were countered by remifentanil, indicating that participants were in a generally more positive state during remifentanil infusion and in the absence of respiratory challenges.

Remifentanil mildly increased sedation by approximately 15%VAS compared to saline infusion. This effect remained present outside the scanner 15 minutes after the infusion was stopped. Sedation did not lead to any functional impairment of participants when performing tasks in the scanner or completing experimental instructions after the scan. Additionally, the increase in sedation during FMRI with remifentanil compared to saline did not correlated with changes in subjective ratings between saline and remifentanil sessions. Hence, we are confident that sedation did not significantly influence the behavioural decrease in unpleasantness scores.

We assume that psychological state did not change significantly throughout one scanning session, hence whilst variations in perceived intensity and unpleasantness might be accompanied or caused by fluctuations in anxiety, general comfort/discomfort and other coping mechanisms in the scanner, they should occur in the same general context (i.e. either with or without the drug). We are not able to dissociate the cause of these variations, but they are in line with fluctuations in dyspnoea perception found in other experimental studies, e.g. see Chapter 3, Figure 3.5.

Learning

A potential confound of the current study is that throughout each session stimulus associations might be relearned to differ between the less unpleasant remifentanil session and

the more unpleasant placebo session. To diminish any such confounding effect, the number of stimulus repeats was kept low (4 mild and 4 strong stimuli per session). Additionally, saline and remifentanil sessions were counterbalanced in order to minimize order effects.

Anticipation

The non-differential nature of the reduction in neural activation during dyspnoea anticipation with remifentanil does not allow us to draw any further conclusions about its origin as dyspnoea specific or non-specific. A future study carefully measuring general anxiety and dyspnoea-specific anticipatory anxiety is needed to dissociate the mechanisms by which remifentanil reduces neural activation during anticipation of dyspnoea.

Combined analysis of trial-by-trial and average dyspnoea perception

In this study, we assessed the trial-by-trial variations in perceived respiratory intensity and unpleasantness within each session for 17 participants. Additionally, we revealed brain activation that correlated with the average ratings of intensity and unpleasantness over all strong respiratory stimuli of a given session for all 19 participants. The two measures were performed in the same analysis. Trial-by-trial variation was analysed at the first level, whilst the correlations of brain activation during application of the strong stimulus with average session intensity and unpleasantness were performed at the third level, the highest analysis level. Results of average intensity and unpleasantness are hence stripped of any potential impact of lower level variations in perception, which was shown in the respective EVs and would hence not be incorporated into the EV showing brain activation related to strong respiratory loading, on which the higher level analysis was performed. This separation should be kept in mind when considering the results relating to average session intensity and unpleasantness and might limit the universality of the results presented in section 3.2.4.

5.4.8 Clinical implications

This study investigated the two factors most important for the treatment of dyspnoea: dyspnoea anticipation that prevents everyday activities and hastens disease progression, and dyspnoea relief with opioids, a potent pharmacological treatment for the reduction of respiratory unpleasantness. Effective dyspnoea treatment should consider both of these factors. For example, pulmonary rehabilitation has been shown to help patients overcome their fear of dyspnoea, which increases their quality of life and encourages them to start performing everyday activities they might have stopped because of fear of dyspnoea (Lacasse et al., 2007). This may, to some extent, work on an anticipatory level. It might be that the most potent treatment of dyspnoea could consist of a multi-level approach in which a proven behavioural therapy like pulmonary rehabilitation is supplemented with a pharmacological agent that further decreases accompanying anxiety.

Opioids may also enhance the relief from dyspnoea. Mu-opioids have been shown to cause a generalized positive shift in affect across the hedonic spectrum from enhancing the pleasantness of food to decreasing perceived pain (see Leknes and Tracey (2008)). It has been suggested that immediate relief from dyspnoea is a rewarding sensation, representing more than the absence of a negative stimuli by being pleasurable (Peiffer et al., 2008). Peiffer and colleagues (Peiffer et al., 2008) used PET imaging to study brain activity during acute dyspnoea induced by respiratory loading and again after its relief in ten healthy male participants. Dyspnoea relief involved characteristic activity in the left ACC, temporal and prefrontal cortices and posterior cerebellum that was distinct from activation during acute dyspnoea in the premotor cortex, left prefrontal cortex and caudate and anterior cerebellum. This activation during dyspnoea relief closely represents areas implicated in reward processing (Leknes and Tracey, 2008), and may be targeted and harnessed in the treatment of dyspnoea.

Interactions between mu-opioid effects and serotonergic transmission are also starting to emerge. Animal studies are starting to uncover potential overlap between opioidergic and serotonergic mechanisms in the spinal cord (Aira et al., 2012) and brainstem (Manzke et al., 2011) and serotonin has shown promise in reversing the respiratory depressant effects of mu-opioids with a neutral or potential enhancing effect of opioid analgesia (Boom et al., 2012). These encouraging results may lead to a focus on serotonergic and opioidergic interactions with the aim of uncovering new pharmacological possibilities to treat opioid-induced respiratory depression without dampening analgesic effects.

5.4.9 Future research

As this is the first study of the anticipation and perception of dyspnoea in one imaging paradigm, future neuroimaging studies are needed to confirm our hypothesis that the unpleasantness of dyspnoea is processed in a widespread network incorporating signals from prefrontal areas, the temporal lobes and the limbic system in cooperation with secondary somatosensory areas. Functional connectivity analyses could elucidate how the brain activation during anticipation of dyspnoea modulates the interplay of different brain areas to reduce or increase perceived dyspnoea. Cognitively modulating the expectation of dyspnoea through a low and a high dyspnoea anticipatory cue that precede dyspnoea of the same strength could help to specifically investigate the modulatory effect of dyspnoea anticipation (Keltner et al., 2006).

Whilst remifentanil reduced perceived unpleasantness, but not intensity of dyspnoea, ratings of intensity and unpleasantness between participants remained highly correlated. Dissociating closely correlated experiences is very difficult. In the study of pain, attention is sometimes modulated to attend to pain unpleasantness or to localization of pain (Kulkarni et al., 2005) in order to dissociate neural networks implicated in affective and sensory processing. As this is

not possible in the study of dyspnoea, cognitively manipulating expectations of dyspnoea intensity and unpleasantness might allow us to dissociate the neural networks involved.

The most important challenge for the study of dyspnoea is to translate findings from research in healthy volunteers to brain activation of patients. A recent study in our laboratory has investigated neural responses of patients with COPD and age-matched healthy controls to the imagination of activities that might induce different levels of dyspnoea (Herigstad in preparation, see Appendix). Older healthy controls activated the DLPFC, the anterior insula and premotor areas in the superior frontal gyrus more strongly in response to activities that they rated as inducing higher dyspnoea, which is very similar to the neural response we have shown in this study. Patients also activated the anterior insula cortex and showed increased activation in the medial prefrontal cortex and the ACC. Activation in emotionally involved medial areas in patients, compared to more evaluative brain activation in the lateral prefrontal cortex in healthy volunteers, shows that imagining performance of dyspnoea inducing activities in patients involves a more emotionally based network. This clearly shows that whilst healthy participants cognitively appraise how breathless a certain activity might make them feel, patients rely on more emotional areas during evaluation of dyspnoea-related activities. Healthy volunteer studies will therefore be useful for generation of specific hypotheses, but will need to be confirmed in patient research.

5.5 Conclusion

This is the first study to investigate the neural signature of both dyspnoea intensity and unpleasantness in the same paradigm. We show that sensory and affective encoding of dyspnoea employs similar mechanisms to encoding of subjective pain. We also show a prominent role of the hippocampus in re-experiencing and evaluating dyspnoea stimuli,

enabled by the differential effects of remifentanil on dyspnoea unpleasantness. A low dose of remifentanil decreases dyspnoea unpleasantness and reduces brain activation in the anterior insula during anticipation, but not subjective perception of acute dyspnoea. We suggest the hippocampus, where learnt emotional associations are processed and updated with regards to subjective evaluation of dyspnoea, to be a key structure in the affective evaluation of dyspnoea. We suggest the hippocampus and the anterior insula, a functionally parsimonious structure in which both anticipatory anxiety and perceived unpleasantness of acute dyspnoea are integrated as key structures important for understanding dyspnoea-specific affect and as potential drug targets for the alleviation of dyspnoea in patients.

Chapter 6

General discussion

Abstract Chapter 6

The aims of this thesis were to determine brain mechanisms of dyspnoea anticipation and acute subjective perception of dyspnoea, to develop the methodologies necessary to accurately induce and assess dyspnoea in an MRI environment and to address potential confounds related to such stimuli. The study presented in Chapter 3 investigated the suitability of a stimulus model inducing dyspnoea through respiratory loading superimposed upon baseline hypercapnia for multi-session FMRI. The study presented in Chapter 4 investigated the effects of respiratory loading on cerebral blood flow that might potentially confound FMRI. The findings from these studies informed the study presented in Chapter 5, which investigated the brain mechanisms of anticipation of dyspnoea and of the reduction of dyspnoea unpleasantness through the opioid remifentanil. In the present chapter, the implications of this research for the laboratory study of dyspnoea and its clinical implications for patient treatment are discussed and avenues for further research are suggested.

6.1 Main findings of this thesis

This thesis set out to use a healthy volunteer model to explore the neural mechanisms of the anticipation and acute perception of dyspnoea. Before this could be accomplished, two methodological studies were performed to better characterise respiratory loading as a stimulus to induce dyspnoea during FMRI.

Chapter 3 assessed the subjective perception of isocapnic respiratory loading. Two major findings emerged. Firstly, whilst hypercapnia enabled the isocapnia beneficial for FMRI analysis, it acted as a mild dyspnoea stimulus and increased perceived respiratory unpleasantness in the absence of respiratory loading. It should hence be kept as low as practically feasible. Secondly, respiratory unpleasantness in response to respiratory loading did not habituate over four sessions on consecutive days, which allows the use of respiratory loading for multi-session FMRI intervention studies.

In Chapter 4, we assessed the potentially confounding impact of inspiratory and expiratory loading on CBF, which might influence the interpretation of the BOLD signal. We showed a small increase of global CBF with inspiratory loading and no change with expiratory loading of approximately plus and minus 9 cmH_2O. This suggests that confounding effects of respiratory loading are small, but might play a significant role at higher loads or in patient populations with altered CBF regulation. The results of Chapters 3 and 4 are important for the induction of dyspnoea during FMRI. Dyspnoea is a difficult symptom to study and its study with FMRI, a promising tool to gain insight into the neural mechanisms underlying this complex symptom, warrants careful consideration of psychological and physiological concepts in order to ensure valid results.

Based on the results from Chapters 3 and 4, Chapter 5 used isocapnic inspiratory loading as a stimulus to dissociate the neural signatures of dyspnoea intensity, unpleasantness and anticipatory anxiety during FMRI. The study highlights the role of the anterior insula for subjective components of anticipatory anxiety and perception of respiratory unpleasantness. Additionally, remifentanil reveals activation in a widespread network of brain areas, including the hippocampus and prefrontal cortex, regions involved in the emotional processing of other aversive stimuli, during subjective perception of dyspnoea.

The direct contributions of each of these studies were discussed in their respective experimental chapters. Here, the wider implications of these studies for the investigation of dyspnoea in the laboratory and their potential impact on the dyspnoea perceived by patients will be discussed to show that this thesis has successfully contributed to the methodological development of dyspnoea research with FMRI and was able to use this methodology to show, for the first time, neural mechanisms related to anticipation and affective perception of dyspnoea.

6.2 Methodological considerations in dyspnoea research

6.2.1 The subjective perception of dyspnoea

The study presented in Chapter 3 investigated repeated subjective responses to dyspnoea induced by respiratory loading during mild isocapnic hypercapnia. This study has two main findings. Firstly, it shows that the combination of respiratory loading with mild hypercapnia avoided the stimulus-locked CO_2 effects that usually occur during administration of respiratory loading, but induced respiratory unpleasantness during periods without respiratory loading and should hence be maintained at the lowest level possible. Secondly, no habituation to the unpleasantness of respiratory loading occurred over four consecutive sessions.

Hypercapnia and respiratory loading

In this thesis, the successful generation of experimenter-controlled isocapnic hypercapnia was initially evaluated during respiratory loading outside the laboratory (Chapter 3) and was then successfully applied during inspiratory loading during FMRI (Chapter 5). Maintaining careful physiological control decreases stimulus-correlated physiological noise that might otherwise contaminate FMRI responses. This is likely to be an important consideration in most, if not all, studies looking at breathing stimuli. Studies using drugs that additionally modulate CO_2 would especially benefit from the cleaner neural signal obtained through this careful control, as we have shown during remifentanil infusion in Chapter 5.

From a behavioural point of view, our results show no effect of hypercapnia on perceived unpleasantness of respiratory loading. This is in contrast with a linear increase in perceived dyspnoea intensity during combination of inspiratory and expiratory loading found by Nishino and colleagues (Nishino et al., 2007). The current study was not designed to investigate the mechanisms behind potential interactions of hypercapnia and respiratory loading, but has highlighted how little we know about perceptual interactions between different laboratory stimuli of dyspnoea. It is crucial to reconcile these findings as addressing the finer details of dyspnoea perception hinges on our ability to deliver reproducible, robust stimuli with predictable response patterns. Below, we discuss mechanisms that might underlie these perceptual interactions, and how these may be tested experimentally.

1) It is possible that dyspnoea intensity and unpleasantness process afferent signals in different fashions, e.g. intensity might be processed in a linear and unpleasantness in a non-linear manner. To test this, one would have to conduct a carefully-controlled study of dyspnoea intensity and unpleasantness in the same experimental setting in

response to compound dyspnoea stimuli (e.g. hypercapnia + inspiratory loading) of a wide range of intensities.

2) It could also be the case that information from different afferent routes (e.g. lung stretch receptors and chemoreceptors) is processed differently and that this accounts for the non-additive effects between these stimuli. This is in line with different neural signatures elicited in response to hypercapnia and respiratory loading (Davenport and Vovk, 2009). Neuroimaging studies using hypercapnia have generally shown higher activations in limbic regions, whilst sensorimotor activations that are prominent during respiratory loading are absent during hypercapnia (for review see (Davenport and Vovk, 2009)). One way to investigate this would be to look at afferent output (recordings from stretch and chemo receptors) and scale those with neural activation (whole brain) and subjective responses.

3) It is also possible that the interaction between stimuli may be influenced by the participants' expectations and the focus of the breathing task. There is evidence that conscious categorization of different stimuli affects perceptions of dyspnoea stimuli (Petersen et al., 2014). Petersen and colleagues investigated this after participants had learnt to categorize high respiratory loads into one and low respiratory loads in a second category. Presentation of these loads during the experiment altered magnitude estimation and unpleasantness in line with categorical expectations. In our experiment, participants were unaware of the application of hypercapnia, whilst they were expecting application of inspiratory loading of a specific strength. Participants might hence simply have evaluated stimuli in an 'on-off' fashion according to whether respiratory loading was applied or not and this conscious process might have overridden any small differences the mild respiratory loading would have made during

application of loading. A behavioural experiment using a similar paradigm as Petersen and colleagues (Petersen et al., 2014) could be devised to include hypercapnia and respiratory loading stimuli of matched unpleasantness and the impact of categorization on such stimuli could be explored.

Habituation of respiratory unpleasantness

Chapter 3 investigated habituation of subjective unpleasantness over four experimental sessions on consecutive days. We found no habituation of subjective unpleasantness to respiratory loads over four half-an hour sessions. This is encouraging for the performance of multi-session FMRI studies like the two-session double-blinded crossover study presented in Chapter 5, and it also lends support to the findings of previous studies using repeated laboratory applications of respiratory loads without controlling for potential habituation.

However, whilst we show no habituation of subjective unpleasantness in response to respiratory loading at a group level, responses of individual participants might differ. It has been shown that differences in trait anxiety influences response habituation to unpleasant respiratory stimuli with more anxious participants habituating less to hypercapnic stimuli (Li et al., 2006). Other psychological differences (e.g. in negative affect (Schon et al., 2008), catastrophising (De Peuter et al., 2007) and perceived control (Craig and Best, 1977)) have been found to influence sensory and affective responses to aversive experiences and could have a similar differentiating effect on habituation to dyspnoea stimuli as anxiety. It would thus be interesting to assess whether (and on what time scale) habituation to respiratory loading occurs at an individual level.

Complimentary to the subjective ratings used in Chapter 3, physiological measures could be used to characterize physiological habituation responses to unpleasant respiratory stimuli

without necessitating conscious evaluation of these stimuli. This might lend greater sensitivity during the investigation of more subtle effects of fear learning before contingency awareness is acquired. Measures of galvanic skin response (GSR) or eye-blink startle responses are two techniques previously used for such purposes (Vrana, 1995) that could be applied to the study of dyspnoea. Electroencephalography (EEG) has shown decreased neural potentials in line with reduced respiratory perceptions in the second half compared to the first half of an experimental session including 12 short inspiratory occlusions (von Leupoldt et al., 2011b), and may also be of use. Together, GSR, EEG and measure of eye-blink startle responses could provide more objective, and perhaps more sensitive, assessments of whether physiological habituation to unpleasant respiratory stimuli occurs over time and whether these responses are reflected in subjective ratings.

Individual variation in subjective response to respiratory loads

As with habituation, subjective responses to respiratory loads may be subject to great inter-individual variation. The studies presented in Chapters 3 and 5 highlighted large individual variation in the perception of (and possibly the ability to perceive) respiratory loads. Such strong inter-individual variation is important in self-medication in asthma sufferers (Dales et al., 1989; Rosenkranz et al., 2005) and should hence be investigated further from a pure clinical point of view.

The large inter-individual variation also needs to be taken into account in the design of neuroimaging studies, in which responses are averaged over multiple participants. Whilst one may adjust stimuli in strength to each individual's subjective perception of unpleasantness (see Chapter 3), such thresholding procedures are long and complicated and cannot currently be easily performed in the scanner. Furthermore, the FMRI environment is different from the normal laboratory environment. It has been shown that dyspnoea is strongly affected by

emotional factors, e.g. anxiety, that might be increased during FMRI. Stimuli derived in a laboratory setting might hence evoke vastly different responses in the scanner. As described in Chapter 3, some of the individual loads necessary to evoke approximately 50%VAS unpleasantness outside of the scanner were so large that we could not exclude profound effects on CBF within the scanner. In Chapter 5, we therefore opted for two stimulus levels that on average evoked the desired responses in individuals during piloting, keeping the physiological load the same and accepting that interindividual variations in perceptions would occur.

6.2.2 Confounding factors of loading on CBF and neural responses to (visual) stimuli

Chapter 4 presents the first ASL study of the effects of respiratory loading on CBF. It shows that inspiratory loading of -9cmH_2O mildly increased CBF by 5%, whilst expiratory loading of +9cmH_2O does not significantly increase CBF. There, inspiratory loading could lead to reduced BOLD signal, especially during large loads.

Nevertheless, general neural responses to other tasks are most likely not affected. Respiratory loading may therefore be a useful tool in dyspnoea research, as long as care is taken to control for CBF (see Chapter 5).

While we have shown that controlling for CBF might be prudent when using inspiratory loading as a dyspnoea stimulus, a few questions remain unanswered.

1) In this study (Chapter 4), we only tested loads of one intensity level and caution is necessary when using higher loads, as we cannot assume linearity of CBF effects with linear increases in loading. Applications of loads at multiple strengths would be necessary to investigate whether effects of loading on CBF are linear or not.

2) We could not determine the cause of CBF increase during inspiratory loading in the work presented in Chapter 4. In the chapter, we discussed the potentially important role of venous return as a main driver of increased CBF during inspiratory loading (due to an accentuated thoracic pump mechanism) that is compensated by increased mean arterial pressure (MAP) during expiratory loading. Whether these mechanistic speculations are true could be assessed with measures of jugular venous pressure and arterial pressure during blood brain flow and CBF recordings. Understanding the nature of the CBF increase induced by inspiratory loading could ensure correct interpretation of both clinical and experimental scans, particularly during neuroimaging in patients with impaired autoregulation.

6.3 Neural mechanisms of acute unpleasantness and anticipation of dyspnoea

Chapter 5 presents the first study looking at the neural signature of anticipation and acute perception of dyspnoea intensity and unpleasantness. Remifentanil decreased brain activations in key areas involved in emotional processing. This study has two main findings:

1) Unpleasantness: During saline administration, behavioural ratings of unpleasantness correlated with brain activity in the hippocampus, temporal pole and somatosensory cortex, whereas brain activity in the anterior insula and the prefrontal cortex correlated with both intensity and unpleasantness. Remifentanil decreased dyspnoea unpleasantness whilst subjective intensity remained constant and also decreased BOLD activation in the prefrontal cortex, periaqueductal grey (PAG), thalamus, amygdala, hippocampus and cerebellum.

2) Anticipation: During the saline condition, we observed brain activity in the anterior insula, midinsula/precentral gyrus and premotor cortex. Remifentanil infusion decreased activity in these regions.

In this study we have used a low-dose opioid to dissociate dyspnoea intensity and unpleasantness, and have for the first time shown brain activation related to both dimensions in the same study. This directly confirms what has long been hypothesised in dyspnoea research (Lansing et al., 2009); that dyspnoea intensity and unpleasantness are processed by distinct neural systems.

We have shown that the anticipation of dyspnoea is related to brain activation in the anterior insula/operculum. These areas are implicated in emotion processing, especially the evaluation of saliency, but without further information we cannot differentiate the cause of this activation. Contingency awareness between visual cues and respiratory stimuli was confirmed, but no subjective ratings of anticipatory anxiety of dyspnoea or implicit measures like skin conductance recordings or eyeblink-startle responses were available. These additional measures could help to further investigate these anticipatory periods. Subjective ratings of dyspnoea-specific anticipatory anxiety would allow the investigation of the modulatory impact of anticipatory anxiety on perceived acute unpleasantness.

This study predominantly recruited participants that scored low on anxiety and negative affect scales. As patients with dyspnoea often suffer from comorbid depression and anxiety (Norwood, 2007; Porzelius et al., 1992) it is crucial to isolate the effects of these psychological dimensions on the perception of acute dyspnoea. This study has looked at general effects of anticipatory anxiety, but for treatment purposes it would be valuable to know whether elevated levels of general anxiety or dyspnoea-specific anxiety are linked to

improved (or attenuated) outcomes of various treatments, i.e. to be able to predict which patients would benefit more from particular treatments, especially pulmonary rehabilitation. We might also be able to assess which participants will benefit from opioids through assessing dyspnoea processing and reward-responsiveness. Differences in endogenous opioids might correlate with reward-responsive traits and affect the size of the response to externally administered opioids (Wanigasekera et al., 2012).

This study was performed in a double-blind manner, in order to abolish effects of expectation of dyspnoea relief on acute dyspnoea (Schenk et al., 2014). Dyspnoea in real-life scenarios is not perceived in such a controlled manner and anticipation of treatment effect is likely to play a significant part in treatment outcome, similar to placebo and nocebo effects in pain (Tracey, 2010). In order to maximize treatment benefits for patients, it would be interesting to investigate how anticipation of dyspnoea relief can be used to boost treatment in a clinical setting, or to counter acquired negative mind-sets in patients accustomed to deterioration and unsuccessful palliation.

The experimental work presented in this thesis solely uses healthy volunteers. This gives us a great opportunity to test hypotheses quickly and in a homogenous pool of patients, but care needs to be taken when translating these findings into patients. The brain mechanisms of patients with chronic dyspnoea might be altered in response to chronic differences in respiratory stimulation and affective response. The first study using neuroimaging in patients with COPD has been performed in our laboratory. Brain activation in response to imagined dyspnoea was largely similar between patients and healthy age-matched volunteers, but also showed increased activations of more medial prefrontal areas that might be involved in emotional processing (Herigstad et al., see Appendix 2). It would be ideal to directly induce unpleasant respiratory sensations in patients during FMRI and to measure evoked brain

responses in order to compare these to activations obtained from healthy volunteers, but only a subset of patients with chronic respiratory disease tolerate FMRI and fewer will tolerate respiratory challenges during FMRI.

Within the next decade, a detailed understanding of the neural mechanisms of dyspnoea might be used to identify different sensations pertaining to breathing difficulties, e.g. perceived threat, anxiety, sensory intensity of dyspnoea or perceived unpleasantness, even in the absence of subjective ratings. This could offer an opportunity to assess dyspnoea relief in patients that are unable to voice their sensations, e.g. sedated patients at the end of life. The work presented in Chapter 5 has started to pave the path for this process by showing distinct neural signatures related to different dyspnoea dimensions at a group level, but much more research will be needed in order to fully characterize neural activation during dyspnoea perception, before such investigations can be meaningful at an individual level.

6.4 Conclusion

In this thesis we rigorously tested the psychological sensations and FMRI effects of an experimental model to induce dyspnoea during FMRI. We then successfully used this model to reveal the neural signature during the anticipation of dyspnoea and the neural activation that correlated with acute dyspnoea intensity and unpleasantness. Methodologically, we showed that respiratory loading combined with mild hypercapnia is a meaningful and consistent stimulus that can be applied during multi-session FMRI intervention studies. Additionally, we showed that cerebral blood flow (CBF) effects of respiratory loading are very small and do not affect CBF changes that originate from simultaneous visual stimulation (Chapter 4). This shows that respiratory loading is a stimulus that is suitable for the study of

dyspnoea with FMRI. Most importantly, we used isocapnic respiratory loading to reveal the previously unknown neural signatures relating to the anticipation of dyspnoea, acute dyspnoea intensity and acute dyspnoea unpleasantness. The (at least partial) separation of respiratory intensity and unpleasantness during neural processing has long been hypothesized, but this thesis contains the first study that directly correlated respiratory intensity and unpleasantness with BOLD activation. We also showed that remifentanil differentially reduces the BOLD response related to perceived, as well as anticipated dyspnoea, which shows a potential close relationship between these affective components and gives us an insight into opioid mechanisms important for reduction of dyspnoea unpleasantness. The work presented in this thesis successfully contributes to the study of the neural mechanisms of experimental dyspnoea in healthy volunteers, with the aim of identifying new targets for the alleviation of dyspnoea suffering in large patient groups.

Bibliography

Abe, M., Hanakawa, T., 2009. Functional coupling underlying motor and cognitive functions of the dorsal premotor cortex. Behav Brain Res 198, 13-23.

Abernethy, A.P., Currow, D.C., Frith, P., Fazekas, B.S., McHugh, A., Bui, C., 2003. Randomised, double blind, placebo controlled crossover trial of sustained release morphine for the management of refractory dyspnoea. BMJ 327, 523-528.

Abidov, A., Rozanski, A., Hachamovitch, R., Hayes, S.W., Aboul-Enein, F., Cohen, I., Friedman, J.D., Germano, G., Berman, D.S., 2005. Prognostic significance of dyspnea in patients referred for cardiac stress testing. N Engl J Med 353, 1889-1898.

Aira, Z., Buesa, I., Garcia del Cano, G., Salgueiro, M., Mendiable, N., Mingo, J., Aguilera, L., Bilbao, J., Azkue, J.J., 2012. Selective impairment of spinal mu-opioid receptor mechanism by plasticity of serotonergic facilitation mediated by 5-HT2A and 5-HT2B receptors. Pain 153, 1418-1425.

Albuquerque, S.C., Carvalho, E.R., Lopes, R.S., Marques, H.S., Macedo, D.S., Pereira, E.D., Hyphantis, T.N., Carvalho, A.F., 2011. Ego defense mechanisms in COPD: impact on health-related quality of life and dyspnoea severity. Qual Life Res 20, 1401-1410.

Alius, M.G., Pane-Farre, C.A., Von Leupoldt, A., Hamm, A.O., 2013. Induction of dyspnea evokes increased anxiety and maladaptive breathing in individuals with high anxiety sensitivity and suffocation fear. Psychophysiology 50, 488-497.

Aloisi, A.M., Bonifazi, M., 2006. Sex hormones, central nervous system and pain. Horm Behav 50, 1-7.

American Thoracic Society, 1999. Dyspnea. Mechanisms, assessment, and management: a consensus statement. Am J Respir Crit Care Med 159, 321-340.

Apkarian, A.V., Bushnell, M.C., Treede, R.D., Zubieta, J.K., 2005. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain 9, 463-484.

Badre, D., 2008. Cognitive control, hierarchy, and the rostro-caudal organization of the frontal lobes. Trends Cogn Sci 12, 193-200.

Ballard, I.C., Murty, V.P., Carter, R.M., MacInnes, J.J., Huettel, S.A., Adcock, R.A., 2011. Dorsolateral prefrontal cortex drives mesolimbic dopaminergic regions to initiate motivated behavior. J Neurosci 31, 10340-10346.

Bantick, S.J., Wise, R.G., Ploghaus, A., Clare, S., Smith, S.M., Tracey, I., 2002. Imaging how attention modulates pain in humans using functional MRI. Brain 125, 310-319.

Banzett, R.B., Adams, L., O'Donnell, C.R., Gilman, S.A., Lansing, R.W., Schwartzstein, R.M., 2011. Using laboratory models to test treatment: morphine reduces dyspnea and hypercapnic ventilatory response. Am J Respir Crit Care Med 184, 920-927.

Banzett, R.B., Garcia, R.T., Moosavi, S.H., 2000. Simple contrivance "clamps" end-tidal PCO(2) and PO(2) despite rapid changes in ventilation. J Appl Physiol (1985) 88, 1597-1600.

Banzett, R.B., Lansing, R.W., Evans, K.C., Shea, S.A., 1996. Stimulus-response characteristics of CO2-induced air hunger in normal subjects. Respir Physiol 103, 19-31.

Banzett, R.B., Pedersen, S.H., Schwartzstein, R.M., Lansing, R.W., 2008. The affective dimension of laboratory dyspnea: air hunger is more unpleasant than work/effort. Am J Respir Crit Care Med 177, 1384-1390.

Baumgärtner, U., Buchholz, H.-G., Bellosevich, A., Magerl, W., Siessmeier, T., Rolke, R., Höhnemann, S., Piel, M., Rösch, F., Wester, H.-J., Henriksen, G., Stoeter, P., Bartenstein, P.,

Treede, R.-D., Schreckenberger, M., 2006. High opiate receptor binding potential in the human lateral pain system. Neuroimage 30, 692-699.

Baydur, A., Behrakis, P.K., Zin, W.A., Jaeger, M., Milic-Emili, J., 1982. A simple method for assessing the validity of the esophageal balloon technique. Am Rev Respir Dis 126, 788-791.

Behan, M., Zabka, A.G., Thomas, C.F., Mitchell, G.S., 2003. Sex steroid hormones and the neural control of breathing. Respir Physiol Neurobiol 136, 249-263.

Beitz, A.J., 1982. The sites of origin brain stem neurotensin and serotonin projections to the rodent nucleus raphe magnus. J Neurosci 2, 829-842.

Bell, C., Kowalchuk, J.M., Paterson, D.H., Scheuermann, B.W., Cunningham, D.A., 1999. The effects of caffeine on the kinetics of O2 uptake, CO2 production and expiratory ventilation in humans during the on-transient of moderate and heavy intensity exercise. Exp Physiol 84, 761-774.

Benarroch, E.E., 2012. Endogenous opioid systems: current concepts and clinical correlations. Neurology 79, 807-814.

Bingel, U., Wanigasekera, V., Wiech, K., Ni Mhuircheartaigh, R., Lee, M.C., Ploner, M., Tracey, I., 2011. The effect of treatment expectation on drug efficacy: imaging the analgesic benefit of the opioid remifentanil. Sci Transl Med 3, 70ra14.

Binks, A.P., Moosavi, S.H., Banzett, R.B., Schwartzstein, R.M., 2002. "Tightness" sensation of asthma does not arise from the work of breathing. Am J Respir Crit Care Med 165, 78-82.

Bishop, C.C., Powell, S., Rutt, D., Browse, N.L., 1986. Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. Stroke; a journal of cerebral circulation 17, 913-915.

Bloch-Salisbury, E., Shea, S.A., Brown, R., Evans, K., Banzett, R.B., 1996. Air hunger induced by acute increase in PCO2 adapts to chronic elevation of PCO2 in ventilated humans. J Appl Physiol (1985) 81, 949-956.

Bogaerts, K., Notebaert, K., Van Diest, I., Devriese, S., De Peuter, S., Van den Bergh, O., 2005. Accuracy of respiratory symptom perception in different affective contexts. J Psychosom Res 58, 537-543.

Bogaerts, K., Wan, L., Diest, I.V., Stans, L., Decramer, M., Van den Bergh, O., 2012. Peak-end memory bias in laboratory-induced dyspnea: a comparison of patients with medically unexplained symptoms and healthy controls. Psychosom Med 74, 974-981.

Boom, M., Niesters, M., Sarton, E., Aarts, L., Smith, T.W., Dahan, A., 2012. Non-analgesic effects of opioids: opioid-induced respiratory depression. Curr Pharm Des 18, 5994-6004.

Booth, S., Moosavi, S.H., Higginson, I.J., 2008. The etiology and management of intractable breathlessness in patients with advanced cancer: a systematic review of pharmacological therapy. Nat Clin Pract Oncol 5, 90-100.

Bourdy, R., Barrot, M., 2012. A new control center for dopaminergic systems: pulling the VTA by the tail. Trends Neurosci 35, 681-690.

Bowden, J.A., To, T.H., Abernethy, A.P., Currow, D.C., 2011. Predictors of chronic breathlessness: a large population study. BMC Public Health 11, 33.

Brannan, S., Liotti, M., Egan, G., Shade, R., Madden, L., Robillard, R., Abplanalp, B., Stofer, K., Denton, D., Fox, P.T., 2001. Neuroimaging of cerebral activations and deactivations associated with hypercapnia and hunger for air. Proc Natl Acad Sci U S A 98, 2029-2034.

Britton, M., 2003. The burden of COPD in the U.K.: results from the Confronting COPD survey. Respir Med 97 Suppl C, S71-79.

Broderick, J.E., Stone, A.A., Calvanese, P., Schwartz, J.E., Turk, D.C., 2006. Recalled pain ratings: a complex and poorly defined task. J Pain 7, 142-149.

Brooks, J.C., Beckmann, C.F., Miller, K.L., Wise, R.G., Porro, C.A., Tracey, I., Jenkinson, M., 2008. Physiological noise modelling for spinal functional magnetic resonance imaging studies. Neuroimage 39, 680-692.

Brown, G.G., Eyler Zorrilla, L.T., Georgy, B., Kindermann, S.S., Wong, E.C., Buxton, R.B., 2003. BOLD and perfusion response to finger-thumb apposition after acetazolamide administration: differential relationship to global perfusion. J Cereb Blood Flow Metab 23, 829-837.

Brown, S., Ngan, E., Liotti, M., 2008. A larynx area in the human motor cortex. Cereb Cortex 18, 837-845.

Carrier, J., Monk, T.H., 2000. Circadian rhythms of performance: new trends. Chronobiol Int 17, 719-732.

Carrieri-Kohlman, V., Gormley, J.M., Eiser, S., Demir-Deviren, S., Nguyen, H., Paul, S.M., Stulbarg, M.S., 2001. Dyspnea and the affective response during exercise training in obstructive pulmonary disease. Nurs Res 50, 136-146.

Carter, R.M., O'Doherty, J.P., Seymour, B., Koch, C., Dolan, R.J., 2006. Contingency awareness in human aversive conditioning involves the middle frontal gyrus. Neuroimage 29, 1007-1012.

Carver, C.S., White, T.L., 1994. Behavioral-Inhibition, Behavioral Activation, and Affective Responses to Impending Reward and Punishment - the Bis Bas Scales. J Pers Soc Psychol 67, 319-333.

Casey, K.L., Minoshima, S., Morrow, T.J., Koeppe, R.A., Frey, K.A., 1995. Imaging the Brain in Pain - Potentials, Limitations, and Implications. Pain and the Brain 22, 201-211.

Chen, Y., Parrish, T.B.T.B., 2009. Caffeine dose effect on activation-induced BOLD and CBF responses. Neuroimage 46, 577-583.

Choi, J.C., Chung, M.I., Lee, Y.D., 2012. Modulation of pain sensation by stress-related testosterone and cortisol. Anaesthesia 67, 1146-1151.

Cieslik, E.C., Zilles, K., Caspers, S., Roski, C., Kellermann, T.S., Jakobs, O., Langner, R., Laird, A.R., Fox, P.T., Eickhoff, S.B., 2013. Is there "one" DLPFC in cognitive action control? Evidence for heterogeneity from co-activation-based parcellation. Cereb Cortex 23, 2677-2689.

Clark, R.E., Manns, J.R., Squire, L.R., 2002a. Classical conditioning, awareness, and brain systems. Trends Cogn Sci 6, 524-531.

Clark, W.C., Yang, J.C., Tsui, S.L., Ng, K.F., Bennett Clark, S., 2002b. Unidimensional pain rating scales: a multidimensional affect and pain survey (MAPS) analysis of what they really measure. Pain 98, 241-247.

Cohen, E.R., Ugurbil, K., Kim, S.G., 2002. Effect of basal conditions on the magnitude and dynamics of the blood oxygenation level-dependent fMRI response. J Cereb Blood Flow Metab 22, 1042-1053.

Cooke, W.H., Lurie, K.G., Rohrer, M.J., Convertino, V.a., 2006. Human autonomic and cerebrovascular responses to inspiratory impedance. The Journal of trauma 60, 1275-1283.

Corfield, D.R., Fink, G.R., Ramsay, S.C., Murphy, K., Harty, H.R., Watson, J.D., Adams, L., Frackowiak, R.S., Guz, A., 1995. Evidence for limbic system activation during CO2-stimulated breathing in man. J Physiol 488 (Pt 1), 77-84.

Craig, A.D., 2003. A new view of pain as a homeostatic emotion. Trends Neurosci 26, 303-307.

Craig, a.D.B., 2009. How do you feel--now? The anterior insula and human awareness. Nature reviews. Neuroscience 10, 59-70.

Craig, K.D., Best, A.J., 1977. Perceived control over pain: individual differences and situational determinants. Pain 3, 127-135.

Crisson, J.E., Keefe, F.J., 1988. The relationship of locus of control to pain coping strategies and psychological distress in chronic pain patients. Pain 35, 147-154.

Currow, D.C., Plummer, J.L., Crockett, A., Abernethy, A.P., 2009. A community population survey of prevalence and severity of dyspnea in adults. J Pain Symptom Manage 38, 533-545.

Dai, W., Garcia, D., de Bazelaire, C., Alsop, D.C., 2008. Continuous flow-driven inversion for arterial spin labeling using pulsed radio frequency and gradient fields. Magn Reson Med 60, 1488-1497.

Dales, R.E., Spitzer, W.O., Schechter, M.T., Suissa, S., 1989. The influence of psychological status on respiratory symptom reporting. Am Rev Respir Dis 139, 1459-1463.

Davenport, P.W., Vovk, A., 2009. Cortical and subcortical central neural pathways in respiratory sensations. Respir Physiol Neurobiol 167, 72-86.

