Neuroimaging pain and analgesia mechanisms in experimental and chronic pain states

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Abstract

Chronic pain is a common and debilitating condition, affecting up to half of the UK population. Despite the use of both pharmacological and psychological/cognitive interventions, it is difficult to treat, and there is a clear need for new, effective therapies. The subjective nature of pain reporting makes the discovery and validation of new treatments challenging, but this could be improved by the presence of an objective biomarker of analgesic efficacy.

In this thesis I looked at the use of functional magnetic resonance imaging (fMRI) as such a biomarker, and as a technique for understanding the mechanisms by which interventions might achieve therapeutic effects. This was achieved through the investigation of two novel treatments for pain, the first being a pharmacological agent, and the second a cognitive therapy.

A randomised controlled trial was set up comparing ABX-1431, a first-in-class pharmacological agent that acts on the endocannabinoid system, with both placebo and nothing. Although it was not possible to show analgesic efficacy using behavioural outcomes, the presence of sedation in the active drug arm indicated a likely pharmacodynamic effect. It was possible to confirm this effect using functional magnetic resonance imaging (fMRI). Since the mechanism of action did not match that of existing analgesic agents, it was not possible to confirm analgesic efficacy, but the presence of altered activity in and connectivity between regions known to have a key role in descending pain modulation, including the lateral prefrontal cortex and right amygdala, indicates that ABX-1431 could impact chronic pain through these mechanisms, and that further trials are warranted.

Next, a prospective study of mindfulness-based cognitive therapy for irritable bowel syndrome (MBCT-IBS) was performed, in which behavioural data showed that it was an effective therapy for chronic visceral pain. Imaging showed that like ABX-1431, MBCT-IBS led to altered activity in regions of the brain known to have a role in descending pain modulation, including the right amygdala and dorsolateral prefrontal cortex. There was evidence of modulated functional connectivity between these regions and other cortical and subcortical regions important for pain perception. Activity and connectivity within the default mode network (DMN) was also affected, as was connectivity between the DMN and sensory cortices, indicating that MBCT-IBS might act by modulating intrinsic attentional networks as well as by influencing the top-down control of pain.

In combination, these studies have shown that fMRI is a valuable tool for elucidating the mechanisms of novel analgesic therapies across both pharmacological and cognitive modalities. The fact that neither intervention produced the imaging pattern seen in classical analgesia meant that fMRI was unable to provide an objective biomarker of efficacy on this occasion, but in both cases it was possible to show clear mediation of neural function in regions relating to descending pain control. It may be that in chronic pain, an objective biomarker of efficacy will look different to that seen in acute pain, and this is something worthy of further investigation.

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List of abbreviations

2-AG	2-arachidonoyl glycerol
ACC	Anterior cingulate cortex
AC-PC	Anterior commissure – posterior commissure
ADD	Attention deficit disorder
ADHD	Attention deficit hyperactivity disorder
ADR	Adverse drug reaction
AE	Adverse event
AEA	Arachidonoyl ethanolamine
aINS	Anterior insula
ALFF	Amplitude of low-frequency fluctuations
ALS	Advanced life support
aMCC	Anterior midcingulate cortex
ANOVA	Analysis of variance
ASL	Arterial spin labelling
B_0	Static magnetic field
BAS	Behavioural activation system
BASIL	Bayesian Inference for Arterial Spin Labelling MRI
BET	Brain extraction tool
BOLD	Blood oxygen level dependent
BIS	Behavioural inhibition system
BLA	Basolateral amygdala
BPI	Brief pain inventory (short form)
BW	Bandwidth
CB1	Cannabinoid receptor type 1

CB2	Cannabinoid receptor type 2
CBF	Cerebral blood flow
CBT	Cognitive behavioural therapy
CeA	Central nucleus of the amygdala
CEN	Central executive network
CHEPS	Contact heat evoked potential stimulator
CI	Confidence interval
CNS	Central nervous system
CPM	Conditioned pain modulation
CrCl	Creatinine clearance
CSF	Cerebrospinal fluid
CTA	Clinical Trials Authorisation
dlPAG	Dorsolateral periaqueductal gray
dlPFC	Dorsolateral prefrontal cortex
DMN	Default mode network
dmPFC	Dorsomedial prefrontal cortex
DNIC	Diffuse noxious inhibitory controls
dpINS	Dorsal posterior insula
DRt	Dorsal reticular nucleus
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	Electrocardiogram
EPI	Echo-planar imaging
FAAH	Fatty acid amide hydrolase
FCA	Fear-conditioned analgesia
FDA	U.S. Food and Drug Administration

FEAT	FMRI expert analysis tool
FGID	Functional gastrointestinal disease
FILM	FMRIB's improved linear model
FIX	FMRIB's ICA-based Xnoiseifier
FLAME	FMRIB's local analysis of mixed effects
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	Functional magnetic resonance imaging
FMRIB	Oxford Centre for Functional MRI of the Brain
FNIRT	FMRIB's nonlinear image registration tool
FOV	Field of view
FSL	FMRIB's software library
FWHM	Full width at half maximum
GABA	Gamma-aminobutyric acid
GCP	Good clinical practice
GLM	General linear model
GP	General practitioner
GSRS	Gastrointestinal symptom rating scale
GUI	Graphical user interface
HADS	Hospital anxiety and depression scale
HADS-A	HADS anxiety subscale
HADS-D	HADS depression subscale
HRA	Health Research Authority
5-HT	5-Hydroxytrptamine
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome

IBS-IS	Irritable bowel syndrome impact scale
ICA	Independent component analysis
IMP	Investigational medicinal product
IRAS	Integrated research application system
LC	Locus coeruleus
lPAG	Lateral periaqueductal gray
MBCT	Mindfulness-based cognitive therapy
MBCT-IBS	Mindfulness-based cognitive therapy for irritable bowel syndrome
MBSR	Mindfulness-based stress reduction
MCC	Midcingulate cortex
McFlirt	Motion correction using FMRIB's linear image registration tool
MCS	Mental component summary
MIA	Mechanically insensitive afferent
MELODIC	Multivariate exploratory linear optimized decomposition into independent components
MGLL	Monoacylglycerol lipase
MHRA	Medicines and healthcare products regulatory agency
MI	Primary motor cortex
MNI	Montreal Neurological Institute
mPFC	Medial prefrontal cortex
MRI	Magnetic resonance imaging
NAc	Nucleus accumbens
NHS	National Health Service

NRS	Numerical rating scale
NSAID	Non-steroidal anti-inflammatory drug
OCD	Obsessive compulsive disorder
OFC	Orbitofrontal cortex
OUH	Oxford University Hospitals
PAG	Periaqueductal gray
PBN	Parabrachial nucleus
pCASL	Pseudocontinuous arterial spin labelling
PCC	Posterior cingulate cortex
PK	Pharmacokinetics
PCS	Physical component summary
PET	Positron emission tomography
PFC	Prefrontal cortex
pINS	Posterior insula
PNM	Physiological noise monitoring
rACC	Rostral anterior cingulate cortex
RCT	Randomised controlled trial
REC	Research ethics committee
ROI	Region of interest
RVM	Rostral ventromedial medulla
SD	Standard deviation
SEM	Standard error of the mean
SF-36	36 item short form survey
SI	Primary somatosensory cortex
SIA	Stress-induced analgesia

SII	Secondary somatosensory cortex
SN	Salience network
SPSS	Statistical Package for the Social Sciences
SpV	Trigeminal nucleus
STAI	State-trait anxiety inventory
STT	Spinothalamic tract
$T_{1/2}$	Half life
tDCS	Transcranial direct current stimulation
TE	Echo time
TFCE	Threshold-free cluster enhanced
THC	Tetrahydrocannabinol
Tmax	Time to maximum plasma concentration
TMF	Trial master file
TOPS	The Over-volunteering Prevention System
TR	Repetition time
TRPV1	Transient receptor potential vanilloid 1
VAS	Visual analogue scale
VLM	Caudal ventrolateral medulla
vlPAG	Ventrolateral periaqueductal gray
vlPFC	Ventrolateral prefrontal cortex
VSI	Visceral sensitivity index

Chapter 1: Introduction

Research into pain and analgesia is vitally important. This chapter will firstly introduce the reasons for this, before outlining the basic biology of pain, focussing on supraspinal pain processing and modulation. It will then discuss the current management of pain, and the need for better analgesics. Finally, it will address the challenges associated with the discovery of new pain treatments, and consider how an objective biomarker of therapeutic efficacy might assist with this.

1.1 Why is the study of pain and analgesia important?

Pain is defined as “an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage” (IASP, 2020). Acute pain is important for survival. It enables the rapid detection and avoidance of a potential threat that may lead to tissue injury. Indeed, individuals with congenital analgesia, a hereditary condition that renders them incapable of feeling pain, experience shortened life expectancy due to repeated injury (Bennett & Woods, 2014).

However, if pain becomes chronic it can become maladaptive. Chronic pain is pain that persists or recurs for more than three months. It can be a disease in its own right, or can be secondary to another condition (Nicholas et al., 2019). Pain may persist due to an ongoing nociceptive input, but often it can persist beyond the point where the underlying pathology has resolved. In some cases, there is no clear physical cause found, and it is often then referred to as ‘functional pain’. A range of mechanisms contribute to the development of chronic pain, including central sensitization, descending facilitation, and reduced descending inhibition.

Chronic pain is a major global health problem. A 2016 meta-analysis estimated that a third to half of the UK population are affected by chronic pain (Fayaz, Croft, Langford, Donaldson, & Jones, 2016), and it is one of the leading causes of disability worldwide (Vos et al., 2015). Existing treatments for chronic pain are often unsatisfactory. In a large European survey, 40% of chronic pain sufferers reported inadequate pain management (Breivik, Collett, Ventafridda, Cohen, & Gallacher, 2006). Therefore, the development of effective treatments for chronic pain is of the utmost importance. This can be challenging, for reasons outlined later in this chapter. However, neuroimaging has the potential to assist in this process through uncovering the mechanisms of pain and analgesia as well as providing an objective means of assessing treatment responses.

1.2 The biology of pain

The pain experience is a composite of nociceptive input and the multiple factors that influence it from the periphery to the brain. Therefore, the relationship between input and perception is non-linear, and a consistent input may be perceived very differently between individuals. Factors that can influence the perception of a noxious stimulus include peripheral and central sensitization; emotional state, including mood and anxiety; and cognitive processes, such as attention, expectation, and reappraisal (Katja Wiech, Ploner, & Tracey, 2008). These factors can affect sensory-discriminative aspects of pain perception, such as intensity and location; as well as motivational-affective aspects, such as unpleasantness and the need to seek relief. The way that some of these factors work to make pain a unique experience will be explored in more detail below.

Somatic pain and visceral pain have different characteristics (Bielefeldt & Gebhart, 2013; Van Oudenhove et al., 2020). Visceral pain tends to be diffuse and poorly localised, and is commonly referred to distant cutaneous regions of the body. It is also less well correlated with stimulus intensity than somatic pain. It is associated with strong emotional and autonomic responses. These differences relate to differences in pain processing at various levels, from the periphery to the brain.

