Diffuse Noxious Inhibitory Control of Cough and the Urge-to-Cough

Abubakar B Abubakar

BSc (Hons), MSc

A thesis submitted for the degree of *Doctor of Philosophy* at
Monash University in 2022

Department of Medical Imaging and Radiation Sciences
School of Primary and Allied Health Care

Copyright notice

Abstract

The abstract should outline the main approach and findings of the thesis and must not be more than 500 words.

Background

Cough is a necessary reflex mechanism that protects the airways in response to stimuli. Cough is also a distressing symptom for people when it becomes chronic. Before patients cough, they can experience a sensation called the "urge-to-cough" (UTC). Recent evidence suggests that both cough and the UTC are susceptible to behavioral and neural modulation via neuronal networks that are widely distributed throughout the brain. Current therapies have been ineffective in reducing persistent cough, and better cough suppressant methods are needed. A greater understanding of the neural brain regions associated with cough and the UTC is needed to develop novel suppression therapies. Exploring mechanisms with the potential to modulate cough, such as conditioned pain modulation (CPM) or the diffuse noxious inhibitory control (DNIC) effect could be especially informative. This mechanism is known to incorporate cortical and subcortical regions to reduce the perception of pain felt at one site in the body during the concurrent application of pain at a distant site. The studies in this thesis investigated CPM-related modulation of cough and the UTC using behavioural measures and functional magnetic resonance imaging (fMRI).

Methods

In three distinct experiments, healthy adult participants underwent a series of psychophysical tests and regional blood oxygen-level-dependent (BOLD) fMRI of the brain to detect the modulation of UTC and cough in humans. The first experiment (Chapter 2) involved each participant inhaling capsaicin to evoke an UTC and cough during either the absence or application of painful conditioning. Ratings of UTC and cough frequency were recorded during the trials followed by BOLD fMRI scans of the brain under both contingencies (pain and no pain). This protocol tested behavioural outcomes of painful conditioning on cough and the UTC including measurement of regional BOLD signal intensities during the experimental contingencies to determine whether the responses of higher brain regions within the capsaicin inhalation network were modified by painful conditioning. As the lower brain is a smaller and more difficult region to probe with fMRI, a second experiment (Chapter 3) was employed and optimised for assessing regional

brainstem responses to the inhalation of capsaicin with and without painful conditioning stimuli. The third experiment (Chapter 4) assessed regional brainstem responses associated with differences in the intensity of the UTC evoked by two concentrations of capsaicin (high and low).

Results

Painful conditioning resulted in significant reductions in participants' UTC ratings and cough frequencies. In addition, painful conditioning during fMRI was associated with widespread decreases in neural activity in networks within the capsaicin-evoked regions in the higher brain. fMRI optimised for the brainstem showed capsaicin-evoked activations within the pons and rostral medulla. However, there were no regions that showed an increase in activations during conditioning events compared to no pain events. Similar findings were observed during fMRI analysis for regional brainstem responses associated with differences in the intensity of UTC evoked by high and low concentrations of capsaicin.

Conclusion

The novel findings in this thesis demonstrated that pain can modify cough-related behavioural and neural processing in humans which is consistent with mechanisms associated with the DNIC phenomenon. The neural outcomes observed in the brain hemispheres also provided evidence of a DNIC-related modulation of incoming sensory input from the airways; most likely via a descending inhibition of such inputs from brainstem nuclei. However, the findings of the current thesis are yet to prove this modulation at the brainstem level, most likely due to the limits of fMRI imaging of the brainstem region. Future research of brainstem mechanisms involved in the DNIC-related modulation of airway inputs may identify regions that could be utilised as potential therapeutic targets for drug development in a clinical setting. The findings of such investigations may aid in addressing unwanted chronic cough, yielding a positive impact on the millions of people worldwide suffering from this disease.

Publications during enrolment

Journal Paper

Abubakar AB, Bautista TG, Dimmock MR, Mazzone SB, Farrell MJ. Behavioral and regional brain responses to inhalation of capsaicin modified by painful conditioning in humans. *Chest.* 2021. Mar;159(3):1136-1146. doi: 10.1016/j.chest.2020.08.2105. Epub 2020 Sep 11. PMID: 32926869

Peer-reviewed international conference abstracts

Abubakar Abubakar, Matthew Dimmock, Stuart Mazzone, Michael Farrell. Pain conditioning of behavioural and regional brain responses to tussive stimuli 2020. Virtual poster presentation at the Organization for Human Brain Mapping 23th Annual Meeting (Poster #1197), Montreal, Canada.

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 1 original paper published in peer reviewed journals, 1 conference paper and no submitted publications. The core theme of the thesis is diffuse noxious inhibitory control of urge-to-cough and cough. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Department of Medical Imaging & Radiation Sciences under the supervision of Dr Matthew R Dimmock and Associate Professor Michael J. Farrell.

(The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.)

In the case of Chapter 2, my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision, submitted)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
2	Behavioral and regional brain responses to inhalation of capsaicin modified by painful conditioning in humans	*Accepted*	*60%. Concept and design, collecting data, data analysis and manuscript write-up*	*1) 25% Michael J. Farrell, concept and design, data collection, manuscript write up* *2) 5% Matthew R. Dimmock, input into the design, data collection, manuscript write up* *3) 5% Stuart B. Mazzone, manuscript write up* *4) 5% Tara G. Bautista, manuscript write up*	*No*

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: **Date:** 26/03/2022

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: **Date:** 26/03/2022

Acknowledgements

First of all, I would like to extend my sincere gratitude to my supervisors, A/Prof. Michael Farrell and Dr Matthew Dimmock. I sincerely thank you both for your continuous generosity and support throughout my PhD journey. You both always had your doors open for many of my endless questions during my studies. A special thanks to Michael for always being patient with me and taking the time to explain and guiding me through the topic and teaching me the knowledge of data analysis about imaging. This is a skill that I have acquired with your help, guidance and expertise that I will forever be in debt to you. Matthew, thank you for always being there for me, pushing me to be my best, constant encouragement and getting the best out of me especially during hard times.

A special thanks also goes to Prof. Stuart Mazzone for all your kind support and guidance during my PhD. Even though our interaction was limited, your support meant a lot to me. I would also like to thank all the Monash Biomedical Imaging (MBI), Monash University team for their support during the preparation and data collection for my studies. In particular, I would like to thank Mr. Richard McIntyre for your technical support, scanning my participants and for the fun times I've had down at the scanner. I would also like to thank all the participants who volunteered their time to be part of this study.

A big thank you to all my friends and fellow students at MBI especially Anjan, Winnie, Sharon and Paul. Thank you for all the countless coffee and lunch outings including the late-night studies we enjoyed together. Our friendship and bond will always be appreciated. I can say that I have made friends for life through this journey no matter where life takes us.

Last but not the least, I would like to thank my family (my parents, siblings, wife and children), especially my wife – Zainab. Thank you for your support and encouragement throughout my PhD journey over the years. You have giving up a lot to support me during this time.

Thank you so much everyone and wish everyone all the best in the future.

Table of Contents

Copyright notice 2
Abstract 3
Publications during enrolment 5
Thesis including published works declaration 6
Acknowledgements 7
List of Figures 10
List of Tables 11
List of Abbreviations 12
Chapter 1 – Introduction 15
Cough 15
Chronic Cough: Clinical Indications, Medications, Disorders & Implications 15
Idiopathic Cough 16
Urge to cough 17
Circuitry involved in processing the Urge to Cough 18
Functional MRI of brain activity associated with Urge to cough 20
Modulation of the UTC and cough in humans 26
Diffuse Noxious Inhibitory Control (DNIC) 29
Neuronal control associated with DNIC 31
Investigation of the DNIC effect using functional brain imaging 37
Factors affecting DNIC and its relevance in clinical pain syndromes 43
Summary 48
Aims 48
Experiment 1 49
Experiment 2 49
Experiment 3 50
Significance 51
Chapter 2 - Behavioral and regional brain responses to inhalation of capsaicin modified by painful conditioning in humans 52
Introduction 52
Conclusion 64
Chapter 3 - Modulation of cough-related neural processing in the brainstem by conditioning painful stimulation in humans 65
Introduction 67
Methods 69
Results 75
Discussion 86
Conclusion 92
Chapter 4 - Regional brainstem responses of urge to cough based on the intensity of capsaicin concentration. 94
Introduction 96
Methods 98

Results 101
Discussion 111
Conclusion 115
Chapter 5- Discussion 117
Summary 117
Behavioural response of cough and the UTC 118
Neural interaction between pain and the UTC 119
Conclusion 121
Chapter 6 -Conclusion 122
Summary of Findings 122
Implication of Studies 122
Limitations of Experimental Methods and Future Directions for Research 123
Conclusions 126
References 127
Supplementary Material (Chapter 2) 142

List of Figures

Figure 1. Schematic representation of the haemodynamic response in brain tissue during rest (top) and stimulated state (bottom). 22
Figure 2. fMRI images obtained from the scans showing areas of excitations throughout the brain. 24
Figure 3. Functional brain maps of sensorimotor activations after capsaicin inhalation in humans in both axial and sagittal planes. 25
Figure 4. fMRI images showing brain activations during control trials and placebo administration trials. 27
Figure 5. Schematic illustration of the pain processing pathway in rodents. 34
Figure 6. Figure showing the effect of noCPM versus CPM in participants based on 8 test stimuli. 40
Figure 7. CPM brainstem sites. 41
Figure 8. Plots showing the percentage differences in intensity changes of participants for periods of CPM and noCPM in all three brainstem regions (PB, SRD, SpVc). 41
Figure 9. A comparison of BOLD signal changes associated with capsaicin inhalation (green) and painful thermal somatosensory stimulation (blue). 42
Figure 10. fMRI protocol during scanning. 72
Figure 11. Psychophysical results of the UTC and cough frequency. 76
Figure 12. Regional brainstem responses elicited during capsaicin inhalation challenges. 80
Figure 13. Brain responses within the field of view for CN and CN>CP. 82
Figure 14. fMRI imaging protocol during scanning. 101
Figure 15. Behavioural results between high and low capsaicin concentrations. 102
Figure 16. Regional brainstem activations during capsaicin inhalation challenges. 104
Figure 17. Brainstem regional response for Hi>Sal. 106
Figure 18. Regional higher brain responses for Hi>Sal and Hi>Lo. 107
Figure 19. Regional higher brain responses for Hi>Sal and Hi>Lo. 108

List of Tables

Table 1. Individual psychophysical data. 78
Table 2. Regional brainstem activations during capsaicin inhalation (CN). 81
Table 3. Regional higher brain activations during capsaicin inhalation with no pain (CN). .. 84
Table 4. Regional higher brain activations for CN>CP. 85
Table 5. fMRI power calculation table for sample and effect size for regions of interest. 86
Table 6. Regional brainstem activations during inhalation of high concentration of capsaicin. 105
Table 7. Regional higher brain activations during high capsaicin concentrations greater than saline inhalations (Hi>Sal). 109
Table 8. Regional higher brain activations during high capsaicin concentrations greater than low concentrations of capsaicin (Hi>Lo). 110
Table 9. fMRI power calculations showing sample and effect size required to elicit difference for selective regions of interest. 111

List of Abbreviations

ACC	anterior cingulate cortex
AMCC	anterior midcingulate cortex
Amy	amygdala
Abd	nucleus abducens
AST	archi-spino-thalamic tract
ATP	adenosine triphosphate
BA44	brodmann area 44 of the primary somatosensory cortex
BET	brain extraction tool
BOLD	blood oxygen level dependent
Cb	cerebellum
CH	chronic hypersensitivity
CN	capsaicin inhalation with no pain
CP	capsaicin inhalation during pain
CPM	conditioned pain modulation
CSF	cerebrospinal fluid
Cu	lowest concentration to elicit an urge to cough sensation
C2	capsaicin concentration required to elicits two or more cough
DHSC	dorsal horn of the spinal cord
DLPFC	dorsolateral pre-frontal cortex
DMPFC	dorsomedial prefrontal cortex
DNIC	Diffuse noxious inhibitory control
DR	dorsal raphe nuclei
DRt	dorsal reticular nucleus
EPI	echo planar images
FEAT	fMRI Expert Analysis Tool
FLAME	FSL's Local Analysis of Mixed Effects
FLIRT	FMRIB linear registration tool
fMRI	functional magnetic resonance imaging
FUGUE	FMRIB's utility for Geometrically Unwarping EPI
GLM	general linear model
Hb	deoxygenated haemoglobin
HbO_2	oxygenated haemoglobin
Hi	Concentration of capsaicin that elicits a high UTC

HRF	hemodynamic response function
Hypo	hypothalamus
Hz	hertz
IBS	irritable bowel syndrome
ICA	independent component analysis
IFG	Inferior frontal gyrus
In	insula
ISN	ipsilateral substantia nigra
LC	locus coeruleus
LF	longitudinal fasiculus
LFG	left inferior temporal gyrus
Lo	Concentration of capsaicin that elicits a low UTC
LORETA	low-resolution brain electromagnetic tomography
mACC	mid-anterior cingulate cortex
MCG	midcingulate gyrus
MD	mediodorsal nucleus of the thalamus
MFG	middle frontal gyrus
Mes5	mesencephalic trigeminal tract and nucleus
MR	median raphe nuclei
ms	milli-seconds
nTS	nucleus of the solitary tract
OFC	orbitofrontal cortex
OP	operculum
Pa5	paratrigeminal nuclei
PAG	periaqueductal gray
ParCG	paracingulate gyrus
PB	parabrachial nucleus
PosCG	postcentral gyrus
PM	premotor area
PreCG	precentral gyrus
PST	paleo-spina-thalamic tract
rACC	rostral anterior cingulate cortex
RFG	right inferior frontal gyrus
Rp	midline raphe
RIII reflex	withdrawal reflex

RVM	rostral ventro-medial medulla
s	seconds
Sal	saline
SD	standard deviation
SI	primary somatosensory cortex
SII	secondary somatosensory cortex
SMA	supplementary motor area
S_{max}	maximum inhaled capsaicin concentration without coughing for 20 seconds
SMG	supramarginal gyrus
SN	saline inhalation with no pain
SP	saline inhalation during pain
SPT	spinothalamic tract
Sp5	spinal trigeminal
Sp5C	pars caudalis of the spinal trigeminal
Sp5I	par interpolaris of the spinal trigeminal
SpVc	spinal trigeminal nuclei caudalis
STG	superior temporal gyrus
TE	echo time
Th	thalamus
TMD	temporomandibular disorder
TR	repetition time
UTC	urge-to-cough
vlPFC	ventrolateral prefrontal cortex
VIIN	facial motor nucleus
VPM	ventral posteromedial nucleus of the thalamus
XIIN	hypoglossal nucleus
F(df1,df2)	(hypothesis degree of freedom, error degree of freedom)
t(df)	(t-test degree of freedom)
(μ±σ)	(mean± standard deviation)
μM	micromolar
3T	three tesla
5thal	trigeminothalmic tract
°C	degree Celsius

Chapter 1 – Introduction

Cough

The cough reflex is essential for protecting against both blockage of and damage to the airways [1]. Additionally, it is tightly regulated in humans and animals as it is critical for the protection of respiration [1]. The presence of mechanical and chemical stimulants within the airways leads to the activation of several subsets of airway afferent nerves which often trigger coughing. The airway afferents then relay information via ascending pathways to regions of the brainstem initiating a cough reflex and the subsequent clearance of material from the airways [2]. There are three phases associated with the cough reflex mechanism: an initial inspiration, followed by compression and then an expulsion [3]. Furthermore, cough is similar to other respiratory reflexes in that it can also be initiated voluntarily or behaviourally. Behavioural cough is usually a repetitive cough in the presence of an underlying disease [4]. In humans, cough can be voluntarily initiated on demand as well as voluntarily suppressed [5]. Notably, recent research has shown that the cough reflex can also be influenced by internal factors such as attention and anxiety and external factors such as social context [6-8]. This implies that there is a contribution to cough control from the cortex, in addition to the brainstem mediated reflex loop of cough [9]. These observations contributed to the current interest in the neural processes involved in the voluntary control of cough. Despite recent advances in our understanding of the mechanisms involved in the regulation of cough, little is known about the neural modulation associated with cough reduction and how it operates in humans.

Chronic Cough: Clinical Indications, Medications, Disorders & Implications

Cough lasting for more than eight weeks is described as chronic cough. More than 30 million patients presented with chronic cough at primary care facilities in the United States in 2010 and between 9 and 33% of the adult population in the developed world suffers from this condition [10-12]. The treatment and management of chronic coughing has mainly been via the prescription of codeine and dextromethorphan [13]. These antitussive drugs suppress cough by acting on the central nervous system [14]. However, recent studies have shown that both codeine and dextromethorphan are not significantly more effective than placebo (a substance or treatment of no intended therapeutic value) at suppressing cough [15-18]. A meta-analysis of 25 randomised placebo-controlled experiments, performed by Smith et al (2008), concluded that “there is no good evidence for or against the

effectiveness of over the counter medicines in acute cough" [19]. Given the ineffectiveness of current treatments of this chronic condition, there has been growing demand for, and an increase in the awareness of, the need for research into the development of better cough suppressants.

There are many disorders associated with chronic cough and persistent UTC. Chronic obstructive pulmonary disease, asthma, gastro-oesophageal reflux and bronchitis are amongst the most common disorders associated with chronic cough [20]. Additionally, chronic cough is associated with many complications including vomiting, rib fractures, loss of sleep and urinary incontinence [21]. The long-term effects of chronic cough include social isolation and embarrassment, anxiety and depression [22,23].

Idiopathic Cough

There is a proportion of patients that suffer from idiopathic chronic cough [24]. Despite extensive clinical studies, the aetiology remains largely unexplained. Some studies suggest that such individuals may suffer from an abnormal or overly sensitised cough reflex [25,26]. A review study conducted by O'Neill et al (2013) indicated that an infection or disease may cause a remodelling of the cough reflex regulatory network [25]. This is known as neuronal plasticity which can lead to heightened responses to normally innocuous stimuli although it is not confined to idiopathic cases of chronic cough [25]. Plasticity of neural responses may lead to hypersensitivity caused by dysregulation of sensory neural pathways and central processing in cough reflex regulation causing exaggerated coughing [27]. However, the precise pathways and mechanism associated with idiopathic cough needs further research as most studies involving cough neurophysiology have been undertaken in animal models. Traditional treatment via antitussives such as dextromethorphan tend to be ineffective for individuals suffering from idiopathic cough. Other forms of treatment such as opioid based cough suppressants often lead to habituation in the long term including the risk of developing side effects [28].

In addition, acute airways pathologies such as upper respiratory infection can lead to cough hypersensitivity [29]. However, the hypersensitivity in these patients may be stimulus specific rather than universal in nature [1]. A number of potential explanations for such discrepancy include decline in attention to sensation of the UTC or differential mechanisms. For instance, speaking, laughing, drinking and or eating may trigger cough in

patients with cough hypersensitivity [30]. The exact reason for the discrepancy remains unclear which further highlights the complexity of the neural processes governing chronic cough disorder. Therefore, it is hoped that further research into the central pathways involved in the generation of a cough reflex may be of use. It is possible that behavioral or pharmaceutical modification to restore or enhance the function of the inhibitory circuits involved in the cough reflex may lead to better therapeutic outcomes than the currently available treatment options.

Urge to cough

Cough is often accompanied by a sensation known as UTC. This sensation is usually described as a feeling of tickle or itch in the back of the throat [31]. The primary complaint of UTC is the constant sense of irritation in the airways and one way to relieve this is by physically coughing [32]. However, previous research has also indicated that the somatosensory irritation of an airway may not necessarily correlate closely with the affective sensation of a perceived UTC [31,33]. Furthermore, Hilton et al (2015) studied the experiences of sensations and relievers associated with UTC amongst chronic cough patients. The findings showed that 91% of patients would cough in response to an UTC. On the other hand, only 69% of patients agreed that they would always experience an UTC before coughing. In addition, participants reported several ways for relieving the UTC which included drinking fluids, sucking sweets, chewing gum and expectorating sputum [1]. In some instances, the motor response of cough due to a persistent UTC does not necessarily lead to cough relief [31]. Notably, these findings are consistent with reports of similar patients in previous studies indicating that not all chronic cough patients experience an UTC before coughing [34,35].

Cough can either be generated reflexively before a sensation of UTC is felt, or it can be produced via a conscious (voluntarily) sensory process; the latter mechanism is where the stimulation of the airways causes a recognition of a desire to cough depending on both the intensity and context of the stimulus [32]. When the mechanism is conscious, the eventual production of cough relies on a series of events, beginning with the relay of sensory input from the airways to subcortical nuclei within the brainstem. Brainstem neurons in receipt of this sensory input then relay this information to the cortex where cognitive, emotional and contextual information is integrated [36]. Descending pathways then feed-back to brainstem regions that initiate or suppress the motor response of cough [36-38]. Notably, the primary

somatosensory cortex has been widely implicated in recent studies to be an important region involved in the generation of voluntary cough [39].

Investigating the neural mechanisms associated with this sensory experience of the UTC, rather than the cough reflex itself, would likely provide a better alternative perspective that could be useful in understanding chronic cough disorders. With this in mind, therapeutic strategies that aim to block the basic cough reflex (including the reflex defensive cough that protects the airways from harmful substances) may not be useful in part, because the absence of cough in humans may result in other clinical problems and may also do nothing to target the central mechanisms associated with excessive coughing or behavioral cough. More desirable outcomes may be achieved with the development of new therapeutic models that target and reduce the UTC or mimic the endogenous inhibitory pathways that help regulate cough in humans. Understanding the neural substrates involved in sensing and responding to airways irritations that evoke an UTC is necessary for identifying novel approaches to cough reduction in chronic cough patients.

Circuitry involved in processing the Urge to Cough

The sensation of an UTC is initiated by peripheral nerve receptors found in the respiratory tract, particularly the bronchi, trachea and larynx. Mechanical and chemical stimulation leads to the activation of subpopulations of these sensory neurons [38]. Capsaicin and other substances such as bradykinin, adenosine triphosphate (ATP) and citric acid activate chemical receptors while mechanical cough receptors found within the linings of the epithelia react to physical stimulation such as particulate matter [38,40,41]. There are two distinct groups of neurons which reach the airways and receive airway afferent input via different vagal branches [42]. The cell bodies of these neurons are found in the nodose ganglion and the jugular ganglion. The neurons from the two ganglia have distinct phenotypes and anatomical projections to the brainstem via different vagal branches[38].

Retrograde tracing has been used to visualise the origin and terminal branches of airway afferents with cell bodies in the nodose and jugular ganglia [43]. The sensory afferents with cell bodies in the jugular ganglion innervate the proximal airways (upper trachea and larynx) while sensory afferents with cell bodies in the nodose ganglion innervate the same airway levels with the inclusion of lower airway segments (lungs). Neurons from both ganglia have projections to the brainstem via the vagus nerve which is a subsidiary of the

cranial nerve [43-45]. The afferents with cell bodies in the nodose and jugular ganglia terminate at the nucleus of the solitary tract (nTS) and the paratrigeminal nuclei (Pa5) of the brainstem respectively. These neurons in turn innervate second-order neurons which either project to other nuclei within the brainstem, or ascend to higher order circuits or descend to the spinal cord [46]. One outcome associated with second order neurons that project to brainstem nuclei is the inducement of reflex motor responses such as cough or autonomic outflow. However, sensory neurons that ascend to higher brain circuits terminate at the pontine, midbrain and a variety of subcortical and cortical sites that encode various involuntary and voluntary cough motor responses and sensations subsequent to airway sensory nerve stimulation [38].

Recent studies have investigated brainstem inputs from airway afferents that ascend to higher brain circuits. Neurons transmitting airway inputs synapse with circuits that ascend the neuro-axis and terminate at several brainstem and higher brain regions. The regions include the thalamus, insula cortex, primary somatosensory cortex and the amygdala [47,48]. However, these circuits have distinct pathways based on whether they project from the nTS or Pa5 brainstem nuclei. For instance, animal studies have shown that airway specific nodose relays via the nTS terminate in the amygdala, hypothalamus and the zona incerta [46]. In contrast, airway-jugular related circuits are relayed via the Pa5 and have broader representation within the medulla, pons and thalamus [42,46]. These fundamentally different circuits of the Pa5 and nTS, identified from animal studies, likely contribute to distinct functional behaviors associated with vagal afferent stimulation leading to the reasonable question as to whether such findings are representative of that involved in human airway sensation. This query was addressed to some extent in human studies where it was shown that differential patterns of human brainstem activations exist between the nTs and Pa5 during inhalation of capsaicin versus ATP (adenosine-triphosphate). ATP inhalation only showed activations in the nTS while inhalation of capsaicin activated both the nTS and the Pa5 [49]. These fundamental differences in the nodose and jugular relay of airway afferents into higher brain sites combined with their distinct sensory processing of different stimuli, suggests that they may have distinct roles in the evocation of cough and the UTC in animals and more recently in humans. However, research is lacking regarding whether one or both of these nuclei are involved in the modulation of UTC and cough in humans. There is evidence suggesting that the nTS serves as the primary relay nucleus for the processing of airway inputs leading to the likelihood that it may play a role in modifying the UTC sensation [38]. However, no research has yet confirmed this hypothesis. Therefore, it is

worthwhile to investigate the brainstem and higher brain responses associated with the modulation of UTC to gain a better understanding of the underlying circuitry involved in this process.

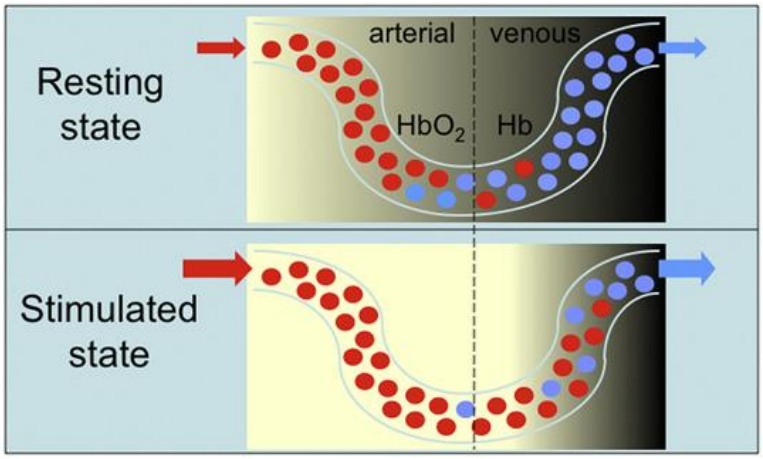
Functional MRI of brain activity associated with Urge to cough

Since its development in the 1990s, functional magnetic resonance imaging (fMRI) has become a leading research tool for mapping brain activity [50-52]. This mapping of brain activity in fMRI is based on the link between neuronal function and associated cerebrovascular hemodynamic changes [53-55]. Early use of fMRI produced brain maps of motor cortex activation after developing human echo planar imaging (EPI) capability [53,56-59]. It wasn't until more recently that fMRI became a widely used tool in neuroimaging studies involved in investigating the cerebral changes in brain activity during emotional and cognitive experiences [52]., These investigations led to better understanding of the neural mechanisms underlying perception in humans and non-human primates [55].

During the performance of a task or specific condition, rapid acquisition of successive EPI of the brain allowed for the mapping of brain regions that are selectively active as a result of changes in neural activity [60]. Neural activity requires oxygen which influences local vessel diameter to induce an increase in oxygenated haemoglobin (HbO_2) compared to deoxygenated haemoglobin (Hb), as arterial blood flow to the region is enhanced [61]. This leads to an over-supply of oxygen in the activated regions and a decrease in deoxygenated haemoglobin (Figure 1) [62]. This process involves an initial build-up of Hb and then a decrease in HbO_2 in the intra- and extra vascular spaces. This decrease in HbO_2 is then followed within a second or two by a vasodilatory response that reverses the situation to result in an increase in HbO_2 and decrease in Hb over that in the resting condition. This sequence of processes leads to an increase in local signal and is referred to as the hemodynamic response [62-64]. HbO_2 is diamagnetic and is magnetically indistinguishable from brain tissue. However, Hb is paramagnetic. This paramagnetism leads to changes in local gradients in magnetic fields whose strength depends on the Hb concentration [65]. The differences in the magnetic properties and ratio of Hb and HbO_2 in the blood is known as blood oxygen level dependant (BOLD) contrast in fMRI [65,66].

The aim of task activation fMRI is to induce different neural states in the brain as the stimulus is manipulated during the scan. A mean regional increase in BOLD signal intensity over successive EPI during a task of interest represents changes in neural activity [67]. EPI is the process of obtaining whole brain scans in a given time frame. In general, most fMRI is performed using an EPI method which can collect data for a 2-dimensional image in approximately 60 milli-seconds (ms) at typical spatial and temporal resolutions (3.4 x 3.4 x 4 mm^2 voxel size). Whole brain scans are typically acquired with a repetition time of (TR) 2 seconds per volume [62]. Activation maps are then derived by using statistical tests that compare the signals recorded during the task of interest to alternative states during the experiment [62,67]. The statistical test for creating activation maps can use a general linear model (GLM) or other forms of data analysis such as cross-correlation with a modelled regressor [62]. fMRI studies that use a GLM approach for the statistical analysis of EPI images obtained from scans include several steps [67]. Prior to GLM analysis, a pre-processing step involving spatial smoothing of EPI is undertaken in order to improve signal to noise ratio. GLM analysis includes co-alignment and co-registration techniques as regressors in the model to reduce noise. This approach substantially increases the number of true positives during statistical analysis making GLM the preferred method for fMRI analysis [68]. The first step involves setting up a design matrix by convolving the external stimulus with a pre-specified hemodynamic response function (HRF) over the duration of a scan. The second step involves fitting the linear model to a single run of fMRI data allowing for spatially varying autocorrelated errors [69,70]. This output is then combined from separate analyses from different runs within a session, different sessions on the same subject and across subjects within a population using a random effect analysis [70,71]. A t-stat threshold sets the threshold for peak and cluster size detection using random theory. The combined output from all the separate analysis is then registered to an atlas standard [67]. The resultant output from the process is then combined with output from other individuals to create a group result and an activation map for the events of interest [67].

Figure 1. Schematic representation of the haemodynamic response in brain tissue during rest (top) and stimulated state (bottom).



Red and blue circles represent red blood cells that are fully oxygenated (HbO2) and fully deoxygenated (Hb), respectively. The MRI signal is depressed in the venous side of the capillary due to the paramagnetic susceptibility of the Hb acting as an endogenous contrast agent (shown darker). In the stimulated condition, increased blood flow causes the Hb to be swept out and replaced by HbO2, causing a BOLD signal increase. Adapted from Glover (2011) [62].

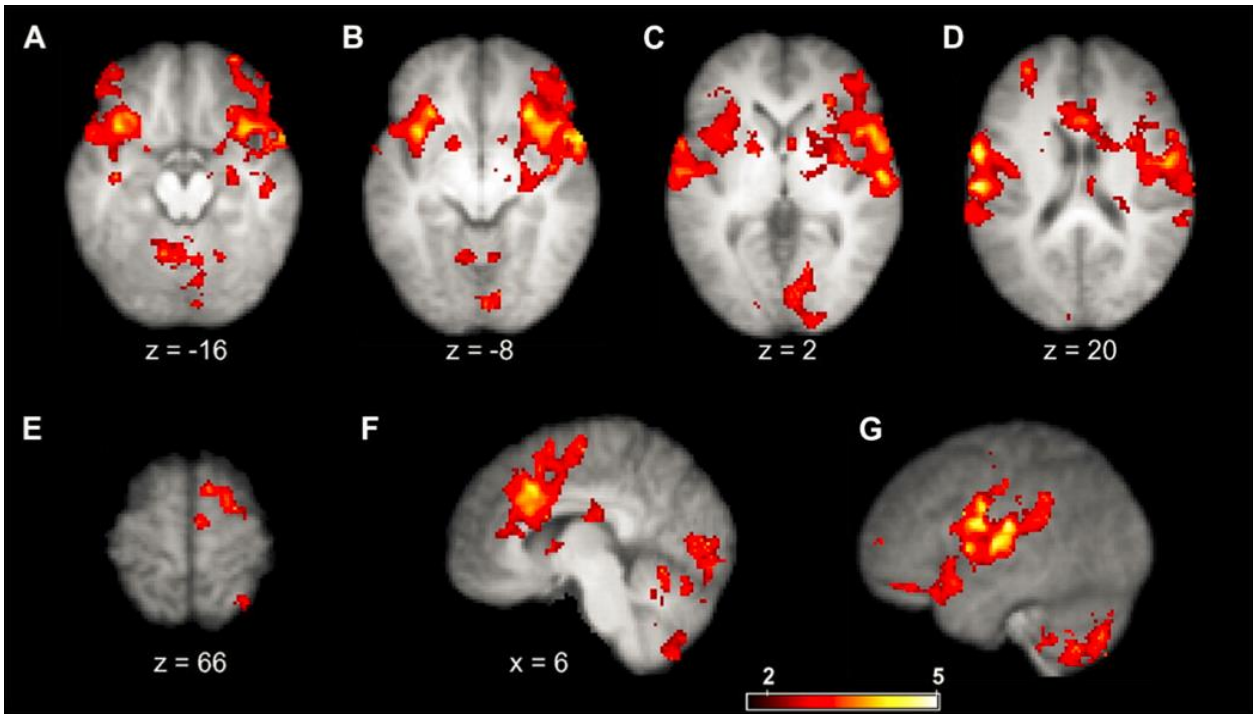
Experimental studies conducted in animals and humans have provided a detailed list of the candidate brain regions that comprise the broader neural network involved in sensing and responding to airway stimuli. For instance, studies analysing neuronal activation following airway sensory stimulation via the use of c-Fos protein expression in animals (cats & guinea pigs) following airway irritation have suggested that cough neural pathways represent a widely distributed network in the brain [8,72-75]. Additionally, previous fMRI studies whereby the inhalation of an irritating airway stimulus (capsaicin) was used to evoke human airway sensation provided insight into some of the constituents of the brain circuitry involved in cough related processing [76-78]. The list of candidate brain regions observed was extensive with activations seen in the primary sensory (somatosensory), cingulate, premotor, motor and orbitofrontal cortices. Other regions that also showed activations include the insula (In), medulla, pons, thalamus (Th) and the cerebellum (Figure 2) [78]. Some insight into circuit organisation associated with airway irritation has been provided in neuroanatomical tracing studies that employed techniques whereby the use of neurotropic viruses allowed for the mapping of interconnected networks of neurons [42,46,79]. As previously described, vagal sensory afferents innervating the airways terminate in the nTS and Pa5 with connections from these medullary nuclei having been traced to

several midbrain nuclei which also have connections to cortical regions such as the insula, cingulate and somatosensory cortices [79]. Using these approaches, at least two ascending airway sensory pathways have been proposed; one which project from the thalamo-limbic pathway projecting via the mediodorsal thalamus onto the anterior insula, cingulate and orbital cortices and another which projects from medullary nuclei to the ventro-basal thalamus onto the somatosensory cortices [38,77]. These outcomes are compatible with the complex phenomenology of cough indicating the existence of multiple neural circuits in the brain that differently coordinate the unique aspects of sensory, motor and cognitive processing of cough and the UTC. Defining these distinct neural substrates requires carefully considered methodologies that provide insights into their organisation from both a functional and anatomical viewpoint.

In behavioural studies, airway irritation is associated with processes that allow for the spatial localisation of the stimulus (*where is it coming from?*) including the assessment of stimuli (*what is the "magnitude" of a given stimulus?*) and the related perceptual consequences (*what level of urge is felt?*). Therefore, it is expected that different regions of the brain encode these different components of the UTC. This hypothesis was tested in fMRI investigations that 'deconstructed' the UTC network into distinct components that appear to encode the different sensory discriminative aspects of the UTC during inhalation of capsaicin [76]. fMRI outcomes showed activity in the anterior insula and the primary somatosensory cortices to be associated with the magnitude of the stimulus (intensity dependent), while activations in the primary somatosensory cortex were correlated with the perception of airway irritation (UTC dependent) in individuals (Figure 3) [77]. In addition, activations in the pre-frontal and posterior parietal cortices were associated with the localisation of the intensity of given stimuli [77]. This is consistent with other sensory paradigms that similarly suggest that prefrontal and posterior regions are important for determining qualitative rather than quantitative aspects of a stimulus [76].

Furthermore, active suppression of urge-related cough was shown to be associated with activations in the dorsomedial prefrontal cortex (DMPFC), dorsolateral prefrontal cortex (DLPFC), right inferior frontal gyrus (IFG), right anterior insula cortex, anterior mid-cingulate cortex (aMCC) and the SMA [37,76,77,80,81]. Such investigation may provide novel insights into the neural mechanisms involved in the modulation of the UTC network and can aid in identifying regions that can act as targets for future development of therapies to address chronic cough disorders.

Figure 2. fMRI images obtained from the scans showing areas of excitations throughout the brain.



The slices coordinates were generated according to the Montreal Neurological Institute (MNI) convention. Regional activations were seen to be distributed in both hemispheres including the orbitofrontal cortex (A), inferior frontal gyrus (A & B), anterior insula (B, C), superior temporal gyrus (C), and primary motor and somatosensory cortices (D, G). Capsaicin activations within the middle of the brain included the supplementary motor area (E), and the anterior midcingulate cortex (F). Axial slice image (D) shows activations within the caudal end of the central sulci in both left and right hemispheres and is associated with capsaicin activations in the primary motor and somatosensory cortices [78].

Figure 3. Functional brain maps of sensorimotor activations after capsaicin inhalation in humans in both axial and sagittal planes.

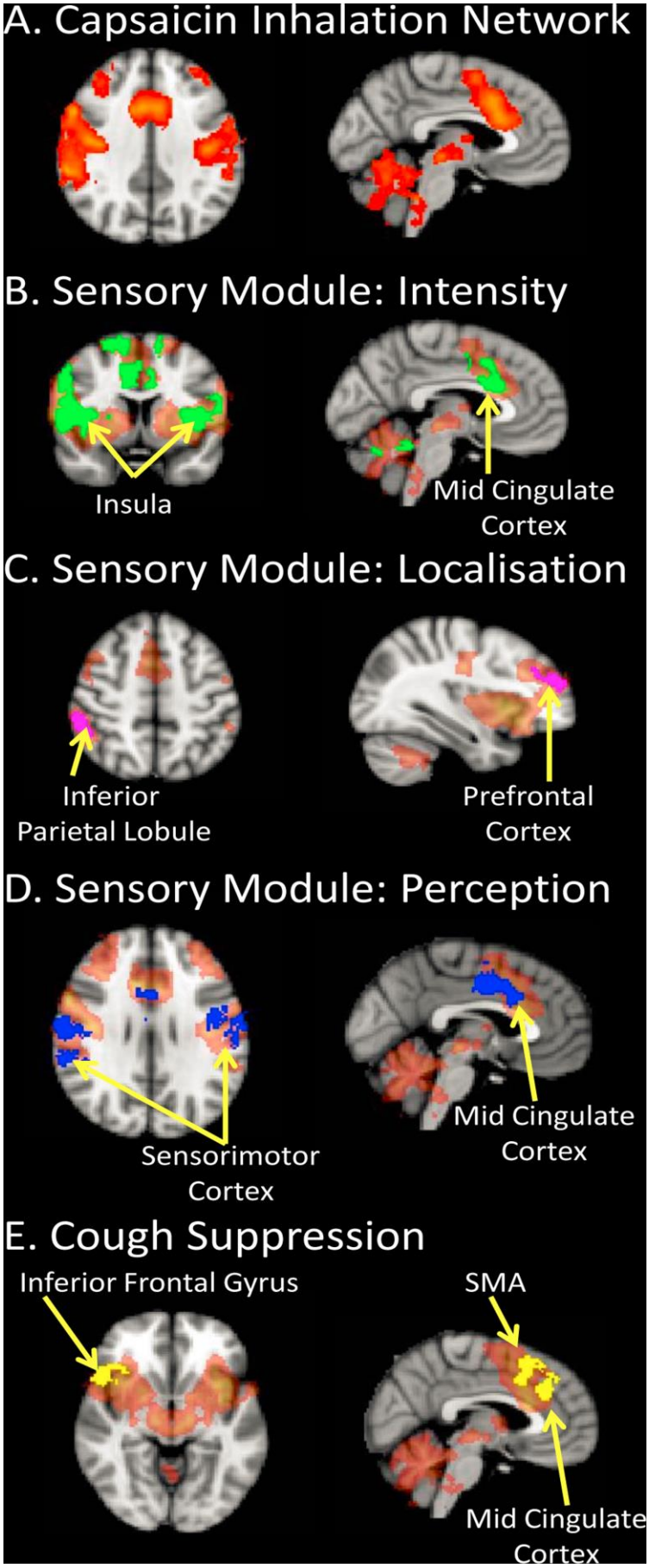
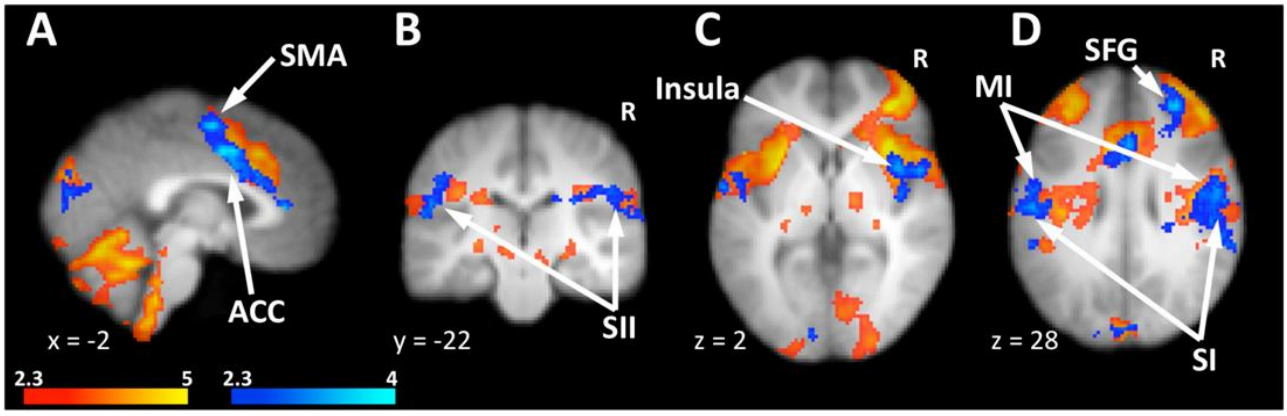


Image (A) showing several activation networks associated with capsaicin inhalation; image (B) shows encode stimulus intensity of the sensory module and is highlighted in green color; image (C) identifies stimulus localisation while image (D) shows activation of areas associated with perceptual experiences and image (E) shows areas activated due to cough suppression. The green, blue, pink and yellows highlighted areas indicated areas of superimposition (B to E) in comparison with capsaicin inhalation network (A) which is highlighted in orange [77].

Modulation of the UTC and cough in humans

Our understanding of the underlying mechanism and neurobiology involved in the modulation of the UTC and cough in humans has recently been evolving. However, very few studies have investigated the behavioural and neural outcomes associated with cough reduction. Studies have shown that both cough and the UTC are markedly reduced by placebo antitussive treatments suggesting the presence of inhibitory mechanisms that can modify processing of sensory inputs [82,83]. Psychophysical trials showed a reduction of 42% in UTC ratings by participants during placebo trials compared with control trials. fMRI results of the control trials showed similar regions of brain activation associated with UTC comparable to previous studies (Figure 4) [82]. Additionally, there were widespread reductions in activation in several brain regions of the UTC network as a result of placebo administration. These regions include the In, SMA, anterior cingulate cortex (ACC) and the primary motor and somatosensory cortices. Furthermore, several regions also showed an increase in activation during placebo administration when compared to control trials. These regions include the posterior parietal cortex, middle frontal gyrus (MFG) (specifically with the region containing DLPFC) and the precentral gyrus (PreCG) [82]. Specifically, the right MFG showed placebo antitussive-related levels of activation that correlated with the size of behavioral placebo effects measured with UTC ratings. The placebo related decreases and increases in activations in several brain networks were consistent with a top-down process. Networks that showed decreases in activation can be explained as a reduction in the encoding of incoming sensory information that leads to a predictable lower rate of an UTC. However, regions that showed increased activity are likely as a result of putative influences of higher brain circuits related to beliefs, expectations or conditioning mechanisms [82]. Also, placebo responses are a result of cognitive processes whereby knowledge that an individual may be taking new therapy and expectation that this therapy may be beneficial act to reduce the sensation of UTC or cough [83,84]. The data presented from this study reinforces the notion that multiple central inhibitory mechanisms exist for modifying cough in humans.

Figure 4. fMRI images showing brain activations during control trials and placebo administration trials.



The regions highlighted in red and yellow indicate regions of capsaicin activation during control trials. A contrast between the control and placebo antitussive trials was undertaken to show regions of reduced activation after placebo administration to suppress cough. This is seen rendered as the blue highlighted regions which are on top of the structural images of the control trials. Image A shows a sagittal slice with the supplementary motor area (SMA), and anterior cingulate cortex (ACC) as regions of reduced activation following placebo administration. Image B shows a coronal slice with reduction in activation of the secondary somatosensory cortex (SII) regions in both hemispheres located the parietal operculum area. Image C is an axial slice showing the insula as reduced regions of activation in both hemispheres of the brain after placebo administration. The primary motor (MI) and somatosensory (SI) cortices and the superior frontal gyrus (SFG) showed reduction in activation of capsaicin associated regions after placebo administration as seen in image D [82].

In pain studies, the pain modulation of placebo analgesia has been shown to involve the descending control of nociceptive inputs to the dorsal horn of the spinal cord from supraspinal regions [85-87]. This descending control of pain inputs involves brain regions such as the ACC, hypothalamus amygdala and the periaqueductal gray (PAG) including the brainstem region known as the rostral ventro-medial medulla or nucleus ventromedialis (RVM) [86,87]. The output from this descending control of the RVM influences spinal nociceptive processing and its input into higher brain regions according to the particular behavioural circumstance. Importantly, this mechanism involves the endogenous opioid system [85-87]. Pain related activity in the human spinal cord was shown to be markedly reduced during placebo analgesia suggesting that cognitive factors mediate their effects

early in the nociceptive pathway and that spinal inhibition is one possible mechanism during placebo analgesia [88-90]. fMRI studies have also provided evidence that this system of inhibitory descending modulation is also a major route by which cognitive and contextual influences change in the experience of pain including that activity in these regions correlate to the behavioural reductions in pain experience [91-94]. In addition, frontal and limbic brain regions have also been shown to be involved in the cognitive control of pain and since these regions are reciprocally connected to the brainstem, they also can exert a descending influence on spinal nociception [91,95,96]. These outcomes support a descending ACC-PAG-pons-medulla pain modulating circuit as being involved in placebo analgesia suggesting a prefrontal limbic-PAG-RVM-dorsal horn of the spinal cord (DHSC) pathway [85,88,97,98].