Davies, H.W., Haldane, J.S., Priestley, J.G., 1919. The response to respiratory resistance. J Physiol 53, 60-69.

de Marco, R., Locatelli, F., Sunyer, J., Burney, P., 2000. Differences in incidence of reported asthma related to age in men and women. A retrospective analysis of the data of the European Respiratory Health Survey. Am J Respir Crit Care Med 162, 68-74.

De Peuter, S., Lemaigre, V., Van Diest, I., Verleden, G., Demedts, M., Van den Bergh, O., 2007. Differentiation between the sensory and affective aspects of histamine-induced bronchoconstriction in asthma. Respir Med 101, 925-932.

De Peuter, S., Van Diest, I., Lemaigre, V., Li, W., Verleden, G., Demedts, M., Van den Bergh, O., 2005. Can subjective asthma symptoms be learned? Psychosom Med 67, 454-461.

De Peuter, S., Van Diest, I., Lemaigre, V., Verleden, G., Demedts, M., Van den Bergh, O., 2004. Dyspnea: the role of psychological processes. Clin Psychol Rev 24, 557-581.

de Torres, J.P., Casanova, C., de Garcini, A.M., Jaime, A.A., Celli, B.R., 2007. COPD heterogeneity: gender differences in the multidimensional BODE index. Int J Chron Obstruct Pulmon Dis 2, 151-155.

Detre, J.A., Wang, J., 2002. Technical aspects and utility of fMRI using BOLD and ASL. Clin Neurophysiol 113, 621-634.

Di Marco, F., Verga, M., Reggente, M., Maria Casanova, F., Santus, P., Blasi, F., Allegra, L., Centanni, S., 2006. Anxiety and depression in COPD patients: The roles of gender and disease severity. Respir Med 100, 1767-1774.

Douma, S.L., Husband, C., O'Donnell, M.E., Barwin, B.N., Woodend, A.K., 2005. Estrogen-related mood disorders: reproductive life cycle factors. ANS Adv Nurs Sci 28, 364-375.

Droste, D.W., Ludemann, P., Anders, F., Kemeny, V., Thomas, M., Krauss, J.K., Ringelstein, E.B., 1999. Middle cerebral artery blood flow velocity, end-tidal pCO2 and blood pressure in patients with obstructive sleep apnea and in healthy subjects during continuous positive airway pressure breathing. Neurol Res 21, 737-741.

Duong, T.Q., Yacoub, E., Adriany, G., Hu, X., Ugurbil, K., Vaughan, J.T., Merkle, H., Kim, S.G., 2002. High-resolution, spin-echo BOLD, and CBF fMRI at 4 and 7 T. Magn Reson Med 48, 589-593.

Eckenhoff, J.E., Oech, S.R., 1960. The effects of narcotics and antagonists upon respiration and circulation in man. A review. Clin Pharmacol Ther 1, 483-524.

Eippert, F., Bingel, U., Schoell, E., Yacubian, J., Buchel, C., 2008. Blockade of endogenous opioid neurotransmission enhances acquisition of conditioned fear in humans. J Neurosci 28, 5465-5472.

el-Manshawi, A., Killian, K.J., Summers, E., Jones, N.L., 1986. Breathlessness during exercise with and without resistive loading. J Appl Physiol (1985) 61, 896-905.

Elliott, M.W., Adams, L., Cockcroft, A., MacRae, K.D., Murphy, K., Guz, A., 1991. The language of breathlessness. Use of verbal descriptors by patients with cardiopulmonary disease. Am Rev Respir Dis 144, 826-832.

Evans, K.C., Banzett, R.B., Adams, L., McKay, L., Frackowiak, R.S., Corfield, D.R., 2002. BOLD fMRI identifies limbic, paralimbic, and cerebellar activation during air hunger. J Neurophysiol 88, 1500-1511.

Evans, K.C., Simon, N.M., Dougherty, D.D., Hoge, E.A., Worthington, J.J., Chow, C., Kaufman, R.E., Gold, A.L., Fischman, A.J., Pollack, M.H., Rauch, S.L., 2009. A PET study

of tiagabine treatment implicates ventral medial prefrontal cortex in generalized social anxiety disorder. Neuropsychopharmacology 34, 390-398.

Fairhurst, M., Wiech, K., Dunckley, P., Tracey, I., 2007. Anticipatory brainstem activity predicts neural processing of pain in humans. Pain 128, 101-110.

Fanselow, M.S., 1998. Pavlovian conditioning, negative feedback, and blocking: mechanisms that regulate association formation. Neuron 20, 625-627.

Fletcher, C.M., Elmes, P.C., Fairbairn, A.S., Wood, C.H., 1959. The significance of respiratory symptoms and the diagnosis of chronic bronchitis in a working population. Br Med J 2, 257-266.

Fox, P.T., Raichle, M.E., 1985. Stimulus rate determines regional brain blood flow in striate cortex. Annals of neurology 17, 303-305.

Frostad, A., Soyseth, V., Andersen, A., Gulsvik, A., 2006. Respiratory symptoms as predictors of all-cause mortality in an urban community: a 30-year follow-up. J Intern Med 259, 520-529.

Gandevia, S.C., Killian, K., McKenzie, D.K., Crawford, M., Allen, G.M., Gorman, R.B., Hales, J.P., 1993. Respiratory sensations, cardiovascular control, kinaesthesia and transcranial stimulation during paralysis in humans. J Physiol 470, 85-107.

Gandevia, S.C., Killian, K.J., Campbell, E.J., 1981. The effect of respiratory muscle fatigue on respiratory sensations. Clin Sci (Lond) 60, 463-466.

Gauthier, C.J., Madjar, C., Tancredi, F.B., Stefanovic, B., Hoge, R.D., 2011. Elimination of visually evoked BOLD responses during carbogen inhalation: implications for calibrated MRI. Neuroimage 54, 1001-1011.

Gerlach, Y., Williams, M.T., Coates, A.M., 2013. Weighing up the evidence-a systematic review of measures used for the sensation of breathlessness in obesity. Int J Obes (Lond) 37, 341-349.

Gifford, A.H., Mahler, D.A., Waterman, L.A., Ward, J., Kraemer, W.J., Kupchak, B.R., Baird, J.C., 2011. Neuromodulatory effect of endogenous opioids on the intensity and unpleasantness of breathlessness during resistive load breathing in COPD. Copd-Journal of Chronic Obstructive Pulmonary Disease 8, 160-166.

Gift, A.G., 1989. Validation of a vertical visual analogue scale as a measure of clinical dyspnea. Rehabil Nurs 14, 323-325.

Gift, A.G., Narsavage, G., 1998. Validity of the numeric rating scale as a measure of dyspnea. Am J Crit Care 7, 200-204.

Gigliotti, F., 2010. Mechanisms of dyspnea in healthy subjects. Multidiscip Respir Med 5, 195-201.

Glover, G.H., Li, T.Q., Ress, D., 2000. Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. Magn Reson Med 44, 162-167.

Gorman, R.B., McKenzie, D.K., Gandevia, S.C., 1999. Task failure, breathing discomfort and CO2 accumulation without fatigue during inspiratory resistive loading in humans. Respir Physiol 115, 273-286.

Gray, T.S., Magnuson, D.J., 1992. Peptide immunoreactive neurons in the amygdala and the bed nucleus of the stria terminalis project to the midbrain central gray in the rat. Peptides 13, 451-460.

Gu, X., Hof, P.R., Friston, K.J., Fan, J., 2013. Anterior insular cortex and emotional awareness. J Comp Neurol 521, 3371-3388.

Guyatt, G.H., Townsend, M., Berman, L.B., Keller, J.L., 1987. A comparison of Likert and visual analogue scales for measuring change in function. J Chronic Dis 40, 1129-1133.

Gysels, M., Bausewein, C., Higginson, I.J., 2007. Experiences of breathlessness: a systematic review of the qualitative literature. Palliat Support Care 5, 281-302.

Hall, J.E., Guyton, A.C., 2011. Guyton and Hall textbook of medical physiology, 12th ed. Saunders/Elsevier, Philadelphia, PA.

Hardy, S.G., Leichnetz, G.R., 1981. Cortical projections to the periaqueductal gray in the monkey: a retrograde and orthograde horseradish peroxidase study. Neurosci Lett 22, 97-101.

Harper, R.M., Poe, G.R., Rector, D.M., Kristensen, M.P., 1998. Relationships between hippocampal activity and breathing patterns. Neurosci Biobehav Rev 22, 233-236.

Harris, A.D., Ide, K., Poulin, M.J., Frayne, R., 2006. Control of end-tidal PCO2 reduces middle cerebral artery blood velocity variability: implications for physiological neuroimaging. Neuroimage 29, 1272-1277.

Harvey, A.K., Pattinson, K.T.S., Brooks, J.C.W., Mayhew, S.D., Jenkinson, M., Wise, R.G., 2008. Brainstem functional magnetic resonance imaging: disentangling signal from physiological noise. Journal of magnetic resonance imaging : JMRI 28, 1337-1344.

Heim, E., Blaser, A., Waidelich, E., 1972. Dyspnea: psychophysiologic relationships. Psychosom Med 34, 405-423.

Herigstad, M., Hayen, A., Wiech, K., Pattinson, K.T., 2011a. Dyspnoea and the brain. Respir Med 105, 809-817.

Herigstad, M., Hayen, A., Wiech, K., Pattinson, K.T.S.S., 2011b. Dyspnoea and the brain. Respiratory Medicine 105, 1-9.

Ho, S.F., O'Mahony, M.S., Steward, J.A., Breay, P., Buchalter, M., Burr, M.L., 2001. Dyspnoea and quality of life in older people at home. Age Ageing 30, 155-159.

Holtz, K., Pane-Farre, C.A., Wendt, J., Lotze, M., Hamm, A.O., 2012. Brain activation during anticipation of interoceptive threat. Neuroimage 61, 857-865.

Hornstein, D., Ledsome, J.R., 1984. Alveolar gas pressure changes with resistive loads in man. Respiration 45, 347-352.

Horton, R., Rocker, G., 2010. Contemporary issues in refractory dyspnoea in advanced chronic obstructive pulmonary disease. Curr Opin Support Palliat Care 4, 56-62.

Hoyert, D.L.X., Jiaquan, 2012. Deaths: Preliminary data for 2011. Division of Vital Statistics.

Hu, J., Meek, P., 2005. Health-related quality of life in individuals with chronic obstructive pulmonary disease. Heart Lung 34, 415-422.

Huang, T.Y., Moser, D.K., Hsieh, Y.S., Gau, B.S., Chiang, F.T., Hwang, S.L., 2013. Moderating effect of psychosocial factors for dyspnea in Taiwanese and American heart failure patients. J Nurs Res 21, 49-58.

Hughes, J., Kosterlitz, H.W., 1983. Opioid Peptides: introduction. Br Med Bull 39, 1-3.

Ide, K., Eliasziw, M., Poulin, M.J., 2003. Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. Journal of Applied Physiology 95, 129.

Isaev, G., Murphy, K., Guz, A., Adams, L., 2002a. Areas of the brain concerned with ventilatory load compensation in awake man. The Journal of Physiology 539, 935-945.

Isaev, G., Murphy, K., Guz, A., Adams, L., 2002b. Areas of the brain concerned with ventilatory load compensation in awake man. J Physiol 539, 935-945.

Isono, S., Nishino, T., Sugimori, K., Mizuguchi, T., 1990. Respiratory effects of expiratory flow-resistive loading in conscious and anesthetized humans. Anesth Analg 70, 594-599.

Jack, S., Kemp, G.J., Bimson, W.E., Calverley, P.M., Corfield, D.R., 2010. Patterns of brain activity in response to respiratory stimulation in patients with idiopathic hyperventilation (IHV). Adv Exp Med Biol 669, 341-345.

Janssens, T., De Peuter, S., Stans, L., Verleden, G., Troosters, T., Decramer, M., Van den Bergh, O., 2011. Dyspnea perception in COPD: association between anxiety, dyspnea-related fear, and dyspnea in a pulmonary rehabilitation program. Chest 140, 618-625.

Jenkinson, M., Bannister, P., Brady, M., Smith, S., 2002a. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17, 825-841.

Jenkinson, M., Bannister, P., Brady, M., Smith, S., 2002b. Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images. Neuroimage 17, 825-841.

Jennings, A.L., Davies, A.N., Higgins, J.P., Gibbs, J.S., Broadley, K.E., 2002. A systematic review of the use of opioids in the management of dyspnoea. Thorax 57, 939-944.

Jensen, D., O'Donnell, D.E., Li, R., Luo, Y.M., 2011. Effects of dead space loading on neuro-muscular and neuro-ventilatory coupling of the respiratory system during exercise in healthy adults: implications for dyspnea and exercise tolerance. Respir Physiol Neurobiol 179, 219-226.

Jezzard, P., Matthews, P.M., Smith, S.M., 2001. Functional MRI : an introduction to methods. Oxford University Press, Oxford ; New York.

Kalliomaki, J., Luo, X.L., Yu, Y.B., Schouenborg, J., 1998. Intrathecally applied morphine inhibits nociceptive C fiber input to the primary somatosensory cortex (SI) of the rat. Pain 77, 323-329.

Kamal, A.H., Maguire, J.M., Wheeler, J.L., Currow, D.C., Abernethy, A.P., 2011. Dyspnea review for the palliative care professional: assessment, burdens, and etiologies. J Palliat Med 14, 1167-1172.

Katz, N., Mazer, N.A., 2009. The impact of opioids on the endocrine system. Clin J Pain 25, 170-175.

Keltner, J.R., Furst, A., Fan, C., Redfern, R., Inglis, B., Fields, H.L., 2006. Isolating the modulatory effect of expectation on pain transmission: a functional magnetic resonance imaging study. J Neurosci 26, 4437-4443.

Kendrick, K.R., Baxi, S.C., Smith, R.M., 2000. Usefulness of the modified 0-10 Borg scale in assessing the degree of dyspnea in patients with COPD and asthma. J Emerg Nurs 26, 216-222.

Ketelaars, C.A., Schlosser, M.A., Mostert, R., Huyer Abu-Saad, H., Halfens, R.J., Wouters, E.F., 1996. Determinants of health-related quality of life in patients with chronic obstructive pulmonary disease. Thorax 51, 39-43.

Killian, K.J., Watson, R., Otis, J., St Amand, T.A., O'Byrne, P.M., 2000. Symptom perception during acute bronchoconstriction. Am J Respir Crit Care Med 162, 490-496.

Kim, S.G., Rostrup, E., Larsson, H.B., Ogawa, S., Paulson, O.B., 1999. Determination of relative CMRO2 from CBF and BOLD changes: significant increase of oxygen consumption rate during visual stimulation. Magn Reson Med 41, 1152-1161.

Kjellstrom, B., van der Wal, M.H., 2013. Old and new tools to assess dyspnea in the hospitalized patient. Curr Heart Fail Rep 10, 204-211.

Kolbitsch, C., Lorenz, I.H., Hörmann, C., Schocke, M., Kremser, C., Zschiegner, F., Felber, S., Benzer, a., 2000. The impact of increased mean airway pressure on contrast-enhanced MRI measurement of regional cerebral blood flow (rCBF), regional cerebral blood volume (rCBV), regional mean transit time (rMTT), and regional cerebrovascular resistance (rCVR) in human vol. Human brain mapping 11, 214-222.

Kruger, G., Glover, G.H., 2001. Physiological noise in oxygenation-sensitive magnetic resonance imaging. Magn Reson Med 46, 631-637.

Kulkarni, B., Bentley, D.E., Elliott, R., Youell, P., Watson, A., Derbyshire, S.W., Frackowiak, R.S., Friston, K.J., Jones, A.K., 2005. Attention to pain localization and unpleasantness discriminates the functions of the medial and lateral pain systems. Eur J Neurosci 21, 3133-3142.

Lacasse, Y., Martin, S., Lasserson, T.J., Goldstein, R.S., 2007. Meta-analysis of respiratory rehabilitation in chronic obstructive pulmonary disease. A Cochrane systematic review. Eura Medicophys 43, 475-485.

Lansing, R.W., Gracely, R.H., Banzett, R.B., 2009. The multiple dimensions of dyspnea: review and hypotheses. Respir Physiol Neurobiol 167, 53-60.

Lansing, R.W., Im, B.S., Thwing, J.I., Legedza, A.T., Banzett, R.B., 2000. The perception of respiratory work and effort can be independent of the perception of air hunger. Am J Respir Crit Care Med 162, 1690-1696.

Lareau, S.C., Meek, P.M., Press, D., Anholm, J.D., Roos, P.J., 1999. Dyspnea in patients with chronic obstructive pulmonary disease: does dyspnea worsen longitudinally in the presence of declining lung function? Heart Lung 28, 65-73.

Laurin, C., Lavoie, K.L., Bacon, S.L., Dupuis, G., Lacoste, G., Cartier, A., Labrecque, M., 2007. Sex differences in the prevalence of psychiatric disorders and psychological distress in patients with COPD. Chest 132, 148-155.

Lee, M.C., Ploner, M., Wiech, K., Bingel, U., Wanigasekera, V., Brooks, J., Menon, D.K., Tracey, I., 2013. Amygdala activity contributes to the dissociative effect of cannabis on pain perception. Pain 154, 124-134.

Leknes, S., Tracey, I., 2008. A common neurobiology for pain and pleasure. Nat Rev Neurosci 9, 314-320.

Levenson, H., 1973. Multidimensional locus of control in psychiatric patients. J Consult Clin Psychol 41, 397-404.

Ley, R., 1999. The modification of breathing behavior. Pavlovian and operant control in emotion and cognition. Behav Modif 23, 441-479.

Ley, R., 2001. Respiratory psychophysiology and behavior modification. Behav Modif 25, 491-494.

Li, W., Daems, E., Van de Woestijne, K.P., Van Diest, I., Gallego, J., De Peuter, S., Bogaerts, K., Van den Bergh, O., 2006. Air hunger and ventilation in response to hypercapnia: effects of repetition and anxiety. Physiol Behav 88, 47-54.

Liotti, M., Brannan, S., Egan, G., Shade, R., Madden, L., Abplanalp, B., Robillard, R., Lancaster, J., Zamarripa, F.E., Fox, P.T., Denton, D., 2001. Brain responses associated with consciousness of breathlessness (air hunger). Proc Natl Acad Sci U S A 98, 2035-2040.

Loggia, M.L., Kim, J., Gollub, R.L., Vangel, M.G., Kirsch, I., Kong, J., Wasan, A.D., Napadow, V., 2013. Default mode network connectivity encodes clinical pain: an arterial spin labeling study. Pain 154, 24-33.

Lorenz, I.H., Kolbitsch, C., Hormann, C., Schocke, M., Kremser, C., Zschiegner, F., Felber, S., Benzer, A., 2001. Increasing mean airway pressure reduces functional MRI (fMRI) signal in the primary visual cortex. Magn Reson Imaging 19, 7-11.

Lorenz, J., Cross, D.J., Minoshima, S., Morrow, T.J., Paulson, P.E., Casey, K.L., 2002. A unique representation of heat allodynia in the human brain. Neuron 35, 383-393.

Lorenz, J., Minoshima, S., Casey, K.L., 2003. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain 126, 1079-1091.

Macey, P.M., Woo, M.A., Macey, K.E., Keens, T.G., Saeed, M.M., Alger, J.R., Harper, R.M., 2005. Hypoxia reveals posterior thalamic, cerebellar, midbrain, and limbic deficits in congenital central hypoventilation syndrome. J Appl Physiol (1985) 98, 958-969.

MacIntosh, B.J., Pattinson, K.T., Gallichan, D., Ahmad, I., Miller, K.L., Feinberg, D.A., Wise, R.G., Jezzard, P., 2008. Measuring the effects of remifentanil on cerebral blood flow and arterial arrival time using 3D GRASE MRI with pulsed arterial spin labelling. J Cereb Blood Flow Metab 28, 1514-1522.

Mahler, D.A., 2013. Opioids for refractory dyspnea. Expert Rev Respir Med 7, 123-134; quiz 135.

Mahler, D.A., Harver, A., 2000. Do you speak the language of dyspnea? Chest 117, 928-929.

Mahler, D.A., Harver, A., Lentine, T., Scott, J.A., Beck, K., Schwartzstein, R.M., 1996. Descriptors of breathlessness in cardiorespiratory diseases. Am J Respir Crit Care Med 154, 1357-1363.

Mahler, D.a., Murray, J.a., Waterman, L.a., Ward, J., Kraemer, W.J., Zhang, X., Baird, J.C., 2009. Endogenous opioids modify dyspnoea during treadmill exercise in patients with COPD. Eur Respir J 33, 771-777.

Mahler, D.A., Selecky, P.A., Harrod, C.G., Benditt, J.O., Carrieri-Kohlman, V., Curtis, J.R., Manning, H.L., Mularski, R.A., Varkey, B., Campbell, M., Carter, E.R., Chiong, J.R., Ely, E.W., Hansen-Flaschen, J., O'Donnell, D.E., Waller, A., 2010. American College of Chest Physicians consensus statement on the management of dyspnea in patients with advanced lung or heart disease. Chest 137, 674-691.

Mahler, D.A., Weinberg, D.H., Wells, C.K., Feinstein, A.R., 1984. The measurement of dyspnea. Contents, interobserver agreement, and physiologic correlates of two new clinical indexes. Chest 85, 751-758.

Main, J., Moss-Morris, R., Booth, R., Kaptein, A.A., Kolbe, J., 2003. The use of reliever medication in asthma: the role of negative mood and symptom reports. J Asthma 40, 357-365.

Mannino, D.M., 2002. COPD: epidemiology, prevalence, morbidity and mortality, and disease heterogeneity. Chest 121, 121S-126S.

Mansour, A., Fox, C.A., Akil, H., Watson, S.J., 1995. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci 18, 22-29.

Manto, M., Bower, J.M., Conforto, A.B., Delgado-Garcia, J.M., da Guarda, S.N., Gerwig, M., Habas, C., Hagura, N., Ivry, R.B., Marien, P., Molinari, M., Naito, E., Nowak, D.A., Oulad Ben Taib, N., Pelisson, D., Tesche, C.D., Tilikete, C., Timmann, D., 2012. Consensus paper: roles of the cerebellum in motor control--the diversity of ideas on cerebellar involvement in movement. Cerebellum 11, 457-487.

Manzke, T., Niebert, M., Koch, U.R., Caley, A., Vogelgesang, S., Bischoff, A.M., Hulsmann, S., Ponimaskin, E., Muller, U., Smart, T.G., Harvey, R.J., Richter, D.W., 2011. [Serotonin receptor 1A-modulated dephosphorylation of glycine receptor alpha3: a new molecular mechanism of breathing control for compensation of opioid-induced respiratory depression without loss of analgesia]. Schmerz 25, 272-281.

Mauk, M.D., Thompson, R.F., 1987. Retention of classically conditioned eyelid responses following acute decerebration. Brain Res 403, 89-95.

McKenzie, D.K., Allen, G.M., Butler, J.E., Gandevia, S.C., 1997. Task failure with lack of diaphragm fatigue during inspiratory resistive loading in human subjects. J Appl Physiol (1985) 82, 2011-2019.

McNally, G.P., Pigg, M., Weidemann, G., 2004a. Blocking, unblocking, and overexpectation of fear: a role for opioid receptors in the regulation of Pavlovian association formation. Behav Neurosci 118, 111-120.

McNally, G.P., Pigg, M., Weidemann, G., 2004b. Opioid receptors in the midbrain periaqueductal gray regulate extinction of pavlovian fear conditioning. J Neurosci 24, 6912-6919.

Meek, P.M., Banzett, R., Parshall, M.B., Gracely, R.H., Schwartzstein, R.M., Lansing, R., 2012. Reliability and validity of the multidimensional dyspnea profile. Chest 141, 1546-1553.

Meek, P.M., Lareau, S.C., Anderson, D., 2001. Memory for symptoms in COPD patients: how accurate are their reports? Eur Respir J 18, 474-481.

Mehendale, A.W., Goldman, M.P., Mehendale, R.P., 2013. Opioid overuse pain syndrome (OOPS): the story of opioids, prometheus unbound. J Opioid Manag 9, 421-438.

Melgert, B.N., Ray, A., Hylkema, M.N., Timens, W., Postma, D.S., 2007. Are there reasons why adult asthma is more common in females? Curr Allergy Asthma Rep 7, 143-150.

Menon, V., Uddin, L.Q., 2010. Saliency, switching, attention and control: a network model of insula function. Brain Struct Funct 214, 655-667.

Miller, K.L., Luh, W.M., Liu, T.T., Martinez, A., Obata, T., Wong, E.C., Frank, L.R., Buxton, R.B., 2001. Nonlinear temporal dynamics of the cerebral blood flow response. Hum Brain Mapp 13, 1-12.

Minto, C.F., Schnider, T.W., Egan, T.D., Youngs, E., Lemmens, H.J., Gambus, P.L., Billard, V., Hoke, J.F., Moore, K.H., Hermann, D.J., Muir, K.T., Mandema, J.W., Shafer, S.L., 1997a. Influence of age and gender on the pharmacokinetics and pharmacodynamics of remifentanil. I. Model development. Anesthesiology 86, 10-23.

Minto, C.F., Schnider, T.W., Shafer, S.L., 1997b. Pharmacokinetics and pharmacodynamics of remifentanil. II. Model application. Anesthesiology 86, 24-33.

Montes de Oca, M., Rassulo, J., Celli, B.R., 1996. Respiratory muscle and cardiopulmonary function during exercise in very severe COPD. Am J Respir Crit Care Med 154, 1284-1289.

Moosavi, S.H., Banzett, R.B., Butler, J.P., 2004. Time course of air hunger mirrors the biphasic ventilatory response to hypoxia. J Appl Physiol 97, 2098-2103.

Moosavi, S.H., Golestanian, E., Binks, A.P., Lansing, R.W., Brown, R., Banzett, R.B., 2003. Hypoxic and hypercapnic drives to breathe generate equivalent levels of air hunger in humans. J Appl Physiol (1985) 94, 141-154.

Moulton, E.A., Elman, I., Pendse, G., Schmahmann, J., Becerra, L., Borsook, D., 2011. Aversion-related circuitry in the cerebellum: responses to noxious heat and unpleasant images. J Neurosci 31, 3795-3804.

Moy, M.L., Woodrow Weiss, J., Sparrow, D., Israel, E., Schwartzstein, R.M., 2000. Quality of dyspnea in bronchoconstriction differs from external resistive loads. Am J Respir Crit Care Med 162, 451-455.

Muris, P., Merckelbach, H., 1996. The short version of the Defense Style Questionnaire: Factor structure and psychopathological correlates. Personality and Individual Differences 20, 123-126.

Naberan, K., Azpeitia, A., Cantoni, J., Miravitlles, M., 2012. Impairment of quality of life in women with chronic obstructive pulmonary disease. Respir Med 106, 367-373.

Nelson, M.E., Rejeski, W.J., Blair, S.N., Duncan, P.W., Judge, J.O., King, A.C., Macera, C.A., Castaneda-Sceppa, C., 2007. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 39, 1435-1445.

Ng, T.P., Niti, M., Tan, W.C., Cao, Z., Ong, K.C., Eng, P., 2007. Depressive symptoms and chronic obstructive pulmonary disease: effect on mortality, hospital readmission, symptom burden, functional status, and quality of life. Arch Intern Med 167, 60-67.

Nici, L., Donner, C., Wouters, E., Zuwallack, R., Ambrosino, N., Bourbeau, J., Carone, M., Celli, B., Engelen, M., Fahy, B., Garvey, C., Goldstein, R., Gosselink, R., Lareau, S., MacIntyre, N., Maltais, F., Morgan, M., O'Donnell, D., Prefault, C., Reardon, J., Rochester, C., Schols, A., Singh, S., Troosters, T., 2006. American Thoracic Society/European Respiratory Society statement on pulmonary rehabilitation. Am J Respir Crit Care Med 173, 1390-1413.

Nielsen, A.N., Lauritzen, M., 2001. Coupling and uncoupling of activity-dependent increases of neuronal activity and blood flow in rat somatosensory cortex. Journal of Physiology-London 533, 773-785.

Ninot, G., Fortes, M., Poulain, M., Brun, A., Desplan, J., Prefaut, C., Varray, A., 2006. Gender difference in coping strategies among patients enrolled in an inpatient rehabilitation program. Heart Lung 35, 130-136.

Nishino, T., 2011. Dyspnoea: underlying mechanisms and treatment. Br J Anaesth 106, 463-474.

Nishino, T., Ide, T., Sudo, T., Sato, J., 2000. Inhaled furosemide greatly alleviates the sensation of experimentally induced dyspnea. Am J Respir Crit Care Med 161, 1963-1967.

Nishino, T., Isono, S., Ishikawa, T., Shinozuka, N., 2007. An additive interaction between different qualities of dyspnea produced in normal human subjects. Respir Physiol Neurobiol 155, 14-21.

Nishino, T., Isono, S., Shinozuka, N., Ishikawa, T., 2005. Effects of naloxone on respiratory sensation before and after a removal of severe respiratory stress. Japanese Journal of Physiology 55, 117-126.

Nishino, T., Sugimori, K., Ishikawa, T., 1996. Changes in the period of no respiratory sensation and total breath-holding time in successive breath-holding trials. Clin Sci (Lond) 91, 755-761.

Norwood, R.J., 2007. A review of etiologies of depression in COPD. Int J Chron Obstruct Pulmon Dis 2, 485-491.

O'Donnell, C.R., Schwartzstein, R.M., Lansing, R.W., Guilfoyle, T., Elkin, D., Banzett, R.B., 2013. Dyspnea affective response: comparing COPD patients with healthy volunteers and laboratory model with activities of daily living. BMC Pulm Med 13, 27.

O'Donnell, D.E., Bertley, J.C., Chau, L.K., Webb, K.A., 1997. Qualitative aspects of exertional breathlessness in chronic airflow limitation: pathophysiologic mechanisms. Am J Respir Crit Care Med 155, 109-115.

O'Donnell, D.E., Hong, H.H., Webb, K.A., 2000. Respiratory sensation during chest wall restriction and dead space loading in exercising men. J Appl Physiol (1985) 88, 1859-1869.

O'Donnell, D.E., Webb, K.A., 1992. Breathlessness in patients with severe chronic airflow limitation. Physiologic correlations. Chest 102, 824-831.

O'Reilly, J.X., Beckmann, C.F., Tomassini, V., Ramnani, N., Johansen-Berg, H., 2010. Distinct and overlapping functional zones in the cerebellum defined by resting state functional connectivity. Cereb Cortex 20, 953-965.

Olin, J.T., Dimmen, A.C., Subudhi, A.W., Roach, R.C., 2012. A simple method to clamp end-tidal carbon dioxide during rest and exercise. Eur J Appl Physiol 112, 3439-3444.

Olson, I.R., Plotzker, A., Ezzyat, Y., 2007. The Enigmatic temporal pole: a review of findings on social and emotional processing. Brain 130, 1718-1731.

Parshall, M.B., Meek, P.M., Sklar, D., Alcock, J., Bittner, P., 2012a. Test-retest reliability of multidimensional dyspnea profile recall ratings in the emergency department: a prospective, longitudinal study. BMC Emerg Med 12, 6.

Parshall, M.B., Schwartzstein, R.M., Adams, L., Banzett, R.B., Manning, H.L., Bourbeau, J., Calverley, P.M., Gift, A.G., Harver, A., Lareau, S.C., Mahler, D.A., Meek, P.M., O'Donnell, D.E., American Thoracic Society Committee on, D., 2012b. An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. Am J Respir Crit Care Med 185, 435-452.

Parsons, L.M., Egan, G., Liotti, M., Brannan, S., Denton, D., Shade, R., Robillard, R., Madden, L., Abplanalp, B., Fox, P.T., 2001. Neuroimaging evidence implicating cerebellum in the experience of hypercapnia and hunger for air. Proc Natl Acad Sci U S A 98, 2041-2046.

Pasco, J.A., Williams, L.J., Jacka, F.N., Brennan, S.L., Berk, M., 2013. Obesity and the relationship with positive and negative affect. Aust N Z J Psychiatry.

Pattinson, K.T., Governo, R.J., MacIntosh, B.J., Russell, E.C., Corfield, D.R., Tracey, I., Wise, R.G., 2009. Opioids depress cortical centers responsible for the volitional control of respiration. J Neurosci 29, 8177-8186.

Pattinson, K.T., Rogers, R., Mayhew, S.D., Tracey, I., Wise, R.G., 2007. Pharmacological FMRI: measuring opioid effects on the BOLD response to hypercapnia. J Cereb Blood Flow Metab 27, 414-423.

Paulus, M.P., Flagan, T., Simmons, A.N., Gillis, K., Kotturi, S., Thom, N., Johnson, D.C., Van Orden, K.F., Davenport, P.W., Swain, J.L., 2012. Subjecting elite athletes to inspiratory breathing load reveals behavioral and neural signatures of optimal performers in extreme environments. PLoS One 7, e29394.

Payne, S.J., 2006. A model of the interaction between autoregulation and neural activation in the brain. Math Biosci 204, 260-281.

Peiffer, C., Costes, N., Herve, P., Garcia-Larrea, L., 2008. Relief of dyspnea involves a characteristic brain activation and a specific quality of sensation. Am J Respir Crit Care Med 177, 440-449.

Peiffer, C., Poline, J.B., Thivard, L., Aubier, M., Samson, Y., 2001. Neural substrates for the perception of acutely induced dyspnea. Am J Respir Crit Care Med 163, 951-957.

Perna, G., Caldirola, D., Namia, C., Cucchi, M., Vanni, G., Bellodi, L., 2004. Language of dyspnea in panic disorder. Depress Anxiety 20, 32-38.

Petersen, S., Ritz, T., 2011. The role of fearful beliefs in the relationship between situational self-awareness and report of breathing-related sensations. Br J Health Psychol 16, 359-372.

Petersen, S., Schroijen, M., Molders, C., Zenker, S., Van den Bergh, O., 2014. Categorical interoception: perceptual organization of sensations from inside. Psychol Sci 25, 1059-1066.

Petrovic, P., Kalso, E., Petersson, K.M., Ingvar, M., 2002. Placebo and opioid analgesia--imaging a shared neuronal network. Science 295, 1737-1740.

Peyron, R., Garcia-Larrea, L., Gregoire, M.C., Costes, N., Convers, P., Lavenne, F., Mauguiere, F., Michel, D., Laurent, B., 1999. Haemodynamic brain responses to acute pain in humans: sensory and attentional networks. Brain 122 (Pt 9), 1765-1780.

Peyron, R., Laurent, B., Garcia-Larrea, L., 2000. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol Clin 30, 263-288.

Phelps, E.A., 2004. Human emotion and memory: interactions of the amygdala and hippocampal complex. Curr Opin Neurobiol 14, 198-202.

Plaufcan, M.R., Wamboldt, F.S., Holm, K.E., 2012. Behavioral and characterological self-blame in chronic obstructive pulmonary disease. J Psychosom Res 72, 78-83.

Ploghaus, A., Narain, C., Beckmann, C.F., Clare, S., Bantick, S., Wise, R., Matthews, P.M., Rawlins, J.N., Tracey, I., 2001. Exacerbation of pain by anxiety is associated with activity in a hippocampal network. J Neurosci 21, 9896-9903.

Ploghaus, A., Tracey, I., Gati, J.S., Clare, S., Menon, R.S., Matthews, P.M., Rawlins, J.N., 1999. Dissociating pain from its anticipation in the human brain. Science 284, 1979-1981.

Ploner, M., Lee, M.C., Wiech, K., Bingel, U., Tracey, I., 2010. Prestimulus functional connectivity determines pain perception in humans. Proc Natl Acad Sci U S A 107, 355-360.

Poe, G.R., Kristensen, M.P., Rector, D.M., Harper, R.M., 1996. Hippocampal activity during transient respiratory events in the freely behaving cat. Neuroscience 72, 39-48.

Porzelius, J., Vest, M., Nochomovitz, M., 1992. Respiratory function, cognitions, and panic in chronic obstructive pulmonary patients. Behav Res Ther 30, 75-77.

Price, D.D., 2000. Psychological and neural mechanisms of the affective dimension of pain. Science 288, 1769-1772.

Price, D.D., 2002. Central neural mechanisms that interrelate sensory and affective dimensions of pain. Mol Interv 2, 392-403, 339.

Price, D.D., Von der Gruen, A., Miller, J., Rafii, A., Price, C., 1985. A psychophysical analysis of morphine analgesia. Pain 22, 261-269.

Quirarte, G.L., Galvez, R., Roozendaal, B., McGaugh, J.L., 1998. Norepinephrine release in the amygdala in response to footshock and opioid peptidergic drugs. Brain Res 808, 134-140.

Racine, M., Tousignant-Laflamme, Y., Kloda, L.A., Dion, D., Dupuis, G., Choiniere, M., 2012. A systematic literature review of 10 years of research on sex/gender and pain perception - part 2: do biopsychosocial factors alter pain sensitivity differently in women and men? Pain 153, 619-635.

Radloff, L.S., 1977. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. Applied Psychological Measurement 1, 385-401.

Raux, M., Tyvaert, L., Ferreira, M., Kindler, F., Bardinet, E., Karachi, C., Morelot-Panzini, C., Gotman, J., Pike, G.B., Koski, L., Similowski, T., 2013. Functional magnetic resonance imaging suggests automatization of the cortical response to inspiratory threshold loading in humans. Respir Physiol Neurobiol 189, 571-580.

Reinhard, M., Hetzel, A., Hinkov, V., Lücking, C.H., 2000. Cerebral haemodynamics during the Mueller manoeuvre in humans. Clinical physiology (Oxford, England) 20, 292-303.

Reiss, S., Peterson, R.A., Gursky, D.M., Mcnally, R.J., 1986. Anxiety Sensitivity, Anxiety Frequency and the Prediction of Fearfulness. Behav Res Ther 24, 1-8.

Reynolds, M., Wells, A., 1999. The Thought Control Questionnaire--psychometric properties in a clinical sample, and relationships with PTSD and depression. Psychol Med 29, 1089-1099.

Rietveld, S., van Beest, I., 2007. Rollercoaster asthma: when positive emotional stress interferes with dyspnea perception. Behav Res Ther 45, 977-987.

Robertson, D., Frolich, J.C., Carr, R.K., Watson, J.T., Hollifield, J.W., Shand, D.G., Oates, J.A., 1978. Effects of caffeine on plasma renin activity, catecholamines and blood pressure. N Engl J Med 298, 181-186.

Rohrbach, M., Perret, C., Kayser, B., Boutellier, U., Spengler, C.M., 2003. Task failure from inspiratory resistive loaded breathing: a role for inspiratory muscle fatigue? Eur J Appl Physiol 90, 405-410.

Rosenkranz, M.A., Busse, W.W., Johnstone, T., Swenson, C.A., Crisafi, G.M., Jackson, M.M., Bosch, J.A., Sheridan, J.F., Davidson, R.J., 2005. Neural circuitry underlying the interaction between emotion and asthma symptom exacerbation. Proc Natl Acad Sci U S A 102, 13319-13324.