1.2.1 Peripheral nociception

1.2.1.1 Somatic nociception

Pain detection starts with a noxious stimulus at the nociceptor. Nociceptors are the free nerve endings of primary sensory neurons which terminate in laminae I, II and V of the dorsal horn of the spinal cord (or in the trigeminal nucleus [SpV] for facial stimuli), where they synapse with second-order neurons that project to the brain. These primary afferents are polymodal (responding to thermal, mechanical and chemical noxious stimuli), though some types are more suited to the detection of certain stimulus types. When a noxious stimulus comes into contact with a nociceptor, it activates ion channels on the nerve ending, causing depolarisation and the propagation of an action potential along the primary afferent. The frequency of the action potential varies according to the intensity of the stimulus (Kendroud, Fitzgerald, Murray, & Hanna, 2022). Nociceptors are excitatory neurons, and release glutamate and neuropeptide neurotransmitters onto ascending second-order neurons. Primary afferents also synapse with inhibitory interneurons which use gamma-aminobutyric acid (GABA) and/or glycine to produce local inhibition of nociceptive input at the level of the dorsal horn (Hughes & Todd, 2020).

The two main classes of nociceptor are the C fibres, and the Aδ fibres. Both types have high activation thresholds, meaning that they are specific to noxious rather than non-noxious stimuli. Aβ fibres also exist, but these large diameter, myelinated fibres have a low activation threshold, and therefore respond to non-noxious stimuli such as light touch.

Aδ fibres are lightly myelinated and have a medium diameter. They conduct faster than C fibres and invoke a rapid, well-localised pain sensation. They can be divided into two subtypes. Type I fibres have a high heat threshold (>53°C) but are sensitive to mechanical stimuli, while type II fibres have a higher mechanical threshold and a lower thermal threshold (Ringkamp, Raja, CAmpbell, & Meyer, 2013). Working together, the two subtypes are ideally placed to provide the early pain response to a noxious stimulus that warns of immediate danger. Aδ fibres release the excitatory neurotransmitter glutamate onto second-order neurons (Kendroud et al., 2022).

C fibres are unmyelinated and small in diameter, resulting in slower conduction. They are responsible for a slower, longer-lasting and more diffuse burning sensation which warns of ongoing noxious input (Ringkamp et al., 2013). C fibres release neuropeptide neurotransmitters onto second-order neurons (Kendroud et al., 2022).

1.2.1.2 Visceral nociception

Mechanical visceral pain tends to result from distension of hollow organs and traction on the mesentery rather than from a direct mechanical insult such as crushing of the organ. Other stimuli that can trigger visceral pain include thermal stimuli, ischaemia, and chemicals produced during inflammation (Bielefeldt & Gebhart, 2013).

Like somatic afferents, most primary visceral afferents synapse with second order spinal neurons in the in the dorsal horn of the spinal cord. They travel to the spinal cord alongside sympathetic efferents within the splanchnic nerves (or alongside parasympathetic efferents in the case of the distal colon). However, a feature specific to visceral afferents is that many of them pass through prevertebral and paravertebral ganglia prior to entering the spinal cord. In the prevertebral ganglia, they give off collaterals that synapse with secretory and motor neurons. From the paravertebral ganglia the afferents can travel up or down the sympathetic trunk and enter distant segments of the cord (Dostrovsky & Craig, 2013). Additionally, a small number of visceral afferents are capable of providing input to a large number of second order neurons (Dostrovsky & Craig, 2013; Giamberardino, Affaitati, Lerza, & De Laurentis, 2002). This divergence of afferents contributes to the diffuse nature of visceral pain.

Visceral input to the spinal cord is also characterised by convergence. Spinal neurons may receive input from multiple visceral sites, contributing further to pain diffusivity and poor localisation. Neurons receiving input from visceral afferents may also receive somatic input (Dostrovsky & Craig, 2013). This explains the occurrence of referred pain, where visceral pain is felt in a distant somatic site.

Unlike the skin, the viscera are also innervated by vagal afferents. Vagal afferents are thought to be important for mediating autonomic and emotional responses to noxious stimuli (Grundy, 2002), though they may also have a role in the modulation of visceral nociception (S. L. Chen et al., 2008). The majority of vagal afferents terminate in the medulla, specifically the nucleus of the solitary tract (Bielefeldt & Gebhart, 2013; Critchley & Harrison, 2013). From there, second order neurons travel to the

parabrachial nucleus (PBN), the periaqueductal gray (PAG) and the thalamus, from where there are outputs to cortical and subcortical regions (Ruffle et al., 2018).

Like cutaneous nociceptors, visceral nociceptive afferents consist of thinly-myelinated Aδ fibres and unmyelinated C fibres. They respond to mechanical, thermal and chemical input. Mechanoreceptors are the best understood of these, and can be divided into two main classes of receptor. High-threshold mechanoreceptors are nociception-specific. Low threshold mechanoreceptors are intensity-coding receptors, responding to a wide range of both noxious and innocuous stimuli (Sikandar & Dickenson, 2012).

1.2.1.3 Silent nociceptors

Mechanically insensitive afferents (MIAs) are a subgroup of C-fibre nociceptors which do not fire under normal circumstances, but acquire mechanosensitivity following tissue injury (Foreman, 2004; Sikandar & Dickenson, 2012). They comprise approximately 30% of the C-fibres found in viscera and joints, and less than a quarter of those in skin (Middleton et al., 2021; Nees et al., 2022), indicating their importance in visceral nociception. MIAs may be sensitized by tonic pressure or by exposure to chemical inflammatory mediators (Namer et al., 2015; Prato et al., 2017; Schmidt, Schmelz, Torebjörk, & Handwerker, 2000). This ability to acquire mechanosensitivity is likely to be important in the development of hyperalgesia/hypersensitivity in disease states. In mouse models of neuropathic pain and chemically-induced colorectal hypersensitivity, there is increased activity in MIAs (Feng et al., 2012; Serra et al., 2012). Meanwhile, people with painful peripheral neuropathy or fibromyalgia have a

higher percentage of sensitized C-nociceptors compared to controls (Kleggetveit et al., 2012; Serra et al., 2013).

MIAs become sensitized in response to the administration of intracutaneous capsaicin, and the timing of this activation matches the timing of the capsaicin-induced pain (Schmelz, Schmid, Handwerker, & Torebjörk, 2000), showing that MIAs have an important role in capsaicin-induced hyperalgesia. This is a phenomenon that is commonly used as a model of central sensitization in human pain research.

1.2.2 Ascending spinal tracts

Nociceptive information is transmitted from the dorsal horn to the brain via multiple ascending tracts (Dostrovsky & Craig, 2013).

1.2.2.1 Spinothalamic tract

The spinothalamic tract (STT) originates in the dorsal horn, where primary afferent fibres synapse with second order neurons. The axons of the second order neurons decussate via the anterior commissure within one to two segments of their origin, and ascend in the contralateral spinal cord. They synapse with third order neurons in the PAG and thalamic nuclei, which project to pain-related regions in the cortex. Thalamic neurons often receive convergent input from somatic neurons, and some receive convergent visceral input.

The STT has lateral and anterior components. The lateral STT projects mainly from the superficial dorsal horn (lamina I). It is somatotopically organised, with axons from rostral areas ascending medially, and those from caudal areas ascending more

laterally. Nociceptive neurons from lamina I are of two main types. Nociceptive specific neurons receive input primarily from Aδ fibres, while polymodal neurons receive input primarily from C fibres. Both have high thresholds and are specific to noxious stimuli. The anterior STT projects mainly from deeper layers of the dorsal horn (laminae IV-V). Most are wide dynamic range neurons that respond to a range of stimulus intensities, and are not specific to the detection of noxious stimuli. They receive input from Aβ fibres in addition to nociceptors.

1.2.2.2 Spinobulbar tracts

Spinobulbar projections originate from laminae I, IV and V of the dorsal horn and project to areas of the brainstem, including the PAG, the PBN, the reticular formation, and areas containing catecholamine cell groups such as the locus coeruleus (LC) and the nucleus of the solitary tract. These projections influence both spinal and forebrain activity, and may be important for the integration of nociception with mechanisms for homeostasis and behavioural control (Dostrovsky & Craig, 2013; Tracey & Mantyh, 2007).

1.2.3 The brainstem and its role in the intrinsic control of pain states

Descending control systems involve the brainstem, and many are controlled by higher centres (Lockwood & Dickenson, 2020). These are the systems by which the brain is able to control pain perception by influencing pain processing at the level of the spinal cord. ‘Top-down’ endogenous pain control can be facilitatory or inhibitory, and the balance between the two is vital in determining how noxious inputs are perceived. This balance can be pathologically altered in chronic pain conditions (Ossipov, Morimura, & Porreca, 2014).

1.2.3.1 The periaqueductal gray – rostral ventromedial medulla – dorsal horn pathway

The PAG – rostral ventromedial medulla (RMV) - dorsal horn pathway is thought to be the most dominant pathway for the central modulation of peripheral nociceptive input, although multiple other brainstem nuclei also form part of this complex system. The PAG is situated around the cerebral aqueduct in the midbrain, while the RVM is located in the medulla. In animals, electrical stimulation of both regions causes reduced nociceptive behaviour (H. Fields & Heinricher, 2013; Reynolds, 1969).

In animals, the PAG is comprised of four functional columns (Coulombe, Erpelding, Kucyi, & Davis, 2016; Ezra, Faull, Jbabdi, & Pattison, 2015). The small size and position of the PAG makes it difficult to study in humans (Ezra et al., 2015; Satpute et al., 2013), but there is evidence that these columns are present (Coulombe et al., 2016; Ezra et al., 2015). Although they may appear similar to the columns seen in animal models, evidence regarding their connectivity to cortical and subcortical structures is mixed. One study of structural connectivity found that in humans the ventrolateral PAG (vlPAG) is the predominant source of efferents from the PAG to the prefrontal cortex (PFC), amygdala and hypothalamus, while in animals it is the dorsolateral PAG (dlPAG; Ezra et al., 2015). However, a functional connectivity study showed that like animals, the human PFC receives most of its PAG output from the dlPAG (Coulombe et al., 2016).

Preclinical studies have suggested that opioid-mediated analgesia involves the ventrolateral column, which is also responsible for passive coping mechanisms such as quiescence and reduced blood pressure. Meanwhile lateral (lPAG) and dlPAG

columns are involved in the active 'fight or flight' response, and are thought to produce analgesia through non-opioid-mediated mechanisms (Bandler & Shipley, 1994). In humans, there is some evidence to support this distinction, with stimulation of the ventral PAG causing reduced blood pressure and altered heart rate variability, indicating parasympathetic activity (Green et al., 2007; Pereira et al., 2010). However, the distinction between opioid and non-opioid mediated analgesia in the human PAG is rather unclear, with some human studies indicating endogenous opioid release to be mediated via the dorsal PAG (Crawford et al., 2021; E. A. Pereira et al., 2013; Sims-Williams et al., 2017).

The lPAG receives somatotopic input from the dorsal horn and SpV, with afferents from the head projecting rostrally, and those from the legs caudally (Benarroch, 2012). There is also evidence that input from somatic and visceral regions is column-specific. Cutaneous Aδ fibres project to the lPAG and dlPAG, while cutaneous C-fibres and afferents from muscles and viscera project to the vlPAG (Napadow, Sclocco, & Henderson, 2019). Visceral sensory afferents travel to the PAG via the nucleus of the solitary tract, a collection of sensory nuclei in the medulla.

The RVM is thought to be the main output node for descending pain modulation. The PAG itself does not project directly to the spinal cord (Boadas-Vaello et al., 2016), and lesioning of the RVM prevents the effect of PAG stimulation on pain-type responses, indicating that the RVM acts as a relay point (H. Fields & Heinricher, 2013). The PAG has reciprocal connectivity with the RVM, which in turn has descending projections to the dorsal horn and SpV, where it modulates the excitability of nociceptive neurons through serotonergic activity.