The outcomes of the previous and recent studies have advanced our understanding of the neural regulation and modulation of the UTC in humans [76-78,80,82,99]. It is also evident that airway processing involves a complex circuitry that can be manipulated to reduce cough and the UTC in humans as observed in placebo studies [82,83]. The advances in knowledge observed in the placebo related modulation of the UTC provided an opportunity to exploit the modulatory influences without enlisting placebo protocols. If airway afferents can be subject to placebo modulation, then other forms of modulatory influences for nociceptive inputs may also operate for airway inputs. With limited research and gaps in knowledge regarding the modulation of the UTC and cough in humans, exploring the central and neural mechanisms that can inhibit airway inputs leading to a reduction in persistent UTC is needed. One method to further investigate the behavioural and neural modulation of the UTC and cough is via a process known as diffuse noxious inhibitory control (DNIC), and more recently termed CPM (conditioned pain modulation). There is already preliminary evidence regarding the DNIC effect in relation to both the perception of airway irritation (UTC) and cough in humans. Studies conducted by Young et al (2011) and Hilton et al (2020) have shown that painful conditioning in the form of cold water significantly reduces the UTC and cough frequency amongst healthy participants [84,100]. The findings are similar to the inhibitory influences of the placebo effect on the UTC [82,83]. These outcomes suggest that the pathways involved in the coding of somatic and pulmonary inputs interact with each other to induce a DNIC effect leading to meaningful changes in the sensory experience of airway irritation. However, the neural mechanisms of a DNIC effect on the UTC in humans is yet to be explored. Further exploration of the cough circuitry via a DNIC process would help reveal the networks and mechanisms involved in the modulation of the

UTC and cough in humans. Outcomes from such investigations would provide novel insights into which circuitry is involved in the downregulation of airway sensory inputs in humans paving the way for better therapeutic outcomes in the clinical context.

Diffuse Noxious Inhibitory Control (DNIC)

The phenomenon of DNIC emerged many decades ago [101,102]. A DNIC effect occurs when the response from one painful stimulus is inhibited by another, often distant noxious stimulus [103]. Behaviourally, the DNIC effect can be defined as the ability to reduce the sensory experience of one painful stimulus due to application of a second painful stimulus [103]. Previously, DNIC was originally used in animal research to describe a specific lower brainstem mediated inhibitory mechanism. However, recent studies have adopted the use of a different term known as CPM which specifically describes the psychophysical paradigms in which a conditioning stimulus is used to affect a test stimulus [104-107]; this was a result of psychophysically based human studies that involve the use of a variety of methods and terms regarding the modulation of pain by conditioning stimuli leading to the need for unified use of methods and terms that accurately encompass distinctive aspects of animals versus human experimentation [105,107].

Behavioural studies of the DNIC effect have mainly used laboratory experiments to measure the psychophysical responses for a test stimulus before, during and after the application of noxious conditioning stimulus. The effect is considered DNIC if the results show a reduction in the magnitude of the test response during the concurrent application of the conditioning stimuli [108]. Several studies have used different approaches in healthy individuals to evoke the DNIC effect and determine other factors affecting this effect via psychological outcomes [109].

A literature review by Pud et al (2009) identified the commonly used experimental methods to evoke a DNIC effect in young healthy individuals [108]. A wide range of experimental techniques such as electrical, chemical, mechanical and thermal stimulation were used as test-pain or conditioning stimuli [108]. The approximated median magnitude of the DNIC effect evoked by painful conditioning across the studies was 29% indicating a significant reduction in pain felt by participants during conditioning [108]. Other findings of the review include that the DNIC effect gradually fades with time [109-112]. Such outcomes led to the

conclusion that the DNIC effect is higher for parallel testing in comparison to sequential testing and that ongoing pain may exhaust this phenomenon due to increased facilitation through descending pathways [113]. In parallel testing, conditioning stimulus is applied during the application of the test stimulus [114,115]. On the other hand, sequential testing involves assessing the DNIC effect by recording pain ratings of the test stimulus after conditioning stimulus is applied after a specified period of time [114,116,117].

The majority of DNIC studies have involved somatic pain as both test and conditioning stimuli with limited research related to how DNIC operates when a visceral noxious sensation is used as test stimulus. The reduction in the sensory experience of a test stimulus as a result of a DNIC-effect observed in many DNIC-related studies associated with somatic pain provides merit for investigating DNIC-effects on noxious visceral sensations such as cough and the UTC. There is already preliminary evidence that is limited to few studies that have investigated the DNIC-related effects during noxious visceral sensations such as dyspnoea and cough with limited insights into the mechanisms involved in regulating visceral pain. For instance, recent studies have shown that DNIC can significantly reduce the sensory experience of both cough and the UTC while dyspnoea has also been shown to trigger a DNIC effect in humans [84,100,118,119]. The findings of such studies provide evidence that endogenous inhibitory pain pathways can be activated to inhibit visceral sensations such as cough and the UTC...

It is evident that a DNIC-effect is particularly effective at reducing cough and the UTC in humans according to findings from previous studies [84,100]. Despite these advancements in knowledge, several questions regarding the psychophysical modulation of the DNIC-effect on the UTC and cough remain unanswered. This provides merit for further investigating the modulation of the UTC and cough based on a pain-centric paradigm including providing an opportunity to gain a better understanding of the mechanisms associated with cough reduction in humans. For example, it is yet to be determined whether the reduction in cough frequency during a DNIC effect is proportionate to decreases in the UTC ratings. One study by Farrell et al (2012) had demonstrated that increases in doubling concentrations during capsaicin inhalations led to a linear increase in UTC ratings and an exponential increase in cough frequency [76]. Investigating the disparity in behavioural outcomes for DNIC-related effects on the sensory experience of the UTC versus the motor act of coughing could have therapeutic implications for the treatment of chronic cough condition in the clinical environment. Furthermore, no studies to date have yet investigated

the distinct neural substrates associated with the modulatory influences of DNIC on airway processing in humans. Although it seems likely that the behavioural DNIC-related outcomes on the UTC during conditioning stimuli likely entail cortical and subcortical modulation of the sensory pathways involved in discerning an UTC, these networks have yet to be studied under experimental conditions in humans. Uncovering the functional neuroanatomy associated with a DNIC-related modulation in the UTC under fMRI experimental conditions may help gain novel insights into the neural mechanisms and brain regions involved in the descending inhibition of sensory airway inputs. These outcomes may help us understand the processes involved in the DNIC-related downregulation of the UTC and may aid in identifying brain regions involved in this process. Such regions may act as targets for future therapeutic intervention to help reduced abnormal or persistent UTC in humans.

Neuronal control associated with DNIC

A DNIC effect may be triggered when conditioning stimuli are applied to a body site distant from the actual excitatory receptive field under study, provided that the stimulus is noxious [120]. In experimental animals, the DNIC mechanism has been shown to completely inhibit incoming nociceptor signals at the primary synapse indicating that DNIC effects do not only have psychophysical outcomes but also have neurological outcomes [101,102,121]. Early studies of the DNIC effect have sought to investigate the multi-receptive neurons associated with responding to noxious and mechanical stimuli. The first study into the neuronal control of the DNIC effect was undertaken by Le Bars and his colleagues in the late 1970s [101,102]. Further research by Le Bars (2002) provided an insight into the neuronal control of the DNIC effect, including the sensory tracts involved in the relay of painful stimuli [120].

Previous animal studies including those mentioned in the review by Le Bars (2002) found that neurons involved with the sensory regulation of the DNIC effect are located in the dorsal horn of the spinal cord as well as the trigeminal nuclei, caudalis and oralis [120,122,123]. Such neurons are multi-receptive in nature meaning that they receive signals from the viscera, joints and muscles throughout the body [124]. Some neurons are deep while others are found in the superficial layers of the dorsal horn. In addition, a gradient of sensitivity is exhibited by these receptive neurons where mechanical stimuli (small hair movements, light touch) no matter how small can activate the neurons centrally. On the contrary,

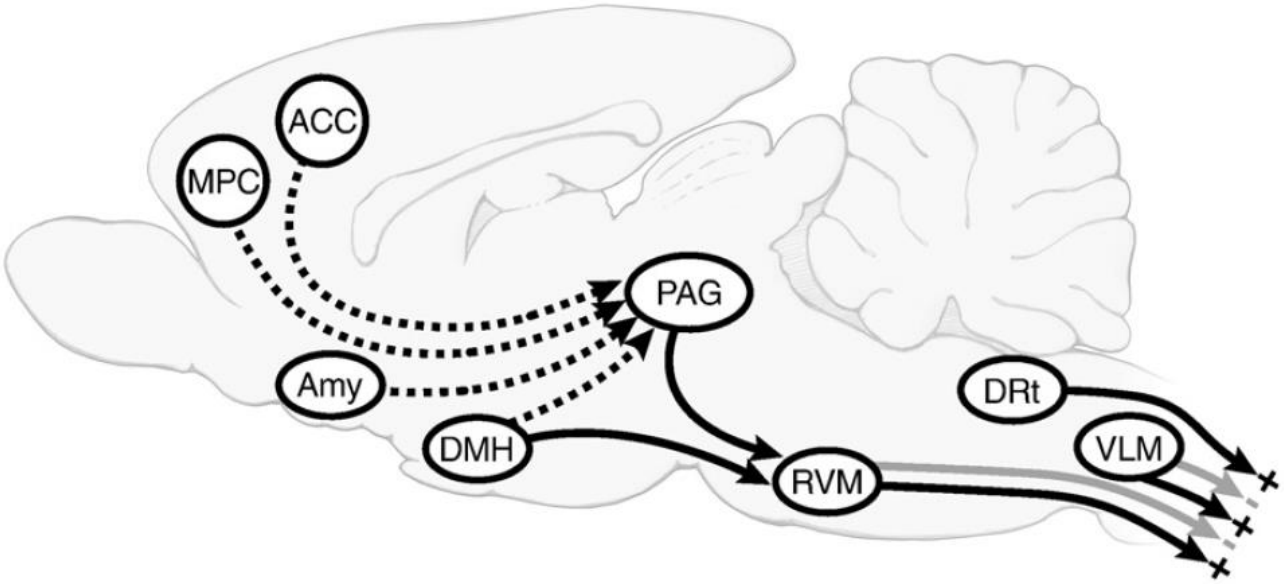
intense stimuli lead to neuronal responses at the periphery of the receptive fields of individual neurons [120]. In addition, these neurons are activated by Aδ (delta) - fibres which respond to mechanical stimuli and are responsible for initial reflex to acute pain; and C - fibres which respond to chemical, mechanical and thermal stimuli [103]. The activation of Aδ – fibres leads to rapid and sharp pain experiences in humans while the activation of C - fibres causes slow and burning pain [103]. The neurons project to other brainstem nuclei via ascending pathways such as the spino-reticular and spino-thalamic tracts to give polysynaptic responses due to a DNIC effect caused by the application of other noxious stimuli [125-127]. The remaining neurons project to the thalamus while others are only involved in spinal reflexes [128]. Notably, one study has indicated that signals responsible for triggering DNIC in animals are essentially confined to the spino-reticular tract with no active role played by the spino-thalamic tract [129-134].

The spinal cord compartments involved in DNIC have also been widely investigated [129,135]. Lesioning of the dorsal, dorsolateral and ventromedial parts of the cervical spinal cord in rats was found not to affect inhibitory processes triggered by DNIC. However, bilateral lesioning of the ventrolateral quadrants of the spinal cord was shown to completely diminish the inhibitory influences of DNIC in rats [129]. The dorsolateral fasciculi of the spinal cord has also been shown to be involved in the DNIC triggering [135]. These outcomes strongly suggest the involvement of the ventrolateral quadrant and the dorsolateral fasciculi of the spinal cord in the triggering of DNIC [129]. In addition, animal studies have shown that the ascending pathways involved in triggering DNIC are mainly crossed but also have a significant uncrossed component [129]. This was evident where the restriction of the lesion to the left anterolateral quadrant strongly diminished DNIC effects during painful stimulation to the right hind-paw in rats whereas DNIC elicited by stimulation to the left hind-paw was not affected. To further investigate the crossed nature of the DNIC pathways responsible for the inhibition inputs, the effects of lumbar commissurotomy were investigated. The findings showed that DNIC responses were strongly depressed (but not completely) whether triggering came from the left or right hind-paw in rats further suggesting that uncrossed components also plays a role in the inhibitory processes of DNIC origin.

As previously mentioned, Aδ- and C-fibres transmit pain signals into the spinal cord and terminate in the external part of the dorsal horn of the spinal cord. They both synapse onto neurons which are located in the dorsal horn of the spinal cord [136]. These specific

nociceptive neurons which receive Aδ- and C-fibres combine to form the spinothalamic tract (also known as the neo-spinal-thalamic tract) (SPT) while the non-specific nociceptive neurons acquire mainly non-nociceptive stimuli and receive all fibres including Aδ, C, Aα(alpha) and Aβ(beta). The axons from the non-specific neurons form the paleo-spina-thalamic tract (PST) while a third tract known as the archi-spino-thalamic tract (AST) carries multi-synaptic neurons (specific and non-specific nociceptive from the whole body) into higher brain circuits. Neurons from the SPT tract project to the thalamus and primary somatosensory cortex of the brain contra-laterally via the medulla oblongata to convey pain sensations from the face, temperature and touch [137]. SPT neurons also have projections to the midbrain and brainstem structures such as the PAG and the RVM (Figure 5) [138,139]. It is believed that these structures return efferent signals downwards to various levels of the spinal cord where, with the help of inhibitory inter-neurons, they produce a diffuse inhibition [120]. This is evident in studies where it was shown that the DNIC effect disappears in animals with a sectioned spinal cord, suggesting that DNIC mechanisms are most likely not confined to the spinal cord but appear to involve supraspinal regions [101,120,138,140-144]. A similar outcome was also observed in human studies where the DNIC effect was absent in patients with complete spinal cord transection [137,141].

Figure 5. Schematic illustration of the pain processing pathway in rodents.



The PAG-RVM system exerts bidirectional nociceptive modulation from the midbrain to the dorsal horn of the spinal cord and the DRt and the ventrolateral medulla (VLM) in the caudal medulla. The PAG especially but also the RVM, DRt and VLM (not shown) receive important direct and indirect inputs from limbic forebrain structures that include the ACC, amygdala (AMY), dorsomedial nucleus of the hypothalamus (DMH) and medial prefrontal cortex (MPC) [139].

Furthermore, there exists a system of descending pain-control pathways that is associated with the DNIC effect. These are neural pathways that descend from the central structures of the nervous system and diminish pain signals travelling up the ascending pathways from the body to the brain. It can therefore be suggested that pain pathways are a direct link between pain receptors in the body and pain centres in the brain [103]. Thus, incoming noxious information can be modulated leading to pain becoming less of a reflexive response to an injury. For instance, an individual can get injured or wounded but practically feel no pain in the heat of the action suggesting that pain [145].

As depicted in early studies, DNIC involves a spinal-medullary-spinal pathway with ascending information projecting from the ventrolateral quadrant of the spinal cord towards supraspinal centres and descending projections from the supraspinal centres via the dorsolateral funiculi of the spinal cord to neurons of the dorsal horn [141,146,147]. Additionally, early animal studies have implicated several supraspinal regions of the brain that are typically involved in the processing of pain input to be involved in the DNIC mechanism.

These include the PAG, RVM, cuneiform nucleus, parabrachial area and the locus coeruleus [142,148]. However, the presence of lesions in these regions do not appear to modify DNIC responses in rats suggesting that such regions may not likely to be involved in the inhibition of inputs associated with the DNIC mechanism [142,148,149]. Other cortical and subcortical structures such as the thalamus were also shown to not be directly involved in DNIC. This is evident in studies whereby DNIC was not affected by the presence of thalamic lesions in patients [149]. Instead, the dorsal reticular nucleus or subnucleus reticularis dorsalis (DRt or SRD) which is also known to be involved the processing of pain signals has been found to be critically involved in DNIC [134,146,149-151]. Such findings were supported in patients with Wallenberg syndrome where DNIC was shown to be absent as a result of unilateral lesions restricted to the medulla [137]. The DRt has reciprocal connections with the dorsal horn of the spinal cord and is preferentially activated by nociceptive stimuli arising from any part of the body [150,152]. Further evidence that the supraspinal loop sustaining DNIC is confined with the most caudal part of the medulla and involves the DRt was confirmed in a series of experiments where the potency of DNIC was tested in animals with complete sections at different levels of the brainstem [120,136]. The tests showed that DNIC disappears once the tests exceed the caudal part of the brainstem. These outcomes suggest that the DRt is one of the key relay stations of DNIC compared to the prefrontal limbic-PAG-RVM-DHSC pathway of other cognitive modulatory influences such as placebo, distraction and attention.

Furthermore, studies have also investigated whether DNIC is partly mediated via an opioidergic mechanism. The involvement of the opioidergic circuitry in DNIC effects has been confirmed in several studies whereby opioidergic neurons in the DRt were shown to activate during DNIC in rodents [153-157]. Further evidence of the requirement of opioidergic signalling in DNIC was shown when DNIC was abolished by naloxone (opiate antagonists) injection into the DRt in sham rats but was unaltered in neuropathic rats; since these experiments, deficient or reduced efficacy of DNIC effects has been widely associated in neuropathic pain syndromes [104,134,158-160]. These outcomes suggest that DRt involvement in DNIC requires endogenous opioidergic mechanism. In human studies, the interaction between opioid use and CPM effects has also been explored. Ram et al (2009) showed that opioid-treated patients had less efficient CPM compared non-opioid treated patients (non-opioid analgesics), further suggesting that opioids decrease the activity of descending pathways in humans [161]. Findings from another study conducted by Nielsen et al (2007) obtained similar conclusions that DNIC is probably opioid-dependent [162]. Notably,

other modulatory effects such as placebo analgesia have also been shown to be associated with activation of the endogenous opioid system, further suggesting the similarities between DNIC and placebo modulation of pain in humans [85,98,163-169].

Furthermore, assumptions have previously been made that DNIC-related effects may be due to distraction or attention caused by the application of painful conditioning. In pain studies, cognitive distraction has been shown to be an effective pain coping strategy suggesting that it may employ similar descending endogenous inhibition of input signals as seen in DNIC experiments [85,170]. For example, attention was shown to modulate spinal cord responses to pain further providing evidence of the inhibition of incoming pain signals in the spinal cord which a similar observation seen in DNIC scenarios [171-175]. However, this narrative has been shown to not be case in several studies that confirmed the hypothesis that DNIC and distraction are independent of each other and that the modulation of pain via DNIC is not due to cognitive attention recruitment to the conditioning pain [84,85,95,170,176-179]. For instance, distraction showed significant increases in activation in the DLPFC, the ACC as well as the PAG including covariation analysis revealing functional interaction between these structures [170]. This is consistent with reports of increased activation of the DLPFC and the ACC during the placebo-related conditioning of UTC in humans [82]. These outcomes imply that the two regions may exert a top-down influence on the PAG to gate pain modulation during distraction and placebo-conditioning. It is noteworthy that other factors such as emotion and attentional control can also reduce the experience of pain [85]. Several psychophysical and neuroimaging studies have shown that negative and positive expectations have powerful influence on the perception of pain [178,180]. It is apparent that emotional, cognitive and placebo effects on pain share similar mechanisms, in that they recruit a top-down process to modulate pain in humans. On the contrary, DNIC or CPM effects result in only decreases in neural activity in higher brain regions implying that this mechanism employs a bottom-up process that involves the descending inhibition of input signals at the lower levels of the brain leading to reduced sensation of pain in the hemispheres. The data reported from several studies have confirmed that DNIC-like effects observed in humans are not simply due to attentional distraction or cognitive processes such as emotion [95,170,178].

Collectively, the literature suggests that the excitatory and inhibitory process of a DNIC effect is distinct from other modulatory effects related to the cognitive influences on pain. The classic prefrontal limbic-PAG-RVM-DHSC pathway has been suggested to be the

likely pathway that produces of cognitive influences on pain such as placebo, distraction and attention [181]. Additionally, there is evidence of the involvement of key higher brain regions such as the ACC including its enhanced functional connectivity with the PAG during the modulation of pain as a result of the cognitive influences of attention, distraction and placebo [84,95,170,177,178,182]. On the contrary, studies have shown DNIC to rather involve a DHSC-DRt-DHSC pathway which is independent of the involvement of the brain hemispheres [120,134,142,146]. For instance, the infralimbic (ILC) of the medial prefrontal cortex of the hemispheres was shown to not be directly involved in DNIC suggesting that DNIC is not dependent on higher brain regions [134]. Additionally, there is preliminary evidence that painful conditioning can modulate and reduce one's UTC and cough frequency behaviourally in humans [84,100]. However, the neural mechanisms associated with this psychophysical influence on the UTC is yet to be determined. Possibilities include the prefrontal limbic-PAG-RVM-dorsal horn of the spinal cord which resembles a placebo modulation or rather a DHSC-DRt-DHSC that is consistent with a DNIC modulation of airway inputs. To date, no imaging studies have explored the descending inhibitory pathways associated with the reduction in the perception of the UTC and the motor act of coughing in humans. Whether the neural substrates associated with DNIC modulation of the UTC are similar to those observed in placebo studies remains unknown. Therefore, further investigations of the circuitry involved in the modulation of the UTC during an induced DNIC effect will provide a better understanding of which brainstem and higher brain regions are associated with this descending modulation of airway inputs. Identifying these circuits could pave the way for future research where such regions could be targets for therapeutic outcomes that will help address chronic cough in humans.

Investigation of the DNIC effect using functional brain imaging

The majority of DNIC related-studies in humans have mainly described the DNIC effect in terms of rating bias where participants labelled their experience of pain as less intense, or adjusted their ratings with the expectations of researchers [106,108,183,184]. Previous studies on dissected rats identified brainstem regions involved in the mediation of the DNIC effect in animals [146]. However, the mechanisms involved in the inhibition of the DNIC effect in humans are yet to be fully established. Youssef et al (2016) investigated the neural mechanisms associated with the DNIC effect in portions of the brain in healthy humans using fMRI [107]. A test stimulus was applied with a thermode to the right side of the mouth

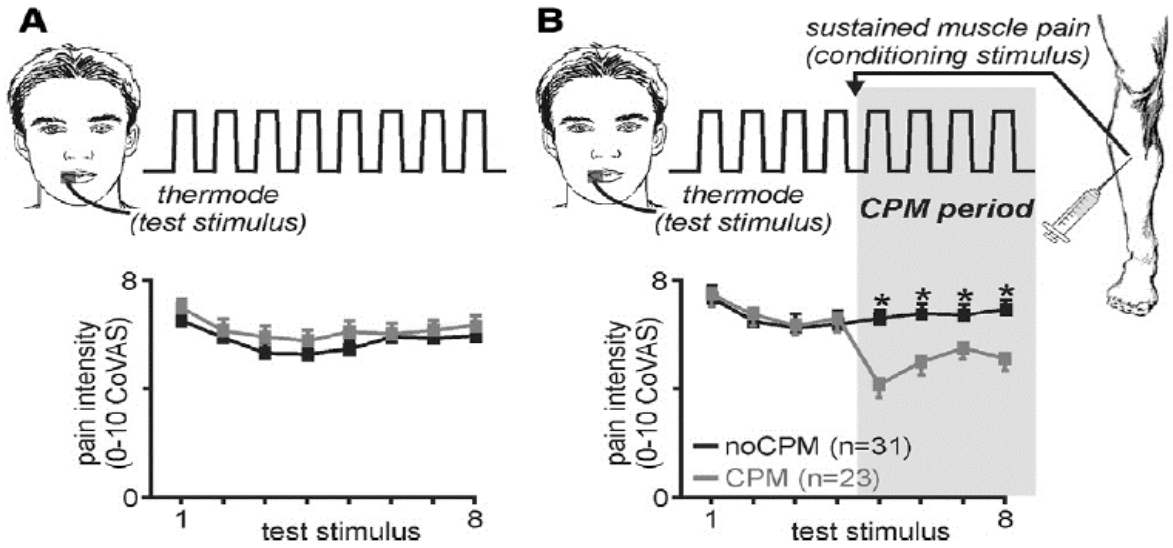
in the form of noxious heat stimuli on eight occasions to evoke a moderate level of pain (rating 6 out of 10) (Figure 6). This paradigm was repeated, and hypertonic saline was injected into the leg at the fourth noxious heat stimulus to evoke a DNIC effect. In 23 of the 54 subjects, the conditioning stimulus resulted in a significant reduction in pain intensity of the test stimulus (CPM). For the remaining 31 subjects, the conditioning stimulus had no effect on the pain intensity of the test heat stimulus (noCPM) (Figure 6). Additionally, there was no significant difference in test stimuli thermode temperature (noCPM: 47.7 ± 0.2 °C, CPM 47.4 ± 0.2 °C, $p>0.05$) between the noCPM and CPM groups.

Psychophysical results from the study showed significant decreases in the rating of pain intensity during times of DNIC (CPM) (-29.0 ± 2.9% i.e.: a reduction of 29% in pain intensity) compared to non-DNIC (noCPM) (3.7 ± 3.2%) trials (Figure 6) [107]. Brainstem activity was also recorded during the non-DNIC and DNIC trials. Initial fMRI results showed evidence that the expression of pain correlated with changes in brainstem activity in both the DNIC and non-DNIC groups once the test noxious stimulus was applied. These regions included the dorsolateral pons encompassing the parabrachial nucleus (PB), spinal trigeminal nuclei caudalis (SpVc), ipsilateral substantia nigra (ISN), SRD (DRt) and the PAG. These findings suggest that noxious inputs are not being processed differentially within the brainstem in these two subject groups supporting the notion that the conditioning effect of CPM is most likely not influenced by psychophysical factors (Figure 7) [107]. Notably, the SpVc is a primary afferent synapse region where orofacial nociceptor afferents terminate suggesting why this region is also activated during the application of painful stimulus. BOLD signal intensity from these regions (SpVc, ISN, PB, DRt) acquired during the fMRI scans showed increased signal intensity for CPM subjects when compared to noCPM subjects (Figure 8) [107]. In addition, there was a decrease in signal intensity for CPM subjects in the presence of conditioning stimulus in the SpVc, PB and the DRt which was not the case for the noCPM subjects. No significant differences between noCPM and CPM subjects in terms of changes in signal intensity were observed in the PAG or the medullary raphe nuclei [107].

These findings further support data from animal studies and reinforce the notion that the circuitry involved in the inhibitory process evoked by a DNIC effect lies within certain brainstem nuclei in humans including strengthening the role of the DRt while adding the notion that the PAG is not necessarily involved in this process. It is worthwhile mentioning that the decrease in BOLD signal intensity observed in the DRt during conditioning was not

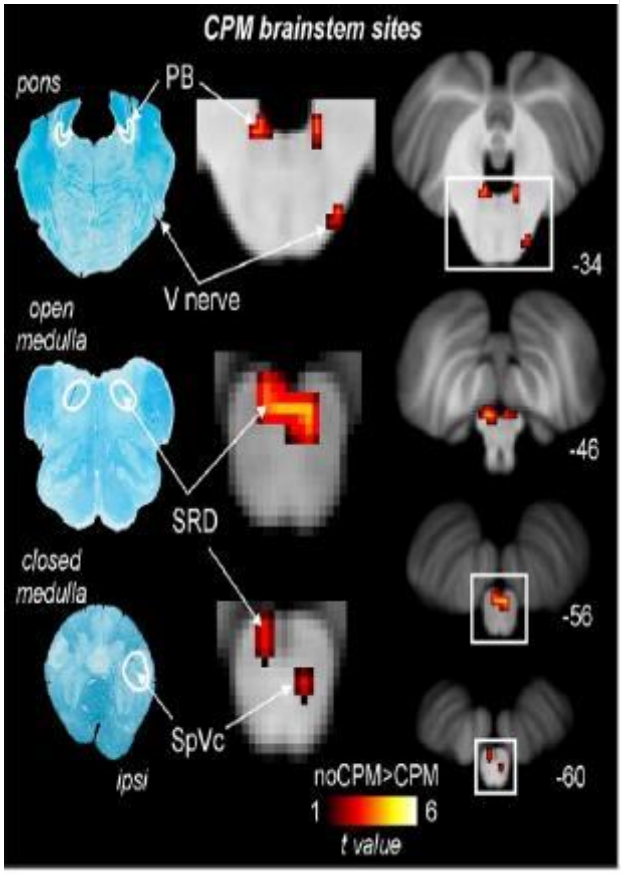
in line with expectations of the direction of signal change given that this region acts as an effector of DNIC-related sensory changes rather than being influenced by a DNIC phenomenon [107,120]. It is expected that the DRt would produce a DNIC effect through widespread projections to nociceptive neurones in the spinal cord and analogous brainstem regions during the application of conditioning stimuli. Therefore, the presumed hypothesises is that BOLD signals measured from the DRt would show an increase in signal intensity given that this region is a driver of DNIC effects, in line with literature regarding the inhibitory mechanisms associated with the DRt during a DNIC effect [120,129,146]. One explanation for such an outcome may be related to the experimental methodology employed during the study. For instance, conditioning a stimulus was applied for a duration of 15 s which is considered a long period of time in the context of fMRI making it difficult to genuinely understand how conditioning changed the BOLD signal intensities of the DRt region. It is known that ongoing application of painful stimuli may exhaust the inhibitory DNIC-related effects due to increased facilitation through descending pathways [113]. Further research that incorporates suitable fMRI protocols is needed to genuinely assess the DNIC-related effects in terms of changes in BOLD signal intensity in the DRt region of the brainstem. Another important finding from this study was that no significant differences in CPM responses between males and females were observed [107]. Other studies have shown greater CPM in men compared to females [107,183,185,186]. However, several studies have refuted this notion and believe that these differences are small and depends on experimental and analytical methodology [106,107,187,188].

Figure 6. Figure showing the effect of noCPM versus CPM in participants based on 8 test stimuli.



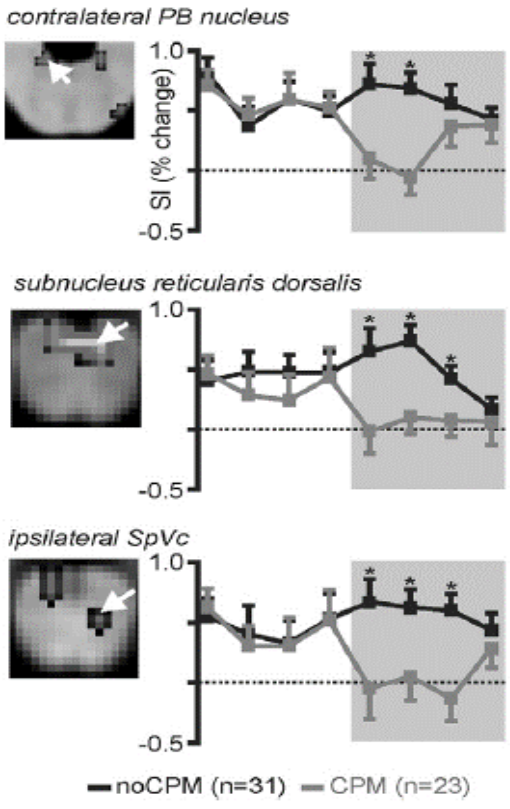
A: *Participants experienced 8 noxious heat stimuli (test stimuli) showing consistent pain ratings between noCPM (non-DNIC) and CPM (DNIC) participants.* ***B:*** *Participants experienced 8 noxious heat stimuli (test stimuli) but with test stimuli 4 and 5, the conditioning stimulus was applied to the tibialis anterior muscle to cause mild/moderate pain. Significant reductions during the CPM (DNIC) period for CPM participants compared to no CPM is shown on the plot graph. The pain rate scale used ranged from 0 (no pain) to 10 (most pain)* [107]*.*

Figure 7. CPM brainstem sites.



A series of axial T1 fMRI slices obtained from DNIC (CPM) participants showing reduced signal intensity in the brainstem regions (PB, SRD, SpVc) [107].

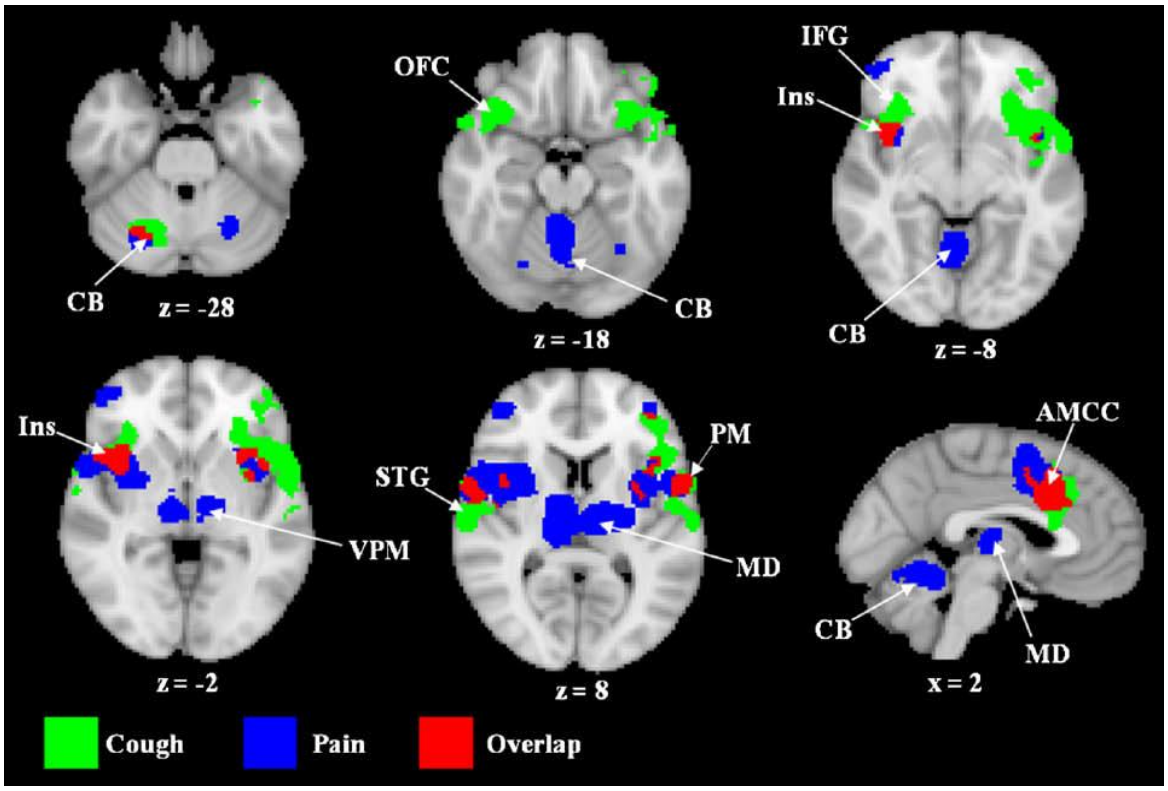
Figure 8. Plots showing the percentage differences in intensity changes of participants for periods of CPM and noCPM in all three brainstem regions (PB, SRD, SpVc).



A significant reduction in signal intensity was observed in all three brainstem regions PB, SRD and SpVc where the grey shades indicate the period when the test stimulus was applied in the presence of the conditioning stimulus [107].

As previously mentioned, research in the field of pain has explored the subcortical and cortical regions involved in the neural processing and relay of inputs during pain stimulation. Higher brain regions constantly highlighted by painful stimuli are those such as the ACC, Th, PreCG, MFG, right inferior frontal gyrus (RFG), left inferior temporal gyrus (LFG) including networks embedded in brainstem regions such as the PAG, DRt (SRD) and the RVM [189-193]. Strikingly, these regions are similar to those observed in studies involving airway irritation undertaken by our group, further supporting the notion that pain and airway processing share similarities in their processing and control of incoming pain inputs (Figure 9) [76-78,80,82,99,194].

Figure 9. A comparison of BOLD signal changes associated with capsaicin inhalation (green) and painful thermal somatosensory stimulation (blue).



Regions of overlapping activations appear red. Data obtained from Mazzone et al., 2007 (capsaicin inhalation) and Farrell et al., 2005 (meta-analysis of thermal pain). Abbreviations: AMCC, anterior midcingulate cortex; CB, cerebellum; IFG, inferior frontal gyrus; Ins, insula; MD, mediodorsal nucleus of the thalamus; OFC, orbitofrontal cortex; PM, premotor area; STG, superior temporal gyrus; VPM, ventral posteromedial nucleus of the thalamus [194].

Collectively, fMRI studies have shown that the brainstem and its subregions are likely involved with the descending inhibition of sensory inputs as a result of a DNIC effect in humans particularly the DRt [107,134,142]. This inhibition in turn affects higher order circuits that process sensory inputs resulting in the reduced sensation of noxious stimuli. The circuitry involved in the processing of pain has also been shown to share similarities with networks involved in the coding of airway inputs [194,195]. This similarity in networks means that the neural pathways for both pain and airway processing share significant homology and thus warrants further investigation on the neural substrates as a result of the interactions between cough-related processing and pain. Such an investigation may uncover novel insights into the functional neuroanatomy and underlying mechanisms involved in the down-regulation of cough and the UTC in humans.

Factors affecting DNIC and its relevance in clinical pain syndromes

There is a growing body of knowledge on the factors that affect the extent of DNIC or CPM in both humans and animals. Recent studies have explored the altered function of endogenous pain modulation in various chronic, idiopathic and neuropathic pain syndromes [104,149,196-198]. CPM has been shown to be less efficient in several of the idiopathic pain syndromes such as fibromyalgia, acute migraine and headache [185,196,199-203]. A similar outcome was observed in animals where a loss of DNIC was observed in rats that experienced prolonged exposure to migraine treatments [203]. Other animal studies have reached similar conclusions where neuropathic pain was associated with deficient DNIC [204]. Additionally, patients with irritable bowel syndrome (IBS), temporomandibular disorder (TMD) and atypical facial pain have been shown to have lower CPM efficiency in comparison to healthy controls [197,205-207]. Neural correlates of DNIC effects in IBS patients showed altered brain activation patterns amongst the pain processing centres of the higher brain and brainstem, when compared to control subjects, implying a dysfunction in the modulation of nociceptive inputs in these patients [208]. Other studies of patients diagnosed with chronic pain syndromes such as chronic fatigue syndrome and chronic pancreatitis have identified the presence of less efficient CPM compared to controls [209,210]. An important question regarding whether a deficient DNIC response may be indicative of developing chronic pain was also recently addressed. Impaired DNIC response was shown to be present in patients with painful osteoarthritis of the hip with restoration of DNIC function 6 to 14 months after successful surgery in a pain-free state. This outcome supports the notion that impairment of DNIC was maintained by the chronic pain state [211].

Opioid-treated patients have also been associated with less efficient CPM compared to non-opioid-treated patients [161]. These findings suggest that pain syndromes decrease the activity of descending inhibition pathways. Neuropathic pain has also been shown to be related to decreased CPM in patients after spinal cord injury [212,213]. However, other studies have shown no difference in CPM versus controls for neurological & psychiatric disorders whose main manifestation is not pain such as Parkinson's disease [214,215]. Alternatively, patients with rheumatoid arthritis and trapezius myalgia had normal CPM efficiency whereas painful knee arthritis patients were associated with low CPM efficiency [216-218]. This disparity in findings suggest that DNIC is deficient predominantly in medically unexplained syndromes [104]. However, more research is warranted before firm conclusions are drawn. There is also evidence that non-painful chronic conditions such as impaired sleep continuity and sleep architecture may be associated with impaired efficacy of DNIC in humans further implying the effect of chronic conditions on DNIC efficacy [219,220].

Furthermore, several studies have reported the altered effects of DNIC or CPM based on several characteristics such as age, gender and ethnicity. Many studies have demonstrated more efficient DNIC effects in men [152,183,185,186,221-224]. However, other studies did not find differences in the DNIC effect between the sexes whereas no studies found a more efficient effect in women [106,188,225-228]. An overview of studies on the sex differences in DNIC responses were found in about 50% of DNIC studies [149]. Interestingly, a study conducted by Staud et al (2003) suggested that sex differences in DNIC response are more consistently found in C-fiber evoked pain conditions as opposed to Aδ-fiber evoked pain. These results indicate increased prominence of the pain modulatory system in males compared to females. Age related differences in the DNIC response during evoked pain has also been reported in several studies [229,230]. For instance, age differences in CPM responses in the form of decreased sensitivity to pain in older adults (mean age ≈ 78 years) compared to young adults (mean age ≈ 23 years) were observed [231]. Another study showed similar outcomes where DNIC was reduced in middle-aged adults (between 40 and 55 years) compared to young adults (between 20 and 35 years) whereas DNIC was absent in older adults (between 60 and 75 years) [232]. Edwards et al (2000) demonstrated age-associated decrements in DNIC responses for older subjects compared to younger subjects after painful cold-water immersion (5°C) indicating differences in central modulation of pain amongst the two groups [233,234]. These concurrent findings suggest that DNIC starts declining at middle age and diminishes at older age [149]. There has also been recent interest in whether DNIC functions differently in individuals with

distinct ethnicity or lifestyle choices [149]. One study assessed pain sensitivity in African Americans and non-Hispanic Whites during stress-induced analgesia as a mechanism of DNIC. The study found DNIC was regulated by cortisol levels which were found to be lower in African Americans compared to their counterpart population [235]. However, several studies failed to show a correlation between DNIC and cortisol levels or between perceived stress and DNIC amongst various populations [188,209,211,236-239].

Moreover, recent studies have investigated whether the site, strength, intensity, type of stimuli and duration of conditioned pain has an effect on the extent of DNIC response [149,183,240]. A stronger and more intense conditioning stimulus has been shown to elicit a greater DNIC effect via increasing painful water temperatures (>45°C) [241]. These outcomes were supported by more recent studies that showed a stronger conditioning stimulus results in a greater DNIC effect during the use of different thermal intensities, muscle pain by saline injection, rectal or gastric injections [221,226,228,242-245]. However, other studies have shown no correlation between the pain scores of conditioning stimuli and the DNIC effect [106,227]. A similar disparity is seen in studies that reported whether the conditioning stimulus needs to be painful [183]. Most studies have shown that only painful stimuli elicit a DNIC effect while few studies found that non-painful stimuli also induced a DNIC effect [120,183,200,241,242]. It has also been shown that a non-painful but strong stimulus can activate DNIC when applied for a longer time, possibly by inducing wind-up or increasing the amount of nociceptive signal in the dorsal root and supraspinal regions [200,226,239,242,243]. Additionally, the intensity of perceived pain appears to be unrelated to the magnitude of the DNIC effect as depicted in several studies [183,224,226,227]. Reports regarding how long the DNIC effect may last after the administration of a painful stimulus have also been investigated [149]. These reports concluded that pain-suppressive effects of DNIC are maximal during the application of the conditioning stimulus and return to baseline values within 5 - 8 minutes after stimulation [110,161,188,211,223,236,246]. The site of a conditioning stimulus also has an effect on DNIC responses. DNIC effects have generally been found only in heterotopic (contralateral) or extra-segmental regions and not homotopic (ipsilateral adjacent) regions [111,247]. Recently, the temporal and spatial changes associated with CPM have also been investigated at the cortical level using a low-resolution brain electromagnetic tomography (LORETA) [240]. A LORETA study by Moont et al (2011) explored the neurophysiological sequence of cortical events underlying CPM in healthy humans, since fMRI is unable to provide an accurate picture of the sequence of cortical events in short time window [240]; this is due to the fact that the hemodynamic response

detected in fMRI peaks approximately 5 seconds after neuronal firings begin in an area [248]. The findings of the study showed increased initial activity in the OFC and amygdala 250-300 ms post stimulus followed by reduced activations in several pain-perceiving and processing cortex areas such as the primary and secondary somatosensory cortex (SI & SII), SMA, insula and ACC [240]. These results indicate the importance of the OFC and amygdala in the modulation of pain [240,249].

Investigations into whether DNIC effects can be influenced by cognitive processes such as emotional and psychological states have recently been examined [104]. Notably, DNIC has been shown to be independent of changes in attention, distractions and expectations [84,170]. Pain modulation during expectations or distractions involves higher brain regions whereas DNIC is independent of higher brain regions and rather a brainstem mediated mechanism [85,95,177,178]. Outcomes of recent studies examining the modulating effects of expectation on the DNIC effect show convincing evidence that expectations can modulate DNIC responses [232,250]. However, only two studies agree with the notion that expectations can modulate DNIC responses leading to urgent need for more research to support these findings.

Furthermore, the intensity, location and strength of conditioning pain including expectations and distractions also have an effect on DNIC including the neural implications associated with temporal changes associated with the DNIC effect. Notably, most studies included in the above literature involve somatic pain as conditioning stimulus with few studies investigating the modulation of DNIC during the administration of visceral pain as test stimulus. For instance, whether the DNIC effects on the sensory experience of the UTC and the motor act of coughing is the same remains unknown. Additionally, it is yet to be confirmed whether a certain threshold of UTC is required to elicit a cough reflex in humans (ie is the sensory threshold of UTC the same as the threshold that would elicit a cough reflex). In pain studies, is has been shown that the sensory threshold for pain is lower than the threshold that elicits a withdrawal reflex [251]. Also, whether the DNIC response for airway irritation is dependent on the intensity of the UTC sensation remains unknown. In other words, would a stronger airway stimulus elicit stronger DNIC-effects for both cough and the UTC.

Collectively, several factors including the presence of pain syndromes in patients, gender, and ethnicity have the potential to influence the extent or efficacy of DNIC in humans. These factors may also have implications for the DNIC-related effects on individuals suffering with chronic cough. For instance, several studies have shown that chronic cough is more prevalent in the elderly compared to younger individuals [252,253]. One explanation for the prevalence of chronic cough in elderly patients may be related to the increased presence of respiratory comorbidities such as asthma and non-respiratory comorbidities such as hypertension [253]. It is widely known that the presence of comorbidities in a patient suffering from chronic pain syndromes may lead to impairment of the descending pain inhibition system in humans [254]. These findings further support the notion that the prevalence of chronic cough in the elderly population may be related to reduced activity of descending inhibition pathways similar to the findings associated with reduced DNIC efficiency in the elderly. In addition, several other studies have also shown chronic cough to be more common in females than in males [21,253,255,256]. However, sex-related differences in the DNIC-effect for sufferers of chronic cough, similar to that observed for somatic pain, is yet to be determined. It is possible to speculate that sex and age-related changes in DNIC-effects may have implications for the epidemiology of cough in ways that correspond with reported prevalence of chronic cough. Factors such as age and gender including the intensity of a stimulus have been shown to be associated with differences in responses related to DNIC-effects as observed in several pain studies. Such factors may have implications for the DNIC-related modulation of the UTC and cough in humans which are yet to be explored. Further investigation of the DNIC effect on visceral pain such as cough and the UTC will further our understanding of the modulatory mechanisms associated with airways processing and in humans.

Summary

The circuitry involved in the sensory and motor processing of cough and its associated sensation of UTC share similarities with those involved in pain processing. In addition, the modulation of the cough reflex and UTC involves the inhibition of several cortical sensory pathways within the cough network as observed in various studies. In placebo investigations, UTC is reduced behaviourally and was accompanied with neural outcomes with regions showing decreased activity within the UTC network including other regions showing increased neural activity. There is preliminary evidence that the modulatory influences of DNIC also lead to a reduction in UTC and cough in humans. However, whether this downregulation of UTC and cough is as a result of a descending inhibition similar to that observed in the case of placebo or rather that which is consistent to a DNIC mechanism was yet to be determined in humans. Therefore, an experimental design whereby a DNIC effect in the form of somatic painful stimulus during evoked UTC via the inhalation of capsaicin was selected to investigate the psychophysical outcomes and to measure regional brain activity associated with the DNIC-related modulation for UTC. It was expected that the primary sites for the relay of airway afferent such as the nTS and the Pa5 in the brainstem would show DNIC-related changes as a result of the modulation of airway inputs which would manifest into regional brain responses in the hemispheres of the brain. Brain regions identified in this modulatory inhibition of airway afferents can consequently constitute prospective targets for intervention that in future development of cough reduction therapies.