Rosenkranz, M.A., Busse, W.W., Sheridan, J.F., Crisafi, G.M., Davidson, R.J., 2012. Are there neurophenotypes for asthma? Functional brain imaging of the interaction between emotion and inflammation in asthma. PLoS One 7, e40921.

Rosenkranz, M.A., Davidson, R.J., 2009. Affective neural circuitry and mind-body influences in asthma. Neuroimage 47, 972-980.

Ruit, K.G., Neafsey, E.J., 1988. Cardiovascular and respiratory responses to electrical and chemical stimulation of the hippocampus in anesthetized and awake rats. Brain Res 457, 310-321.

Saaresranta, T., Polo, O., 2002. Hormones and breathing. Chest 122, 2165-2182.

Scharf, S.M., 1991. Cardiovascular effects of airways obstruction. Lung 169, 1-23.

Schenk, L.A., Sprenger, C., Geuter, S., Buchel, C., 2014. Expectation requires treatment to boost pain relief: an fMRI study. Pain 155, 150-157.

Schneider, B.K., Pickett, C.K., Zwillich, C.W., Weil, J.V., McDermott, M.T., Santen, R.J., Varano, L.A., White, D.P., 1986. Influence of testosterone on breathing during sleep. J Appl Physiol 61, 618-623.

Schon, D., Dahme, B., von Leupoldt, A., 2008. Associations between the perception of dyspnea, pain, and negative affect. Psychophysiology 45, 1064-1067.

Schreckenberger, M., Klega, A., Grunder, G., Buchholz, H.G., Scheurich, A., Schirrmacher, R., Schirrmacher, E., Muller, C., Henriksen, G., Bartenstein, P., 2008. Opioid receptor PET reveals the psychobiologic correlates of reward processing. J Nucl Med 49, 1257-1261.

Sehlmeyer, C., Schoning, S., Zwitserlood, P., Pfleiderer, B., Kircher, T., Arolt, V., Konrad, C., 2009. Human fear conditioning and extinction in neuroimaging: a systematic review. PLoS One 4, e5865.

Simon, P.M., Schwartzstein, R.M., Weiss, J.W., Fencl, V., Teghtsoonian, M., Weinberger, S.E., 1990. Distinguishable types of dyspnea in patients with shortness of breath. Am Rev Respir Dis 142, 1009-1014.

Simon, S.T., Higginson, I.J., Booth, S., Harding, R., Bausewein, C., 2010. Benzodiazepines for the relief of breathlessness in advanced malignant and non-malignant diseases in adults. Cochrane Database Syst Rev, CD007354.

Smith, J., Albert, P., Bertella, E., Lester, J., Jack, S., Calverley, P., 2009. Qualitative aspects of breathlessness in health and disease. Thorax 64, 713-718.

Smith, S.M., 2002. Fast robust automated brain extraction. Hum Brain Mapp 17, 143-155.

Smoller, J.W., Pollack, M.H., Otto, M.W., Rosenbaum, J.F., Kradin, R.L., 1996. Panic anxiety, dyspnea, and respiratory disease. Theoretical and clinical considerations. Am J Respir Crit Care Med 154, 6-17.

Solano, J.P., Gomes, B., Higginson, I.J., 2006. A comparison of symptom prevalence in far advanced cancer, AIDS, heart disease, chronic obstructive pulmonary disease and renal disease. J Pain Symptom Manage 31, 58-69.

Spielberger, C.D., 2010. State-Trait Anxiety Inventory. The Corsini Encyclopedia of Psychology. John Wiley & Sons, Inc.

Sripada, R.K., Marx, C.E., King, A.P., Rampton, J.C., Ho, S.S., Liberzon, I., 2013. Allopregnanolone elevations following pregnenolone administration are associated with enhanced activation of emotion regulation neurocircuits. Biol Psychiatry 73, 1045-1053.

Stoodley, C.J., Schmahmann, J.D., 2009. Functional topography in the human cerebellum: a meta-analysis of neuroimaging studies. Neuroimage 44, 489-501.

Sturdy, G.A., Hillman, D.R., Green, D.J., Jenkins, S.C., Cecins, N.M., Eastwood, P.R., 2004. The effect of learning on ventilatory responses to inspiratory threshold loading in COPD. Respir Med 98, 1-8.

Supinski, G., Dimarco, A., Bark, H., Chapman, K., Clary, S., Altose, M., 1990. Effect of codeine on the sensations elicited by loaded breathing. Am Rev Respir Dis 141, 1516-1521.

Tenholder, M.F., South-Paul, J.E., 1989. Dyspnea in pregnancy. Chest 96, 381-388.

Tiecks, F.P., Douville, C., Byrd, S., Lam, A.M., Newell, D.W., 1996. Evaluation of Impaired Cerebral Autoregulation by the Valsalva Maneuver. Stroke 27, 1177-1182.

Tracey, I., 2010. Getting the pain you expect: mechanisms of placebo, nocebo and reappraisal effects in humans. Nat Med 16, 1277-1283.

Tracey, I., Mantyh, P.W., 2007. The cerebral signature for pain perception and its modulation. Neuron 55, 377-391.

Tracey, I., Ploghaus, A., Gati, J.S., Clare, S., Smith, S., Menon, R.S., Matthews, P.M., 2002. Imaging attentional modulation of pain in the periaqueductal gray in humans. J Neurosci 22, 2748-2752.

Traupe, T., Stettler, C.D., Li, H., Haas, E., Bhattacharya, I., Minotti, R., Barton, M., 2007. Distinct roles of estrogen receptors alpha and beta mediating acute vasodilation of epicardial coronary arteries. Hypertension 49, 1364-1370.

Treede, R.D., Kenshalo, D.R., Gracely, R.H., Jones, A.K., 1999. The cortical representation of pain. Pain 79, 105-111.

Vallejo, R., de Leon-Casasola, O., Benyamin, R., 2004. Opioid therapy and immunosuppression: a review. Am J Ther 11, 354-365.

van Beek, A.H., Claassen, J.A., Rikkert, M.G., Jansen, R.W., 2008. Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. J Cereb Blood Flow Metab 28, 1071-1085.

Vincent, K., Tracey, I., 2010. Sex hormones and pain: the evidence from functional imaging. Curr Pain Headache Rep 14, 396-403.

Vogt, B.A., 2005. Pain and emotion interactions in subregions of the cingulate gyrus. Nat Rev Neurosci 6, 533-544.

Vogt, B.A., Watanabe, H., Grootoonk, S., Jones, A.K.P., 1995. Topography of diprenorphine binding in human cingulate gyrus and adjacent cortex derived from coregistered PET and MR images. Hum Brain Mapp 3, 1-12.

von Leupoldt, A., Chan, P.Y., Esser, R.W., Davenport, P.W., 2013. Emotions and neural processing of respiratory sensations investigated with respiratory-related evoked potentials. Psychosom Med 75, 244-252.

von Leupoldt, A., Dahme, B., 2005. Differentiation between the sensory and affective dimension of dyspnea during resistive load breathing in normal subjects. Chest 128, 3345-3349.

von Leupoldt, A., Sommer, T., Kegat, S., Baumann, H.J., Klose, H., Dahme, B., Buchel, C., 2008. The unpleasantness of perceived dyspnea is processed in the anterior insula and amygdala. Am J Respir Crit Care Med 177, 1026-1032.

von Leupoldt, A., Sommer, T., Kegat, S., Baumann, H.J., Klose, H., Dahme, B., Buchel, C., 2009a. Dyspnea and pain share emotion-related brain network. Neuroimage 48, 200-206.

von Leupoldt, A., Sommer, T., Kegat, S., Eippert, F., Baumann, H.J., Klose, H., Dahme, B., Buchel, C., 2009b. Down-regulation of insular cortex responses to dyspnea and pain in asthma. Am J Respir Crit Care Med 180, 232-238.

von Leupoldt, A., Taube, K., Henkhus, M., Dahme, B., Magnussen, H., 2010. The impact of affective states on the perception of dyspnea in patients with chronic obstructive pulmonary disease. Biol Psychol 84, 129-134.

von Leupoldt, A., Taube, K., Lehmann, K., Fritzsche, A., Magnussen, H., 2011a. The impact of anxiety and depression on outcomes of pulmonary rehabilitation in patients with COPD. Chest 140, 730-736.

von Leupoldt, A., Taube, K., Schubert-Heukeshoven, S., Magnussen, H., Dahme, B., 2007. Distractive auditory stimuli reduce the unpleasantness of dyspnea during exercise in patients with COPD. Chest 132, 1506-1512.

von Leupoldt, A., Vovk, A., Bradley, M.M., Lang, P.J., Davenport, P.W., 2011b. Habituation in neural processing and subjective perception of respiratory sensations. Psychophysiology 48, 808-812.

Vrana, S.R., 1995. Emotional modulation of skin conductance and eyeblink responses to startle probe. Psychophysiology 32, 351-357.

Wagner, K.J., Willoch, F., Kochs, E.F., Siessmeier, T., Tolle, T.R., Schwaiger, M., Bartenstein, P., 2001. Dose-dependent regional cerebral blood flow changes during remifentanil infusion in humans: a positron emission tomography study. Anesthesiology 94, 732-739.

Wan, L., Van Diest, I., De Peuter, S., Bogaerts, K., Van den Bergh, O., 2009. Repeated breathlessness experiences induced by hypercapnia: differential effects on intensity and unpleasantness. Chest 135, 455-461.

Wang, J., Aguirre, G.K., Kimberg, D.Y., Roc, A.C., Li, L., Detre, J.A., 2003. Arterial spin labeling perfusion fMRI with very low task frequency. Magn Reson Med 49, 796-802.

Wanigasekera, V., Lee, M.C., Rogers, R., Kong, Y., Leknes, S., Andersson, J., Tracey, I., 2012. Baseline reward circuitry activity and trait reward responsiveness predict expression of opioid analgesia in healthy subjects. Proc Natl Acad Sci U S A 109, 17705-17710.

Ward, T., Scheid, V., Tuffrey, V., 2010. Women's mid-life health experiences in urban UK: an international comparison. Climacteric 13, 278-288.

Watson, D., Clark, L., 1994. The PANAS-X: Manual for the Positive and Negative Affect Schedule - Expanded Form. Springer Publishing, New York.

Weil, J.V., McCullough, R.E., Kline, J.S., Sodal, I.E., 1975. Diminished ventilatory response to hypoxia and hypercapnia after morphine in normal man. N Engl J Med 292, 1103-1106.

Weingartner, V., Bausewein, C., Higginson, I.J., Scheve, C., Murtagh, F.E., Voltz, R., Simon, S.T., 2013. Characterizing episodic breathlessness in patients with advanced disease. J Palliat Med 16, 1275-1279.

Weisenbach, S.L., Rapport, L.J., Briceno, E.M., Haase, B.D., Vederman, A.C., Bieliauskas, L.A., Welsh, R.C., Starkman, M.N., McInnis, M.G., Zubieta, J.K., Langenecker, S.A., 2014. Reduced emotion processing efficiency in healthy males relative to females. Soc Cogn Affect Neurosci 9, 316-325.

Weiss, S.T., 2005. Obesity: insight into the origins of asthma. Nat Immunol 6, 537-539.

Westbrook, R.F., Greeley, J.D., Nabke, C.P., Swinbourne, A.L., 1991. Aversive conditioning in the rat: effects of a benzodiazepine and of an opioid agonist and antagonist on conditioned hypoalgesia and fear. J Exp Psychol Anim Behav Process 17, 219-230.

Wiech, K., Lin, C.S., Brodersen, K.H., Bingel, U., Ploner, M., Tracey, I., 2010. Anterior insula integrates information about salience into perceptual decisions about pain. J Neurosci 30, 16324-16331.

Wiech, K., Tracey, I., 2009. The influence of negative emotions on pain: behavioral effects and neural mechanisms. Neuroimage 47, 987-994.

Williams, J.T., Ingram, S.L., Henderson, G., Chavkin, C., von Zastrow, M., Schulz, S., Koch, T., Evans, C.J., Christie, M.J., 2013. Regulation of mu-opioid receptors: desensitization, phosphorylation, internalization, and tolerance. Pharmacol Rev 65, 223-254.

Wise, R.G., Bulte, D.P., Chiarelli, P.A., Mayhew, S.D., Balanos, G.M., Pragnell, T.R., Robbins, P.A., Tracey, I., Jezzard, P., 2007a. Dynamic forcing of end-tidal carbon dioxide and oxygen applied to functional magnetic resonance imaging. Interface, 1521-1532.

Wise, R.G., Ide, K., Poulin, M.J., Tracey, I., 2004. Resting fluctuations in arterial carbon dioxide induce significant low frequency variations in BOLD signal. Neuroimage 21, 1652-1664.

Wise, R.G., Pattinson, K.T., Bulte, D.P., Chiarelli, P.A., Mayhew, S.D., Balanos, G.M., O'Connor, D.F., Pragnell, T.R., Robbins, P.A., Tracey, I., Jezzard, P., 2007b. Dynamic forcing of end-tidal carbon dioxide and oxygen applied to functional magnetic resonance imaging. J Cereb Blood Flow Metab 27, 1521-1532.

Woolrich, M.W., Behrens, T.E.J., Beckmann, C.F., Jenkinson, M., Smith, S.M., 2004. Multilevel linear modelling for FMRI group analysis using Bayesian inference. Neuroimage 21, 1732-1747.

World Health Organization, 2013. Obesity and overweight. Fact sheet no. 311.

Yaguez, L., Coen, S., Gregory, L.J., Amaro, E., Jr., Altman, C., Brammer, M.J., Bullmore, E.T., Williams, S.C., Aziz, Q., 2005. Brain response to visceral aversive conditioning: a functional magnetic resonance imaging study. Gastroenterology 128, 1819-1829.

Yellowlees, P.M., Ruffin, R.E., 1989. Psychological defenses and coping styles in patients following a life-threatening attack of asthma. Chest 95, 1298-1303.

Yorke, J., Moosavi, S.H., Shuldham, C., Jones, P.W., 2010. Quantification of dyspnoea using descriptors: development and initial testing of the Dyspnoea-12. Thorax 65, 21-26.

Appendix

Overview Appendix I

Two versions of the Multidimensional Dyspnoea Profile (MDP) were used throughout this thesis:

1) Appendix Ia: Older version of the MDP as used in Chapters 3 and 4.

2) Appendix Ib: Newer version of the MDP as used in Chapter 5.

Appendix Ia: Older version of the MDP as used in Chapters 3 and 4

1) How **short of breath** were you?

0 1 2 3 4 5 6 7 8 9 10

NOT AT ALL THE MOST I CAN IMAGINE

2) Use this scale to rate the **unpleasantness** of your breathing sensations, how **good** or **bad** your breathing felt.

0 1 2 3 4 5 6 7 8 9 10

PLEASANT NEUTRAL UNBEARABLE

3) Below are phrases or terms arranged in groups of similar meaning. Decide whether each statement applies to your breathing experience (tick does or does not apply). In the second step, please mark **ONE** term that best describes what you felt.

	DOES NOT APPLY	**DOES APPLY**	**BEST DESCRIBES**
My breathing muscles are working hard			
I am not getting enough air, or, I am smothering			
My breathing requires mental effort or concentration			
My chest and lungs feel tight or constricted			
Other: _____			

4) Use these scales to rate the **intensity** of the breathing sensations you felt, that is, how strong the sensation was, not necessarily how unpleasant. Please first tick the items that do *not* apply, then rate the items that do apply.

	DOES NOT APPLY	**VERY SLIGHT**									**AS INTENSE AS IT CAN GET**
My breathing muscles are working hard		1	2	3	4	5	6	7	8	9	10
I am not getting enough air, or, I am smothering		1	2	3	4	5	6	7	8	9	10
My breathing requires mental effort or concentration		1	2	3	4	5	6	7	8	9	10
My chest and lungs feel tight or constricted		1	2	3	4	5	6	7	8	9	10
I am breathing rapidly		1	2	3	4	5	6	7	8	9	10
I am breathing deeply		1	2	3	4	5	6	7	8	9	10
Other (from above)		1	2	3	4	5	6	7	8	9	10

Appendix Ib: Newer version of the MDP as used in Chapter 5

1) Use this scale to rate the **overall intensity** of your breathing sensation, that is, how strong the sensation was, not necessarily how unpleasant:

0 1 2 3 4 5 6 7 8 9 10

NONE MODERATE MAXIMUM

2) Use this scale to rate the **unpleasantness** of your breathing sensations, how **good** or **bad** your breathing felt:

0 1 2 3 4 5 6 7 8 9 10

NEUTRAL ANNOYING UNBEARABLE

3) Use these scales to rate the **intensity** of the breathing sensations you felt:

	DOES NOT APPLY									**AS INTENSE AS I CAN IMAGINE**
My breathing requires muscle work or effort	0	1	2	3	4	5	6	7	8	9	10
I am breathing a lot (breathing rapidly, deeply or heavily)	0	1	2	3	4	5	6	7	8	9	10
My chest and lungs feel tight or constricted	0	1	2	3	4	5	6	7	8	9	10
I am not getting enough air, I feel hunger for air, or I am smothering	0	1	2	3	4	5	6	7	8	9	10
My breathing requires mental effort or concentration	0	1	2	3	4	5	6	7	8	9	10

4) Please rate how much you felt each of the following emotions on the scales below:

ANGRY

0 1 2 3 4 5 6 7 8 9 10

NONE THE MOST SEVERE IMAGINABLE

FRUSTRATED

0 1 2 3 4 5 6 7 8 9 10

NONE THE MOST SEVERE IMAGINABLE

ANXIOUS

0 1 2 3 4 5 6 7 8 9 10

NONE THE MOST SEVERE IMAGINABLE

AFRAID

0 1 2 3 4 5 6 7 8 9 10

NONE THE MOST SEVERE IMAGINABLE

DEPRESSED

0 1 2 3 4 5 6 7 8 9 10

NONE THE MOST SEVERE IMAGINABLE

Overview Appendix II

Manuscripts containing parts of this work

Parts of the work presented in this thesis have previously been published. This Appendix contains peer-reviewed publications that contain or are based on parts of work from this thesis. Additionally, a manuscript describing a study from our laboratory that investigates dyspnoea in patients with chronic obstructive pulmonary disease (COPD) in comparison to healthy controls is included.

Peer reviewed articles

1) Appendix IIa: Understanding dyspnoea as a complex individual experience.
   Citation: Hayen A, Herigstad M, Pattinson KTS, 2013. Understanding dyspnea as a complex individual experience. *Maturitas,* 76 (1), 45-50.
2) Appendix IIb: The effects of altered intrathoracic pressure on resting cerebral blood flow and its response to visual stimulation.
   Citation: Hayen A, Herigstad M, Kelly M, Okell TW, Murphy K, Wise RG, Pattinson KTS, 2012. The effects of altered intrathoracic pressure on resting cerebral blood flow and its response to visual stimulation. *Neuroimage,* 66: 479-488.
3) Appendix IIc: Dyspnoea and the brain.
   Citation: Herigstad M, Hayen A, Wiech K, Pattinson KTS, 2011. Dyspnoea and the brain. *Respiratory medicine*, 105 (6), 809-817.

Abstracts

1) Appendix IId: Effects of inspiratory and expiratory loading upon global and stimulus evoked CBF.
   Citation: Hayen A, Herigstad M, Wise R, Pattinson KTS, 2011. Effects of inspiratory and expiratory loading upon global and stimulus evoked CBF. *Proc ISMRM 19*, 1562.
2) Appendix IIe: Murphy K, Hayen A, Herigstad M, Pattinson KTS, 2013. Physiological noise reduction for multi-inversion time ASL. *Proc ISMRM 21*, 2537.

Appendix IIa: Understanding dyspnea as a complex individual experience

Maturitas 76 (2013) 45–50

Contents lists available at SciVerse ScienceDirect

Maturitas

journal homepage: www.elsevier.com/locate/maturitas

Review

Understanding dyspnea as a complex individual experience☆

Anja Hayen*, Mari Herigstad, Kyle T.S. Pattinson

Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, University of Oxford, Oxford, OX3 9DU, United Kingdom

ARTICLE INFO

Article history:
Received 29 May 2013
Accepted 5 June 2013

Keywords:
Dyspnea
Psychology
Neuroimaging
Gender
Obesity
Aging

ABSTRACT

Dyspnea is the highly threatening experience of breathlessness experienced by patients with diverse pathologies, including respiratory, cardiovascular, and neuromuscular diseases, cancer and panic disorder. This debilitating symptom is especially prominent in the elderly and the obese, two growing populations in the Western world. It has further been found that women suffer more strongly from dyspnea than men. Despite optimization of disease-specific treatments, dyspnea is often inadequately treated. The immense burden faced by patients, families and the healthcare system makes improving management of chronic dyspnea a priority.

Dyspnea is a multidimensional sensation that encompasses an array of unpleasant respiratory sensations that vary according to underlying cause and patient characteristics. Biopsychological factors beyond disease pathology exacerbate the perception of dyspnea, increase symptom severity and reduce quality of life. Psychological state (especially comorbid anxiety and depression), hormone status, gender, body weight (obesity) and general fitness level are particularly important. Neuroimaging has started to uncover the neural mechanisms involved in the processing of sensory and affective components of dyspnea.

Awareness of biopsychological factors beyond pathology is essential for diagnosis and treatment of dyspnea. Increasing understanding the interactions between biopsychological factors and dyspnea perception will enhance the development of symptomatic treatments that specifically address each patient's most pressing needs at a specific stage in life. Future neuroimaging research can provide objective markers to fully understand the role of biopsychological factors in the perception of dyspnea in the hope of uncovering target areas for pharmacologic and non-pharmacologic therapy.

© 2013 The Authors. Published by Elsevier Ireland Ltd. All rights reserved.

Contents

1. Introduction 45
2. Perception of dyspnea 46
3. The impact of emotions on dyspnea perception 46
4. Dyspnea and cognitive processing 47
5. Challenges for dyspnea management 48
6. Conclusion 49
Contributors 49
Competing interest 49
Funding 49
Provenance and peer review 49
References 49

1. Introduction

Dyspnea is the highly distressing experience of breathlessness that makes simple everyday activities like playing with the grandchildren, going to the shops or simply getting dressed in the morning a major challenge. This chronic debilitating burden affects an increasingly large group of patients with respiratory diseases

☆ This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

* Corresponding author. Tel.: +44 1865 234544; fax: +44 1865 234072.
E-mail address: anja.hayen@ndcn.ox.ac.uk (A. Hayen).

0378-5122/$ – see front matter © 2013 The Authors. Published by Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.maturitas.2013.06.005

like chronic obstructive pulmonary disease (COPD) and asthma, heart failure and terminal cancer [1] and is a main symptom of panic disorder [2]. Dyspnea is a strong predictor of mortality in patients with COPD and heart failure and amongst the most common causes for emergency department visits [3,4]. Due to its chronicity, costly long-term treatment of dyspnea is necessary in many patients. For example, the annual direct treatment costs of COPD, the fifth biggest killer disease in the UK with over 30,000 annual deaths, were estimated at £820 per patient with an additional £820 of secondary economic costs, accumulating to a total societal cost of over £2.1 billion [5]. Similar figures are reported from the USA [6].

Dyspnea itself often influences the course of disease and symptoms often persist after medical treatment options have been exhausted [7]. Dyspnea can lead to early cessation of exercise, and fear of dyspnea might discourage patients from engaging in daily activities, resulting in reduced cardiovascular fitness and muscle strength, which again increase the feeling of dyspnea, thus initiating and perpetuating a spiral of decline [3]. Patients, family and friends endure great emotional suffering when facing loss of independence and increasing physical distress [8]. Many patients, especially those who have smoked, are obese or do not engage in regular exercise, feel that they have brought their symptoms upon themselves, which makes them feel guilty and less likely to seek appropriate medical help [9].

This review will highlight variations in perception and treatment success of dyspnea that present challenges for dyspnea management. Evidence from clinical and laboratory studies will be reviewed to show how emotions affect the perception of dyspnea and how understanding the neural mechanisms behind these modulations can aid dyspnea management. The challenges of managing dyspnea in older people, obese people and women, three populations particularly vulnerable to dyspnea, will be highlighted and we will suggest how future research can help to understand the neural mechanisms involved in the perception of dyspnea in order to better target treatment. Increased understanding of the neural mechanisms behind dyspnea will allow us to individually address the most important aspects of dyspnea suffering in each patient. While research on the neural mechanisms of dyspnea is still in its infancy, it can reveal important directions for improvements of dyspnea therapy and future research.

2. Perception of dyspnea

Dyspnea is not one experience, but encompasses a whole range of sensations (e.g. air hunger, feeling of increased effort, rapid breathing) that are highly subjective. Individual variation between patients in dyspnea perception and resulting impairment is large and reaches beyond the underlying cause [10]. Treatment of the primary cause of dyspnea is essential, but despite optimum treatment, patients often continue to suffer from dyspnea and the associated decrease in quality of life [7]. In conjunction with disease-specific treatments, symptomatic treatments can help to manage dyspnea. To improve and optimally target these treatments, a better understanding of the mechanisms underlying dyspnea is necessary because "one size does not fit all".

One model of dyspnea perception describes a primary sensory component (intensity) and a primary affective component (unpleasantness) [11]. These components can vary independently [12]. These primary components are followed by a secondary, more cognitive affective component that leads to long-term emotional responses (suffering) that affect future dyspnea-related behavior [11]. Dyspnea is a subjective experience and while animal models have given us insight into mechanisms behind specific pathologies, it is difficult to derive information about the subjective experience of dyspnea from animal models; these models are ill equipped

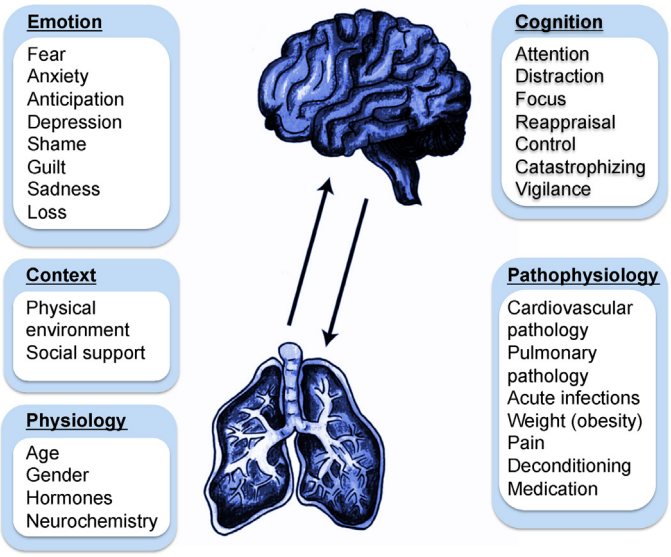


Fig. 1. Dyspnea is a multidimensional set of experiences that is closely linked to almost every aspect of a patient's physiological and psychological state. The brain is the main relay station integrating these complex internal and external experiences with physiological respiratory signals.

to study the mechanisms underlying dyspnea perception. Much research on dyspnea mechanisms has hence been performed in healthy individuals exposed to laboratory dyspnea. Replicating the emotional component of dyspnea in a laboratory environment is difficult as laboratory dyspnea does not cause the existential fears dyspnea sufferers encounter in daily life, hence patient studies will be necessary in order to fully comprehend all aspects of dyspnea.

Neuroimaging research has confirmed the important role of emotional processing in the perception of dyspnea. There is evidence for a common emotion-related human brain network that underlies the perception of aversive bodily sensations such as dyspnea and pain [13]. When respiratory stimuli are applied to healthy volunteers in a non-threatening laboratory context, they primarily activate somatomotor areas [14]. When laboratory dyspnea is evoked, the limbic system, an array of brain structures involved in emotional processing, and paralimbic areas like the insula (implicated in interoception, emotion and cognition) are activated [15]. This research hints at different neuronal networks being responsible for the different aspects of dyspnea perception, but future research in clinical populations is necessary to disentangle the neuronal processes implicated in the sensory and affective processing of chronic dyspnea.

3. The impact of emotions on dyspnea perception

Based on the concept of total pain proposed by Dame Cicerly Saunders in the 1960s, a model of total dyspnea has highlighted the vicious circle in which dyspnea affects and is affected by physical, psychological, spiritual and social aspects of a patient's life [16]. While an array of potential modulators for the perception of dyspnea is presented in Fig. 1, this review will focus on the impact of negative emotions, and the challenges that age, obesity and gender present to dyspnea management and research.

An individual's emotional disposition, current mood, level of general anxiety and anticipation of dyspnea, as well as the increased attention paid to one's respiration can all influence how dyspnea is perceived. Depression and anxiety are two major comorbidities of respiratory disease (e.g. approx. 40% of patients with COPD suffer from depression [17] and approx. 30% have comorbid anxiety disorders [18]) and have been shown to almost double mortality

rates, diminish social and physical functioning by approximately 25%, increase frequency of hospitalization by 10% and length of stay by approximately 3 days [19].

Emotions are powerful modulators of the perception of dyspnea. This has been shown in asthmatics, where strong emotions (both positive and negative) decrease pulmonary function, but positive emotions during a rollercoaster ride led to reduced dyspnea besides lower lung function [20]. High negative emotionality, as present in depression, was shown to increase the strength of reported dyspnea in response to laboratory stimuli in individuals with normal lung function [21] and is associated with increased symptom reporting in patients with respiratory disease [22], especially when sensations are ambiguous or low in intensity [3]. There is evidence that dyspnea improvement during pulmonary rehabilitation is correlated with a decrease in experience of negative emotions [23], but the driving force behind this interaction is unknown.

Neuroimaging can be used to identify specific neuronal mechanisms of respiratory disease that can help us to understand the processing of dyspnea in particular conditions. Evidence from functional magnetic resonance imaging (FMRI) suggests that emotional processing might be altered in asthma. The anterior cingulate cortex and the insula, both implicated in the regulation of the sensitivity of the response to respiratory stimuli [24], might be hyperresponsive in individuals with asthma and other stress-related conditions [25], leading to a perception of increased severity of respiratory stimuli, increased use of medication [26] and increased frequency of emergency hospital visits. A weak perception of dyspnea in asthmatics, as e.g. occurs when positive emotion is experienced, can lead to a dangerous lack of necessary treatment [20]. Dyspnea perception might vary between patients and within the same patient at different times, which might have severe consequences. Neuroimaging might be able to provide a biological marker for the subjective perception of dyspnea. A better understanding of the neuronal mechanisms involved in the multidimensional perception of respiratory symptoms can highlight particularly vulnerable patients and dangerous situations in order to minimize the risks associated with over- and under-treatment.

Besides the strong negative impact of emotions on dyspnea perception, few studies have looked at the specific contributions of particular emotions on the perception of dyspnea. Much research still needs to be done to fully understand the impact of emotions on the perception of dyspnea. The following section will summarize knowledge about the intricate relationship between anxiety and dyspnea. In the context of dyspnea, we divide anxiety into general anxiety and anxiety relating directly to breathing and breathlessness (dyspnea-specific anxiety). High general anxiety increases the perception of dyspnea in patients [27] and is associated with decreased quality of life [28]. High anxiety in turn induces maladaptive breathing that intensifies the unpleasant sensations elicited by respiratory stimuli [29], generating a positive-feedback loop. Dyspnea-related anxiety was shown to have a mediating effect on the relationship between anxiety and dyspnea in patients [30] and increased perception of dyspnea in response to respiratory stimuli in healthy volunteers, while high general anxiety did not increase dyspnea perception in the same experiment [29]. The importance of dyspnea-specific anxiety might explain why anxiolytics (e.g. benzodiazepines), besides having shown positive results for managing dyspnea in individual patients, have not been successful for improving dyspnea in clinical group trials in patients with increased general anxiety but without measures of dyspnea-specific anxiety [31]. This indicates that patients with dyspnea-specific anxiety might benefit more from a dyspnea-focused treatment, like pulmonary rehabilitation, which has been shown to decrease both exercise-induced dyspnea and dyspnea-specific anxiety in COPD patients [30].

Anticipatory anxiety is the anxiety felt when anticipating specific future events, so in the case of dyspnea, it is acute dyspnea-specific anxiety. Patients remember triggers of their dyspnea and anticipate dyspnea and alter their behavior when encountering these situations in the future [32], e.g. when dyspnea is experienced while walking up the stairs, patients will likely be wary of using the stairs and try to avoid walking upstairs in the future. An initially neutral stimulus (e.g. the stairs) begins to carry the negative associations related to dyspnea, i.e. the patient becomes conditioned to this association. If negatively cued stimuli cannot be avoided, learnt anticipation of dyspnea in response to a stimulus might increase the negative perception of respiratory stimuli [33], so patients are more likely to feel out-of breath because they know they are climbing the stairs and they expect to feel breathless. The fear of future dyspnea and resulting avoidance of activities that might induce dyspnea generate a positive feedback loop that drives the spiral of decline and leads to increased perception of disability at comparable pulmonary function [34]. On the neuronal level, brain activation during anticipation of dyspnea was found to correlate with brain activation during the perception of dyspnea [35]. Anticipation of pain has further shown that brain activation during anticipation can alter brain activity during pain perception and can amplify pain perception [36]. The power of anticipation to amplify the subsequent experience makes understanding the negative impact of acute dyspnea-specific anxiety vital for introducing targeted treatments to break the spiral of decline and to increase quality of life in affected patients. FMRI could be used to provide neurobiological markers for potential anxiolytic effects. Drug intervention studies suggest that the medial prefrontal cortex, an area central to conscious threat appraisal, might be a useful target area for dyspnea and anxiety interactions [37,38].

4. Dyspnea and cognitive processing

Emotion modulates the neural processing of respiratory sensations on a higher-order level [39] and might hence interact with non-emotional mental processes, such as attention, expectation or catastrophizing, which have been found to modulate aversive experiences like pain or dyspnea [40]. Limited attentional resources are allocated according to emotional salience. Hence, if attention is used to focus on the threat of dyspnea, lack of cognitive resources might impair performance on everyday tasks. There is evidence that high dyspnea-specific anxiety might reduce an individuals' ability to allocate attention to cognitive tasks and that this impairment is enhanced in a negative emotional context relative to a neutral emotional context [39]. Reduced cognitive performance might impair a patient's quality of life. While exercise has been proven beneficial to cognitive function, it needs to be investigated whether this effect stretches to dyspnoeic patients.

The allocation of attention plays an important role in treatment. During exacerbations of their conditions, patients with lung disorders are often told to focus on their breathing. Laboratory evidence has shown that interoceptive self-awareness does decrease the strength of perceived dyspnea [41], but that this positive cognitive manipulation is susceptible to interruptions by negative emotions, [41]. This makes these maneuvers more challenging for dyspnea patients with comorbid depression and/or anxiety disorders. In apparent contradiction to the positive effects of interoceptive self-awareness, external distraction has been shown to alleviate the unpleasantness of dyspnea during exercise [42] and could therefore be used to improve exercise performance. Distraction from pain was shown to decrease brain activity in areas commonly implicated in the processing of pain intensity, whilst increasing activity in the frontal cortex and the cingulate cortex [43]. Similar mechanisms could be employed during

distraction from dyspnea. Understanding the important role of attention in the modulation of dyspnea could lead to relatively straight-forward and cost-effective interventions that may help relieve dyspnea.

5. Challenges for dyspnea management

Besides being affected by psychological state, dyspnea perception can be shaped by an individual's particular life situation (e.g. context). A growing population of patients suffering from chronic dyspnea are elderly, with approximately a third of elderly people (over 75) at home being affected by dyspnea [44]. Comorbid medical conditions, low fitness levels, and/or functional limitations in older adults [45] make this population especially vulnerable to deconditioning and deterioration. Additionally, the onset of chronic dyspnea is gradual and in older patients it is often disguised as a normal part of aging. Mistaking dyspnea due to respiratory disease as a normal sign of aging delays diagnosis of underlying medical conditions and start of treatment. Increasing public awareness and patient education are vital to encourage elderly patients to see dyspnea as potentially serious and to present early with dyspnea symptoms. Understanding the neural processes leading to particular dyspnea sensations has the potential to improve its management in elderly patients.

Improving the management of dyspnea in an aging Western population with an unhealthy diet, increasingly sedentary lifestyle and growing rates of obesity is of vital importance. In 2008, the World Health Organization estimated that 1.4 billion adults were overweight, including 200 million men and 300 million women who were obese [46]. Dyspnea is strongly associated with increased body mass index and decreased physical activity [47]. Obesity mechanically affects respiration by decreasing tidal volume and residual capacity [48]. It also leads to altered hormone levels (e.g. increased estrogen), but whether and how obesity-induced hormonal changes increase dyspnea is not yet understood [48]. There is a strong association between asthma and obesity and losing weight has been shown to improve asthma [48]. Additional comorbidities (e.g. joint pain) makes obese dyspnea patients especially prone to deconditioning and there is evidence indicating that obese individuals more frequently experience negative emotions [49], which could further increase dyspnea perception and suffering. A holistic view toward improving both dyspnea and obesity are vital for optimum long-term patient care. Neuroimaging could be a useful tool to understand the role the burdens of obesity (e.g. deconditioning, altered hormone levels and negative emotions) play in modulating dyspnea with a view of better targeting treatments.

Respiratory disease affects both men and women [50–53], but there is evidence for emotions playing a bigger role in dyspnea perception in women. Female COPD patients are twice as likely to suffer from comorbid anxiety and depression [54]. The perception of dyspnea in women with respiratory disease seems to be more strongly influenced by socio-emotional factors and less related to physiological measures compared to men [55]. Women with dyspnea also perceive poorer quality of life compared to men with the same level of dyspnea [22,53]. Women respond more emotionally (e.g. anger toward self) to their dyspnea than men, which is associated with lower general wellbeing [56] and might amplify the negative consequences of dyspnea in women [57].

It is currently not clear whether gender differences in dyspnea perception arise from differences in physiology, hormonal make-up, social and cultural experiences and/or emotional processing strategies. Neuroimaging studies of dyspnea have not (yet) investigated gender differences in the neural mechanisms of its perception, but there is evidence that men and women use different neural pathways when processing emotions [58]. Inconclusive results of behavioral and neuroimaging studies investigating responses to complex multifactorial stimuli (e.g. pain) might indicate that so-called 'gender effects' on the perception of these stimuli might be due to variations in other biopsychological factors that often vary with gender [59].

The effects of sex hormones on respiration, emotion and pain perception make it likely that differences in dyspnea perception between men and women might be related to differences in sex hormone levels. The three major sex hormones (estrogen, progesterone, and testosterone) have been shown to act at sites throughout the body, including the central nervous system [60] and the cardiovascular [61] system. Testosterone, while present at low levels in women, is predominant in men. The effects of testosterone on respiration are complex (increased ventilation response to hypoxia [62] and worsening of obstructive sleep apnoea [63]). Testosterone elevates mood [64], has been found to decrease during stress, including anticipatory stress and to increase pain perception [65], but its effects on dyspnea have not been investigated.