Stimulation of the RVM can be both antinociceptive and pronociceptive, indicating that it can produce both facilitatory and inhibitory descending control. The neural basis for this is thought to be ON cells and OFF cells within the RVM. OFF cells are inhibited by a peripheral noxious stimulus, and they are key for descending inhibition. Meanwhile, ON cells are excited by noxious stimuli and are responsible for the facilitation of nociceptive inputs. OFF cell firing is activated by opioid agonists, while the reverse is true of ON cell firing (H. L. Fields, Vanegas, Hentall, & Zorman, 1983; Heinricher, Morgan, & Fields, 1992). The injection of opioids into the PAG has an antinociceptive effect, and the analgesic effect of PAG stimulation is blocked by naloxone, an opioid antagonist (Mehta, Halder, Khanna, Tandon, & Sharma, 2012). Additionally, injection of opioids into the amygdala causes an antinociceptive effect that is blocked by the administration of local anaesthetic to the PAG or RVM (Tershner & Helmstetter, 2000). These findings indicate not only that endogenous opioids are likely to have an important role in descending modulatory control, but that higher brain structures engage these mechanisms.

Opioidergic mechanisms are not the only means of modulating ON and OFF cell activity. Endogenous cannabinoids also have an important role. Like opioids, cannabinoid type 1 (CB1) receptor agonists produce antinociceptive behaviour when injected into the PAG or RVM (Finn et al., 2003; W. J. Martin, Tsou, & Walker, 1998). Systemic injection of a cannabinoid agonist leads to increased OFF cell activity and reduced ON cell activity, and this effect is not blocked by opioid antagonism (Meng, Manning, Martin, & Fields, 1998). The role of endocannabinoids in endogenous pain modulation is discussed further in section 1.2.5.

Cortical input to the PAG comes from a wide range of regions known to be involved in pain processing, including the amygdala, the anterior cingulate cortex (ACC), the insular cortex, and the hypothalamus (Heinricher, Tavares, Leith, & Lumb, 2009; C. Lu et al., 2016; Mills et al., 2018). The RVM also has rich connectivity with the cortex, with input coming from the amygdala, hypothalamus and PFC. The connectivity with these higher brain regions is likely to reflect the presence of top-down regulation in response to a range of cognitive and emotional factors that influence perceived pain intensity and aversiveness (Q. Chen & Heinricher, 2019).

1.2.3.2 The role of other brainstem nuclei in descending control

Although the PAG and RVM are the best characterised components of the pain modulatory system in the brainstem, other brainstem nuclei are involved in the modulation of nociceptive input.

The PAG has reciprocal connectivity with the LC in the pons, which projects to the dorsal horn, where it exerts inhibitory control through noradrenergic activity (Boadas-Vaello et al., 2016). The LC also acts through noradrenergic projections to the dorsal reticular nucleus (DRt), enabling facilitation via α1-adrenoceptors (Mills, Keay, & Henderson, 2021). Like the PAG and RVM, opioid receptors are highly expressed in the LC, and endogenous opioids act on LC neurons to influence their descending modulatory activity (Tavares, Costa-Pereira, & Martins, 2021).

The medullary reticular formation forms part of the reticulo-thalamo-cortical ascending nociceptive pathway. The medullary reticular formation comprises two other regions in addition to the RVM. These are the caudal ventrolateral medulla (VLM) and the DRt.

There are strong reciprocal connections between the three areas, and it is thought that they work as a triad to help fine-tune descending nociceptive control (Martins & Tavares, 2017). Unlike the RVM, the VLM and DRt receive ascending projections from the dorsal horn in addition to providing descending projections. Like the RVM, the VLM contains ON- and OFF-cells, indicating that it has both facilitatory and inhibitory effects on nociception (Martins & Tavares, 2017). The DRt also has bidirectional effects, and is a key region for conditioned pain modulation (a measure of the efficiency of descending pain control), during which it has an inhibitory effect on nociceptive input (Mills et al., 2021). The DRt receives afferents from brainstem nuclei (including the PAG and LC) and from higher cortical regions involved in descending control, including the ACC and amygdala as well as sensorimotor areas (Martins & Tavares, 2017).

RVM and PAG activity can both be modulated by ascending input from the parabrachial complex, which forms part of the spinoparabrachial tract, and receives input from the dorsal horn in response to noxious stimulation (Yanes & Akintola, 2022). It contributes to the effective-motivational aspects of pain perception, as shown by its involvement in aversive learning (Reilly & Trifunovic, 2000). Projections from the parabrachial complex to the lPAG are excitatory, leading to analgesia (Chiang et al., 2020), while those to the RVM can be either excitatory or inhibitory (Yanes & Akintola, 2022). The parabrachial complex is also reciprocally connected to the central nucleus of the amygdala, a higher brain region important for affective aspects of pain that will be discussed in more detail below (section 1.2.4.1.5).

The nucleus cunieformis is a component of the mesencephalic pontine reticular formation, which is a key area for central sensitization (M. C. Lee, Zambreanu, Menon,

& Tracey, 2008). It is situated next to the PAG in the midbrain and receives input from the dorsal horn. It is a major source of input to the RVM, and shows increased activity during hyperalgesia (Zambreanu, Wise, Brooks, Iannetti, & Tracey, 2005). It is another region in which ON and OFF cells are present, indicating its role in pain facilitation and inhibition (Napadow et al., 2019).

1.2.3.3 The role of monoamines

The effect of descending control from the brainstem is thought to be mediated predominantly through monoaminergic mechanisms (Ossipov, Dussor, & Porreca, 2010). The RVM sends serotonergic projections to the dorsal horn (Tavares et al., 2021), and stimulation of the RVM causes the release of serotonin into cerebrospinal fluid (CSF; Bravo, Llorca-Torralba, Berrocoso, & Mico, 2019; Cui, Feng, McAdoo, & Willis, 1999). Spinal serotonin can have inhibitory or facilitatory effects on nociceptive transmission depending on the receptor subtype activated (Dogrul, Ossipov, & Porreca, 2009). Activation of 5-HT2A and 5-HT3 receptors in the dorsal horn causes facilitation of spinal nociceptive transmission, while that of 5-HT1A/B and 5-HT7 receptors causes inhibition (Rahman, Bannister, Bee, & Dickenson, 2011; Tavares et al., 2021).

Descending noradrenergic projections to the dorsal horn originate predominantly from the LC as well as the A5 and A7 noradrenergic cell groups in the pons (Bannister & Dickenson, 2016; Bravo et al., 2019; Tavares et al., 2021). Stimulation of these areas is anti-nociceptive (Bannister & Dickenson, 2016). This is largely due to the activation of α2-adrenoceptors on peripheral nociceptors (presynaptic) and spinal neurons (postsynaptic) leading to the inhibition of nociceptive input. (Budai, Harasawa, &

Fields, 1998; Pertovaara, 2006). Additionally, α1-adrenoceptors are expressed in lamina II of the dorsal horn, where they cause anti-nociception through the excitation of inhibitory interneurons (Baba et al., 2000).

1.2.4 The pain experience and the representation of pain in the brain

The pain experience is multidimensional. Therefore, the perception of pain involves a network of cortical and subcortical structures rather than a single 'pain region' in the brain. The advent of modern imaging techniques has facilitated huge advances in the understanding of how these regions function and interact, by enabling in vivo spacial and temporal localisation during pain perception.

Broadly, the pain experience is the result of the integration of the sensory aspects of pain, such as location and intensity; the affective-motivational aspects of pain, such as unpleasantness; and the cognitive processes affecting pain, such as attention and expectation (Melzack & Casey, 1968). Different parts of the pain network are optimised to deal with these different aspects of pain, enabling the interpretation and modulation of nociceptive input. Cognitive and emotional factors are able to influence pain perception by two overarching mechanisms: exerting top-down control over the facilitation and inhibition of nociceptive input, which is mediated primarily but not solely by structures in the brainstem, as described above; and altering the interpretation of these nociceptive signals once they reach areas of higher cognition. Therefore, the relationship between nociceptive input and pain perception is a non-linear one. Factors that may mediate this relationship include attention, expectation, previous experience, context, anxiety and perceived threat level, and reappraisal (Katja Wiech et al., 2006; Katja Wiech, Ploner, et al., 2008).

1.2.4.1 Pain regions in the brain

The brain regions most commonly activated in studies of acute pain are the primary and secondary somatosensory cortices (SI and SII), the ACC, the insular cortex, the PFC, and the thalamus (A. V. Apkarian, Bushnell, Treede, & Zubieta, 2005). However, other regions that are also frequently identified, depending on the nature of the study, include the amygdala, basal ganglia, hippocampus, cerebellum, and parts of the parietal and temporal cortices (Tracey & Mantyh, 2007; figure 1.1). The role of some of these key regions in pain perception are discussed in more detail below.

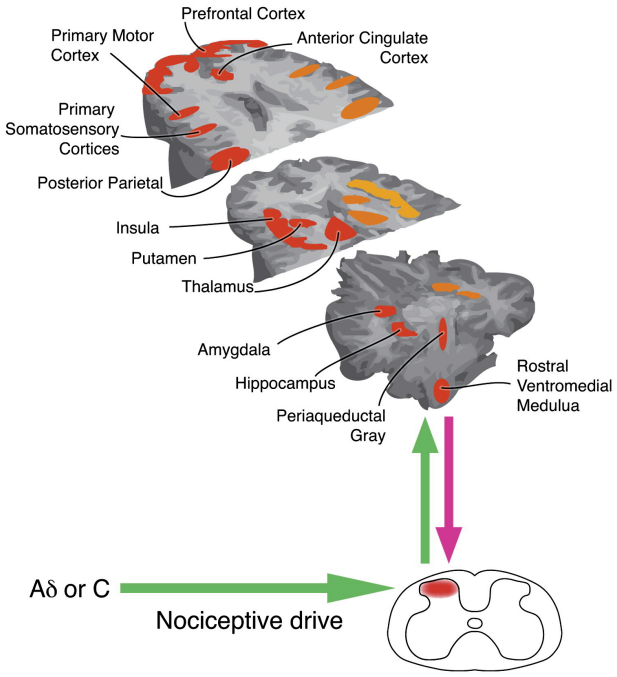


Figure 1.1: Brain regions that are consistently found to be active during a painful experience. From Tracey and Mantyh (2007).

1.2.4.1.1 Thalamus

The thalamus is crucial in transmitting sensory information, including nociceptive input, from the spinal cord to higher brain regions. However, rather than simply relaying this information, it can filter, modulate and integrate the input, and is a vital hub for

sensorimotor processing. It also receives extensive cortical and subcortical input, enabling descending feedback (Ward, 2013).

1.2.4.1.2 Somatosensory cortices

SI is located in the postcentral gyrus while SII lies in the parietal lobe within the lateral sulcus. Both are somatotopically organised, and both are well connected to other pain-related regions. SI and SII receive both noxious and innocuous input from the thalamus, with which they have reciprocal connections. Both also have connections with the insula, which then projects to the amygdala. SII has direct connections to the amygdala and other limbic areas (Kropf, Syan, Minuzzi, & Frey, 2019).

SI is important for the sensory-discrimination aspects of touch and pain but does not on its own modulate the affective domain (Case et al., 2016; Ploner, Freund, & Schnitzler, 1999; Uhelski, Davis, & Fuchs, 2012). SII is thought to have a role in sensorimotor integration (Kropf et al., 2019), as well as the identification of the noxious nature of a nociceptive stimulus (Timmermann et al., 2001).

1.2.4.1.3 Insular cortex

The insular cortex is situated within the lateral sulcus and is divided into anterior and posterior components. The posterior insula (pINS) receives the most dense STT-thalamocortical input in the cortex (Dum, Levinthal, & Strick, 2009), and has strong connectivity with somatosensory regions (K. Wiech, Jbabdi, Lin, Andersson, & Tracey, 2014). It is important for encoding stimulus properties (A. V. Apkarian, Bushnell, & Schweinhardt, 2013). Activity in one particular region of the pINS, the dorsal posterior

insula, has been found to track the perceived intensity of a noxious stimulus over time (Segerdahl, Mezue, Okell, Farrar, & Tracey, 2015).