Aims

The aim of this project was to measure regional brain and brainstem responses associated with the modulation of airway irritation as a result of painful conditioning in healthy human volunteers. Initially, the behavioural responses in the experience of capsaicin-evoked UTC and cough during the absence and presence of painful conditioning for different levels of capsaicin concentrations was recorded. The results were compared to determine the psychophysical outcomes of a DNIC effect on UTC and cough frequency in humans. In addition, fMRI was used to identify regional brain networks involved in the modulation of UTC in higher brain and brainstem regions as a result of painful conditioning. Additionally, regional brainstem responses associated with the differences in the intensity of UTC evoked by two concentrations of capsaicin (high and low) were assessed.

Experiment 1

The initial experiment (Chapter 2) involved a psychophysical analysis of whether the presence of pain during the inhalation of capsaicin interacted with both UTC and cough in humans. The experiment also explored the regional brain responses associated with this modulation in the UTC using fMRI. It was hypothesized that the application of somatic pain during capsaicin challenges would evoke a DNIC effect and subsequently reduce the experience of one's UTC and cough frequency compared to an absence of pain. It was further hypothesized that this reduction in behavioural UTC would be accompanied by changes in neural activity within the cough network consistent with the DNIC phenomenon. The changes in neural activity in the nTS and Pa5 of the brainstem including the airway processing sites of the hemispheres would most likely be singular in direction (only reductions in neural activity) as opposed to the increase and decrease in neural activity observed in the neuroanatomical studies of placebo effects on airway processing. If this hypothesis were true, then the neural outcomes of DNIC-related modulation of the UTC is indeed distinct to placebo effects further implying that the DNIC effect involves distinct mechanisms and different circuitry in the inhibition of airway inputs. Brain regions identified from such investigation could act as targets for therapeutic intervention in future research to address chronic cough in humans.

Experiment 2

The second experiment (Chapter 3) involved replicating the methods of the previous study with a different focus on the psychophysical and neural outcomes in the interaction between pain and airway irritation. The psychophysical analysis incorporated various doubling concentrations of capsaicin challenges to assess whether pain had an equal or disproportionate effect on the sensory experience of UTC and the motor act of coughing. Additionally, the first experiment was not optimised for the brainstem and thus, did not provide any putative modulatory regions associated with the DNIC effect since such regions are confined to the brainstem. Therefore, fMRI optimised for the brainstem allowed better assessment of subcortical regions and nuclei at lower levels of the brain associated with the modulation of the UTC. It was hypothesized that DNIC related modulation of airway inputs would manifest as changes in neural activity in the form of regional brainstem responses as a result of painful conditioning. This approach allowed for the exploration of the exact circuitry involved in the modulation of inputs and related outcomes that were observed in the first experiment. It was expected that DNIC-related modulation

of airway inputs, which leads to the behavioural reduction in the UTC, is confined to the nTS and Pa5 in the form of reduced BOLD signal intensities of these primary regions for the relay of afferent airway inputs. However, the outcomes of this experiment did not show any evidence of DNIC-related changes in the neural activity of the nTS and the Pa5 associated with the modulation of airways inputs at the brainstem level. This led to further exploration of the brainstem for regional brainstem responses using a different approach.

Experiment 3

The third experiment (Chapter 4) aimed at further investigating regional brainstem responses associated with intensity-related changes in the UTC. The absence of the expected effects in the form of reduced BOLD signal intensity in the nTS and the Pa5 as a result of painful conditioning observed in the previous experiment (Chapter 3) raised a fundamental question regarding the acuity of fMRI of the brainstem to differentiate intensity-related BOLD signal variance in humans. One possibility was that the absence of an effect in the nTS and Pa5 was an accurate outcome in that these two sites may not necessarily be influenced by DNIC. Another possibility was that limitations associated with fMRI of the brainstem may have led to the absence of DNIC-related effects in terms of reduced BOLD signal intensities of the nTS and the Pa5 regions as previously hypothesized. This led to further investigation of regional brainstem responses related to airway processing by pointedly manipulating the intensity of the airway stimulus. This approach allowed for determining whether large behavioural differences in the UTC ratings would manifest as changes in BOLD signal intensity in the brainstem rather be consistent with the absence of regional brainstem responses as observed from the previous study (Experiment 2). It was expected that the inhalation of low concentrations of capsaicin compared to high concentrations which would result in significant intensity differences in UTC ratings. It was hypothesized that differences in the intensity of UTC would be accompanied by changes in regional brainstem responses specifically in the nTS and Pa5. Again, no regional brainstem responses in the nTS and the Pa5 nor in other brainstem nuclei were observed in the present experiment. These findings suggest that the absence of regional brainstem responses is most likely as a result of the limitations associated with fMRI of the brainstem using the current experimental methods.

Significance

The proposed research project aimed at gaining a better understanding of the behavioural and neural regulation involved in the DNIC-related modulation of both cough and the UTC in humans. Despite advancements in understanding of the networks associated with cough regulation in animal studies and recent human investigations, there is still a lack of evidence regarding the functional coordination of cortical and brainstem regions involved in the reduction of UTC in humans. Given the lack of effective medical treatment for chronic cough disease, it is hoped that exploring other modulatory influences observed for nociceptive inputs such as DNIC could also provide evidence of distinct inhibitory regions associated with the modulation of airway afferents. Investigating this mechanism could open up the possibility of using knowledge gained from these experiments by exploiting the modulatory influences of DNIC without enlisting DNIC protocols. This approach may also prove useful in developing future studies to create better therapeutic treatments for chronic cough.

Chapter 2 - Behavioral and regional brain responses to inhalation of capsaicin modified by painful conditioning in humans

Introduction

The cough reflex is a vital protective mechanism for clearing the airways in response to foreign matter in order to prevent damage and maintain normal airway function [33]. In humans, cough can become a distressing symptom when it becomes chronic. It is often accompanied by a sensory experience known as the UTC [31]. Recent research has begun to focus on this sensory aspect of cough with the aim of understanding how this information is integrated in the central nervous system. Given the lack of effective antitussive treatments currently available, there is a pressing need for research in this area.

The neural networks involved in the processing and generating of the UTC has been widely investigated [38]. Recent fMRI and neuroanatomical studies in humans have investigated the placebo-related modulation of this network [82]. The outcomes showed that placebo-related effects significantly reduced an individual's UTC in healthy humans. fMRI results showed that this reduction in UTC ratings was accompanied with placebo-related decreases in selective regional brain responses within the UTC network [82]. These findings suggest that there is a possibility that other modulatory influences may also employ similar inhibitory mechanisms associated with the reduction in UTC and cough in humans. One method to further investigate the behavioural and neural modulation of cough and the UTC is via a process known as DNIC. In pain studies, DNIC has shown to reduce the perception of pain felt at a different body site due to the application of painful stimuli at another body region [120]. Animal studies have shown that the mechanisms involved in the DNIC-related modulation of neural inputs involves the DRT of the brainstem which incorporates the descending inhibition of sensory information as a result of painful conditioning [103,120,142,146,257]. Therefore, the present study (Chapter 2) aimed at exploring the behavioural and neural outcomes associated with the DNIC-related modulation of cough and the UTC. Outcomes of such investigation would help provide novel insights into the circuitry involved in the downregulation of airway inputs in humans paving the way for better future therapeutic outcomes from a clinical perspective.

CHEST

Behavioral and Regional Brain Responses to Inhalation of Capsaicin Modified by Painful Conditioning in Humans

Abubakar B. Abubakar, BSc Hons; Tara G. Bautista, PhD; Matthew R. Dimmock, PhD; Stuart B. Mazzone, PhD; and Michael J. Farrell, PhD

BACKGROUND: Cough is a defense mechanism that protects the airways and lungs in response to airway irritation. The sensory neurons involved in detecting airway irritants and the neural pathways mediating cough share similarities with those that encode pain from the body. Painful conditioning stimuli applied to one body site are known to reduce the perception of pain at another. However, whether the neural regulation of cough is influenced by painful stimuli is not known.

RESEARCH QUESTION: What are the behavioral and neural outcomes of painful conditioning stimuli on urge-to-cough (UTC) and cough evoked by inhaled capsaicin?

STUDY DESIGN AND METHODS: Sixteen healthy participants underwent psychophysical testing and functional MRI while completing a series of capsaicin inhalations to induce UTC and cough. The responses associated with capsaicin inhalation without pain were compared with those after the application of painful conditioning stimuli.

RESULTS: Significant decreases were seen behaviorally of 18.7% ± 17.3% ($P < .001$) and 47.0% ± 30.8% ($P < .001$) in participants' UTC ratings and cough frequencies, respectively, during the application of pain. UTC ratings were reduced by 24.2% ± 36.5% ($P < .005$) and increased by 67% ± 40% ($P < .001$) for capsaicin and saline inhalation, respectively, during the scanning session. Painful conditioning stimuli were associated with widespread decreases in regional brain responses to capsaicin inhalation ($P < .001$). Several brain regions showed levels of reduced activation attributable to painful conditioning that correlated with related changes in behavioral responses during scanning ($R^2 = 0.53$).

INTERPRETATION: Pain-related decreases of cough and UTC are accompanied by widespread changes in brain activity during capsaicin inhalation, suggesting that pain can modify the central processing of inputs arising from the airways. A mechanistic understanding of how cough and pain processing interact within the brain may help develop more effective therapies to reduce unwanted coughing. CHEST 2021; 159(3):1136-1146

KEY WORDS: conditioned pain modulation; cough; heterotrophic noxious conditioning; neural control of cough; urge to cough

FOR EDITORIAL COMMENT, SEE PAGE 904

ABBREVIATIONS: BOLD = blood oxygen level dependent; CN = capsaicin inhalation with no pain; CP = capsaicin inhalation during pain; fMRI = functional MRI; In = insula; OP = operculum; ParCG = paracingulate gyrus; PosCG = postcentral gyrus; PreCG = precentral gyrus; SMA = supplementary motor area; S_{max} = maximum inhaled capsaicin concentration without coughing for 20 seconds; SN = saline inhalation with no pain; SP = saline inhalation during pain; UTC = urge-to-cough

AFFILIATIONS: From the Department of Medical Imaging and Radiation Sciences (Mr Abubakar; Drs Dimmock and Farrell) and the Department of Anatomy and Neuroscience (Drs Bautista and Mazzone), The University of Melbourne, Parkville, Australia;

The airways receive a rich sensory neural supply from the vagus nerves that in part serves a protective role by monitoring the pulmonary environment for potentially harmful stimuli and regulating defensive behaviors, including cough.[1] Many of these sensory neurons are nociceptive, sharing functional similarities with the somatosensory nociceptors that encode pain from skin, muscles, and other body tissues. When activated, the vagal sensory neurons input to complex neural networks in the brain, which not only regulate reflex coughing but also contribute to generating the sensation of an urge-to-cough (UTC) and allow for behavioral control over cough motor patterning.[2-4] Altered activity within peripheral and central processing pathways has been implicated in chronic cough in disease, and therefore understanding in more detail how these neural networks are regulated is of interest.[5,6]

Functional MRI (fMRI) and neuroanatomical studies in humans and animals have provided insight into the central (brain and brainstem) sensory and motor networks involved in regulating cough and the UTC.[1,7] These regions include widespread cortical and subcortical activations that encompass sensory, motor, premotor, and limbic structures.[1] Strikingly these brain networks also share important similarities to those involved in regulating somatic pain.[8] For example, airway irritation evoked by inhaling capsaicin in healthy humans induces neural activity in several brain regions that are also activated by noxious painful stimuli applied to the skin.[9] Placebo conditioning in humans dramatically reduces the perception of the UTC and the associated neural activations in the cortical cough network. Like placebo analgesia, this depends on increased activity in the dorsolateral prefrontal cortex.[10,11] Additionally, in rodents, the ascending neural pathways arising from the airways share significant homology with somatosensory pain pathways.[1] However, despite these commonalities in the neural networks regulating pulmonary and somatic nociception, little is known regarding whether functional interactions occur between cough and pain and, if so, where these interactions may occur in the brain.

In the pain field, it is well known that painful stimuli applied to one body region can reduce the perception of pain felt at a different body site through a process known as conditioned pain modulation or heterotopic noxious conditioning.[12] The mechanisms involved in conditioned pain modulation include brainstem and higher brain neural networks that are recruited by conditioning painful stimuli. These networks, in turn, contribute descending inhibition to sensory processing throughout the spinal cord, such that the processing of painful stimuli applied to sites away from the conditioned region is down-regulated.[13,14] Given the homology between cough and pain neural networks, we hypothesized that conditioning pain may similarly interact with pulmonary nociceptor processing and reduce the perception of airway irritation or cough. Thus, in this study we employed psychophysical measures in conjunction with fMRI in healthy humans to explore novel interactions between cough and pain processing in the brain.

Methods

Participant Recruitment and Experimental Protocol

Sixteen healthy participants were recruited via advertisements posted on the campus of Monash University (Melbourne, Australia) (nine men and seven women; mean ages, 24.94 ± [SD] 4.39 years). Exclusion criteria included claustrophobia, pregnancy, metal implants, and dental braces. Consent was obtained from the participants in compliance with procedures approved by the Monash University Human Research Ethics Committee (approval 2019.18292) and was consistent with the Declaration of Helsinki.

Psychophysical Testing Session

Thresholds for two coughs (C2) were determined using single tidal inhalations of capsaicin vapor prepared in doubling concentrations (0.12-125 μM) as previously described.[2-4,6,15] The C2 level and two dose increments above the C2 (C2 + 2) were used as test stimuli in a conditioning pain experiment. Increasing levels of pressure were applied to the left thumbnail for a period of 5 seconds to identify the stimulus needed to elicit a rating of five on an 11-point numerical rating scale (0-10) of pain intensity.[16,17] The moderately painful pressure was subsequently used as the conditioning stimulus in the first experiment. Participants inhaled single tidal volumes of the C2 and C2 + 2 concentrations on four occasions each in a random order in which they were not aware which of the two concentrations were delivered. Moderately painful pressure was

and Monash Biomedical Imaging (Dr Farrell), Monash University, Clayton, Australia.

FUNDING/SUPPORT: This research was supported by grants to Drs Mazzone and Farrell from the National Health and Medical Research Council (NHMRC) of Australia (APP1042528). Dr Bautista was funded by an Early Career Fellowship from The Garnett Passe and Rodney Williams Memorial Foundation of Australia and a University of Melbourne Early Career Researcher grant.

CORRESPONDENCE TO: Stuart B. Mazzone, PhD, Department of Anatomy and Neuroscience, School of Biomedical Science, The University of Melbourne, Parkville, VIC, 3010, Australia; e-mail: stuart.mazzone@unimelb.edu.au

DOI: https://doi.org/10.1016/j.chest.2020.08.2105

applied before the initiation of inhalation of nebulized vapor and was sustained until after the end of expiration; this process occurred on half of the eight occasions of tussive challenge. Audible coughs were counted during the 10 seconds after each tidal breath. Participants rated UTC after each inhalation, using a modified Borg scale as previously described.[2,3,10] Sufficient duration before the next capsaicin challenge allowed participants' sensation of an UTC to return to the baseline during the session. This resting period was subject dependent.

The second experiment involved initial testing for individual levels of capsaicin concentration that elicited moderate UTC and pressure required to elicit moderate pain. Participants then inhaled increasing concentrations of capsaicin for 20 seconds at a time to determine the highest concentration that could be inhaled without coughing (S_{max}), as previously described.[6] The S_{max} concentration was used as the test stimulus during fMRI scanning. The thumbnail pressure that elicited moderate pain at the conclusion of a 40-second period of stimulation was determined. Pressure during the 40-second period was applied onto the left thumbnail for 5 seconds at a time, followed by 1 second of no pressure to reduce the degree of temporal summation. This pressure was used as the conditioning stimulus during fMRI scanning. This approach was taken to minimize the increased pain perception that can result from temporal summation during prolonged stimulation.[18]

fMRI Protocol

Scanning was performed at Monash Biomedical Imaging (Clayton, Australia), using a MAGNETOM Skyra 3 T scanner (Siemens). The fMRI imaging protocol involved a block design incorporating four stimulus contingencies during the acquisition of blood oxygen level-dependent (BOLD) images. The four different contingencies were 20 seconds of inhalation of saline with no pain (SN), 20 seconds of inhalation of saline during 40 seconds of moderately painful left thumbnail pressure (SP), 20 seconds of capsaicin inhalation with no pain (CN), and 20 seconds of capsaicin inhalation during 40 seconds of moderately painful left thumbnail pressure (CP) (Fig 1). The onsets of painful pressure blocks were 10 seconds before the onsets of inhalations of saline or capsaicin. Each of the four contingencies was followed by a no-stimulus period of 40 seconds, during which participants responded to visual cues to rate UTC and pain. The order of delivery of test stimuli was pseudo-randomized and double-blinded in nature, whereby participants were not aware of when either capsaicin or saline were delivered (e-Fig 1A, 1B). Painful conditioning was averaged across the session such that a conditioning event was only followed by a no-conditioning event (e-Fig 1A). Two blocks of each contingency occurred during the scans of 8.34 minutes of duration. Each participant underwent three scanning runs (e-Fig 1).

Analysis

Repeated measures analysis of variance using SPSS Statistics software (IBM Corporation) was used to test for the effects of

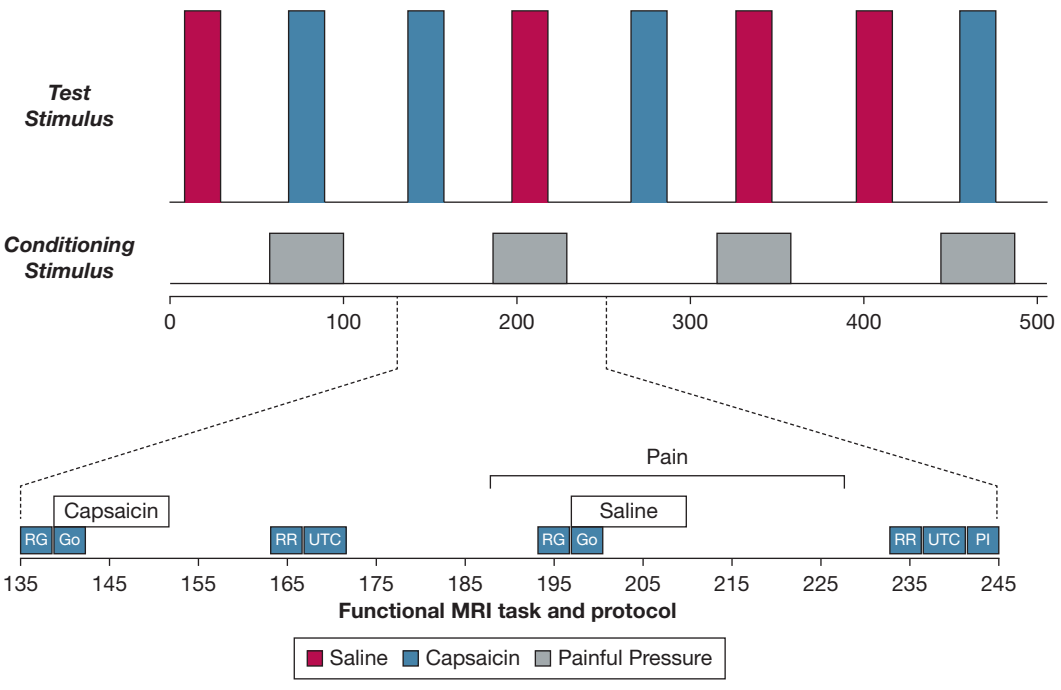


Figure 1 – *Participants underwent three fMRI scans of 8 minutes' and 26 seconds' duration each. Conditioning stimuli of painful thumbnail pressure were applied for 40 seconds on four occasions during the scan. Test stimuli were inhaled for 20 seconds on eight occasions, and were either saline or the maximum suppressible concentration of capsaicin. The timing of the respective stimuli meant that the test stimuli were associated with no-pain or pain with equal frequency. The expanded panel shows one of the four cycles of stimuli delivered during an fMRI scan. A visual cue of 3 seconds' duration was used to prepare participants before the onset of all inhaled stimuli (RG = ready to go). This strategy reduces the risk of uncontrolled coughing. Go cues (Go) for capsaicin and saline challenges ensured that participants initiated their inhalations at consistent time points throughout the sequence. Visual cues prompted participants to prepare to provide ratings (RR = ready to rate), after which numerical rating scales (0-10) appeared on screen. Participants rated UTC and pain intensity (PI). The sequence of test stimuli was different for each of the three scans and can be found in e-Figure 1A. The sequence in the figure corresponds to the first of the three fMRI scans. A detailed description of fMRI task and protocol can be found in e-Figure 1. fMRI = functional MRI; UTC = urge-to-cough.*

conditioning (pain, no-pain) and test stimulus concentration (C2, C2 + 2) on UTC and cough frequency recorded during the psychophysical session. The effects of inhalation stimuli (saline, S_{max}) and conditioning (pain, no-pain) on UTC during scanning were also assessed with repeated-measures analysis of variance. Analysis of fMRI data was performed as previously described,[3,4,10] with a detailed description of analysis found in e-Appendix 1. First-level analyses of participants' fMRI scans tested for regional brain BOLD signal responses to the four stimulus contingencies (CN, CP, SN, SP) as well as contrasts of CN > SN, CN > CP, and CP > CN. The negative and positive interactions between the four contingencies were also tested. Group analyses involved *t* tests to show regional activation for the contrasts of contingencies, and their interactions. A group analysis was performed to test for correlation between regional CN > CP activation levels and a behavioral measure of conditioning-related changes in UTC. This behavioral measure consisted of subtraction of a participant's average UTC rating for CP blocks from the average UTC rating for the CN blocks. Significant group regional brain responses were determined using a single-voxel inclusion of Z > 3.09 and a cluster-level family-wise error corrected threshold of $P_{corr} < .05$ based on the Functional Magnetic Resonance Imaging of the Brain Software Library Expert Analysis Tool implementation of random field theory (Functional Magnetic Resonance Imaging of the Brain Software Library version 6.0.3).[19,20]

Results

Psychophysical Results

The geometric means of C2 and S_{max} were 0.69 μM and 0.23 μM, respectively. Levels of stimuli for conditioning pain were 5.3 ± 1.3 kg/cm^2 during the psychophysical session and 3.8 ± 0.9 kg/cm^2 during fMRI scanning.

Both cough (F[1,15] = 30.3; $P < .001$) and UTC (F[1,15] = 22.2; $P < .001$) showed conditioning-related decreases in the psychophysical session that averaged (μ ± σ) 47.0% ± 30.8% and 18.7% ± 17.3%, respectively, which was a significant difference between the two outcome variables (t[15] = 3.2; $P < .005$) (Fig 2A, 2B, e-Table 1A, 1F). Conditioning and test concentration interacted for cough frequency (F[1,15] = 23.8; $P < .001$), with greater conditioning-related decreases in cough frequency at the higher concentration (Fig 2B), whereas UTC levels did not show a significant interaction for the two factors (F[1,15] = 1.9; $P = .2$).

A conditioning-related effect on UTC was seen during scanning (F[1,15) = 5.9: $P < .05$), which interacted with inhalation stimulus (F[1,15) = 19.5; $P < .001$). Ratings of UTC decreased by 1.2 ± 1.3 on the Borg scale (average, 24.2% ± 36.5%) during conditioning pain for capsaicin inhalations compared with no-pain events (t[15] = 3.5; $P < .005$), whereas UTC ratings in response

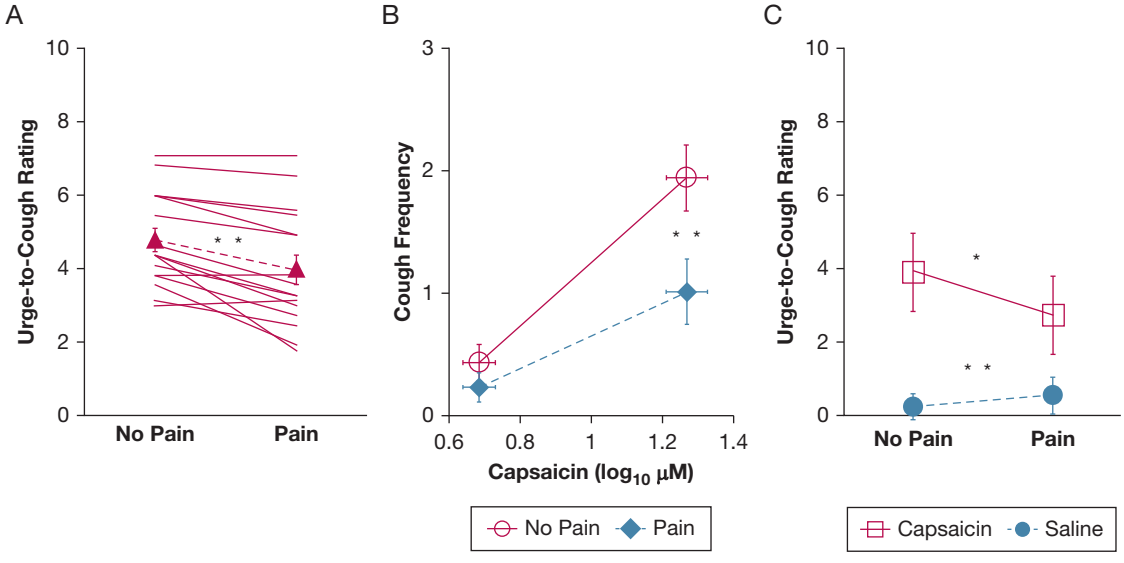


Figure 2 – *A, UTC ratings after capsaicin inhalation were averaged during the psychophysical session according to the concurrent application of conditioning (pain) or the no-pain contingency. Each unbroken line in the graph represents the ratings from a single participant. A downward slope to the right indicates a reduction in UTC ratings when conditioning pain was applied. The average effect across the group is represented by triangles connected by the broken line. B, Coughs were recorded from participants inhaling two concentrations of capsaicin with and without conditioning during the psychophysical session. A significant decrease in cough frequency in association with conditioning was seen for the higher of the two concentrations of test stimuli. C, UTC ratings were recorded after each test stimulus of either saline or capsaicin during fMRI scans. Concurrent application of the conditioning stimulus of painful thumbnail pressure was associated with significant decreases in UTC ratings after capsaicin inhalation, whereas the conditioning was associated with a significant increase in UTC ratings for saline. Error bars represent SEs of the mean. *P < .01; **P < .001. See Figure 1 legend for expansion of abbreviations.*

to saline inhalations increased from 0.2 ± 0.3 during no-pain events to 0.6 ± 0.5 during conditioning pain (t [15] = 4.2; $P < .001$) (Fig 2C, e-Table 2A, 2E). Additionally, no significant difference ($P = .236$) was seen in the average pain ratings (μ ± σ) between when saline (3.10 ± 2.30) and capsaicin (3.26 ± 3.50) were inhaled during the fMRI session. A detailed description of the multivariate model of UTC ratings and cough frequency in both the psychophysical and fMRI sessions is outlined in the online supplement (e-Table 1A-J, e-Table 2A-E).

fMRI Results

Regional brain responses during capsaicin inhalation that were increased compared with saline inhalation (CN > SN) were widely distributed and included the insula (In), operculum (OP), precentral gyrus (PreCG), postcentral gyrus (PosCG), midcingulate cortex, paracingulate gyrus (ParCG), middle frontal gyrus, supplementary motor area (SMA), supramarginal gyrus, thalamus, and cerebellum (Fig 3, e-Fig 2, e-Table 3).

Regions within the network of CN > SN activation showed increased responses to capsaicin inhalation during no-pain events compared with conditioning pain events (CN > CP). The CN > CP activated regions included the bilateral OP, PreCG, PosCG, ParCG, SMA, cerebellum, and right In (Fig 3, Table 1). No regions showed activation for the contrast of CP > CN.

Regions within the CN > SN network showed levels of CN > CP activation in the participants that were positively correlated with differences in UTC levels for the two contingencies (CN-CP). Regions showing a correlation between activation and behavior were found bilaterally in the PreCG, superior frontal gyri, SMA, and the left ParCG (Fig 4, Table 2, e-Fig 3).

Regions within the CN > SN network showed a negative interaction between conditioning and inhalation stimuli

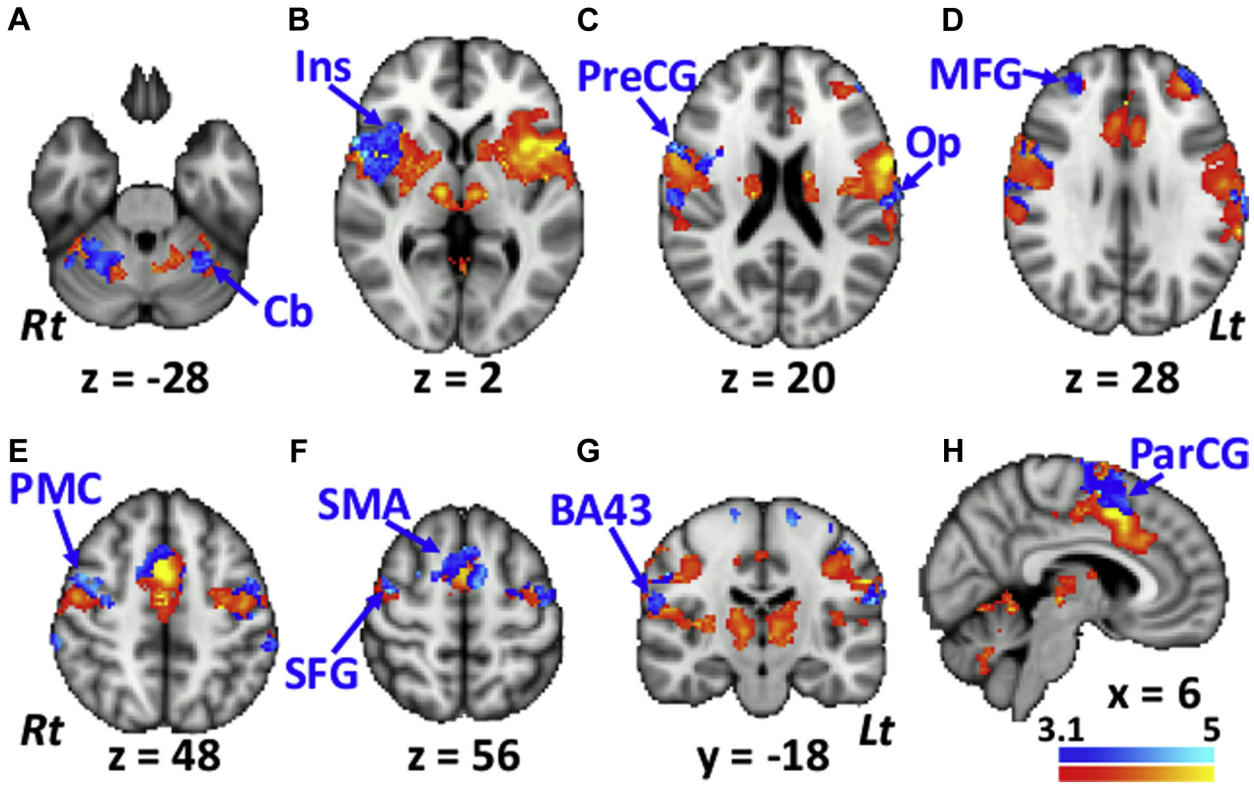


Figure 3 – *Brain regions where responses to capsaicin inhalation during no-pain were greater than saline with no-pain are rendered in red-yellow (CN > SN). The blue rendering represents regions where capsaicin inhalation responses were significantly decreased during pain conditioning compared with the no-pain contingency (CN > CP). A, Bilateral cerebellar (Cb) regions showed a decrease in capsaicin inhalation activation when painful thumbnail pressure was applied. Conditioning-related decreases in capsaicin inhalation activation were also seen in B, the right insula (Ins), C, bilateral operculum (Op), right precentral gyrus (PreCG), D, bilateral middle frontal gyri (MFG), E, bilateral premotor cortices (PMC), F, supplementary motor area (SMA), bilateral superior frontal gyri (SFG), G, the most caudal part of the primary somatosensory cortex corresponding to Brodmann area 43 (BA43), H, and the paracingulate gyrus (ParCG). Activations are rendered on the Montreal Neuroscience Institute template brain that is displayed in radiological convention (right side of brain on left of image).*

TABLE 1] Regional Brain Activations for Inhalation of Capsaicin With No Pain Greater Than Capsaicin With Pain (CN>CP)

Region	Peak Voxel Coordinates[a]				
	BA	x	y	z	*z* Score
Supplementary motor area	6	4	-8	70	6.04
	6	10	4	64	5.52
Superior frontal gyrus	6	24	2	62	5.11
	6	-10	4	64	4.69
Middle frontal gyrus	9	-36	52	26	5.40
	9	32	50	26	4.25
Paracingulate gyrus	6	-2	16	50	4.56
	6	4	16	52	4.18
Precentral gyrus	6	-46	2	44	4.91
	6	42	-2	40	5.02
	4	42	-6	56	5.02
	4	-48	-6	52	4.51
Postcentral gyrus	2	-66	-18	28	4.65
	43	-62	-18	20	4.88
	2	66	-18	26	4.27
	43	62	-14	20	4.27
Inferior parietal lobule	40	-56	-28	50	5.03
	40	60	-32	44	4.84
Frontal operculum	44	52	16	0	4.82
	44	-54	18	-4	5.32
Rolandic operculum	4	46	12	-2	4.91
	4	-58	12	0	4.27
Insula	13	36	4	14	5.43
	13	40	20	4	5.40
Cerebellum		28	-56	-30	4.33
		-28	-56	-32	4.58

BA = Brodmann area

[a]The coordinates correspond to the Montreal Neuroscience Institute standard brain template where *x* values are distance in millimeters to the left (negative *x* values) or right (positive *x* values) from the anterior commissure; *y* represents millimeter distance anterior (positive) or posterior (negative) from the anterior commissure, and *z* is millimeter distance superior (positive) or inferior (negative) from the anterior commissure.

(CN, CP, SN, SP), whereas no regions showed a positive interaction. Regions showing a negative interaction included the bilateral OP, SMA, In, right ParCG, superior frontal gyri, and left PosCG (Fig 5, Table 3, e-Fig 4A, 4B).

Discussion

In this study, we investigated interactions between pain and cough. Painful conditioning stimuli significantly reduced both UTC ratings and cough frequency among individuals inhaling capsaicin. This behavioral effect was accompanied by reductions in BOLD signal responses in brain regions activated by capsaicin inhalation. These consonant findings of behavioral and regional brain responses are likely to represent a modulation of sensory inputs at lower levels of the neuroaxis that leads to a reduction in UTC and cough in humans.

Behavioral Response of Cough and UTC

Our finding that the application of somatic pain significantly reduced both the perception of airway irritation and evoked cough is consistent with data reported from a previous study. Young et al[21] reported in a published conference abstract that cold pain applied to the hand of healthy participants significantly reduced cough frequency, and a trend for reduced UTC, during inhalations of half maximal effective concentration doses of capsaicin (inducing at least 50% maximum cough frequency).[21] Furthermore, dyspnea, which is a

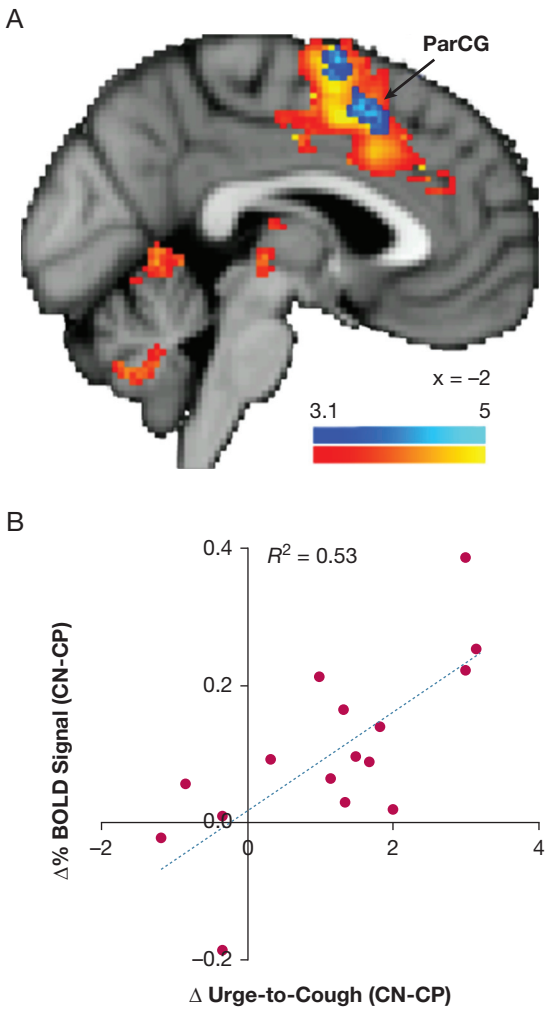


Figure 4 – *Regions where CN > SN are rendered in red-yellow. The contrast of CN > CP was tested for associations with behavioral results using the difference score of UTC ratings (CN-CP) during the scanning session. Regions with a significant positive correlation between the related differences are rendered in blue. A, A region in the left paracingulate gyrus (ParCG) had conditioning-related changes in capsaicin activation that were associated with changes in UTC. B, The scattergram illustrates the relationship between conditioning-related changes in regional signal responses from the paracingulate gyrus and UTC levels. See e-Figure 3 for additional regional responses showing positive correlation between related differences of UTC ratings. The sagittal brain slice is the Montreal Neuroscience Institute template brain.*

related unpleasant sensation that is thought to involve pulmonary nociceptor sensory pathways, has been shown to modulate the perception of pain.[22-25] Collectively, these observations support the hypothesis that nociceptive pathways of somatic and pulmonary tissues functionally interact to induce meaningful changes in sensory-evoked behavior.

Our data suggest a disproportionate effect of pain on the motor act of coughing compared with the perception of UTC. UTC scores were reduced by 19% during conditioning pain, compared with a 47% reduction in cough frequency among participants inhaling capsaicin. This may suggest a differential effect of conditioning pain on sensory vs motor neural activity. An alternate possibility is that subjective (UTC) and objective (cough frequency) measures are not tightly correlated. Indeed, we have previously reported that ratings of UTC increase linearly with doubling concentrations of capsaicin, whereas the frequency of coughing shows an accelerating function in response to the same concentrations of inhaled capsaicin.[26] Consequently, small changes in sensory processing (ie, UTC) may lead to larger changes in motor output (ie, coughing.) However, cough frequencies were smaller compared with UTC ratings in this study, therefore having disproportionate contributions to the calculation of percentage changes, which could have a bearing on the differential values across the two outcome variables. The low absolute levels of cough in response to inhaling the C2 and C2 + 2 concentrations during the first session was likely due to adaptation.[27,28] Future assessments of pain conditioning of responses to capsaicin inhalation may benefit from using a wide range of concentrations for tussive test stimuli to test the effects of conditioning on cough and UTC across the stimulus-response function. Conditioning pain affected UTC ratings in response to lower concentrations of capsaicin that infrequently evoked coughing under either contingency of conditioning (ie, either pain or no-pain), reinforcing the conclusion that the conditioning stimulation was likely to have exerted an influence on sensory processing.

Pain was used as the conditioning intervention in this study of modulation of responses to tussive stimuli. Necessarily, the design of the experiment limited opportunities to examine the possibility that pain responses might have been influenced by capsaicin inhalation. Nevertheless, two observations with respect to pain reports warrant mention. First, we noted that the stimulus intensity needed to elicit moderate pain during the fMRI session was typically lower compared with that used during the initial psychophysical testing. However, the painful stimuli were applied for a substantially longer duration during fMRI. Pain perception is influenced by both stimulus magnitude and stimulus duration in humans, and likely temporal summation accounts largely for the differences in psychophysical and fMRI stimulus thresholds in the current study.[18] Second, no difference was found in pain ratings between when saline and capsaicin were inhaled during the fMRI session. This suggests that a bidirectional interaction

TABLE 2] Regional Brain Activations That Show a Correlation Between Regional CN > CP Activations and Conditioning Pain Related Changes in Urge to Cough

Region	Peak Voxel Coordinates[a]					R^2
	BA	x	y	z	*z* Score	
Supplementary motor area	6	-2	2	66	4.69	0.54
	6	10	-2	66	4.67	0.51
Paracingulate gyrus	32	-4	14	48	4.52	0.53
	6	2	10	50	4.48	0.58
Superior frontal gyrus	6	-26	-6	58	4.47	0.60
	6	-16	4	58	4.20	0.57
	6	18	-2	60	4.57	0.59
Precentral/premotor cortex	4	-38	-10	44	4.57	0.60
	6	-26	-12	56	4.50	0.53
	4	40	-10	48	4.64	0.64
	4	46	-6	48	4.27	0.56

Regions of interest (ROI) were defined as activated voxels contiguous with the peaks reported in the table. The average %BOLD signal change from voxels in ROI were estimated for each participant and regressed against the change in urge-to-cough ratings associated with the conditioning. The resulting R^2 values are reported in the right column, and are statistically significant at a Bonferroni corrected threshold of $P < .005$. BA = Brodmann area.
[a]The coordinates correspond to the Montreal Neuroscience Institute standard brain template, where *x* values are distance in millimeters to the left (negative *x* values) or right (positive *x* values) from the anterior commissure; *y* represents millimeter distance anterior (positive) or posterior (negative) from the anterior commissure, and *z* is millimeter distance superior (positive) or inferior (negative) from the anterior commissure.

between airway sensation and pain is unlikely given the current experimental design. An alternative experimental protocol would be required to further explore putative conditioning of pain responses by tussive inhalations.

Cough and Pain Neural Interactions

We have previously demonstrated that the UTC in humans is contingent on neural activation in distributed brain regions that process incoming sensory information from the airways. The regional activation pattern during capsaicin inhalation in the current study is highly consistent with our previous reports of this network.[2-4,10] Such regional brain networks are also activated in other sensorimotor paradigms such as noxious stimulation of cutaneous tissue and visceral sensory modalities.[29,30]

Painful conditioning was associated with widespread changes in regional brain responses to capsaicin inhalation. These changes, observed throughout the hemispheres of the brain, were always decreases in capsaicin-related BOLD signal changes during conditioning pain compared with the no-pain contingency. Additionally, variability in the levels of conditioning-related reductions of capsaicin-inhalation activation throughout the network were predicted by the associated changes in UTC ratings. The specificity of the conditioning-related decreases in activation levels for capsaicin vs saline inhalations were also distributed throughout the brain regions showing responses to the inhaled substances. This is notably a significant physiological outcome given that both the behavioral and corresponding neural measures were significantly impacted by concomitant painful stimulation. Whether this translates to a clinically significant outcome awaits studies in patient populations. Collectively, the distributed nature of the conditioning-related changes throughout the capsaicin-inhalation network is consistent with modulation of the sensory inputs at lower levels of the nervous system, such as the brainstem nuclei that respond to noxious tussive stimuli.[31]

Confirming the circuitry that is involved in this putative descending inhibition is difficult with the current study design, which was not optimized for brainstem imaging. One possibility is that conditioning pain may enlist suprabulbur networks and enhance activity of the descending analgesia system. Placebo suppression of pain and cough likely operates via a network that originates in cortical areas that influence primary sensory processing areas of the brainstem and spinal cord via midbrain opioidergic circuits.[10] Thus, placebo-related modification of sensory experience is an acknowledged top-down process, which involves higher-order regional brain responses associated with beliefs and expectations that are thought to influence sensory processing.[10] Conceivably, conditioning pain also could

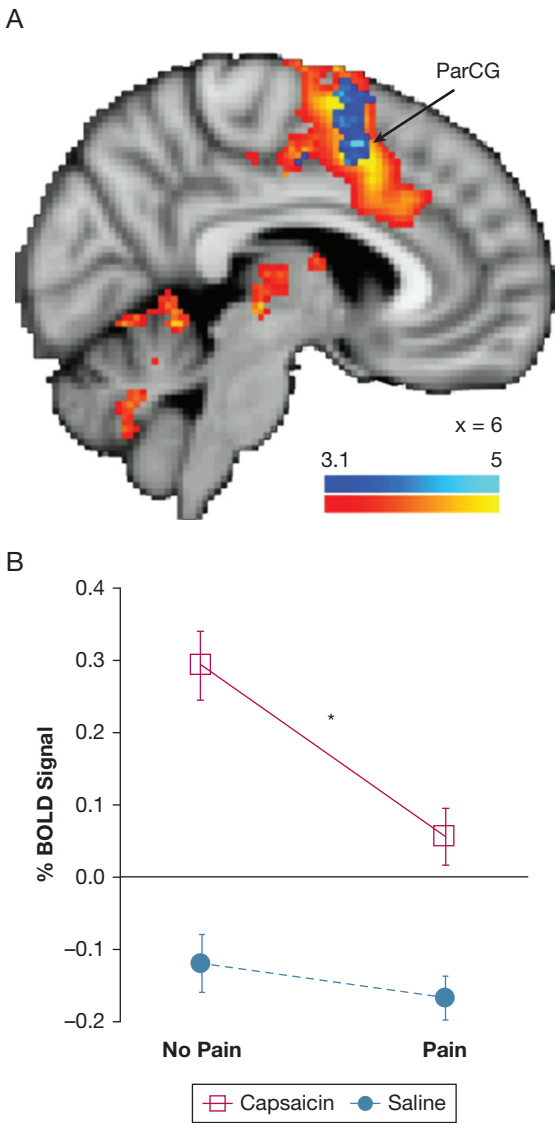


Figure 5 – *Interactions were tested between conditioning (pain, no-pain) and test (saline, capsaicin) stimuli to identify brain regions where the effects of concurrent pain on activation levels was more pronounced for one type of test stimulus. Brain regions rendered in blue had conditioning-related changes for capsaicin responses that were increased compared with saline (CN, CP, SN, SP). Regions showing CN > SN activation are rendered in red yellow. A, The left paracingulate gyrus (ParCG) region showed an interaction in responses to the test and conditioning stimuli. B, The nature of the interaction between conditioning and test stimuli for BOLD signal changes in ParCG can be seen in the graph. BOLD signal changes during capsaicin inhalation were significantly decreased during pain compared with the no-pain contingency, whereas responses to saline inhalation did not show a conditioning-related change. See e-Figure 4A and e-Figure 4B for additional regional responses showing interaction in response to the test and conditioning stimuli. Error bars are SEs of the mean. *P < .001.*

recruit this network to modulate pulmonary sensory processing. However, the singular direction (reductions) of conditioning pain-related regional brain responses to capsaicin inhalation seen in this study is in distinction to placebo-related regional brain responses during capsaicin inhalation, in which we reported both increases and decreases in regional BOLD signals. Regional brain activation associated with top-down processes would be expected to show increases during conditioning, as is observed during placebo-related modification of responses to capsaicin inhalation.[10] Similarly, any putative influences of higher-order processing in association with noxious conditioning, such as attention-related mechanisms, would also be expected to manifest as increased regional brain responses during the conditioning pain vs no-pain contingencies, but this did not occur in the current study.