Progesterone and estrogen are predominant in women, with low concentrations in men. During the menstrual cycle, progesterone levels are low during the first half of the cycle, and then increase, peaking in the middle of the luteal phase [66]. Progesterone has been shown to stimulate respiration [62] and elevate mood [64]. Estrogen peaks during ovulation and is increased during the luteal phase of the cycle [66]. Estrogen increases progesterone-induced respiratory stimulation [67] and elevates mood [64]. These strong variations in estrogen and progesterone levels throughout a woman's cycle will likely affect her respiration, mood and perception of dyspnea. The neural effects of these hormones on dyspnea perception have not been shown, but evidence from pain studies suggests that increases in estrogen and progesterone seem to be associated with enhancement of the affective component of the pain experience, but the neural mechanism are not understood [66].

Pregnancy and menopause are two important life events in women that are often associated with dyspnea. Over 60% of healthy pregnant women are affected by dyspnea [68]. During pregnancy, the body changes: the diaphragm is elevated, metabolism increases, levels of estrogen and progesterone increase and ventilation and respiratory drive increase. Mood changes, fatigue and stress associated with the pregnancy make pregnancy an emotionally challenging time for many women. When a pregnant woman complains of dyspnea, the clinician should determine whether her symptoms are due to an underlying disease or present a normal physiologic response to pregnancy. If no pathology is found, cognitive reappraisal of dyspnea from a life-threatening warning sign to a normal part of the physical and emotional burden of pregnancy decreases suffering. Understanding the neuronal mechanisms behind this reappraisal might inform the study of the brain mechanisms behind the emotional threat in more chronic dyspnea.

Dyspnea was found to have a prevalence of 20% in menopausal women in the UK [69]. During menopause, estrogen and progesterone decrease, which might negatively affect mood [64] lead to increased anxiety and could in turn increase dyspnea perception. Additionally, pharmacological treatment of hot flushes and emotional symptoms occurring during menopause might in itself induce dyspnea. Being aware of hormonal changes, medications and their interactions and potential side-effects is vital for managing dyspnea in menopausal women. Addressing how hormonal changes influence brain chemistry and emotional processing in the limbic system might help to better manage dyspnea during menopause in future.

6. Conclusion

In this review, we have shown that dyspnea is a complex, subjective sensation, and that its successful treatment depends upon viewing dyspnea as a composite, assessing psychological comorbidities, hormonal changes and mood changes as well as the underlying respiratory cause. Future research should aim to address the role of modulating factors and to improve our knowledge of the mechanisms involved in dyspnea processing, particularly the effects of attention, self-awareness and distraction. The important role that aging, gender and obesity play in the modulation of dyspnea is under-researched and warrants further attention. Integrating behavioral measures and carefully designed neuroimaging protocols will allow us to clearly define intricacies of the neural pathways and spatial organization of the respiratory network involved in dyspnea perception and its modulation. Accurate diagnosis and a greater awareness and understanding of modulating factors can facilitate targeted treatments of dyspnea and subsequently dramatically improve clinical outcomes.

Contributors

Anja Hayen: conceptualized and wrote the manuscript, conceptualized and generated the figure. Mari Herigstad: conceptualized and wrote the manuscript, conceptualized the figure. Kyle Pattinson: conceptualized and wrote the manuscript, conceptualized the figure.

Competing interest

The authors declare no conflict of interest.

Funding

Funding was received by Anja Hayen, Mari Herigstad and Kyle Pattinson from the Medical Research Council (MRC) for this article. AH and KP were supported by the National Institute for Health Research (NIHR), Oxford Biomedical Research Centre based at Oxford University Hospitals, NHS Trust and University of Oxford.

Provenance and peer review

Commissioned and externally peer reviewed.

References

[1] Solano JP, Gomes B, Higginson IJ. A comparison of symptom prevalence in far advanced cancer, AIDS, heart disease, chronic obstructive pulmonary disease and renal disease. J Pain Symptom Manage 2006;31(1):58–69.
[2] Smoller JW, Pollack MH, Otto MW, Rosenbaum JF, Kradin RL. Panic anxiety, dyspnea, and respiratory disease. Theoretical and clinical considerations. Am J Respir Crit Care Med 1996;154(1):6–17 [Epub 1996/07/01].
[3] American Thoracic Society. Dyspnea. Mechanisms, assessment, and management: a consensus statement. Am J Respir Crit Care Med 1999;159(1):321–40.
[4] Abidov A, Rozanski A, Hachamovitch R, et al. Prognostic significance of dyspnea in patients referred for cardiac stress testing. N Engl J Med 2005;353(18):1889–98 [Epub 2005/11/04].
[5] Britton M. The burden of COPD in the U.K.: results from the confronting COPD survey. Respir Med 2003;97(Suppl C):S71–9 [Epub 2003/03/22].
[6] Hoyert DL, Jiaquan X. Deaths preliminary data for 2011. Division of Vital Statistics; 2012. Contract no. 6.
[7] Mahler DA, Selecky PA, Harrod CG, et al. American College of Chest Physicians consensus statement on the management of dyspnea in patients with advanced lung or heart disease. Chest 2010;137(3):674–91.
[8] Gysels M, Bausewein C, Higginson IJ. Experiences of breathlessness: a systematic review of the qualitative literature. Palliat Support Care 2007;5(3):281–302.
[9] Plaufcan MR, Wamboldt FS, Holm KE. Behavioral and characterological self-blame in chronic obstructive pulmonary disease. J Psychosom Res 2012;72(1):78–83 [Epub 2011/12/28].
[10] Mahler DA, Harver A, Lentine T, Scott JA, Beck K, Schwartzstein RM. Descriptors of breathlessness in cardiorespiratory diseases. Am J Respir Crit Care Med 1996;154(5):1357–63 [Epub 1996/11/01].
[11] Lansing RW, Gracely RH, Banzett RB. The multiple dimensions of dyspnea: review and hypotheses. Respir Physiol Neurobiol 2009;167(1):53–60.
[12] Banzett RB, Pedersen SH, Schwartzstein RM, Lansing RW. The affective dimension of laboratory dyspnea: air hunger is more unpleasant than work/effort. Am J Respir Crit Care Med 2008;177(12):1384–90.
[13] von Leupoldt A, Sommer T, Kegat S, et al. Dyspnea and pain share emotion-related brain network. NeuroImage 2009;48(1):200–6.
[14] Hayen A, Herigstad M, Kelly M, et al. The effects of altered intrathoracic pressure on resting cerebral blood flow and its response to visual stimulation. NeuroImage 2012;66C:479–88 [Epub 2012/10/31].
[15] Herigstad M, Hayen A, Wiech K, Pattinson KT. Dyspnoea and the brain. Respir Med 2011;105(6):809–17.
[16] Kamal AH, Maguire JM, Wheeler JL, Currow DC, Abernethy AP. Dyspnea review for the palliative care professional: assessment, burdens, and etiologies. J Palliat Med 2011;14(10):1167–72.
[17] Norwood RJ. A review of etiologies of depression in COPD. Int J Chronic Obstructive Pulm Dis 2007;2(4):485–91.
[18] Porzelius J, Vest M, Nochomovitz M. Respiratory function, cognitions, and panic in chronic obstructive pulmonary patients. Behav Res Therapy 1992;30(1):75–7.
[19] Ng TP, Niti M, Tan WC, Cao Z, Ong KC, Eng P. Depressive symptoms and chronic obstructive pulmonary disease: effect on mortality, hospital readmission, symptom burden, functional status, and quality of life. Arch Intern Med 2007;167(1):60–7 [Epub 2007/01/11].
[20] Rietveld S, van Beest I. Rollercoaster asthma: when positive emotional stress interferes with dyspnea perception. Behav Res Ther 2007;45(5):977–87.
[21] Bogaerts K, Notebaert K, Van Diest I, Devriese S, De Peuter S, Van den Bergh O. Accuracy of respiratory symptom perception in different affective contexts. J Psychosom Res 2005;58(6):537–43.
[22] Di Marco F, Verga M, Reggente M, et al. Anxiety and depression in COPD patients: the roles of gender and disease severity. Respir Med 2006;100(10):1767–74.
[23] von Leupoldt A, Taube K, Lehmann K, Fritzsche A, Magnussen H. The impact of anxiety and depression on outcomes of pulmonary rehabilitation in patients with COPD. Chest 2011;140(3):730–6 [Epub 2011/04/02].
[24] Rosenkranz MA, Davidson RJ. Affective neural circuitry and mind-body influences in asthma. NeuroImage 2009;47(3):972–80.
[25] Rosenkranz MA, Busse WW, Johnstone T, et al. Neural circuitry underlying the interaction between emotion and asthma symptom exacerbation. Proc Natl Acad Sci USA 2005;102(37):13319–24.
[26] Main J, Moss-Morris R, Booth R, Kaptein AA, Kolbe J. The use of reliever medication in asthma: the role of negative mood and symptom reports. J Asthma 2003;40(4):357–65.
[27] Dales RE, Spitzer WO, Schechter MT, Suissa S. The influence of psychological status on respiratory symptom reporting. Am Rev Respir Dis 1989;139(6):1459–63.
[28] Huang TY, Moser DK, Hsieh YS, Gau BS, Chiang FT, Hwang SL. Moderating effect of psychosocial factors for dyspnea in Taiwanese and American heart failure patients. J Nurs Res 2013;21(1):49–58.
[29] Alius MG, Pane-Farre CA, Von Leupoldt A, Hamm AO. Induction of dyspnea evokes increased anxiety and maladaptive breathing in individuals with high anxiety sensitivity and suffocation fear. Psychophysiology 2013.
[30] Janssens T, De Peuter S, Stans L, et al. Dyspnea perception in COPD: association between anxiety, dyspnea-related fear, and dyspnea in a pulmonary rehabilitation program. Chest 2011;140(3):618–25 [Epub 2011/04/16].
[31] Simon ST, Higginson IJ, Booth S, Harding R, Bausewein C. Benzodiazepines for the relief of breathlessness in advanced malignant and non-malignant diseases in adults. Cochrane Database Syst Rev 2010;1:CD007354 [Epub 2010/01/22].
[32] Ley R. The modification of breathing behavior. Pavlovian and operant control in emotion and cognition. Behav Modif 1999;23(3):441–79.
[33] De Peuter S, Van Diest I, Lemaigre V, et al. Can subjective asthma symptoms be learned? Psychosom Med 2005;67(3):454–61.
[34] Yellowlees PM, Ruffin RE. Psychological defenses and coping styles in patients following a life-threatening attack of asthma. Chest 1989;95(6):1298–303.
[35] Paulus MP, Flagan T, Simmons AN, et al. Subjecting elite athletes to inspiratory breathing load reveals behavioral and neural signatures of optimal performers in extreme environments. PLoS ONE 2012;7(1):e29394.
[36] Fairhurst M, Wiech K, Dunckley P, Tracey I. Anticipatory brainstem activity predicts neural processing of pain in humans. Pain 2007;128(1–2):101–10 [Epub 2006/10/31].
[37] Sripada RK, Marx CE, King AP, Rampton JC, Ho SS, Liberzon I. Allopregnanolone elevations following pregnenolone administration are associated with enhanced activation of emotion regulation neurocircuits. Biol Psychiatry 2013.
[38] Evans KC, Simon NM, Dougherty DD, et al. A PET study of tiagabine treatment implicates ventral medial prefrontal cortex in generalized social anxiety disorder. Neuropsychopharmacology 2009;34(2):390–8 [Epub 2008/06/10].
[39] von Leupoldt A, Chan PY, Esser RW, Davenport PW. Emotions and neural processing of respiratory sensations investigated with respiratory-related evoked potentials. Psychosom Med 2013;75(3):244–52 [Epub 2013/03/06].

[40] Wiech K, Tracey I. The influence of negative emotions on pain: behavioral effects and neural mechanisms. NeuroImage 2009;47(3):987–94 [Epub 2009/06/02].
[41] Petersen S, Ritz T. The role of fearful beliefs in the relationship between situational self-awareness and report of breathing-related sensations. Br J Health Psychol 2011;16(Pt 2):359–72.
[42] von Leupoldt A, Taube K, Schubert-Heukeshoven S, Magnussen H, Dahme B. Distractive auditory stimuli reduce the unpleasantness of dyspnea during exercise in patients with COPD. Chest 2007;132(5):1506–12.
[43] Bantick SJ, Wise RG, Ploghaus A, Clare S, Smith SM, Tracey I. Imaging how attention modulates pain in humans using functional MRI. Brain 2002;125(Pt 2):310–9 [Epub 2002/02/15].
[44] Ho SF, O'Mahony MS, Steward JA, Breay P, Buchalter M, Burr ML. Dyspnoea and quality of life in older people at home. Age Ageing 2001;30(2):155–9 [Epub 2001/06/08].
[45] Nelson ME, Rejeski WJ, Blair SN, et al. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 2007;39(8):1435–45 [Epub 2007/09/01].
[46] World Health Organization. Obesity and overweight. Fact sheet no. 311; 2013. http://www.who.int/mediacentre/factsheets/fs311/en/index.html [accessed 15.05.13].
[47] Gerlach Y, Williams MT, Coates AM. Weighing up the evidence-a systematic review of measures used for the sensation of breathlessness in obesity. Int J Obes (Lond) 2013;37(3):341–9 [Epub 2012/04/26].
[48] Weiss ST. Obesity: insight into the origins of asthma. Nat Immunol 2005;6(6):537–9 [Epub 2005/05/24].
[49] Pasco JA, Williams LJ, Jacka FN, Brennan SL, Berk M. Obesity and the relationship with positive and negative affect. Aust N Z J Psychiatry 2013 [Epub 2013/03/26].
[50] de Marco R, Locatelli F, Sunyer J, Burney P. Differences in incidence of reported asthma related to age in men and women. A retrospective analysis of the data of the European Respiratory Health Survey. Am J Respir Crit Care Med 2000;162(1):68–74.
[51] Mannino DM. COPD: epidemiology, prevalence, morbidity and mortality, and disease heterogeneity. Chest 2002;121(5 Suppl), 121S–6S.
[52] Melgert BN, Ray A, Hylkema MN, Timens W, Postma DS. Are there reasons why adult asthma is more common in females? Curr Allergy Asthma Rep 2007;7(2):143–50.
[53] Naberan K, Azpeitia A, Cantoni J, Miravitlles M. Impairment of quality of life in women with chronic obstructive pulmonary disease. Respir Med 2012;106(3):367–73.
[54] Laurin C, Lavoie KL, Bacon SL, et al. Sex differences in the prevalence of psychiatric disorders and psychological distress in patients with COPD. Chest 2007;132(1):148–55 [Epub 2007/05/17].
[55] de Torres JP, Casanova C, de Garcini AM, Jaime AA, Celli BR. COPD heterogeneity: gender differences in the multidimensional BODE index. Int J Chronic Obstructive Pulm Dis 2007;2(2):151–5.
[56] Ketelaars CA, Schlosser MA, Mostert R, Huyer Abu-Saad H, Halfens RJ, Wouters EF. Determinants of health-related quality of life in patients with chronic obstructive pulmonary disease. Thorax 1996;51(1):39–43.
[57] Ninot G, Fortes M, Poulain M, et al. Gender difference in coping strategies among patients enrolled in an inpatient rehabilitation program. Heart Lung J Crit Care 2006;35(2):130–6.
[58] Weisenbach SL, Rapport LJ, Briceno EM, et al. Reduced emotion processing efficiency in healthy males relative to females. Social Cogn Affect Neurosci 2012.
[59] Racine M, Tousignant-Laflamme Y, Kloda LA, Dion D, Dupuis G, Choiniere M. A systematic literature review of 10 years of research on sex/gender and pain perception – part 2: do biopsychosocial factors alter pain sensitivity differently in women and men? Pain 2012;153(3):619–35.
[60] Aloisi AM, Bonifazi M. Sex hormones, central nervous system and pain. Horm Behav 2006;50(1):1–7 [Epub 2006/01/21].
[61] Traupe T, Stettler CD, Li H, et al. Distinct roles of estrogen receptors alpha and beta mediating acute vasodilation of epicardial coronary arteries. Hypertension 2007;49(6):1364–70 [Epub 2007/05/02].
[62] Saaresranta T, Polo O. Hormones and breathing. Chest 2002;122(6):2165–82 [Epub 2002/12/12].
[63] Schneider BK, Pickett CK, Zwillich CW, et al. Influence of testosterone on breathing during sleep. J Appl Physiol 1986;61(2):618–23 [Epub 1986/08/01].
[64] Douma SL, Husband C, O'Donnell ME, Barwin BN, Woodend AK. Estrogen-related mood disorders: reproductive life cycle factors. ANS Adv Nurs Sci 2005;28(4):364–75 [Epub 2005/11/18].
[65] Choi JC, Chung MI, Lee YD. Modulation of pain sensation by stress-related testosterone and cortisol. Anaesthesia 2012;67(10):1146–51 [Epub 2012/07/19].
[66] Vincent K, Tracey I. Sex hormones and pain: the evidence from functional imaging. Curr Pain Headache Rep 2010;14(5):396–403 [Epub 2010/08/11].
[67] Behan M, Zabka AG, Thomas CF, Mitchell GS. Sex steroid hormones and the neural control of breathing. Respir Physiol Neurobiol 2003;136(2–3):249–63 [Epub 2003/07/11].
[68] Tenholder MF, South-Paul JE. Dyspnea in pregnancy. Chest 1989;96(2):381–8.
[69] Ward T, Scheid V, Tuffrey V. Women's mid-life health experiences in urban UK: an international comparison. Clim J Int Menopause Soc 2010;13(3):278–88.

Appendix IIb: The effects of altered intrathoracic pressure on resting cerebral blood flow and its response to visual stimulation

NeuroImage 66 (2013) 479–488

Contents lists available at SciVerse ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

The effects of altered intrathoracic pressure on resting cerebral blood flow and its response to visual stimulation

Anja Hayen [a,*], Mari Herigstad [a], Michael Kelly [a], Thomas W. Okell [a], Kevin Murphy [b], Richard G. Wise [b], Kyle T.S. Pattinson [a]

[a] *Nuffield Division of Anaesthetics and Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB), Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, University of Oxford, Oxford, OX3 9DU, UK*
[b] *Cardiff University Brain Research Imaging Centre (CUBRIC), School of Psychology, Cardiff University, Park Place, CF10 3AT, Cardiff, UK*

ARTICLE INFO

Article history:
Accepted 6 October 2012
Available online 27 October 2012

Keywords:
Arterial spin labeling
Cerebral blood flow
Dyspnea
Functional magnetic resonance imaging
Respiratory loading
Transcranial Doppler sonography

ABSTRACT

Investigating how intrathoracic pressure changes affect cerebral blood flow (CBF) is important for a clear interpretation of neuroimaging data in patients with abnormal respiratory physiology, intensive care patients receiving mechanical ventilation and in research paradigms that manipulate intrathoracic pressure. Here, we investigated the effect of experimentally increased and decreased intrathoracic pressures upon CBF and the stimulus-evoked CBF response to visual stimulation.

Twenty healthy volunteers received intermittent inspiratory and expiratory loads (plus or minus 9 cmH_2O for 270 s) and viewed an intermittent 2 Hz flashing checkerboard, while maintaining stable end-tidal CO_2. CBF was recorded with transcranial Doppler sonography (TCD) and whole-brain pseudo-continuous arterial spin labeling magnetic resonance imaging (PCASL MRI).

Application of inspiratory loading (negative intrathoracic pressure) showed an increase in TCD-measured CBF of 4% and a PCASL-measured increase in grey matter CBF of 5%, but did not alter mean arterial pressure (MAP). Expiratory loading (positive intrathoracic pressure) did not alter CBF, while MAP increased by 3%. Neither loading condition altered the perfusion response to visual stimulation in the primary visual cortex. In both loading conditions localized CBF increases were observed in the somatosensory and motor cortices, and in the cerebellum.

Altered intrathoracic pressures, whether induced experimentally, therapeutically or through a disease process, have possible significant effects on CBF and should be considered as a potential systematic confound in the interpretation of perfusion-based neuroimaging data.

© 2012 Elsevier Inc. All rights reserved.

Introduction

The brain regulates its blood supply in order to ensure adequate delivery of oxygen and nutrients in the face of changing systemic blood pressure and regional changes in metabolic demand. Cerebral autoregulation is the hemodynamic process that keeps cerebral blood flow (CBF) within tight physiological limits during changes in perfusion pressure via adaptations in cerebrovascular resistance (van Beek et al., 2008), while neurovascular coupling matches local CBF to changes in underlying neuronal activity (Nielsen and Lauritzen, 2001).

CBF can be affected by physiological modulations or pharmacological agents, which in turn can affect the CBF-derived blood oxygen level dependent (BOLD) response (Brown et al., 2003; Cohen et al., 2002). Altered intrathoracic pressures could potentially affect CBF via direct pressure effects on the great arteries and veins of the chest, causing systemic changes in arterial blood pressure and in venous return from the brain (Kolbitsch et al., 2000).

Classical physiology teaching states that CBF is affected by systemic factors (e.g. systemic vascular resistance, arterial blood pressure, venous return, arterial carbon dioxide and oxygen tensions) and by local factors within the brain (e.g. autoregulation). It is therefore important to examine how these effects might confound the interpretation of CBF-derived functional neuroimaging methods, such as arterial spin labeling (ASL) or BOLD contrast, when intrathoracic pressures are changed. In order to correctly interpret FMRI to examine brain activity of patients with altered intrathoracic pressure or of healthy volunteers undergoing respiratory loading, we need to establish the effects of respiratory loading, both on baseline CBF and on CBF changes during neuronal activation. This understanding of the effects of altered intrathoracic pressure on CBF may be important in a number of clinical and experimental scenarios.

* Corresponding author at: John Radcliffe Hospital, Nuffield Department of Clinical Neurosciences, 6th Floor, West Wing, Headley Way, OX3 9DU, UK. Fax: +44 1865 234072.
E-mail addresses: anja.hayen@ndcn.ox.ac.uk (A. Hayen), mari.herigstad@ndcn.ox.ac.uk (M. Herigstad), mkelly@fmrib.ox.ac.uk (M. Kelly), tokell@fmrib.ox.ac.uk (T.W. Okell), murphyk2@cardiff.ac.uk (K. Murphy), WiseR2@cardiff.ac.uk (R.G. Wise), kyle.pattinson@ndcn.ox.ac.uk (K.T.S. Pattinson).

1053-8119/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2012.10.049

The ability to quantify CBF regulation in patients following neurological injury is commonly required in both research and clinical practice. As these patients can often require intensive care interventions such as sedation and mechanical ventilation, changes in intrathoracic pressure may potentially affect measurements of CBF. During mechanical ventilation, gas is forced into the lungs, increasing intrathoracic pressures to +15 to +30 cmH_2O. Additionally, positive end expiratory pressure (PEEP) of up to +10 cmH_2O is often applied to prevent lung collapse. Any known effect of mechanical ventilation on CBF would need to be taken into consideration when interpreting CBF measures.

Changes in intrathoracic pressure might also be a potential confound when interpreting functional neuroimaging in patients with respiratory diseases. There is currently much interest in using FMRI to understand the relevance of brain processes in these often chronic and debilitating conditions. In COPD, there is fixed obstruction of airways and inspiratory pressure of approximately −13 cmH_2O, and expiratory pressures of +3 cmH_2O have been reported (Montes de Oca et al., 1996). Acute bronchoconstriction in asthma can generate negative intrathoracic pressures of −30 cmH_2O (Stalcup and Mellins, 1977). In addition to clinical asthma, methacholine, a compound that is inhaled to create bronchoconstriction, is used to test for bronchial hyperreactivity or model asthma for experimental purposes, and could conceivably be used to investigate brain mechanisms of asthma with FMRI.

Furthermore, intrathoracic pressure is altered by breathing against an external resistive respiratory loads as an integral part of laboratory models used to investigate the neuronal mechanisms involved in the control of breathing and in the perception of breathlessness, e.g. (Banzett et al., 2008; Sturdy et al., 2004). These models can be used to gain an understanding of the neuronal mechanisms involved in the perception of breathlessness with FMRI (Banzett et al., 2008; von Leupoldt et al., 2007). Although the profound CBF effects of the Valsalva maneuver (Tiecks et al., 1996) and Mueller maneuver (Reinhard et al., 2000) have been well described, these techniques, which involve forced expiration or inspiration against a closed glottis, are extreme, unphysiological, and induce other physiological processes beyond changing thoracic pressure (e.g. hypercapnea). Therefore, to better understand the effects of altered intrathoracic pressure on cerebral physiology, a controlled experimental paradigm is required.

In this study we examined the effects of inspiratory and expiratory loading on CBF measured in two ways, with ASL FMRI and with transcranial Doppler sonography (TCD). We also determined whether or not respiratory loading induces CBF changes that might alter the perfusion response to visual stimulation. Informed by previous studies using TCD during inspiratory loading (decreased inspiratory pressure), we hypothesize that inspiratory loading will lead to increased CBF and that expiratory loading (increased expiratory pressure) will lead to a decrease in CBF. If the induced CBF changes are relatively large, these could potentially alter the CBF response to visual stimulation, similar to the way in which CBF changes affect evoked BOLD responses (e.g. increased arterial CO_2 increases CBF, which decreases the BOLD response to visual activation (Cohen et al., 2002)). We furthermore expect inspiratory and expiratory loading to show locally increased perfusion in brain areas that are engaged in the control of breathing.

Materials and methods

Brief overview

The study consisted of three sessions: a training session, a laboratory session in which CBF was measured indirectly (with TCD) with simultaneous continuous non-invasive mean arterial blood pressure (MAP) measurements and a third session in the MRI scanner during which CBF was measured across the whole brain with ASL FMRI.

TCD enables the beat-by-beat investigation of local blood velocity changes in the middle cerebral artery (MCAV), which reliably correlate with changes in CBF (Bishop et al., 1986) and hence can be used as an indirect measure of CBF, as long as a stable diameter of the insonated vessel and constant blood vessel resistance and permeability throughout the brain can be assumed.

ASL allows serial measurement of relative CBF to be taken over several minutes from the whole brain (Dai et al., 2008). During ASL, water molecules in flowing blood are magnetically labeled at the level of the neck. An image is acquired in the brain once this labeled blood water has exchanged from the vasculature into the brain tissue. Tissue perfusion, or CBF, can then be assessed by subtracting this labeled image from a control image in which the inflowing blood water is not magnetically labeled. We used a recently developed variant of ASL, PCASL (Dai et al., 2008), which provides enhanced labeling efficiency and a resultant increase in the signal-to-noise ratio of the CBF measurements.

Participants

Twenty volunteers (mean age 27 (SD ± 6) years, 6 females) participated in this study after giving written informed consent in accordance with the Oxfordshire Research Ethics Committee. Participants were healthy right-handed non-smokers with no history of significant neurological, pulmonary or cardiovascular disease and free from acute illness (e.g. acute upper respiratory tract infection). Participants abstained from excessive consumption of alcohol (24 h), caffeine (6 h) and heavy meals (2 h) before the experiment and had sufficient sleep the preceding night. Female participants either used hormonal contraception or were at the same stage of their menstrual cycle during both parts of the experiment.

The 20 volunteers were drawn from a total of 27 participants, seven of which were excluded for the following reasons: one did not tolerate the visual stimulus, four did not comply with the study protocol, and two displayed excessive head motion during FMRI. These complete datasets were excluded from all analyses.

Respiratory loading

The same experimental paradigm was performed in both experimental sessions (Fig. 1). In a supine position, participants wore a nose clip (Air Safety Ltd, Morecambe, UK) and standard foam earplugs while breathing room air through a mouthpiece connected to a custom-built respiratory circuit (shown in Fig. 2). Participants were exposed to intermittent inspiratory or expiratory loads of either minus or plus 9 cmH_2O, which was induced by partially occluding either the inspiratory or the expiratory limb of the breathing circuit with a balloon that was remotely controlled by a hydraulic system. Intrathoracic pressure can be inferred through non-invasive measurements of mouth pressure, which is approximately equivalent to measurements of esophageal pressure, the common way to determine intrathoracic pressure (Baydur et al., 1982).

Respiratory control

As a strong cerebrovascular vasodilator, CO_2 has been shown to alter CBF and to induce changes in the BOLD FMRI signal (Cohen et al., 2002). Therefore, participants were trained to maintain the partial pressure of end-tidal CO_2 ($PETCO_2$) stable throughout this study. A $PETCO_2$ baseline value was determined for each individual at the beginning of each experimental session by monitoring $PETCO_2$ over two minutes after an 8-minute habituation period. A $PETCO_2$ target value was set 0.1 kPa lower than the baseline. During the training session, participants learnt to keep their $PETCO_2$ stable. At the beginning of each session, participants were instructed to keep their $PETCO_2$ stable within 1 kPa of their target value throughout the paradigm via

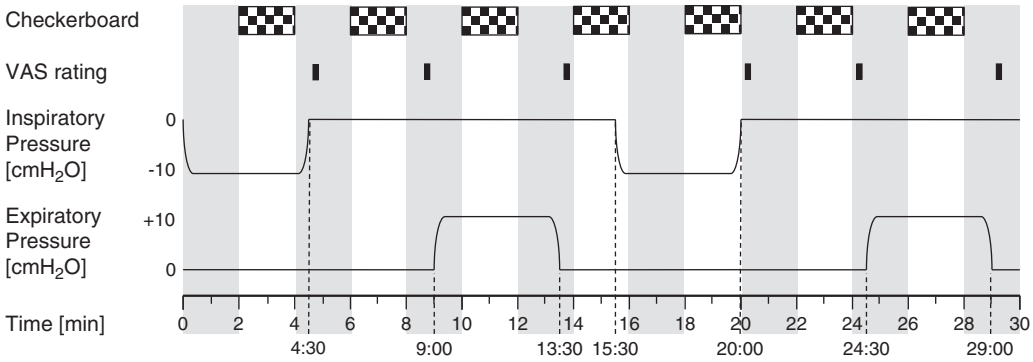


Fig. 1. Timeline of experimental paradigm. A 4½-minute block of inspiratory resistance of −9 cmH_2O is followed by 4½ min of unloaded breathing at 4:30 min and 4½ min of expiratory resistance of +9 cmH_2O at 9:00 min. After a normal breathing period of 2 min at 13½ min, this is followed by a second cycle of inspiratory loading (4½ min from 15½ min onwards), unloaded breathing (4½ min, starting at 20:00) and expiratory resistance (4½ min from 24½ min onwards). 10 s after each resistance block and 10 s before the end of the unloaded breathing blocks, participants were presented with a VAS scale for 10 s to rate their average breathlessness over that block. A 2 Hz black and white flashing checkerboard was presented for 120 s every 240 s, starting at 2 min. $PETCO_2$ values remained visible throughout baseline and visual stimulation.

numerical visual feedback that was back-projected to participants onto the centre of a screen behind them, visible via a mirror (similar set-up to Pattinson et al. (2007)).

All physiological data were sampled at 50 Hz and were logged via a Power1401 (CED, Cambridge, UK) using Spike2.7 (CED, Cambridge, UK). $PETCO_2$ was calculated on a breath-by-breath basis through an online script in Spike2.7 and was fed back to the participant via a stimulus presentation program (Presentation, Neurobehavioral Systems, Albany, USA) on a second computer.

Behavioral responses were made using a custom-made button box and were represented on a horizontal visual analog scale (VAS). This scale allowed participants to answer the question "How strong was your breathlessness averaged over the last 4½ min block?" with the anchors 'none' on the left and 'unbearable' on the right. Participants were told to interpret the bar as a scale from 0 to 10, where 9 would indicate severe breathlessness and the wish to terminate the experiment.

Every 4 min, participants were shown a 2 Hz flashing checkerboard for 2 min (see Fig. 1). A 2 Hz flashing checkerboard induces robust activations of the visual cortex, hence allowing us to detect potential intrathoracic pressure related changes in the perfusion increase to visual activation with high sensitivity.

The numerical $PETCO_2$ feedback was continuously displayed during the whole experimental period in a small box with a black background displayed in the centre of the screen. The only time $PETCO_2$ feedback was not shown, was whilst participants gave subjective ratings on a VAS-scale (six periods of ten seconds).

Recordings during laboratory session

A 2.0 MHz pulsed wave Doppler transducer (DWL Elektronische Systeme GMbH, Sipplingen, Germany), held over the right temporal bone window, provided continuous MCAV. Standard vessel identification criteria were used and the signal was sampled at the individual's optimum depth between 50 and 55 mm. Continuous non-invasive blood pressure was measured with a Finapres blood pressure monitor (Ohmeda 2300, BOC Health Care, USA). The cuff was applied to the midphalanx of the middle or ring finger of the non-dominant hand and placed at heart level. Beat-to-beat systolic and diastolic pressures,

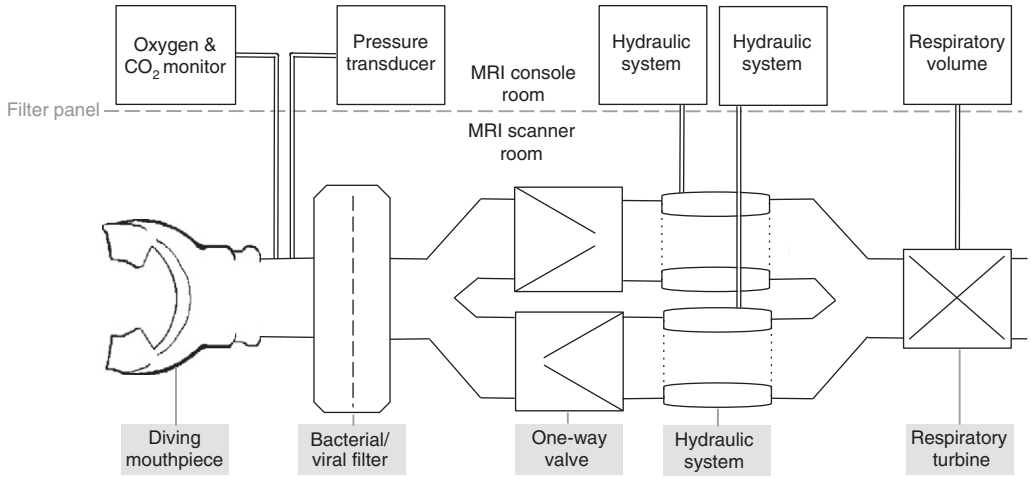


Fig. 2. Schematic diagram of respiratory circuit. A diving mouthpiece (Scubapro UK Ltd, Mitcham, UK) connects to a bacterial and viral filter (Vitalograph, Buckingham, UK) from which respiratory gases and respiratory pressure are sampled via polyethylene extension tubing (Vygon SA, Ecouen, France). One sampling line leads to a pressure transducer (MP 45, ±50 cmH_2O, Validyne Corp., Northridge, CA, USA) connected to an amplifier (Pressure transducer indicator, PK Morgan Ltd, Kent, UK). During the physiology session, the second sampling line connects to a gas analyzer that samples oxygen and CO_2 (Capnomac Ultima, Datex Ohmeda, Helsinki, Finland), while during the scanning session tidal CO_2 was measured with a rapidly responding gas analyzer (CD-3A and S-3A; AEI Technologies, Pittsburgh, PA, USA). The filter connects to a Y-piece, creating an inspiratory and an expiratory limb through one-way valves (Hans Rudolf, Kansas City, MO, USA). The diameter of a 5 cm long section of the inspiratory and the expiratory channel can be altered remotely via a hydraulic system. Each modifiable section of tubing is lined with a rubber party balloon connected via non-distensible plastic tubing to a 20 ml syringe filled with water and used for inflation and deflation of the balloon. The inspiratory and expiratory tubes recombine through a Y-piece and an attached turbine (VMM-400, Interface Associates, Aliso Viejo, CA, USA) records inspiratory (V_i) and expiratory (V_e) volumes.

as well as MAP, were computed. MAP was calculated as one-third of systolic blood pressure (SBP) plus two-thirds of diastolic blood pressure (DBP). Pulse pressure (PP) was calculated as the difference between SBP DBP. Cerebrovascular resistance (CVR) was calculated with the formula CVR = MAP/MCAV. Blood pressure was recorded during the laboratory session only, due to the lack of availability of an MRI compatible device. Heart rate was measured with a pulse oximeter (Capnomac Ultima, Datex Ohmeda, Helsinki, Finland). Respiratory measures were obtained as explained in Fig. 2.

Recordings during MRI session

Participants were scanned with a 3 tesla Siemens Verio MRI scanner (Siemens, Erlangen, Germany) with a 32-channel radiofrequency receive coil, using foam inserts to minimize head motion. A whole-brain PCASL sequence was utilized (Dai et al., 2008) with a gradient-echo echo-planar (EPI) readout (PCASL module flip angle 20°, labeling duration = 1.4 s, post labeling delay = 1 s, EPI readout flip angle 90°, TR = 4.0 s, TE = 14 ms, 6/8 k-space). We acquired twenty-two axial slices in ascending order (450 volumes, $3.8 \times 3.8 \times 5.0$ mm voxels, interslice gap = 1.75 mm,). Pre-saturation of the imaging region was performed just prior to labeling to suppress static tissue signals, reducing the sensitivity to subject motion and physiological noise. A time-of-flight angiography scan was acquired in the neck to allow visualization of the main feeding arteries. The PCASL labeling plane was positioned approximately halfway between the upper and lower contortions of the vertebral arteries, so that the plane was perpendicular to both the vertebral and carotid arteries, thereby optimizing the labeling efficiency. For each subject, an MPRAGE structural image was acquired to enable registration of functional data. Continuous heart rate was recorded with a pulse oximeter (MR multigas monitor, MR Equipment, model 9500) and respiratory measures were obtained as described in Fig. 2.

Data analysis

Physiological recordings

Data were exported to MatLab (MathWorks Inc., Natick, MA, USA) and analyzed using custom-written scripts. Respiratory parameters (CO_2 partial pressures and respiratory pressures), circulatory parameters (heart rate and MAP) and MCAV were averaged within subject across each of the six respiratory resistance periods (unloaded breathing, inspiratory loading and expiratory loading [×2]). No difference between the first and the second repeat of a condition were found and the results for both repeats were combined.

PCASL

Analysis of the PCASL FMRI images to identify regions exhibiting significant perfusion changes was carried out using tools from the FMRIB Software Library (FSL, www.fmrib.ox.ac.uk/fsl). Full perfusion signal modeling was performed in FEAT (FMRI Expert Analysis Tool in FSL), with the ASL tag-control difference and the stimulus time courses modeled as separate explanatory variables (EV) and perfusion change obtained from their interaction. Non-brain structures (i.e. skull and surrounding tissues) were removed using BET (Smith, 2002) and 4D images were motion corrected using MCFLIRT (Jenkinson et al., 2002). Spatial smoothing using a Gaussian kernel of full-width-half-maximum 5 mm and high pass temporal filtering were performed (Gaussian-weighted least-squares straight line fitting with a high-pass-filter cutoff of 12 s). After preprocessing, the functional scans were co-registered to the individual's structural scan (linear registration) and then to the MNI152 standard space (average T1 brain image constructed from 152 normal subjects at the Montreal Neurological institute, Montreal, QC, Canada) using nonlinear registration. ASL images were corrected for physiological noise using RETROICOR (Glover et al., 2000; Harvey et al., 2008). RETROICOR involves fitting low-order Fourier series to the EPI data based on the time of each image acquisition relative to the phase of the cardiac (3 terms) and respiratory cycles (4 terms) and their interaction (1 term).