The anterior insula (aINS) is a key region for the affective-motivational dimension of pain. It receives sensory input from the thalamus (A. V. Apkarian et al., 2013), and has high levels of connectivity with prefrontal regions (K. Wiech et al., 2014). It has an important role in the conscious appraisal of sensory information (Uddin, 2015). The contrasting roles of the pINS and aINS in nociception and pain perception are illustrated by a positron emission tomography (PET) study that showed that the actual temperature of a thermal stimulus was related to activity in the pINS, while perceived thermal intensity correlated with aINS activity (Craig, Chen, Bandy, & Reiman, 2000).

There is good evidence that the aINS has a vital role in interoceptive attention (X. Wang et al., 2019) and in emotional processing (Uddin, Nomi, Hébert-Seropian, Ghaziri, & Boucher, 2017), making it ideally placed to integrate external and internal sensory information with emotional responses. Together with other structures, including the anterior midcingulate cortex (aMCC), the aINS is a key component of the salience network, which is critical in determining which internal and external stimuli to attend at any given moment (Menon & Uddin, 2010), and in using the context of a stimulus to assess its significance and determine its subsequent perception (Katja Wiech et al., 2010). In the presence of a salient event, the aINS can mediate network interactions by activating the central executive network and deactivating the default mode network (Sridharan, Levitin, & Menon, 2008), thereby facilitating an appropriate cognitive response.

1.2.4.1.4 Cingulate cortex

The cingulate cortex can be divided into anterior, middle, posterior and retrosplenial components (Vogt, 2019b). The anterior and middle divisions are consistently activated during nociceptive stimulation (Vogt, 2005), and will be discussed here. The anterior cingulate cortex (ACC) is primarily activated during emotional tasks, while the midcingulate cortex (MCC) is primarily activated during cognitive tasks (Vogt, 2019b), and this reflects their roles in pain.

1.2.4.1.4.1 Anterior cingulate cortex

The ACC is associated with emotion and autonomic regulation (Vogt, 2005), and has a key role in the affective dimension of pain. It is also the region most commonly activated by noxious stimuli (A. V. Apkarian et al., 2005). The ACC can be subdivided into the pregenual, or rostral ACC (rACC), and the subgenual ACC (Vogt, 2019b). In patients with chronic pain, cingulotomy has been shown to remove the affective response to their symptoms, while preserving the sensory component (Foltz & White, 1962). A study in rabbits showed that while ACC neurons respond to multiple types of nociceptive stimulation, they do not encode location (Sikes & Vogt, 1992). Lesioning the rACC in rodents leads to the abolition of aversive learning in response to pain without affecting sensory thresholds (Johansen, Fields, & Manning, 2001; Yan et al., 2012), while activation of the rACC induces aversive behaviour (Johansen & Fields, 2004). The ACC is known to be important in fear conditioning and anxiety (Bliss, Collingridge, Kaang, & Zhuo, 2016), and preclinical evidence shows that synaptic plasticity in the ACC may contribute to the close relationship between anxiety and chronic pain (Kohei Koga et al., 2015; M. Zhou, 2016). In humans, the rACC is implicated in studies that elicit both pain and negative affect (Shackman et al., 2011),

and negative emotional states consistently alter pain-evoked activity in the ACC (Bushnell, Ceko, & Low, 2013). The ACC has anatomical connectivity with the amygdalae, which are also regions implicated in fear and anxiety (M. Zhou, 2016). Functional connectivity between the ACC and amygdalae increases during placebo analgesia (U. Bingel, Lorenz, Schoell, Weiller, & Büchel, 2006), indicating that this circuitry has a role in the emotional processing of pain and analgesia.

The role of endogenous opioids in the processing of affective aspects of pain in the rACC is highlighted by the fact that the rACC has the highest level of opioid receptor binding in the cingulate cortex (Vogt, Watanabe, Grootoonk, & Jones, 1995), and by preclinical data showing that the release of endogenous opioids in the rACC is required for the relief of pain aversiveness (E. Navratilova et al., 2015). Meanwhile, injecting morphine into the rACC of rats does not affect tactile withdrawal reflexes, indicating that sensory aspects of pain are not modulated by this mechanism (Gomtsian et al., 2018). The effects of opioidergic activity in the ACC on pain aversiveness are mediated by the subsequent downstream engagement of dopamine signalling in the nucleus accumbens (NAc), a key part of the reward system (E. Navratilova et al., 2015).

Preclinical studies have shown that the ACC is also an important part of the descending pain modulatory system, with stimulation or activation of glutamate receptors in the ACC leading to the facilitation of nociception at the level of the spinal cord (Calejesan, Kim, & Zhou, 2000; T. Chen et al., 2018; M. Zhou, 2017). This top-down engagement may take place via multiple pathways. There are direct excitatory projections from the ACC to the dorsal horn, and these have been shown to potentiate

nociceptive input independently of the RVM (T. Chen et al., 2018). However, there is also evidence for the involvement of modulatory nuclei in the brainstem, including the RVM, which has been shown to act as a relay between the ACC and the spinal cord in some studies (Calejesan et al., 2000).

In people with chronic pain, increased functional connectivity is seen between the ACC and the LC, and the strength of this connectivity correlates positively with reported pain intensity (Mills et al., 2018). This indicates the possible involvement of noradrenergic as well as serotonergic circuitry. Other ACC changes consistently seen in chronic pain include reduced grey matter volume, reduced white matter integrity, and reduced opioid binding (Bushnell et al., 2013).

1.2.4.1.4.2 Midcingulate cortex

The MCC can be divided into anterior and posterior regions (Vogt, 2019). Both regions are activated during nociception. The anterior MCC (aMCC) is critical for response selection (Vogt, 2019a), and although activity in the aMCC is associated with fear and the prediction of aversive consequences (Andrzejewski, Greenberg, & Carlson, 2019; Vogt, Berger, & Derbyshire, 2003), its role is in cognition rather than emotion (Vogt, 2019a). It responds to pain-related fear by mediating nocifensive behaviours (Vogt, 2005, 2016; Vogt et al., 2003). It receives input from the amygdala, a region known to have a role in fear-related learning (Vogt, 2005). Its role in generating avoidance behaviours is consistent with the fact that it is reliably activated by experiments eliciting a combination of negative affect, pain and cognitive control (Shackman et al., 2011). Unlike the aMCC, the posterior MCC has does not show emotion-related activity (Vogt,

2005, 2019b). It is involved in attentional orientation and motor withdrawal in response to painful inputs (Vogt, 2019b).

The aMCC is commonly affected in chronic pain, with human studies showing reduced gray matter volume or cortical thickness (Hubbard et al., 2014; Schmidt-Wilcke et al., 2005), as well as changes in functional activity (Cottam, Condon, Alshuft, Reckziegel, & Auer, 2016). Animal data implicate the MCC in descending pain facilitation via a pathway from the MCC to the pINS, and subsequently to the brainstem, where descending pathways are engaged (Tan et al., 2017). This MCC-pINS pathway is capable of generating and maintaining sensory hypersensitivity independent of the peripheral stimulus, thus giving a potential pathway for the exacerbation of pain by psychosocial factors as opposed to nociceptive input. This also gives insight into the possible involvement of the MCC in the transition from acute to chronic pain. In humans, deep brain stimulation of the aMCC at a frequency likely to be high enough to have a lesion-like effect (130Hz) alleviates refractory chronic neuropathic pain, demonstrating the region's potential as a therapeutic target (Boccard et al., 2017).

1.2.4.1.5 Amygdala

The amygdala is commonly modulated in both preclinical models and human functional imaging studies of pain processing (Simons et al., 2014; Thompson & Neugebauer, 2017). It is situated in the medial temporal lobe, and is an important region for the affective component of pain perception and for pain modulation. It contains multiple nuclei, broadly grouped into the basolateral group, the central nucleus, and the medial and superficial nuclei. The central nucleus (CeA) is the main output nucleus. It receives integrated information from the thalamus, insula and cortex,

primarily via the basolateral and lateral nuclei, though it does also receive some cortical and thalamic input directly. It also receives nociceptive input directly from the spinal cord and PBN. It projects to the brainstem (including PAG, PBN and reticular formation), the hypothalamus, the thalamus and other regions of the amygdala (Veinante, Yalcin, & Barrot, 2013), as well as sending some reciprocal projections to cortical regions including the PFC (Ong, Stohler, & Herr, 2019). The basolateral amygdala (BLA) receives input from the spinal cord via the PBN (Huang et al., 2019), and projects to multiple brain regions, including the hippocampus, the NAc, and the PFC, with which it has reciprocal connectivity (McGarry & Carter, 2017).

In rodent studies, electrical stimulation of the amygdala at 60Hz causes reduced nociceptive behaviours in response to tonic and phasic experimental pain (Mena, Marthur, & Nayar, 1995). Lesioning or inhibiting the CeA reduces the analgesic effects of systemic morphine and cannabinoids (B. H. Manning, Martin, & Meng, 2003; Barton H. Manning & Mayer, 1995), and injecting opioids into the amygdala produces analgesia, as well as increased OFF cell and decreased ON cell firing in the RVM (McGaraughty & Heinricher, 2002). The analgesic effects of morphine receptor activation in the amygdala can be inhibited by lesioning the PAG or RVM (Helmstetter, Tershner, Poore, & Bellgowan, 1998; Tershner & Helmstetter, 2000), indicating that the amygdala has a role in the descending modulation of nociceptive input. This is supported by the finding that in rats with neuropathic pain who have impaired diffuse noxious inhibitory controls (DNIC; a measure of the efficiency of descending pain modulation), descending modulation is restored by the injection of opioids into the CeA (E. Navratilova et al., 2020).

Work in rats has shown that a pathway from the BLA to PAG via the medial PFC (mPFC; an area thought to be functionally equivalent to the dlPFC in humans) is key in the development of hypersensitivity after peripheral nerve injury. Huang and colleagues (2019) showed that injury led to weakened endocannabinoid signalling in the mPFC, causing reduced inhibition of excitatory inputs from the BLA to inhibitory interneurons in the mPFC. This led to feedforward inhibition of signalling from the mPFC to the PAG, and subsequently to reduced monoaminergic modulation of nociceptive input via the RVM and LC. Like the amygdala, the dlPFC is an important region for pain processing in humans (see section 1.2.4.1.6).

The amygdala is important for processing aversive and threatening experiences (Bach, Hurlemann, & Dolan, 2015), and is often referred to as the 'fear centre' due to its role in fear-related learning (LaBar, Gatenby, Gore, Ledoux, & Phelps, 1998; Phan, Wager, Taylor, & Liberzon, 2002; Whalen et al., 2001). However, it also has a role in the processing of positive emotions, including learning about stimulus-reward values (Baxter & Murray, 2002). As mentioned in section 1.2.4.1.4.1, there is connectivity between the amygdala and the ACC, and placebo analgesia is associated with increased functional connectivity between the two (U. Bingel et al., 2006; M. Zhou, 2016). Like the rACC, opioidergic activity in the CeA has been shown to contribute to pain aversiveness via dopamine release at the NAc. However, this is abolished by the blockade of opioid signalling in the rACC, indicating that the effect of the amygdala on affective aspects of pain perception is likely to be mediated by its effect on opioidergic activity in the rACC (E. Navratilova et al., 2020).

Given the role of the amygdala in learning about stimulus values and in pain modulation, it is ideally placed to use information about the emotional significance of a stimulus to initiate appropriate physiological, behavioural and affective responses, including the descending modulation of a noxious input.