An alternative explanation is that conditioning-related decreases in the UTC are attributable to a bottom-up process. In animals, a form of conditioning pain modulation has been described, known as diffuse noxious inhibitory controls, that does not involve the higher brain but rather is dependent on conditioning pain-activating brainstem neurons in the caudal medulla that in turn inhibit nociception in the spinal cord.[13] This circuitry may exist in humans[32] and could conceivably also regulate visceral nociception at the level of the brainstem sensory relay sites for vagal sensory neurons. In addition, other studies have demonstrated that patients with chronic pain show diminished conditioning pain modulation, an effect that is thought to reflect loss of central inhibitory controls.[33] Whether this impacts cough regulation in these patients is not known. Whether patients with chronic cough similarly have diminished conditioning painful modulation also is not known, although recent data[34] suggest they may have reductions in the activity of other central cough suppression networks. The images acquired in this study were optimized for the hemispheres of the brain and consequently were not suitable for showing responses in small structures in the brainstem. Further investigation of the brainstem using fMRI optimized for this region will be worthwhile to gain a better understanding of mechanisms contributing to pain conditioning of cough and UTC.

Interpretation

Concurrent pain has a strong influence on coughing and UTC in healthy humans, effects that are associated with widespread changes in regional brain responses to capsaicin inhalation. This confluence of behavior and brain activity points toward a down-regulation of sensory inputs in the lower brainstem that is perhaps consistent with mechanisms of the diffuse noxious

TABLE 3] Regional Brain Activations With a Negative Interaction Between Conditioning (No-Pain, Pain) and Test (Saline, Capsaicin) Stimulation

Region	Peak Voxel Coordinates[a]				
	BA	x	y	z	*z* Score
Supplementary motor area	6	0	4	64	4.58
	6	-4	2	58	4.53
	6	4	2	60	4.95
	6	6	4	66	4.49
Paracingulate gyrus	6	6	8	50	5.29
	6	10	4	50	4.87
Superior frontal gyrus	6	16	-2	68	4.03
Pars opercularis	44	-48	4	18	4.43
	44	54	6	14	4.36
Operculum	43	-56	12	0	5.55
	43	-54	18	0	4.99
	43	58	4	4	5.06
Postcentral gyrus	2	-62	-26	24	4.82
	43	-60	-20	20	4.43
Insula	13	-28	14	2	4.03
	13	36	10	6	4.04

BA = Brodmann area.
[a]The coordinates correspond to the Montreal Neuroscience Institute standard brain template, where *x* values are distance in millimeters to the left (negative *x* values) or right (positive *x* values) from the anterior commissure; *y* represents millimeter distance anterior (positive) or posterior (negative) from the anterior commissure, and *z* is millimeter distance superior (positive) or inferior (negative) from the anterior commissure.

inhibitory controls phenomenon. These outcomes have special significance and constitute a promising target for future studies whereby specific brain network activities could be therapeutically targeted to reduce excessive cough in patients. Further studies of the brainstem have the potential to expand our understanding of the mechanisms involved in contributing to pain modulation of UTC and cough.

Acknowledgments

Author contributions: M. J. F. is the guarantor of this study and takes responsibility of the manuscript. A. B. A had full access to the study data and vouches for the integrity of the data analysis. A. B. A. contributed to study design, data collection, analysis, and manuscript preparation. T. G. B. contributed to study design and manuscript preparation. M. R. D. contributed to study design and manuscript preparation. S. B. M. contributed to study conceptualization, design, data interpretation, and manuscript preparation. M. J. F. contributed to study conceptualization, design, data collection, analysis, interpretation, and manuscript preparation.

Financial/nonfinancial disclosures: The authors reported to *CHEST* the following: S. B. M declares personal fees from Merck and NeRRe Therapeutics and grant support from Merck. None declared (A. B. A., T. G. B. M. R. D., M. J. F.).

Role of sponsors: The sponsors had no role in the design of the study, the collection and analysis of the data, or in the preparation of the manuscript.

Additional information: The e-Appendix, e-Figures, and e-Tables can be found in the Supplemental Materials section of the online article.

References

1. Mazzone SB, Undem BJ. Vagal afferent innervation of the airways in health and disease. *Physiol Rev*. 2016;96(3):975-1024.
2. Mazzone SB, McLennan L, McGovern AE, Egan GF, Farrell MJ. Representation of capsaicin-evoked urge-to-cough in the human brain using functional magnetic resonance imaging. *Am J Respir Crit Care Med*. 2007;176(4):327-332.
3. Mazzone SB, Cole LJ, Ando A, Egan GF, Farrell MJ. Investigation of the neural control of cough and cough suppression in humans using functional brain imaging. *J Neurosci*. 2011;31(8):2948-2958.
4. Farrell MJ, Cole LJ, Chiapoco D, Egan GF, Mazzone SB. Neural correlates coding stimulus level and perception of capsaicin-evoked urge-to-cough in humans. *Neuroimage*. 2012;61(4):1324-1335.
5. Mazzone SB, McGovern AE, Farrell MJ. Endogenous central suppressive mechanisms regulating cough as potential targets for novel antitussive therapies. *Curr Opin Pharmacol*. 2015;22:1-8.
6. Ando A, Smallwood D, McMahon M, Irving L, Mazzone SB, Farrell MJ. Neural correlates of cough hypersensitivity in humans: evidence for central sensitisation and dysfunctional inhibitory control. *Thorax*. 2016;71(4):323-329.
7. Ando A, Farrell MJ, Mazzone SB. Cough-related neural processing in the brain: a roadmap for cough dysfunction? *Neurosci Biobehav Rev*. 2014;47:457-468.
8. Xu A, Larsen B, Baller EB, et al. Convergent neural representations of experimentally-induced acute pain in healthy volunteers: a large-scale fMRI meta-analysis. *Neurosci Biobehav Rev*. 2020;112:300-323.

9. Mazzone SB, McGovern AE, Koo K, Farrell MJ. Mapping supramedullary pathways involved in cough using functional brain imaging: comparison with pain. *Pulm Pharmacol Ther*. 2009;22(2):90-96.

10. Leech J, Mazzone SB, Farrell MJ. Brain activity associated with placebo suppression of the urge-to-cough in humans. *Am J Respir Crit Care Med*. 2013;188(9):1069-1075.

11. Atlas LY, Wager TD. A meta-analysis of brain mechanisms of placebo analgesia: consistent findings and unanswered questions. *Handb Exp Pharmacol*. 2014;225:37-69.

12. Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. *Curr Opin Anaesthesiol*. 2010;23(5):611-615.

13. Le Bars D. The whole body receptive field of dorsal horn multireceptive neurones. *Brain Res Rev*. 2002;40(1-3):29-44.

14. Sprenger C, Bingel U, Buchel C. Treating pain with pain: supraspinal mechanisms of endogenous analgesia elicited by heterotopic noxious conditioning stimulation. *Pain*. 2011;152(2):428-439.

15. Leech J, Mazzone SB, Farrell MJ. The effect of placebo conditioning on capsaicin-evoked urge to cough. *Chest*. 2012;142(4):951-957.

16. Cole LJ, Farrell MJ, Duff EP, Barber JB, Egan GF, Gibson SJ. Pain sensitivity and fMRI pain-related brain activity in Alzheimer's disease. *Brain*. 2006;129(Pt 11):2957-2965.

17. Farrell MJ, Egan GF, Zamarripa F, et al. Unique, common, and interacting cortical correlates of thirst and pain. *Proc Natl Acad Sci U S A*. 2006;103(7):2416-2421.

18. Nie H, Arendt-Nielsen L, Andersen H, Graven-Nielsen T. Temporal summation of pain evoked by mechanical stimulation in deep and superficial tissue. *J Pain*. 2005;6(6):348-355.

19. 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(6):900-918.

20. Eklund A, Nichols TE, Knutsson H. Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. *Proc Natl Acad Sci U S A*. 2016;113(28):7900-7905.

21. Young EC, Houghton LA, Holt KJ, Woodcock AA, Smith JA. S144a endogenous inhibition of experimentally induced cough in healthy subjects. *Thorax*. 2011;66(Suppl 4):A66.

22. Morelot-Panzini C, Demoule A, Straus C, et al. Dyspnea as a noxious sensation: inspiratory threshold loading may trigger diffuse noxious inhibitory controls in humans. *J Neurophysiol*. 2007;97(2):1396-1404.

23. Morelot-Panzini C, Mayaux J, Hug F, Willer JC, Similowski T. Analgesic effects of dyspnoea: "Air hunger" does not inhibit the spinal nociception reflex in humans. *Respir Physiol Neurobiol*. 2014;190:81-85.

24. Morelot-Panzini C, Corvol JC, Demoule A, et al. Intravenous adenosine activates diffuse nociceptive inhibitory controls in humans. *J Appl Physiol (1985)*. 2013;115(5):697-703.

25. Nishino T, Shimoyama N, Ide T, Isono S. Experimental pain augments experimental dyspnea, but not vice versa in human volunteers. *Anesthesiology*. 1999;91(6):1633-1638.

26. Farrell MJ, Mazzone SB. Are neural pathways processing airway inputs sensitized in patients with cough hypersensitivity? *Pulm Pharmacol Ther*. 2019;57:101806.

27. Hilton EC, Baverel PG, Woodcock A, Van Der Graaf PH, Smith JA. Pharmacodynamic modeling of cough responses to capsaicin inhalation calls into question the utility of the C5 end point. *J Allergy Clin Immunol*. 2013;132(4):847-855.

28. Pullerits T, Ternesten-Hasseus E, Johansson EL, Millqvist E. Capsaicin cough threshold test in diagnostics. *Respir Med*. 2014;108(9):1371-1376.

29. von Leupoldt A, Sommer T, Kegat S, et al. Dyspnea and pain share emotion-related brain network. *Neuroimage*. 2009;48(1):200-206.

30. Coen SJ, Gregory LJ, Yaguez L, et al. Reproducibility of human brain activity evoked by esophageal stimulation using functional magnetic resonance imaging. *Am J Physiol Gastrointest Liver Physiol*. 2007;293(1):G188-G197.

31. Bautista TG, Leech J, Mazzone S, Farrell MJ. Regional brainstem activations during capsaicin inhalation using functional magnetic resonance imaging in humans. *J Neurophysiol*. 2019;121(4):1171-1182.

32. Youssef AM, Macefield VG, Henderson LA. Pain inhibits pain; human brainstem mechanisms. *Neuroimage*. 2016;124(Pt A):54-62.

33. O'Brien AT, Deitos A, Trinanes Pego Y, Fregni F, Carrillo-de-la-Pena MT. Defective endogenous pain modulation in fibromyalgia: a meta-analysis of temporal summation and conditioned pain modulation paradigms. *J Pain*. 2018;19(8):819-836.

34. Cho PSP, Fletcher HV, Turner RD, Jolley CJ, Birring SS. Impaired cough suppression in chronic refractory cough. *Eur Respir J*. 2019;53(5).

Conclusion

Painful conditioning has a strong influence on both cough and the UTC in humans. The reduction in the sensory experience of the UTC was accompanied widespread reduction in regional brain responses within the capsaicin inhalation network. The neural outcomes of the present study (Chapter 2) is consistent with the DNIC-related modulation of airway inputs most likely at the level of the brainstem. The findings also provide insight into brain networks that could act as targets for therapeutic intervention to help reduce persistent UTC and unwanted cough in humans. Further exploration of the brainstem regions associated with the DNIC-related modulation of the airway inputs has the potential to further out understanding of the mechanisms involved in the down-regulation of cough and the UTC.

Chapter 3 - Modulation of cough-related neural processing in the brainstem by conditioning painful stimulation in humans

Preamble

The neural networks involved in the processing and generating of the UTC has been widely investigated [36].In humans, recent studies have investigated the modulation of this network via placebo. Placebo effects were shown to significantly reduce an individual's UTC by 45% in healthy people [83]. This reduction in the behavioural outcomes of the UTC was accompanied with selective decreases in brain responses within the UTC and cough network suggesting that beliefs about treatment can modify the central processing of inputs arising from the airways. By contrast, placebo trials also showed regions of increased activations in regions such as the MFG and PreCG when compared to controls indicating the involvement of higher brain regions during the placebo-related modulation of airway inputs [82]. These findings suggest that placebo-related modulation of UTC is likely a top-down process due to the involvement of higher brain regions that showed heightened activity during placebo trials. This is consistent with outcomes of placebo-related modulation of pain signals shown in many pain studies where the ACC showed increased activation associated with placebo analgesia [98,166].

The advances in knowledge gained from the placebo studies regarding modulation of the UTC meant that there is a possibility that other modulatory influences may also employ similar descending inhibitory mechanisms associated with the reduction in UTC and cough in humans. Therefore, the previous study (Chapter 2) investigated the behavioural and neural outcomes associated with painful conditioning which is a phenomenon commonly known as DNIC. This form of modulation is known to involve a distinct circuitry for the descending inhibition of neural inputs at the level of the brainstem [103,120,137,146,258,259]. Literature from animal and human studies have suggested a circuitry that is independent of higher brain regions where the inhibition of neural inputs occurs between the DRt and the dorsal horn of the spinal cord [134,136]. This is in contrast with the placebo-related modulation of pain signals which is an acknowledged prefrontal-limbic-PAG-RVM-DHSC pathway suggesting the involvement of higher brain regions in this process [85,166]. The behavioural findings of the previous study (Chapter 2) were in line with reported decreases in the UTC and cough frequency observed in the placebo and recent DNIC-related studies. In addition, the neural findings of the previous study (Chapter 2) were consistent with the DNIC-modulation of the UTC and distinct to the placebo-related modulation of the UTC.

The singular direction (reductions) of DNIC-related regional brain responses to capsaicin inhalation seen the previous study (Chapter 2) is in distinction to the placebo-related modulation of the UTC where both increases and decreases in regional BOLD signals were observed. However, the fMRI images obtained in the previous (Chapter 2) were not optimized for the hemispheres of the brain and thus, were not suitable for showing regional responses in small structures of the brainstem. Therefore, the present study aimed at investigating the regional brainstem responses associated with the descending inhibition of airway inputs consistent with the DNIC mechanism. The present study also explored evidence of a DNIC-related modulation of airway inputs in the brainstem that may explain the regional higher brain outcomes observed in the previous study (Chapter 2). It is expected that painful conditioning would be associated with less activation in brainstem nuclei involved in the relay of sensory airway information. Possibilities include brainstem nuclei such as the nTS and Pa5 given that the two act as primary airway afferent sites for the relay and processing of airway inputs. This information could assist in assessing and targeting interventions in chronic cough patients aimed at reducing the sensation of the UTC without impairing reflex cough.

Introduction

The novel findings of the previous chapter (Chapter 2) provided the first insight into the neural interaction between pain and airway processing. The study implicated several higher brain regions distributed within the cough network that interacted with pain via the process known as DNIC [260]. This interaction was in the form of widespread reduction in neural activity amongst regions in the capsaicin inhalation network. Additionally, there were no regions that showed increases in neural activity within the capsaicin inhalation network as a result of painful conditioning. These findings were consistent with the notion that the DNIC mechanism involves an endogenous inhibitory process that recruits brainstem nuclei to effect inhibition of the dorsal horn cells of the spinal cord responding to nociceptive inputs [120]. This process occurs as a result of the concurrent application of a second (ie conditioning) stimulus during the presence of an existing nociceptive (test) stimulus. The inputs that ascend the spinal cord as a result of the application of the conditioning stimulus initiate a DNIC effect. In animal studies, the circuitry sustaining DNIC has been to shown to be confined to the DRt whereby the potency of DNIC was tested in animals with complete sections at different levels of the brainstem [120,136]. Such findings confirmed that the DRt is the key inhibiting region for the DNIC-related modulation of neural inputs and that this mechanism encompasses a DHSC-DRt-DHSC pathway [129,136,142]. This is in contrast to the acknowledged prefrontal limbic-PAG-RVM-DHSC pathway which is consistent with modulation associated with placebo and other cognitive influences such as attention and distraction [85,95,104,142,170,175,183]. The neural outcomes of the previous study (Chapter 2) confirmed the different modulatory influences of placebo effects compared to DNIC effects on airway processing. Furthermore, the behavioural outcomes showed that painful stimulus was associated with a reduction in the perceived UTC sensation and cough frequency of participants. However, the behavioural results showed a disproportionate effect of painful conditioning on the UTC versus cough in the previous study (Chapter 2). UTC ratings were reduced by 19% compared to 47% for cough frequency suggesting a differential effect of conditioning on the sensory experience of UTC versus the motor act of cough. This finding also suggests that differential effects of pain conditioning on the sensory versus motor neural activity may exist. Alternatively, this disparity in conditioning related responses of the UTC versus cough frequency may suggest that the two measures are not tightly regulated in humans. Investigating pain conditioning responses to capsaicin inhalation may benefit from using a wide range of capsaicin concentrations for tussive test stimuli to test the effects of conditioning on the

UTC and cough across the stimulus-response function. The findings would also identify whether increases and conditioning-related decreases in UTC ratings and cough frequencies as a result of increases in doubling concentrations during capsaicin inhalation challenges would follow a linear path or rather an exponential path as observed in previous studies [49,76]. Additionally, this approach may also provide other insights into the differential DNIC-related effects on cough and the UTC. For instance, previous studies involving airway irritation showed that airway stimulation with ATP which only activates the nTS evoked less frequent coughing compared to stimulation with capsaicin which activates both the nTS and the Pa5 [49]. Such findings suggest that airway stimulation that activates the both nuclei may differently contribute to cough and the UTC compared to stimulation that activates only the nTS. These outcomes may have implications in the relative efficacy of DNIC for cough and the UTC in that the different levels of DNIC responses for the sensory experience of the UTC versus motor act of cough might be related to the respective nuclei.

The outcomes of the previous study (Chapter 2) acknowledged that the behavioural and neural conditioning-related decreases of UTC and cough are most likely as a result of the DNIC-related modulation of airway inputs at the brainstem level. This proposed gating mechanism whereby inhibition at the first synaptic level of sensory integration in the brainstem causes decreased activation in all regions of cough sensory processing as observed in the previous study (Chapter 2). Despite this advancement in understanding the DNIC-related modulation and its influence on higher order processing of airway-irritant related to sensory information of airway inputs, the study was not optimized for brainstem imaging [260]. Therefore, whether an interaction between pain and cough-related neural processing at the level of the brainstem exists remains unknown. Additionally, evidence of a DNIC-related modulation of airway inputs observed in the previous study (Chapter 2) at the level of brainstem was yet to be confirmed including the precise networks involved in this modulation.

Interpreting brainstem activations collected during standard whole brain fMRI similar to the previous study (Chapter 2) is challenging due to several factors. One factor is the presence of poor signal-to-noise ratios due to the proximity of tissue boundaries that may disturb the homogeneity of the magnetic field [261,262]. In addition, the small size of brainstem nuclei and cardiorespiratory noise would make it difficult to accurately interpret brainstem activations during whole brain imaging scanning [262]. Therefore, the objective of the present

study was to perform a direct survey of the brainstem region using image acquisition parameters that was optimized for the brainstem to further investigate the mechanisms and subcortical networks involved in the modulation of airway inputs including providing evidence of whether a DNIC-related modulation of the UTC is involved in this process.

As previously mentioned, research has identified two distinct nuclei known as the nTS and the Pa5 to be the main connections for receiving afferent sensory input from the airways making them the ideal candidates involved in the modulation of airway inputs at the subcortical level [79,263]. Therefore, the aim of the present study (Chapter 3) was to determine whether these (nTS and Pa5) brainstem nuclei could potentially be involved in the DNIC-related modulation of airway sensory inputs before they ascend to the higher cortex. If a DNIC-effect leads to the inhibition of neural inputs at the brainstem level as previously hypothesized, we would expect to see decreases in activations in brainstem nuclei involved in cough related neural processing such as the nTS and Pa5. Whether one or both nuclei are involved in the DNIC-related modulation of airway inputs is yet to be confirmed. If responses in these nuclei are inhibited, then the cough reflex would presumably also be attenuated. fMRI optimized of the brainstem was done to assist in identifying whether this proposed hypothesis is true regarding the DNIC-related modulation of airway inputs including providing evidence of the modulation of airway inputs that led to the neural outcomes of the hemispheres of the brain observed in the previous study (Chapter 2). It was therefore hypothesized that DNIC-related changes will manifest as reduced BOLD signal intensities of the nTS and the Pa5 nuclei. This information could assist in identifying regions that could be targets for therapeutic interventions aimed at reducing the sensation of UTC in chronic cough patients without impairing the cough reflex.

Methods

Participant recruitment and experimental protocol

Twenty-three healthy individuals were recruited to participate in the study via advertisements distributed across the campus of Monash University (Melbourne, Australia) (12 males and 11 females, mean age ±SD 28.37 ± 8.58). Prior to inclusion, all subjects were screened via a questionnaire to exclude any history or medical conditions such as respiratory and heart disease, brain injury, claustrophobia, pregnancy and metal implants.

Consent was obtained from the participants to be involved in the study which is in compliance with procedures approved by Monash University Human Research Ethics Committee (approval 2018.11644).

Psychophysical testing session

Participants inhaled a series of different concentrations of capsaicin in the form of a single tidal volume of capsaicin vapour prepared in doubling concentrations (0.12 – 125 µM) via an MRI-compatible nebuliser. Their ratings of UTC using a Borg scale (0=no urge to cough to 10= maximum urge to cough) were recorded. In addition, any cough events within a period of 8 seconds after every inhalation challenge was also noted. The first lowest concentration of capsaicin to elicit an UTC sensation was recorded as (Cu). The concentration that elicited two or more coughs was recorded as the cough threshold (C2). A further one increment of concentration of capsaicin (C2+1) were delivered to participants. The subsequent further two (C2+2) and (C2+3) three increments of concentration of capsaicin from C2 were also recorded.

Participants also underwent mild to moderate painful stimulation in the form of mechanical pressure applied in a slow and step-wise fashion onto the left thumbnail. This was achieved via the use of a custom-made device that placed controllable weight directly onto the left thumbnail starting at low levels and gradually increasing in increments of 500 g [195,264]. Pressure was applied for 5 seconds at a time and the weight (range from 500 g to 8 kg) that produced a moderate level of pain on an 11-point numerical rating scale (0=no pain, 5=moderate pain, 10=extreme pain) was recorded. This moderately painful pressure was used as the conditioning stimulus during the first experiment of the psychophysical session.

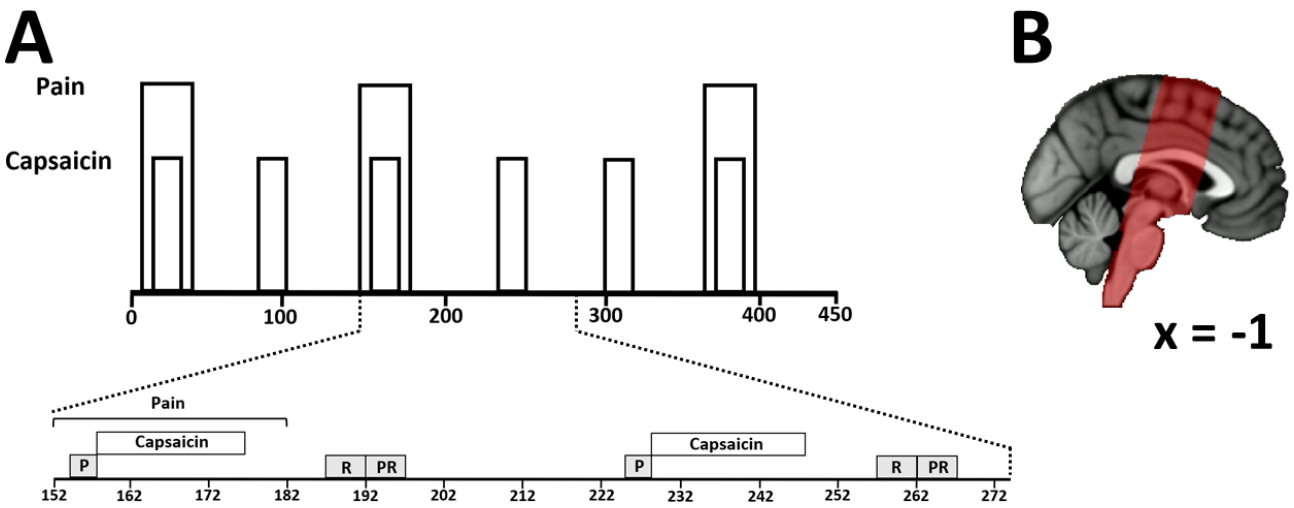
Subsequently, the influence of painful conditioning on UTC and cough was recorded. This was determined by the contrast of UTC ratings and cough frequency in response to single tidal volume inhalation of capsaicin concentrations of C2, C2+1, C2+2 and C2+3 during either the absence or concurrent application of painful stimulus in a randomised order. Each concentration (total of 16 for all concentrations) was inhaled on four occasions with painful stimuli applied on two occasions of the tussive challenge. Moderate painful pressure was applied prior to inhalation of capsaicin vapour and sustained until the end of expiration. UTC ratings and cough frequencies were recorded after each inhalation challenge.

Furthermore, the second experiment of the psychophysical session involved participants undergoing trials to determine the weight and capsaicin concentration that would be used in the subsequent fMRI session. The maximum capsaicin concentration (S_{max}) that each individual was able to inhale repeatedly for 20 seconds (s) without causing coughing was recorded. The weight that achieved a moderate level of pain (5 from the 11-point scale) when pressure was applied onto the left thumbnail was also recorded. This pressure was applied in 5 s intervals with 1 s resting period during a total duration of 40 s. This approach aimed at reducing temporal summation when applying pressure onto the left thumbnail [265]. The weight that evoked a moderate pain level during sustained application of painful stimulus was recorded and used as the conditioning stimulus while the S_{max} was used as the capsaicin concentration for inhalations during the fMRI session.

fMRI testing session

The fMRI imaging protocol involved a block design incorporating two different contingencies during scanning. These were a contribution of capsaicin inhalation during either the presence or absence of painful stimuli. Testing during these blocks involved random inhalation of nebulised capsaicin with or without painful stimuli. The first block involved inhalation of capsaicin for a duration of 20 s combined with the application of a moderate level of painful stimuli for a total duration of 40 s onto the left thumbnail. Painful stimulus was applied 10 s prior to inhalation of capsaicin and concluded 10 s after inhalation (Figure 10). The second block only involved capsaicin inhalation for a total duration of 20 s. Participants were informed via a projector running Presentation software of an impending presentation of a stimulus 3 s before stimulus onset. Participants were then required to rate their UTC and pain ratings 5 s following stimulus offset. There was a resting period of 28 s between the two blocks. The two blocks were repeated on three occasions in a randomised order for each run totalling a duration of 7.50 minutes per scan run (Figure 10). Rating was performed using the Borg scale via a trackball of the mouse placed on the right hand of each participant. Each participant underwent testing for a total of four runs. Painful conditioning was averaged across the session such that a no conditioning event was not followed by another no conditioning event.

Figure 10. fMRI protocol during scanning.



(A) *Participants underwent four fMRI scans for a total duration of 7 minutes and 30 seconds each. Conditioning stimulus was applied in the form of thumbnail pressure for a period of 30 seconds on three occasions during each scan. Capsaicin was inhaled as test stimuli for a period of 20 seconds on six occasions. The expanded panel shows one of the three cycles of stimuli delivered during an fMRI scan. Participants prepared for capsaicin challenges of three seconds via visual cue (P) to ensure commencement of inhalations were consistent throughout the sequence. Visual cues also prompted participants to provide ratings of UTC (R) and pain intensity (PR) in which numerical rating scales (0-10) appeared on the screen. The sequence of capsaicin inhalation was different for each of the four fMRI scans. The sequence in the figure corresponds to the first fMRI scan.* ***(B)*** *Sagittal cross section showing the field of view (in red) optimized for brainstem imaging (overlaid on a standard MNI template) during the fMRI session.*

Image acquisition parameters

Scanning was performed at Monash Biomedical Imaging (Melbourne, Australia) using a MAGNETOM Skyra 3 T scanner (Siemens, Erlangen, Germany) with a 32-channel head coil. Structural T1-wieghted images were acquired in the sagittal plane (192 slices, 1 mm slice thickness, 1 × 1 mm^2 in plane resolution, echo time (TE) 2.07 ms, repetition time (TR) 1900 ms, flip angle 9^0). BOLD contrast images were acquired in trans-axial plane (34 slices, 4.5 mm slice thickness, 3 × 3 mm^2 in plane resolution, TE 31 ms, TR 2000 ms, flip angle 90^0), producing a total of 222 sequential volumes in 7.50 minutes of scanning time. A total of four whole brain echo planar imaging (EPI) volumes were collected from all participants. The mean of these volumes was used during analysis for registration

purposes. Furthermore, additional phase and magnitude images of the B0 field were acquired to create fieldmaps.

Analysis

Psychophysical analysis

Statistical analysis of behavioural responses was performed using SPSS version 25.0 (IBM, Armok, NY, USA) for the behavioural results in both the psychophysical and fMRI scanning sessions. The capsaicin concentration values of C2, C2+1, C2+2 and C2+3 were tested for stimulus response to determine the effect of pain on the ratings of UTC and cough frequency as a form of behavioural measure using a repeated measure statistical analysis (ANOVA) and post hoc dependent t-tests. ANOVA and post hoc dependent t-tests were also used to test whether the stimuli (capsaicin), presentation order of stimuli during fMRI scans and scan order had an effect on the variance of UTC ratings. ANOVA was also used to assess the interaction between the test stimulus and the conditioning stimulus (moderate pain) and whether it had an effect on the magnitude of UTC ratings during scanning.

fMRI registration & analysis

fMRI analysis was carried out using the FMRIB suite of software tools including the fMRI Expert Analysis Tool (FEAT) [266]. Anatomical and functional images were stripped of non-brain voxels using the brain extraction tool (BET) [266]. The outcome of BET was manually assessed for quality with necessary adjustments made if required. This approach allowed for extending the BET outcome caudally to include the full extent of the brainstem distal to the obex which is not invariable retained by the automated BET processing. Pre-processing of functional EPI. FUGUE (FMRIB's utility for Geometrically Unwarping EPI) was employed to correct the geometric distortion of functional data and mean EPI images at the brainstem level. This process involved production of two fieldmaps using additional phase and magnitude images taken from the B0 field during image acquisition. Brainstem fieldmaps were created to enable unwarping of the 4D data while whole brain fieldmaps were created for unwarping of the mean EPI images which was to be used later in analysis for initial registration.

Pre-processing of functional EPI data involved two stages. The first stage involved motion correct using MCFLIRT. The second stage involved unwarping the motion correct data using the brainstem fieldmap produced earlier. Functional data acquired during scanning

was then spatially smoothed with a 4mm full-width half maximum Gaussian kernel and high pass filtered with a 100s cut-off.

A multiple stage registration approach was used to transform partial brainstem volumes to the standard MNI152 template brain. This was performed using FLIRT (FMRIB linear registration tool). Each individual's motion corrected and functional (unwarped) EPI data was linearly registered to the whole brain (unwarped) mean EPI. The whole brain mean EPI was then registered with the individual's high resolution T1 volume and the resultant volume later registered to the standard MNI152 space template brain (1 mm resolution). A transformation matrix of the resultant image was also created. Since registration using FLIRT was originally developed for the cerebral hemispheres, an additional registration step was undertaken to aid in optimized registration of the brainstem. This was achieved by registration of the high resolution T1 images to the standard MNI template brain with weighting of the brainstem [267]. This method incorporated a custom-made mask of the MNI template brain with voxels of the brainstem giving a weighting of 2 while other brain and outside voxels were weighted 1 and 0 respectively. The resultant transformation matrix from this step was concatenated with the initial transformation matrix for use in the second and third (group) level analysis [267].

The first level of image analysis involved constructing regressors for events of interest such as CP and CN. These regressors and their derivatives were convolved with a hemodynamic response function and used to generate a model for each run in the general linear model. Regressors for other events such as prepare to inhale (P), prepare to rate UTC (PR), rate urge to cough (R), prepare to rate UTC (R) and pain (PR) were also constructed. In addition, other regressors of no interest were included to account for noise such as residual respiratory motion as previously described [261]. These included time-courses from cerebrospinal fluid (CSF) within the lateral ventricles, motion correction parameters, voxels of high standard deviation in the sagittal sinus and mean global signal from non-activated areas of the brain [268]. Planned contrast resulting from the included two events (CN, CP) were analysed as well as CN>CP and CP>CN. Second level analysis involved fixed effects to amalgamate results from all four runs for each subject. Mixed effects analysis incorporated in the group analysis used "FSL's Local Analysis of Mixed Effects" (FLAME) across individuals to generate a final group result. All fMRI group image analyses were determined using a single voxel inclusion of Z>2.3 and a cluster-wise family

error (FEW)-corrected threshold of p_{cor}<0.05 based on the FMRIB software library expert analysis Tool (FEAT) implementation of random field theory [269]

The localisation of ROI for the nTS and Pa5 were inferred using the Duvernoy (for rostral areas) and Paxinos (for ventral areas) atlases of the brainstem [270,271]. Corresponding functional regions to ventral areas of activation were interpreted with caution as the horizontal slices from the atlases were cut at a different angle to that of the standard MNI152 brain template. This was not an issue for rostral areas of the brainstem as the Duvernoy atlas incorporated the same horizontal slice angle as the standard MNI152 brain template. The regions that were defined were further analysed using FEAT query. Mean activation of ROIs during each event (CN or CP) were expressed as BOLD signal changes from the baseline (Figure 12 and Figure 13). Finally, fMRI power calculations based on the BOLD signals between the two events were obtained to determine sample and effect size that may yield regional brainstem responses based on CN>CP (Table 5).

Results

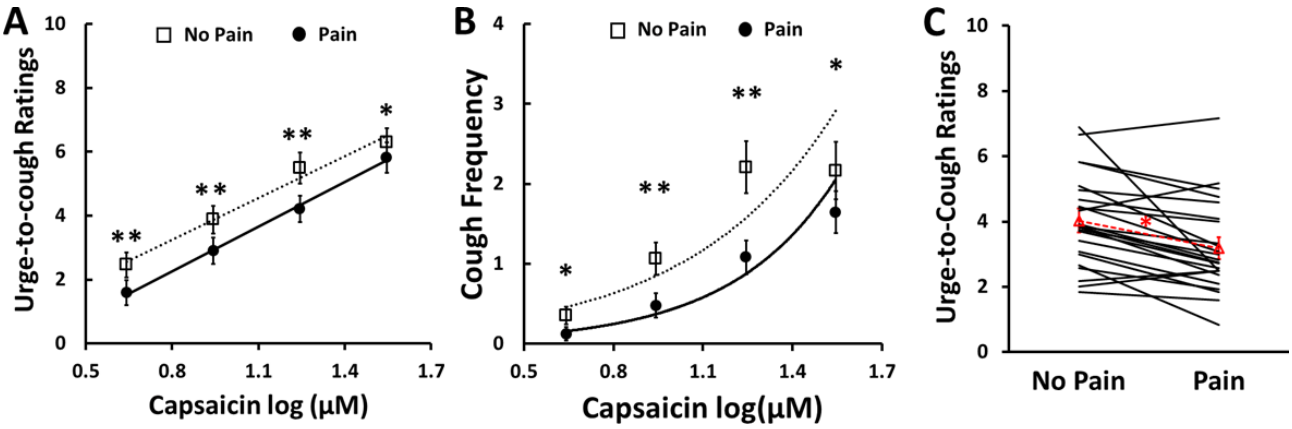
Psychophysical results

The geometric means of Cu, C2, C2+1, C2+2, C2+3 and S_{max} were 0.13 µM, 0.64 µM, 0.94 µM, 1.24 µM, 1.55 µM and 0.50 µM respectively. Levels of stimuli for conditioning pain during the psychophysical and fMRI session were 4.02±0.35 kg/cm^2 and 3.33±0.26 kg/cm^2. Individual threshold levels for the different capsaicin concentrations (C2, C2+1, C2+2, C2+3) to test the effect of painful conditioning on UTC and cough during the psychophysical session is shown in Table 1. The S_{max} concentrations including the weight to evoke moderate painful stimulus administered during the psychophysical and fMRI session are also shown in Table 1.

Both UTC ($F(1,22)=22.6$, $p<0.001$) and cough ($F(1,22)=20.6$, $p<0.001$) showed conditioning related decreases during the psychophysical session that averaged 19.9±3.15% and 42.5±33.9% respectively across all concentrations which was showed significant differences between the two outcome variables ($t(22)=4.0$, $p<0.001$) (Figure 11A & 11B). Painful conditioning reduced cough frequencies by 64.7±21.2%, 54.9±25.0%, 50.9±35.2% and 24.0±26.8% for the C2, C2+1, C2+2 and C2+3 concentrations respectively. For UTC ratings, painful conditioning was associated with reductions of 35.2±0.71%, 25.3±6.4%, 23.3±15.4% and 7.6±9.9% for the C2, C2+1, C2+2 and C2+3 concentrations respectively. In addition, test concentration and conditioning interacted for

cough frequency (F(3,20)=4.05, p<0.05) for higher test concentrations showing greater conditioning related decreases (Figure 11A & 11B) while UTC ratings showed no significant interaction for the two factors (F(3,20)=1.83, p=0.25). There were no significant differences between males (UTC CN=4.88; CP=3.82, Cough CN=1.51; CP=0.95) vs females (UTC CN=4.48; CP=3.67, Cough CN=1.47; CP=0.78) for UTC ratings (t(22)=0.62, p=0.54) and cough frequencies (t(22)=1.2, p=0.25) as a result of painful conditioning during the psychophysical session. In addition, gender differences were not significant between male (Cu=0.10; C2=0.62) & females (Cu=0.10; C2=0.68) for the Cu (t(22)=2.5, p=0.91) and C2 (t(22)=1.2, p=0.67) thresholds. UTC ratings showed conditioning related effects during scanning (F(1,22)=15.4, p<0.001). Decreases in UTC ratings averaged 0.85±0.21 on the Borg scale (average 21.0±1.4%) during conditioning pain compared to no pain events (t(22)=3.9, p<0.001) (Figure 11C).

Figure 11. Psychophysical results of the UTC and cough frequency.



(A) *Ratings of UTC after capsaicin inhalation for concentrations of C2, C2+1, C2+2, C2+3 were averaged during the psychophysical session. The average ratings of UTC during the concurrent application of conditioning (pain) is represented by circles and solid line. Average ratings of UTC during the no pain contingency is represented by triangles and broken line. All concentrations of capsaicin showed significant reduction in UTC ratings as a result of painful conditioning after capsaicin inhalation.* ***(B)*** *Coughs were recorded after inhalation of all four concentrations of capsaicin with and without conditioning during the psychophysical session. The average cough frequency during conditioning (pain) is represented by circles and solid line while the no pain contingency is represented by triangles and broken line. The reduction in cough frequency becomes more prominent as the concentration of capsaicin increases.* ***(C)*** *UTC ratings were recorded during each capsaicin challenge with and without conditioning during the fMRI scans. Concurrent*

application of painful conditioning was associated with a significant decrease in UTC ratings. The average reduction in UTC ratings across all participants is represented by triangles and broken line.
*Error bars represent standard errors of the mean. * p<0.05 **p<0.01*

Table 1. Individual psychophysical data.

Subject #	C2 (µM)	C2+1 (µM)	C2+2 (µM)	C2+3 (µM)	S_{max} (µM)	Weight (kg)*	Weight (kg)**
1	7.81	15.63	31.25	62.5	-0.31	3.25	2.25
2	3.91	7.81	15.63	31.25	0.59	6	4.75
3	7.81	15.63	31.25	62.5	0.29	5	4.00
4	15.63	31.25	62.5	125	1.19	6	3.75
5	7.81	15.63	31.25	62.5	0.89	3	2.63
6	7.81	15.63	31.25	62.5	0.89	8	6.00
7	7.81	15.63	31.25	62.5	0.29	5.5	4.00
8	1.95	3.91	7.81	15.63	-0.09	1.25	1.19
9	3.91	7.81	15.63	31.25	0.89	2	2.00
10	15.63	31.25	62.5	125	1.49	4	3.25
11	3.91	7.81	15.63	31.25	0.59	3.5	2.88
12	3.91	7.81	15.63	31.25	0.59	5	4.00
13	7.81	15.63	31.25	62.5	1.19	2	1.69
14	3.91	7.81	15.63	31.25	0.29	4.5	3.88
15	7.81	15.63	31.25	62.5	0.89	6	5.50
16	0.98	1.95	3.91	7.81	0.29	2	2.00
17	3.91	7.81	15.63	31.25	0.59	6	5.00
18	1.95	3.91	7.81	15.63	-0.01	4.5	4.25
19	3.91	7.81	15.63	31.25	0.89	3	2.63
20	1.95	3.91	7.81	15.63	-0.01	2	2.00
21	1.95	3.91	7.81	15.63	-0.31	3	3.00
22	3.91	7.81	15.63	31.25	0.59	4	4.00
23	7.81	15.63	31.25	62.5	0.29	3	2.50
24	0.98	1.95	3.91	7.81	-0.01	4	2.75
Mean	0.64	0.94	1.24	1.55	0.50	4.02	3.33

** Weight used as painful stimulus during the psychophysical session.*

*** Weight used as painful stimulus during the fMRI session.*

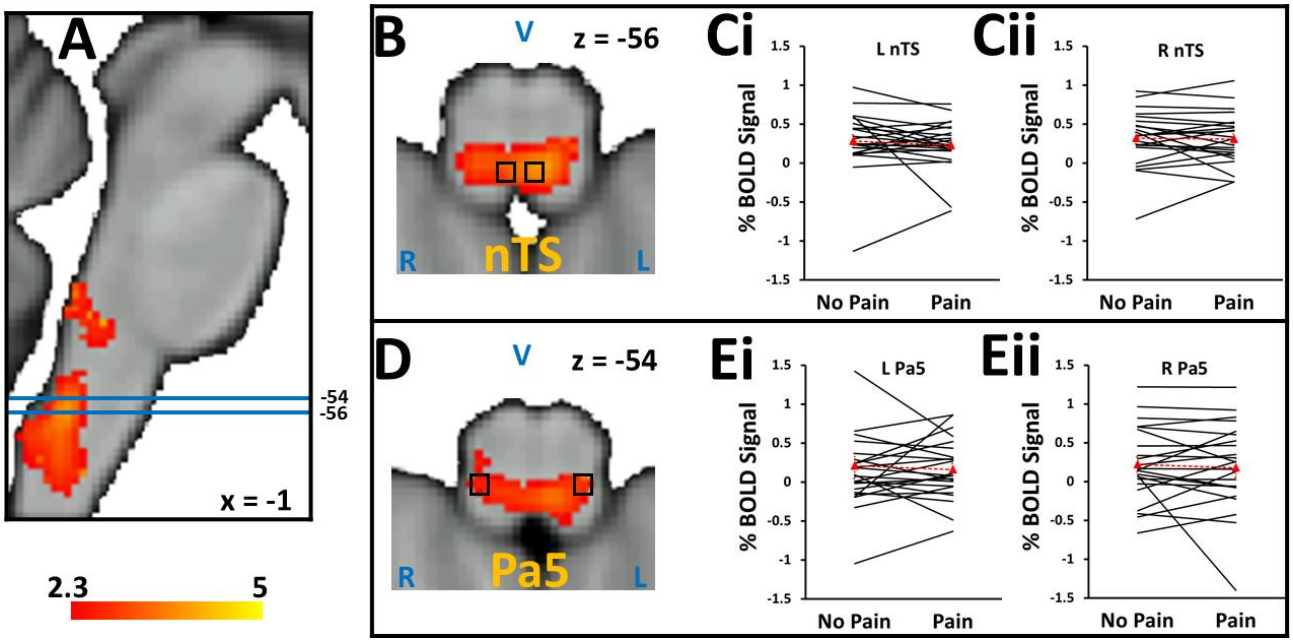
Individual data for the different capsaicin (log µM) concentrations used during the psychophysical session to test the effect of painful conditioning on UTC and cough. S_{max} was the capsaicin concentration given to participants during the fMRI session and is the maximum concentration that elicits a strong UTC without coughing. The weights used for the application of painful stimulus during both the psychophysical and fMRI session is also illustrated.

fMRI results

Regional brainstem activation during capsaicin inhalation

Capsaicin-evoked activations were widely distributed at almost all rostrocaudal levels of the brainstem during no pain events (CN) (Figure 12A, 12B and 12D and Table 2). At the level of the obex (z = - 58), capsaicin related activations were equally distributed bilaterally and mainly confined to the dorsal half of the medulla. These regions likely encompass structures including the spinal trigeminal (Sp5) and its par interpolaris (Sp5I) and pars caudalis (Sp5C), nTS and the DRt (dorsal reticular nucleus). Rostrally, activation was observed bilaterally distributed across the medullary midline encompassing regions such as the nTS, Pa5, Sp5, Sp5I and hypoglossal nucleus. Similar regions were activated during conditioning pain events (CP). Below the obex (z = < -58), medullary activation in response to capsaicin inhalation were observed in the region that encompasses the DRt. There were no regions that showed increased responses during no pain events compared to conditioning events (CN>CP) or vice versa (CP>CN).

Figure 12. Regional brainstem responses elicited during capsaicin inhalation challenges.



(A) *Sagittal view of the brainstem region areas activated during capsaicin inhalation (CN) without conditioning are rendered in red-yellow. Axial slices rendered in red-yellow showing regional activations of the nTS* ***(B)*** *and Pa5* ***(D)*** *during capsaicin inhalation without conditioning. There were no regions where capsaicin inhalation responses were significantly reduced during pain conditioning compared to the no pain contingency (CN>CP). Line graphs showing the mean percentage BOLD signal change within boxed regions of the nTS* ***(Ci and Cii)*** *and the Pa5* ***(Ei and Eii)*** *for all participants during pain and no pain events. Activations are rendered in the Montreal Neuroscience Institute template of the brain (1mm resolution).*

Table 2. Regional brainstem activations during capsaicin inhalation (CN).