FEAT (http://www.fmrib.ox.ac.uk/fsl/feat5/index.html) was used to compare the relative CBF change in response to inspiratory and expiratory resistance to CBF while breathing was unloaded. The general linear model (GLM) included the following EVs: Mouth pressure during inspiration (P_{min}), mouth pressure during expiration (P_{max}), $PETCO_2$, visual stimulation, the act of rating, breathlessness score and six motion parameters. Interactions between inspiratory loading and visual activation and expiratory loading and visual activation were modeled. On the second level (group level), FEAT was used to perform a voxel-wise statistical analysis using a mixed effects model to combine the GLM results of individual participants while accounting for intersubject variance. A cluster threshold of $Z > 2.3$ and a (corrected) cluster significance threshold of $P = 0.05$ were used.

An anatomical mask of the primary visual cortex (V1) was created using the Juelich histological atlas within FSLView (http://www.fmrib.ox.ac.uk/fsl/fslview/index.html) with a probability threshold applied at 0.35. This mask was used to determine perfusion changes in the visual cortex in response to visual stimulation and the interactions between visual stimulation and respiratory loading.

A region of interest approach was used to calculate mean fractional perfusion changes in response to inspiratory and expiratory loading in the whole brain grey matter (grey matter masks determined individually by segmenting participants' structural scans with FAST (FMRIB's Automated Segmentation Tool, http://www.fmrib.ox.ac.uk/analysis/research/fast/)), and in the individual brain areas with significantly increased perfusion demonstrated in the voxel-wise analysis.

It is possible that localized CBF increases (e.g. activation due to the work of breathing against load etc.) might elevate the general global CBF change. It is also possible that a small collinearity between respiratory loading and the visual stimulation could inflate the global grey matter increase. Therefore, to account for both of these potential effects, we performed a second region of interest analysis specifically excluding localized CBF increases (i.e. sensorimotor cortices and cerebellum) that were assumed to be task specific and also excluding V1.

In order to compare our results to the well-known effects of CO_2 on cerebral perfusion (Cohen et al., 2002), we included the natural fluctuations in $PETCO_2$ in the statistical model (i.e. although participants attempted to keep $PETCO_2$ constant, spontaneous fluctuations and small imperfections in performing this task were present (Wise et al., 2004)).

To retrospectively evaluate the sensitivity of our study design, we used the study sample size and the standard deviations from our region of interest analyses to statistically determine the smallest grey matter and V1 perfusion increases that our study is able to detect with 80% confidence.

Results

Laboratory session

Physiological results of the laboratory session are reported in the top part of Table 1. There was no significant difference between the first and second block of each stimulation condition, hence values are presented as averages over both blocks. Mean arterial pressure increased from 90.7 ± 15.2 mm Hg to 93.8 ± 15.7 mm Hg ($p < 0.01$), during expiratory loading, while $PETCO_2$ and HR remained stable for both loading conditions. The time courses of MCAV, $PETCO_2$ and minimum and maximum mouth pressure values throughout the first half of the laboratory session are illustrated in Fig. 3.

MRI session

The mean changes in relative CBF ($\pm$SD) in the grey matter and in V1 are reported in Table 2. Areas in which perfusion was increased during inspiratory loading are shown in the top panel of Fig. 4. Areas

Table 1
The top part of the table shows physiological measures during the laboratory session during unloaded breathing, inspiratory loading and expiratory loading. The lower part of the table shows physiological measures collected during the scanning session.

	Unloaded breathing	Inspiratory loading	Expiratory loading
Physiology session			
MCAV [cm/s]	60.6 ± 6.2	62.6 ± 7.0*	60.7 ± 6.1
CVR [mmHg/cm/s]	1.5 ± .30	1.4 ± .30**	1.53 ± .31
MAP [mmHg]	90.7 ± 15.2	89.8 ± 15.4	93.8 ± 15.7**
SBP [mmHg]	134.8 ± 19.1	134.1 ± 18.6	138.4 ± 17.9**
DBP [mmHg]	68.7 ± 14.6	67.8 ± 14.6	71.4 ± 16.0*
Pulse Pressure [mmHg]	66.1 ± 11.6	66.2 ± 9.8	67.0 ± 11.8
HR [bpm]	59.0 ± 7.3	59.3 ± 6.6	59.0 ± 6.7
Minute ventilation [l/min]	6.3 ± 2.0	5.9 ± 2.8	6.3 ± 2.0
Resp. Rate [breaths/min]	18.2 ± 11.5	16.3 ± 11.3	15.9 ± 10.0
$PETO_2$ [kPa]	14.7 ± 0.8	14.6 ± 0.8	14.7 ± 1.1
$PETCO_2$ [kPa]	5.5 ± 0.5	5.5 ± 0.5	5.5 ± 0.6
P_{min} [cmH_2O]	−0.1 ± 1.5	−8.8 ± 2.6***	0.2 ± 1.7
P_{max} [cmH_2O]	2.0 ± 0.7	1.6 ± 1.1*	9.3 ± 1.9***
Breathlessness [VAS 0-10]	0.9 ± 1.2	4.1 ± 1.6***	3.6 ± 2.0***
MRI session			
$HR_{(scan)}$ [bpm]	55.9 ± 7.8	56.7 ± 7.3	56.6 ± 7.3
$PETCO_{2\ (scan)}$ [kPa]	41.3 ± 5.3	41.3 ± 5.3	41.3 ± 0.8
$P_{min\ (scan)}$ [mmHg]	−0.8 ± 0.4	−9.8 ± 1.7***	0.2 ± 0.9**
$P_{max\ (scan)}$ [mmHg]	1.0 ± 0.5	0.3 ± 0.9***	9.1 ± 2.0***
$Breathlessness_{(scan)}$ [VAS 0-10]	0.7 ± 1.0	4.1 ± 1.9***	3.9 ± 2.1***

Stars indicate statistically significant differences between respective loading condition and unloaded breathing. *p<.05, **p<.01, ***p<.001. Mean ± SD MCAV (middle cerebral artery velocity), MAP (mean arterial pressure), SBP (systolic blood pressure), DBP (diastolic blood pressure), pulse pressure, CVR (cerebrovascular resistance), HR (heart rate), $PETCO_2$ (partial pressure of end-tidal CO_2), $PETO_2$ (partial pressure of end-tidal oxygen), minute ventilation, resp. rate (respiratory rate), P_{min} (minimum pressure at mouth), P_{max} (maximum pressure at mouth) and breathlessness rating averaged over both 4½ min blocks for each respiratory loading condition and during unloaded breathing. $_{(scan)}$ indicates that data was collected inside the MRI scanner.

in which perfusion was increased during expiratory loading are shown in the bottom panel of Fig. 4. Locations of peak perfusion increase, Z scores of peak voxels (Z_{max}) and % perfusion increase during inspiratory and expiratory loading within the respective activity masks are provided in Table 3. No relative CBF decreases were seen in any of these regions of interest during the different loading conditions.

In response to inspiratory loading, significant regional increases in perfusion were found in the right primary and secondary somatosensory cortices, the right premotor and primary motor cortices and in cerebellar lobe VI. Significant perfusion increases in the somatomotor cluster extended over the primary and secondary somatosensory cortices and the primary motor cortex and the premotor cortex. The locations and Z scores (Z_{max}) of local peak voxels are shown in Table 3. Additional areas commonly activated in respiratory studies using BOLD showed increased perfusion in our data only when no cluster threshold was applied. Table 4 shows the location of peak voxels in clusters that showed a significant Z score of above 2.3, but that did not survive the application of the cluster threshold.

Even when the localized brain activity related to increased breathing effort and V1 was excluded from analysis, global CBF remained increased during inspiratory loading, which indicates a small, but significant increase in baseline CBF (+0.5% per cmH_2O, p<.05, Table 2).

During application of expiratory loading, relative CBF was increased in the bilateral somatosensory and motor cortices and in the cerebellar lobe VI. The locations of signal maxima and Z scores are shown in Table 3.

The voxel-wise analysis demonstrated a robust bilateral increase in CBF in the visual cortex in response to visual stimulation (Z_{max} = 7.11, not shown). We calculated a 14.7% increase in relative in CBF response to visual stimulation compared to baseline in a standard anatomic mask of the V1. No significant interactions were observed between the relative CBF response to visual stimulation and inspiratory or expiratory loading (Table 2).

Results of the physiological recordings during the scanning session are reported in the lower part of Table 1 and show stable $PETCO_2$ and HR for all respiratory conditions. As during the laboratory session, there were no differences between the first and second block of each condition, hence values are presented as averages over both blocks.

Retrospective estimation of the power of the study based on sample size and standard deviations from the region of interest analysis demonstrated that the study can detect a perfusion change of 0.5% of global grey matter perfusion with 80% confidence. A change of 1.3% of the perfusion increase in response to visual stimulation can be detected with 80% confidence to determine possible interactions between visual stimulation and inspiratory or expiratory resistance in V1.

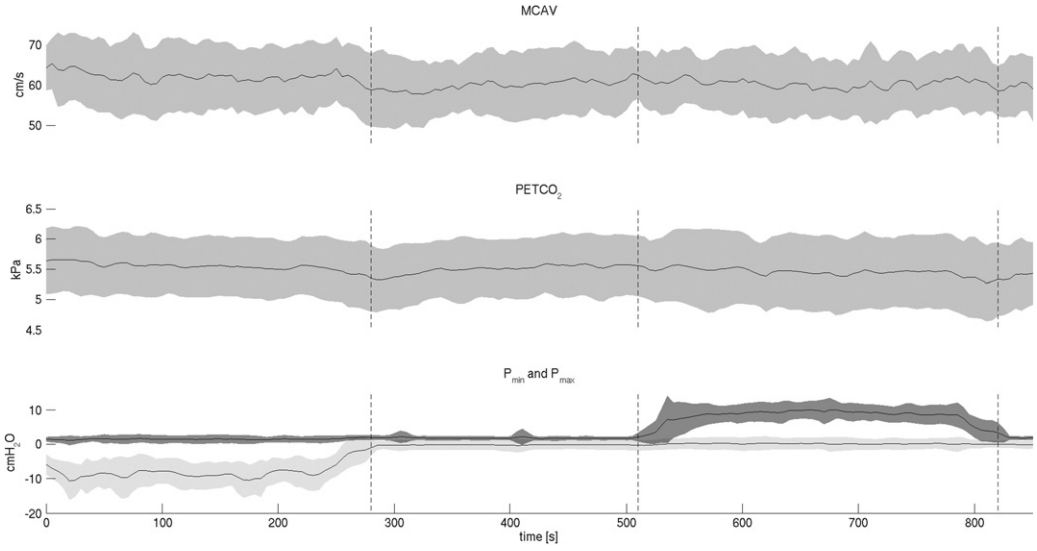


Fig. 3. Middle cerebral artery velocity (MCAV), partial pressure of end-tidal CO_2 ($PETCO_2$), minimum pressure at the mouth (Pmin, light grey SD) and maximum pressure at the mouth (Pmax, dark grey SD) averaged across 20 participants and plotted over the first half of the physiological recording session (mean ± SD).

Table 2
Mean ± SD relative CBF changes measured with arterial spin labeling as percentage change from baseline in the global grey matter (total GM), the mean grey matter CBF excluding areas specifically activated in response to the loading task (non-task GM), the mean grey matter excluding areas specifically activated in response to the loading task and excluding the primary visual cortex (V1; non-task GM–V1) and in an anatomical mask of V1 in response to inspiratory and expiratory loading, CO_2 , visual stimulation and the interaction between visual stimulation and inspiratory or expiratory loading.

Condition	Brain region	CBF change
Inspiratory load	Total GM	$+0.5 \pm 0.8^{*}$ [%/cmH_2O]
	Non-task GM–V1	$+0.5 \pm 0.8^{*}$ [%/cmH_2O]
Expiratory load	Total GM	$+0.3 \pm 0.8$ [%/cmH_2O]
	Non-task GM–V1	$+0.2 \pm 0.8$ [%/cmH_2O]
CO_2	Total GM	$+24.0 \pm 11.3^{***}$ [%/kPa]
Visual stimulation	V1	$+14.7 \pm 5.8^{***}$ [%]
Interaction: visual and inspiratory loading	V1	$+0.1 \pm 1.9$ [%]
Interaction: visual and expiratory loading	V1	$+0.6 \pm 1.9$ [%]

Stars indicate statistically significant differences between respective loading condition and unloaded breathing. * p<.05, ** p<.01, ***p<.001.

Discussion

Summary

The key findings of this study are:

1) Inspiratory loading caused an increase in relative CBF of approximately 0.5% per cmH_2O load pressure, but did not have any effect upon arterial blood pressure. This global increase in relative CBF remained evident even when localized CBF increases in brain areas thought to be responsible for performing the breathing tasks and the primary visual cortex were excluded in a subsequent region of interest analysis.
2) Expiratory loading had no effect on global CBF (either including or excluding task-specific regions of interest and the primary visual cortex), but was associated with an increase in mean arterial pressure from 90.7 ± 15.2 mm Hg to 93.8 ± 15.7 mm Hg (approximately 0.3% per cmH_2O, p<0.01).
3) There was no generalized modulation of the CBF response to visual stimulation in the primary visual cortex by either inspiratory or expiratory resistive loading.
4) Localized perfusion increases were observed in the somatosensory and motor cortices and the cerebellum during inspiratory and expiratory loading.

CBF changes with inspiratory and expiratory loading

This study was motivated by the need to understand how the well-described effects of respiratory loading on systemic arterial and venous systems (Scharf, 1991) might affect CBF. Other studies of respiratory loading employing a variety of different techniques have shown varying effects on CBF (Cooke et al., 2006; Droste et al., 1999; Kolbitsch et al., 2000; Lorenz et al., 2001; Reinhard et al., 2000). The conflicting results of previous studies may possibly depend on the specific methods used to generate respiratory loads, the differing techniques used to measure CBF and the fact that studies tended to only apply either inspiratory or expiratory loading and the effects might therefore not be directly comparable. With ASL, we have additionally been able to examine the effect of regional changes in CBF across the whole brain and to test how the CBF response to neural activation might be affected by respiratory loading.

Arterial blood pressure and CBF

Inspiratory loading elicited no changes in blood pressure (although CBF increased), while expiratory loading caused a small increase in blood pressure (but was not associated with altered CBF). As both experimental sessions were otherwise performed in an identical manner, and as the physiology measurements and CBF changes in both sessions were comparable, we assume that similar blood pressure changes occurred during the MRI scanning session. In terms of understanding CBF regulation, our data therefore suggest that small changes in blood pressure are directly caused by expiratory loading, but that a

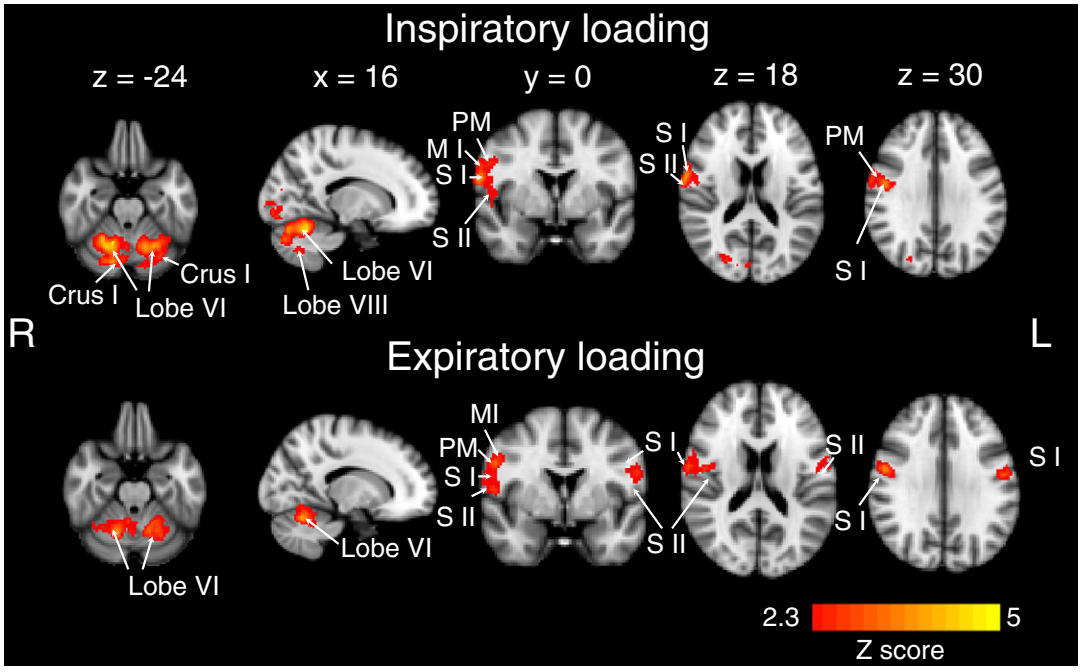


Fig. 4. Significant CBF increases during inspiratory and expiratory loading compared to unloaded breathing. The images consist of a colour-rendered statistical map superimposed upon a standard (MNI) brain. Significant regions are displayed with a threshold of Z>2.3 and a cluster probability threshold of p<0.05 (corrected for multiple comparisons). PM = premotor area, M1 = motor cortex, S I = primary somatosensory cortex, S II = secondary somatosensory cortex.

Table 3
Peak voxel locations, peak voxel Z scores (Z_{max}) and % perfusion increase (averaged over cluster) in activation clusters during inspiratory and expiratory loading.

Condition	Region	Peak voxel location	Z_{max}	Perfusion increase [%/cmH_2O]
Inspiratory loading	Right somatosensory cortex	X = 62, Y = 0, Z = 18	4.13	1.3 ± 0.9***
		X = 48, Y = −4, Z = 30	3.96	
		X = 62, Y = −4, Z = 30	3.26	
		X = 52, Y = −2, Z = 10	3.13	
	Cerebellum	X = 16, Y = −58, Z = −24	5.09	1.5 ± 1.0***
Expiratory loading	Primary motor cortex	Right: X = 54, Y = −4, Z = 32	Right: 4.01	1.0 ± 0.9***
		X = 50, Y = −10, Z = 38	3.95	
		X = 50, Y = −8, Z = 34	3.86	
		Left: X = −50, Y = −8, Z = 40	Left: 3.78	
		X = −52, Y = −8, Z = 40	3.63	
		X = −44, Y = −12, Z = 38	3.41	
	Somatosensory cortex	Right: X = 62, Y = −6, Z = 16	Right: 3.35	
		X = 44, Y = −8, Z = 22	3.25	
		X = 56, Y = 0, Z = 10	3.23	
		Left: X = −56, Y = −8, Z = 26	Left: 3.53	
		X = −58, Y = −4, Z = 24	3.49	
		X = −52, Y = −6, Z = 20	3.12	
	Cerebellum	X = 18, Y = −60, Z = −22	4.23	1.4 ± 1.3***

Stars indicate statistically significant differences between respective loading condition and unloaded breathing. ***p<.001. A cluster probability threshold of p<.05 was applied to correct for multiple comparisons.

different mechanism is responsible for the increase in CBF observed during inspiratory loading. We speculate that the increase in blood pressure during expiratory loading might occur due to a primary localized direct pressure effect of increased intrathoracic pressure on the great arteries of the chest, e.g. the aorta, as other cardiovascular parameters (heart rate, pulse pressure) were unchanged, making it unlikely that the increased blood pressure was secondary to systemic factors, such as increased sympathetic activation or increased systemic vascular resistance.

Venous return

Venous return from the brain to the heart is important for the regulation of the cerebral circulation. In an upright position, gravity maintains a slightly negative pressure in the jugular veins, resulting in unimpeded venous drainage from the brain. Venous drainage is further assisted by the "thoracic pump", the slight negative intrathoracic pressure generated during normal inspiration that is transmitted to the great veins in the thorax (e.g. the superior vena cava). In a supine position like in this study, gravity no longer has such a profound effect and jugular venous pressure (JVP) increases to approximately 8 mm Hg (Leonard et al., 2008), potentially impeding venous outflow from the brain. Decreased intrathoracic pressure caused by inspiratory loading in a supine position decreases JVP through direct pressure effects on the great veins in the thorax that are in direct continuity with the jugular veins. Inspiratory loading therefore may act to accentuate the normal thoracic pump mechanism and will therefore increase the arterio-venous pressure gradient across the brain, leading to increased CBF.

Increased intrathoracic pressure during expiratory loading has the opposite effect on the central venous circulation and will tend to elevate the pressure in the jugular vein, decreasing the arterio-venous pressure gradient across the brain. Without an effective compensatory mechanism, this would decrease CBF. We therefore speculate that this reduction in venous return would be compensated by the increased MAP during expiratory loading, leading to unchanged CBF. Jugular venous pressure was not measured during this study (due to the invasive nature of this measurement), but the effects of intrathoracic pressure on JVP have been well documented (Hall and Guyton, 2011).

Systemic vascular resistance

Another possibility is that alterations in atrial transmural pressure induced by respiratory loading stimulate the release of atrial natriuretic peptide, a powerful vasodilator that decreases systemic vascular resistance. The difference between the systolic and diastolic blood pressures (i.e. pulse pressure) is proportional to systemic vascular resistance. Although we observed an increase in mean arterial pressure during expiratory loading, this was associated with equivalent increases in diastolic and systolic pressures, while pulse pressure remained constant. Therefore, the increase in CBF during inspiratory loading is unlikely to originate from altered systemic arterial tone

Table 4
Z scores (Z_{max}) of peak voxels and coordinates of clusters of relative CBF increase in regions previously implicated in respiratory studies that did not survive the application of our cluster threshold during inspiratory and expiratory loading (data with uncorrected Z stats).

Condition	Region	Peak voxel location	Z_{max}
Inspiratory loading	Left somatosensory cortex	X = −58, Y = −6, Z = 36	3.78
	Amygdala	Right: X = 30, Y = 2, Z = −16	Right: 2.3
		Left: X = −14, Y = −4, Z = −22	Left: 2.2
	Right anterior cingulate gyrus	X = 0, Y = −12, Z = 42	2.2
	Left posterior cingulate gyrus	X = −4,Y = −4, Z = 4	2.4
	Right anterior insula	X = 40, Y = 80, Z = −2	2.5
	Right posterior insula	X = 40, Y = −6, Z = 2	2.4
Expiratory loading	Right posterior insula	X = 38, Y = −14, Z = 6	2.7
	Amygdala	Right: X = 24, Y = −12, Z = −12	Right: 2.6
		X = 30, Y = 2, Z = −16	2.0
		Left: X = −14, Y = −2, Z = −18	Left: 2.3
	Right anterior cingulate gyrus	X = 12, Y = 40, Z = 2	2.2
	Posterior cingulate gyrus	Right: X = 6, Y = −14, Z = 46	Right: 2.3
		Left: X = −2, Y = −48, Z = 2	Left: 2.7

and we therefore conclude that alterations in venous return may explain the increased CBF during inspiratory loading.

Arterial CO_2

Additionally, arterial CO_2 tension has a strong impact on the regulation of CBF. For each 1 mm Hg change in $PaCO_2$, there is a corresponding CBF change in the same direction of approximately 2.5% in hypocapnia, and 4.5% in hypercapnia (Ide et al., 2003) and there are correspondingly profound effects on the BOLD response, which is decreased in hypercapnia and increased in hypocapnia. As changes in respiration potentially affect $PaCO_2$ (and therefore CBF), we have paid meticulous attention to the maintenance of constant CO_2 levels. We trained our research participants to maintain their $PETCO_2$ constant through a visual feedback system. Without this training, respiratory loading, equivalent to that used here during spontaneous breathing, is well documented to cause hypercapnia due to decreases in ventilation (Davies et al., 1919), of between 1 and 3 mm Hg, therefore further increasing CBF by approximately 4.5% to 13.5%. Such a CBF increase would confound the interpretation of our results. As we have maintained constant $PETCO_2$, we can be confident that the CBF increase during inspiratory loading is a true primary physiological effect, that taken in isolation is small, but could contribute to a more profound systematic confound due to secondary effects of loading on ventilation.

Such careful control of CO_2 levels is important not only in respiratory studies, but also in any other paradigm that may alter respiration. One example of the importance of respiratory confounds is the caffeine-induced decrease of CBF (Chen and Parrish, 2009), which confounds the BOLD response. Caffeine stimulates both the respiratory system (causing hypocapnia (Bell et al., 1999)) and the cardiovascular system (increasing blood pressure (Robertson et al., 1978)), both important contributors to CBF regulation. We are not aware of any current reports of caffeine's effect on CBF having obtained what we consider essential measures of systemic physiology and its direct effects on CBF are therefore not clear.

Interactions between respiratory loading and visual stimulation

Inspiratory and expiratory loading did not modulate the stimulus-evoked perfusion increase in V1. Therefore, the theoretical confounding effects of ABP and of baseline CBF on stimulus-evoked CBF responses as suggested by Payne (2006) were not observed. This finding is in agreement with a recent paper by Gauthier et al. (2011), demonstrating a profound effect of carbogen on baseline CBF and the visually-evoked BOLD response in the visual cortex during simultaneous carbogen inhalation and visual stimulation, which were evident at the lowest level of carbogen inhalation (2.5% CO2, 38.25% O2, balance N_2), while relative CBF increases in the visual cortex induced by visual stimulation remained unaffected during carbogen inhalation.

The interpretation of the BOLD signal, which is a composite signal comprising CBF, cerebral blood volume and the cerebral metabolic rate of oxygen (Kim et al., 1999), is highly susceptible to changes in blood deoxyhemoglobin level and to the size of the perfusion response to activation (Gauthier et al., 2011). A small CBF increase, as seen during inspiratory loading in our study, could hence lead to a lower fraction of deoxyhemoglobin, an elevated BOLD baseline and a reduced BOLD response to functional tasks. Pooling of venous blood due to increased venous pressure might lead to a further decrease in the BOLD response (Lorenz et al., 2001). As such, CBF effects might confound the comparison of BOLD activations across different levels of inspiratory loading, e.g. in von Leupoldt et al. (2009), where the CO_2 effects of loading will affect CBF in addition to the direct effects of negative intrathoracic pressure. Similarly, Evans et al. (1999) compared positive pressure ventilation with spontaneous breathing, hence their results might be confounded due to opposing effects on CBF and venous drainage.

As we only used ASL during this study, we can only comment on the CBF component of the BOLD signal. One approach to control for potential CBF influences on the BOLD response is to acquire a measure of baseline CBF, which can be used as a regressor to aid the differentiation of local metabolic effects from purely vascular effects when interpreting BOLD signal change (Pattinson et al., 2009). Intrathoracic pressure changes might additionally affect other components of the BOLD signal, which could be investigated in future studies.

Implications for the understanding of respiratory control

Activation in somatosensory, motor and cerebellar areas has previously been found to be implicated in neuroimaging studies of modified respiration (reviewed in Herigstad et al. (2011)). Increased perfusion in the premotor, motor and supplementary motor cortices is likely to indicate stronger mechanical breathing effort during loading periods, as these areas are implicated in conscious breathing (Pattinson et al., 2007, 2009). In the primary motor cortex, the location of the peak perfusion increase during expiratory loading was near to locations of peak activation in the orofacial region close to areas previously identified to activate in the performance of non-speech tasks involving the lips and tongue (reviewed by Takai et al. (2010)). While respiration is classically located in the trunk area of the homunculus (Penfield and Rasmussen, 1950; Penfield and Roberts, 1959), respiratory activation is more complex. A second, more ventrally located peak has been identified that shows the location of the larynx to overlap with the location of the expiratory muscles (Loucks et al., 2007). Inspiratory and expiratory loading in our study activate within this orofacial area, indicating the strong activation of respiratory muscles during these manipulations.

In the somatosensory cortex, inspiratory and expiratory loading each activated a distinct peak in the inferior precentral gyrus. Activation relating to inspiratory loading was unilateral (right side of the brain). On the right side the peak activations were spatially separated, although relatively close to each other (inspiratory loading: $x = 62$, $y = 0$, $z = 18$, expiratory loading: $x = 54$, $y = -4$, $z = 32$,). These perfusion maxima are in a similar position to those identified during voluntary hyperpnea (McKay et al., 2003). However, these activation peaks are located considerably more ventrally in the somatosensory cortex when compared with the location of peak sites of activation in neuroimaging studies that manipulated respiration without employing a paced voluntary breathing task (Evans et al., 1999; von Leupoldt et al., 2008), indicating the importance of exact task specifications when placing respiratory-related perceptions in the somatosensory cortex. Our findings suggest that different aspects of respiratory sensation are processed within distinct areas within the somatosensory cortex, and that a more detailed study of this structure in relation to respiratory control would be worthwhile.

We have also shown increased perfusion in cerebellar lobe VI, which has previously been shown during resistive loading (Isaev et al., 2002), but its role in respiratory control is unclear. Based on increased BOLD signal in cerebellar lobe VI found during other sensorimotor tasks and during the application of aversive stimuli in other studies (Moulton et al., 2011), we speculate that this activity might be due to the sensorimotor control of breathing (sensorimotor cerebellum) and might also represent the negative psychological affect experienced during respiratory loading.

CBF increases that did not survive cluster thresholding were identified in the insula, amygdala and the anterior and posterior cingulate gyri in response to both inspiratory and expiratory loading. These brain areas have consistently been identified in neuroimaging studies using resistive loading to induce breathlessness (reviewed in Herigstad et al. 2011). It may be that the poorer signal to noise ratio of ASL explains why these CBF increases have not reached our statistical thresholds or that our experimental paradigm did not induce sufficient breathlessness to activate these structures. Furthermore, in a study by von Leupoldt et

al. (2008) data analysis necessitated small volume correction to identify respiratory-related activity in these structures. We have chosen not to follow this analysis approach, as identifying respiratory-related activity in these structures was not a primary aim of this study.

Clinical implications

The increase in CBF of ~5% caused by an inspiratory load of −10 cmH_2O has potential implications in the interpretation of functional neuroimaging data in a variety of clinical and experimental settings.

Negative intrathoracic pressure is the hallmark of respiratory obstruction in asthma and COPD. Neuroimaging in these conditions might be aimed at understanding how the brain contributes to respiratory obstruction (e.g. in asthma, higher cortical centres mediate increased activity in the vagus nerve, which innervates the bronchial musculature (Rosenkranz et al., 2005)) and to the perception of breathlessness (Banzett et al., 2008; Herigstad et al., 2011). Furthermore, experimental manipulations of the respiratory system in these patients might exacerbate negative intrathoracic pressures, e.g. by the administration of methacholine challenges in the investigation of asthma (Birnbaum and Barreiro, 2007), or by use of external respiratory loading to investigate breathlessness in both asthma and COPD.

Therefore, care should be taken when measuring and interpreting cerebral perfusion in patients with altered respiratory physiology. The small CBF increase caused by inspiratory loading might add to a change in underlying baseline physiology and could potentially alter FMRI results. Experimental interventions might affect CBF in other ways (e.g. by an effect on $PETCO_2$ which was carefully controlled in the present study) that also need careful consideration. In these instances, care should be taken to include physiological monitoring (e.g. of $PETCO_2$) and awareness and if possible measurements of potentially altered respiratory pressures during imaging analysis.

In the present study, although expiratory loading of +10 cmH_2O increased mean arterial blood pressure by a small amount, it did not significantly alter CBF, suggesting that at this level of loading autoregulation effectively compensates. Therefore, interventions such as CPAP (usually up to +10 cmH_2O) and the slightly raised expiratory intrathoracic pressure in COPD (+3 cmH_2O) are unlikely to be important confounds in CBF interpretation. Mechanical ventilation (e.g. in intensive care patients) generates higher positive thoracic pressures (e.g. up to +30 cmH_2O, but usually +15 to +25 cmH_2O) and it is conceivable that a greater load might affect CBF. In intensive care patients with brain injury (e.g. subarachnoid hemorrhage, stroke or traumatic brain injury), autoregulation might be impaired (Rowland et al., 2012) and it is therefore possible that small increases in blood pressure caused by mechanical ventilation will be translated directly into CBF increases. Adjustments of ventilator settings over time might additionally confound comparisons of CBF within those patients. It should also be considered that expiratory loading theoretically affects venous return and may have independent effects on BOLD responsiveness irrespective of CBF. It is furthermore worth noting that whilst stimulus-evoked ASL responses appear to be relatively robust in the face of baseline CBF changes, BOLD is more sensitive to such alterations in baseline CBF (Brown et al., 2003).

Research involving altered respiration can also benefit from using ASL in combination with careful $PETCO_2$ control. The advantage of using ASL to detect respiratory-related CBF responses is that ASL gives higher temporal signal stability during longer stimulus blocks than BOLD FMRI (Wang et al., 2003), while not requiring the radioactive isotopes necessary for positron emission tomography (PET). The advantage of longer stimulus blocks is that longer periods of modulated breathing are more realistic with respect to patient experiences, while experimental respiratory sensations take some time to build-up (Evans et al., 2002). Furthermore, ASL allows the investigation of a single physiological parameter (i.e. CBF) as opposed to BOLD, which is a compounded signal encompassing different underlying physiological components, and which can therefore be influenced by physiological changes beyond CBF.

Conclusion

Inspiratory loading at 9 cmH_2O increases CBF by ~5%. We have demonstrated that this does not affect the ASL perfusion contrast, but we cannot exclude a systemic vascular effect that could potentially dampen the BOLD response in patients with clinical conditions involving decreased intrathoracic pressures. It appears that expiratory loading is better compensated for, as it does not change CBF when autoregulation is intact. Taking into account direct effects of intrathoracic pressure, especially during inspiratory loading, will allow the comparison of patients with altered respiratory physiology to healthy controls. Understanding how physiological factors may affect CBF in diseases such as COPD will level the path towards understanding disease mechanisms and drug action in the brains of patients with altered cerebrovascular and respiratory physiology.

Disclosure/Conflict of interest

All authors declared that they have no conflicts of interest.

Acknowledgments

The authors thank Dan Bulte, Jon Brooks and Matt Rowland for their critical reviews of earlier versions of this paper. This study was supported by the Medical Research Council (UK). AH was supported by the DAAD (Germany) and MK and AH were supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre based at The Oxford University Hospitals Trust Oxford. KM was supported by the Wellcome Trust. TO was supported by the Dunhill Medical Trust and the UK Stroke Association.

References

Banzett, R.B., Pedersen, S.H.S.H., Schwartzstein, R.M.R.M., Lansing, R.W.R.W., 2008. The affective dimension of laboratory dyspnea: air hunger is more unpleasant than work/effort. Am. J. Respir. Crit. Care Med. 177, 1384–1390.

Baydur, A., Behrakis, P.K., Zin, W.A., Jaeger, M., Milic-Emili, J., 1982. A simple method for assessing the validity of the esophageal balloon technique. Am. Rev. Respir. Dis. 126, 788–791.

Bell, C., Kowalchuk, J.M., Paterson, D.H., Scheuermann, B.W., Cunningham, D.A., 1999. The effects of caffeine on the kinetics of O2 uptake, CO2 production and expiratory ventilation in humans during the on-transient of moderate and heavy intensity exercise. Exp. Physiol. 84, 761–774.

Birnbaum, S., Barreiro, T.J., 2007. Methacholine challenge testing: identifying its diagnostic role, testing, coding, and reimbursement. Chest 131, 1932–1935.

Bishop, C.C., Powell, S., Rutt, D., Browse, N.L., 1986. Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. Stroke 17, 913–915.

Brown, G.G., Eyler Zorrilla, L.T., Georgy, B., Kindermann, S.S., Wong, E.C., Buxton, R.B., 2003. BOLD and perfusion response to finger-thumb apposition after acetazolamide administration: differential relationship to global perfusion. J. Cereb. Blood Flow Metab. 23, 829–837.

Chen, Y., Parrish, T.B.T.B., 2009. Caffeine dose effect on activation-induced BOLD and CBF responses. NeuroImage 46, 577–583.

Cohen, E.R., Ugurbil, K., Kim, S.-G., 2002. Effect of basal conditions on the magnitude and dynamics of the blood oxygenation level-dependent fMRI response. J. Cereb. Blood Flow Metab. 22, 1042–1053.

Cooke, W.H., Lurie, K.G., Rohrer, M.J., Convertino, V.A., 2006. Human autonomic and cerebrovascular responses to inspiratory impedance. J. Trauma 60, 1275–1283.

Dai, W., Garcia, D., Bazelaire, C., Alsop, D., 2008. Continuous flow driven inversion for arterial spin labeling using pulsed radiofrequency and gradient fields. Magn. Reson. Med. 60, 1488–1497.

Davies, H.W., Haldane, J.S., Priestley, J.G., 1919. The response to respiratory resistance. J. Physiol. 53, 60–69.

Droste, D.W., Ludemann, P., Anders, F., Kemeny, V., Thomas, M., Krauss, J.K., Ringelstein, E.B., 1999. Middle cerebral artery blood flow velocity, end-tidal pCO2 and blood pressure in patients with obstructive sleep apnea and in healthy subjects during continuous positive airway pressure breathing. Neurol. Res. 21, 737–741.

Evans, K.C., Shea, S.A., Saykin, A.J., 1999. Functional MRI localisation of central nervous system regions associated with volitional inspiration in humans. J. Physiol. 520 (2), 383–392.

Evans, K.C., Banzett, R.B., Adams, L., McKay, L., Frackowiak, R.S., Corfield, D.R., 2002. BOLD fMRI identifies limbic, paralimbic, and cerebellar activation during air hunger. J. Neurophysiol. 88, 1500–1511.

Gauthier, C.J., Madjar, C., Tancredi, F.B., Stefanovic, B., Hoge, R.D., 2011. Elimination of visually evoked BOLD responses during carbogen inhalation: implications for calibrated MRI. NeuroImage 54, 1001–1011.

Glover, G.H., Li, T.Q., Ress, D., 2000. Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. Magn. Reson. Med. 44, 162–167.

Hall, J.E., Guyton, A.C., 2011. Guyton and Hall Textbook of Medical Physiology, 12th ed. Saunders/Elsevier, Philadelphia, PA.

Harvey, A.K., Pattinson, K.T.S., Brooks, J.C.W., Mayhew, S.D., Jenkinson, M., Wise, R.G., 2008. Brainstem functional magnetic resonance imaging: disentangling signal from physiological noise. J. Magn. Reson. Imaging 28, 1337–1344.

Herigstad, M., Hayen, A., Wiech, K., Pattinson, K.T.S., 2011. Dyspnoea and the brain. Respir. Med. 105, 1–9.

Ide, K., Eliasziw, M., Poulin, M.J., 2003. Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic–hypercapnic range in humans. J. Appl. Physiol. 95, 129.