1.2.4.1.6 Prefrontal cortex

Although the PFC is consistently activated in pain imaging studies, pain-evoked activity does not correlate linearly with stimulus intensity (Coghill, Sang, Maisog, & Iadarola, 1999). An early study found that electrical stimulation of the PFC at 10Hz in rats led to a reduction in midbrain responses to noxious stimulation (Hardy & Haigler, 1985), indicating a role for the PFC in top-down pain modulation. Since then, human imaging studies have confirmed the role of the PFC in the cognitive manipulation of pain perception though modulation of descending pathways and other cortical regions. Subregions of the PFC have a range of functions in pain, and the dlPFC, ventrolateral PFC (vlPFC) and orbitofrontal cortex (OFC) will be discussed here.

Increased dlPFC activity is seen during noxious stimulation in healthy individuals (Lorenz, Minoshima, & Casey, 2003). It is thought to have a role in pain inhibition, and is often implicated in placebo analgesia (Hashmi et al., 2012). In a PET study of capsaicin-induced heat allodynia, the bilateral dlPFC were the only regions in which activity was negatively correlated with perceived pain intensity and unpleasantness (Lorenz et al., 2003). As discussed above, the aINS is a key region for processing affective aspects of pain, and right dlPFC activity is negatively associated with the correlation between aINS activity and perceived pain intensity and unpleasantness during a noxious stimulus (Lorenz et al., 2003). There is also evidence for reduced

pain intensity and reduced connectivity between the dlPFC and aINS when pain is controllable versus uncontrollable (Bräscher, Becker, Hoeppli, & Schweinhardt, 2016), indicating that interactions between the dlPFC and the aINS may be involved in the cognitive control of pain. Structural connectivity between the dlPFC and the PAG passes through the thalamus (Hadjipavlou, Dunckley, Behrens, & Tracey, 2006; Lin et al., 2017). Lorenz et al. (2003) found that activity in the left dlPFC correlated with reduced pain unpleasantness as well as with coupling between the midbrain and the medial thalamus, and between the midbrain and rACC during a noxious stimulus. They noted that when left dlPFC activity was low, there was a positive correlation between rACC activity and unpleasantness, whereas when dlPFC activity was high, the correlation was negative. They hypothesised that by modulating cortico-cortical and cortico-subcortical connectivity, the left dlPFC may have an important role in mediating the affective responses to pain, and governing the resultant cognitive manipulation of pain. The idea that the dlPFC is able to modulate the perception of pain by influencing other cortical and subcortical parts of the pain network is supported by the finding that anodal (facilitatory) transcranial direct current stimulation (tDCS) of the left dlPFC induces analgesia and suppresses activity in the thalamus and pINS. The degree of analgesia that is achieved correlates with structural and functional connectivity between the dlPFC and the thalamus (Lin et al., 2017). Furthermore, anodal tDCS of the dlPFC modulates the functional connectivity of the thalamus to both sensory and affective networks, and this modulation is greater in individuals with high pain tolerance (Sankarasubramanian et al., 2017).

As outlined above (section 1.2.4.1.5), animal data has shown that a pathway from the amygdala to the mPFC (thought to be functionally equivalent to the human dlPFC) and

then to the PAG is implicated in the descending facilitation of pain following peripheral nerve injury. It is mediated by the effect of inhibitory interneurons in the mPFC (Huang et al., 2019). This demonstrates a mechanism by which the human dlPFC might exert direct top down control over nociceptive input.

Like the dlPFC, the vlPFC is involved in the modulation of nociceptive input. Electrical stimulation of a homologous brain region at 100Hz in rats leads to reduced pain behaviour in response to a noxious stimulus, and this effect is eliminated by lesioning the PAG (Y. Q. Zhang, Tang, Yuan, & Jia, 1997), confirming the top-down nature of the modulation. The vlPFC is well-connected to other regions of the descending modulatory system, including the amygdala, MCC and dlPFC (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Ray & Zald, 2012; Katja Wiech, Ploner, et al., 2008).

The vlPFC has a role in emotion regulation, and is implicated in the reappraisal of negative emotions (Tor D. Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008). With respect to pain perception specifically, there is evidence that the vlPFC is activated when pain is downregulated in the context of a belief that pain will be reduced, leading to the hypothesis that the vlPFC has a role in the reappraisal of pain. Examples of this include placebo analgesia, religious beliefs and perceived control over pain, all of which lead to a reduction in perceived pain that correlates with increased right vlPFC activity (Lieberman et al., 2004; Katja Wiech, Farias, et al., 2008; Katja Wiech et al., 2006).

The OFC encodes the reward value of sensory stimuli, both received and anticipated (Kahnt, Heinzle, Park, & Haynes, 2010), and has been implicated in the inhibition of

pain by reward (Becker, Gandhi, Pomares, Wager, & Schweinhardt, 2017). OFC activity has also been shown to be increased when a stimulus is perceived as painful or anticipated to be painful, compared to a non-noxious stimulus (Brodersen et al., 2012). It has reciprocal connections with multiple other pain-related regions, including the insula, MCC and somatosensory regions, and modulation of these supraspinal regions by the OFC may be responsible for controlling the relationship between reward and perceived pain (Becker et al., 2017).

1.2.4.2 Visceral pain and the brain

Visceral pain is perceived as more unpleasant and anxiety-inducing than somatic pain of the same intensity, and unlike somatic pain, may not habituate (L.R. Koenen et al., 2017). It is more salient, leading to greater interference with other cognitions even when unpleasantness, fear and expectation are taken into account (Kleine-Borgmann et al., 2022). It is also associated with a quiescent behavioural response rather than the fight or flight response induced by somatic pain (P. Dunckley et al., 2005).

Although both types of pain share brain representations in core pain regions, clear differences are also seen between the two modalities even when matched for pain intensity or unpleasantness (P. Dunckley et al., 2005; Paul Dunckley et al., 2005; L.R. Koenen et al., 2017; Van Oudenhove et al., 2020). This is to be expected given the aforementioned differences in associated cognitions, as well as the interoceptive nature of visceral pain. The differences identified vary between Individual studies, probably due to differences in methodology. However, a meta-analysis of functional magnetic resonance imaging (fMRI) studies showed that using machine learning across multiple studies, it was possible to reliably discriminate rectal from cutaneous

pain based on the neural signature (Van Oudenhove et al., 2020). In particular, they found that somatic pain showed greater engagement with the somatomotor network, while visceral pain was associated with greater activation of the frontoparietal network. Visceral pain also produced greater activity in parts of the default mode network, a key network for interoceptive awareness; and limbic regions, which are involved in the emotional processing of pain.

1.2.4.3 The placebo effect

The modulation of pain perception by cognitive processes is demonstrated by the placebo effect. Cognitive processes such as attention, expectation and reappraisal are important mechanisms for generating placebo (Katja Wiech, Ploner, et al., 2008). Classical conditioning, in which associative learning takes place unconsciously over a period of time, is also a key mechanism (Schafer, Geuter, & Wager, 2018; Tracey, 2010).

Placebo analgesia engages the descending modulatory system. The rACC, PAG and RVM are consistently activated in placebo-induced analgesia, and placebo is linked to increased functional connectivity between the rACC and both the PAG and RVM, (U. Bingel et al., 2006; Eippert et al., 2009; Frisaldi, Shaibani, & Benedetti, 2020; Tracey, 2010) as well as the amygdalae (U. Bingel et al., 2006). It has been suggested that the rACC links higher order cognition to the descending pain modulatory system by recruiting subcortical regions (U. Bingel et al., 2006). This is supported by a study showing that stronger connectivity between the rACC and the PAG is associated with greater RVM activity and a larger placebo effect (Eippert et al., 2009). In keeping with multiple other studies that have shown inhibition of the placebo effect by opioid

receptor antagonism (Frisaldi et al., 2020), these effects are reduced by naloxone (Eippert et al., 2009), showing that they are opioid-mediated. A PET binding study found that placebo caused increased binding of endogenous opioids to μ-opioid receptors in the dlPFC, rACC, insula and NAc. The degree of opioid receptor binding in the rACC was correlated with the reported reduction in pain scores, as well as the increase in the noxious stimulus required to maintain a consistent pain intensity (Zubieta et al., 2005).

There is evidence that the PFC (and more specifically the lateral PFC) is responsible for initiating and controlling the placebo response (Benedetti et al., 2006; Frisaldi et al., 2020; Krummenacher, Candia, Folkers, Schedlowski, & Schönbächler, 2010; Katja Wiech, Ploner, et al., 2008). It is ideally positioned to do so as it receives multimodal sensory information, and has connectivity to limbic structures involved in the affective-motivational aspects of pain as well as motor regions involved in behavioural responses (Katja Wiech, Ploner, et al., 2008). The dlPFC has been shown to be of particular importance in the effect of expectation and attention on pain (T.D. Wager et al., 2004). Opioid receptor binding in the dlPFC has been shown to correlate with expected analgesia (Zubieta et al., 2005), and dlPFC disruption leads to the abolition of expectation-induced placebo (Krummenacher et al., 2010). It is known to modulate the connectivity of other cortical and subcortical regions implicated in pain, as well as affecting the link between both the rACC and the insulae with behavioural pain outcomes (Lorenz et al., 2003). Meanwhile, activation of the vlPFC, an area implicated in the reappraisal of a stimulus (Katja Wiech, Ploner, et al., 2008), is correlated with the strength of placebo analgesia (Lieberman et al., 2004). The dlPFC and vlPFC are

highly connected to one another, and are likely to interact during cognitive pain modulation (Katja Wiech, Ploner, et al., 2008).

Despite the fact that placebo is largely mediated by endogenous opioidergic mechanisms, nonopioid placebo analgesia does exist. In people who undergo conditioning with a non-steroidal anti-inflammatory drug that blocks cyclooxygenase activity, placebo is blocked by a CB1 receptor antagonist (Benedetti, Amanzio, Rosato, & Blanchard, 2011), indicating involvement of the endocannabinoid system. It has been suggested that the component of the placebo response that relates to expectation is opioid-dependent, while conditioned analgesia that is independent of expectations may be mediated by endocannabinoids, and that these may act on the pathway from the amygdala to modulatory systems in the brainstem (Schafer et al., 2018).

1.2.5 The endocannabinoid system in endogenous pain control

The role of endocannabinoids in placebo (section 1.2.4.3) alludes to the fact that the endocannabinoid system has increasingly become recognised alongside opioidergic mechanisms as an important endogenous pain control pathway (Woodhams, Chapman, Finn, Hohmann, & Neugebauer, 2017).

Endocannabinoids bind to two main types of receptor, cannabinoid type 1 (CB1) and cannabinoid type 2 (CB2). CB1 receptors are expressed throughout the body, but are found in particularly high concentrations in the central nervous system (CNS), especially in cortex, hippocampus, amygdala, basal ganglia and cerebellum (Mackie, 2005). Cortical areas with high receptor concentrations include the cingulate gyrus

and the middle frontal gyrus (dlPFC), while the somatosensory cortex has a medium concentration (Bloomfield et al., 2019). Receptors are also found in the PAG, RVM and PBN, as well as in the dorsal horn and dorsal root ganglia, meaning that they are distributed throughout multiple regions associated with pain perception and modulation (Finn et al., 2021). In contrast, CB2 receptors are found primarily on peripheral immune cells. However, they can also be found on neurons in pain-relevant regions, including peripheral nerve endings and the dorsal horn of the spinal cord; and on glial cells throughout the CNS (Finn et al., 2021).