	Peak voxel coordinates*				
Region	*x*	*y*	*z*	*Z-score*	Possible structures
Medulla	-1	-43	-60	3.43	DRt, Sp5, Sp5C
	7	-41	-58	3.31	Pa5, Sp5
	6	-42	-54	3.22	nTS, XIIn
	-1	-43	-58	3.25	Pa5, Sp5
	2	-43	-56	3.57	nTS, XIIn
Pons	10	-37	-44	4.27	Rp
	7	-36	-42	2.84	Rp, Abd
	8	-35	-40	3.14	VIIn, Sp5, Sp5O

Definition of abbreviation: BA = Brodmann area

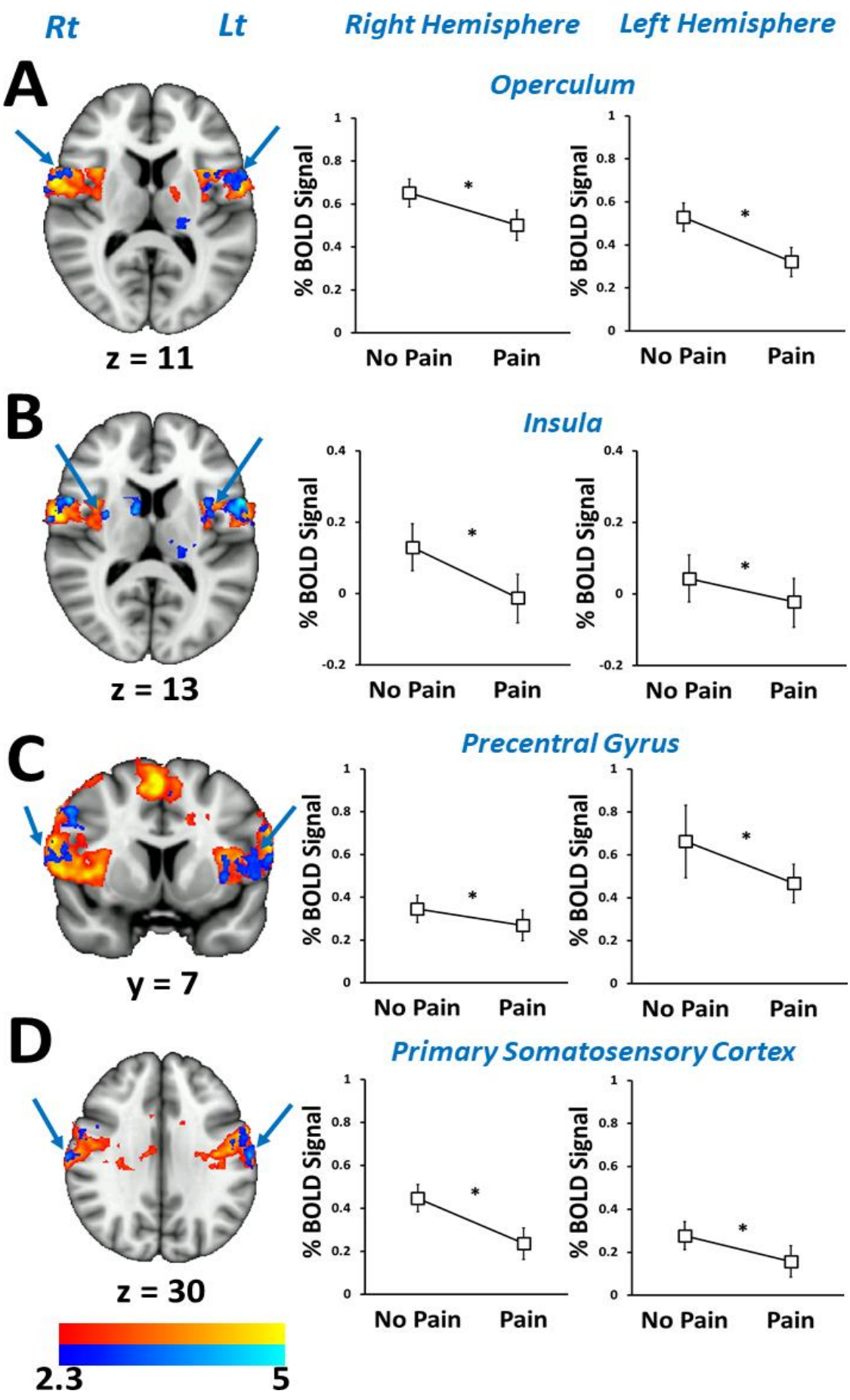
**The coordinates correspond to the Montreal Neuroscience Institute standard brain template where x values are distance in millimetres to the left (negative x values) or right (positive x values) from the anterior commissure; y represents millimetre distance anterior (positive) or posterior (negative) from the anterior commissure, and z is millimetre distance superior (positive) or inferior (negative) from the anterior commissure.*

Abbreviations: Abd – nucleus abducens, DRt – dorsal reticular nucleus, nTS – nucleus of the solitary tract and solitary tract, LF – longitudinal fasiculus (medial & dorsal parts), Mes5 – mesencephalic trigeminal tract and nucleus, Pa5 – paratrigeminal nucleus, Pon – pontine nuclei, Rp – midline raphe, Sp5 – spinal trigeminal tract and nucleus, Sp5C – spinal trigeminal nucleus pars caudalis, *Sp5O – spinal trigeminal nucleus pars oralis, VIIn – facial motor nucleus, XIIn – hypoglossal motor nucleus, 5thal – trigeminothalmic tract.*

Regional higher brain activations during capsaicin inhalation

Regional brain responses during (CN) within the restricted field of view were widely distributed bilaterally in the cerebrum and included the operculum (OP), insula (In), precentral gyrus (PreCG) and the primary somatosensory cortex that corresponds to Brodmann area 44 (BA44) (Figure 13 and Table 3). Regions that showed increased responses to capsaicin inhalation during no pain events compared to conditioning pain events (CN>CP) included the bilateral OP, PreCG, primary somatosensory cortex (BA44) and the bilateral In (Figure 13 and Table 4).

Figure 13. Brain responses within the field of view for CN and CN>CP.



Brain regions where responses to capsaicin inhalation during no pain are rendered in red-yellow (CN). Regions where capsaicin inhalation responses were significantly decreased during pain conditioning compared to the no pain contingency (CN>CP) are rendered in blue. ***(A)*** *Bilateral operculum showed a decrease in capsaicin inhalation during the application of thumbnail pressure. Conditioning-related decreases in capsaicin inhalation activation were also seen in the* ***(B)*** *insula,* ***(C)*** *precentral gyrus and the* ***(D)*** *primary somatosensory cortex corresponding the Brodmann area 44 (BA43). Line graphs showing significant decrease in the mean percentage BOLD signal intensity within the CN>CP regions.*

Activations are rendered in the Montreal Neuroscience Institute template of the brain (1mm resolution).

*Error bars represent standard errors of the mean. * p<0.01*

Table 3. Regional higher brain activations during capsaicin inhalation with no pain (CN).

		Peak coordinates*			
Region	BA	x	y	z	z-score
Precentral	44	-60	1	18	7.23
	44	64	4	12	5.7
Paracingulate	6	5	10	53	6.38
	6	-4	14	49	5.13
Supplementary motor area	6	5	2	63	6.19
	6	-2	-3	60	5.03
Postcentral	3	62	-7	16	5.33
	3	-59	-7	15	4.29
Insula	13	35	4	14	3.54
	13	-34	-9	14	3.07
Central Operculum	44	52	3	6	4.01
	44	-46	6	3	3.11

Definition of abbreviation: BA = Brodmann area

**The coordinates correspond to the Montreal Neuroscience Institute standard brain template where x values are distance in millimetres to the left (negative x values) or right (positive x values) from the anterior commissure; y represents millimetre distance anterior (positive) or posterior (negative) from the anterior commissure, and z is millimetre distance superior (positive) or inferior (negative) from the anterior commissure.*

Table 4. Regional higher brain activations for CN>CP.

		Peak coordinates*			
Region	BA	x	y	z	z-score
Precentral	3	-58	-2	34	4.43
	6	53	5	38	2.84
Supplementary motor area	6	-2	-5	61	2.7
	6	2	1	63	3
Postcentral	1	-59	-10	38	3.07
Insula	13	-35	7	13	3
Central Operculum	44	-36	8	11	2.71

Definition of abbreviation: BA = Brodmann area

**The coordinates correspond to the Montreal Neuroscience Institute standard brain template where x values are distance in millimetres to the left (negative x values) or right (positive x values) from the anterior commissure; y represents millimetre distance anterior (positive) or posterior (negative) from the anterior commissure, and z is millimetre distance superior (positive) or inferior (negative) from the anterior commissure.*

Table 5. fMRI power calculation table for sample and effect size for regions of interest.

Region	*Sample Size*	*Effect Size*
Left nTS	267	0.13
Right nTS	2494	0.04
Left Pa5	1690	0.07
Right Pa5	864	0.08
Left Op	10	0.30
Right Op	17	0.28
Left Insula	20	0.14
Right Insula	12	0.31
Left PreCG	11	0.45
Right PreCG	20	0.20
Left BA44	10	0.53
Right BA44	10	0.62

Power calculations indicating the sample and effect (Cohen's d) size required to elicit a difference in regional brain responses as a result on painful conditioning (Cohen's d: 0.2 = small effect size; 0.5 = medium effect size; 0.8 and greater = large effect size).

Discussion

This study has demonstrated that painful conditioning can interact with and modify cough and the UTC sensations during capsaicin-evoked inhalations. Painful conditioning was associated with significant reductions in UTC ratings and cough frequencies for a wide range of capsaicin concentrations (C2, C2+1, C2+2 and C2+3). Differential effect in decreases associated with painful conditioning on the UTC and cough was observed across a stimulus response function. These psychophysical outcomes were also consistent with decreased BOLD signal responses in higher brain regions that were within the field of view during fMRI scanning. However, the same outcome was not observed in regional brainstem responses providing no evidence of a spinal-medullary-spinal modulation of UTC and cough at the subcortical level. The absence of regional brainstem responses indicate that activations associated with changes in the sensory processing or

coding of airway inputs is most likely not well represented at the subcortical level using the current methodological approach.

Psychophysical results

The findings of the present study confirmed previous reports that painful conditioning significantly reduces the sensory perception of the UTC and the motor act of cough in humans [100,260]. In addition, the results showed a disproportionate reduction in UTC ratings compared to cough frequency for the different concentrations of capsaicin. This is evident where conditioning pain led to an average reduction of UTC by 19% compared to the 42.5% reduction in cough frequency across the four concentrations of capsaicin. Individual results also showed a similar pattern whereby pain resulted in a reduction of 25% versus 55% for UTC rating and cough frequency respectively for the C2+2 capsaicin concentration. It is apparent from these findings that a small alteration in the sensory processing of UTC leads to a larger proportionate alteration in the motor act of cough. Increases in UTC ratings with doubling concentrations (from C2 to C2+3) followed a linear path across the stimulus response function compared to the exponential increases in cough frequencies in the present study. Similarly, reductions in UTC ratings and cough frequencies followed a similar linear versus a non-linear pathway across the stimulus response function respectively. This narrative is parallel with previous data where the increase in UTC ratings were linear compared to the non-linear increase in cough frequencies based on the same doubling concentrations of capsaicin [49,76]. It is also worthwhile to note that such observations may be as result of the differences in scale between actual cough frequencies compared to UTC ratings. The smaller cough frequency may have led to the disproportioned differences in values compared to the larger ratings of UTC. Also, the conditioning effects of pain appear to saturate for the motor act of cough at the highest concentrations of capsaicin (C2+3). This suggests that the modulation of airway inputs may have a saturation point at which the DNIC effect of the UTC may become exhausted. Collectively, these outcomes may suggest a differential effect of conditioning pain on sensory versus motor neural activity that resulted in disproportionate conditioning related effect on the behavioural outcomes of the UTC and cough. It is also possible that subjective (UTC) and objective (cough frequency) measures may not be tightly regulated in humans.

Cough and pain neural interactions

Regional activations of capsaicin-induced inhalations observed in the present study were widely distributed within brainstem networks. These regions are consistent with previous reports of this network in brainstem studies associated with responses to inhaled chemical irritant stimulus in humans [76,78,82,99,272]. Furthermore, activations were observed in the nTS and Pa5 which are known to be the primary sites for receiving airway afferents before this input is relayed to higher brain regions for processing. The pattern of regional activations in the higher brain within the field of view were similar to those observed in previous cough related whole brain studies [37,76,80,82,260,273,274]. Notably, many of these regions are also involved in the sensory neural processing of somatic and visceral pain [107,275].

Widespread changes were observed within the restricted field of view (FOV) in regional higher brain responses as a result of painful conditioning. These changes within the capsaicin inhalation network were also consistent with reductions in BOLD signal intensities during painful conditioning compared to the absence of painful conditioning events. These outcomes are highly consistent with results of the previous study (Chapter 2) regarding the effect of pain on capsaicin evoked regional brain responses in a fMRI study [260]. This widespread effect is consistent with the notion that there is a putative descending inhibition of incoming sensory information most likely at the level of the brainstem. Given that the reduction in pain-related regional brain responses is steady throughout the higher cortex suggests that the modulation of airway sensory input is likely representative of a DHSC-medullary-DHSC descending inhibition as that observed in DNIC studies. However, the present study was optimized for the brainstem region and thus, the findings of the present study does not allow for the same contentions to be made in relation to the previous study (Chapter 2). Therefore, careful consideration should in interpreting the outcomes of higher brain regions in the present study. Also, the limited field of view of higher brain regions means that other networks in the hemispheres may have different activation patterns associated with conditioning related effects that was not measurable due to the exclusion of such regions in the fMRI analysis. The present study aimed at identifying conditioning related responses at the brainstem level making it difficult to derive similar conclusions as those observed in the previous study (Chapter 2). Nevertheless, conditioning related activations observed in the hemispheres is consistent with the notion that painful conditioning does lead to widespread changes in neural activity in the capsaicin inhalation network.

Regional brainstem responses for both the no-pain and pain events showed similar activation patterns during fMRI scanning. However, regional brainstem responses as result of the difference between the pain and no pain contingencies were not observed (CN>CP) neither for CP>CN. This was an unexpected finding given that higher brain responses that were within the field of scanning in the present study including the outcomes of the previous study (Chapter 2) suggest that DNIC-related modulation of incoming airway inputs is most likely at the level of the brainstem [260]. Furthermore, BOLD signal changes extracted from regions of interest such as nTS and Pa5 showed mixed results. The majority of individuals showed a decrease in activation during painful conditioning with some showing an increase in BOLD signal intensity. In addition, bilateral fMRI power analysis of BOLD signal intensity from these two nuclei showed that the effect size (left nTS = 0.13; right nTS=0.04; left Pa5=0.07; right Pa5=0.08; Cohen's D effect size analysis) required to show conditioning related differences statistically were very small to non-existent (Table 5). On the contrary, fMRI power analysis of selective higher brain regions within the field of view showed moderate and larger effect sizes required to show conditioning-related activations (Table 5). For instance, the effect size for left and right PreCG was 0.45 and 0.20 respectively. These are moderate effect sizes required to show differences in activations between the pain and no pain contingencies. These outcomes discourage the proposition that the absence of a significant effect between the no-pain and pain events was related to the smaller than requisite sample size but rather consistent with the absence of DNIC-related effect at the brainstem level under the experimental conditions. Notably, fMRI analysis was not confined to the nTS and Pa5 giving that other nuclei within the brainstem are also known to be involved in the processing of airway inputs. A positive outcome in the form of changes in regional brainstem responses associated with painful conditioning would have yielded other brainstem regions that may have been involved in the DNIC-related modulation of airway inputs.

The absence of regional brainstem responses described above raises several questions regarding the conditioning-related modulation of airway inputs in humans. One possibility is that the findings of the present study is an accurate interpretation of the DNIC-related modulation of airway inputs. This may suggest that the modulation of airway inputs may not necessarily involve brainstem regions giving that changes in activations were only observed in the hemispheres of the brain in the present and previous study (Chapter 2). However, this is not in line with how the circuitry employed by a DNIC effect operates within the brainstem. The mechanism involved in this circuitry is well established in animal

and human studies where activations of certain nuclei lead to the inhibition of nociceptive input in the spinal cord [103,257]. The current literature strongly suggests that the DNIC-related modulation of noxious inputs is confined to the DRt regions of the brainstem [120]. Therefore, it is highly unlikely that the pain interaction effect observed in the current study is due to higher order processing which is more consistent with other forms of modulation such as placebo or distraction [85].

Another explanation for the lack of differences in regional brainstem responses between the pain and no pain contingency could be due to the limitations of fMRI in the present study. The findings could be misleading in that the output of brainstem responses were modulated but were not detectable under the current methods. This raises two possibilities that lead to the absence of regional brainstem responses associated with limits of fMRI scanning. One possibility could be that there is indeed a physiological change in the neural activity in the brainstem that leads to changes in activations observed in higher brain regions. However, the BOLD signal measures were not sufficiently sensitive enough to detect such changes in neural activity. This may be due to the small or subtle change in the sensation of UTC (19% decrease) is unable to be detected by BOLD signal measures at the brainstem level. Another possibility could be that the absence of regional brainstem responses may represent both a reduction in brainstem nuclei activity, but also a presumptive increase in the local inhibitory inputs to the nuclei. The sum total of reductions and increases in neural activity (less in output neurons and more in local inhibitory influences) could be similar to the activity in the absence of a DNIC scenario. In other words, the sum total of reductions as well an increase in neural activity at the same time could lead to the hemodynamic response and BOLD signal intensity to be unchanged leading to absence in activations associated with differences between pain and no pain events. These assumptions are supported by recent studies regarding variations in BOLD signal responses during fMRI. Indeed, BOLD signal responses obtained during fMRI data acquisition are believed to be as a result of alterations in neural activity mainly associated with postsynaptic activity of principal cells within local brain regions [276-281]. However, recent findings have shown that substantial postsynaptic activity of cells within the field of image acquisition do not necessarily generate significant BOLD responses [276,282]. In addition, it was also reported that contributions of pre-synaptic and post-synaptic neural activity was accompanied by variations in the resultant BOLD signal responses in fMRI [61,276,278,279]. Moreover, several studies have provided evidence of the disproportionate relationship between neurophysiological outcomes and hemodynamic responses in fMRI BOLD signals

responses in certain brain regions [277,283,284]. For instance, one study conducted by Hill et al (2021) showed that fMRI BOLD signal responses were negatively correlated with neural activity in the hippocampus [283]. This outcome was consistent with prior research reporting the functional dissociation between BOLD signal responses and encoding-related brain activity in the form of intracranial electrophysiological recordings in local field potentials that were measured during the administration of high and low gamma-band frequencies [284-290]. However, the same study reported a robust and positively correlated relationship between BOLD signal responses and neural activity across much of the neocortex (mainly the frontal and parietal cortices) which is consistent with findings reported in several other studies [283,291-294]. It is evident that changes in neural activity within nuclei in the hippocampus of the human brain does not necessarily correlate with changes in fMRI BOLD signal responses. The findings from these investigations may have implications for the negatively covaried relationship between the BOLD signal responses and neural activity in the brainstem region as observed in the present study. It may therefore explain the absence in DNIC-related differences in the BOLD signal intensities in the nTS and the Pa5 despite reported significant DNIC-related differences in the behavioural outcomes for the UTC ratings and the DNIC-related neural outcomes observed in the hemispheres of the brain.

Alternatively, fMRI studies of the bulbar region can be challenging due to several technical factors. The relatively small sizes of bulbar nuclei in the brainstem and small voxel sizes used in functional imaging may lead to spatial constraints [295,296]. Geometric distortion related to the front-to-back phase coding may give rise to activation patterns that are not necessarily symmetrical in either side of the midline and have a negative influence on the signal-to-noise ratio [297]. Brainstem motion originating from cardiac and pulmonary activities may also lead to geometric distortions in the form of displacements between caudal and rostral structures of the brainstem [296,298,299]. These factors could lead to signal loss in regions of interest including poor signal to noise ratio of BOLD signals resulting in differential outcomes of regional fMRI brainstem responses [296,300-302]. However, this claim can be refuted on the basis that primary capsaicin inhalation activations for both contingencies (CN and CP) were observed in the present study. The activations were consistent with expectations of neuroanatomy observed in previous capsaicin inhalation brainstem studies [49,99].

Despite additional steps taken to optimise registration of the brainstem in order to improve the outcome of data analysis, it still proved difficult to identify activations in several regions with high precision. Therefore, it is noteworthy that some degree of caution should be taken in the interpretation of regional brainstem activations in the present study as a result of the interaction between image distortion and precision of regional anatomy. Future imaging studies with better image optimization of the brainstem to minimize image distortion during registration may be beneficial in gaining a better understanding of the neural modulation of UTC and cough in humans.

In light of the outcomes of the present study, it is imperative to endeavour in further investigations of brainstem regions in a capsaicin-evoked UTC protocol to advance our knowledge of the mechanisms involve in cough modulation and suppression. This can be achieved by incorporating a protocol that explores regional brainstem responses based on the differences in the intensity of capsaicin concentrations. Future imaging studies may benefit from identifying regions showing altered activations associated with differences in regional brainstem activity as a result of inhaling high versus low concentrations of capsaicin. In human experiments related to pain paradigms, activations associated with functional connectivity based on fluctuating pain intensity manifested in changes in local brainstem regions typically involved in pain processing [303]. Another study also showed significant CPM related reductions in BOLD signal responses compared to non-CPM subjects [193]. However, whether these changes in neural activity related to pain modulation will also be observed for airway modulation is yet to be known. Nevertheless, combining this approach with the use of ultrahigh field strengths and improved optimisation of the brainstem regions during fMRI data registration will certainly aid in this investigation and provide precise and improved accuracy in the interpretations of BOLD signal responses. The results of such investigation may provide further evidence whether the modulation of airway sensory inputs manifest as changes in neural activity at the level of the brainstem. Future therapies targeting cough suppression mechanisms could benefit from the outcome of such findings.

Conclusion

Overall, the findings of this study show that pain has a strong influence on both UTC and cough. Substantial reductions in UTC and cough were matched with parallel decrease in regional higher brain responses as previously reported consistent with the DNIC-related modulation of inputs at the brainstem level. However, the evidence of a DNIC-related

modulation of airway inputs consistent with the DHSC-DRt-DHSC pathway was not observed in the brainstem. These outcomes suggest that limitations associated with fMRI of the brainstem are most likely to result in the absence of regional brainstem responses associated with the DNIC-related modulation of airway inputs. Therefore, the findings of the presented warranted further investigation of brainstem regions using different methods that may yield an appropriate analysis of modulation of neural activity involved in airway processing. With improved imaging protocols, further brainstem investigations may benefit from intensity-related modulations of airway inputs similar to those observed in pain studies. This approach has the potential to expand our understanding of the circuitry involved in the inhibitory modulation of UTC in humans. From a clinical perspective, such outcome may prove useful in developing novel therapies that target brainstem regions to reduced unwanted cough especially in sufferers of chronic pulmonary disease.

Chapter 4 - Regional brainstem responses of urge to cough based on the intensity of capsaicin concentration.

Preamble

.

The neural outcomes presented in Chapter 3 showed a lack of differences in the regional brainstem responses between the no pain and pain events. Interestingly, higher brain responses that were within the field of view showed significant reductions in neural activity during pain events compared to no pain events. This observation of higher regional brain responses was consistent with the findings in Chapter 2. The absence in regional brainstem responses between the two contingencies was unexpected and resulted in the lack of credible evidence of a DNIC related modulation of airway inputs. One conclusion was that absent regional brainstem responses between the two contingencies (CN>CP) observed in Chapter 3 were an accurate outcome leading to suggestions that DNIC modulation of airway inputs may not necessarily involve in brainstem and instead, DNIC in humans can only manifest as changes in neural activity in the hemispheres. Since DNIC disappears in animals with sectioned spinal cord including studies showing that DNIC is confined in the most caudal part of the medulla, it is most likely that the underlying DNIC mechanisms in humans should also involve inhibition of inputs at the brainstem level [103,140,143,144]. Alternatively, this outcome might be misleading suggesting that the absence of activations related to differences for CN>CP may be due to technical factors associated with the limits of fMRI of the brainstem. One possibility is that the BOLD signal measures may be unable to detect small behavioural differences in UTC (21% reduction) ratings between CN and CP at the brainstem level. In a previous study by Youssef et al (2016), painful conditioning led to a 29% reduction in the behavioural outcomes of pain ratings including significant reduction in BOLD signal intensity in several key brain regions involved in processing pain inputs such as the DRt [107]. Whether the small reduction in UTC ratings observed in Chapter 3 may have had an effect on the outcome of BOLD signal responses remains unknown. Alternatively, the sum total of all neural activity associated with the modulation of airway inputs in terms of BOLD signal intensity may be null and present as an absence of DNIC as a result of DNIC-related reductions in the activity of brainstem nuclei occur simultaneously with increases in local inhibitory inputs to the nuclei. This may suggest that the fidelity of BOLD signals to detect inhibition-related synaptic activity is rather ambiguous. This narrative is supported by numerous studies where it was shown that changes in neural activity do not correlate with variations in fMRI BOLD signal responses in brain regions such as the hippocampus [276,277,283,284,304]. Such findings may

also relate to the brainstem in that changes in DNIC-related neural activity of several brainstem nuclei does not manifest as changes in BOLD signal intensity as observed in the previous study (Chapter 3). The most likely hypothesis was therefore that the absence of DNIC-related modulation of airway inputs is likely to be as a result of null changes in the hemodynamic response and BOLD signal intensity due to the simultaneous DNIC-related reductions and increases in the local inhibitory inputs to the nuclei.

In order to gain further insight into any limitations, Chapter 4 aimed at gathering evidence that might help explain the absence of regional brainstem responses associated with differences between pain and no pain events that were observed in the previous brainstem study (Chapter 3). The present study involved data acquisition of regional brainstem responses by incorporating a different method that may yield an appropriate analysis of the modulation of airway processing in humans. The aim of the present study (Chapter 4) was to explore the neural modulation of the UTC based on the differences in the intensity of the UTC evoked by inhalations of high and low capsaicin concentrations. The concentrations of capsaicin were manipulated in order to determine whether the large and significant behavioural differences in UTC ratings between the two concentrations would be reflected in regional brainstem responses. Activations associated with differences between capsaicin inhalation of high versus low concentrations were expected to show up in the primary regions commonly involved in the relay of airway inputs i.e., the nTS and Pa5. It was expected that inhalations of high concentration of capsaicin would elicit significantly larger BOLD signal responses compared to inhalations of low concentrations of capsaicin. However, an absence in activations associated with differences in the intensity-related modulation of the UTC would likely indicate that variation in neural activity in the brainstem are not well represented in the form of BOLD signal responses under the current experimental conditions. This hypothesis is supported by several studies that showed negative correlation between varied neural activity and BOLD signal responses in certain brain regions such as the hippocampus [193,276,277,283,303]. Investigating fMRI BOLD signal outcomes related to regional brainstem responses associated with intensity-related changes may provide acknowledgment that variations in neural activity in brainstem nuclei is not positively correlated to changes in BOLD signal responses.

Introduction

The findings of the first study concluded that the DNIC-related modulation of the UTC is likely as a result of endogenous inhibition of neural inputs at the brainstem level due to widespread reduction in neural activity within the UTC network in the higher cortex of the brain. This endogenous inhibition of sensory inputs most likely arose from nuclei embedded in the brainstem since this is the site that receives airway afferent inputs from the respiratory tract before projecting information into higher brain circuits. This is consistent with the DHSC-DRT -DHSC pathway associated with the DNIC-related modulation of inputs at lower levels of the brainstem [104,120,142,303]. The second study (Chapter 3) further investigated the effects of painful conditioning on the UTC with optimized imaging of the brainstem. However, the findings showed an absence in the regional brainstem responses between the no pain and pain events. inconsistent with the well-established brainstem mechanisms associated with the DNIC phenomenon in animal and human studies [103,107,142]. Therefore, it was concluded that the result was likely due to the limitations of fMRI of the brainstem and the protocol in the previous study (Chapter 3) including various challenges associated with optimized imaging of the brainstem. This led to two possibilities of what might explain the absence in activations between the two contingencies (CN>CP). One possibility could be that the BOLD signal measures were unable to detect physiological changes in the neural activity of brainstem nuclei. Another possibility was that the absence of activations for CN>CP was most likely as a result of the BOLD signal intensity being unchanged due to both conditioning-related reductions in neural activity in conjunction with increases in the local inhibitory influences at the same time. This hypothesis is supported in pain studies where brainstem regions showed no changes in absolute levels of activity but rather that these changes are associated with changes in brainstem coupling [303]. It is possible that detecting BOLD signal changes associated with intensity coding for “inhibition related” synaptic activity in the brainstem via fMRI is ambiguous as suggested by recent studies [193]. This narrative is consistent with reports in several studies that showed a weak and negative correlation between variations in electrophysiological neural activity and BOLD signal changes within local nuclei of the hippocampus region of the brain [276,277,283]. This finding may also be true for the brainstem in that changes in neural activity within brainstem nuclei does not manifest into meaningful changes in fMRI BOLD signal responses.

Despite the outcomes of Chapter 3, the present study (Chapter 4) aimed at exploring the regional brainstem responses of UTC by manipulating the intensity of the capsaicin concentrations (High versus low) inhaled by participants. This would lead to large and significant behavioural differences in the intensity of the UTC ratings between the two concentrations. The aim of the present study was to determine whether the substantial differences in the behavioural responses will also be reflected in regional brainstem responses. There is some evidence in one study that showed significant changes in BOLD signal intensity associated with the gradual changes in the frequency of the stimulus (2Hz, 10Hz, 25Hz, 100Hz) in several pain-processing brainstem regions [305]. The painful stimulus used during the study was aimed to be a moderately strong but not painful sensation. This moderate painful stimulus was constant during respiratory gated auricular vagal afferent nerve stimulation (RAVANS) across the different frequencies [305]. Results showed that the highest frequency (100Hz) was associated with the highest fMRI responses and activation clusters in several brainstem regions such as the locus coeruleus (LC), dorsal and median raphe nuclei (DR, MR), PAG, and the nTS [305]. Notably, the painful stimulus was constant while the frequencies were varied during the study suggesting that the outcomes may not necessarily be described as intensity related changes in the brainstem. Furthermore, a recent brainstem study has shown intensity-related changes associated with inter-subject variance for capsaicin inhalation activations that was explained by the relative UTC ratings [99]. However, these regional brainstem responses were observed at higher levels of the brainstem where the PAG nucleus is located rather than lower levels of the brainstem where primary airway nuclei are typically located [99]. If regional brainstem responses in the present study manifest as a result of the differences in the intensity of the UTC (High versus low), then it could be hypothesized that the neural outcomes of the previous study (Chapter 3) may be due to BOLD signal measure's inability to detect the small behavioural reductions in UTC ratings (21%) compared to the large behavioural differences in UTC between the two concentrations. However, an absence of regional brainstem responses will support the alternative hypothesis that intensity coding in the form of changes in local synaptic activity may not necessarily lead to changes in fMRI BOLD signal responses at lower levels of the brainstem as suggested in several studies [193,277,303]. This might also explain the lack of regional brainstem responses for CN>CP observed in the previous study (Chapter 3) compared to the profound changes in BOLD signal responses for CN>CP in the hemispheres.

Methods

Participant recruitment and experimental protocol

Fifteen healthy individuals were recruited to participate in the study at the Murdoch Children's Research Institute (Melbourne, Australia) (6 males; mean age ±SD 24.20 ± 4.80). The exclusion criteria included any history of smoking, chronic respiratory disease or infections, claustrophobia, pregnancy and the presence of any neurological abnormalities in the brain. Written consent was obtained from all participants. All aspects of the study were approved by the Melbourne Health Human Research Ethics Committee (2011.100).

Capsaicin Sensitivity Testing

Participants underwent a series of capsaicin challenges in the form of inhaled vapour via pressure-driven nebulizers. Capsaicin concentrations were prepared in doubling concentrations (0.12 µM to 125 µM). Participants were asked to rate their UTC on a modified Borg scale (0 – 10) after each single vital capacity inhalation to determine their individual sensitivity. The capsaicin concentrations that elicits a low (2 -3) and high (8-10) UTC rating on the Borg scale were recorded. The two capsaicin concentrations were inhaled by participants in a random order during fMRI trials via a MRI compatible pressure-driven nebulizers.

fMRI session

During fMRI data acquisition, participants lay on the scanner bed with their head immobilized with foam padding. Participants were fitted with hearing protection as well as a facemask connected to a nebulising apparatus via which either capsaicin (high or low) or saline were delivered. The addition of a line fixed to the nebulisers and connected to a syringe allowed for the delivering of capsaicin concentrations or saline during scanning. Each participant underwent a series of trials where they inhaled test stimuli in the form of either nebulised saline (Sal), high (Hi) or low (Lo) concentrations of capsaicin in a pseudo-randomised order (Figure 14). This meant that no subsequent trial involved the same test stimulus from the previous trial. Each test stimulus was inhaled for a total duration of 15 seconds and administered on three occasions during each run of fMRI acquisition. Participants were asked to indicate their UTC ratings 6 seconds after the conclusion of

each trial using the fingers of their right hand (scale 0-10). The duration for participants to rate their UTC lasted for 4 seconds followed by a resting period of 15 seconds before the next trial of test stimulus. Each participant completed two scanning runs that lasted a total of 6 minutes and 90 seconds during the fMRI session. Instructions were given via a computer screen before the commencement of each trail to prepare participants for inhalations and before they rate their UTC.

Image acquisition parameters

Images of the brain were acquired using a Siemens Trio 3T scanner (Siemens, Erlangen, Germany) at the Murdoch Children's Research Institute (Melbourne, Australia). T1-weighted images in a sagittal plane were obtained for registration purposes (TR 1900 ms, TE 2.59 ms, 192 slices with 0.9mm thickness, 0.9 × 0.9 mm^2 in plane resolution). Functional BOLD images were obtained in trans-axial plane (TR 2000 ms, TE 32 ms, 4.5 mm slice thickness, 1.88 × 1.88 mm^2 in plane resolution, flip angle 90°) producing a total of 216 sequential volumes in 6 minutes and 9 seconds. A total of two functional runs optimized for the brainstem were obtained. Additional phase and magnitude images of the B0 field were acquired to create fieldmaps.

Analysis (Behavioural & fMRI)

Statistical analysis of UTC ratings was performed using SPSS version 25.0 (IBM, Armok, NY, USA). Paired t-test were used to determine whether the behavioural differences between the average UTC ratings of high concentrations of capsaicin versus low concentrations was significant during the fMRI session.

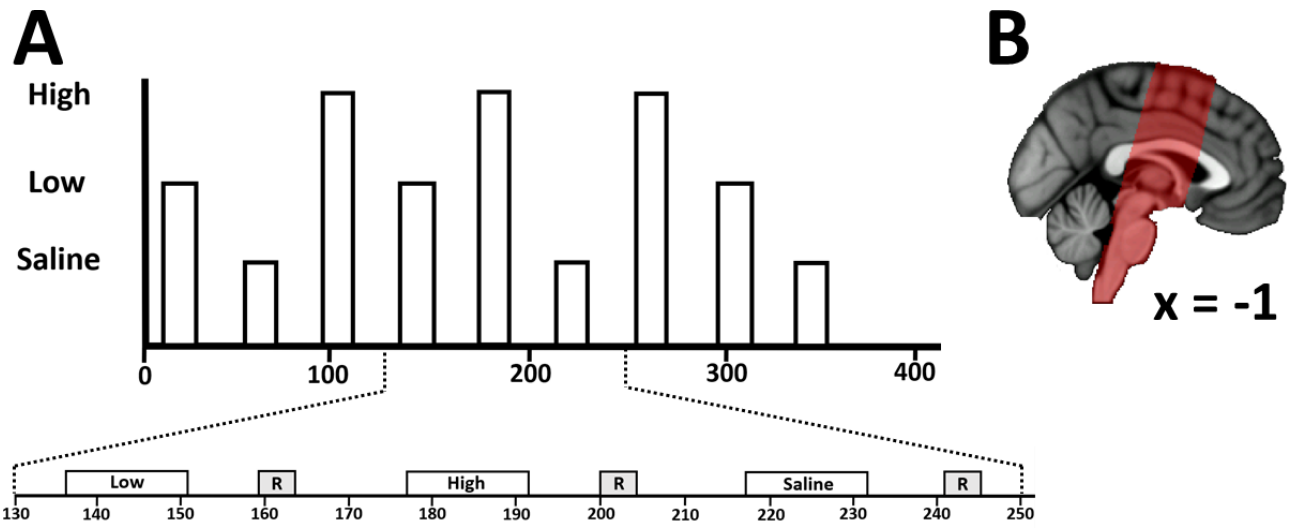
fMRI analysis was performed using FEAT version 6.0 based on the FMRIB software [269]. Pre-processing of functional EPI data involved several steps with the first step using BET to remove non-brain tissue. The outcome of BET was manually assessed for quality with necessary adjustments made if required. This approach allowed for extending the BET outcome caudally to include the full extent of the brainstem distal to the obex which is not invariably retained by the automated processing. FUGUE was then employed to correct geometric distortion of functional data and mean EPI images. This process involved the production of two fieldmaps using the additional magnitude and phase images obtained from the B0 field during fMRI scanning. A brainstem fieldmap was created to enable unwarping of functional 4D data while whole brain fieldmap was created for unwarping of the mean EPI image. Furthermore, realignment of the EPI images to correct motion using

rigid body transformation (MCFLIRT) was done. This step was followed by “unwarping” the motion corrected data using the brainstem fieldmap produced earlier. Functional data then underwent spatial smoothing with a 4mm full-width half maximum Gaussian kernel and high pass filtered with a cut-off of 0.5Hz.

The brainstem EPI images were transformed to the standard MNI152 template brain (1mm) using FLIRT in a multistage registration approach. Firstly, each individual’s motion corrected mean functional EPI data was linearly registered to the whole brain mean EPI. The resultant mean EPI was then registered to the individual’s high resolution T1 volume. The outcome of this transformation was then registered the standard MNI152 template brain (1mm resolution). Additional registration was undertaken to aid in optimized registration of the brainstem where the high resolution T1 image was registered to the standard MNI152 template with weighting of the brainstem as previously described (Chapter 3) [99,267].

Regressors were constructed for the inhalation of each test stimuli (Sal, Hi and Lo) including the rating and resting periods. The first level of analysis involved these regressors being convolved with a hemodynamic response function in order to generate a model for each run. Additional regressors for events of no interest such as motion correction parameters, time-courses for CSF within the ventricles, the sagittal sinus and the mean global signal from non-activated areas of the brain were also included in the general linear model. This allowed for any residual respiratory noise to be accounted for within the model of each run [261,268]. Contrasts for each of the main events (Sal, Hi and Lo) including contrasts between the differences of the main events such as high capsaicin concentration inhalation greater than saline (Hi>Sal), low concentration inhalation of capsaicin greater than saline (Lo>Sal), high concentration of capsaicin greater than low concentration of capsaicin inhalation (Hi>Lo), low concentration of capsaicin greater than high concentration of capsaicin inhalation (Lo>Hi) were obtained in the first level analysis. Fixed effect analysis was used in the second level analysis to amalgamate results from the two runs of each subject. Group level analysis involved transformation of each individual’s T1 weighted images to the standard MNI152 template. The group results incorporated mixed effect analysis using FLAME across all subjects. All group analysis covering the entire FOV was determined using a single voxel inclusion of $Z>2.3$ and a cluster probability of $p<0.05$ [269].

Figure 14. fMRI imaging protocol during scanning.



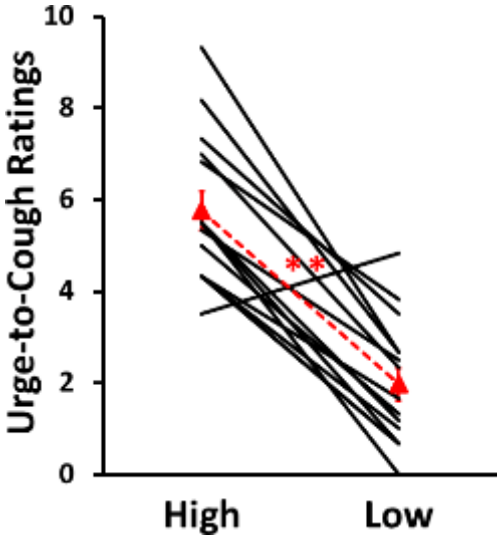
(A) *Participants inhaled test stimulus of either saline, high or low concentrations of capsaicin via a nebulizer for a duration 15 seconds during stimulus trials for each run of fMRI brain image acquisition lasting 6 minutes and 90 seconds. Each test stimuli were administered on three occasions during each fMRI scanning run. All participants completed two scanning runs. The expanded panel shows one of the two cycles of stimuli delivered during an fMRI scan. At the conclusion of each trial, participants were asked to indicate their UTC ratings (scale 0-10) for the preceding test stimulus using the fingers of the right hand. The duration for rating their UTC was for 4 seconds followed by a resting period of 15 seconds before the next inhalation challenge.* ***(B)*** *Sagittal cross section showing the field of view (in red) optimized for brainstem imaging (overlaid on a standard MNI template) during the fMRI scanning runs.*

Results

Psychophysical results

The geometric means of high and low concentrations of capsaicin were 1.66µM and 0.39µM respectively during the fMRI scanning runs. The average UTC ratings for saline, low and high capsaicin concentrations were 0.9, 2 and 5.7 respectively during fMRI image acquisition. Paired t-tests showed a significant decrease in UTC ratings from high to low concentrations of capsaicin ($F(1,14)=8.13$, $p<0.001$) (Figure 15). On average, there was 66% reduction in UTC ratings from high to low concentrations of capsaicin.

Figure 15. Behavioural results between high and low capsaicin concentrations.



Ratings of UTC after capsaicin challenges for both the high and low concentrations of capsaicin were averaged across the two cycles of fMRI scans for each participant. The graph shows individual UTC ratings for both concentrations of capsaicin. The average reduction in UTC ratings from high to low concentrations of capsaicin was significant and is represented by the triangles and broken line in red color.

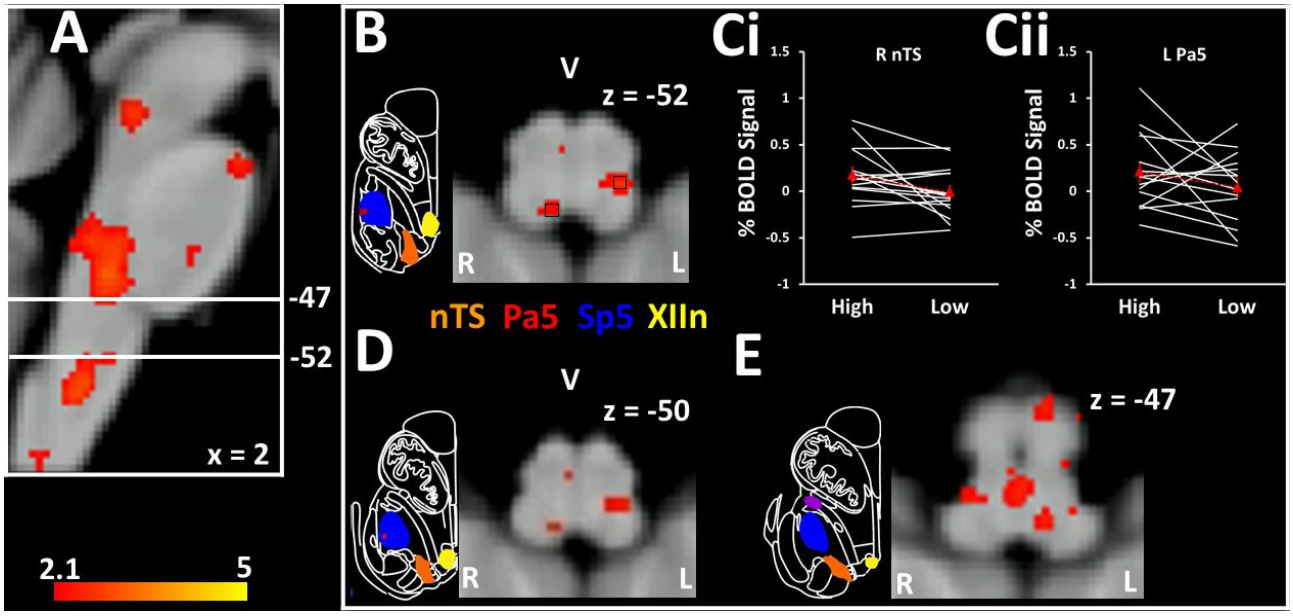
*Error bars represent standard errors of the mean. **p<0.001*

fMRI results

Regional brainstem activations during high and low capsaicin concentrations

Hi>Sal outcomes were associated with widespread activations at almost all levels of the brainstem (Figure 16 and Table 6). At the level of the obex (z = -58), Hi>Sal related activations were distributed in the nucleus of solitary tract (nTS), paratrigeminal nucleus (Pa5), spinal trigeminal (Sp5) and the hypoglossal nucleus (XIIN). There were no regional brainstem activations for Lo>Sal at the level of obex. Above the level of the obex (z = >-58), medullary activations in response to both Hi>Sal and Lo>Sal were distributed bilaterally similarly (between z = -50 & z = -12) (Figure 17 and Table 6). These regions include the bilateral nucleus abducens (Abd), left mesencephalic trigeminal tract and nucleus (Mes5), bilateral trigeminothalmic tract (5thal), bilateral midline raphe (Rp), bilateral longitudinal fasiculus (LF), bilateral facial motor nucleus (VIIn). Regional higher brain responses for Hi>Lo were observed in regions as those shown in the previous study (Chapter 2) (Figure 18 & 19 and Table 8). There were no regions that showed increased responses throughout the brainstem as a result of the differences between inhalation of high concentrations of capsaicin events compared to low concentrations of capsaicin events (Hi > Lo).

Figure 16. Regional brainstem activations during capsaicin inhalation challenges.



(A) *Sagittal view of the brainstem showing regions activated during inhalation of high concentration of capsaicin greater than saline (Hi>Sal) are rendered in red-yellow. Axial slices rendered in red-yellow showing activations of the nTS and Pa5 including other nuclei such as the Sp5 and XIIn* ***(B, D, E)*** *during inhalation of high concentrations of capsaicin. Line graphs showing the mean percentage BOLD signal change within boxed regions of the nTS* ***(Ci)*** *and Pa5* ***(Cii)*** *for all participants during high compared to low inhalations of capsaicin concentration. Similar regional brainstem activations were observed for the low inhalations of capsaicin concentration.*

Activations are rendered in the Montreal Neuroscience Institute template of the brain (1mm resolution).

Abbreviations: nTS – nucleus of the solitary tract and solitary tract, Pa5 – paratrigeminal nucleus, Sp5 – spinal trigeminal tract and nucleus, XIIn – hypoglossal motor nucleus.

Table 6. Regional brainstem activations during inhalation of high concentration of capsaicin.