Isaev, G., Murphy, K., Guz, A., Adams, L., 2002. Areas of the brain concerned with ventilatory load compensation in awake man. J. Physiol. 539, 935–945.

Jenkinson, M., Bannister, P., Brady, M., Smith, S., 2002. Improved optimization for the robust and accurate linear registration and motion correction of brain images. NeuroImage 17, 825–841.

Kim, S.G., Rostrup, E., Larsson, H.B., Ogawa, S., Paulson, O.B., 1999. Determination of relative CMRO2 from CBF and BOLD changes: significant increase of oxygen consumption rate during visual stimulation. Magn. Reson. Med. 41, 1152–1161.

Kolbitsch, C., Lorenz, I.H., Hörmann, C., Schocke, M., Kremser, C., Zschiegner, F., Felber, S., Benzer, A., 2000. The impact of increased mean airway pressure on contrast-enhanced MRI measurement of regional cerebral blood flow (rCBF), regional cerebral blood volume (rCBV), regional mean transit time (rMTT), and regional cerebrovascular resistance (rCVR) in human vol. Hum. Brain Mapp. 11, 214–222.

Leonard, A.D., Allsager, C.M., Parker, J.L., Swami, A., Thompson, J.P., 2008. Comparison of central venous and external jugular venous pressures during repair of proximal femoral fracture. Br. J. Anaesth. 101 (2), 166–170.

Lorenz, I.H., Kolbitsch, C., Hörmann, C., Schocke, M., Kremser, C., Zschiegner, F., Felber, S., Benzer, A., 2001. Increasing mean airway pressure reduces functional MRI (fMRI) signal in the primary visual cortex. Magn. Reson. Imaging 19, 7–11.

Loucks, T.M., Poletto, C.J., Simonyan, K., Reynolds, C.L., Ludlow, C.L., 2007. Human brain activation during phonation and exhalation: common volitional control for two upper airway functions. NeuroImage 36, 131–143.

McKay, L.C., Evans, K.C., Frackowiak, R.S., Corfield, D.R., 2003. Neural correlates of voluntary breathing in humans. J. Appl. Physiol. 95, 1170–1178.

Montes de Oca, M., Rassulo, J., Celli, B.R., 1996. Respiratory muscle and cardiopulmonary function during exercise in very severe COPD. Am. J. Respir. Crit. Care Med. 154, 1284–1289.

Moulton, E.A., Elman, I., Pendse, G., Schmahmann, J., Becerra, L., Borsook, D., 2011. Aversion-related circuitry in the cerebellum: responses to noxious heat and unpleasant images. J. Neurosci. 31, 3795–3804.

Nielsen, A.N., Lauritzen, M., 2001. Coupling and uncoupling of activity-dependent increases of neuronal activity and blood flow in rat somatosensory cortex. J. Physiol. (Lond.) 533, 773–785.

Pattinson, K.T.S., Rogers, R., Mayhew, S.D., Tracey, I., Wise, R.G., 2007. Pharmacological FMRI: measuring opioid effects on the BOLD response to hypercapnia. J. Cereb. Blood Flow Metab. 27, 414–423.

Pattinson, K., Governo, R., MacIntosh, B., Russell, E.C., Corfield, D.R., Tracey, I., Wise, R.G., 2009. Opioids depress cortical centers responsible for the volitional control of respiration. J. Neurosci. 29 (25), 8177–8186.

Payne, S.J., 2006. A model of the interaction between autoregulation and neural activation in the brain. Math. Biosci. 204, 260–281.

Penfield, W., Rasmussen, T., 1950. The cerebral cortex of man : a clinical study of localization of function. Macmillan, New York.

Penfield, W., Roberts, L., 1959. Speech and Brain-mechanisms. Princeton University Press, Princeton.

Reinhard, M., Hetzel, A., Hinkov, V., Lücking, C.H., 2000. Cerebral haemodynamics during the Mueller manoeuvre in humans. Clin. Physiol. 20, 292–303.

Robertson, D., Frolich, J.C., Carr, R.K., Watson, J.T., Hollifield, J.W., Shand, D.G., Oates, J.A., 1978. Effects of caffeine on plasma renin activity, catecholamines and blood pressure. N. Engl. J. Med. 298, 181–186.

Rosenkranz, M.A., Busse, W.W., Johnstone, T., Swenson, C.A., Crisafi, G.M., Jackson, M.M., Bosch, J.A., Sheridan, J.F., Davidson, R.J., 2005. Neural circuitry underlying the interaction between emotion and asthma symptom exacerbation. Proc. Natl. Acad. Sci. U. S. A. 102, 13319–13324.

Rowland, M.J., Hadjipavlou, G., Kelly, M., Westbrook, J., Pattinson, K.T., 2012. Delayed cerebral ischaemia after subarachnoid haemorrhage: looking beyond vasospasm. Br. J. Anaesth. 109, 315–329.

Scharf, S.M., 1991. Cardiovascular effects of airways obstruction. Lung 169, 1–23.

Smith, S.M., 2002. Fast robust automated brain extraction. Hum. Brain Mapp. 17, 143–155.

Stalcup, S.A., Mellins, R.B., 1977. Mechanical forces producing pulmonary edema in acute asthma. N. Engl. J. Med. 297, 592–596.

Sturdy, G.A., Hillman, D.R., Green, D.J., Jenkins, S.C., Cecins, N.M., Eastwood, P.R., 2004. The effect of learning on ventilatory responses to inspiratory threshold loading in COPD. Respir. Med. 98, 1–8.

Takai, O., Brown, S., Liotti, M., 2010. Representation of the speech effectors in the human motor cortex: somatotopy or overlap? Brain Lang. 113, 39–44.

Tiecks, F.P., Douville, C., Byrd, S., Lam, A.M., Newell, D.W., 1996. Evaluation of impaired cerebral autoregulation by the Valsalva maneuver. Stroke 27, 1177–1182.

van Beek, A.H., Claassen, J.A., Rikkert, M.G., Jansen, R.W., 2008. Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. J. Cereb. Blood Flow Metab. 28, 1071–1085.

von Leupoldt, A., Seemann, N., Gugleva, T., Dahme, B., Leupoldt, A.V., 2007. Attentional distraction reduces the affective but not the sensory dimension of perceived dyspnea. Respir. Med. 101, 839–844.

von Leupoldt, A., Sommer, T., Kegat, S., Baumann, H.J., Klose, H., Dahme, B., Büchel, C., 2008. The unpleasantness of perceived dyspnea is processed in the anterior insula and amygdala. Am. J. Respir. Crit. Care Med. 177, 1026–1032.

von Leupoldt, A., Sommer, T., Kegat, S., Eippert, F., Baumann, H.J., Klose, H., Dahme, B., Büchel, C., 2009. Down-regulation of insular cortex responses to dyspnea and pain in asthma. Am. J. Respir. Crit. Care Med. 180, 232–238.

Wang, J., Aguirre, G.K., Kimberg, D.Y., Roc, A.C., Li, L., Detre, J.A., 2003. Arterial spin labeling perfusion fMRI with very low task frequency. Magn. Reson. Med. 49, 796–802.

Wise, R.G., Ide, K., Poulin, M.J., Tracey, I., 2004. Resting fluctuations in arterial carbon dioxide induce significant low frequency variations in BOLD signal. NeuroImage 21, 1652–1664.

Appendix IIc: Dyspnoea and the brain

Respiratory Medicine (2011) **105**, 809–817



available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed



REVIEW

Dyspnoea and the brain

Mari Herigstad [a,*], Anja Hayen [a], Katja Wiech [a,b], Kyle T.S. Pattinson [a]

[a] *Nuffield Department of Clinical Neuroscience, University of Oxford, John Radcliffe Hospital, Level 6, West Wing, Oxford OX3 9DU, UK*
[b] *Department of Psychology, University of Leuven, Leuven, Belgium*

Received 23 August 2010; accepted 28 December 2010
Available online 3 February 2011

KEYWORDS
Dyspnoea;
Neuroimaging;
Brain mechanisms

Summary
Chronic dyspnoea is a devastating symptom that debilitates millions of people worldwide. It causes a large burden on both patient and carer, and significant costs to society and health services. Treatment options are limited. Much effort has been directed at optimising lung function and improving exercise capacity, however, the brain mechanisms underlying dyspnoea perception have received less attention.

In this review, we focus on cognitive and affective aspects of dyspnoea and discuss how novel neuroimaging methods can provide quantitative measures of these subjective sensations. We draw parallels with the more advanced field of chronic pain, and explain some of the challenges faced when imaging dyspnoea.

To date, brain mechanisms of dyspnoea have been investigated in a handful of studies by a limited number of authors. These have found consistent activation in the insular cortex, the anterior cingulate cortex and the amygdala. Novel neuroimaging methods and an improved understanding of perceptual mechanisms underlying dyspnoea now position us to transform dyspnoea research. Future research should investigate how brain regions associated with dyspnoea interact, as well as accurately correlate this neuronal activation with reliable behavioural measures.

A better understanding of the brain processes underlying dyspnoea perception will lead to new therapies that will improve quality of life for a very large group of patients.

Contents

Introduction 810
Addressing the multidimensionality of dyspnoea 810

* Corresponding author. Tel.: +44 1865234544.
E-mail addresses: mari.herigstad@nda.ox.ac.uk (M. Herigstad), anja.hayen@nda.ox.ac.uk (A. Hayen), kwiech@fmrib.ox.ac.uk (K. Wiech), kyle.pattinson@nda.ox.ac.uk (K.T.S. Pattinson).

0954-6111/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2010.12.022

Borrowing ideas from pain research . . . 811
Neuroimaging of dyspnoea . . . 811
Pain research as a roadmap . . . 813
The challenges and potential of imaging . . . 813
Conclusions . . . 814
Funding . . . 814
Conflict of interest . . . 814
Acknowledgements . . . 814
References . . . 814

Introduction

Dyspnoea is the uncomfortable breathlessness suffered by millions of patients with illnesses as diverse as cardiac failure, cancer and chronic obstructive pulmonary disease (COPD). Few sensations can match dyspnoea for unpleasantness, and even with an artificial dyspnoea stimulus delivered under controlled laboratory conditions, subjects have reported a feeling of impending death.[1] In COPD, dyspnoea is an independent predictor of mortality as well as a cause for morbidity.[2] For simplicity, dyspnoea is often described in terms of air hunger, chest tightness, respiratory effort or a combination of these,[3] although the clinical state is in fact more complicated. Air hunger is the conscious sensation of being starved of air,[4] respiratory effort is frequently described in terms of work[4] and chest tightness appears to be associated with bronchoconstriction.[5] These can be independently manipulated in an experimental model of dyspnoea.[6]

Most healthy individuals will experience breathlessness, for example during physical exercise or conscious hyperventilation. These transient, reversible sensations are likely to be qualitatively different from the sensations of breathlessness experienced by dyspnoeic patients, which are, by their very nature, associated with threat to survival and linked to disease-related fear and anxieties. Terms such as 'rewarded' breathing (respiratory effort is matched by ventilation) and 'unrewarded' breathing (respiratory effort is not matched by ventilation, leading to an unpleasant sensation) may be used to differentiate between the normal breathlessness experienced by healthy individuals and the breathlessness suffered by patients.

The symptoms of dyspnoea vary between patients and are not fully explained by differences in disease severity (e.g. spirometric measures of lung function). Patients may exhibit low disease severity but report high levels of dyspnoea,[7] or *vice versa*.[8] Indeed, in idiopathic hyperventilation, no lung abnormalities are identified.[9] This suggests that the perception of dyspnoea is not necessarily linearly related to the sensory input, but is modulated by cognitive and affective factors.[10] Cognitive factors include attention, expectation and catastrophising, and affective factors include depression, anxiety and short-lasting moods.

Up until recently, most research into dyspnoea has focused on the lungs and muscles, while the cognitive and affective aspects have been less investigated.[11] In this paper, we will outline our current understanding of cognitive and affective factors in dyspnoea, and address how neuroimaging may provide objective measures of brain mechanisms in dyspnoea.

Addressing the multidimensionality of dyspnoea

A growing body of literature, including consensus statements from the American Thoracic Society[12] and the American College of Chest Physicians,[13] supports the view that chronic dyspnoea has a cognitive and affective dimension that is (partly) separate from the sensory intensity of breathlessness,[10,14,15] i.e. a multidimensional model (Fig. 1). However, our understanding of the cognitive and affective dimension remains limited.

Chronic dyspnoea has been linked to depression and anxiety,[16,17] and may be exacerbated by negative emotions,[18,19] including worry and fear.[6] Negative emotions and anxiety have also been linked to the perception of dyspnoea in healthy volunteers.[20] In COPD patients, high depression scores are consistently associated with poor exercise performance,[21] although the causal relationship has yet to be determined. Distraction may improve exercise tolerance in COPD patients,[22,23] suggesting a role for attention in the modulation of dyspnoea perception. Furthermore, expectation and affective factors may provide a possible explanation for variation in symptom perception that cannot be explained by severity of physical symptoms (reviewed by Jansson et al.[15]). Studies using electroencephalography (EEG) during brief inspiratory occlusions (less than 1 s) demonstrate that attention[24,25] and affective states[26] may modulate respiratory-related evoked potentials (RREP), specifically the P300/P3 peak of the RREP. This further supports the importance of attention and affective states in respiratory perception.

Pulmonary rehabilitation is a therapy that successfully alleviates the feeling of dyspnoea in patients suffering from COPD, yet does not affect lung function. A 2007 Cochrane review, taking into account 31 randomised controlled trials, found no significant change in maximal exercise capacity following pulmonary rehabilitation, but reported significant reduction in perception of dyspnoea and fatigue, and improvements in functional exercise capacity that were less striking.[27] More importantly, Carrieri-Kohlman et al.[28] have found that an exercise programme (one component of pulmonary rehabilitation) lowers dyspnoea-related anxiety to a greater extent than the dyspnoea itself. If the improvements seen with pulmonary rehabilitation were solely due to muscle training, then one would expect an equal decrease in dyspnoea intensity and dyspnoea-related anxiety in these patients. Exercise programmes have also been linked to improvements in cognitive function in COPD patients.[29] In addition, pulmonary rehabilitation includes

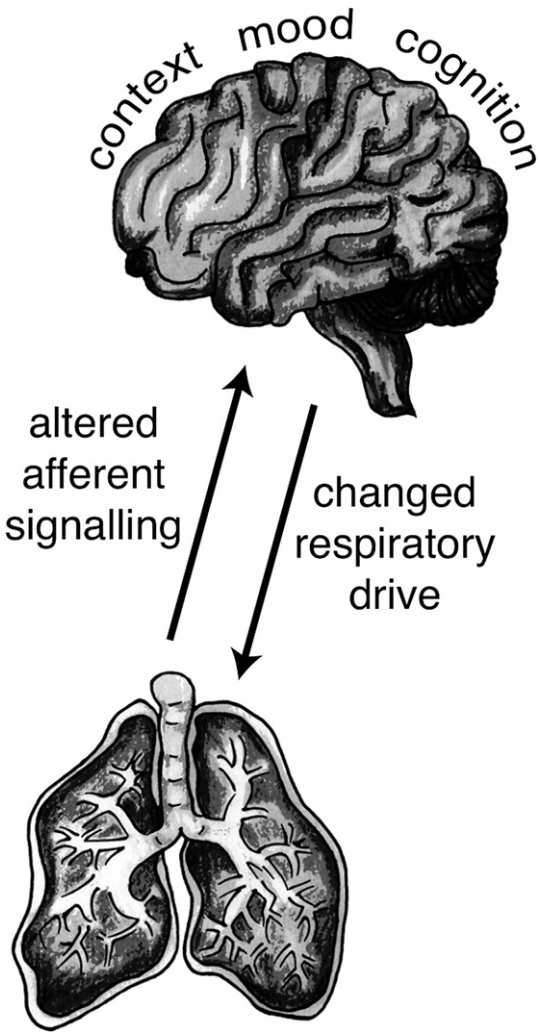


Figure 1 Schematic illustrating factors that influence afferent respiratory signalling to affect the perception of dyspnoea and respiratory drive.

cognitive and behavioural treatment paradigms, such as providing coping strategies, giving information and undertaking group therapy, which are components also found in cognitive behavioural therapy, an established treatment program for chronic pain.[30] As a therapy for chronic pain, cognitive behavioural therapy lowers anxiety levels and reduces symptom perception[31] without altering the underlying disease process. Pulmonary rehabilitation may reduce dyspnoea in a similar fashion for COPD.

Borrowing ideas from pain research

Comparing pain and dyspnoea has recently garnered attention,[32,33] including comparing the multidimensionality of dyspnoea with the more established multidimensional pain model.[34] Pain, like dyspnoea, is a highly subjective sensation, often associated with negative emotions.[35] Both dyspnoea and pain are closely linked to chronic and often severe conditions and the sensory intensity level in both conditions may vary irrespective of disease state. While studies on dyspnoea to date have largely focussed on anxiety and depression, the multidimensional pain model has grown to distinguish between and incorporate several centrally mediated components.[36,37] Expectation[38] and threat perception[39] may heighten the pain experience (and also anxiety levels[40]), and pain-related fear is a better predictor for disability than the sensory intensity itself.[41] Positive emotions may reduce while negative emotions may increase perceived pain,[42,43] similar to what has been observed in dyspnoea.[18,19] Attention may modulate pain perception i.e. distraction may reduce the perception of pain intensity and *vice versa*.[44] In pain, attention may alter both neural activation and its functional integration,[36] and this modulation has been linked to activation in the periaqueductal grey (PAG) of the brainstem.[45]

Addressing cognitive and affective factors that are well known in the pain field could provide further insight into the morbidity of chronic dyspnoea. Indeed, the novel dyspnoea-12 questionnaire specifically includes items on the affective dimension of dyspnoea,[46] studies on asthmatic patients have recently begun to incorporate assessments of catastrophic thinking in addition to anxiety assessments,[47] and the role of attention states has also garnered some interest.[22] Furthermore, based on the role of anxiety in the treatment of COPD using pulmonary rehabilitation,[27] it is reasonable to expect that previous experiences and expectations (i.e. memory and learning) are important to a similar extent in dyspnoea as has been shown in pain.[48] Indeed, studies using conditioning paradigms, such as different odours coupled with various respiratory challenges, have shown that previous experiences can subsequently alter respiratory responses and perception.[49–52]

While pain and dyspnoea certainly share some common features, it would be equally interesting to explore the brain mechanisms by which pain and dyspnoea differ, by addressing the factors that are unique to dyspnoea. Some of these differences have already been outlined in an earlier review.[35] To fully understand the cognitive and affective dimension of dyspnoea, we should investigate the brain mechanisms underlying these factors rather than treating the brain as a black box, and to do this, neuroimaging is one of our best bets.

Neuroimaging of dyspnoea

To date, neuroimaging studies on dyspnoea are rare. Early neuroimaging studies have demonstrated the importance of the insular cortex[53–58] and also implied a role for the anterior cingulate cortex[58,59] and the amygdala[54–56,58,60] in experimental dyspnoea (Table 1).

It is difficult to draw solid conclusions based on these results due to both the limited number of studies and to the great variation in experimental methods used in these studies (for the studies listed in Table 1, no less than three different types of respiratory stimuli have been employed). For example, while it is possible that the activation of the anterior cingulate reported by Evans et al.[58] may have been a consequence of using a constantly changing stimulus, it is equally possible that the absence of activation in the same region observed by Von Leupoldt et al.[60] could have been due to a type 2 error (perhaps physiological noise[61]). As such, we may still only speculate on the role of the anterior cingulate in dyspnoea. Interpretation of results is further complicated as these experimental methods may to various degrees also cause changes in neural control of breathing

Table 1 Brain regions identified in neuroimaging studies of dyspnoea.

Author(s)	Year	Brain region activations reported	Method	Stimulus
Liotti et al.[55] Brannan et al.[54] Parsons et al.[56]	2001	Amygdala/periamygdala Anterior insula/claustrum Bilateral anterior temporal poles Bilateral lingual gyri Cerebellum Cingulate cortex Dorsal anterior/middle cingulate gyrus Fusiform gyri Hypothalamus Hippocampus Inferior pre- and orbitofrontal cortex[a] Left inferior parietal lobule[a] Left lentiform/caudate nuclei[a] Left thalamus[a] Lingual/middle temporal gyrus Midbrain tegmentum Parahippocampal regions Periaqueductal grey Pons Pulvinar Right middle insula[a] Right precentral gyrus[a] Right dorsal prefrontal cortex	PET	Hypercapnia, CO_2 baseline raised from 41.0 mmHg to 66.1 mmHg for ~3 min
Banzett et al.[53]	2000	Mid/anterior insula	PET	Hypercapnia, P_{ETCO_2} constant at 44.0 mmHg and high and low tidal volume for 210 s
Peiffer et al.[57]	2001	Cerebellar vermis Left medial pons Right anterior insula/claustrum	PET	Inspiratory and expiratory resistive loads, 13.3 cmH_2O for 90 s
Evans et al.[58]	2002	Amygdala Anterior cingulate Anterior insula Basal ganglia Cerebellum Operculum Prefrontal cortex Thalamus	BOLD FMRI	Mechanical ventilation, P_{ETCO_2} constant at 41.0 mmHg and high and low tidal volume for 60 s
Peiffer et al.[59]	2008	Anterior cingulate cortex[b] Caudate nucleus Cerebellum Prefrontal cortex[a] Premotor cortex	PET	Inspiratory and expiratory resistive loads, 19.4 cmH_2O for 100 s
Von Leupoldt et al.[60]	2008	Amygdala[c] Bilateral insular cortex Right anterior insular cortex[c] Sensorimotor cortex Supplemental motor area	BOLD FMRI	Inspiratory resistive load, 13.3 cmH_2O for 24 s
Von Leupoldt et al.[84]	2009	Insula Periaqueductal gray	BOLD FMRI	Inspiratory resistive load, 13.3 cmH_2O (patients) and 12.2 cmH_2O (controls) for 24 s
Von Leupoldt et al.[83]	2009	Amygdala Dorsal/rostral anterior cingulate cortex Insula Medial thalamus Operculum/Somatosensory cortex II Sensorymotor cortex Supplementary motor area	BOLD FMRI	Inspiratory resistive load, 12.2 cmH_2O for 24 s

(PET, Positron Emission Tomography; BOLD, Blood Oxygen Level Dependent; FMRI, Functional Magnetic Resonance Imaging, P_{ETCO_2}, end-tidal PCO_2).

[a] deactivation.

[b] associated with relief from dyspnoea.

[c] associated with perceived unpleasantness of dyspnoea.

that is not specific to dyspnoea. Last but certainly not least, all neuroimaging studies to date have investigated acute experimental dyspnoea in healthy volunteers, but no studies have yet looked at chronic dyspnoea. Acute experimental dyspnoea induced in healthy volunteers is likely to be qualitatively very different from the chronic dyspnoea experienced by patients, since the former is experienced in a safe, experimental setting while the latter is often influenced by disease-related fear and anxiety. This is an area that requires further study.

There have also been a few neuroimaging studies investigating brain mechanisms of control of respiration other than dyspnoea. Brainstem activation has been observed both during volitional control of breathing (breath hold, voluntary hyperpnoea, Valsalva manoeuvre)[57,62–64] and during automatic or unconscious control of breathing (chemo-stimulated ventilation).[65,66] Studies of volitional breathing have suggested the involvement of a range of cortical areas involved in breathing,[62,64,67,68] of which some, including the insular cortex[64,67] and caudate nucleus,[67] have also been identified in dyspnoea.[58,59] Similarly, neuroimaging studies on chemo-stimulated increases in ventilation have shown activation in the basal ganglia, parietal cortex, cerebellum, midbrain, occipital lobe, the frontal cortex[69,70] and the limbic system.[66,70] While not addressing dyspnoea directly, imaging studies on respiratory control may assist in identifying and separating the brain regions that are involved in other breathing responses from those that are unique for dyspnoea.

Pain research as a roadmap

Whereas neuroimaging of dyspnoea is in its infancy, these techniques have revolutionised our understanding of mechanisms underlying chronic pain perception. Neuroimaging has led to the identification of what has (perhaps unfortunately[71]) been termed the pain matrix, which includes the thalamus and the primary and secondary somatosensory, insular, anterior cingulate and prefrontal cortices.[72,73] These brain regions are associated with, but not unique to, pain, and comprise a network which activation may be altered depending on the factors involved in pain perception.[71] Furthermore, several modulatory networks that may control nociceptive processing in the central nervous system have also been identified. The most extensively investigated of these is the descending pain modulatory system, which is thought to underlie the analgesia observed during fight-or-flight responses and includes the hypothalamus, the frontal lobe, anterior cingulate cortex, insula, amygdala, PAG, nucleus cuneiformis and the rostral ventromedial medulla.[72]

In light of the similarities between dyspnoea and pain, it is not surprising that the brain regions that so far have been associated with dyspnoea (the insular cortex, anterior cingulate cortex and the amygdala) have also been implicated in pain perception.[73,74] The insula plays a central role in the conscious awareness of body state[75] and is important in pain perception[76]. The anterior cingulate cortex, which is involved in emotional processing,[77] has been widely implicated in cognitive and affective pain processing.[37,78] The amygdala, which has a well-established involvement in emotion processing[79] and conditioning,[80] is thought to be central to the modulation of pain,[81] possibly through attention.[82] Interactions between brain regions are central to most sensory perception, and identifying networks that govern higher-order functions is therefore crucial to further our understanding of dyspnoea. One could reasonably speculate that the brain regions associated with dyspnoea are part of a complex network that is shared in part with other types of sensations, including pain. Indeed, early observational studies have shown some commonalities in brain activation between pain and dyspnoea,[83–85] highlighting the insular cortex, anterior cingulate cortex, amygdala, PAG and thalamus. To better address the clinical aspects of dyspnoea, this line of investigation may be continued using longer, more realistic, dyspnoea stimuli as well as sophisticated methods for physiological noise correction. Intervention studies that carefully link brain imaging results to reliable behavioural measures may also improve our current understanding of dyspnoea. This would generate a well-controlled, more detailed picture of the brain mechanisms involved in dyspnoea specifically, and assist in disentangling these from brain areas that are merely generally associated with unpleasant sensations.

It is our opinion, however, that dyspnoea is not merely a carbon copy of pain. Patients suffering from dyspnoea often have very different pathologies, and it is likely that the psychological components of these conditions are also not identical. As such, blindly copying pain research will most likely not be fruitful in dyspnoea research. Nevertheless, considering the commonalities between dyspnoea and pain, using the more advanced pain field as a roadmap for dyspnoea research might be a reasonable strategy,[33,35] a point which was raised as early as 1956.[86] For example, the insula may play a part in mediating the influence of expectations (including anxiety) on the experience of dyspnoea, as has been suggested for pain.[37] A study on relief from dyspnoea[59] has suggested the presence of a dyspnoea modulatory network, similar to what has been observed in pain. Investigating relief and reward processing may aid the development of new therapeutic targets and would mirror research previously done in pain.[87] Furthermore, results from pain research have highlighted brain regions that may be prospective targets in future dyspnoea research. For example, one might also expect the prefrontal cortex to be important for dyspnoea (as in pain[88,89]). Indeed, Evans et al.[58] did report activation in the prefrontal cortex, however, other studies have failed to repeat this observation.

The challenges and potential of imaging

Magnetic resonance imaging (MRI) may be crucial to the further identification of brain mechanisms in dyspnoea. Two techniques in particular are considered to hold a lot of potential; blood oxygen level dependant (BOLD) functional MRI (FMRI)[90] and arterial spin labelling (ASL).[91]

BOLD FMRI is an indirect measure of changes in cerebral blood flow, while ASL is a measure of cerebral blood flow in absolute units using an endogenous tracer (magnetically labelled water protons). BOLD FMRI offers high spatial resolution, but has limited sensitivity for low-frequency tasks and cannot be used to measure a tonic state, such as chronic dyspnoea. ASL does not share this limitation, and may therefore be important to further investigations of chronic conditions. Compared with BOLD FMRI, ASL has

certain drawbacks such as smaller spatial coverage, relatively low signal-to-noise ratio (which in turn introduces a need for more subjects and/or longer imaging time), however, novel acquisition schemes are addressing these limitations,[92] making ASL a much more attractive imaging technique.

The successful further investigation of brain mechanisms underlying dyspnoea depends on several factors. Firstly, it is important to use a realistic dyspnoea stimulus that is compatible with FMRI. Physiological noise relating to respiration degrades MR image quality[61] and minor alterations in blood gases may affect the interpretation of the FMRI signal.[65,93,94] Satisfactorily addressing these challenges is crucial to future dyspnoea research. Secondly, it is important to look beyond experimentally induced dyspnoea. This, however, introduces practical concerns such as the reduced ability of some patients to be supine for an extended period of time. Furthermore, some patients suffer primarily from exertion-related dyspnoea, which may be difficult to induce in a manner that is compatible with FMRI. Finally, it is highly important that future research uses appropriate experimental paradigms in order to accurately correlate neuronal activation with reliable behavioural measures. Future research should therefore employ predictive, and thus testable, models of brain function (e.g.[95]), rather than aimlessly search for static "blobs" of activation.

Measuring electrical activity in the brain with magnetoencephalography (MEG) and EEG also holds great potential for investigating dyspnoea mechanisms. Synaptic transmission creates an electrical current in the brain (measured by EEG), which in turn generates magnetic field variations (measured by MEG). As such, MEG and EEG are direct measures of synaptic transmission in the brain, unlike FMRI, which is indirect due to its haemodynamic nature. MEG and EEG have very high temporal resolution (i.e. milliseconds), are non-invasive and can be conducted in an upright position, which may be of benefit when studying patients suffering from severe orthopnoea.[96] MEG is less susceptible to signal loss across the skull than EEG and displays better spatial resolution, but is more expensive than EEG by far. However, both MEG and EEG are ill equipped to measure subcortical activation and cannot provide structural information.

With the advent of even more sophisticated acquisition schemes, such as more powerful MRI scanners (3T and above), neuroimaging will become even more central to dyspnoea research.

Conclusions

Dyspnoea research lags years behind pain research. In light of the similarities between chronic dyspnoea and chronic pain, a number of concepts successfully used in the study of pain might reasonably be applied to guide the future approaches to dyspnoea research. However, one should be careful not to automatically assume similarities between the two conditions. It is highly important for the future of dyspnoea research and, ultimately, dyspnoea treatment, to invest time and effort into identifying and addressing factors that are unique to dyspnoea. These factors must be seen in the context of dyspnoea as a multidimensional disease, in which there may often be a mismatch between disease severity and symptom perception. Cognitive and affective factors, including how threat and disease-related anxiety affect symptom perception, are thus crucial to our understanding of dyspnoea and should be incorporated in future research. Investigating brain mechanisms underlying dyspnoea perception is likely to aid the development of novel therapies. This includes identifying new drug targets, building the capacity to non-invasively measure responses to novel treatments, i.e. making quantitative measures of hitherto subjective sensations, and also paving the way for individualised treatments. In order to identify novel treatments, the cognitive and affective dimension of dyspnoea must be better understood, building both on clinical observations and on new neuroimaging techniques.

Funding

KP & MH are supported by the UK Medical Research Council. This work was supported by the National Institute of Health Research (NIHR).

Conflict of interest

None.

Acknowledgements

Thank you to Professor Robert Davies, Dr Annabel Nickol, Dr Stuart McKechnie and Professor Richard Wise for commenting on an earlier draft of this paper.

References

1. Banzett RB, Lansing RW, Brown R, Topulos GP, Yager D, Steele SM, Londono B, Loring SH, Reid MB, Adams L, et al. 'Air hunger' from increased PCO_2 persists after complete neuromuscular block in humans. *Respir Physiol* 1990;**81**:1—17.
2. Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, Pinto Plata V, Cabral HJ. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. *N Engl J Med* 2004;**350**:1005—12.
3. Schwartzstein RM, Manning HL, Weiss JW, Weinberger SE. Dyspnea: a sensory experience. *Lung* 1990;**168**:185—99.
4. Lansing RW, Im BS, Thwing JI, Legedza AT, Banzett RB. The perception of respiratory work and effort can be independent of the perception of air hunger. *Am J Respir Crit Care Med* 2000;**162**:1690—6.
5. Moy ML, Woodrow Weiss J, Sparrow D, Israel E, Schwartzstein RM. Quality of dyspnea in bronchoconstriction differs from external resistive loads. *Am J Respir Crit Care Med* 2000;**162**:451—5.
6. Banzett RB, Pedersen SH, Schwartzstein RM, Lansing RW. The affective dimension of laboratory dyspnea: air hunger is more unpleasant than work/effort. *Am J Respir Crit Care Med* 2008;**177**:1384—90.
7. Teeter JG, Bleecker ER. Relationship between airway obstruction and respiratory symptoms in adult asthmatics. *Chest* 1998;**113**:272—7.
8. Banzett RB, Dempsey JA, O'Donnell DE, Wamboldt MZ. Symptom perception and respiratory sensation in asthma. *Am J Respir Crit Care Med* 2000;**162**:1178—82.
9. Jack S, Rossiter HB, Pearson MG, Ward SA, Warburton CJ, Whipp BJ. Ventilatory responses to inhaled carbon dioxide,

hypoxia, and exercise in idiopathic hyperventilation. *Am J Respir Crit Care Med* 2004;**170**:118—25.
10. De Peuter S, Van Diest I, Lemaigre V, Verleden G, Demedts M, Van den Bergh O. Dyspnea: the role of psychological processes. *Clin Psychol Rev* 2004;**24**:557—81.
11. O'Donnell DE, Banzett RB, Carrieri-Kohlman V, Casaburi R, Davenport PW, Gandevia SC, Gelb AF, Mahler DA, Webb KA. Pathophysiology of dyspnea in chronic obstructive pulmonary disease: a roundtable. *Proc Am Thorac Soc* 2007;**4**:145—68.
12. Dyspnea. Mechanisms, assessment, and management: a consensus statement. American Thoracic Society. *Am J Respir Crit Care Med* 1999;**159**:321—40.
13. Mahler DA, Selecky PA, Harrod CG, Benditt JO, Carrieri-Kohlman V, Curtis JR, Manning HL, Mularski RA, Varkey B, Campbell M, Carter ER, Chiong JR, Ely EW, Hansen-Flaschen J, O'Donnell DE, Waller A. American College of Chest Physicians consensus statement on the management of dyspnea in patients with advanced lung or heart disease. Chest; 137:674—91.
14. von Leupoldt A, Dahme B. Psychological aspects in the perception of dyspnea in obstructive pulmonary diseases. *Respir Med* 2007; **101**:411—22.
15. Janssens T, Verleden G, De Peuter S, Van Diest I, Van den Bergh O. Inaccurate perception of asthma symptoms: a cognitive-affective framework and implications for asthma treatment. *Clin Psychol Rev* 2009;**29**:317—27.
16. Hill K, Geist R, Goldstein RS, Lacasse Y. Anxiety and depression in end-stage COPD. *Eur Respir J* 2008;**31**:667—77.
17. Carrieri-Kohlman V, Donesky-Cuenco D, Park SK, Mackin L, Nguyen HQ, Paul SM. Additional evidence for the affective dimension of dyspnea in patients with COPD. *Res Nurs Health* 2010;**33**:4—19.
18. Von Leupoldt A, Riedel F, Dahme B. The impact of emotions on the perception of dyspnea in pediatric asthma. *Psychophysiology* 2006;**43**:641—4.
19. Bogaerts K, Notebaert K, Van Diest I, Devriese S, De Peuter S, Van den Bergh O. Accuracy of respiratory symptom perception in different affective contexts. *J Psychosom Res* 2005;**58**: 537—43.
20. Wan L, Van Diest I, De Peuter S, Bogaerts K, Oyen N, Hombroux N, Van de Woestijne K, Gallego J, Van den Bergh O. Repeated experiences of air hunger and ventilatory behavior in response to hypercapnia in the standardized rebreathing test: effects of anxiety. *Biol Psychol* 2008;**77**:223—32.
21. Spruit MA, Watkins ML, Edwards LD, Vestbo J, Calverley PM, Pinto-Plata V, Celli BR, Tal-Singer R, Wouters EF. Determinants of poor 6-min walking distance in patients with COPD: the ECLIPSE cohort. *Respir Med* 2010;**104**:849—57.
22. Bauldoff GS, Hoffman LA, Zullo TG, Sciurba FC. Exercise maintenance following pulmonary rehabilitation: effect of distractive stimuli. *Chest* 2002;**122**:948—54.
23. Thornby MA, Haas F, Axen K. Effect of distractive auditory stimuli on exercise tolerance in patients with COPD. *Chest* 1995;**107**:1213—7.
24. Davenport PW, Chan PY, Zhang W, Chou YL. Detection threshold for inspiratory resistive loads and respiratory-related evoked potentials. *J Appl Physiol* 2007;**102**:276—85.
25. Webster KE, Colrain IM. The respiratory-related evoked potential: effects of attention and occlusion duration. *Psychophysiology* 2000;**37**:310—8.
26. Von Leupoldt A, Vovk A, Bradley MM, Keil A, Lang PJ, Davenport PW. The impact of emotion on respiratory-related evoked potentials. *Psychophysiology* 2010;**47**:579—86.
27. Lacasse Y, Martin S, Lasserson TJ, Goldstein RS. Meta-analysis of respiratory rehabilitation in chronic obstructive pulmonary disease. A Cochrane systematic review. *Eur Medicophys* 2007; **43**:475—85.
28. Carrieri-Kohlman V, Gormley JM, Eiser S, Demir-Deviren S, Nguyen H, Paul SM, Stulbarg MS. Dyspnea and the affective response during exercise training in obstructive pulmonary disease. *Nurs Res* 2001;**50**:136—46.
29. Dodd JW, Getov SV, Jones PW. Cognitive function in COPD. *Eur Respir J* 2010;**35**:913—22.
30. Morley S, Eccleston C, Williams A. Systematic review and meta-analysis of randomized controlled trials of cognitive behaviour therapy and behaviour therapy for chronic pain in adults, excluding headache. *Pain* 1999;**80**:1—13.
31. Leeuw M, Goossens ME, Linton SJ, Crombez G, Boersma K, Vlaeyen JW. The fear-avoidance model of musculoskeletal pain: current state of scientific evidence. *J Behav Med* 2007; **30**:77—94.
32. von Leupoldt A, Dahme B. Experimental comparison of dyspnea and pain. *Behav Res Methods* 2007;**39**:137—43.
33. Banzett RB, Moosavi SH. Dyspnea and pain: similarities and contrasts between two very unpleasant sensations. *APS Bull* 2001;**11**:1—6.
34. Lansing RW, Gracely RH, Banzett RB. The multiple dimensions of dyspnea: review and hypotheses. *Respir Physiol Neurobiol* 2009;**167**:53—60.
35. Gracely RH, Undem BJ, Banzett RB. Cough, pain and dyspnoea: similarities and differences. *Pulm Pharmacol Ther* 2007;**20**: 433—7.
36. Wiech K, Ploner M, Tracey I. Neurocognitive aspects of pain perception. *Trends Cogn Sci* 2008;**12**:306—13.
37. Wiech K, Tracey I. The influence of negative emotions on pain: behavioral effects and neural mechanisms. *Neuroimage* 2009; **47**:987—94.
38. Fairhurst M, Wiech K, Dunckley P, Tracey I. Anticipatory brainstem activity predicts neural processing of pain in humans. *Pain* 2007; **128**:101—10.
39. Van Damme S, Crombez G, Van Nieuwenborgh-De Wever K, Goubert L. Is distraction less effective when pain is threatening? An experimental investigation with the cold pressor task. *Eur J Pain* 2008;**12**:60—7.
40. Arntz A, Claassens L. The meaning of pain influences its experienced intensity. *Pain* 2004;**109**:20—5.
41. Crombez G, Vlaeyen JW, Heuts PH, Lysens R. Pain-related fear is more disabling than pain itself: evidence on the role of pain-related fear in chronic back pain disability. *Pain* 1999;**80**: 329—39.
42. de Wied M, Verbaten MN. Affective pictures processing, attention, and pain tolerance. *Pain* 2001;**90**:163—72.
43. Weisenberg M, Raz T, Hener T. The influence of film-induced mood on pain perception. *Pain* 1998;**76**:365—75.
44. Villemure C, Bushnell MC. Cognitive modulation of pain: how do attention and emotion influence pain processing? *Pain* 2002; **95**:195—9.
45. Tracey I, Ploghaus A, Gati JS, Clare S, Smith S, Menon RS, Matthews PM. Imaging attentional modulation of pain in the periaqueductal gray in humans. *J Neurosci* 2002;**22**: 2748—52.
46. Yorke J, Moosavi SH, Shuldham C, Jones PW. Quantification of dyspnoea using descriptors: development and initial testing of the dyspnoea-12. *Thorax* 2010;**65**:21—6.
47. De Peuter S, Lemaigre V, Van Diest I, Verleden G, Demedts M, Van den Bergh O. Differentiation between the sensory and affective aspects of histamine-induced bronchoconstriction in asthma. *Respir Med* 2007;**101**:925—32.
48. Gedney JJ, Logan H. Pain related recall predicts future pain report. *Pain* 2006;**121**:69—76.
49. Van den Bergh O, Stegen K, Van de Woestijne KP. Memory effects on symptom reporting in a respiratory learning paradigm. *Health Psychol* 1998;**17**:241—8.
50. Stegen K, De Bruyne K, Rasschaert W, Van de Woestijne KP, Van den Bergh O. Fear-relevant images as conditioned stimuli for somatic complaints, respiratory behavior, and reduced end-tidal PCO_2. *J Abnorm Psychol* 1999;**108**:143—52.