Arachidonoyl ethanolamine (AEA) and 2-arachidonoyl glycerol (2-AG) are the main endogenous ligands of the cannabinoid receptors. 2-AG has high affinity for both CB1 and CB2, while AEA has more affinity for CB1 than CB2 (H. C. Lu & MacKie, 2016). Levels of AEA and 2-AG are regulated predominantly by the serine hydrolase enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGLL) respectively (Blankman, Simon, & Cravatt, 2007; Cravatt et al., 2001). These enzymes are responsible for the degradation of the endocannabinoids by hydrolysis. Inhibition of FAAH and MGLL results in reduced endocannabinoid degradation, thereby increasing levels of AEA and 2-AG, and facilitating more signalling via CB1 and CB2 receptors (Figure 1.2). Since products of endocannabinoid degradation include arachidonic acid and eicosanoids, the inhibition of degradation may also have an anti-inflammatory effect through reduced production of pro-inflammatory mediators (Nomura et al., 2011).

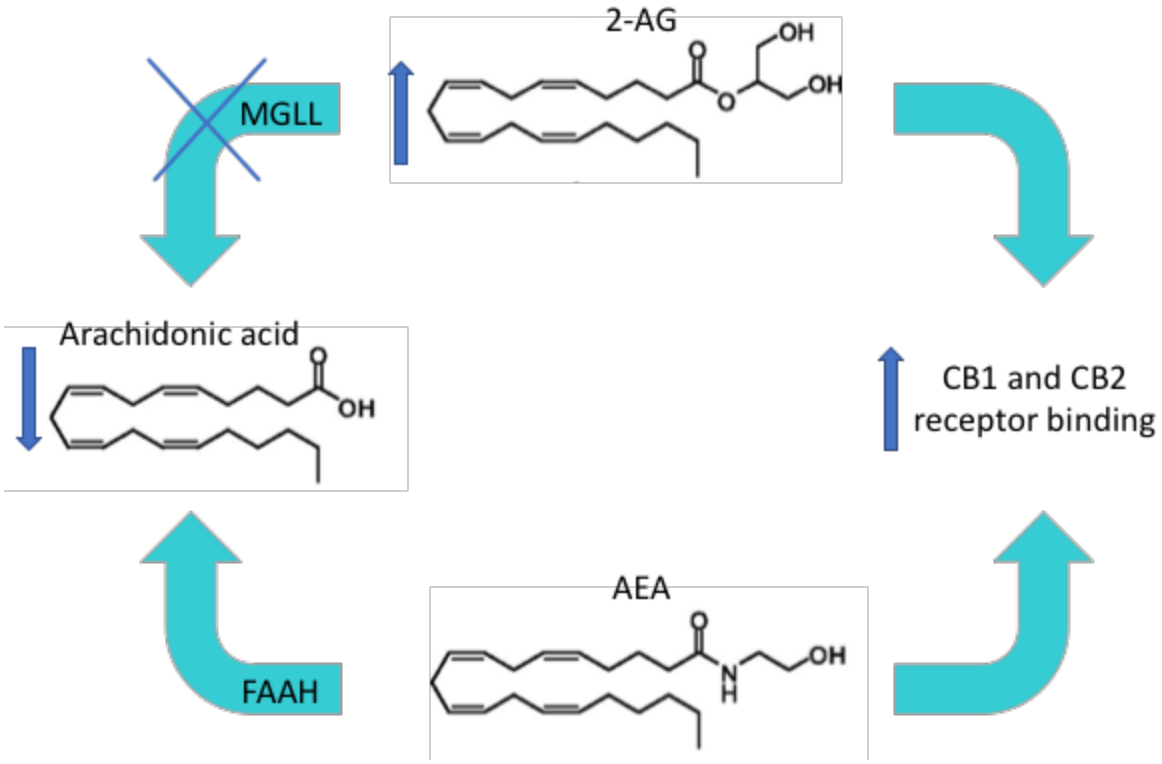


Figure 1.2: The endocannabinoid system. 2-AG (2-arachidonoyl glycerol) and AEA (Arachidonoyl ethanolamine) are degraded by MGLL (monoacylglycerol lipase) and FAAH (fatty acid amide hydrolase) respectively. The effects of MGLL inhibition are shown in dark blue. Inhibiting MGLL causes a rise in the concentration of 2-AG, leading to increased cannabinoid receptor type 1 and 2 (CB1 and CB2) signalling. Meanwhile arachidonic acid production is reduced.

Endocannabinoids cause antinociception through retrograde transmission. Increased postsynaptic synthesis of endocannabinoids takes place in pain-related regions, leading to presynaptic CB1 agonism, which inhibits presynaptic voltage gated calcium channels, suppressing GABA release and therefore disinhibiting transmission (Finn et al., 2021; Ohno-Shosaku, Maejima, & Kano, 2001). The duration of this effect is limited by the rapid degradation of endocannabinoids by MGLL and FAAH.

There is strong preclinical evidence for the role of endocannabinoids in endogenous descending pain modulation. In rodents, the injection of CB1 antagonists into the RVM prevents the analgesia associated with systemic cannabinoid administration, and also induces hyperalgesia through inhibition of endogenous cannabinoids. The effect of CB1 agonism on RVM ON and OFF cells is similar to that of endogenous opioids, but is unaffected by opioid antagonism (Meng et al., 1998). At the level of the dorsal horn, the systemic administration of a cannabinoid agonist leads to the inhibition of nociceptive transmission (Hohmann, Martin, Tsou, & Walker, 1995), while spinal CB1 antagonism leads to the facilitation of nociceptive responses in dorsal horn neurons (V. Chapman, 1999).

While the effect of endocannabinoids on acute pain is largely CB1-mediated, CB2 receptors on immune cells and glial cells appear to have an important role in chronic pain (Woodhams et al., 2017). In rodent models, CB2 is upregulated in the CNS in response to injury (Finn et al., 2021). CB2 agonists reduce mechanical allodynia as well as reactive gliosis in the spinal cord in neuropathic pain models (Leichsenring, Andriske, Bäcker, Stichel, & Lübbert, n.d.), and also reduce pain responses in inflammatory models (Soliman et al., 2021). Meanwhile, CB2 knockout mice with sciatic nerve injury develop contralateral pain and increased glial cell expression in the contralateral dorsal horn (Racz et al., 2008).

Stress or fear can lead to antinociception. This is referred to as stress-induced analgesia (SIA). Fear-conditioned analgesia (FCA) is a form of SIA in which the ‘stressor’ becomes aversive through conditioning (R.K. Butler & Finn, 2009). SIA can be opioid or non-opioid mediated, and recruits descending modulatory pathways.

Non-opioid SIA is associated with increased endocannabinoid levels in the PAG (Hohmann et al., 2005). It is reduced by the injection of a CB1 antagonist into the RVM, PAG or BLA (Connell, Bolton, Olsen, Piomelli, & Hohmann, 2006; Hohmann et al., 2005; Woodhams et al., 2017). Conversely it is potentiated by the injection of drugs that inhibit endocannabinoid degradation into the PAG or RVM (Hohmann et al., 2005; Woodhams et al., 2017).

In rats, FCA is abolished by either CB1 receptor antagonism or opioid receptor antagonism (Ryan K. Butler, Rea, Lang, Gavin, & Finn, 2008; Finn et al., 2004), and CB1 knockout mice do not exhibit opioid-mediated SIA (Valverde, Ledent, Beslot, Parmentier, & Roques, 2000). FCA is enhanced by reducing endocannabinoid degradation through FAAH inhibition, but this effect is prevented by antagonism of CB1, CB2 or opioid receptors (Ryan K. Butler et al., 2008). FCA is also enhanced by inhibiting MGLL in the ACC, leading to increased 2-AG levels. This effect is blocked by a CB2 receptor antagonist (Corcoran, Mattimoe, Roche, & Finn, 2020).

These findings indicate that intrinsic endocannabinoid tone may be important for the regulation of descending pain modulation, and that drugs that increase that tone might enable enhanced descending inhibition (Hohmann et al., 2005). They also show that opioid and cannabinoid systems interact, and that the endocannabinoid system mediates both opioid and non-opioid dependent forms of stress-induced endogenous pain inhibition (Woodhams et al., 2017).

Preclinical data has shown that repeated stress causes increased endocannabinoid levels in the amygdala and the mPFC, an area thought to be functionally equivalent

to the dlPFC in humans (Rademacher et al., 2008). As introduced in section 1.2.4.1.5, endocannabinoids have a role in the altered forebrain-amygdala circuitry that is seen in pain states (Huang et al., 2019; Woodhams et al., 2017). In rodent models of pain mechanisms, decreased mPFC output occurs due to the increased glutamatergic activation of inhibitory GABAergic interneurons by projections from the BLA. This leads to feedforward inhibition of output from mPFC pyramidal cells .CB1 is expressed in GABAergic interneurons, and in normal circumstances, control of synaptic inhibition of mPFC pyramidal output is achieved by 2-AG signalling. In pain models, there is a lack of available 2-AG, meaning that there is reduced signalling from the mPFC to the descending modulatory system, resulting in reduced pain inhibition (Huang et al., 2019; Kiritoshi, Ji, & Neugebauer, 2016; Woodhams et al., 2017).

Not only does the amygdala control frontal output in preclinical models of pain, but the frontal cortex controls amygdala output. The activation of presynaptic CB1 receptors and postsynaptic glutamate receptors in the mPFC leads not only to increased descending inhibition, but also to increased glutamatergic output from the mPFC to the amygdala. This causes the activation of inhibitory interneurons in the amygdala which inhibit output from the CeA (G. Ji & Neugebauer, 2014; Woodhams et al., 2017).

Overall, the evidence suggests that increasing the concentration of 2-AG available in the mPFC should lead to enhanced descending pain inhibition, and reduced pain-related amygdala output, thereby influencing sensory and affective aspects of pain. Indeed, administration of CB1 and glutamate receptor agonists into the mPFC of

arthritic rats leads to a reduction in behaviours associated with both pain intensity and pain aversiveness (Woodhams et al., 2017).

In humans with neuropathic pain, the administration of an exogenous cannabinoid, tetrahydrocannabinol (THC), leads to reduced connectivity between the dlPFC and the rest of the pain network, the degree of which correlates with the amount of analgesia achieved. The analgesic response to THC also correlates with a reduction in functional connectivity between the aMCC and sensorimotor cortex. This could indicate that analgesia is achieved by altering the interactions between regions involved in sensory (sensorimotor) and affective (cingulate cortex) aspects of pain perception (Weizman et al., 2018).

1.2.6 Peripheral and central sensitization

Neuronal plasticity in pain pathways is thought to contribute to the development and maintenance of chronic pain. It occurs in response to tissue injury, and takes place both centrally and in the periphery.

1.2.6.1 Peripheral sensitization

Peripheral sensitization refers to the reduced threshold, increased magnitude of response and increased spontaneous activity that is seen at peripheral nociceptive nerve endings in response to the chemical mediators released by tissue injury. It leads to primary hyperalgesia, which is increased sensitivity to pain at the site of injury (Lamotte, Thalhammer, Torebjork, & Robinson, 1982). Sensitization of sensory neurons induces neurogenic inflammation, leading to increased release of

inflammatory mediators and subsequently further sensitization (Bhave & Gereau, 2004).

The mechanisms that underlie peripheral sensitization can be transcriptional/translational or posttranslational. Alterations in the transcription or translation of proteins cause changes in gene expression. An example of this is the upregulation of transient receptor potential vanilloid 1 (TRPV1) receptors in response to the presence of nerve growth factor (Vardeh & Naranjo, 2017; X. Zhang, Huang, & McNaughton, 2005). An example of a posttranslational mechanism is the effect of some inflammatory mediators on signalling pathways that modulate the gating of existing ion channels in peripheral nociceptor terminals. The efficiency of these ion channels is then affected by lowering the threshold for opening or increasing the length of opening, leading to increased depolarisation of sensory afferents (Vardeh & Naranjo, 2017).