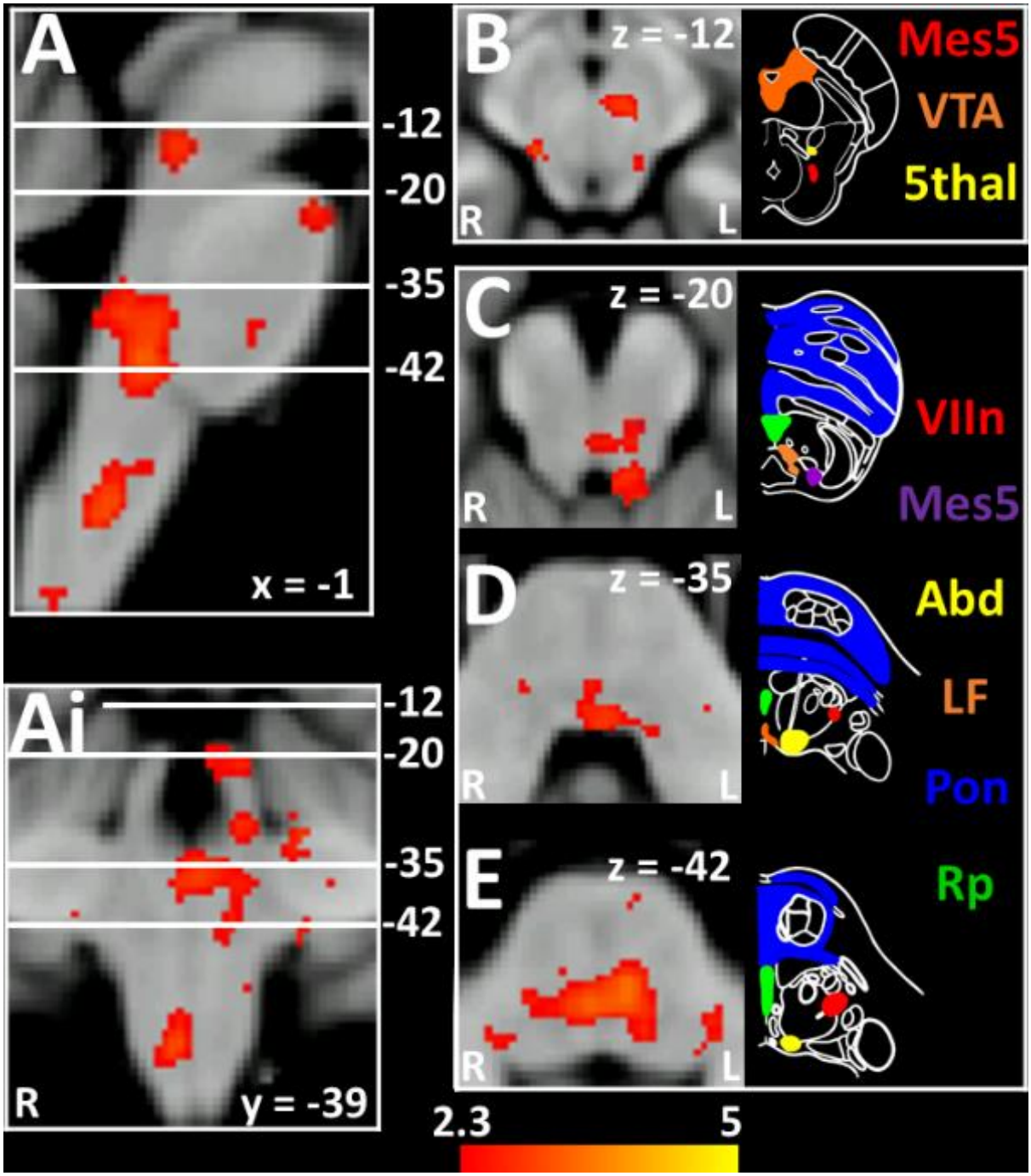
	Peak voxel coordinates*				
Region	*x*	*y*	*z*	*Z-score*	Possible structures
Medulla	3	-40	-58	3.07	DRt, Sp5, Pa5
	5	-40	-57	2.74	nTs, Sp5
	2	-40	-56	3.60	nTS, XIIn, SP5
	-2	-43	-54	2.93	nTS, XIIn
	2	-36	-45	2.83	Rp
Pons	0	-37	-42	4.27	Rp
	-1	-36	-35	2.94	Rp, LF
	-6	-33	-18	3.00	Mes5, LF
	-5	-18	-14	3.26	VTA
	-7	-24	-14	2.91	Mes5, 5thal

Definition of abbreviation: BA = Brodmann area

**The coordinates correspond to the Montreal Neuroscience Institute standard brain template where x values are distance in millimetres to the left (negative x values) or right (positive x values) from the anterior commissure; y represents millimetre distance anterior (positive) or posterior (negative) from the anterior commissure, and z is millimetre distance superior (positive) or inferior (negative) from the anterior commissure.*

Abbreviations: Abd – nucleus abducens, DRt – dorsal reticular nucleus, nTS – nucleus of the solitary tract and solitary tract, LF – longitudinal fasiculus (medial & dorsal parts), Mes5 – mesencephalic trigeminal tract and nucleus, Pa5 – paratrigeminal nucleus, Pon – pontine nuclei, Rp – midline raphe, Sp5 – spinal trigeminal tract and nucleus, VIIn – facial motor nucleus, XIIn – hypoglossal motor nucleus, 5thal – trigeminothalmic tract.

Figure 17. Brainstem regional response for Hi>Sal.



Regional brain activations as a result of inhalations for the high concentrations of capsaicin were widespread across the remainder of the brainstem. These regions likely include the Mes5, VTA, Rp, VIIn, Abd, LF and the 5thal and are rendered in red-yellow. Similar activations were observed for low concentrations of capsaicin.

Abbreviations: Abd – nucleus abducens, DRt – dorsal reticular nucleus, nTS – nucleus of the solitary tract and solitary tract, LF – longitudinal fasiculus (medial & dorsal parts), Mes5 – mesencephalic trigeminal tract and nucleus, Pa5 – paratrigeminal nucleus, Pon –

pontine nuclei, Rp – midline raphe, Sp5 – spinal trigeminal tract and nucleus, VIIn – facial motor nucleus, XIIn – hypoglossal motor nucleus, 5thal – trigeminothalmic tract.

Figure 18. Regional higher brain responses for Hi>Sal and Hi>Lo.

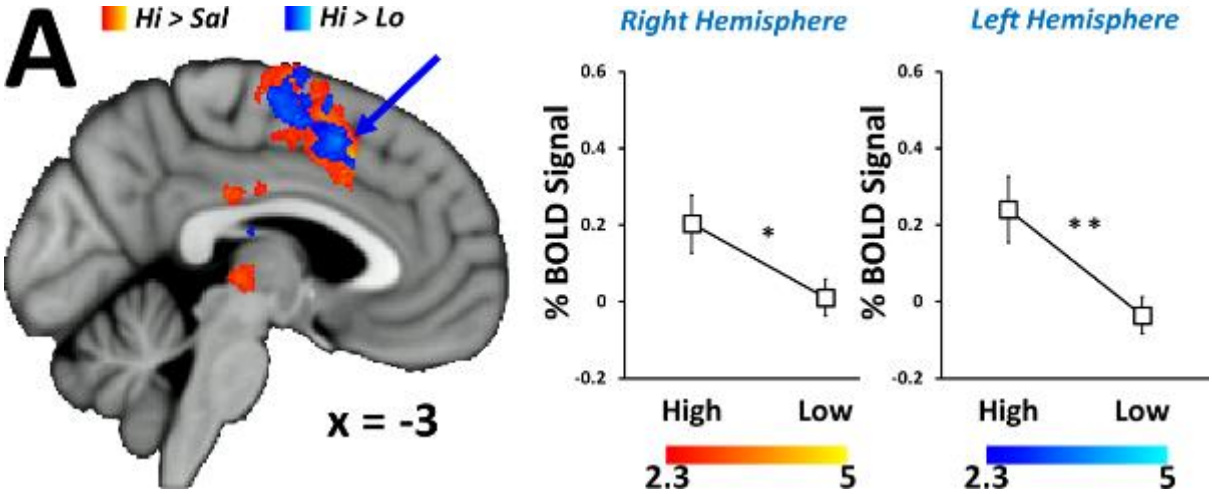
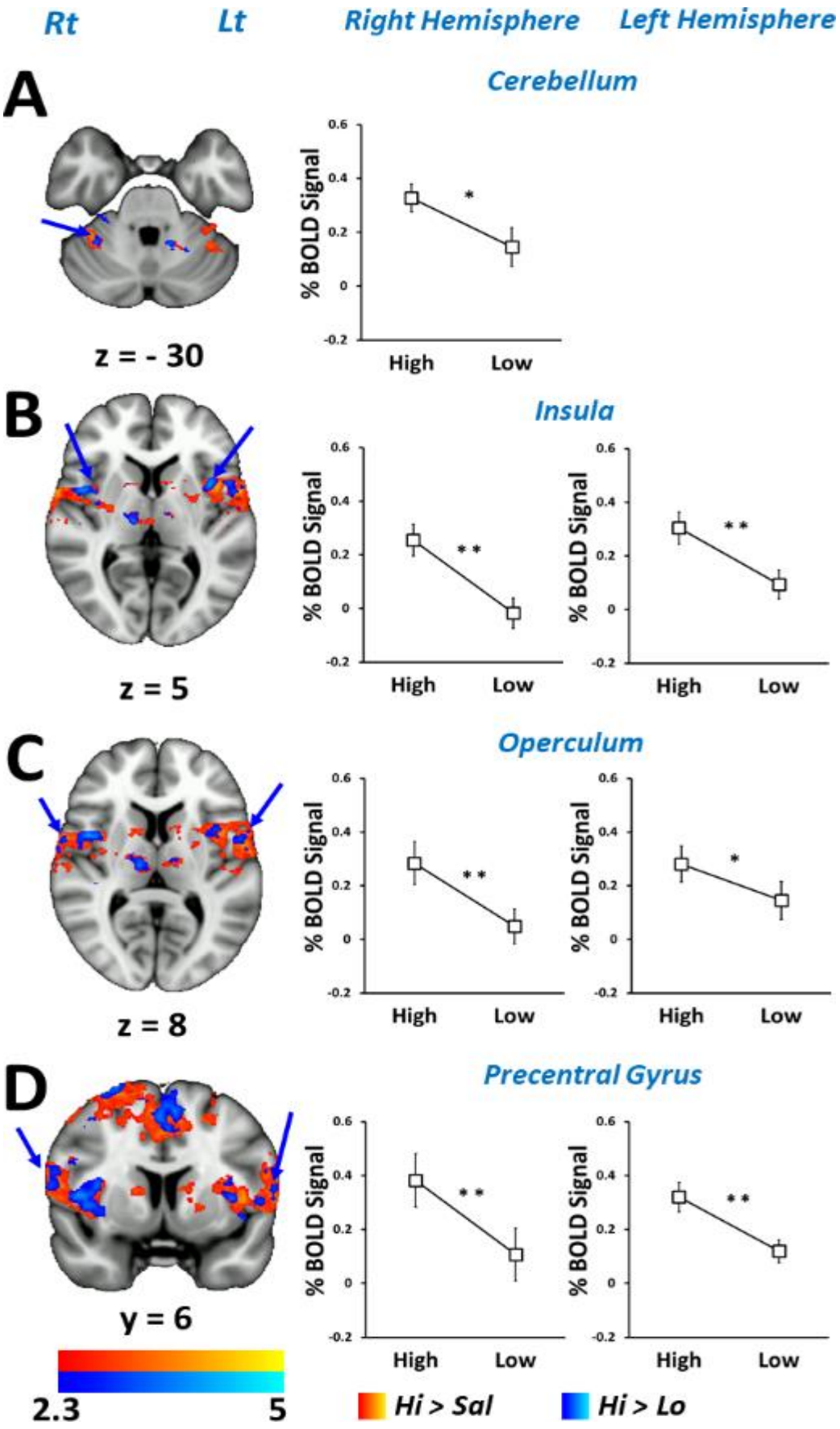


Figure 19. Regional higher brain responses for Hi>Sal and Hi>Lo.



Regional higher brain activations for Hi>Sal and Hi>Lo. Regions where responses for high concentration of capsaicin inhalation were greater than saline (Hi>Sal) are rendered in red-yellow. The blue regions represent regions where high concentration of capsaicin inhalation were greater than the low concentration of capsaicin (Hi>Lo). The regions showing greater activation during Hi>Lo include the (A) bilateral paracingulate gyrus (Fig 23). Other regions include (A) right cerebellum, (B) bilateral insula cortex, (C) bilateral operculum and (D) and bilateral precentral gyrus (Fig 24).

Table 7. Regional higher brain activations during high capsaicin concentrations greater than saline inhalations (Hi>Sal).

		Peak coordinates*			
Region	BA	x	y	z	z-score
Precentral	3	61	-13	31	3.50
	3	-59	-5	31	3.47
Paracingulate	6	-1	12	48	4.16
	6	7	9	48	3.30
Supplementary motor area	6	-1	10	55	3.61
	6	-4	-3	55	3.35
Postcentral	2	58	-20	47	3.16
	3	-47	-18	40	3.15
Insula	13	36	4	14	3.11
	13	-31	6	14	3.17
Central Operculum	44	36	7	11	3.23
	44	-35	11	11	3.47
Cerebellum		38	-46	-34	3.44
		-36	-56	-31	3.79

Definition of abbreviation: BA = Brodmann area

**The coordinates correspond to the Montreal Neuroscience Institute standard brain template where x values are distance in millimetres to the left (negative x values) or right (positive x values) from the anterior commissure; y represents millimetre distance anterior (positive) or posterior (negative) from the anterior commissure, and z is millimetre distance superior (positive) or inferior (negative) from the anterior commissure.*

Table 8. Regional higher brain activations during high capsaicin concentrations greater than low concentrations of capsaicin (Hi>Lo).

		Peak coordinates*			
Region	BA	x	y	z	z-score
Precentral	44	58	10	24	3.19
	44	-60	-4	27	3.54
Paracingulate	6	-3	9	43	3.10
	6	5	16	43	3.20
Supplementary motor area	6	3	8	52	3.30
	6	-1	9	52	3.74
Postcentral	3	62	-19	20	3.79
	3	-59	-15	22	2.97
Insula	13	36	-5	8	3.24
	13	-33	9	8	2.93
Central Operculum	44	36	9	11	3.11
	44	-31	13	11	3.71
Cerebellum		-38	-52	-32	3.00
		38	-48	-32	3.1

Definition of abbreviation: BA = Brodmann area

**The coordinates correspond to the Montreal Neuroscience Institute standard brain template where x values are distance in millimetres to the left (negative x values) or right (positive x values) from the anterior commissure; y represents millimetre distance anterior (positive) or posterior (negative) from the anterior commissure, and z is millimetre distance superior (positive) or inferior (negative) from the anterior commissure.*

Table 9. fMRI power calculations showing sample and effect size required to elicit difference for selective regions of interest.

Region	*Sample Size*	*Effect Size*
Right nTS	26	0.21
Left Pa5	75	0.20
Left Op	36	0.47
Right Op	14	0.47
Left Insula	10	0.60
Right Insula	12	1.00
Left PreCG	13	1.07
Right PreCG	13	0.70
Left ParCG	10	0.71
Right ParCG	15	0.80
Left Cerebellum	51	0.48
Right Cerebellum	17	0.70

Power calculations indicating the sample and effect (Cohen's d) size required to elicit a difference in regional brain responses for high versus low concentrations of capsaicin (0.2 = small effect size, 0.5 = medium effect size; 0.8 and greater = large effect size).

Discussion

The present study investigated regional brainstem responses associated with the differences in the intensity of the UTC stimulus (Hi>Lo) in the form of capsaicin concentrations in a group of healthy individuals. Acquired brain images were optimized for the brainstem including the use additional technical parameters to improve analyses of regional brainstem responses. The resultant analysis has identified primary capsaicin (Hi, Lo) and saline (Sal) inhalation-related activations in regions throughout the midbrain, pons and the medulla of the brainstem. Notably, low inhalations of capsaicin did not show primary activations at the level of the obex. No regional brainstem responses were observed for the differences between the two capsaicin concentrations (Hi>Lo). Additionally, the study also identified primary regional responses in the higher cortex of the brain that were within the FOV of the fMRI scanning protocol. Interestingly, regional

responses for Hi>Lo were widely spread amongst the capsaicin inhalation network in the hemispheres.

Regional brainstem & higher brain responses

Primary regional brainstem and higher brain activations for both the Hi and Lo capsaicin inhalations including Hi>Sal were observed in regions known to be involved in the neural encoding of afferent sensory airway information. Brainstem regions included nuclei such as the nTS, Pa5 and the Sp5 including several other nuclei of the pons and the midbrain with the exception for Lo inhalations of capsaicin and Lo>Sal where no activations at the level of the obex and regions below this level were observed. These regions were consistent with previous reports of brainstem activations related to airway processing in humans and animal studies [38,42,99]. Activations observed in regions of the higher brain limited to the FOV were also consistent with those reported in Chapter 2 and previous findings [76-78,82,83]. Regional responses in the higher brain as a result of the difference between Hi and Lo (Hi>Lo) was widespread amongst the capsaicin inhalation network. This change in neural activity in capsaicin related activations was consistent with the significant reductions in BOLD signal intensity for Lo inhalations compared to Hi. The identified regions were consistent with the regions showing activations associated with conditioning-related changes responses in the previous findings (Chapter 2 and 3) and suggest that the neural modulation of airway inputs related to UTC observed in the higher brain as a result of differences between Hi and Lo was most likely to occur at lower levels of the brain [38,260].

Brainstem fMRI analysis showed no regional brainstem responses as a result of the differences between Hi and Lo capsaicin inhalations (Hi>Lo). Such findings were unexpected giving the significant differences in the intensity of the UTC ratings between the two capsaicin concentrations. It was expected that intensity related-differences between Hi and Lo (Hi>Lo) would yield regional brainstem responses especially in nuclei such as the nTS and Pa5 giving that these are apparent sites known to encode and relay sensory inputs during mechanical irritation of the airways. Surprisingly, the lack in activations for Hi>Lo amongst the nTS and Pa5 nuclei were also consistent with the absence of regional brainstem responses observed in our previous study involving painful conditioning (Chapter 3). In the case of the first pain study (Chapter 2), it was concluded that the DNIC-related modulation of airway inputs as a result of painful conditioning was most likely to occur at the level of the brainstem which is consistent with the DNIC

mechanism [101-103,137,146,306,307]. This led to further investigation of the brainstem region in the next study to provide evidence of a DNIC-related modulation of airway inputs (Chapter 3) that may explain the neural outcomes observed in the first study (Chapter 2) [104,120,142]. However, the findings of the study showed no regional brainstem responses (Chapter 3) associated with the DNIC-related modulation of airway inputs. Similarly, the present study (Chapter 4) also showed an absence in regional brainstem responses associated with the intensity-related modulation of airway inputs.

The absence of regional brainstem responses in the present study could be related to several factors. As previously mentioned, technical challenges and limitations related to fMRI of the brainstem may be one explanation for the outcomes observed in the present study. The small sizes of brainstem nuclei including the relatively small voxel sizes used in fMRI may lead to spatial constraints [296]. Geometric distortion associated with the right to left phase coding may lead to unsymmetrical distribution of activation patterns from the midline [297]. Physiological noise related to cardiac and pulmonary activities including brainstem motion may also lead to significant geometric distortions which can influence the sensitivity of brainstem fMRI [298,300-302]. These technical limitations may lead to signal loss and thus, diminishing the ability for fMRI to detect small changes in regional brainstem responses despite the large differences in UTC ratings between Hi and Lo [296,297]. A similar outcome was thought to be the case in the previous brainstem study related to painful conditioning (Chapter 3). However, this claim can be refuted based on the fact that primary activations were observed in several brainstem regions for the main events ie, Hi, Lo in the present study and activations for CN and CP in the previous study (Chapter 3). This makes it highly unlikely that technical factors such as brainstem nuclei size, geometric distortion and spatial constraints contribute to the absence of regional brainstem responses in both the present and previous study (Chapter 3).

In addition, fMRI power analysis of BOLD signal intensity from the right nTS and left Pa5 showed that the effect size (right nTS = 0.21; left Pa5=0.20; Cohen's D effect size analysis) required to show conditioning related differences statistically were very small to non-existent (Table 9). On the contrary, fMRI power analysis of selective higher brain regions within the field of view showed moderate and larger effect sizes required to show conditioning-related activations (Table 9). This suggests that brainstem fMRI may not necessarily represent changes in neural activity in the form of variations in BOLD signal

responses associated with intensity-related changes under the current experimental conditions.

Another explanation for the lack of regional brainstem responses observed in the previous and present study may due to the fact that BOLD signal measures may not be sensitive enough to detect physiological change in brainstem nuclei. The differences in UTC ratings between Hi and Lo capsaicin concentrations were considerably large (66%) compared to DNIC-related reductions in the UTC ratings in the previous study (21%) (Chapter 3). Despite this large behavioural difference in the UTC ratings between the Hi and Lo, the behavioural outcomes did not manifest into regional brainstem responses. This suggests that BOLD signal measures are not dependent on the size of the of behavioural difference and that differences in the intensity of airway inputs may not necessarily result in neural outcomes at the brainstem level. Therefore, the alternative hypothesis that was earlier presumed to result in the absence of regional brainstem responses may likely be the most logical and accurate explanation for the neural outcomes observed in the previous (Chapter 3) and present brainstem studies. It was hypothesized that the fidelity of BOLD signals measures to detect inhibition-related synaptic activity in local brainstem nuclei is ambiguous during BOLD fMRI data acquisition. In other words, the sum total of all neural activity associated with the modulation of airway inputs in terms of BOLD signal may be null and present as an absence of differences between two contingencies (Hi>Lo and CN>CP) as a result of related reductions in activity in brainstem nuclei occur at the same time as increase in local inhibitory inputs of airway information to the nuclei. This narrative is supported by recent studies where it was shown that changes in neural activity in the hippocampus is not positively correlated to changes in BOLD signal responses in this region [277,284,304,308]. It was suggested that the neural activity derived by BOLD signal responses in the hippocampus may be representative a combination of constant presynaptic and post-synaptic activity in nuclei leading which could also be the case for the brainstem region [278,309,310]. This hypothesis is also supported in a recent study where no change in the absolute levels of activity in brainstem regions was observed during pain intensity fluctuations suggesting spontaneous changes in pain are rather associated with changes in coupling of brainstem regions and not overall activity levels [303]. Similar findings were observed in another recent study which led to further calls into question of what exactly fMRI signal intensity is measurement. The study refuted the prevailing thought that BOLD fMRI is a measure primarily of post-synaptic transmission due to the absence of regional brainstem responses associated with changes in the intensity of noxious stimuli

[193,311]. The findings from these studies combined with brainstem outcomes from the present (Chapter 4) and previous study (Chapter 3) indicate that BOLD fMRI may not be the adequate tool to assess or show intensity-related differences for inhibition-related synaptic activity. However, further investigations of regional brainstem responses using different protocols or methods compared to those used in the present studies is required before making such conclusions.

Nevertheless, the present study (Chapter 4) and previous pain studies (Chapter 2 and 3) used a 3T MRI scanner and therefore, it remains unknown whether an ultra-high field strength MRI scanner such as a 7T may yield a different outcome due several differences in the technical aspects between the two scanners. For instance, the typical in-plane fMRI spatial resolution is 2 to 4mm at 3T compared to the spatial resolution in the order of 1mm isotropic for 7T [312-322]. The effectiveness in the superiority in spatial resolution for 7T has been shown in several pain studies where activations in several pain-processing brainstem regions was only found in 7T compared to 3T for painful vs innocuous stimulation [312,323,324]. Therefore, future brainstem imaging studies investigating the modulation of UTC may benefit from the use of such ultra-high field MRI scanners as they provide images with higher resolution, decreased relative noise level and improved sensitivity of fMRI responses as described in several studies [319,325,326].

Despite the outcomes of the present study, further investigation of regional brainstem responses associated with the neural modulation of UTC in humans is needed. Till now, little is known regarding which nuclei may be involved in this neural modulation of airway inputs at the brainstem level before it is relayed into higher brain circuits. With improved optimization of brainstem regions, the incorporation of suitable experimental protocols and the use of ultra-high field strength MRI scanners will help aid in providing better analysis and precise interpretations of this regional responses of this delicate region. The results will advance our understanding of the neural regulation of UTC in humans which can lead to therapeutic implications in the near future.

Conclusion

Collectively, the outcomes of the present study suggest that differences in the intensity of airway inputs has neural implications in humans. Inhalations of low concentrations of capsaicin was matched with decreases in neural activity when compared to high capsaicin inhalations in the higher brain regions within the UTC network. Such findings were consistent with previous reports related to regions associated with the modulation of the UTC via painful conditioning at higher levels of the brain as observed in the previous studies (Chapter 2 and 3). However, no differences in regional brainstem responses were observed between the two capsaicin concentrations (Hi>Lo) consistent with the absence of activations for CN>CP observed in the previous pain study (Chapter 3). It was assumed that brainstem fMRI is limited in its ability to detect BOLD signal response associated with intensity-related changes in neural activity. Therefore, the findings of the present study suggest that a different approach such as the use ultra-high field MRI scanners may benefit from investigating differences in regional brainstem responses associated with the changes in the intensity of airway inputs. An outcome of such study may lead to gaining a better understanding of the brainstem nuclei involved in the regulation and modulation of UTC in humans. This could lead to novel therapies being developed in order to address unwanted cough amongst individuals suffering from chronic pulmonary conditions worldwide.

Chapter 5- Discussion

Summary

The cough reflex acts a protective mechanism to prevent damage to the airways and lungs in response to irritant stimuli within the respiratory system [36,38,77,327]. The sensation of perceived airway irritation is known as the UTC [24,29,31-33,328]. This sensory experience may or may not result in a cough reflex [31,33]. Investigations regarding the modulation of the UTC have been limited to few studies (placebo) with no therapeutic outcome yet to be achieved [82,83]. Given that airway afferents are subject to placebo modulation, then it was deemed reasonable to test the proposition that other modulatory influences observed for nociceptive inputs may also be operable for airway inputs. There is preliminary evidence that DNIC does modulate the UTC and cough reflex in an experimental setting [84,100]. This modulation was in the form painful conditioning that lead to significant reductions in the UTC ratings and cough frequencies amongst participants [84,100]. However, the neural mechanisms involved in this conditioning related modulation remained unknown. Therefore, the present studies aimed at providing the first insight into the DNIC-related neural modulation of the UTC and cough. This investigation also provided evidence that pain-related modulation of airway irritation involves the inhibition of airway inputs most likely at the level of the brainstem. However, the studies of the brainstem presented in this thesis were unable to provide evidence of a DNIC-related modulation which encompasses a DHSC-DRt-DHSC pathway including regions involved in this inhibition of airway inputs; this suggests that BOLD fMRI may not be a suitable tool in showing intensity-related changes in neural activity of brainstem nuclei. Identifying these regions would have been beneficial in that they may act as potential targets for future development of therapeutic treatments that may aid in addressing chronic cough disorders.

Painful conditioning was associated with significant reductions in the behavioural outcomes for cough and the UTC. This decrease was associated with widespread reductions in the neural activity within the capsaicin-inhalation network which is normally involved in the processing of sensory airway irritation. These findings imply that pain can cause the inhibition of afferent airway inputs at the subcortical level of the brain leading to reduced perception of UTC in higher brain centres associated with coding airway information. However, neither painful conditioning, nor capsaicin intensity-related changes manifested in regional brainstem responses in the subsequent brainstem-related studies (Chapter 3 and 4).

Behavioural response of cough and the UTC

Significant decreases were observed behaviourally for both the UTC and cough as a result of painful conditioning (Chapter 2 and 3). Interestingly, there was a disproportionate effect of pain on the perception of the UTC compared to the motor act of cough. Cough frequency was reduced by 47% compared to 19% for UTC scores in the first study (Chapter 2). A similar outcome was observed in the second study (Chapter 3) where UTC scores were reduced by 19% compared to 42.5% for cough frequency. These findings suggest a differential effect of conditioning pain on the sensory experience versus the motor act of cough. This is also consistent with previous reports where ratings of UTC increase linearly with increased doubling concentrations of capsaicin while the increase in cough frequency was exponential [49,76]. These outcomes imply that the regulation of cough and the UTC in humans may not be tightly regulated giving the differential effect of DNIC on cough and the UTC. Such findings suggest the existence of a complex model involved in the processing of sensory input versus the motor output of airway irritation. Additionally, it was observed that the capsaicin concentrations required to elicit a cough reflex (C2) were higher than concentrations required to detect perceived sensation of an UTC (Cu). For instance, the average C2 concentration was 0.64 µM compared to the average Cu concertation of 0.13 µM. In pain studies, similar findings were observed whereby the RIII reflex (withdrawal reflex) is only activated at higher levels of pain compared to the sensory threshold for pain in humans (initial sensory experience of pain) [251]. Additionally, the present studies showed no significant differences in the behavioural outcomes of the UTC and cough as a result of painful conditioning between the two genders (male vs female). This is in line with several reports of no DNIC-related differences between the two genders [106,188,225-228]. Gender differences for the Cu and C2 thresholds were also not significant which is consistent with previous reports [32].

The DNIC-related behavioural outcomes observed in the present studies support the hypothesis that nociceptive pathways of somatic and pulmonary tissues functionally interact to induce meaningful changes in sensory evoked behaviour. The findings also reinforce the notion that painful conditioning was likely to exert an influence on the sensory processing of airway inputs. Importantly, conditioning pain resulted in differential DNIC-related effect on the sensory experience of the UTC versus the motor act of cough. This differential modulatory influence of DNIC on the UTC and cough observed in the present studies could be exploited without enlisting DNIC protocols to substantially reduce

persistent cough in an individual. Future therapeutic interventions could be designed to similarly engage in DNIC-related mechanisms without applying conditioning pain. From a clinical perspective, individuals suffering from chronic cough may benefit from such interventions to reduce their UTC and cough frequency without impairing the cough reflex.

Neural interaction between pain and the UTC

Painful conditioning was associated with widespread reductions in activity within the capsaicin-inhalation network in the higher brain regions (Chapter 2). This pattern of reduced neural activity pointed towards the descending inhibition of airway inputs at the brainstem level. These reductions in activations were consistent with reduced BOLD signal intensity for the capsaicin related changes during conditioning pain compared to no pain events. This is consistent with how the DNIC mechanism operates in animals and other human studies, whereby brainstem regions act as gateways that incorporate the inhibition of sensory inputs before reaching higher brain centres [107,120]. This implies that the perception of the UTC reaching higher circuits for processing is reduced and thus leads to the reduced motor act of coughing. In placebo studies, regions within the capsaicin-inhalation network showed both decreases and increases in neural activity during placebo conditioning which is highly consistent with the acknowledged prefrontal limbic-PAG-RVM-DHSC pathway of placebo-related modulation. However, no regions of increased activity were observed in the present studies during painful conditioning. This suggests that the presence of only-reduced activations in the higher brain is likely as a result of a DNIC-related inhibition of airway inputs at lower levels of the brainstem without the need for intervention from higher order circuits. In animal studies, the supraspinal loop sustaining DNIC was confirmed to be confined to the DRt [120,136,142]. The neural outcomes of the present thesis confirmed that the DNIC-related modulation of airway inputs is distinct to that of placebo-related modulation and is consistent with the known DHSC-DRt-DHSC pathway of the DNIC mechanism as observed in animal studies [104,120,142].

The neural findings of the first study (Chapter 2) led to further investigation of the brainstem mechanisms involved in the DNIC-related modulation of airway inputs in the subsequent study (Chapter 3). However, there were no regional brainstem responses as a result of the differences between the pain conditioning and the no pain contingency (CN>CP) (Chapter 3). One explanation for the lack of regional brainstem responses for CN>CP was that the outcomes of the brainstem study may be an accurate and that DNIC-

related modulation may only manifest as changes in higher brain regions. However, this is inconsistent with the findings of the previous study (Chapter 2) and in contrast with how the DNIC mechanism operates in animals and humans. These results were also unexpected and point towards assessing the modulation of neural inputs at the level of the brainstem using a different approach. However, a similar outcome was also observed where no regional brainstem responses were identified as a result of differences between high and low concentrations of capsaicin (Chapter 4). The large behavioural differences between the two concentrations (66%) did not manifest as changes in BOLD signal responses at the brainstem level. Higher brain regions within the field of view showed intensity related differences between pain vs no pain events “CN>CP” (Chapter 3) and high vs low “Hi>Lo” concentrations of capsaicin inhalation (Chapter 4) suggesting that BOLD signal measures associated with intensity-related changes are not detectable at the lower levels of the brainstem. fMRI power analysis showed minimal to non-existent effect sizes required to elicit intensity-related activations in several brainstem nuclei of interest such as the nTS and Pa5. It was therefore hypothesized that the fidelity of BOLD signal measures to detect inhibition-related synaptic activity in local brainstem nuclei is ambiguous during BOLD fMRI data acquisition. Several pain studies support this hypothesis where no change in the absolute levels of activity in brainstem nuclei were observed during pain intensity fluctuations [193,303]. Other studies have also shown electrophysiological activity to be negatively correlated with changes in BOLD signal responses in the hippocampus region compared to the robust and positively correlated variations in BOLD signal responses with changes in neural activity in the hemispheres of the brain [276,277,283,287]. This could also be the case for the brainstem region in that encoding-related differences in the neural activity of local brainstem nuclei does not positively correlate to changes in fMRI BOLD signal responses. This narrative is most likely to be a suitable explanation for the lack of regional brainstem responses associated with intensity-related changes in neural activity in the brainstem region observed in the brainstem studies of the present thesis.

Another narrative that may explain the absence of regional brainstem responses observed in the present thesis could be related to the state of activation of different sensory inputs that generate an UTC. Fundamental to the gating of an UTC or cough is the integration of multiple sensory inputs which may be active or inactive depending on the state of activation of different types of afferent inputs [8]. Alteration in the activity of the different afferent modalities changes the integrated state of the gate nucleus which is at the level of

the brainstem [8]. Therefore, the net activity of the brainstem may not change but the specific integrated signal that opens or closes the gate nuclei may change leading to the alteration of the neural input that reaches higher brain centres that that are involved in the processing of the sensory input of an UTC. This may help explain the absence of changes in the form of BOLD signal intensities at the brainstem level while the gating nuclei is changing state and signalling an interoceptive event. However, this narrative is yet to be proven and thus, further investigation is required before reaching to such conclusions regarding the brainstem outcomes observed in the present thesis.

Despite the brainstem outcomes of the present studies (Chapter 3 and 4), further investigation into the neural mechanisms involved in the modulation of airway inputs is needed. Studies showing gradual intensity related activations at the brainstem level are lacking. With current treatments for chronic cough disorders being widely ineffective, increased interest in research that may yield future therapeutic outcomes is needed. The outcomes of such investigations may lead to the development of new medications that may alleviate persistent cough without impairing the cough reflex. The present studies used 3T for data acquisition and thus, future studies may benefit from the use of ultras-high field strength such as 7T giving superiority in spatial resolution including increased BOLD signal to noise ratio attributes [312,323,329,330].

Conclusion

Painful conditioning leads to significant decreases in the sensory experience of the UTC and the motor act of coughing. It is also associated with significant reduction in neural activity within the capsaicin-inhalation network in the hemispheres. The results of the present studies show that the neural implications of the DNIC effect on the UTC is most likely as a result of descending endogenous inhibition of brainstem regulatory regions related to the control of airway inputs. Further research into the functional processing of airway sensory inputs at the level of the brainstem may lead to novel insights into of the neural mechanism associated with the modulation and regulation of the UTC. These regions could be targets for developing effective antitussive therapies aimed at addressing persistent UTC and chronic cough disease in the future.

Chapter 6 -Conclusion

Summary of Findings

Persistent UTC is a common medical symptom that accompanies chronic cough in disease. Treatment is via antitussive drugs have been widely ineffective in dealing with chronic cough and its associated symptoms. Previous research has provided insight into the neural regulation of UTC and cough in humans [37,49,76,77,82,99,260,274]. Recent studies have also explored the neural modulation of UTC in experimental settings involving placebo and painful conditioning [82]. Placebo was shown to manipulate and reduce the behavioural outcome for UTC and cough frequency in humans. This was accompanied with changes in neural activity at higher brain regions as a result of placebo conditioning [82]. However, placebo is regarded as a form of deception leading to limited therapeutic outcomes in a clinical context. Additionally, pain studies have shown that painful conditioning in a DNIC setting reduces both the UTC and cough behaviourally [84,100]. Despite this advancement in knowledge regarding the modulation of UTC and cough via painful conditioning, the neural outcomes involved in this process remained unknown.

The present experimental studies in this thesis aimed at investigating the behavioural and neural outcomes of painful conditioning on cough and the UTC. It also provided the first evidence of the DNIC-related neural modulation of the UTC that is associated with intensity related changes at the cortical level. The neural outcomes from the present thesis showed that DNIC-related modulation of the UTC is distinct from placebo-related modulation of cough related processing [260]. The findings of the present thesis also highlight the difficulty of brainstem fMRI in showing variations in intensity-related changes in neural activity amongst brainstem nuclei in the form of BOLD signal responses.

Implication of Studies

The outcomes of the present studies in this thesis reaffirm the notion that pain has a strong influence on the behavioural and neural outcomes on cough and the UTC. The substantial decreases in the neural outcomes of the UTC due to painful conditioning further illustrates the ability of the central nervous system to employ cough suppression mechanisms in humans. These findings suggest that afferent airway inputs can be subjective to DNIC-related modulation via an endogenous inhibitory and/or facilitatory mechanism before they reach higher circuits of the brain [104,107,303,312].

The targeting of the central nervous system as a novel approach to reduce coughing in humans is arguably not a novel concept. Previously, opiates such as morphine and codeine including dextromethorphan have been widely used as cough suppressants. However, they have been shown to be ineffective and have proven to be no better than placebo conditioning [15,18]. Furthermore, there is evidence that pharmacological approaches associated with the management of pain can also be effective in reducing cough. This approach involves the administration of drugs that target higher order circuits associated with the processing of pain. Treatments have been trialled using gabapentin and amitriptyline with results showing significant reduction in cough frequency amongst chronic cough patients [331-334]. However, the absence of a notable effect on the UTC ratings and thresholds of capsaicin challenges from the gabapentin trials raised doubts regarding its effectiveness. It was concluded that gabapentin could lead to the sensitisation of circuits that would normally be involved in the down-regulation of sensory inputs. Combining the outcomes of these trials and the results of the present studies in this thesis lament the concept of central targets for cough reduction in humans. Further investigations of the brainstem regions showing altered activation as a result of reduced UTC could present opportunities for the development of novel therapies. These regions could be localised and used as targets for selective drug trials with possible implication for novel drug developments.

Limitations of Experimental Methods and Future Directions for Research

There were several limitations in the current studies that warrant mentioning. Firstly, the studies were limited to experimental cough and the UTC induced via the inhalation of capsaicin. Other tussive substances such as ATP (Adenosine Tri-Phosphate) and citric acid are available airway irritants that can evoke similar cough and UTC responses observed during capsaicin challenges [335]. Contrasting the influence of pain on other forms of inducible cough stimuli would be helpful in determining whether these substances also share similar behavioural and neural outcomes compared to those observed in the present capsaicin studies. Previous studies have shown that different airway irritants activate different fibres within the respiratory system. For instances, it was shown that the inhalation of ATP results in brainstem activations limited to the nTS [49]. The findings of such investigations would further our understanding of how other airway irritants interact with pain. Similarly, there is no evidence of the neural outcomes associated with intensity

related changes for other cough irritants. It could be that the regional higher brain and brainstem responses observed in the present studies may be different when using other airway irritant such as ATP or citric acid are inhaled. These contrasting outcomes may shed light on other underlying mechanisms involved in the inhibition and modulation of sensory airway inputs.

Additionally, the second and third studies (Chapters 3 and 4) did not yield regional brainstem response as a result of painful conditioning nor for capsaicin-related intensity changes. It is evident that significant behavioural differences in UTC ratings may not necessarily translate into regional brainstem responses. One explanation for these outcomes is likely related to the technical challenges associated with fMRI imaging of the brainstem. The brainstem comprises of small nuclei which may lead to spatial constraints due to the relatively small voxel sizes. Geometric distortion combined with noise could mean that small changes in airway inputs are not detectable using the current data acquisition methods [99,312]. However, primary activations for events of interest (CN, CP, Hi>Sal, Lo>Sal) were shown in both brainstem studies suggesting that geometric distortion and spatial constraints may not necessarily be related to the absence of intensity-related regional brainstem responses. Power analysis of BOLD signals from regions of interest such as the nTS and Pa5 also indicated effect sizes from the differences were small to non-existent. Therefore, repeating similar experiments with larger sample sizes may not be a viable option. It was therefore concluded that changes in the neural activity of local brainstem nuclei does not correlate to variations in fMRI BOLD signal responses of this region compared to higher brain regions. This hypothesis is supported by numerous findings related to the negative correlation between changes in electrophysiological neural activity and BOLD signal responses in the hippocampus region which was not the case for higher brain regions [276,283,292].

Despite the absence of regional brainstem responses observed in the present thesis, future brainstem investigations related the modulation of airway inputs may benefit from the use of ultra-high field strength MRI scanners for better imaging acquisition and analysis of brainstem responses. There is evidence that 7T MRI scanners yield better regional brainstem responses in human studies due to superior spatial and temporal resolution including increased BOLD signal-to-noise ratio [305,312,319,326]. In pain studies, 7T has shown better regional activations in brainstem medullary components of the descending pain modulatory system such as the PAG, nTS, RVM when compared to 3T

[312,316,323]. It could be that a 7T scanner may be able to detect inhibition-related synapse activity in brainstem nuclei involved in the descending inhibition of neural inputs which could be beneficial for future research.

Notably, the present studies assessed the conditioning-related DNIC effects in healthy individuals. Whether the findings in the present thesis also translate to similar outcomes in chronic cough patients remain unknown. Chronic, idiopathic and neuropathic pain syndromes have been associated with less efficient or impaired DNIC [104]. King et al (2009) showed deficient DNIC in patients with IBS where the pain felt by individuals is a form of visceral pain [197]. This was supported with evidence of reduced brainstem inhibition during anticipated visceral pain in women with IBS [336]. These findings suggest that pain syndromes decrease the activity of endogenous inhibition pathways. This may well be the case in the chronic cough population whereby deficiency in the pain modulating system may lead to persistent UTC and uncontrollable cough in these patients. Future studies may benefit from investigating the behavioural outcomes of DNIC in chronic cough patients in conjunction with fMRI protocols to identify the regions of impaired or reduced neural activity. The findings may enlist brainstem regions that be targets for therapeutic interventions in the future.

Another interesting finding of the present thesis is the lack of bilateral painful conditioning-related responses within the insula cortex. In the first study (Chapter 2), the right insula showed pain-related reduction in neural activity which was not observed in the left insula. This disparity in bilateral pain-related activations was not observed in other brain regions. The DNIC-related inhibition of neural inputs is known to be diffuse leading to bilateral effects in the hemispheres of the brain. One explanation for such finding could be related to the unilateral application of painful stimulus on the left thumbnail. We know that the right hemisphere of the brain receives and processes somatic sensory inputs from the left side of the body [337]. This could potentially explain the unilateral DNIC-related effects of the right insula cortex. However, previous animal studies have suggested that the ascending pathways involved in triggering DNIC are mainly crossed but have a significant uncrossed component [129]. The application of thumbnail pressure bilaterally in a similar experimental design employed by the current studies (Chapters 2 and 3) would further explore whether DNIC-related effects of the insula region are side dependent. Furthermore, exploring the spatial summation of painful conditioning might be of interest. It could be that increasing the surface area of a conditioning stimulus may lead to greater effects on the behavioural

and neural outcomes of cough and the UTC in humans. In the field of pain, it has been shown that increasing the surface area of stimulation by a painful stimulus amplifies the pain experience [338-342]. These outcomes will further our understanding regarding the modulation, interaction and influence of pain on cough and the UTC.

Conclusions

The findings of the present studies provided novel outcomes regarding the interaction between pain and airway processing. For the first time, the neural outcomes associated with the endogenous inhibition of airway inputs have been identified. In addition, there is substantial evidence that the DNIC-related modulation of airway inputs results in changes in the neural activity within the airway processing network in the hemispheres. This modulation is most likely as a result of DNIC-related inhibition of airway inputs from brainstem regions. However, the present brainstem studies were unable to provide evidence of a DNIC-related modulation of airway inputs. It is also clear that the perception of airway irritation involves a complex process and employs several circuits at the subcortical and cortical levels. Furthermore, the lack of regional brainstem responses indicates that the modulation of airway inputs at the subcortical level is difficult to prove using the current methods. Although the present thesis has not provided substantial brainstem outcomes, this body of work has yielded impetus towards better image acquisition and analysis of the brainstem region. Future experiments investigating the endogenous inhibitory mechanisms associated with reduced perception of the UTC should provide novel insights into the factors that proposed therapies for chronic cough and other respiratory disorders should address.

References

1. Hilton E, Marsden P, Thurston A, Kennedy S, Decalmer S, Smith JA. Clinical features of the urge-to-cough in patients with chronic cough. *Respir Med.* 2015;109(6):701-707.
2. Satoh I, Shiba K, Kobayashi N, Nakajima Y, Konno A. Upper airway motor outputs during sneezing and coughing in decerebrate cats. *Neurosci Res.* 1998;32(2):131-135.
3. Vernon M, Leidy NK, Nacson A, Nelsen L. Measuring cough severity: Perspectives from the literature and from patients with chronic cough. *Cough.* 2009;5:5.
4. Magni C, Chellini E, Lavorini F, Fontana GA, Widdicombe J. Voluntary and reflex cough: similarities and differences. *Pulm Pharmacol Ther.* 2011;24(3):308-311.
5. Udezue E. Lidocaine inhalation for cough suppression. *Am J Emerg Med.* 2001;19(3):206-207.
6. Van den Bergh O, Van Diest I, Dupont L, Davenport PW. On the psychology of cough. *Lung.* 2012;190(1):55-61.
7. von Leupoldt A, Keil A, Chan PY, Bradley MM, Lang PJ, Davenport PW. Cortical sources of the respiratory-related evoked potential. *Respir Physiol Neurobiol.* 2010;170(2):198-201.
8. Davenport PW, Vovk A. Cortical and subcortical central neural pathways in respiratory sensations. *Respir Physiol Neurobiol.* 2009;167(1):72-86.
9. Young EC, Brammer C, Owen E, et al. The effect of mindfulness meditation on cough reflex sensitivity. *Thorax.* 2009;64(11):993-998.
10. Chung KF, Pavord ID. Prevalence, pathogenesis, and causes of chronic cough. *Lancet.* 2008;371(9621):1364-1374.
11. Pavord ID, Chung KF. Management of chronic cough. *Lancet.* 2008;371(9621):1375-1384.
12. Survey NAMC. Summary Tables. 2010.
13. Pratter MR, Brightling CE, Boulet LP, Irwin RS. An empiric integrative approach to the management of cough: ACCP evidence-based clinical practice guidelines. *Chest.* 2006;129(1 Suppl):222S-231S.
14. Vertigan AE, Gibson PG. Chronic refractory cough as a sensory neuropathy: evidence from a reinterpretation of cough triggers. *J Voice.* 2011;25(5):596-601.
15. Bolser DC. Cough suppressant and pharmacologic protussive therapy: ACCP evidence-based clinical practice guidelines. *Chest.* 2006;129(1 Suppl):238S-249S.
16. Paul IM, Yoder KE, Crowell KR, et al. Effect of dextromethorphan, diphenhydramine, and placebo on nocturnal cough and sleep quality for coughing children and their parents. *Pediatrics.* 2004;114(1):e85-90.
17. Taylor JA, Novack AH, Almquist JR, Rogers JE. Efficacy of cough suppressants in children. *J Pediatr.* 1993;122(5 Pt 1):799-802.
18. Eccles R, Morris S, Jawad M. Lack of effect of codeine in the treatment of cough associated with acute upper respiratory tract infection. *J Clin Pharm Ther.* 1992;17(3):175-180.
19. Smith SM, Schroeder K, Fahey T. Over-the-counter medications for acute cough in children and adults in ambulatory settings. *Cochrane Database Syst Rev.* 2008(1):CD001831.
20. Morice A. Chronic cough: epidemiology. *Chron Respir Dis.* 2008;5(1):43-47.
21. French CL, Irwin RS, Curley FJ, Krikorian CJ. Impact of chronic cough on quality of life. *Arch Intern Med.* 1998;158(15):1657-1661.
22. Dicpinigaitis PV, Tso R, Banauch G. Prevalence of depressive symptoms among patients with chronic cough. *Chest.* 2006;130(6):1839-1843.
23. McGarvey LP, Morice AH. Clinical cough and its mechanisms. *Respir Physiol Neurobiol.* 2006;152(3):363-371.
24. Davenport PW, Vovk A, Duke RK, Bolser DC, Robertson E. The urge-to-cough and cough motor response modulation by the central effects of nicotine. *Pulm Pharmacol Ther.* 2009;22(2):82-89.
25. O'Neill J, McMahon SB, Undem BJ. Chronic cough and pain: Janus faces in sensory neurobiology? *Pulm Pharmacol Ther.* 2013;26(5):476-485.
26. Woodcock A, Young EC, Smith JA. New insights in cough. *Br Med Bull.* 2010;96:61-73.
27. Song WJ, Morice AH. Cough Hypersensitivity Syndrome: A Few More Steps Forward. *Allergy Asthma Immunol Res.* 2017;9(5):394-402.