51. De Peuter S, Van Diest I, Lemaigre V, Li W, Verleden G, Demedts M, Van den Bergh O. Can subjective asthma symptoms be learned? *Psychosom Med* 2005;**67**:454—61.
52. Meek PM. Influence of attention and judgment on perception of breathlessness in healthy individuals and patients with chronic obstructive pulmonary disease. *Nurs Res* 2000;**49**:11—9.
53. Banzett RB, Mulnier HE, Murphy K, Rosen SD, Wise RJ, Adams L. Breathlessness in humans activates insular cortex. *Neuroreport* 2000;**11**:2117—20.
54. Brannan S, Liotti M, Egan G, Shade R, Madden L, Robillard R, Abplanalp B, Stofer K, Denton D, Fox PT. Neuroimaging of cerebral activations and deactivations associated with hypercapnia and hunger for air. *Proc Natl Acad Sci U S A* 2001;**98**:2029—34.
55. Liotti M, Brannan S, Egan G, Shade R, Madden L, Abplanalp B, Robillard R, Lancaster J, Zamarripa FE, Fox PT, Denton D. Brain responses associated with consciousness of breathlessness (air hunger). *Proc Natl Acad Sci U S A* 2001;**98**:2035—40.
56. Parsons LM, Egan G, Liotti M, Brannan S, Denton D, Shade R, Robillard R, Madden L, Abplanalp B, Fox PT. Neuroimaging evidence implicating cerebellum in the experience of hypercapnia and hunger for air. *Proc Natl Acad Sci U S A* 2001;**98**:2041—6.
57. Peiffer C, Poline JB, Thivard L, Aubier M, Samson Y. Neural substrates for the perception of acutely induced dyspnea. *Am J Respir Crit Care Med* 2001;**163**:951—7.
58. Evans KC, Banzett RB, Adams L, McKay L, Frackowiak RS, Corfield DR. BOLD fMRI identifies limbic, paralimbic, and cerebellar activation during air hunger. *J Neurophysiol* 2002;**88**:1500—11.
59. Peiffer C, Costes N, Herve P, Garcia-Larrea L. Relief of dyspnea involves a characteristic brain activation and a specific quality of sensation. *Am J Respir Crit Care Med* 2008;**177**:440—9.
60. von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, Buchel C. The unpleasantness of perceived dyspnea is processed in the anterior insula and amygdala. *Am J Respir Crit Care Med* 2008;**177**:1026—32.
61. Harvey AK, Pattinson KT, Brooks JC, Mayhew SD, Jenkinson M, Wise RG. Brainstem functional magnetic resonance imaging: disentangling signal from physiological noise. *J Magn Reson Imaging* 2008;**28**:1337—44.
62. McKay LC, Evans KC, Frackowiak RS, Corfield DR. Neural correlates of voluntary breathing in humans. *J Appl Physiol* 2003;**95**:1170—8.
63. Henderson LA, Woo MA, Macey PM, Macey KE, Frysinger RC, Alger JR, Yan-Go F, Harper RM. Neural responses during Valsalva maneuvers in obstructive sleep apnea syndrome. *J Appl Physiol* 2003;**94**:1063—74.
64. McKay LC, Adams L, Frackowiak RS, Corfield DR. A bilateral cortico-bulbar network associated with breath holding in humans, determined by functional magnetic resonance imaging. *Neuroimage* 2008;**40**:1824—32.
65. Pattinson KT, Mitsis GD, Harvey AK, Jbabdi S, Dirckx S, Mayhew SD, Rogers R, Tracey I, Wise RG. Determination of the human brainstem respiratory control network and its cortical connections in vivo using functional and structural imaging. *Neuroimage* 2009;**44**:295—305.
66. Evans KC, Dougherty DD, Schmid AM, Scannell E, McCallister A, Benson H, Dusek JA, Lazar SW. Modulation of spontaneous breathing via limbic/paralimbic-bulbar circuitry: an event-related fMRI study. *Neuroimage* 2009;**47**:961—71.
67. Pattinson KT, Governo RJ, MacIntosh BJ, Russell EC, Corfield DR, Tracey I, Wise RG. Opioids depress cortical centers responsible for the volitional control of respiration. *J Neurosci* 2009;**29**:8177—86.
68. Brown S, Ngan E, Liotti M. A larynx area in the human motor cortex. *Cereb Cortex* 2008;**18**:837—45.
69. McKay LC, Critchley HD, Murphy K, Frackowiak RS, Corfield DR. Sub-cortical and brainstem sites associated with chemo-stimulated increases in ventilation in humans. *Neuroimage* 2010;**49**:2526—35.
70. Corfield DR, Fink GR, Ramsay SC, Murphy K, Harty HR, Watson JD, Adams L, Frackowiak RS, Guz A. Evidence for limbic system activation during CO_2-stimulated breathing in man. *J Physiol* 1995;**488**(Pt 1):77—84.
71. Tracey I. Nociceptive processing in the human brain. *Curr Opin Neurobiol* 2005;**15**:478—87.
72. Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. *Neuron* 2007;**55**:377—91.
73. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. *Eur J Pain* 2005;**9**:463—84.
74. Casey KL. Forebrain mechanisms of nociception and pain: analysis through imaging. *Proc Natl Acad Sci U S A* 1999;**96**:7668—74.
75. Craig AD. How do you feel—now? The anterior insula and human awareness. *Nat Rev Neurosci* 2009;**10**:59—70.
76. Treede RD, Kenshalo DR, Gracely RH, Jones AK. The cortical representation of pain. *Pain* 1999;**79**:105—11.
77. Beckmann M, Johansen-Berg H, Rushworth MF. Connectivity-based parcellation of human cingulate cortex and its relation to functional specialization. *J Neurosci* 2009;**29**:1175—90.
78. Villemure C, Bushnell MC. Mood influences supraspinal pain processing separately from attention. *J Neurosci* 2009;**29**:705—15.
79. Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron* 2005;**48**:175—87.
80. Chavez CM, McGaugh JL, Weinberger NM. The basolateral amygdala modulates specific sensory memory representations in the cerebral cortex. *Neurobiol Learn Mem* 2009;**91**:382—92.
81. Neugebauer V, Li W, Bird GC, Han JS. The amygdala and persistent pain. *Neuroscientist* 2004;**10**:221—34.
82. Zald DH. The human amygdala and the emotional evaluation of sensory stimuli. *Brain Res Brain Res Rev* 2003;**41**:88—123.
83. von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, Buchel C. Dyspnea and pain share emotion-related brain network. *Neuroimage* 2009;**48**:200—6.
84. von Leupoldt A, Sommer T, Kegat S, Eippert F, Baumann HJ, Klose H, Dahme B, Buchel C. Down-regulation of insular cortex responses to dyspnea and pain in asthma. *Am J Respir Crit Care Med* 2009;**180**:232—8.
85. Schon D, Rosenkranz M, Regelsberger J, Dahme B, Buchel C, von Leupoldt A. Reduced perception of dyspnea and pain after right insular cortex lesions. *Am J Respir Crit Care Med* 2008;**178**:1173—9.
86. Comroe JH. Dyspnea. *Mod Concepts Cardiovasc Dis* 1956;**25**:347—9.
87. Leknes S, Brooks JC, Wiech K, Tracey I. Pain relief as an opponent process: a psychophysical investigation. *Eur J Neurosci* 2008;**28**:794—801.
88. Mohr C, Leyendecker S, Mangels I, Machner B, Sander T, Helmchen C. Central representation of cold-evoked pain relief in capsaicin induced pain: an event-related fMRI study. *Pain* 2008;**139**:416—30.
89. Bingel U, Tracey I. Imaging CNS modulation of pain in humans. *Physiology (Bethesda)* 2008;**23**:371—80.
90. Matthews PM, Jezzard P. Functional magnetic resonance imaging. *J Neurol Neurosurg Psychiatry* 2004;**75**:6—12.
91. Williams DS, Detre JA, Leigh JS, Koretsky AP. Magnetic resonance imaging of perfusion using spin inversion of arterial water. *Proc Natl Acad Sci U S A* 1992;**89**:212—6.
92. Dai W, Garcia D, de Bazelaire C, Alsop DC. Continuous flow-driven inversion for arterial spin labeling using pulsed radio frequency and gradient fields. *Magn Reson Med* 2008;**60**:1488—97.

93. Wise RG, Pattinson KT, Bulte DP, Chiarelli PA, Mayhew SD, Balanos GM, O'Connor DF, Pragnell TR, Robbins PA, Tracey I, Jezzard P. Dynamic forcing of end-tidal carbon dioxide and oxygen applied to functional magnetic resonance imaging. *J Cereb Blood Flow Metab* 2007;**27**:1521—32.
94. Pattinson KT, Rogers R, Mayhew SD, Tracey I, Wise RG. Pharmacological FMRI: measuring opioid effects on the BOLD response to hypercapnia. *J Cereb Blood Flow Metab* 2007;**27**:414—23.
95. Behrens TE, Hunt LT, Woolrich MW, Rushworth MF. Associative learning of social value. *Nature* 2008;**456**:245—9.
96. Dorman S, Jolley C, Abernethy A, Currow D, Johnson M, Farquhar M, Griffiths G, Peel T, Moosavi S, Byrne A, Wilcock A, Alloway L, Bausewein C, Higginson I, Booth S. Researching breathlessness in palliative care: consensus statement of the national cancer research Institute palliative care breathlessness subgroup. *PaS200lliat Med* 2009;**23**:213—27.

Appendix IId: Effects of inspiratory and expiratory loading upon global and stimulus evoked CBF

Effects of inspiratory and expiratory loading upon global and stimulus evoked CBF

A. Hayen[1,2], M. Herigstad[1,2], R. G. Wise[3], and K. T. Pattinson[1,2]

[1]Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, Oxfordshire, United Kingdom, [2]Oxford Centre for Functional Magnetic Resonance Imaging of the Brain, Oxford, Oxfordshire, United Kingdom, [3]School of Psychology, Cardiff University, CUBRIC, Cardiff, United Kingdom

Introduction: Cerebral blood flow (CBF) is affected by alterations in intrathoracic pressure, via effects on arterial blood pressure (ABP), heart rate (HR) and venous outflow from the brain. These effects (illustrated in Figure 1) are clearly observed during a Valsalva manoeuvre [1] and the Mueller manoeuvre [2]. Alterations in intrathoracic pressure occur in disease states such as asthma and chronic lung disease. In order to understand the neural processing in a wide range of respiratory disorders it is important to understand the CBF effects of altered intrathoracic pressure, and how such changes may interact with stimulus-evoked signal changes. The aim of this study was to investigate how resistive inspiratory and expiratory loading, a tool for the investigation of breathlessness, affects CBF and the stimulus-evoked CBF response. We used trans-cranial Doppler (TCD) to investigate short-lived temporal effects and whole brain pseudo-continuous ASL to assess longer term and spatial effects.

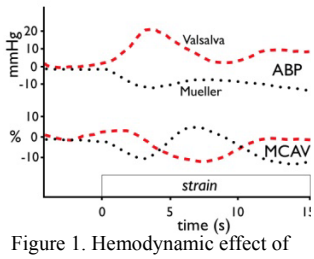


Figure 1. Hemodynamic effect of Valsava and Mueller Maneuvers [1,

Methods: 13 healthy volunteers (2 female; age 28+/-7(SD)) were examined on two occasions, first in a physiology laboratory and second in a Siemens 3T Verio scanner. In both sessions, participants breathed room air via a custom designed breathing system in which the internal diameter of the inspiratory and expiratory limb could be altered remotely by inflation/deflation of a water-filled balloon. Subjects were trained to maintain end-tidal carbon dioxide ($PETCO_2$) constant via real-time visual feedback [3]. Alternating 270-second blocks of inspiratory loading (-10cmH20), expiratory loading (+10cmH20) and no loading were delivered. Subjective ratings of breathlessness (0-10 on a visual analogue scale) were acquired after each block. A 2Hz flashing checkerboard was displayed for 120 seconds during each block. In the first experimental session, continuous recordings were made of middle cerebral artery velocity (MCAV; trans-cranial Doppler) and blood pressure (Finapres). During the second session, pseudo continuous ASL (TE= 14ms, TR= 4000ms, 450 volumes, voxel size 3.8x3.8x5.0mm) was acquired on a Siemens Verio 3T MRI scanner with a 32-channel radiofrequency receive coil. Analysis of the ASL data was carried out using FSL (http://www.fmrib.ox.ac.uk/fsl). A region-of interest approach was used to calculate mean perfusion changes across whole brain grey matter in response to inspiratory and expiratory loading and mean perfusion changes in the visual cortex (anatomical mask based on standard atlas template in FSL) in response to visual stimulation. The interaction between visual stimulation and the two loading conditions was modelled in the first level analysis, and explored using the same ROI approach. Voxel-wise statistical analysis was extended to a second (group) level in a fixed effects analysis using cluster threshold of Z>2.3 and a (corrected) cluster significance threshold of P=0.05. Physiological data were analyzed with a custom written script in Matlab.

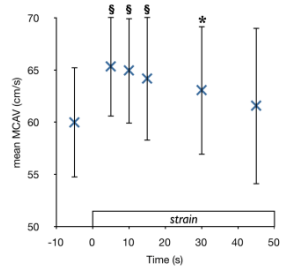


Figure 2: MCAV changes after application of inspiratory resistive load § p<0.01, * p<0.5

Results: ***Laboratory session*:** The main results are depicted in Table 1 and Figure 2. We observed a transient increase in CBF (TCD) in the first 30 seconds following application of an inspiratory load that was associated with an increase in $PETCO_2$, but not in blood pressure. We did not observe physiological changes in response to removal of the inspiratory load nor in response to application or removal of an expiratory load. ***FMRI session*:** Perfusion changes in the regions of interest are demonstrated in Table 2. This shows no effect of inspiratory or expiratory loading upon global CBF. As expected there was a robust CBF response to visual stimulation in the visual cortex, but no interaction was observed between loading and visual stimulation. Physiological data is summarized in Table 3. This shows that $PETCO_2$ was maintained constant across all experimental blocks. The voxel wise analysis (Figure 3) demonstrated robust activation in the visual cortex in response to visual stimulation (not pictured), no significant activations in response to inspiratory loading, activation in the right sensorimotor and bilateral lobe VI of the cerebellum in response to expiratory loading, and activation in the right insula and ventroposterolateral thalamic nucleus that correlated with the subjective dyspnea ratings.

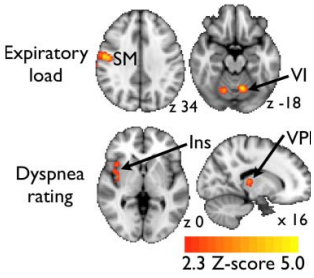


Figure 3. Significant CBF increases during exp loading (top), and in response to subjective dyspnoea ratings (bottom). Abbr: SM sensorimotor cortex, VI cerebellum lobe VI, Ins Insular cortex, VPL ventroposterior thalamic nucleus

Time:	Baseline	5	10	15	30	45
MAP:	96(16)	95(16)	95(17)	94(18)	94(18)	93(16)*
HR:	71(15)	69(12)	70(15)	69(15)	73(18)	72(15)
CO2:	5.5(0.5)	5.7(0.6)*	5.7(0.6)*	5.6(0.5)*	5.6(0.5)	5.6(0.5)

Table 1. Physiological variables over first 45 seconds after inspiratory load

Load	Minimum pressure [cmH_2O]	Maximum pressure [cmH_2O]	PETCO2 [%]	Heart rate [/min]	Dyspnea Rating [0-100]
Insp	-9.0(1.4)*	0.3(0.5)	5.5(0.7)	67(7)*	41(14)***
No	-0.7(0.2)	0.8(0.2)	5.6(0.7)	63(7)	8(12)
Exp	-0.2(0.7)	8.5(2.0)	5.5(0.8)	63(7)	38(14)***

Table 3. Physiological measurements obtained during scanning
* p<0.05, *** p<0.001 compared with baseline

Discussion: These data show that respiratory loading for durations of 270 seconds has no significant effects upon global CBF, nor the stimulus evoked CBF response. This is extremely useful for studies in patients with respiratory disease and for a realistic volunteer model of dyspnoea. However, we have demonstrated significant effects on CBF in the early phase of respiratory loading (i.e. the first 30 seconds). The increase in CBF is most likely due to a raised $PETCO_2$ as subjects find it more difficult to breathe, although rapidly adjusting their breathing pattern. We would expect the negative intrathoracic pressure to cause a transient decrease in CBF (Figure 1), therefore it appears that the transiently increased $PETCO_2$ has a more powerful effect on cerebral perfusion. This finding of altered CBF in the initial stages following an inspiratory load suggests that studies using much shorter breathlessness durations (e.g.[4]) may be confounded by physiological effects upon global cerebral blood flow. The findings of increased CBF in the sensorimotor cortices and lobule VI of the cerebellum (sensorimotor cerebellum) reflect the increased effort participants consciously make. Such changes were not observed with inspiratory loading, which seemed to be more threatening and steered by reflexes. We observed increases in CBF in the right insular cortex and the right ventroposterolateral nucleus of the thalamus that correlated with changes in the subjective ratings of dyspnoea. These findings are supported by other neuroimaging literature on dyspnoea that has used either BOLD FMRI with short stimulus durations (~25 seconds) or positron emission tomography. Arterial spin labeling has the potential to be a useful neuroimaging method for understanding the neural mechanisms dyspnoea, particularly since stimulus durations can be extended to make a more clinically realistic model.

	%ΔCBF (SD)
Grey matter	
Inspiratory loading	0.5(3.5)
Expiratory loading	6.9(2.0)
Visual cortex (V1)	
Visual stimulation	25.2(37.1)
Interaction(insp/vis)	0.3(0.5)
Interaction (exp/vis)	0.1(0.6)

Table 2. Perfusion measurements from ASL* p<0.05 compared wit baseline

References: [1] J Appl Physiol 86: 675-680, 1999. [2] Clin Physiol 20: 292-303, 2000. [3] J Cereb Blood Flow Metab 27: 414-423, 2007. [4] Am J Respir Crit C Med. 177 :1026-32, 2008.

Appendix IIe: Physiological noise reduction for multi-inversion time ASL

2537

Physiological noise reduction for multi-inversion time ASL

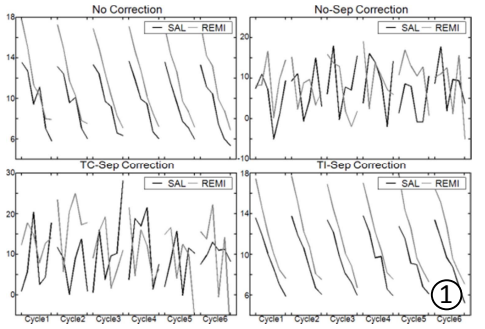
Kevin Murphy[1], Anja Hayen[2], Mari Herigstad[2], and Kyle T.S. Pattinson[2]

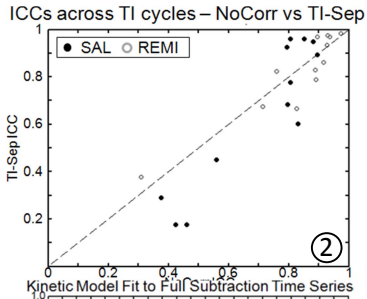
[1]*CUBRIC, School of Psychology, Cardiff University, Cardiff, Wales, United Kingdom,* [2]*Nuffield Dept Clinical Neurosciences & FMRIB Centre, University of Oxford, United Kingdom*

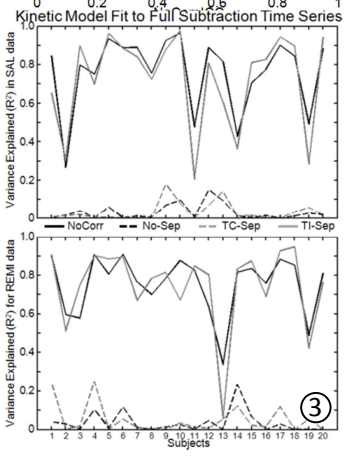
Target Audience Researchers using multi-TI ASL imaging

Purpose Due to the inherently low signal-to-noise ratio (SNR) of arterial spin labelling (ASL) methods, minimisation of noise sources is important for accurate cerebral blood flow (CBF) quantification. One source of noise arises from physiological processes such as respiration and cardiac cycles which introduce fluctuating confounds into the MR time series. Methods have been developed to remove such noise in standard BOLD fMRI imaging such as RETROICOR [1]. Restom and colleagues extended this method to ASL time series [2], demonstrating that the optimal approach to applying this technique is to remove physiological noise from tag and control time series separately before combining them to quantify CBF. The purpose of this study is to extend this finding to determine the optimal approach to removing physiological noise from multiple inversion time (multi-TI) ASL data.

Methods Twenty participants were scanned on two occasions at 3T using a multi-TI pseudo continuous ASL (PCASL) sequence (scan parameters: TIs = 1.65, 1.9, 2.15, 2.4, 2.65, 2.9s; TI cycles = 6; tag duration = 1.4s; TR = 4s; TE = 13ms; num slices = 24; matrix = 64x64; slice thickness = 5mm; num volumes = 72). During one session (REMI), the participants received an intravenous target-controlled infusion of remifentanil for 45 minutes. During the other session (SAL), saline was infused. Respiration and cardiac traces were recorded throughout the scanning with the expectation that physiological traces would differ between the two sessions. Slice-wise RETROICOR physiological noise regressors were calculated using the first and second harmonics of the phase of the respiratory and cardiac cycles at the time of acquisition along with an interaction term. Three versions of RETROICOR were performed: *No-Sep* – time points were not separated before regressing the noise from the signals; *TC-Sep* – the time series were separated into tag and controls before regressing the noise from each individually and then recombining into a single time series (the Restom approach [2]); *TI-Sep* – the time series were separated into tag/controls and by TI to yield 12 time series before noise regression. CBF time series were calculated after motion correction by subtracting successive tag/control pairs, yielding 36 volumes (6 TIs x 6 TI cycles). Gray matter masks were calculated from a structural image, and the average subtraction time series was calculated for each ASL run (SAL and REMI) for each participant. To assess noise removal efficacy, intra-class correlation coefficients (ICCs) were calculated across TI time points. A general kinetic model [3] was fit the 36 point time series. Fits were compared across correction types.







Results Figure 1 demonstrates the effect of noise correction using the different correction schemes for the SAL and REMI data. The across-subject average time series shows that the *No-Sep* and *TC-Sep* methods introduce a large amount of variance into the data. Separating the time series by tag/control and TI (*TI-Sep* method) before removing the physiological noise does not introduce this confound. If physiological noise is removed properly, the repeatability of each TI value across the 6 TI cycles should improve, i.e. ICC values should increase. Figure 2 compares the ICC values with no correction (*NoCorr*) to the *TI-Sep* method for each subject. The mean ICCs for the *NoCorr* method are 0.71±0.19 and 0.83±0.18 and for the *TI-Sep* method are 0.65±0.31 and 0.82±0.18 for SAL and REMI respectively. Figure 2 demonstrates that with the *TI-Sep* method, the ICCs improve for some subjects but not others and therefore no consistent differences to the *NoCorr* method are observed. Figure 3 shows the R^2 fits of the kinetic model to the 36 time point data for each of the 4 methods for each subject. As expected, the fits are poor to the *No-Sep* and *TC-Sep* data. The fits are broadly similar for *NoCorr* and *TI-Sep* for both the SAL and REMI runs with means of approximately 0.75 and no significant difference observed between the methods.

Discussion This study demonstrates that correction of physiological noise in multi-TI ASL data can introduce serious confounds if not performed correctly. Both the naive approach of not separating the data at all (*No-Sep*) and the Restom approach [2] of separating the tags from the controls (*TC-Sep*) introduce far more noise than they remove (Fig 1) resulting in very poor kinetic model fits (Fig 3). This is probably due to the non-linear increase in signal with TI leading to errors in the GLM fit. Separating both TIs and tags/controls alleviates this problem allowing for good repeatability of signal across TIs (Fig 2) and improved fits of the kinetic curve model (Fig 3). However, there is no significant or consistent improvement on any measure with the *TI-Sep* method over the uncorrected data. This is probably due to nature of multi-TI ASL in which data is essential averaged over TI cycles and thus inherently removes noise. However, if multi-TI data are to be used in dynamic fashion rather than through averaging, correction of physiological noise may be beneficial and should be performed with the *TI-Sep* method.

References [1] Glover (2000), MRM:44,162; [2] Restom (2006), NeuroImage:31,1104; [3] Buxton (1998), MRM:40,383

Acknowledgements Funded by: Wellcome Trust; Medical Research Council; NIHR Biomedical Research Centre, Oxford

Overview Appendix III

Manuscript in preparation

1) Appendix III: Dyspnea in COPD is associated with altered processing in the medial prefrontal cortex.
   Citation: Herigstad M, Hayen A, Evans E, Hardinge M, Davies RJ, Wiech K, Pattinson KTS. Dyspnea in COPD is associated with altered processing in the medial prefrontal cortex. Submitted to *Chest.*

Appendix III: Dyspnoea in COPD is associated with altered processing in the medial prefrontal cortex

Authors:

Mari Herigstad [a], Anja Hayen [a], Eleanor Evans [a], Maxine Hardinge [b], Robert J. Davies[b†], Katja Wiech [a,c], Kyle T. S. Pattinson [a]

Affiliations:

[a] FMRIB Centre, Nuffield Department of Clinical Neurosciences, University of Oxford, UK

[b] Oxford Respiratory Trials Unit, Nuffield Department of Medicine, University of Oxford, UK

[c] Centre for Pain Research, University of Bath, UK

† Deceased

At a glance commentary:

Scientific Knowledge on the Subject: The neural mechanisms of dyspnoea are poorly understood and have so far only been investigated in neuroimaging studies performed in healthy volunteers. Increasing clinical evidence implicates fear as a major contributor to dyspnoea. Using functional magnetic resonance imaging (fMRI) in COPD patients and healthy controls, we investigated the relevance and neural underpinnings of anticipatory fear in dyspnoea.

What This Study Adds to the Field: COPD patients showed increased brain activity in the medial prefrontal cortex (mPFC) during the presentation of dyspnoea-related words that correlated with measures of an increased attention to dyspnoea as a cognitive component of fear. We propose that a heightened response to dyspnoea-related material even in the absence of any physical challenge reflects a key process in the experience of dyspnoea in COPD patients.

Keywords: COPD, neuroimaging, dyspnoea, FMRI, breathlessness

ABSTRACT

Rationale: Dyspnoea is the major source of disability in chronic obstructive pulmonary disease (COPD) yet correlates poorly with objective measures of lung function. Psychological research suggests that this discrepancy would be explained by an exacerbation of dyspnoea through heightened fear. To explore whether COPD patients show abnormal fear processing related to dyspnoea, we investigated neural responses to dyspnoea-related words in the absence of any physical challenge.

Methods: Using fMRI, we investigated brain responses to validated dyspnoea-related word cues in 44 COPD patients and 40 healthy age-matched controls. Neural responses were related to physiological and behavioural measures of dyspnoea.

Results: Patients scored higher on measures of anxiety, depression, dyspnoea-related catastrophizing, vigilance and fatigue. Patients showed worse lung function and exercise performance. In both groups dyspnoea-related activation was observed in the left anterior insula and dorsolateral prefrontal cortex (dlPFC). COPD patients demonstrated greater activation in the medial PFC that correlated with measures of vigilance.

Conclusions: Based on behavioural and fMRI findings, we propose that engagement of the medial prefrontal cortex in COPD is indicative of a distortion of dyspnoea processing towards heightened fear processing. Future research will harness these mechanisms to improve the clinical treatment of dyspnoea.

INTRODUCTION

The dyspnoea of chronic obstructive pulmonary disease (COPD) has traditionally been explained in terms of lung damage. However, discrepancies between dyspnoea severity and spirometric measures of lung function are common (1), yet remain unexplained.

Contemporary models of COPD emphasize that the experience of dyspnoea is critically determined by psychological processing (2-5). In particular, fear of dyspnoea has a profound effect on dyspnoea itself (6). Critically, fear might impact on dyspnoea irrespective of lung function and might even be apparent in the absence of any physical challenge.

Anticipation or imagination of previously experienced painful situations can trigger or exacerbate pain by evoking fear-memories (7). In analogy to the findings in pain, there is converging evidence that such responses relating to fear-memories associated with dyspnoea have similar roles in exacerbating the dyspnoea of COPD: In healthy volunteers exposed to an equivalent resistive respiratory load, heightened fear of suffocation at baseline is associated with more unpleasant dyspnoea (8). COPD patients who suffer panic attacks and heightened anxiety report higher dyspnoea ratings for equivalent resistive loads in comparison with patients without panic (9). In COPD patients, dyspnoea ratings during resistive loading are exacerbated when attention is simultaneously focused on recalling a recent clear memory of dyspnoea (10). These studies demonstrate that fear heightens interoceptive sensitivity (the conscious awareness of the physiological state of the body), and that despite equivalent physical challenge dyspnoea can be biased by negative memories or emotional state.

The physical sensation of dyspnoea commonly originates from an array of distinct sensory afferent sources, including the heart, lungs and muscles. The conscious experience of dyspnoea, however, is created within the brain and is therefore subject to influence from multiple psychological factors that influence respiratory perception irrespective of the sensory input (11). A better understanding of the brain processes

contributing to dyspnoea perception in COPD will point the way towards explaining the discrepancy between lung function and dyspnoea severity.

So far, neuroimaging studies of dyspnoea have mainly focused on experimental approaches (3) that have administered artificial breathing manipulations to healthy volunteers. Although these studies have given some insight into the brain processes occurring during perception of a respiratory load, by their very nature they have not been able to unravel the complex psychological processes that may contribute to dyspnoea in a clinical population.

In this study, FMRI was used to investigate brain processes associated with dyspnoea in COPD. We adapted methodologies (12-14) that have successfully been used to understand the role of fear and anticipation in pain. Based on the fear-avoidance model described above, we hypothesised that the long-term changes in cognitive-affective state induced by COPD would be associated with abnormal brain processing of dyspnoea-related triggers. Physiological and behavioural measures were combined with functional magnetic brain imaging (FMRI). FMRI activations were assessed in response to dyspnoea triggers in the form of validated dyspnoea-related word cues.

METHODS

Participants

We recruited 44 (16 F, age 68.2+/-8.3(SD)) patients with COPD and 40 age and sex matched healthy controls (16 F, age 69.1+/-8.1(SD)). See online supplement for exclusion criteria and recruitment procedures. All participants gave written, informed consent. The study was approved by Oxfordshire Research Ethics Committee A.

FMRI

Imaging was performed with a Siemens 3 Tesla TIM-Trio scanner, using a 12-channel head coil. Participants underwent two FMRI scans (BOLD echo-planar image acquisition, repetition time (TR)=3000ms, echo time (TE)=30ms, field-of-view=192x192mm, voxel-size=3x3x3mm, 45 slices, 168 volumes), each lasting 8m20s with a 30s break between. A structural T1-weighted, whole-brain scan

(MPRAGE, TR=2040ms, TE=4.68ms, flip angle=8°, voxel-size=1x1x1mm) was used for image registration.

During scanning, participants were shown a randomised set of validated dyspnoea-related word cues (see online supplement for further details). After the word presentation were asked to rate each item according to how breathless and how anxious it would make them feel on a visual analogue scale (VAS)(scale 0 to 100), presented on screen and controlled via a button box. The words represented situations ranging from those that may induce maximal dyspnoea (e.g. "walking uphill") to those that would not be associated with dyspnoea (e.g. "watching television"). There was no specific instruction to imagine their breathlessness during the word presentation.

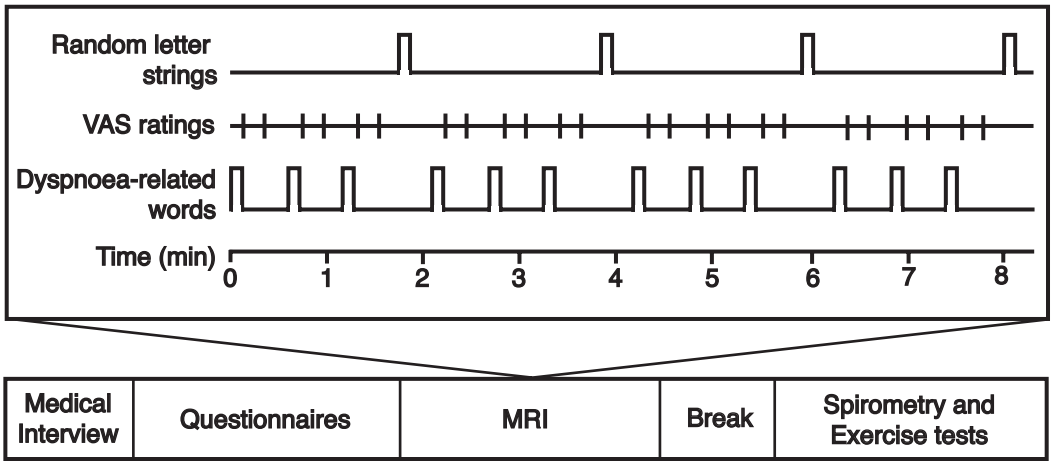


Figure 1. FMRI task. Participants were presented with, and asked to rate, dyspnea-related word cues. Dyspnea-related word cues and ratings were each displayed for 7 seconds. Every block was separated by a fixation cross (12 seconds) and every third block was followed by random letter strings (7 seconds). To accommodate participants that were less able to suppress coughs and/or lie flat for an extended period of time, the paradigm was undertaken in two instalments, each lasting 8 minutes 20 seconds, as presented.

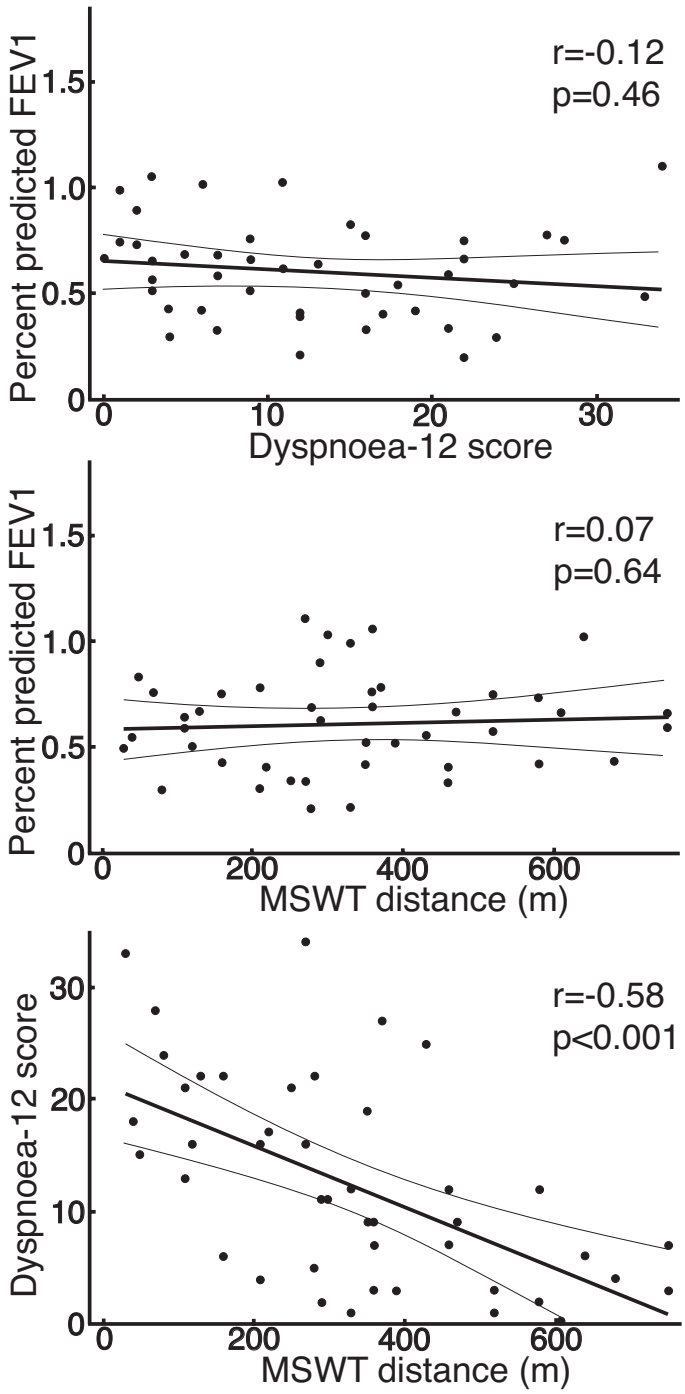


Figure 2: Percent predicted FEV1 plotted against Dyspnoea-12 score and distance walked on modified shuttle walking test (MSWT) for patient group. Dyspnoea-12 score plotted against distance walked on MSWT for patient group. Simple linear model representing a least-squares fit of the data (line) with confidence bounds (dotted lines).