1.2.6.2 Central sensitization

Central sensitization is defined as the "increased responsiveness of nociceptive neurons in the CNS to their normal or subthreshold afferent input" (IASP, 2020). It is characterised by secondary hyperalgesia (increased sensitivity to a painful stimulus at a location without injury) and allodynia (pain caused by a stimulus that is not normally painful; Woolf, 2007). Other features include prolonged pain, and temporal summation (Yekkirala, Roberson, Bean, & Woolf, 2017), a term that describes the increase in spinal neuronal responses seen in the presence of repetitive peripheral stimulation. While central sensitization often occurs due to repeated or high intensity peripheral input, it can also exist independently of peripheral injury due to changes originating in

the CNS (Harte, Harris, & Clauw, 2018; Yekkirala et al., 2017). It is a common finding in chronic pain conditions, including functional conditions, for which the underlying pathophysiology is not clear (Harte et al., 2018).

There are multiple mechanisms that can contribute to CS. Improved synaptic efficacy may relate to presynaptic mechanisms, such as the upregulation of presynaptic ion channels and the increased release of excitatory neurotransmitters such as glutamate. It may also involve postsynaptic mechanisms, such as the altered expression and activity of neurotransmitter receptors (Yekkirala et al., 2017). These changes all lead to greater postsynaptic depolarisation in the dorsal horn, and therefore an increase in action potentials within ascending spinal neurons.

Neuroinflammation can act as a driver of central sensitization. Neuroinflammation in the peripheral and central nervous system involves the activation of glial cells to produce proinflammatory mediators. These act as neuromodulators that lead to increased excitatory transmission or decreased inhibitory transmission in the CNS through the mechanisms already noted (R.-R. Ji, Nackley, Huh, Terrando, & Maixner, 2018; Moehring, Halder, Seal, & Stucky, 2018). Some of these inflammatory mediators may also reach dorsal root ganglions and affect gene expression in primary sensory afferents, enabling neuroinflammation in the CNS to trigger peripheral sensitization in addition to central sensitization (R.-R. Ji et al., 2018). T cells have also been shown to be of importance in central sensitization. They infiltrate the CNS after injury, and like glial cells, can produce proinflammatory mediators that facilitate sensitization (Laumet et al., 2019; Vardeh & Naranjo, 2017).

Another mechanism implicated in central sensitization is the loss of inhibitory spinal interneurons in the dorsal horn. This enables the recruitment of subthreshold afferent fibres, including Aβ fibres, and may lead to allodynia and secondary hyperalgesia (Finnerup, Kuner, & Jensen, 2020; Foster et al., 2015; Petitjean et al., 2015; Yekkirala et al., 2017).

In addition to the changes seen at the level of the spinal cord, central sensitization is also associated with structural, chemical and functional changes in brain regions associated with pain processing, including the prefrontal cortices, somatosensory cortices, cingulate, insula and PAG (Baron, Baron, Disbrow, & Roberts, 1999; Freynhagen, Baron, Gockel, & Tölle, 2006; Harte et al., 2018; M. C. Lee et al., 2008; Zambreanu et al., 2005). It is not always clear whether these supraspinal changes are drivers for CS, or whether they are a consequence of increased pain perception. However, the role of descending control from supraspinal regions in modulating the excitability of spinal neurons is well established (see section 1.2.3). One fMRI study showed that the mesencephalic pontine reticular formation and the thalami were the only supraspinal areas in which activity was increased during experimentally-induced central sensitization even when controlling for perceived pain intensity. Activity in the mesencephalic pontine reticular formation, known to be an important region in descending pain modulation (see section 1.2.3.2), also correlated with stimulus strength during central sensitization and not control conditions, indicating that it is a key region for maintaining central sensitization (M. C. Lee et al., 2008). The study controlled for pain intensity, but did not account for affective or cognitive aspects of pain perception, meaning that these results do not rule out the importance of other regions in top-down pain modulation. In fact, the thalamic clusters were functionally

highly connected to the PFC, a key area for descending modulation and the cognitive control of pain (see section 1.2.4.1.6). As outlined above (section 1.2.4.1.4.1 and 1.2.4.1.5), the descending modulatory system also includes the amygdala and ACC. ACC neurons show increased excitatory synaptic transmission following peripheral injury, due to increased presynaptic neurotransmitter release and altered postsynaptic structure and receptor numbers (K. Koga et al., 2018; J.-H. A. Lee, Chen, & Zhou, 2022; Xu et al., 2008). The presence of this plasticity implicates the ACC in central sensitization.

Secondary hyperalgesia can be experimentally induced through the application of topical capsaicin (see section 2.2.1.1), and this provides a useful model for central sensitization that can be used in healthy volunteers.

1.3 The current management of pain

The management of acute pain is primarily pharmacological, and is guided by the aetiology and severity of the pain. Mild non-neuropathic pain is managed with non-opioid drugs such as paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs), which act predominantly by inhibiting cyclooxygenases, and thereby reducing the synthesis of prostaglandins and other inflammatory mediators. A metabolite of paracetamol may also act on TRPV1 and CB1 receptors in the CNS (Ohashi & Kohno, 2020). Ibuprofen, a commonly used NSAID, has been associated with increased activity in descending control circuits during postsurgical pain, indicating that it may also have a central component (Hodkinson et al., 2015). Severe acute pain is treated with opioid analgesics. Opioids act on G-protein-coupled receptors in both the peripheral and central nervous system, where they stimulate

descending inhibitory pathways from the brain as well as acting directly on nociceptive neurons in the dorsal horn and periphery (Chong & Johnson, 2012).

In contrast to acute pain, the management of chronic pain should be multimodal, and take into account the psychosocial contributors to the disease as well as the underlying physiology (Nicholas et al., 2019). Although the recommended management depends on the cause of pain, opioids, which are often a highly effective treatment for severe acute pain, are rarely helpful in chronic pain. When given over long periods they may actually induce hyperalgesia and facilitate pain (Rivat & Ballantyne, 2016). They can also lead to dependence and tolerance, and carry a high incidence of adverse events.

Recent guidance recommends that chronic primary pain (i.e. pain for which there is no clear cause or which is out of proportion to that which would be expected) is managed with a combination of physical activity, psychological therapy, acupuncture and antidepressants (National Institute for Health and Care Excellence, 2021). Meanwhile first-line treatments for chronic neuropathic pain usually comprise an antidepressant, such as amitriptyline or duloxetine, or an antiepileptic, such as pregabalin or gabapentin (National Institute for Health and Care Excellence, 2013). Pregabalin and gabapentin bind to presynaptic voltage gated calcium channels in the dorsal horn and block the release of excitatory neurotransmitters. They are also thought to act through manipulation of descending modulatory pathways and by influencing the affective component of pain, though these mechanisms remain poorly understood (Chincholkar, 2018). The analgesic mechanisms of amitriptyline and duloxetine are also unclear, but since they block the presynaptic reuptake of serotonin and noradrenaline, leading to increased monoamine transmission, they are likely to

act by influencing endogenous pain control (Ammitzbøll et al., 2021; Lawson, 2017; Yarnitsky, Granot, Nahman-Averbuch, Khamaisi, & Granovsky, 2012). The main site of analgesic action for reuptake inhibitors is thought to be the spinal cord (Bannister & Dickenson, 2016; Bannister, Lockwood, Goncalves, Patel, & Dickenson, 2016).

Psychological and cognitive therapies form an important treatment modality for chronic pain, and are often used alongside pharmacological treatments. Of these interventions, cognitive behavioural therapy (CBT) has been the most extensively studied, and the effect size has been shown to be small to medium (Eccleston, Morley, De, & Williams, 2013). Both CBT and acceptance and commitment therapy are recommended in the treatment of chronic primary pain (National Institute for Health and Care Excellence, 2021). Other psychological therapies for chronic pain include mindfulness-based interventions and operant behavioural approaches (Sturgeon, 2014).

For selected patients whose chronic pain is refractory to other measures, interventional procedures may be considered. Such measures include nerve blocks (injections of local anaesthetic and/or corticosteroids around a nerve), denervation, and neuromodulation. Neuromodulation covers a range of interventions, with varying levels of invasiveness, including deep brain stimulation, spinal cord stimulation, peripheral nerve stimulation, transcranial stimulation, and transcutaneous nerve stimulation (Knotkova et al., 2021). Though such treatments show promise in the management of neuropathic pain, and chronic pain more generally, the evidence is mixed, and often of poor quality. Given the risks associated with many of these therapies, further research is required, and careful patient selection is vital.

Given the high proportion of chronic pain sufferers in whom pain management is inadequate (Breivik et al., 2006), the low percentage of patients that experience benefit from individual therapies, and the limited effect size of both pharmacological and non-pharmacological treatments (Scottish Intercollegiate Guidelines Network, 2013), there is a clear need for the development of safe and effective new treatments for chronic pain. The analgesic discovery process can be challenging, and is discussed in the next section.

1.4 The discovery of new analgesics

On average in the USA, it takes 12 years to take a drug from discovery to market. This is a costly process, and promising preclinical studies often fail to translate into successful clinical trials and subsequent approval (Van Norman, 2016). In the field of analgesia, there have been very few effective new drugs discovered since 1960 (Kissin, 2010), and data shows that only 2% of analgesic drugs entering human trials reach the market (Houlton, 2019). Reasons for this are likely to include the inadequacy of animal models of pain and the difficulties associated with analgesic trials in humans.

Preclinical animal models are invaluable for the very early stages of drug development. They give an insight into potential safety and efficacy. However, pain outcomes are difficult to assess in animals, and behavioural models in animals do not predict clinical efficacy well, leading to low rates of translation (Berge, 2011; Clark, 2016). One reason for this is that pain cannot be directly measured in animals, and nociceptive behaviour in non-humans is not necessarily a good proxy for human pain perception (Deuis, Dvorakova, & Vetter, 2017).

Patient populations are highly heterogenous, making it impossible to develop a model that is widely representative. Meanwhile, most preclinical studies use inbred, genetically modified animals of the same age and sex, that are kept in a homogenous environment (Lascelles, Brown, Maixner, & Mogil, 2018). The models themselves may also be problematic, with many of them lacking fidelity to the human condition they are designed to mimic (Abboud et al., 2021). Outcome measures used in preclinical studies are often measures of mechanical or thermal hypersensitivity, a feature that is present in some but not all chronic pain patients (Clark, 2016; Percie Du Sert & Rice, 2014). Many of these outcome measures are reflexes that do not involve cortical structures, making them a poor representation of the complex human pain experience (Duncan, Lascelles, & Flecknell, 2010; Mogil, 2009). Preclinical studies also tend to look at evoked responses rather than the spontaneous pain seen in most chronic disease states (Mogil, 2009). More recently, there has been increased use of models that demonstrate spontaneous or tonic pain, as well as those that look at the effects of higher order cognition. Examples include burrowing (Deuis et al., 2017), conditioned place preference (T. King et al., 2009), facial expression scoring (Fischer-Tenhagen, Meier, & Pohl, 2022; Heinsinger et al., 2020), and the use of a conditioned stimulus to reveal context-dependent hypersensitivity (L. J. Martin et al., 2019). The elevated plus maze test and the light and dark box test are among a range of tests used to assess the presence of anxiety-like behaviour in the presence of pain, while a number of tests also exist to measure depression-like behaviour (Abboud et al., 2021; Kremer, Becker, Barrot, & Yalcin, 2020).