28. Iyer VN, Lim KG. Chronic cough: an update. *Mayo Clin Proc.* 2013;88(10):1115-1126.
29. Dicpinigaitis PV, Bhat R, Rhoton WA, Tibb AS, Negassa A. Effect of viral upper respiratory tract infection on the urge-to-cough sensation. *Respir Med.* 2011;105(4):615-618.
30. Prudon B, Birring SS, Vara DD, Hall AP, Thompson JP, Pavord ID. Cough and glottic-stop reflex sensitivity in health and disease. *Chest.* 2005;127(2):550-557.
31. Davenport PW. Urge-to-cough: what can it teach us about cough? *Lung.* 2008;186 Suppl 1:S107-111.
32. Gui P, Ebihara T, Sato R, Ito K, Kohzuki M, Ebihara S. Gender differences in the effect of urge-to-cough and dyspnea on perception of pain in healthy adults. *Physiol Rep.* 2014;2(8).
33. Davenport PW. Clinical cough I: the urge-to-cough: a respiratory sensation. *Handb Exp Pharmacol.* 2009(187):263-276.
34. Decalmer SC, Webster D, Kelsall AA, McGuinness K, Woodcock AA, Smith JA. Chronic cough: how do cough reflex sensitivity and subjective assessments correlate with objective cough counts during ambulatory monitoring? *Thorax.* 2007;62(4):329-334.
35. Kelsall A, Houghton LA, Jones H, Decalmer S, McGuinness K, Smith JA. A novel approach to studying the relationship between subjective and objective measures of cough. *Chest.* 2011;139(3):569-575.
36. Mazzone SB. An overview of the sensory receptors regulating cough. *Cough.* 2005;1:2.
37. Ando A, Farrell MJ, Mazzone SB. Cough-related neural processing in the brain: a roadmap for cough dysfunction? *Neurosci Biobehav Rev.* 2014;47:457-468.
38. Mazzone SB, Undem BJ. Vagal Afferent Innervation of the Airways in Health and Disease. *Physiol Rev.* 2016;96(3):975-1024.
39. Narula M, McGovern AE, Yang SK, Farrell MJ, Mazzone SB. Afferent neural pathways mediating cough in animals and humans. *J Thorac Dis.* 2014;6(Suppl 7):S712-719.
40. Hansson L, Wollmer P, Dahlback M, Karlsson JA. Regional sensitivity of human airways to capsaicin-induced cough. *Am Rev Respir Dis.* 1992;145(5):1191-1195.
41. Widdicombe J, Singh V. Physiological and pathophysiological down-regulation of cough. *Respir Physiol Neurobiol.* 2006;150(2-3):105-117.
42. McGovern AE, Driessen AK, Simmons DG, et al. Distinct brainstem and forebrain circuits receiving tracheal sensory neuron inputs revealed using a novel conditional anterograde transsynaptic viral tracing system. *J Neurosci.* 2015;35(18):7041-7055.
43. Hunter DD, Undem BJ. Identification and substance P content of vagal afferent neurons innervating the epithelium of the guinea pig trachea. *Am J Respir Crit Care Med.* 1999;159(6):1943-1948.
44. Carr MJ, Hunter DD, Jacoby DB, Undem BJ. Expression of tachykinins in nonnociceptive vagal afferent neurons during respiratory viral infection in guinea pigs. *Am J Respir Crit Care Med.* 2002;165(8):1071-1075.
45. Nassenstein C, Taylor-Clark TE, Myers AC, et al. Phenotypic distinctions between neural crest and placodal derived vagal C-fibres in mouse lungs. *J Physiol.* 2010;588(Pt 23):4769-4783.
46. McGovern AE, Davis-Poynter N, Yang SK, Simmons DG, Farrell MJ, Mazzone SB. Evidence for multiple sensory circuits in the brain arising from the respiratory system: an anterograde viral tract tracing study in rodents. *Brain Struct Funct.* 2015;220(6):3683-3699.
47. Cechetto DF, Saper CB. Evidence for a viscerotopic sensory representation in the cortex and thalamus in the rat. *J Comp Neurol.* 1987;262(1):27-45.
48. Ito S. Visceral region in the rat primary somatosensory cortex identified by vagal evoked potential. *J Comp Neurol.* 2002;444(1):10-24.
49. Farrell MJ, Bautista TG, Liang E, Azzollini D, Egan GF, Mazzone SB. Evidence for multiple bulbar and higher brain circuits processing sensory inputs from the respiratory system in humans. *J Physiol.* 2020;598(24):5771-5787.
50. Field DT, Inman LA. Weighing brain activity with the balance: a contemporary replication of Angelo Mosso's historical experiment. *Brain.* 2014;137(Pt 2):634-639.
51. Sandrone S, Bacigaluppi M, Galloni MR, et al. Weighing brain activity with the balance: Angelo Mosso's original manuscripts come to light. *Brain.* 2014;137(Pt 2):621-633.
52. Sandrone S, Bacigaluppi M, Galloni MR, Martino G. Angelo Mosso (1846-1910). *J Neurol.* 2012;259(11):2513-2514.

53. Bandettini PA. Sewer pipe, wire, epoxy, and finger tapping: the start of fMRI at the Medical College of Wisconsin. *Neuroimage.* 2012;62(2):620-631.
54. Rosen BR, Buckner RL, Dale AM. Event-related functional MRI: past, present, and future. *Proc Natl Acad Sci U S A.* 1998;95(3):773-780.
55. Bandettini PA, Wong EC, Hinks RS, Tikofsky RS, Hyde JS. Time course EPI of human brain function during task activation. *Magn Reson Med.* 1992;25(2):390-397.
56. Kwong KK, Belliveau JW, Chesler DA, et al. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc Natl Acad Sci U S A.* 1992;89(12):5675-5679.
57. Turner R, Jezzard P, Wen H, et al. Functional mapping of the human visual cortex at 4 and 1.5 tesla using deoxygenation contrast EPI. *Magn Reson Med.* 1993;29(2):277-279.
58. Blamire AM, Ogawa S, Ugurbil K, et al. Dynamic mapping of the human visual cortex by high-speed magnetic resonance imaging. *Proc Natl Acad Sci U S A.* 1992;89(22):11069-11073.
59. Frahm J, Bruhn H, Merboldt KD, Hanicke W. Dynamic MR imaging of human brain oxygenation during rest and photic stimulation. *J Magn Reson Imaging.* 1992;2(5):501-505.
60. Logothetis NK. The neural basis of the blood-oxygen-level-dependent functional magnetic resonance imaging signal. *Philos Trans R Soc Lond B Biol Sci.* 2002;357(1424):1003-1037.
61. Logothetis NK. The underpinnings of the BOLD functional magnetic resonance imaging signal. *J Neurosci.* 2003;23(10):3963-3971.
62. Glover GH. Overview of functional magnetic resonance imaging. *Neurosurg Clin N Am.* 2011;22(2):133-139, vii.
63. Buxton RB, Wong EC, Frank LR. Dynamics of blood flow and oxygenation changes during brain activation: the balloon model. *Magn Reson Med.* 1998;39(6):855-864.
64. Davis TL, Kwong KK, Weisskoff RM, Rosen BR. Calibrated functional MRI: mapping the dynamics of oxidative metabolism. *Proc Natl Acad Sci U S A.* 1998;95(4):1834-1839.
65. Ogawa S, Lee TM, Kay AR, Tank DW. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc Natl Acad Sci U S A.* 1990;87(24):9868-9872.
66. Ogawa S, Tank DW, Menon R, et al. Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci U S A.* 1992;89(13):5951-5955.
67. Worsley KJ, Liao CH, Aston J, et al. A general statistical analysis for fMRI data. *Neuroimage.* 2002;15(1):1-15.
68. Bosma RL, Stroman PW. Assessment of data acquisition parameters, and analysis techniques for noise reduction in spinal cord fMRI data. *Magn Reson Imaging.* 2014;32(5):473-481.
69. Liao CH, Worsley KJ, Poline JB, Aston JA, Duncan GH, Evans AC. Estimating the delay of the fMRI response. *Neuroimage.* 2002;16(3 Pt 1):593-606.
70. Cao J. The size of the connected components of excursion sets of χ2, t and F fields. *Advances in Applied Probability.* 1999;31(3):579-595.
71. Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, Evans AC. A unified statistical approach for determining significant signals in images of cerebral activation. *Hum Brain Mapp.* 1996;4(1):58-73.
72. Gestreau C, Bianchi AL, Grelot L. Differential brainstem Fos-like immunoreactivity after laryngeal-induced coughing and its reduction by codeine. *J Neurosci.* 1997;17(23):9340-9352.
73. Jakus J, Poliacek I, Halasova E, et al. Brainstem circuitry of tracheal-bronchial cough: c-fos study in anesthetized cats. *Respir Physiol Neurobiol.* 2008;160(3):289-300.
74. Poliacek I, Halasova E, Jakus J, et al. Brainstem regions involved in the expiration reflex. A c-fos study in anesthetized cats. *Brain Res.* 2007;1184:168-177.
75. Canning BJ, Mori N. An essential component to brainstem cough gating identified in anesthetized guinea pigs. *FASEB J.* 2010;24(10):3916-3926.
76. Farrell MJ, Cole LJ, Chiapoco D, Egan GF, Mazzone SB. Neural correlates coding stimulus level and perception of capsaicin-evoked urge-to-cough in humans. *Neuroimage.* 2012;61(4):1324-1335.
77. Mazzone SB, McGovern AE, Yang SK, et al. Sensorimotor circuitry involved in the higher brain control of coughing. *Cough.* 2013;9(1):7.

78. Mazzone SB, McLennan L, McGovern AE, Egan GF, Farrell MJ. Representation of capsaicin-evoked urge-to-cough in the human brain using functional magnetic resonance imaging. *Am J Respir Crit Care Med.* 2007;176(4):327-332.
79. McGovern AE, Davis-Poynter N, Farrell MJ, Mazzone SB. Transneuronal tracing of airways-related sensory circuitry using herpes simplex virus 1, strain H129. *Neuroscience.* 2012;207:148-166.
80. Mazzone SB, Cole LJ, Ando A, Egan GF, Farrell MJ. Investigation of the neural control of cough and cough suppression in humans using functional brain imaging. *J Neurosci.* 2011;31(8):2948-2958.
81. Mazzone SB, McGovern AE, Farrell MJ. Endogenous central suppressive mechanisms regulating cough as potential targets for novel antitussive therapies. *Curr Opin Pharmacol.* 2015;22:1-8.
82. Leech J, Mazzone SB, Farrell MJ. Brain activity associated with placebo suppression of the urge-to-cough in humans. *Am J Respir Crit Care Med.* 2013;188(9):1069-1075.
83. Leech J, Mazzone SB, Farrell MJ. The effect of placebo conditioning on capsaicin-evoked urge to cough. *Chest.* 2012;142(4):951-957.
84. Hilton E, Satia I, Holt K, Woodcock AA, Belcher J, Smith JA. The Effect of Pain Conditioning on Experimentally Evoked Cough: Evidence of Impaired Endogenous Inhibitory Control Mechanisms in Refractory Chronic Cough. *Eur Respir J.* 2020.
85. Tracey I. Getting the pain you expect: mechanisms of placebo, nocebo and reappraisal effects in humans. *Nat Med.* 2010;16(11):1277-1283.
86. Fields HL, Basbaum AI, Heinricher MM. Central nervous system mechanisms of pain modulation. 2006.
87. Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annual review of neuroscience.* 1984;7:309-338.
88. Eippert F, Finsterbusch J, Bingel U, Buchel C. Direct evidence for spinal cord involvement in placebo analgesia. *Science.* 2009;326(5951):404.
89. Wager TD, Rilling JK, Smith EE, et al. Placebo-induced changes in FMRI in the anticipation and experience of pain. *Science.* 2004;303(5661):1162-1167.
90. Lorenz J, Hauck M, Paur RC, et al. Cortical correlates of false expectations during pain intensity judgments--a possible manifestation of placebo/nocebo cognitions. *Brain Behav Immun.* 2005;19(4):283-295.
91. Wiech K, Ploner M, Tracey I. Neurocognitive aspects of pain perception. *Trends Cogn Sci.* 2008;12(8):306-313.
92. Lee MC, Zambreanu L, Menon DK, Tracey I. Identifying brain activity specifically related to the maintenance and perceptual consequence of central sensitization in humans. *J Neurosci.* 2008;28(45):11642-11649.
93. Tracey I, Ploghaus A, Gati JS, et al. Imaging attentional modulation of pain in the periaqueductal gray in humans. *J Neurosci.* 2002;22(7):2748-2752.
94. 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):310-319.
95. Valet M, Sprenger T, Boecker H, et al. Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain--an fMRI analysis. *Pain.* 2004;109(3):399-408.
96. Seifert F, Bschorer K, De Col R, et al. Medial prefrontal cortex activity is predictive for hyperalgesia and pharmacological antihyperalgesia. *J Neurosci.* 2009;29(19):6167-6175.
97. Petrovic P, Ingvar M. Imaging cognitive modulation of pain processing. *Pain.* 2002;95(1-2):1-5.
98. Eippert F, Bingel U, Schoell ED, et al. Activation of the opioidergic descending pain control system underlies placebo analgesia. *Neuron.* 2009;63(4):533-543.
99. Bautista TG, Leech J, Mazzone S, Farrell MJ. Regional brainstem activations during capsaicin inhalation using functional magnetic resonance imaging in humans. *J Neurophysiol.* 2019.
100. Young EC, Houghton LA, Holt KJ, Woodcock AA, Smith JA. S144a Endogenous inhibition of experimentally induced cough in healthy subjects. *Thorax.* 2011;66(Suppl 4):A66.
101. Le Bars D, Dickenson AH, Besson JM. Diffuse noxious inhibitory controls (DNIC). II. Lack of effect on non-convergent neurones, supraspinal involvement and theoretical implications. *Pain.* 1979;6(3):305-327.

102. Le Bars D, Dickenson AH, Besson JM. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. *Pain.* 1979;6(3):283-304.
103. Le Bars D, Villanueva L, Bouhassira D, Willer JC. Diffuse noxious inhibitory controls (DNIC) in animals and in man. *Patol Fiziol Eksp Ter.* 1992(4):55-65.
104. Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. *Curr Opin Anaesthesiol.* 2010;23(5):611-615.
105. Yarnitsky D, Arendt-Nielsen L, Bouhassira D, et al. Recommendations on terminology and practice of psychophysical DNIC testing. *Eur J Pain.* 2010;14(4):339.
106. Baad-Hansen L, Poulsen HF, Jensen HM, Svensson P. Lack of sex differences in modulation of experimental intraoral pain by diffuse noxious inhibitory controls (DNIC). *Pain.* 2005;116(3):359-365.
107. Youssef AM, Macefield VG, Henderson LA. Pain inhibits pain; human brainstem mechanisms. *Neuroimage.* 2016;124(Pt A):54-62.
108. Pud D, Granovsky Y, Yarnitsky D. The methodology of experimentally induced diffuse noxious inhibitory control (DNIC)-like effect in humans. *Pain.* 2009;144(1-2):16-19.
109. Fujii K, Motohashi K, Umino M. Heterotopic ischemic pain attenuates somatosensory evoked potentials induced by electrical tooth stimulation: diffuse noxious inhibitory controls in the trigeminal nerve territory. *Eur J Pain.* 2006;10(6):495-504.
110. Kakigi R. Diffuse noxious inhibitory control. Reappraisal by pain-related somatosensory evoked potentials following CO2 laser stimulation. *J Neurol Sci.* 1994;125(2):198-205.
111. Svensson P, Hashikawa CH, Casey KL. Site- and modality-specific modulation of experimental muscle pain in humans. *Brain Res.* 1999;851(1-2):32-38.
112. Willer JC, De Broucker T, Le Bars D. Encoding of nociceptive thermal stimuli by diffuse noxious inhibitory controls in humans. *J Neurophysiol.* 1989;62(5):1028-1038.
113. Yarnitsky D, Crispel Y, Eisenberg E, et al. Prediction of chronic post-operative pain: pre-operative DNIC testing identifies patients at risk. *Pain.* 2008;138(1):22-28.
114. Kennedy DL, Kemp HI, Ridout D, Yarnitsky D, Rice ASC. Reliability of conditioned pain modulation: a systematic review. *Pain.* 2016;157(11):2410-2419.
115. Lie MU, Matre D, Hansson P, Stubhaug A, Zwart JA, Nilsen KB. A tonic heat test stimulus yields a larger and more reliable conditioned pain modulation effect compared to a phasic heat test stimulus. *Pain Rep.* 2017;2(6):e626.
116. Olesen SS, van Goor H, Bouwense SA, Wilder-Smith OH, Drewes AM. Reliability of static and dynamic quantitative sensory testing in patients with painful chronic pancreatitis. *Reg Anesth Pain Med.* 2012;37(5):530-536.
117. Valencia C, Kindler LL, Fillingim RB, George SZ. Stability of conditioned pain modulation in two musculoskeletal pain models: investigating the influence of shoulder pain intensity and gender. *BMC Musculoskelet Disord.* 2013;14:182.
118. Morelot-Panzini C, Demoule A, Straus C, et al. Dyspnea as a noxious sensation: inspiratory threshold loading may trigger diffuse noxious inhibitory controls in humans. *J Neurophysiol.* 2007;97(2):1396-1404.
119. Morelot-Panzini C, Mayaux J, Hug F, Willer JC, Similowski T. Analgesic effects of dyspnoea: "Air hunger" does not inhibit the spinal nociception reflex in humans. *Respir Physiol Neurobiol.* 2014;190:81-85.
120. Le Bars D. The whole body receptive field of dorsal horn multireceptive neurones. *Brain Res Brain Res Rev.* 2002;40(1-3):29-44.
121. Bars L, Hecht Y, Beaugrand M, Ferrier JP, Hornstein M. [Peritoneal diffusion of gentamicin in the ascitic cirrhotic]. *Nouv Presse Med.* 1979;8(8):614.
122. Dallel R, Raboisson P, Woda A, Sessle BJ. Properties of nociceptive and non-nociceptive neurons in trigeminal subnucleus oralis of the rat. *Brain Res.* 1990;521(1-2):95-106.
123. Morgan MM, Heinricher MM, Fields HL. Inhibition and facilitation of different nocifensor reflexes by spatially remote noxious stimuli. *J Neurophysiol.* 1994;72(3):1152-1160.
124. Danziger N, Gautron M, Le Bars D, Bouhassira D. Activation of diffuse noxious inhibitory controls (DNIC) in rats with an experimental peripheral mononeuropathy. *Pain.* 2001;91(3):287-296.
125. Falinower S, Willer JC, Junien JL, Le Bars D. A C-fiber reflex modulated by heterotopic noxious somatic stimuli in the rat. *J Neurophysiol.* 1994;72(1):194-213.

126. Nishioka K, Harada Y, Kitahata LM, Tsukahara S, Collins JG. Role of WDR neurons in a hind limb noxious heat evoked flexion withdrawal reflex. *Life Sci.* 1995;56(7):485-489.
127. Schouenborg J, Weng HR, Kalliomaki J, Holmberg H. A survey of spinal dorsal horn neurones encoding the spatial organization of withdrawal reflexes in the rat. *Exp Brain Res.* 1995;106(1):19-27.
128. Dickenson AH, Le Bars D. Diffuse noxious inhibitory controls (DNIC) involve trigeminothalamic and spinothalamic neurones in the rat. *Exp Brain Res.* 1983;49(2):174-180.
129. Villanueva L, Peschanski M, Calvino B, Le Bars D. Ascending pathways in the spinal cord involved in triggering of diffuse noxious inhibitory controls in the rat. *J Neurophysiol.* 1986;55(1):34-55.
130. Kerr FW. The ventral spinothalamic tract and other ascending systems of the ventral funiculus of the spinal cord. *J Comp Neurol.* 1975;159(3):335-356.
131. Mehler WR, Feferman ME, Nauta WJ. Ascending axon degeneration following anterolateral cordotomy. An experimental study in the monkey. *Brain.* 1960;83:718-750.
132. Lapirot O, Chebbi R, Monconduit L, Artola A, Dallel R, Luccarini P. NK1 receptor-expressing spinoparabrachial neurons trigger diffuse noxious inhibitory controls through lateral parabrachial activation in the male rat. *Pain.* 2009;142(3):245-254.
133. Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH. Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nat Neurosci.* 2002;5(12):1319-1326.
134. Patel R, Dickenson AH. A study of cortical and brainstem mechanisms of diffuse noxious inhibitory controls in anaesthetised normal and neuropathic rats. *Eur J Neurosci.* 2020;51(4):952-962.
135. Villanueva L, Chitour D, Le Bars D. Involvement of the dorsolateral funiculus in the descending spinal projections responsible for diffuse noxious inhibitory controls in the rat. *J Neurophysiol.* 1986;56(4):1185-1195.
136. Bouhassira D, Chitour D, Villaneuva L, Le Bars D. The spinal transmission of nociceptive information: modulation by the caudal medulla. *Neuroscience.* 1995;69(3):931-938.
137. De Broucker T, Cesaro P, Willer JC, Le Bars D. Diffuse noxious inhibitory controls in man. Involvement of the spinoreticular tract. *Brain.* 1990;113 (Pt 4):1223-1234.
138. Fields H. State-dependent opioid control of pain. *Nat Rev Neurosci.* 2004;5(7):565-575.
139. Heinricher MM, Tavares I, Leith JL, Lumb BM. Descending control of nociception: Specificity, recruitment and plasticity. *Brain Res Rev.* 2009;60(1):214-225.
140. Villanueva L, Le Bars D. The activation of bulbo-spinal controls by peripheral nociceptive inputs: diffuse noxious inhibitory controls. *Biol Res.* 1995;28(1):113-125.
141. Roby-Brami A, Bussel B, Willer JC, Le Bars D. An electrophysiological investigation into the pain-relieving effects of heterotopic nociceptive stimuli. Probable involvement of a supraspinal loop. *Brain.* 1987;110 (Pt 6):1497-1508.
142. Bouhassira D, Bing Z, Le Bars D. Studies of the brain structures involved in diffuse noxious inhibitory controls: the mesencephalon. *J Neurophysiol.* 1990;64(6):1712-1723.
143. Cadden SW, Villanueva L, Chitour D, Le Bars D. Depression of activities of dorsal horn convergent neurones by propriospinal mechanisms triggered by noxious inputs; comparison with diffuse noxious inhibitory controls (DNIC). *Brain Res.* 1983;275(1):1-11.
144. Morton CR, Maisch B, Zimmermann M. Diffuse noxious inhibitory controls of lumbar spinal neurons involve a supraspinal loop in the cat. *Brain Res.* 1987;410(2):347-352.
145. Bing Z, Villanueva L, Le Bars D. Acupuncture and diffuse noxious inhibitory controls: naloxone-reversible depression of activities of trigeminal convergent neurons. *Neuroscience.* 1990;37(3):809-818.
146. Bouhassira D, Villanueva L, Bing Z, le Bars D. Involvement of the subnucleus reticularis dorsalis in diffuse noxious inhibitory controls in the rat. *Brain Res.* 1992;595(2):353-357.
147. Le Bars D, Willer JC. Higher pain tolerance thresholds. *Pain.* 1988;32(2):259-260.
148. Bouhassira D, Chitour D, Villanueva L, Le Bars D. Morphine and diffuse noxious inhibitory controls in the rat: effects of lesions of the rostral ventromedial medulla. *Eur J Pharmacol.* 1993;232(2-3):207-215.
149. van Wijk G, Veldhuijzen DS. Perspective on diffuse noxious inhibitory controls as a model of endogenous pain modulation in clinical pain syndromes. *J Pain.* 2010;11(5):408-419.

150. Villanueva L, Bouhassira D, Le Bars D. The medullary subnucleus reticularis dorsalis (SRD) as a key link in both the transmission and modulation of pain signals. *Pain.* 1996;67(2-3):231-240.
151. Leite-Almeida H, Valle-Fernandes A, Almeida A. Brain projections from the medullary dorsal reticular nucleus: an anterograde and retrograde tracing study in the rat. *Neuroscience.* 2006;140(2):577-595.
152. Bernard JF, Villanueva L, Carroue J, Le Bars D. Efferent projections from the subnucleus reticularis dorsalis (SRD): a Phaseolus vulgaris leucoagglutinin study in the rat. *Neurosci Lett.* 1990;116(3):257-262.
153. Le Bars D, Chitour D, Kraus E, Clot AM, Dickenson AH, Besson JM. The effect of systemic morphine upon diffuse noxious inhibitory controls (DNIC) in the rat: evidence for a lifting of certain descending inhibitory controls of dorsal horn convergent neurones. *Brain Res.* 1981;215(1-2):257-274.
154. Le Bars D, Chitour D, Kraus E, Dickenson AH, Besson JM. Effect of naloxone upon diffuse noxious inhibitory controls (DNIC) in the rat. *Brain Res.* 1981;204(2):387-402.
155. Martins I, Carvalho P, de Vries MG, et al. GABA acting on GABAB receptors located in a medullary pain facilitatory area enhances nociceptive behaviors evoked by intraplantar formalin injection. *Pain.* 2015;156(8):1555-1565.
156. Wen YR, Wang CC, Yeh GC, et al. DNIC-mediated analgesia produced by a supramaximal electrical or a high-dose formalin conditioning stimulus: roles of opioid and alpha2-adrenergic receptors. *J Biomed Sci.* 2010;17(1):19.
157. Willer JC, Le Bars D, De Broucker T. Diffuse noxious inhibitory controls in man: involvement of an opioidergic link. *Eur J Pharmacol.* 1990;182(2):347-355.
158. Perrotta A, Serrao M, Ambrosini A, et al. Facilitated temporal processing of pain and defective supraspinal control of pain in cluster headache. *Pain.* 2013;154(8):1325-1332.
159. Albusoda A, Ruffle JK, Friis KA, et al. Systematic review with meta-analysis: conditioned pain modulation in patients with the irritable bowel syndrome. *Aliment Pharmacol Ther.* 2018;48(8):797-806.
160. Yarnitsky D, Granot M, Granovsky Y. Pain modulation profile and pain therapy: between pro- and antinociception. *Pain.* 2014;155(4):663-665.
161. Ram KC, Eisenberg E, Haddad M, Pud D. Oral opioid use alters DNIC but not cold pain perception in patients with chronic pain - new perspective of opioid-induced hyperalgesia. *Pain.* 2008;139(2):431-438.
162. Nielsen PR, Norgaard L, Rasmussen LS, Kehlet H. Prediction of post-operative pain by an electrical pain stimulus. *Acta Anaesthesiol Scand.* 2007;51(5):582-586.
163. Wager TD, Scott DJ, Zubieta JK. Placebo effects on human mu-opioid activity during pain. *Proc Natl Acad Sci U S A.* 2007;104(26):11056-11061.
164. Zubieta JK, Bueller JA, Jackson LR, et al. Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *J Neurosci.* 2005;25(34):7754-7762.
165. Petrovic P, Kalso E, Petersson KM, Andersson J, Fransson P, Ingvar M. A prefrontal non-opioid mechanism in placebo analgesia. *Pain.* 2010;150(1):59-65.
166. Petrovic P, Kalso E, Petersson KM, Ingvar M. Placebo and opioid analgesia-- imaging a shared neuronal network. *Science.* 2002;295(5560):1737-1740.
167. Stroman PW, Khan HS, Bosma RL, et al. Changes in Pain Processing in the Spinal Cord and Brainstem after Spinal Cord Injury Characterized by Functional Magnetic Resonance Imaging. *Journal of neurotrauma.* 2016;33(15):1450-1460.
168. Ioachim G, Powers JM, Stroman PW. Comparing Coordinated Networks Across the Brainstem and Spinal Cord in the Resting State and Altered Cognitive State. *Brain connectivity.* 2019;9(5):415-424.
169. Ioachim G, Powers JM, Warren HJM, Stroman PW. Coordinated Human Brainstem and Spinal Cord Networks during the Expectation of Pain Have Elements Unique from Resting-State Effects. *Brain sciences.* 2020;10(9).
170. Moont R, Pud D, Sprecher E, Sharvit G, Yarnitsky D. 'Pain inhibits pain' mechanisms: Is pain modulation simply due to distraction? *Pain.* 2010;150(1):113-120.
171. Sprenger C, Eippert F, Finsterbusch J, Bingel U, Rose M, Buchel C. Attention modulates spinal cord responses to pain. *Curr Biol.* 2012;22(11):1019-1022.

172. Cahill CM, Stroman PW. Mapping of neural activity produced by thermal pain in the healthy human spinal cord and brain stem: a functional magnetic resonance imaging study. *Magn Reson Imaging.* 2011;29(3):342-352.
173. Knudsen L, Petersen GL, Nørskov KN, et al. Review of neuroimaging studies related to pain modulation. *Scand J Pain.* 2018;2(3):108-120.
174. Dobek CE, Beynon ME, Bosma RL, Stroman PW. Music modulation of pain perception and pain-related activity in the brain, brain stem, and spinal cord: a functional magnetic resonance imaging study. *J Pain.* 2014;15(10):1057-1068.
175. Leung RH, Stroman PW. Neural Correlates of Cognitive Modulation of Pain Perception in the Human Brainstem and Cervical Spinal Cord using Functional Magnetic Resonance Imaging: A Review. *Critical reviews in biomedical engineering.* 2016;44(1-2):33-45.
176. Stumpf A, Pfleiderer B, Schneider G, et al. Distraction From Itch Shows Brainstem Activation Without Reduction in Experimental Itch Sensation. *Acta Derm Venereol.* 2017;97(9):1074-1080.
177. Lautenbacher S, Prager M, Rollman GB. Pain additivity, diffuse noxious inhibitory controls, and attention: a functional measurement analysis. *Somatosens Mot Res.* 2007;24(4):189-201.
178. Kong J, Gollub RL, Polich G, et al. A functional magnetic resonance imaging study on the neural mechanisms of hyperalgesic nocebo effect. *J Neurosci.* 2008;28(49):13354-13362.
179. Bosma RL, Ameli Mojarad E, Leung L, Pukall C, Staud R, Stroman PW. Neural correlates of temporal summation of second pain in the human brainstem and spinal cord. *Hum Brain Mapp.* 2015;36(12):5038-5050.
180. Keltner JR, Furst A, Fan C, Redfern R, Inglis B, Fields HL. Isolating the modulatory effect of expectation on pain transmission: a functional magnetic resonance imaging study. *J Neurosci.* 2006;26(16):4437-4443.
181. Crawford L, Mills E, Hanson T, et al. Brainstem mechanisms of pain modulation: a within-subjects 7T fMRI study of Placebo Analgesic and Nocebo Hyperalgesic Responses. *J Neurosci.* 2021.
182. Oliva V, Gregory R, Davies WE, et al. Parallel cortical-brainstem pathways to attentional analgesia. *Neuroimage.* 2021;226:117548.
183. Granot M, Weissman-Fogel I, Crispel Y, et al. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: do conditioning stimulus painfulness, gender and personality variables matter? *Pain.* 2008;136(1-2):142-149.
184. Oono Y, Fujii K, Motohashi K, Umino M. Diffuse noxious inhibitory controls triggered by heterotopic CO2 laser conditioning stimulation decreased the SEP amplitudes induced by electrical tooth stimulation with different intensity at an equally inhibitory rate. *Pain.* 2008;136(3):356-365.
185. Staud R, Robinson ME, Vierck CJ, Jr., Price DD. Diffuse noxious inhibitory controls (DNIC) attenuate temporal summation of second pain in normal males but not in normal females or fibromyalgia patients. *Pain.* 2003;101(1-2):167-174.
186. Ge HY, Madeleine P, Arendt-Nielsen L. Sex differences in temporal characteristics of descending inhibitory control: an evaluation using repeated bilateral experimental induction of muscle pain. *Pain.* 2004;110(1-2):72-78.
187. Popescu A, LeResche L, Truelove EL, Drangsholt MT. Gender differences in pain modulation by diffuse noxious inhibitory controls: a systematic review. *Pain.* 2010;150(2):309-318.
188. France CR, Suchowiecki S. A comparison of diffuse noxious inhibitory controls in men and women. *Pain.* 1999;81(1-2):77-84.
189. Moisset X, Bouhassira D. Brain imaging of neuropathic pain. *Neuroimage.* 2007;37 Suppl 1:S80-88.
190. Linnman C, Moulton EA, Barmettler G, Becerra L, Borsook D. Neuroimaging of the periaqueductal gray: state of the field. *Neuroimage.* 2012;60(1):505-522.
191. Limbrick-Oldfield EH, Brooks JC, Wise RJ, et al. Identification and characterisation of midbrain nuclei using optimised functional magnetic resonance imaging. *Neuroimage.* 2012;59(2):1230-1238.

192. Mainero C, Zhang WT, Kumar A, Rosen BR, Sorensen AG. Mapping the spinal and supraspinal pathways of dynamic mechanical allodynia in the human trigeminal system using cardiac-gated fMRI. *Neuroimage.* 2007;35(3):1201-1210.
193. Henderson LA, Keay KA. Imaging Acute and Chronic Pain in the Human Brainstem and Spinal Cord. *Neuroscientist.* 2018;24(1):84-96.
194. Mazzone SB, McGovern AE, Koo K, Farrell MJ. Mapping supramedullary pathways involved in cough using functional brain imaging: comparison with pain. *Pulm Pharmacol Ther.* 2009;22(2):90-96.
195. Farrell MJ, Egan GF, Zamarripa F, et al. Unique, common, and interacting cortical correlates of thirst and pain. *Proc Natl Acad Sci U S A.* 2006;103(7):2416-2421.
196. Julien N, Goffaux P, Arsenault P, Marchand S. Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. *Pain.* 2005;114(1-2):295-302.
197. King CD, Wong F, Currie T, Mauderli AP, Fillingim RB, Riley JL, 3rd. Deficiency in endogenous modulation of prolonged heat pain in patients with Irritable Bowel Syndrome and Temporomandibular Disorder. *Pain.* 2009;143(3):172-178.
198. Nahman-Averbuch H, Yarnitsky D, Granovsky Y, et al. Pronociceptive pain modulation in patients with painful chemotherapy-induced polyneuropathy. *J Pain Symptom Manage.* 2011;42(2):229-238.
199. Song GH, Venkatraman V, Ho KY, Chee MW, Yeoh KG, Wilder-Smith CH. Cortical effects of anticipation and endogenous modulation of visceral pain assessed by functional brain MRI in irritable bowel syndrome patients and healthy controls. *Pain.* 2006;126(1-3):79-90.
200. Lautenbacher S, Rollman GB. Possible deficiencies of pain modulation in fibromyalgia. *Clin J Pain.* 1997;13(3):189-196.
201. Pielsticker A, Haag G, Zaudig M, Lautenbacher S. Impairment of pain inhibition in chronic tension-type headache. *Pain.* 2005;118(1-2):215-223.
202. Cathcart S, Winefield AH, Lushington K, Rolan P. Noxious inhibition of temporal summation is impaired in chronic tension-type headache. *Headache.* 2010;50(3):403-412.
203. Nation KM, Dodick DW, Navratilova E, Porreca F. Sustained exposure to acute migraine medications combined with repeated noxious stimulation dysregulates descending pain modulatory circuits: Relevance to medication overuse headache. *Cephalalgia.* 2019;39(5):617-625.
204. Patel R, Qu C, Xie JY, Porreca F, Dickenson AH. Selective deficiencies in descending inhibitory modulation in neuropathic rats: implications for enhancing noradrenergic tone. *Pain.* 2018;159(9):1887-1899.
205. Heymen S, Maixner W, Whitehead WE, Klatzkin RR, Mechlin B, Light KC. Central processing of noxious somatic stimuli in patients with irritable bowel syndrome compared with healthy controls. *Clin J Pain.* 2010;26(2):104-109.
206. Piche M, Arsenault M, Poitras P, Rainville P, Bouin M. Widespread hypersensitivity is related to altered pain inhibition processes in irritable bowel syndrome. *Pain.* 2010;148(1):49-58.
207. Leonard G, Goffaux P, Mathieu D, Blanchard J, Kenny B, Marchand S. Evidence of descending inhibition deficits in atypical but not classical trigeminal neuralgia. *Pain.* 2009;147(1-3):217-223.
208. Wilder-Smith CH, Schindler D, Lovblad K, Redmond SM, Nirkko A. Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. *Gut.* 2004;53(11):1595-1601.
209. Meeus M, Nijs J, Van de Wauwer N, Toeback L, Truijen S. Diffuse noxious inhibitory control is delayed in chronic fatigue syndrome: an experimental study. *Pain.* 2008;139(2):439-448.
210. Olesen SS, Brock C, Krarup AL, et al. Descending inhibitory pain modulation is impaired in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol.* 2010;8(8):724-730.
211. Kosek E, Ordeberg G. Lack of pressure pain modulation by heterotopic noxious conditioning stimulation in patients with painful osteoarthritis before, but not following, surgical pain relief. *Pain.* 2000;88(1):69-78.
212. Albu S, Gomez-Soriano J, Avila-Martin G, Taylor J. Deficient conditioned pain modulation after spinal cord injury correlates with clinical spontaneous pain measures. *Pain.* 2015;156(2):260-272.

213. Gagné M, Côté I, Boulet M, Jutzeler CR, Kramer JLK, Mercier C. Conditioned Pain Modulation Decreases Over Time in Patients With Neuropathic Pain Following a Spinal Cord Injury. *Neurorehabilitation and neural repair.* 2020;34(11):997-1008.
214. Mylius V, Engau I, Teepker M, et al. Pain sensitivity and descending inhibition of pain in Parkinson's disease. *J Neurol Neurosurg Psychiatry.* 2009;80(1):24-28.
215. Potvin S, Stip E, Tempier A, et al. Pain perception in schizophrenia: no changes in diffuse noxious inhibitory controls (DNIC) but a lack of pain sensitization. *J Psychiatr Res.* 2008;42(12):1010-1016.
216. Arendt-Nielsen L, Nie H, Laursen MB, et al. Sensitization in patients with painful knee osteoarthritis. *Pain.* 2010;149(3):573-581.
217. Leffler AS, Hansson P, Kosek E. Somatosensory perception in a remote pain-free area and function of diffuse noxious inhibitory controls (DNIC) in patients suffering from long-term trapezius myalgia. *Eur J Pain.* 2002;6(2):149-159.
218. Leffler AS, Kosek E, Lerndal T, Nordmark B, Hansson P. Somatosensory perception and function of diffuse noxious inhibitory controls (DNIC) in patients suffering from rheumatoid arthritis. *Eur J Pain.* 2002;6(2):161-176.
219. Edwards RR, Grace E, Peterson S, Klick B, Haythornthwaite JA, Smith MT. Sleep continuity and architecture: associations with pain-inhibitory processes in patients with temporomandibular joint disorder. *Eur J Pain.* 2009;13(10):1043-1047.
220. Smith MT, Edwards RR, McCann UD, Haythornthwaite JA. The effects of sleep deprivation on pain inhibition and spontaneous pain in women. *Sleep.* 2007;30(4):494-505.
221. Arendt-Nielsen L, Sluka KA, Nie HL. Experimental muscle pain impairs descending inhibition. *Pain.* 2008;140(3):465-471.
222. Goodin BR, McGuire L, Allshouse M, et al. Associations between catastrophizing and endogenous pain-inhibitory processes: sex differences. *J Pain.* 2009;10(2):180-190.
223. Serrao M, Rossi P, Sandrini G, et al. Effects of diffuse noxious inhibitory controls on temporal summation of the RIII reflex in humans. *Pain.* 2004;112(3):353-360.
224. Weissman-Fogel I, Sprecher E, Pud D. Effects of catastrophizing on pain perception and pain modulation. *Exp Brain Res.* 2008;186(1):79-85.
225. Ge HY, Madeleine P, Arendt-Nielsen L. Gender differences in pain modulation evoked by repeated injections of glutamate into the human trapezius muscle. *Pain.* 2005;113(1-2):134-140.
226. Lautenbacher S, Kunz M, Burkhardt S. The effects of DNIC-type inhibition on temporal summation compared to single pulse processing: does sex matter? *Pain.* 2008;140(3):429-435.
227. Pud D, Sprecher E, Yarnitsky D. Homotopic and heterotopic effects of endogenous analgesia in healthy volunteers. *Neurosci Lett.* 2005;380(3):209-213.
228. Tousignant-Laflamme Y, Page S, Goffaux P, Marchand S. An experimental model to measure excitatory and inhibitory pain mechanisms in humans. *Brain Res.* 2008;1230:73-79.
229. Brattberg G, Parker MG, Thorslund M. A longitudinal study of pain: reported pain from middle age to old age. *Clin J Pain.* 1997;13(2):144-149.
230. Gagliese L, Melzack R. Chronic pain in elderly people. *Pain.* 1997;70(1):3-14.
231. Washington LL, Gibson SJ, Helme RD. Age-related differences in the endogenous analgesic response to repeated cold water immersion in human volunteers. *Pain.* 2000;89(1):89-96.
232. Lariviere M, Goffaux P, Marchand S, Julien N. Changes in pain perception and descending inhibitory controls start at middle age in healthy adults. *Clin J Pain.* 2007;23(6):506-510.
233. Edwards RR, Fillingim RB, Ness TJ. Age-related differences in endogenous pain modulation: a comparison of diffuse noxious inhibitory controls in healthy older and younger adults. *Pain.* 2003;101(1-2):155-165.
234. Edwards RR. Individual differences in endogenous pain modulation as a risk factor for chronic pain. *Neurology.* 2005;65(3):437-443.
235. Mechlin MB, Maixner W, Light KC, Fisher JM, Girdler SS. African Americans show alterations in endogenous pain regulatory mechanisms and reduced pain tolerance to experimental pain procedures. *Psychosom Med.* 2005;67(6):948-956.

236. Campbell CM, France CR, Robinson ME, Logan HL, Geffken GR, Fillingim RB. Ethnic differences in diffuse noxious inhibitory controls. *J Pain.* 2008;9(8):759-766.
237. Edwards RR, Ness TJ, Weigent DA, Fillingim RB. Individual differences in diffuse noxious inhibitory controls (DNIC): association with clinical variables. *Pain.* 2003;106(3):427-437.
238. Kosek E, Hansson P. Modulatory influence on somatosensory perception from vibration and heterotopic noxious conditioning stimulation (HNCS) in fibromyalgia patients and healthy subjects. *Pain.* 1997;70(1):41-51.
239. Tuveson B, Leffler AS, Hansson P. Time dependent differences in pain sensitivity during unilateral ischemic pain provocation in healthy volunteers. *Eur J Pain.* 2006;10(3):225-232.
240. Moont R, Crispel Y, Lev R, Pud D, Yarnitsky D. Temporal changes in cortical activation during conditioned pain modulation (CPM), a LORETA study. *Pain.* 2011;152(7):1469-1477.
241. Willer JC, Roby A, Le Bars D. Psychophysical and electrophysiological approaches to the pain-relieving effects of heterotopic nociceptive stimuli. *Brain.* 1984;107 (Pt 4):1095-1112.
242. Lautenbacher S, Roscher S, Strian F. Inhibitory effects do not depend on the subjective experience of pain during heterotopic noxious conditioning stimulation (HNCS): a contribution to the psychophysics of pain inhibition. *Eur J Pain.* 2002;6(5):365-374.
243. Price DD, McHaffie JG. Effects of heterotopic conditioning stimuli on first and second pain: a psychophysical evaluation in humans. *Pain.* 1988;34(3):245-252.
244. Bouhassira D, Sabate JM, Coffin B, Le Bars D, Willer JC, Jian R. Effects of rectal distensions on nociceptive flexion reflexes in humans. *Am J Physiol.* 1998;275(3):G410-417.
245. Bouhassira D, Chollet R, Coffin B, et al. Inhibition of a somatic nociceptive reflex by gastric distention in humans. *Gastroenterology.* 1994;107(4):985-992.
246. Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. *Neuron.* 2007;55(3):377-391.
247. Graven-Nielsen T, Babenko V, Svensson P, Arendt-Nielsen L. Experimentally induced muscle pain induces hypoalgesia in heterotopic deep tissues, but not in homotopic deep tissues. *Brain Res.* 1998;787(2):203-210.
248. Gusnard DA, Raichle ME, Raichle ME. Searching for a baseline: functional imaging and the resting human brain. *Nat Rev Neurosci.* 2001;2(10):685-694.
249. Huang J, Kang XZ, Luo P. [Involvement of amygdala in the emotional processing of pain]. *Sheng Li Ke Xue Jin Zhan.* 2005;36(4):289-294.
250. Goffaux P, Redmond WJ, Rainville P, Marchand S. Descending analgesia--when the spine echoes what the brain expects. *Pain.* 2007;130(1-2):137-143.
251. Micalos PS, Drinkwater EJ, Cannon J, Arendt-Nielsen L, Marino FE. Reliability of the nociceptive flexor reflex (RIII) threshold and association with Pain threshold. *Eur J Appl Physiol.* 2009;105(1):55-62.
252. Song WJ, Won HK, An J, et al. Chronic cough in the elderly. *Pulm Pharmacol Ther.* 2019;56:63-68.
253. Zeiger RS, Xie F, Schatz M, et al. Prevalence and Characteristics of Chronic Cough in Adults Identified by Administrative Data. *Perm J.* 2020;24:1-3.
254. Tao ZY, Wang PX, Wei SQ, Traub RJ, Li JF, Cao DY. The Role of Descending Pain Modulation in Chronic Primary Pain: Potential Application of Drugs Targeting Serotonergic System. *Neural Plast.* 2019;2019:1389296.
255. Morice AH, Jakes AD, Faruqi S, et al. A worldwide survey of chronic cough: a manifestation of enhanced somatosensory response. *Eur Respir J.* 2014;44(5):1149-1155.
256. Colak Y, Nordestgaard BG, Laursen LC, Afzal S, Lange P, Dahl M. Risk Factors for Chronic Cough Among 14,669 Individuals From the General Population. *Chest.* 2017;152(3):563-573.
257. Bouhassira D, Bing Z, Le Bars D. Studies of brain structures involved in diffuse noxious inhibitory controls in the rat: the rostral ventromedial medulla. *J Physiol.* 1993;463:667-687.
258. Bouhassira D, Le Bars D, Bolgert F, Laplane D, Willer JC. Diffuse noxious inhibitory controls in humans: a neurophysiological investigation of a patient with a form of Brown-Sequard syndrome. *Ann Neurol.* 1993;34(4):536-543.