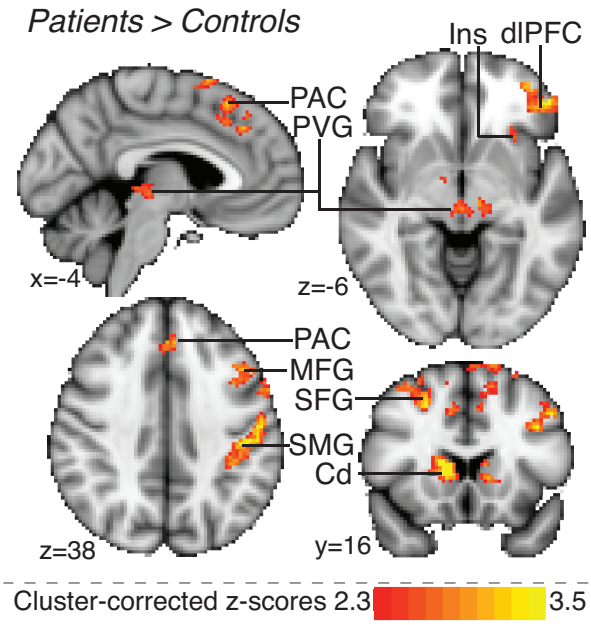


Figure 3: Activation during the presentation of words for patients and controls. Maps are whole-brain analysis, cluster level corrected for multiple comparisons at $p<0.05$. Maps represent comparison between groups (patients>controls). No activation was greater in controls than in patients. PAC (paracingulate gyrus), PVG (periventricular grey), Ins (insula), dlPFC (dorsolateral prefrontal cortex), MFG (middle frontal gyrus), SFG (superior frontal gyrus), SMG (supramarginal gyrus), Cd (caudate).

A control task to assess potential differences in baseline BOLD responsiveness between groups was employed in the form of random letter strings which were presented after every third dyspnoea-related word cue. For an overview of the scanning protocol, see Figure 1. Heart rate (HR) and pulse oximetry (SaO2, multigas monitor, 9500, MR Equipment), respiration (respiratory bellows around the chest) and end-tidal partial pressures of oxygen and carbon dioxide (Datex, Normocap, nasal cannula (Salter Labs)) were continuously measured. All physiological data were sampled at 50Hz and recorded along with scan volume triggers via PowerLab 8 (ADinstruments), using Chart5 (ADinstruments).

Psychological measurements

Prior to scanning, a comprehensive assessment of respiratory perception and impact was obtained using the following self-report questionnaires: St George's Hospital Respiratory Questionnaire (SGRQ) (15), Medical Research Council (MRC) dyspnoea

scale (16), Dyspnoea-12 questionnaire (17), Catastrophic Thinking Scale in Asthma (18) and Pain Awareness and Vigilance Scale (19). The latter two were modified for dyspnoea by substituting the words 'asthma' and 'pain' for 'breathlessness'. Levels of depression, anxiety, fatigue and motivation were measured with the Center for Epidemiologic Studies Depression Scale (CES-D) (20), State-Trait Anxiety Inventory (21), Fatigue Severity Scale (22) and Behavioral Approach System/Behavioral Inhibition System (BIS/BAS) scale (23), respectively.

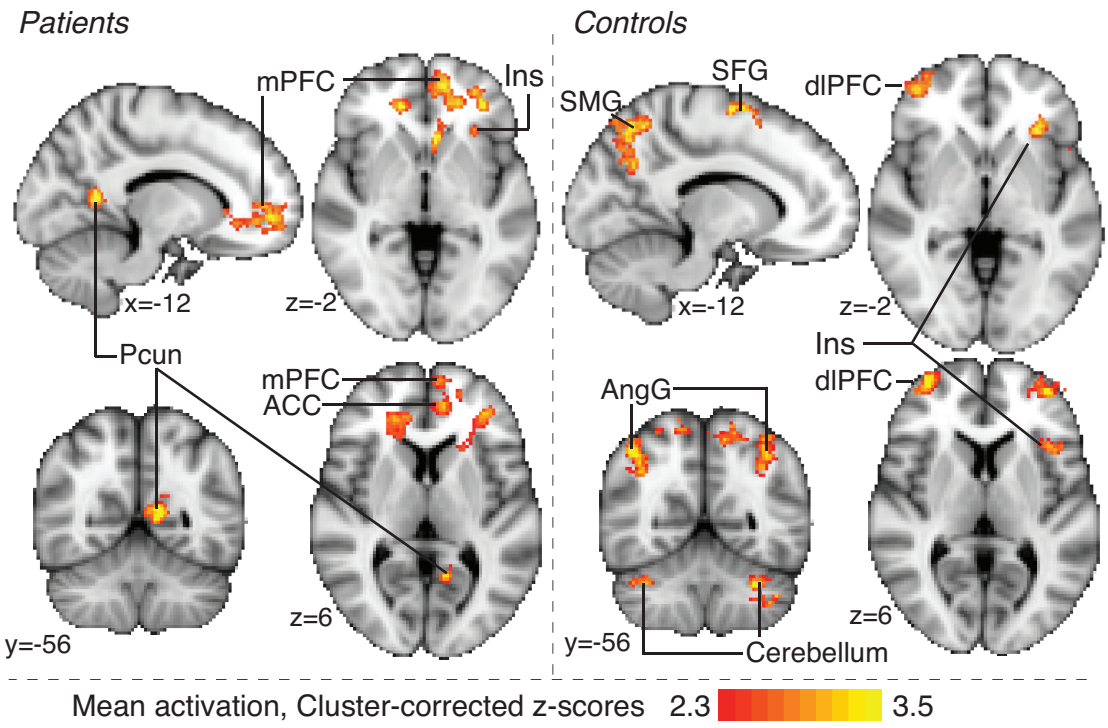


Figure 4: Activation during the presentation of words for patients and controls, weighted by dyspnea ratings. Maps are whole-brain analysis, cluster level corrected for multiple comparisons at $p<0.05$. Maps represent mean activation for both groups. mPFC (medial prefrontal cortex), SMG (supramarginal gyrus), SFG (superior frontal gyrus), dlPFC (dorsolateral prefrontal cortex), Pcun (precuneus cortex), Ins (insula), ACC (anterior cingulate cortex), AngG (angular gyrus), Cerebellum (CrusI and VI).

Spirometry and exercise testing

Participants undertook spirometry, performed by a trained respiratory nurse using Association for Respiratory Technology and Physiology (ARTP) standards, and an exercise test (Modified Shuttle Walking Test (MSWT), performed twice). Before, during and after the MSWT, HR and SaO2 were measured every minute using a

noninvasive fingertip pulse oximeter (Go2, Nonin Medial Inc.) and participants rated their current dyspnoea on a modified Borg scale (24) immediately before and after the MSWT.

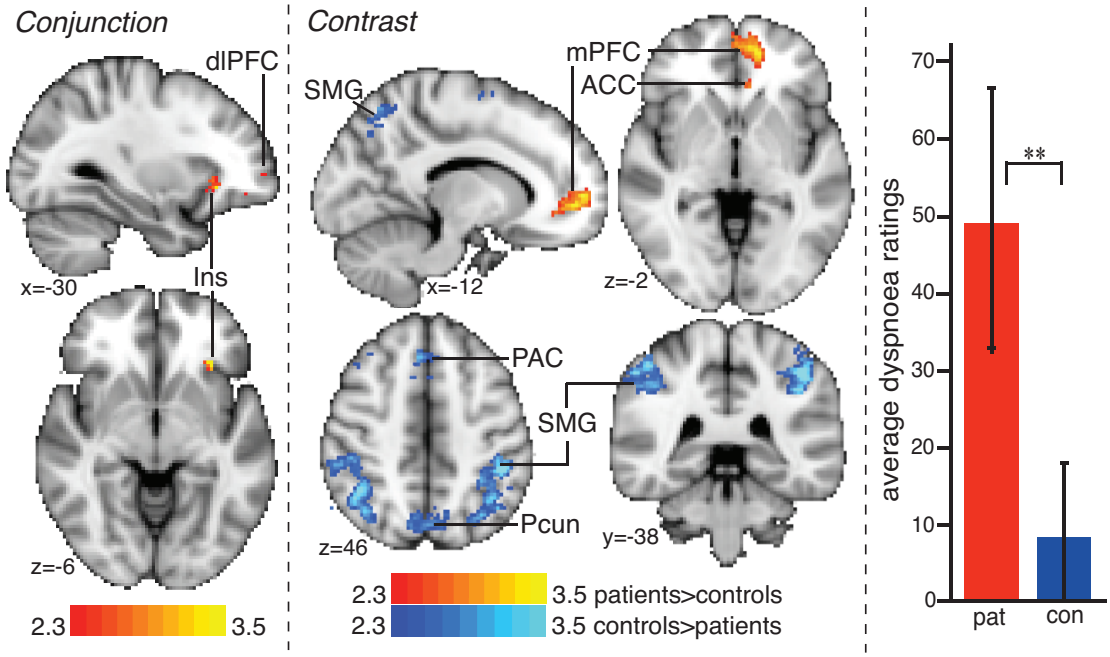


Figure 5: Activation during the presentation of words for patients and controls, weighted by dyspnea ratings. Maps are whole-brain analysis, cluster level corrected for multiple comparisons at $p<0.05$. Maps represent conjugation analysis (activations common to both groups) and comparisons between groups, (patients>controls in red-yellow, controls>patients in blue-lightblue). Bar graph is average+/-SD dyspnea ratings for each group. dlPFC (dorsolateral prefrontal cortex), SMG (supramarginal gyrus), mPFC (medial prefrontal cortex), ACC (anterior cingulate cortex), Ins (insula), PAC (paracingulate gyrus), Pcun (precuneus cortex).

Data analysis

All FMRI data processing was carried out within FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB Software Library www.fmrib.ox.ac.uk/fsl), using a whole brain approach with standard parameters and settings (for details see the online supplement). To correct for multiple comparisons, Z statistic images were thresholded using clusters determined by $Z > 2.3$ and a (corrected) cluster significance threshold of $p = 0.05$. (25).

First-level analysis used a general linear model (GLM) with multiple explanatory variables (EVs), incorporating timing values for the different events. A 6 second haemodynamic delay was assumed.

Contrasts of interest in the group analyses were the presentation of dyspnoea-related word cues and random letter strings from the first lower-level analysis, and dyspnoea and anxiety ratings of the dyspnoea-related word cues from the second lower-level analysis. Age and sex were included as nuisance regressors. To account for inter-individual differences in psychological functioning a multiple regression analysis (IBM SPSS Statistics) was performed upon the questionnaire scores. Dyspnoea-12 scores were the dependent variable. The following major components were identified: State Anxiety, Fatigue Severity Scale, CES-D and Awareness and Vigilance Scale and were included in the GLM (for details see online supplement).

Three group level analyses were conducted, contrast images were used for higher-level analyses as appropriate (26). :

- In the first, the EVs included the presentation of dyspnoea-related word cues, random letter strings and VAS rating scales.
- In the second, the GLM included trial-by-trial dyspnoea and anxiety ratings of dyspnoea-related word cues as additional EVs.
- The third analysis examined the contrast relating to vigilance scores in the second analysis, but in patients only. Vigilance scores were identified as a major contributor to dyspnoea in the regression analysis, are breathlessness-specific and are regarded as an important component of fear-avoidance (27-29) and are therefore relevant to the hypotheses under test. As the depression, state anxiety and fatigue scores were not

dyspnoea specific, we considered these as regressors of no-interest in relation to our primary hypotheses.

We performed a conjunction analysis to determine common areas of brain activation in each group, and unpaired t-tests to determine between group differences.

To assess group differences in generalised BOLD responsiveness, a region-of-interest analysis explored between-group differences in the BOLD response to visual stimulation for random letter strings (30) in the primary visual cortex.

All questionnaires were scored according to their respective manuals. For spirometry, the measurement associated with the highest FEV1 was used and the percentage of value predicted for participant height and age calculated. For the MSWT, the measurement associated with the furthest distance walked was used. Data was compared using a Student's t-test. Correlations between FEV1, MSWT and Dyspnoea-12 scores were performed using a custom written script in Matlab (Mathworks, USA). P values<0.05 were considered significant.

RESULTS

Participants

For participants' demographics, spirometry and MSWT values see Table 1. A summary of patients' medical assessments can be found in the supplementary material. Patients' FEV1 did not correlate with dyspnoea perception (Dyspnoea-12 score, r=-0.12,p=0.46) or exercise performance (MSWT distance, r=-0.07,p=0.64), but a negative correlation was observed between Dyspnoea-12 score and MSWT distance (r=-0.58,p<0.001, Figure 2).

Questionnaires

Patients scored significantly higher than controls on the following questionnaires: Dyspnoea-12, St Georges' Respiratory Questionnaire, Catastrophic Thinking Scale, Awareness and Vigilance Scale, Fatigue Severity Score, and depression and anxiety

scores. There was no significant difference between groups on the BIS/BAS scale (a measure of motivation and reward responsiveness) (Table 2).

On-line ratings obtained during FMRI scanning

Compared to healthy controls, patients rated dyspnoea-related word cues significantly higher for both dyspnoea (mean patients: 52.9+/-13.4(SD); mean controls: 8.4+/-10.4(SD); $p<0.001$) and anxiety (average across patients: 41.6+/-19.1(SD); controls: 5.8+/-10.8(SD); $p<0.001$).

FMRI

Word presentation (not weighted by dyspnoea ratings), conjunction analysis: Both groups exhibited bilateral activation in response to dyspnoea-related word cues in an extended network comprising the visual cortex, prefrontal cortex (PFC), insula, anterior cingulate cortex and the left hippocampus (Table E4, Figure E3).

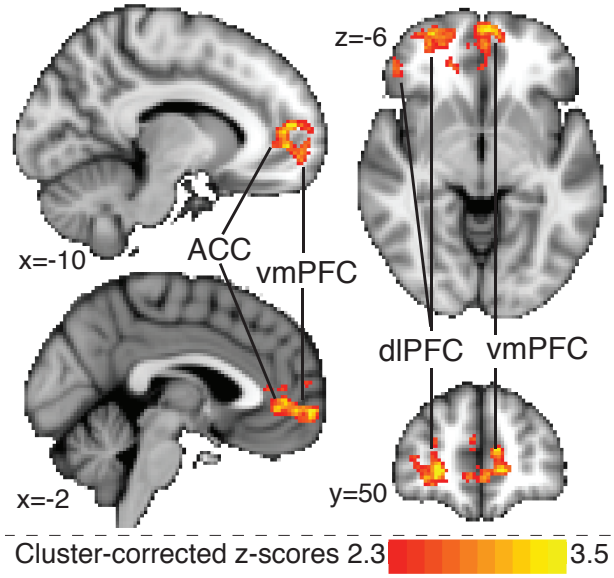


Figure 6: Activation during the presentation of words for patients, weighted by dyspnea ratings, vigilance contrast. Maps are whole-brain analysis, cluster level corrected for multiple comparisons at $p<0.05$. Maps represent conjugation analysis (activations common to both groups) and comparisons between groups, (patients>controls in red- yellow, controls>patients in blue-lightblue). Bar graph is average+/-SD dyspnea ratings for each group. dlPFC (dorsolateral prefrontal cortex), SMG (supramarginal gyrus), mPFC (medial prefrontal cortex), ACC (anterior cingulate cortex), Ins (insula), PAC (paracingulate gyrus), Pcun (precuneus cortex).

Word presentation (not weighted by dyspnoea ratings), differential activations: Compared to healthy controls, the patient group showed stronger activation in the left dorsal and ventrolateral PFC, supramarginal gyrus and insular cortex, and bilateral anterior cingulate cortex (ACC), caudate, thalamus and periventricular grey extending into the periaqueductal grey (Figure 3, Table 3). There were no brain regions where healthy controls showed stronger activation than patients.

Word presentation (weighted by dyspnoea VAS ratings), conjunction analysis: This revealed common activation in the left anterior insula and dlPFC (Figure 5). Group mean activations are presented in Figure 4 and in supplementary table E4.

Word presentation (weighted by dyspnoea VAS ratings), differential activations:
A direct group comparison revealed stronger activation in the left vmPFC and ACC in patients; the reverse contrast showed stronger activation in the supramarginal gyrus, angular gyrus, precuneus and paracingulate gyrus regions in controls (Figure 5).

Word presentation (weighted by dyspnoea VAS ratings) vigilance contrast, patients only: Here we observed significant correlations between vigilance and activation in the vmPFC, ACC and right dlPFC (Figure 6). There was considerable overlap in the vmPFC activation with that observed in the previous analysis (Figure 5)

Word presentation (weighted by dyspnoea VAS ratings and by depression, state anxiety and fatigue), patients only: We did not observe any activation relating to depression scores. In relation to state anxiety, we observed significant correlations in the superior frontal gyrus, right dlPFC and precuneous cortex. In relation to fatigue, we observed significant correlations in the vmPFC, dlPFC, left frontal operculum cortex, inferior frontal gyrus and visual cortex.

Word presentation (weighted by breathlessness-anxiety VAS ratings): Weighing BOLD signal by anxiety VAS ratings did not yield any significant group differences.

Visual activation (control task). The average activation level of the top 20% voxels within the visual cortex (V1) did not differ significantly between groups (p=0.61, see

online supplement for discussion), suggesting that there was no significant between-group difference in BOLD responsiveness.

Table 1: Participant details and physiological data

	Patients (mean (SD))	**Controls (mean (SD))**
Age (years)	68.2 (8.3)	69.1 (8.1)
Sex (females/males)	16/28	16/24
IMD score	11.6 (6.6)	11.7 (9.2)
BMI (kg/m^2)	28.3 (6.6)	25.2 (3.2)*
MRC score (1-5)	3.0 (0.9)	1.0 (0.2)**[a]
Resting Borg score (1-10)	0.8 (1.04)	0.06 (0.2)**
Resting SaO2 (%)	94.4 (2.6)	96.4 (1.3)**
Resting HR (bpm)	82.3 (13.6)	72.2 (11.0)**
MSWT (distance walked, m)	331.4 (193.1)	804.0 (274.5)**
Pack years	41.3 (51.5)	3.1 (6.6)**
FEV1 (% predicted)	61.0 (23.0)	99.4 (24.6)**

*Demographic data for patients and controls (mean results and standard deviation). MRC score (Medical Research Council breathlessness score), IMD score (English Indices of Deprivation score, 2010 data), BMI (body mass index), SaO2 (blood oxygen saturation), MSWT (modified shuttle walking test), FEV1% (ratio of forced expiratory volume in 1 second to forced vital capacity). *p<0.01, ** p<0.001. [a] Mann-Whitney-Wilcoxon*

Table 2: Questionnaire scores

Questionnaire		Patients (mean (SD))	Controls (mean (SD))
Dyspnoea-12		12.7 (9.2)	0.0 (0.0)***
	Physical	7.9 (5.2)	0.0 (0.0)***
	Affective	4.8 (4.4)	0.0 (0.0)***
SGRQ		51.3 (17.0)	6.9 (5.1)***
	Symptom	61.4 (18.4)	2.7 (2.1)***
	Activity	69.0 (22.5)	3.1 (3.1)***
	Impact	38.4 (17.9)	0.6 (1.4)***
Catastrophic Thinking Scale [a]		13.7 (12.4)	0.0 (0.2)***
	Helplessness	5.3 (5.6)	0.0 (0.2)***
	Magnification	3.8 (3.2)	0.0 (0.0)***
	Rumination	4.6 (4.4)	0.0 (0.0)***
Awareness and Vigilance Scale [b]		41.7 (14.6)	12.9 (11.4)***
Fatigue Severity Scale		42.9 (11.2)	22.3 (12.0)***
BIS/BAS		54.0 (8.8)	54.7 (8.5)
	BAS: Drive	10.0 (2.7)	10.5 (2.7)
	BAS: Fun-seeking	9.2 (2.5)	9.4 (2.4)
	BAS: Reward responsiveness	9.7 (2.9)	9.5 (3.1)
	BIS: Inhibition	16.6 (4.0)	16.5 (3.7)
CES-D		14.4 (9.2)	7.2 (6.6)***
State anxiety		34.9 (9.5)	25.6 (7.5)***
Trait anxiety		37.5 (10.5)	29.1 (6.8)***

*Behavioural measurements in patients (n=44) and controls (n=40, mean results and standard deviation). Component scores are included where appropriate. SGRQ (St George's Respiratory Questionnaire), BIS/BAS (Behavioral Approach System/ Behavioral Inhibition System), CES-D (Center for Epidemiologic Studies Depression Scale) Modified from use in asthma [a]. Modified from use in pain [b]. **p<0.01, *** p<0.001.*

Table 3: FMRI activation, group interractions

Contrast	Region	Peak voxel location X,Y,Z (zmax)	
		Left	**Right**
Dyspnoea-related words			
Patients > Controls	PFC (dorsolateral)	-42, 46, 0 (4.53)	
	(ventrolateral)	-44, 42, 0 (4.38)	
	ACC	-10, 24, 24 (4.14)	12, 26, 28 (3.56)
	Paracingulate gyrus	-8, 24, 42 (3.64)	
	MFG	-40, 18, 32 (4.81)	36, 12, 58 (3.54)
	Insular cortex	-30, 26, 6 (3.06)	
	Operculum	-36, 26, 6 (3.16)	
	Premotor cortex	-34, -16, 66 (5.46)	
	SI	-50, -24, 42 (4.76)	
	Primary motor cortex	-34, -20, 50 (3.23)	
	Caudate	-12, 0, 18 (3.36)	12, 14, 10 (4.59)
	Thalamus	-14, -20, -2 (3.98)	12, -8, 14 (3.72)
	Midbrain (PVG)		2, -20, -4 (3.26)
Dyspnoea-weighted words			
Patients > Controls	PFC (ventromedial)	-14, 50, -2 (3.43)	
	(dorsomedial)	-10, 56, 4 (3.68)	
	ACC	-6, 32, -8 (3.30)	
Controls > Patients	MFG		40, 8, 58 (3.30)
	Paracingulate gyrus		6, 28, 46 (3.13)
	Superior LOC	-42, -62, 44 (3.46)	42, -74, 36 (3.38)
	Angular gyrus	-38, -56, 46 (3.35)	42, -50, 52 (3.79)
	Supramarginal gyrus	-42, -38, 38 (3.95)	56, -38, 48 (3.65)
	Superior parietal lobule	-42, -48, 54 (3.52)	
	Premotor cortex	-18, 2, 66 (3.29)	12, 10, 64 (3.78)
	SI	-44, -40, 48 (4.53)	
Random letter strings			
Patients > Controls	PFC (dorsomedial)		2, 64, 20 (4.14)
	(dorsolateral)		36, 58, 10 (4.02)
	Amygdala		22, -4, -18 (3.53)
	Hippocampus		24, -16, -14 (3.02)
	TFG	-44, -44, -8 (3.34)	

Peak voxel locations and peak z scores (zmax) during FMRI, MNI standard space. PFC (prefrontal cortex), ACC (anterior cingulate cortex), MFG (medial frontal gyrus), SI (primary somatosensory cortex), PVG (periventricular grey), LOC (lateral occiptial cortex), TFG (temporal fusiform gyrus)

DISCUSSION

The most important finding of this study is that COPD patients demonstrate markedly stronger activation in the vmPFC and ACC when faced with dyspnoea-relevant cues. Furthermore, the activation in these structures can be explained by the degree of breathlessness vigilance reported by patients.

Response to word cues, not weighted by dyspnoea

The pattern of greater BOLD activity in response to word cues in COPD patients than healthy controls could be interpreted as an anxiety response as the pattern reflects findings in studies of anxiety, threat and fear: Abnormal insular activity is observed in patients with anxiety disorders (31). The ACC (31, 32) and the PFC (32) are implicated in the appraisal of threat associated with pain and with noxious respiratory challenges. The periventricular and periaqueductal grey are associated with anxiety and fight-or-flight escape behaviours (33).The pattern also broadly reflects brain areas activated in FMRI studies of experimentally induced dyspnoea (31, 34-38). Insular activation has been associated with negative emotions in experimentally-induced dyspnoea (34-37, 39). However, to determine how these patterns of brain activity directly relate to dyspnoea, behavioural measures in the form of questionnaires and ratings taken during scanning were incorporated into a separate analysis.

Response to word cues scaled by dyspnoea VAS ratings

Scaling by dyspnoea ratings (Supplementary Figure E1) accounts for the subjective experience of looking at the word cues. This allows a meaningful comparison between patients and the healthy controls with respect to understanding dyspnoea processing.

In response to dyspnoea-weighted word cues, patients and controls display common activation in the left anterior insula and in a small area of the left dlPFC. The insula is strongly implicated in interoception (40), the conscious awareness of bodily sensations. It has been speculated that the insula acts as a salience filter to control behavioural responses to external stimuli, firstly through bottom-up detection of salient stimuli and subsequently through initiation of attentional control signals, facilitation of brain regions associated with cognition, memory and motor responses,

and modulation of autonomic reactivity (41). The dlPFC is known for its role in executive functioning, particularly working memory and planning. Although activation in the anterior insula and dlPFC have been implicated in FMRI studies of dyspnoea (3) and pain (7), activity here is not exclusive to unpleasant or threatening situations, for example these structures are important for the processing of pleasure and reward (42). Furthermore, as we see activation in these areas in our healthy controls (in whom the word cues are not threatening) the findings do suggest we need to look elsewhere in the brain to start to explain how clinical dyspnoea suffered by patients differs from that induced in a healthy volunteer laboratory setting.

We have observed a marked difference in the pattern of brain activation relating to appraisal of dyspnoea-cues between COPD patients and healthy controls. This is most evident in vmPFC (Figures 4 and 5). Furthermore, self reported measures of dyspnoea vigilance explained this cluster the best (Figure 6). Vigilance is an important construct in the fear-avoidance model (27), and although there is behavioural evidence for its relevance in the dyspnoea of COPD (2, 6) here we provide, for the first time, neuroimaging evidence of how the brain processing of dyspnoea vigilance is altered in COPD. The vmPFC has been linked with fear-related memory processes and emotional learning (43-46). During threat assessment, the activity in the vmPFC is implicated in context assessment and behavioural control (46). Models of fear systems in humans have suggested that the vmPFC acts to control midbrain 'fight-or-flight' regions (47). Activity in the vmPFC has not been reported in any dyspnoea or respiratory studies to date, although none have examined anticipatory processes in clinical populations. Our findings are important as they are the first to demonstrate aberrant brain processing in COPD patients in the processing of clinically-relevant dyspnoea.

Based on our imaging and behavioural findings we propose that the vmPFC engagement in COPD patients is indicative of a hypervigilant, fear-related assessment of the word cues that may act to increase the threat value or amplify the sensations of dyspnoea, making it considerably more unpleasant and frightening. It is possible that this emotional processing of dyspnoea in COPD patients to some extent is contingent upon fear-memory and learning, i.e. a prolonged association between fear and breathing, as this activation pattern is not observed in healthy volunteers during

unpleasant and frightening acutely administered experimental dyspnoea (3). This interpretation is supported by increasing body of clinical evidence that highlights the importance of fear and anxiety as a major contributor to the dyspnoea of COPD. For example, in patients scheduled for pulmonary rehabilitation, pre-treatment levels of dyspnoea-related fear correlates with treatment success (improvement in exercise dyspnoea; (6). Furthermore, the beneficial effect of pulmonary rehabilitation appears to be most strongly linked with reductions in dyspnoea-related anxiety/fear than the physical intensity of dyspnoea (48).

Clinical implications

In a 2012 consensus statement, the American Thoracic Society wrote that dyspnoea "derives from interactions among multiple physiological, psychological, social, and environmental factors" (11). Here we provide FMRI evidence of the brain processes that are likely to play a major role in some of the psychological factors described in that statement, particularly pertaining to those that may contribute to fear avoidance in the dyspnoea of COPD.

The best targets for future clinical interventions are likely to be regions unique to dyspneic patients, i.e. regions of 'abnormal' processing of dyspnoea. A fruitful research venue may be intervention studies addressing modulation of activity in these regions, with a particular focus on facilitation of symptom reappraisal and/or alleviation. A neuroimaging biomarker of dyspnoea may allow the testing of interventions using a smaller number of participants than necessary for a full-scale clinical trial, allowing preliminary decisions on effectiveness to be made faster (49).

Conclusions

In summary, dyspnoea in COPD engages a markedly different pattern of brain activity to that observed in healthy controls. Dyspnoea assessment scaled with brain activity in medial PFC in patients and was related to the amount of dyspnoea hypervigilance. In contrast dyspnoea assessment scaled with brain activity in the supramarginal gyrus, angular gyrus and precuneus in healthy controls. Our imaging and behavioural findings together suggest that fear and anticipatory anxiety play an important role in shaping the anticipatory processes associated with dyspnoea in COPD. Understanding the neural processing of dyspnoea in a clinical population is crucial for advances to be

made in its treatment, and the findings presented in this paper may represent important therapeutic targets.

ACKNOWLEDGEMENTS

The authors thank Steve Knight, Bethan Hughes, Isabel Chabata, Debby Nicoll, Tara Harris, Fran Sinfield, Emma Tucker, Rachel Lardner, Julie Young and the Oxfordshire Pulmonary Rehabilitation Team for their continued support to the study; and Richard Wise, Andrea Reinecke, Annabel Nickol and Najib Rahman for their generous academic input and support. This study was supported by the Medical Research Council (UK) and the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre based at The Oxford University Hospitals Trust, Oxford.

REFERENCES:

1. Jones PW. Health status measurement in chronic obstructive pulmonary disease. *Thorax* 2001;56:880-887.
2. Hayen A, Herigstad M, Pattinson KT. Understanding dyspnea as a complex individual experience. *Maturitas* 2013.
3. Herigstad M, Hayen A, Wiech K, Pattinson KT. Dyspnoea and the brain. *Respir Med* 2011;105:809-817.
4. Lansing RW, Gracely RH, Banzett RB. The multiple dimensions of dyspnea: Review and hypotheses. *Respir Physiol Neurobiol* 2009;167:53-60.
5. von Leupoldt A, Kenn K. The psychology of chronic obstructive pulmonary disease. *Current opinion in psychiatry* 2013;26:458-463.
6. Janssens T, De Peuter S, Stans L, Verleden G, Troosters T, Decramer M, Van den Bergh O. Dyspnea perception in copd: Association between anxiety, dyspnea-related fear, and dyspnea in a pulmonary rehabilitation program. *Chest* 2011;140:618-625.
7. Wiech K, Ploner M, Tracey I. Neurocognitive aspects of pain perception. *Trends in cognitive sciences* 2008;12:306-313.
8. Alius MG, Pane-Farre CA, Von Leupoldt A, Hamm AO. Induction of dyspnea evokes increased anxiety and maladaptive breathing in individuals with high anxiety sensitivity and suffocation fear. *Psychophysiology* 2013;50:488-497.
9. Livermore N, Butler JE, Sharpe L, McBain RA, Gandevia SC, McKenzie DK. Panic attacks and perception of inspiratory resistive loads in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2008;178:7-12.
10. Meek PM. Influence of attention and judgment on perception of breathlessness in healthy individuals and patients with chronic obstructive pulmonary disease. *Nursing research* 2000;49:11-19.
11. Parshall MB, Schwartzstein RM, Adams L, Banzett RB, Manning HL, Bourbeau J, Calverley PM, Gift AG, Harver A, Lareau SC, Mahler DA, Meek PM, O'Donnell DE. An official american thoracic society statement: Update on the mechanisms, assessment, and management of dyspnea. *Am J Respir Crit Care Med* 2012;185:435-452.

12. Ogino Y, Nemoto H, Inui K, Saito S, Kakigi R, Goto F. Inner experience of pain: Imagination of pain while viewing images showing painful events forms subjective pain representation in human brain. *Cereb Cortex* 2007;17:1139-1146.
13. Singer T, Seymour B, O'Doherty J, Kaube H, Dolan RJ, Frith CD. Empathy for pain involves the affective but not sensory components of pain. *Science* 2004;303:1157-1162.
14. Corradi-Dell'Acqua C, Hofstetter C, Vuilleumier P. Felt and seen pain evoke the same local patterns of cortical activity in insular and cingulate cortex. *J Neurosci* 2011;31:17996-18006.
15. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation. The st. George's respiratory questionnaire. *The American review of respiratory disease* 1992;145:1321-1327.
16. Bestall JC, Paul EA, Garrod R, Garnham R, Jones PW, Wedzicha JA. Usefulness of the medical research council (mrc) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. *Thorax* 1999;54:581-586.
17. Yorke J, Moosavi SH, Shuldham C, Jones PW. Quantification of dyspnoea using descriptors: Development and initial testing of the dyspnoea-12. *Thorax* 2010;65:21-26.
18. De Peuter S, Lemaigre V, Van Diest I, Van den Bergh O. Illness-specific catastrophic thinking and overperception in asthma. *Health Psychol* 2008;27:93-99.
19. McCracken LM. "Attention" to pain in persons with chronic pain: A behavioral approach. *Behav Ther* 1997;28:271-284.
20. Radloff LS. The ces-d scale. A self-report depression scale for research in the general population. *Applied Psychological Measurement* 1977;1:385-401.
21. Spielberger CD, Gorsuch, R.L., Lushene, R., Vagg P.R., Jacobs, G.A. Manual for the state-trait anxiety inventory. Palo Alto, California, USA: Consulting Psychologists Press; 1983.
22. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Archives of neurology* 1989;46:1121-1123.
23. Carver CS, White TL. Behavioral-inhibition, behavioral activation, and affective responses to impending reward and punishment - the bis bas scales. *J Pers Soc Psychol* 1994;67:319-333.

24. Burdon JGW, Juniper EF, Killian KJ, Hargreave FE, Campbell EJM. The perception of breathlessness in asthma. *American Review of Respiratory Disease* 1982;126:825-828.
25. Worsley KJ, Evans AC, Marrett S, Neelin P. A three-dimensional statistical analysis for cbf activation studies in human brain. *J Cereb Blood Flow Metab* 1992;12:900-918.
26. Beckmann CF, Jenkinson M, Smith SM. General multilevel linear modeling for group analysis in fmri. *NeuroImage* 2003;20:1052-1063.
27. Vlaeyen JW, Linton SJ. Fear-avoidance model of chronic musculoskeletal pain: 12 years on. *Pain* 2012;153:1144-1147.
28. Stoilkova A, Janssen DJ, Franssen FM, Spruit MA, Wouters EF. Coping styles in patients with copd before and after pulmonary rehabilitation. *Respir Med* 2013;107:825-833.
29. Leeuw M, Goossens ME, Linton SJ, Crombez G, Boersma K, Vlaeyen JW. The fear-avoidance model of musculoskeletal pain: Current state of scientific evidence. *Journal of behavioral medicine* 2007;30:77-94.
30. Mitsis GD, Iannetti GD, Smart TS, Tracey I, Wise RG. Regions of interest analysis in pharmacological fmri: How do the definition criteria influence the inferred result? *NeuroImage* 2008;40:121-132.
31. Paulus MP, Stein MB. An insular view of anxiety. *Biol Psychiatry* 2006;60:383-387.
32. Kalisch R, Wiech K, Critchley HD, Dolan RJ. Levels of appraisal: A medial prefrontal role in high-level appraisal of emotional material. *NeuroImage* 2006;30:1458-1466.
33. Brandao ML, Troncoso AC, de Souza Silva MA, Huston JP. The relevance of neuronal substrates of defense in the midbrain tectum to anxiety and stress: Empirical and conceptual considerations. *European journal of pharmacology* 2003;463:225-233.
34. Banzett RB, Mulnier HE, Murphy K, Rosen SD, Wise RJ, Adams L. Breathlessness in humans activates insular cortex. *Neuroreport* 2000;11:2117-2120.
35. Evans KC, Banzett RB, Adams L, McKay L, Frackowiak RS, Corfield DR. Bold fmri identifies limbic, paralimbic, and cerebellar activation during air hunger. *J Neurophysiol* 2002;88:1500-1511.

36. Peiffer C, Poline JB, Thivard L, Aubier M, Samson Y. Neural substrates for the perception of acutely induced dyspnea. *Am J Respir Crit Care Med* 2001;163:951-957.
37. von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, Buchel C. The unpleasantness of perceived dyspnea is processed in the anterior insula and amygdala. *Am J Respir Crit Care Med* 2008;177:1026-1032.
38. Hayen A, Herigstad M, Kelly M, Okell TW, Murphy K, Wise RG, Pattinson KT. The effects of altered intrathoracic pressure on resting cerebral blood flow and its response to visual stimulation. *NeuroImage* 2012;66C:479-488.
39. von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, Buchel C. Dyspnea and pain share emotion-related brain network. *NeuroImage* 2009;48:200-206.
40. Craig AD. How do you feel--now? The anterior insula and human awareness. *Nat Rev Neurosci* 2009;10:59-70.
41. Menon V, Uddin LQ. Saliency, switching, attention and control: A network model of insula function. *Brain structure & function* 2010;214:655-667.
42. Leknes S, Tracey I. A common neurobiology for pain and pleasure. *Nat Rev Neurosci* 2008;9:314-320.
43. Jovanovic H, Perski A, Berglund H, Savic I. Chronic stress is linked to 5-ht(1a) receptor changes and functional disintegration of the limbic networks. *NeuroImage* 2011;55:1178-1188.
44. Parsons RG, Ressler KJ. Implications of memory modulation for post-traumatic stress and fear disorders. *Nat Neurosci* 2013;16:146-153.
45. Urry HL, van Reekum CM, Johnstone T, Kalin NH, Thurow ME, Schaefer HS, Jackson CA, Frye CJ, Greischar LL, Alexander AL, Davidson RJ. Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *J Neurosci* 2006;26:4415-4425.
46. Mobbs D, Marchant JL, Hassabis D, Seymour B, Tan G, Gray M, Petrovic P, Dolan RJ, Frith CD. From threat to fear: The neural organization of defensive fear systems in humans. *J Neurosci* 2009;29:12236-12243.
47. Linnman C, Moulton EA, Barmettler G, Becerra L, Borsook D. Neuroimaging of the periaqueductal gray: State of the field. *NeuroImage* 2012;60:505-522.

48. Carrieri-Kohlman V, Gormley JM, Eiser S, Demir-Deviren S, Nguyen H, Paul SM, Stulbarg MS. Dyspnea and the affective response during exercise training in obstructive pulmonary disease. *Nursing research* 2001;50:136-146.
49. Wise RG, Preston C. What is the value of human fmri in cns drug development? *Drug discovery today* 2010;15:973-980.