259. Le Bars D, Bouhassira D, Villanueva L. Opioids and diffuse noxious inhibitory control (DNIC) in the rat. *Pain and the brain: From nociception to cognition.* New York, NY, US: Raven Press; 1995:517-539.
260. Abubakar AB, Bautista TG, Dimmock MR, Mazzone SB, Farrell MJ. Behavioral and regional brain responses to inhalation of capsaicin modified by painful conditioning in humans. *Chest.* 2020.
261. Birn RM, Murphy K, Handwerker DA, Bandettini PA. fMRI in the presence of task-correlated breathing variations. *Neuroimage.* 2009;47(3):1092-1104.
262. Barry RL, Coaster M, Rogers BP, et al. On the origins of signal variance in FMRI of the human midbrain at high field. *PLoS One.* 2013;8(4):e62708.
263. Shannon R, Baekey DM, Morris KF, Nuding SC, Segers LS, Lindsey BG. Production of reflex cough by brainstem respiratory networks. *Pulm Pharmacol Ther.* 2004;17(6):369-376.
264. Cole LJ, Farrell MJ, Duff EP, Barber JB, Egan GF, Gibson SJ. Pain sensitivity and fMRI pain-related brain activity in Alzheimer's disease. *Brain.* 2006;129(Pt 11):2957-2965.
265. Nie H, Arendt-Nielsen L, Andersen H, Graven-Nielsen T. Temporal summation of pain evoked by mechanical stimulation in deep and superficial tissue. *J Pain.* 2005;6(6):348-355.
266. Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp.* 2002;17(3):143-155.
267. Napadow V, Dhond R, Kennedy D, Hui KK, Makris N. Automated brainstem co-registration (ABC) for MRI. *Neuroimage.* 2006;32(3):1113-1119.
268. Andersson JL. How to estimate global activity independent of changes in local activity. *Neuroimage.* 1997;6(4):237-244.
269. 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(6):900-918.
270. Naidich TP, Haacke EM, Kollias SS, Sorensen AG, Duvernoy HM, Delman BN. Duvernoy's Atlas of the Human Brain Stem and Cerebellum : High-Field MRI: Surface Anatomy, Internal Structure, Vascularization and 3D Sectional Anatomy. 2009.
271. Paxinos G HX-F, Sengul G, and Watson C. *Organization of brainstem nuclei. In: The Human Nervous System 3rd Edition, edited by Mai J, and Paxinos GAcademic Press.* Acadmic Press, Elsevier; 2012.
272. Ando A, Mazzone SB, Farrell MJ. Altered neural activity in brain cough suppression networks in cigarette smokers. *Eur Respir J.* 2019;54(3).
273. Ando A, Smallwood D, McMahon M, Irving L, Mazzone SB, Farrell MJ. Neural correlates of cough hypersensitivity in humans: evidence for central sensitisation and dysfunctional inhibitory control. *Thorax.* 2016;71(4):323-329.
274. Farrell MJ, Mazzone SB. Are neural pathways processing airway inputs sensitized in patients with cough hypersensitivity? *Pulm Pharmacol Ther.* 2019;57:101806.
275. von Leupoldt A, Sommer T, Kegat S, et al. Dyspnea and pain share emotion-related brain network. *Neuroimage.* 2009;48(1):200-206.
276. Scherf T, Angenstein F. Postsynaptic and spiking activity of pyramidal cells, the principal neurons in the rat hippocampal CA1 region, does not control the resultant BOLD response: a combined electrophysiologic and fMRI approach. *J Cereb Blood Flow Metab.* 2015;35(4):565-575.
277. Ekstrom A. How and when the fMRI BOLD signal relates to underlying neural activity: the danger in dissociation. *Brain Res Rev.* 2010;62(2):233-244.
278. Attwell D, Iadecola C. The neural basis of functional brain imaging signals. *Trends Neurosci.* 2002;25(12):621-625.
279. Attwell D, Laughlin SB. An energy budget for signaling in the grey matter of the brain. *J Cereb Blood Flow Metab.* 2001;21(10):1133-1145.
280. Angenstein F. The actual intrinsic excitability of granular cells determines the ruling neurovascular coupling mechanism in the rat dentate gyrus. *J Neurosci.* 2014;34(25):8529-8545.
281. Angenstein F, Krautwald K, Scheich H. The current functional state of local neuronal circuits controls the magnitude of a BOLD response to incoming stimuli. *Neuroimage.* 2010;50(4):1364-1375.

282. Mathiesen C, Caesar K, Akgoren N, Lauritzen M. Modification of activity-dependent increases of cerebral blood flow by excitatory synaptic activity and spikes in rat cerebellar cortex. *J Physiol.* 1998;512 (Pt 2):555-566.
283. Hill PF, Seger SE, Yoo HB, et al. Distinct neurophysiological correlates of the fMRI BOLD signal in the hippocampus and neocortex. *J Neurosci.* 2021.
284. Ekstrom A, Suthana N, Millett D, Fried I, Bookheimer S. Correlation between BOLD fMRI and theta-band local field potentials in the human hippocampal area. *J Neurophysiol.* 2009;101(5):2668-2678.
285. Buzsaki G, Wang XJ. Mechanisms of gamma oscillations. *Annual review of neuroscience.* 2012;35:203-225.
286. Colgin LL, Denninger T, Fyhn M, et al. Frequency of gamma oscillations routes flow of information in the hippocampus. *Nature.* 2009;462(7271):353-357.
287. Colgin LL, Moser EI. Gamma oscillations in the hippocampus. *Physiology (Bethesda).* 2010;25(5):319-329.
288. Ray S, Maunsell JH. Different origins of gamma rhythm and high-gamma activity in macaque visual cortex. *PLoS Biol.* 2011;9(4):e1000610.
289. Colgin LL. Do slow and fast gamma rhythms correspond to distinct functional states in the hippocampal network? *Brain Res.* 2015;1621:309-315.
290. Bieri KW, Bobbitt KN, Colgin LL. Slow and fast gamma rhythms coordinate different spatial coding modes in hippocampal place cells. *Neuron.* 2014;82(3):670-681.
291. Nir Y, Fisch L, Mukamel R, et al. Coupling between neuronal firing rate, gamma LFP, and BOLD fMRI is related to interneuronal correlations. *Curr Biol.* 2007;17(15):1275-1285.
292. Ojemann GA, Corina DP, Corrigan N, et al. Neuronal correlates of functional magnetic resonance imaging in human temporal cortex. *Brain.* 2010;133(Pt 1):46-59.
293. Conner CR, Ellmore TM, Pieters TA, DiSano MA, Tandon N. Variability of the relationship between electrophysiology and BOLD-fMRI across cortical regions in humans. *J Neurosci.* 2011;31(36):12855-12865.
294. Hermes D, Miller KJ, Vansteensel MJ, Aarnoutse EJ, Leijten FS, Ramsey NF. Neurophysiologic correlates of fMRI in human motor cortex. *Hum Brain Mapp.* 2012;33(7):1689-1699.
295. Brooks JC, Faull OK, Pattinson KT, Jenkinson M. Physiological noise in brainstem FMRI. *Front Hum Neurosci.* 2013;7:623.
296. Brooks J, Faull O, Pattinson K, Jenkinson M. Physiological Noise in Brainstem fMRI. *Frontiers in Human Neuroscience.* 2013;7.
297. Wei P, Lan Z, Lv Z, Fan Y. Brainstem fMRI. *Encyclopedia.* 2021;1(1):4-11.
298. Enzmann DR, Pelc NJ. Brain motion: measurement with phase-contrast MR imaging. *Radiology.* 1992;185(3):653-660.
299. Cordes D, Turski PA, Sorenson JA. Compensation of susceptibility-induced signal loss in echo-planar imaging for functional applications. *Magn Reson Imaging.* 2000;18(9):1055-1068.
300. Whedon JM, Glassey D. Cerebrospinal fluid stasis and its clinical significance. *Altern Ther Health Med.* 2009;15(3):54-60.
301. Budgell BS, Bolton PS. Cerebrospinal fluid pressure in the anesthetized rat. *J Manipulative Physiol Ther.* 2007;30(5):351-356.
302. Maier SE, Hardy CJ, Jolesz FA. Brain and cerebrospinal fluid motion: real-time quantification with M-mode MR imaging. *Radiology.* 1994;193(2):477-483.
303. Mills EP, Alshelh Z, Kosanovic D, et al. Altered Brainstem Pain-Modulation Circuitry Connectivity During Spontaneous Pain Intensity Fluctuations. *J Pain Res.* 2020;13:2223-2235.
304. Ekstrom AD. Regional variation in neurovascular coupling and why we still lack a Rosetta Stone. *Philos Trans R Soc Lond B Biol Sci.* 2021;376(1815):20190634.
305. Sclocco R, Garcia RG, Kettner NW, et al. Stimulus frequency modulates brainstem response to respiratory-gated transcutaneous auricular vagus nerve stimulation. *Brain stimulation.* 2020;13(4):970-978.
306. Atkinson-Clement C, Maillet A, LeBars D, et al. Subthalamic nucleus stimulation effects on single and combined task performance in Parkinson's disease patients: a PET study. *Brain Imaging Behav.* 2017;11(4):1139-1153.

307. Bouhassira D, Bing Z, Le Bars D. Effects of lesions of locus coeruleus/subcoeruleus on diffuse noxious inhibitory controls in the rat. *Brain Res.* 1992;571(1):140-144.
308. Angenstein F, Kammerer E, Scheich H. The BOLD response in the rat hippocampus depends rather on local processing of signals than on the input or output activity. A combined functional MRI and electrophysiological study. *J Neurosci.* 2009;29(8):2428-2439.
309. Buzsaki G, Kaila K, Raichle M. Inhibition and brain work. *Neuron.* 2007;56(5):771-783.
310. Ackermann RF, Finch DM, Babb TL, Engel J, Jr. Increased glucose metabolism during long-duration recurrent inhibition of hippocampal pyramidal cells. *J Neurosci.* 1984;4(1):251-264.
311. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. *Nature.* 2001;412(6843):150-157.
312. Napadow V, Sclocco R, Henderson LA. Brainstem neuroimaging of nociception and pain circuitries. *Pain Rep.* 2019;4(4):e745.
313. Wargo CJ, Gore JC. Localized high-resolution DTI of the human midbrain using single-shot EPI, parallel imaging, and outer-volume suppression at 7T. *Magn Reson Imaging.* 2013;31(6):810-819.
314. Sclocco R, Beissner F, Desbordes G, et al. Neuroimaging brainstem circuitry supporting cardiovagal response to pain: a combined heart rate variability/ultrahigh-field (7 T) functional magnetic resonance imaging study. *Philos Trans A Math Phys Eng Sci.* 2016;374(2067).
315. Sclocco R, Beissner F, Bianciardi M, Polimeni JR, Napadow V. Challenges and opportunities for brainstem neuroimaging with ultrahigh field MRI. *Neuroimage.* 2018;168:412-426.
316. Satpute AB, Wager TD, Cohen-Adad J, et al. Identification of discrete functional subregions of the human periaqueductal gray. *Proc Natl Acad Sci U S A.* 2013;110(42):17101-17106.
317. Prats-Galino A, Soria G, de Notaris M, Puig J, Pedraza S. Functional anatomy of subcortical circuits issuing from or integrating at the human brainstem. *Clin Neurophysiol.* 2012;123(1):4-12.
318. Faull OK, Jenkinson M, Clare S, Pattinson KT. Functional subdivision of the human periaqueductal grey in respiratory control using 7 tesla fMRI. *Neuroimage.* 2015;113:356-364.
319. Deistung A, Schafer A, Schweser F, et al. High-Resolution MR Imaging of the Human Brainstem In vivo at 7 Tesla. *Front Hum Neurosci.* 2013;7:710.
320. Cho ZH, Kang CK, Son YD, et al. Pictorial review of in vivo human brain: from anatomy to molecular imaging. *World Neurosurg.* 2014;82(1-2):72-95.
321. Blazejewska AI, Schwarz ST, Pitiot A, et al. Visualization of nigrosome 1 and its loss in PD: pathoanatomical correlation and in vivo 7 T MRI. *Neurology.* 2013;81(6):534-540.
322. Bianciardi M, Toschi N, Edlow BL, et al. Toward an In Vivo Neuroimaging Template of Human Brainstem Nuclei of the Ascending Arousal, Autonomic, and Motor Systems. *Brain connectivity.* 2015;5(10):597-607.
323. Hahn A, Kranz GS, Seidel EM, et al. Comparing neural response to painful electrical stimulation with functional MRI at 3 and 7 T. *Neuroimage.* 2013;82:336-343.
324. Lee J, Mawla I, Kim J, et al. Machine learning-based prediction of clinical pain using multimodal neuroimaging and autonomic metrics. *Pain.* 2019;160(3):550-560.
325. Springer E, Bohndorf K, Juras V, et al. Comparison of Routine Knee Magnetic Resonance Imaging at 3 T and 7 T. *Invest Radiol.* 2017;52(1):42-54.
326. Colizoli O, de Gee JW, van der Zwaag W, Donner TH. Comparing fMRI responses measured at 3 versus 7 Tesla across human cortex, striatum, and brainstem. *bioRxiv.* 2020:2020.2005.2012.090175.
327. Mazzone SB, McGovern AE, Cole LJ, Farrell MJ. Central nervous system control of cough: pharmacological implications. *Curr Opin Pharmacol.* 2011;11(3):265-271.
328. Dicpinigaitis PV, Rhoton WA, Bhat R, Negassa A. Investigation of the urge-to-cough sensation in healthy volunteers. *Respirology.* 2012;17(2):337-341.
329. Moss MS, Basbaum AI. The peptidergic organization of the cat periaqueductal gray. II. The distribution of immunoreactive substance P and vasoactive intestinal polypeptide. *J Neurosci.* 1983;3(7):1437-1449.

330. Moss MS, Glazer EJ, Basbaum AI. The peptidergic organization of the cat periaqueductal gray. I. The distribution of immunoreactive enkephalin-containing neurons and terminals. *J Neurosci.* 1983;3(3):603-616.
331. Bastian RW, Vaidya AM, Delsupehe KG. Sensory neuropathic cough: a common and treatable cause of chronic cough. *Otolaryngol Head Neck Surg.* 2006;135(1):17-21.
332. Jeyakumar A, Brickman TM, Haben M. Effectiveness of amitriptyline versus cough suppressants in the treatment of chronic cough resulting from postviral vagal neuropathy. *Laryngoscope.* 2006;116(12):2108-2112.
333. Lee B, Woo P. Chronic cough as a sign of laryngeal sensory neuropathy: diagnosis and treatment. *Ann Otol Rhinol Laryngol.* 2005;114(4):253-257.
334. Mintz S, Lee JK. Gabapentin in the treatment of intractable idiopathic chronic cough: case reports. *Am J Med.* 2006;119(5):e13-15.
335. Dicpinigaitis PV. Experimentally induced cough. *Pulm Pharmacol Ther.* 2007;20(4):319-324.
336. Berman SM, Naliboff BD, Suyenobu B, et al. Reduced brainstem inhibition during anticipated pelvic visceral pain correlates with enhanced brain response to the visceral stimulus in women with irritable bowel syndrome. *J Neurosci.* 2008;28(2):349-359.
337. Bui T, J MD. Neuroanatomy, Cerebral Hemisphere. *StatPearls*. Treasure Island (FL)2021.
338. Curatolo M. Spatial summation of pain and its meaning to patients. *Scand J Pain.* 2017;17:116-117.
339. Price DD, McHaffie JG, Larson MA. Spatial summation of heat-induced pain: influence of stimulus area and spatial separation of stimuli on perceived pain sensation intensity and unpleasantness. *J Neurophysiol.* 1989;62(6):1270-1279.
340. Machet-Pietropaoli H, Chery-Croze S. Spatial summation of thermal pain in human beings. *Sens Processes.* 1979;3(2):183-187.
341. Kojo I, Pertovaara A. The effects of stimulus area and adaptation temperature on warm and heat pain thresholds in man. *Int J Neurosci.* 1987;32(3-4):875-880.
342. Douglass DK, Carstens E, Watkins LR. Spatial summation in human thermal pain perception: comparison within and between dermatomes. *Pain.* 1992;50(2):197-202.

Supplementary Material (Chapter 2)

EXTENDED METHODS SECTION

Image acquisition

Scanning was performed at Monash Biomedical Imaging (Melbourne, Australia) using a MAGNETOM Skyra 3 T scanner (Siemens, Erlangen, Germany) with a 32 channel head coil. Structural T1-wieghted images were acquired in the sagittal plane (192 slices, 1 mm slice thickness, 1 × 1 mm^2 in plane resolution, echo time (TE) of 2.07 ms, repetition time (TR) 1900 ms, flip angle 9^0). BOLD contrast images were acquired in the transaxial plane (34 slices, 4.5 mm slice thickness, 3 × 3 mm^2 in plane resolution, TE 31 ms, TR 2000 ms, flip angle 90^0), producing a total of 253 sequential volumes during 8 minutes and 26 seconds of scanning time. A total of three BOLD contrast scans were acquired for each participant.

During image acquisition, participants were supine on the scanner bed with their head immobilized with foam padding. Participants were fitted with hearing protection and a facemask connected to two nebulizers via which either capsaicin or saline were delivered. A hydraulic stimulator was used to apply pressure to the left thumbnail. An MRI compatible track ball mouse was placed under the right hand of participants. Participants were instructed to breath normally throughout the scanning session.

Visual stimuli generated with Presentation software (Presentation® V 21.1, Neuro Behavioral Systems 2019) were shown to participants on an MRI compatible screen outside the bore of the scanner that was visible through a mirror mounted on the head coil. Participants were informed of impending deliveries of tussive stimuli 3 s before each stimulus onset (the stimulus type was not disclosed). An 11 point numerical rating scale (0-10) appeared on the screen after each stimulus block. Participants were instructed to point and click on the number representing their rating of urge-to-cough or pain intensity using the MRI compatible mouse. Prompts for urge-to-cough ratings appeared six seconds after the conclusion of all stimulus blocks and remained on screen for five seconds. Prompts for pain ratings, also of five seconds duration, appeared on screen eleven seconds after the conclusion of stimulus blocks that included painful thumbnail pressure.

Image analysis

fMRI analysis was carried out using the fMRI Expert Analysis Tool (FEAT) in version 6.0.3 of the FMRIB Software Library (FSL). Anatomical and functional images were stripped of non-brain voxels using the Brain Extraction Tool (BET) [266]. Briefly, pre-processing of images consisted of realignment to correct for motion using a rigid body transformation, spatial smoothing with a 5mm full-width half maximum Gaussian kernel, and masking to remove non-brain tissue and high-pass temporal filtering with a cut-off of 0.01Hz. Regressors were constructed for each of the four conditions (capsaicin without pain "CN", capsaicin with pain "CP", saline without pain "SN", saline with pain "SP") as well as the rating, go and rest periods. For the first level of analysis, these regressors and their derivatives were convolved with a hemodynamic response function and used to generate a model for each run in the general linear model. In addition, other regressors of no interest were included to account for noise such as residual respiratory motion as described by Birn [261]. These included time-courses from CSF within the lateral ventricles, motion correction parameters, voxels of high standard deviation in the sagittal sinus and mean global signal from non-activated areas of the brain [261]. Contrasts were obtained for each of the four contingencies as well as the differences between CN>SN, CN>CP, SN>SP and SP>SN. Second level analysis involved fixed effects to amalgamate results from all three runs for each subject.

Group level analysis involved transforming individual's high resolution T1 weighted images into a standard space (2mm resolution) based on MNI152 (Montreal Neurological Institute) template. Mixed effects analysis was then performed using FLAME across individuals to generate a group result. All statistical maps used thresholds to include voxels with a Z-value>3.09 and a cluster probability of $p<0.05$, corrected for a multiple comparisons using Gaussian random field theory cluster-based correction as implemented in FEAT [269].

Supplementary Data

<u>Multi-variate analysis</u>

Analyses of urge-to-cough ratings and cough frequency reported in the manuscript included values there were averaged across multiple presentations. This analysis strategy was based on the absence of interactions between the repeated measures and the main experimental factor of conditioning when a full factorial model was used. The following

tables report the outcomes of the full factorial multivariate model for a) urge-to-cough ratings during the psychophysical session, b) cough frequencies during the psychophysical session, c) urge-to-cough ratings during the fMRI scanning sessions. The additional factors included in these models are "Order" (1st, 2nd presentation of stimulus) and Scan (1st, 2nd, and 3rd, fMRI scan). Estimated marginal means (±standard deviation of the mean) are provided for all significant factors and interactions between factors. Where required, post hoc tests of significance are provided to indicate the nature of interaction effects.

Psychophysical session

(Table 1A)

Full factorial multivariate model of urge-to-cough ratings associated with concentration, presentation order and conditioning including interactions between these factors are reported in this table.

	Degrees of Freedom	F-value	p-value
Concentration	(1,15)	113.50	2.16×10^{-6}
Order	(1,15)	5.00	0.041
Conditioning	(1,15)	22.20	2.88×10^{-4}
Concentration*Order	(1,15)	11.94	0.004
Concentration*Conditioning	(1,15)	1.88	0.191
Order*Conditioning	(1,15)	0.17	0.684
Concentration*Order*Conditioning	(1,15)	0.16	0.700

(Table 1B)

Mean urge-to-cough ratings and standard deviations (in brackets) associated with the two levels of capsaicin concentration.

Concentration	Urge-to-cough
C2	2.80 (1.27)
C2+2	5.22 (1.46)

(Table 1C)

Mean urge-to-cough ratings and standard deviations (in brackets) associated with the presentation order of capsaicin inhalations.

Order	Urge-to-cough
First	4.31 (1.59)
Second	3.70 (1.19)

(Table 1D)

Mean urge-to-cough ratings and standard deviations (in brackets) associated with the two levels of conditioning stimulus.

Conditioning	Urge-to-cough
No pain	4.38 (1.18)
Pain	3.63 (1.47)

(Table 1E)

Mean urge-to-cough ratings and standard deviations (in brackets) associated with the presentation order for the two concentration levels of capsaicin inhalations. Paired t-tests indicate the nature of the interaction between the factors of order and concentration.

Concentration	Order	Urge-to-cough	p-value (posthoc paired t-tests)
C2	First	3.51 (1.78)	p = 0.003
	Second	2.08 (1.17)	
C2+2	First	5.11 (1.56)	p = 0.498
	Second	5.33 (1.62)	

(Table 1F)

Full factorial multivariate model of cough frequency associated with concentration, presentation order and conditioning including interactions between these factors are reported in this table.

	Degrees of Freedom	F-value	p-value
Concentration	(1,15)	30.32	6.0×10^{-5}
Order	(1,15)	3.66	0.075
Conditioning	(1,15)	30.30	2.0×10^{-4}
Concentration*Order	(1,15)	5.25	0.037
Concentration*Conditioning	(1,15)	23.80	0.006
Order*Conditioning	(1,15)	3.27	0.091
Concentration*Order*Conditioning	(1,15)	1.27	0.276

(Table 1G)

Mean cough frequency and standard deviations (in brackets) associated with the two levels of capsaicin concentration

Concentration	Cough
C2	0.36 (0.48)
C2+2	1.59 (1.10)

(Table 1H)

Mean cough frequency and standard deviations (in brackets) associated with the two levels of conditioning stimulus

Conditioning	Cough
No Pain	1.28 (0.74)
Pain	0.67 (0.77)

(Table 1I)

Mean cough frequency and standard deviations (in brackets) associated with the presentation order for the two concentration levels of capsaicin inhalations. Paired t-tests indicate the nature of the interaction between the factors of order and concentration.

Concentration	Order	Cough	p-value (posthoc paired t-tests)
C2	First	0.66 (0.91)	p = 0.018
	Second	0.06 (0.17)	
C2+2	First	1.59 (1.10)	p = 1.000
	Second	1.59 (3.82)	

(Table 1J)

Mean cough frequency and standard deviations (in brackets) associated with the level of conditioning for the two levels of concentration stimulus. Paired t-tests indicate the nature of the interaction between the factors of concentration and conditioning.

Concentration	Conditioning	Cough	p-value (posthoc paired t-tests)
C2	No Pain	0.47 (0.62)	p = 0.186
	Pain	0.25 (0.52)	
C2+2	No Pain	2.09 (1.16)	$p = 8.7 \times 10^{-5}$
	Pain	1.09 (1.16)	

fMRI session

(Table 2A)

Full factorial multivariate model of urge-to-cough ratings frequency associated with the type of stimulus, conditioning, presentation order and scan including interactions between these factors are reported in this table.

	Degrees of Freedom	F-value	p-value
Stimulus	(1,15)	153.64	2.77×10^{-9}
Conditioning	(1,15)	5.90	0.044
Order	(1,15)	5.12	0.039
Scan	(1,15)	1.14	0.347
Stimulus*Conditioning	(1,15)	19.50	0.001
Stimulus*Order	(1,15)	3.77	0.071
Conditioning*Order	(1,15)	0.73	0.407
Stimulus*Conditioning*Order	(1,15)	0.04	0.852
Stimulus*Scan	(1,15)	0.92	0.422
Conditioning*Scan	(1,15)	1.12	0.354
Stimulus*Conditioning*Scan	(1,15)	0.37	0.698
Order*Scan	(1,15)	1.86	0.192
Stimulus*Order*Scan	(1,15)	2.35	0.132
Conditioning*Order*Scan	(1,15)	1.14	0.349
Stimulus*Conditioning*Order*Scan	(1,15)	0.80	0.471

(Table 2B)

Mean urge-to-cough ratings and standard deviations (in brackets) associated with the type of inhaled substance (Stimulus).

Stimulus	Urge-to-cough
Capsaicin	3.34 (0.82)
Saline	0.41 (0.41)

(Table 2C)

Mean urge-to-cough ratings and standard deviations (in brackets) associated with the two levels of conditioning stimulus.

Conditioning	Urge-to-cough
No Pain	2.07 (0.55)
Pain	1.68 (0.60)

(Table 2D)

Mean urge-to-cough ratings and standard deviations (in brackets) associated with the presentation order of inhaled stimuli.

Order	Urge-to-cough
First	2.02 (0.54)
Second	1.73 (0.48)

(Table 2E)

Mean urge-to-cough ratings and standard deviations (in brackets) associated with the level of conditioning for the two types of test stimulus. Paired t-tests indicate the nature of the interaction between the factors of stimulus and conditioning.

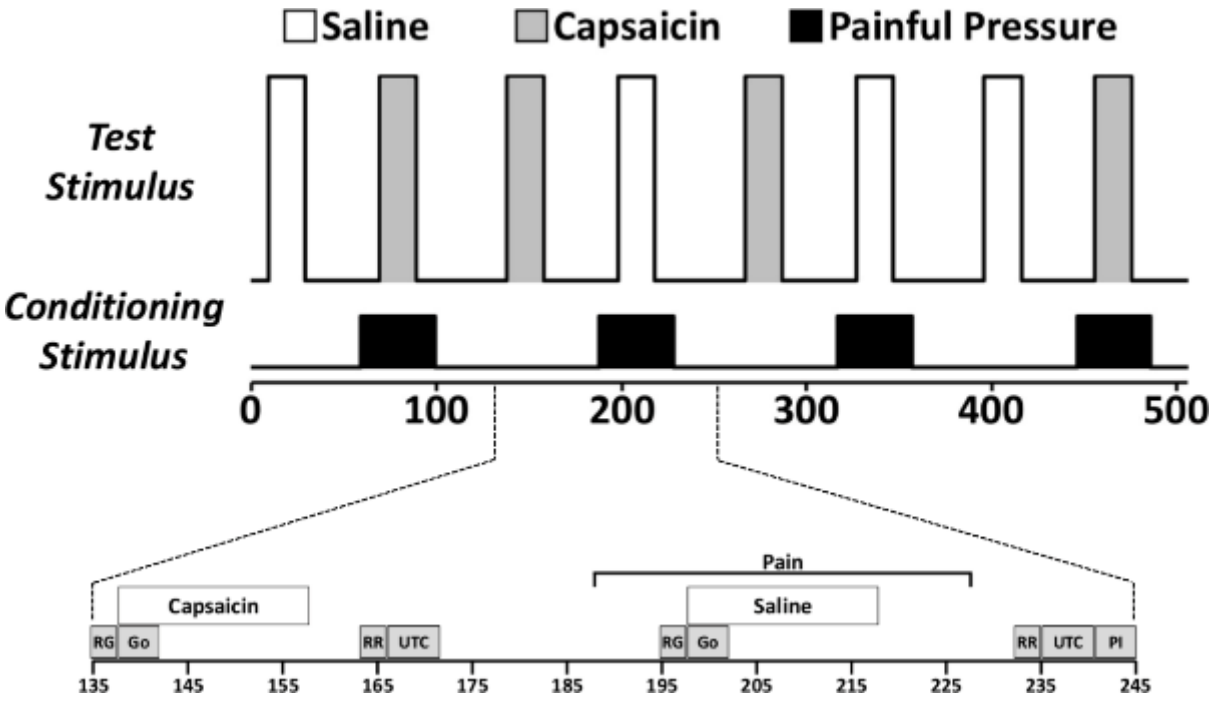
Stimulus	Conditioning	Urge-to-cough	p-value (posthoc paired t-tests)
Capsaicin	No Pain	3.91 (1.30)	p = 0.004
	Pain	2.75 (1.30)	
Saline	No Pain	0.24 (0.34)	p = 0.001
	Pain	0.57 (0.52)	

Table 2. ***Brain regions activated during capsaicin inhalation with saline used as a contrast.***

	MNI coordinates*			
Region	*x*	*y*	*z*	*Z-score*
Operculum	-44	4	16	7.14
	42	20	2	6.40
Cerebellum	36	-60	-26	4.68
	36	-48	-28	4.85
	18	-64	-18	4.92
	6	-50	-8	4.70
	-4	-60	-32	4.75
Thalamus	-6	-16	4	4.42
	8	-4	12	4.29
Insula	-36	10	0	4.89
	40	10	0	4.57
Postcentral	-54	-12	28	4.27
	60	-16	32	4.82
Occipital Fusiform Gyrus	18	-64	-18	4.92
Lingual Gyrus	20	-62	-12	4.69
Frontal Lobe	-36	46	24	5.00
	-28	48	38	5.53
	-24	50	40	4.95
	28	50	26	7.40
	36	48	30	5.11
	32	50	30	4.87
Paracingulate	-6	10	46	3.86
	6	10	46	4.97
Cingulate Cortex	-4	-12	40	6.34
	56	2	46	6.28
Stopplementary Motor Area	-4	0	58	4.87
	4	2	62	4.94
Stopramarginal Gyrus	-62	-24	32	6.23
	64	-24	32	4.74
Precentral Cortex	-52	-10	36	6.53
	56	2	46	6.28

* The coordinates correspond to the Montreal Neuroscience Institute standard brain template where *x* values are distance in millimetres to the left (negative x values) or right (positive x values) from the anterior commissure; y represents millimetre distance anterior (positive) or posterior (negative) from the anterior commissure, and z is millimetre distance superior (positive) or inferior (negative) from the anterior commissure. The magnitudes of the peaks of clusters are represented by Z statistics. The coordinates of the voxel with the highest level of activation within the cluster are listed.

Supplementary Figures



(A) Scan order:

Run 1:	*SN*	*CP*	*CN*	*SP*	*CN*	*SP*	*SN*	*CP*
Run 2:	*CN*	*SP*	*SN*	*CP*	*CN*	*SP*	*SN*	*CP*
Run 3:	*SN*	*SP*	*CN*	*CP*	*SN*	*CP*	*CN*	*SP*

(B) Capsaicin challenge order:

1. C2+ 2 (NP)	*2. C2 (P)*	*3. C2 (NP)*	*4. C2 + 2 (P)*
5. C2 (P)	*6. C2 + 2 (NP)*	*7. C2 (NP)*	*8. C2 + 2 (P)*

Figure 1: **Experimental protocol for fMRI scanning session.** The topper panel shows one example of the three fMRI scans that were performed for each participant. A 2 × 2 factorial design involving the factors of conditioning (pain/no pain) and test stimuli (capsaicin/saline) was used in association with measurement of regional brain BOLD signal changes. The expanded panel shows one of the four cycles of stimuli delivered during an fMRI scan. A visual cue of three seconds duration was used to prepare participants prior to the onset of all inhaled stimuli (RG - ready to go). This strategy reduces the risk of uncontrolled coughing. Go cues (Go) for capsaicin and saline challenges ensured that participants initiated their inhalations at consistent time points throughout the sequence. Visual cues prompted participants to prepare to provide ratings (RR – ready to rate), after which numerical rating scales (0-10) appeared on screen. Participants rated urge-to-cough (UTC) and pain intensity (PI). *(A)* Sequences of stimuli for each of the three scans are shown. This approach in sequencing was used to achieve randomization. There was a 20 second resting period after each cycle of stimuli delivered during the fMRI scan. *(B)* The sequence of capsaicin challenge for C2 and C2+2 were used as test stimuli in a conditioning pain experiment. Urge-to-cough ratings and cough frequency were recorded after each challenge. *(Abbreviations: "SN": saline without pain, "SP": saline with pain, "CN": capsaicin without pain, "CP": capsaicin with pain, "NP": without pain, "P": with pain, "C2": thresholds for two coughs, "C2+2": two dose increments above C2).*

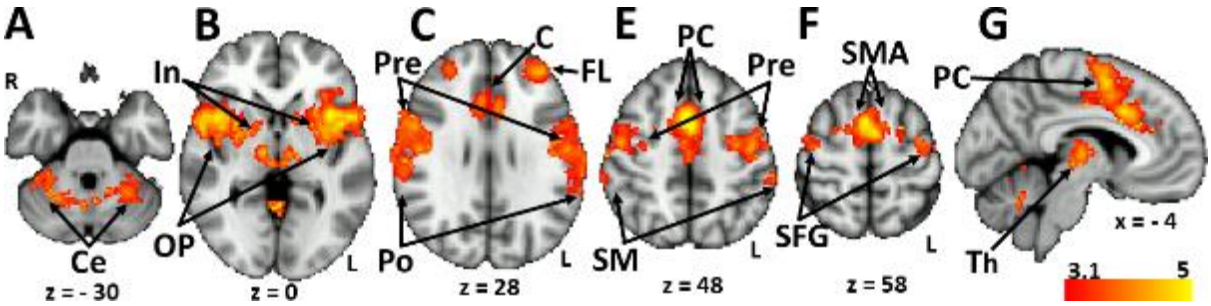


Figure 2: Brain regions activated during capsaicin inhalation compared to saline inhalation (CN>SN). Such regions include the cerebellum (Ce), insula cortex (In), operculum (OP), precentral gyrus (Pre), postcentral gyrus (Po), cingulate gyrus (C), paracingulate gyrus (PC), superior frontal gyrus (SFG), frontal lobe (FL), thalamus (Th) and the supplementary motor area (SMA). These regions were warped to the standard dimensions of the Montreal Neuroscience Institute template brain.

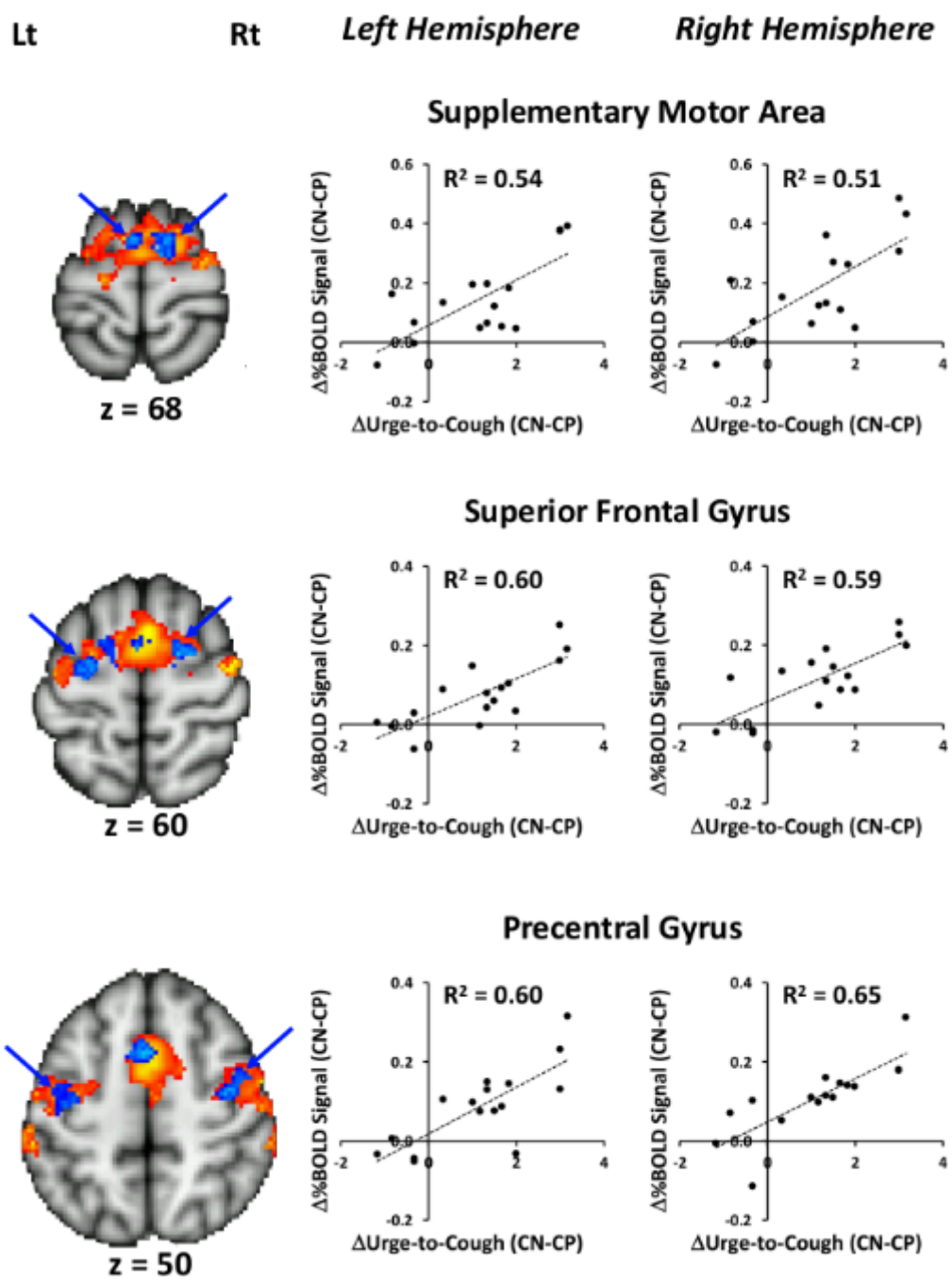


Figure 3: Correlations between regional CN>CP activations and conditioning pain-related changes in UTC. Regions within the CN>SN network showed levels of CN>CP activation in the participants that were positively correlated with differences in UTC levels for the two contingencies (CN-CP). Regions showing a correlation between activation and behavior were found bilaterally in the PreCG, SFG, SMA and the left ParCG. These regions were warped to the standard dimensions of the Montreal Neuroscience Institute template brain.

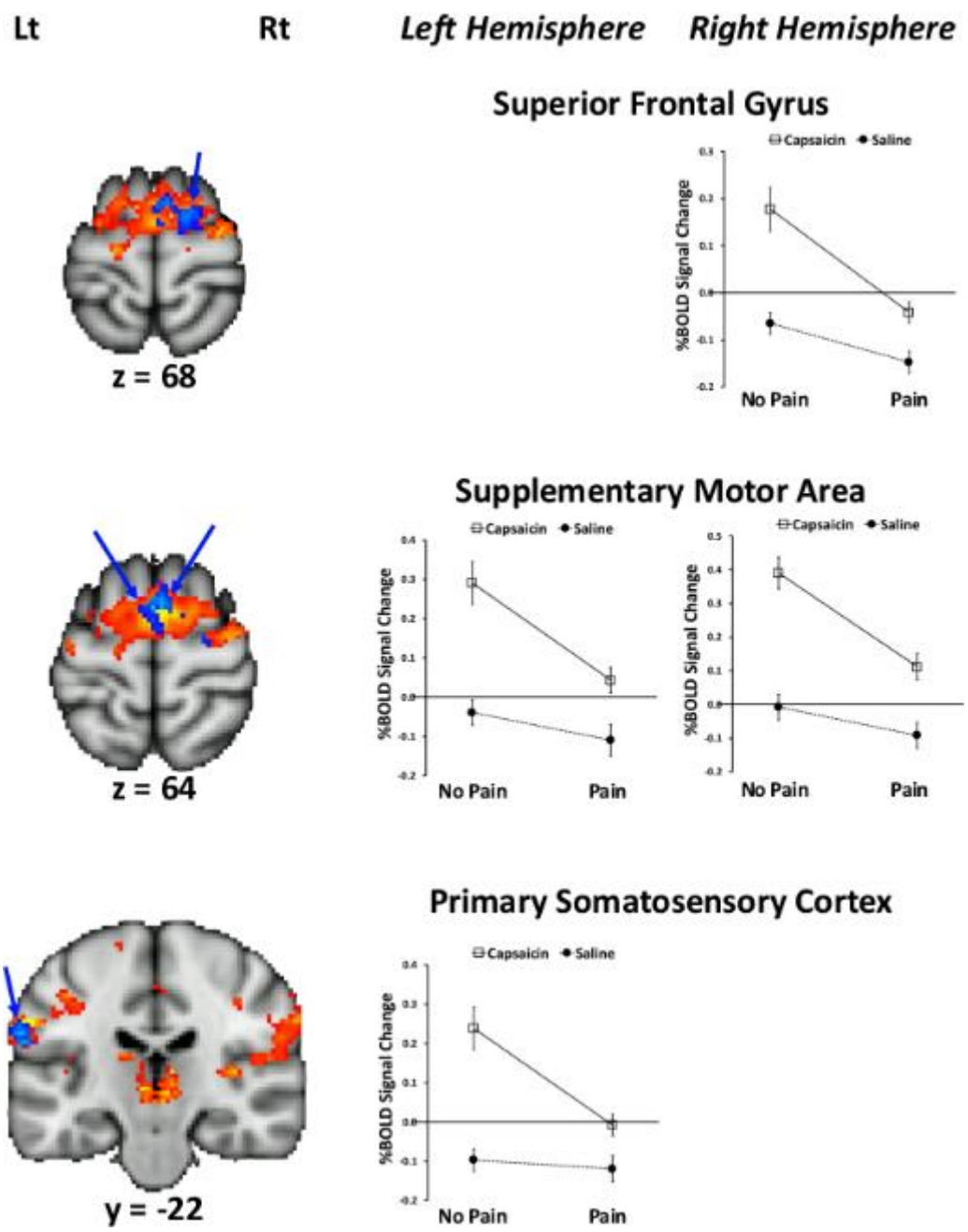


Figure 4A: Brain regions showing negative interaction between CN, CP, SN, SP. Regions within the CN>SN network showed a negative interaction between conditioning and inhalation stimuli, whereas no regions showed a positive interaction. Regions showing a negative interaction included the bilateral OP, SMA and In, right ParCG and SFG, and left PosCG. These regions were warped to the standard dimensions of the Montreal Neuroscience Institute template brain.

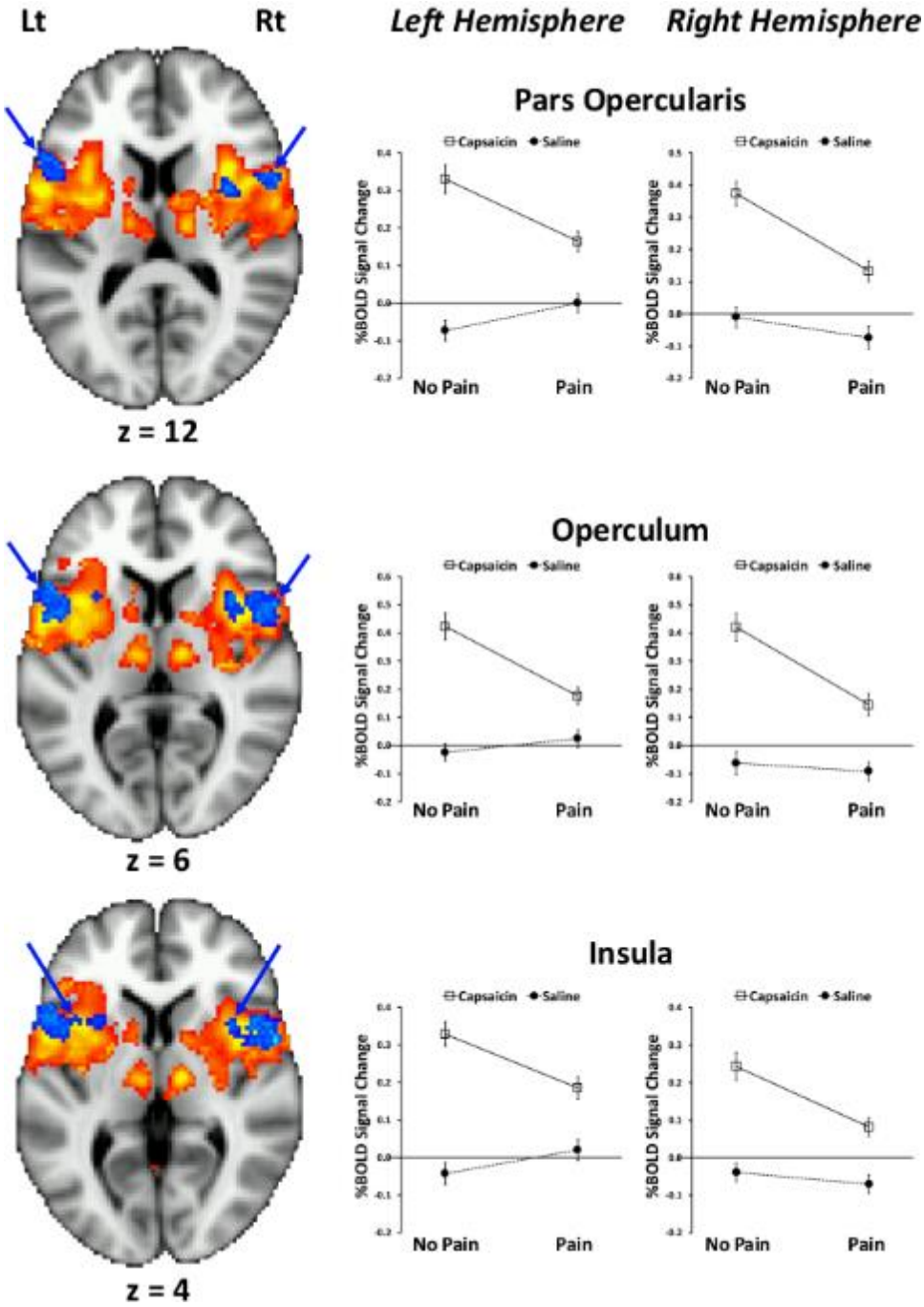


Figure 4B: Brain regions showing negative interaction between CN, CP, SN, SP. Regions within the CN>SN network showed a negative interaction between conditioning and inhalation stimuli, whereas no regions showed a positive interaction. Regions showing a negative interaction included the bilateral OP, SMA and In, right ParCG and SFG, and left PosCG. These regions were warped to the standard dimensions of the Montreal Neuroscience Institute template brain.