Despite the use of measures that do involve cortical processing, the relationship between non-verbal behaviour and actual perception is unlikely to be straightforward

or consistent. Prey animals in particular will avoid showing behaviours that indicate pain due to the risks associated with showing possible injury. Additionally, there may be inter-species differences in pain physiology and anatomy (Craig, 2009), though the presence of qualitative as opposed to quantitative differences is contentious (Mogil, 2019). Taking all this into account, animal models cannot be expected to model all aspects of human pain perception and analgesia with high levels of accuracy.

Human analgesic trials are also challenging. The subjective nature of pain reporting means that self-reported outcome measures show high variability both within and between participants. Consequently, large subject numbers are needed to provide sufficient power for an efficacy trial. Not only does this expose more individuals to the potential risk of an early-phase drug, but it is expensive and time-consuming. A biomarker of analgesia has the potential to solve this problem.

1.4.1 The need for an objective biomarker in analgesic discovery

The U.S. Food and Drug Administration (FDA) have stated that to improve the success rate and efficiency of drug development “we need a whole new generation of biomarkers” in order to give early feedback on which drugs are likely to be successful (U.S. Food and Drug Administration, 2017). The existence of an objective and reliable biomarker of analgesic efficacy would transform analgesic discovery. It would enable the use of small trials to prioritize resources, by providing sufficient information on target engagement and modulation of pain pathways to make early stop/go decisions on new analgesics. Neuroimaging has the potential to provide a non-invasive biomarker of this nature. fMRI has been successfully used in a neuropathic pain model to disambiguate an effective analgesic (gabapentin) from both a non-effective

analgesic (ibuprofen) and a placebo using just 12 datasets, despite no significant difference in subjective pain measures (Vishvarani Wanigasekera, Mezue, Andersson, Kong, & Tracey, 2016).

Using machine learning, Duff et al. (2015) demonstrated that even across highly heterogenous drug trials, with a variety of evoked stimuli, disease states and analgesic types, (opioids, NSAIDs, pregabalin and gabapentin were included; tramadol was also included, but other reuptake inhibitors were absent), it is possible to identify a common effect of pain and analgesia on neural activation during noxious stimulation. They developed a protocol for the fMRI-based assessment of central drug activity in analgesics. The protocol has three stages. First, quality assurance is achieved by checking that the experimental pain model used in the study is functioning correctly. Then, the imaging is assessed for the presence of a reliable drug effect on neural responses, giving an indication of pharmacodynamic effect. Last, clinical efficacy is assessed by determining whether the drug effect matches that of other effective analgesics.

While the ability to use fMRI to distinguish effective from non-effective analgesics has been validated in existing drugs, showing proof of concept, the ultimate aim will be to use this approach in the assessment of novel agents in order to facilitate early decisions on whether to continue trials. Additionally, evoked pain is a useful tool for driving central activity, but it does not fully capture the spontaneous pain experienced by chronic pain patients. Therefore, when looking for analgesics that target this population, it is important to consider whether a composite signature of analgesia might provide a suitable biomarker for drug discovery. For example, this could include

neural activity and resting connectivity during tonic pain in addition to evoked responses (Tracey, Woolf, & Andrews, 2019).

1.4.2 Elucidating the neural mechanisms of pain and analgesics

Unlike self-reported pain measures, imaging can provide information about the neural mechanisms that underlie novel analgesic agents. Increasingly, mechanistic information is becoming a requirement for the involvement of industry in new compounds. It has been shown that drugs for which the mechanism is properly understood are more likely to survive phase II trials. In particular it is important to understand the 'three pillars of survival' for drug development. The first of these pillars is that the compound reaches the site at which the drug is expected to act, the second is that it binds to the target, and the third is that there is evidence of target modulation (Morgan et al., 2012). Therefore, before showing interest in a novel agent, industry partners require a good understanding of the pharmacodynamics of a drug, including in vivo assessment of its target engagement; and ideally a biomarker of drug action or efficacy that can be used in clinical trials alongside behavioural data (Lipton & Nordstedt, 2016).

There is considerable variability in the efficacy of different analgesics between individuals and disease processes (Amanzio, Pollo, Maggi, & Benedetti, 2001), and determining the mechanisms of potential treatments may help to determine which patients are most likely to benefit. It can also be used to disambiguate the placebo effect from the drug effect in a clinical trial setting. The importance of this is discussed further in section 1.5.

Imaging also has the potential to inform the earliest phases of treatment development by determining the neural mechanisms that are associated with increased pain perception in a particular group of patients. An understanding of these mechanisms could give insight into possible new analgesic targets.

1.5 The assumption of additivity in placebo-controlled trials

FDA guidance for analgesic development recommends that new drugs should show superiority over placebo or an active competitor rather than non-inferiority (FDA, 2014). The placebo-controlled trial is considered gold standard. However, the assumption of this model is that the same placebo effect takes place in both groups, and that the drug effect can be obtained by pure subtraction of the placebo arm from the drug arm.

It is well known that expectation is a powerful modulator of analgesic efficacy in both experimental pain and clinical pain (Ulrike Bingel et al., 2011; Kam-Hansen et al., 2014), to the extent that a placebo labelled as an active drug can have a similar subjective effect to the drug labelled as a placebo (Kam-Hansen et al., 2014).

There is also evidence that the expectation effect interacts with the treatment effect such that the effect of expectation on pain relief may be greater in the active treatment arm than in the placebo arm (Schenk, Sprenger, Geuter, & Büchel, 2014). Synergy between the active drug and the placebo component of the drug response can occur when the effects of the drug increase the expectancy that it will work, and the increased expectancy increases the drug effect. Therefore, any drug effect that

influences expectations is likely to cause modulation of the associated placebo response. This includes the presence of side effects (Bjørkedal & Flaten, 2011).

The subtraction of placebo effect from drug effect becomes even more problematic when considering the potential of a centrally-acting drug to modulate the neural effects of the placebo, even in the absence of a change in expectancy. Placebo analgesia engages the inhibitory descending pain modulatory system. An imaging study comparing gabapentin and tramadol to placebo showed that placebo caused increased connectivity between the brainstem and the rACC, which are both components of this descending system. However, this effect was not seen during dosing with gabapentin or tramadol, despite the double-blind nature of the study (V. Wanigasekera et al., 2018). A double dissociation has also been seen when comparing the neural effects of remifentanil and placebo (Petrovic et al., 2010; T. D. Wager & Roy, 2010).

The fact that the size of the placebo response is not equal for all interventions, and that the neural mechanisms of placebo are not necessarily replicated in the drug arm, means that additivity cannot be assumed, and subtraction of the placebo response from the response to the active drug may lead to an underestimation of the drug effect. In a superiority trial, this could easily lead to the failure of an effective drug.

This could be one reason for the low number of new analgesics that have advanced beyond initial efficacy trials in recent years. Neuroimaging helps to untangle this by enabling better identification of the actual neural pathways modulated by a drug. The addition of a third “no treatment” arm to studies may also help with this.

1.6 Thesis outline

The need to develop new, effective therapies for pain is clear, particularly for the treatment of chronic pain. It has previously been shown that fMRI can be used as a biomarker for identifying pharmacodynamic effect and analgesic efficacy in drugs for which efficacy is known and mechanisms are well understood (Duff et al., 2015; Vishvarani Wanigasekera et al., 2016). The aim of this thesis is to translate this to the evaluation of novel therapies in small numbers of participants, in order to assist in their early development and inform decisions on the merits of larger, more resource-intensive trials. This would be of particular benefit to pharmacological therapies, for which the drug development pipeline is long and expensive, but would also help with the assessment of psychological/cognitive therapies, which often lack a good evidence base.

Chapter 2 discusses the general methods that were used across all of the studies in this thesis, including the measurement of self-reported pain; the use of validated questionnaires; and the fundamentals of magnetic resonance imaging (MRI) physics, acquisition and analysis.

Chapter 3 describes the specific methods that were used to conduct an early phase clinical trial of the novel pharmacological agent ABX-1431, an MGLL inhibitor that is the first of its class. Phase I trials such as this have stringent requirements to ensure safety and scientific integrity, and this chapter looks at how these requirements were met.

In chapter 4, the effect of ABX-1431 on self-reported behavioural outcome measures was assessed. The aims were to look for behavioural evidence of a pharmacodynamic effect and of analgesic efficacy. This chapter also considers the possible influence of confounding factors that may have an effect on the power of the study to detect analgesic efficacy.

Chapter 5 investigates whether the use of fMRI as a biomarker enhanced the ability to detect pharmacodynamic effect and analgesic efficacy in ABX-1431 in a small group of participants. Imaging was also used to assess the possible mechanisms by which ABX-1431 might act, and consequently to assess what types of patients may benefit from it as an analgesic.

In chapter 6, irritable bowel syndrome is introduced as an example of a chronic pain condition in which new therapies are needed. A cohort of these patients underwent a course of mindfulness-based cognitive therapy. This is a psychological intervention for which there is currently minimal evidence. Chapter 6 looks at the effect of this intervention on self-reported behavioural outcomes to determine whether it was possible to show a pharmacodynamic effect and analgesic efficacy from this data. Potential mediating factors for its effect are also considered.

Chapter 7 uses fMRI to look for objective evidence that mindfulness-based cognitive therapy modulates central neural processes in chronic pain, and to determine the mechanisms that might underlie its effectiveness.

Finally, chapter 8 discusses the implications of these findings for the use and future development of both of the therapies assessed in this thesis, as well as for the utility of fMRI in the development of novel pharmacological and cognitive therapies for pain as a whole.

Chapter 2: General methods

2.1 Introduction

A mixture of behavioural and imaging-based measures were used across all studies performed. This chapter aims to summarise the methods used. In particular, it will address the different experimental pain models that were used; the validated questionnaires that were administered; the issue of multiplicity during the analysis of behavioural data; and the use of functional MRI (fMRI), including MRI physics, acquisition and analysis. Details of the application of these methods to individual studies will be given in subsequent chapters.

2.2 Experimental pain models

2.2.1 Pain stimuli

A mixture of thermal, chemical and mechanical pain stimuli were used in these experiments.

2.2.1.1 *Chemical stimulation*

Capsaicin cream has been used extensively in pain research as a model of central sensitization, which is a mechanism seen in neuropathic pain (e.g. Evans et al., 2021; M. C. Lee et al., 2013; Vishvarani Wanigasekera et al., 2016). It creates both a tonic pain response, which normally feels like a burning or stinging sensation, and an area of surrounding secondary hyperalgesia. Capsaicin is the active ingredient in chilli peppers. It acts on C-nociceptive fibres by binding to TRPV1 receptors in the skin. Topical capsaicin produces pain, allodynia, and mechanical hyperalgesia, which are

all features of neuropathic pain. However, it does not cause nerve injury, and its effect is reversed following removal of the cream.

Capsaicin can also be administered by intradermal injection. Although this produces a larger and longer-lasting hyperalgesia response than topical capsaicin (Liu, Max, Robinovitz, Gracely, & Bennett, 1998), the main disadvantage of this method is that the tonic pain that it produces subsides after a few minutes (Jantsch et al., 2009; M. C. Lee et al., 2008).

A topical 1% capsaicin cream was supplied by The Specials Laboratory (Northumberland, UK), using Unguentum M as a base cream. The response to capsaicin is variable between individuals, and may also vary depending on the dose of capsaicin applied and the absorbency of the base cream. A small pilot study was performed to ensure that the cream produced nociception, and to assess the time required for an adequate pain response to occur. The findings are outlined in appendix A. Ambient temperature also affects the response to capsaicin, and for this reason air conditioning was used to maintain a consistent temperature during all visits in which capsaicin cream was administered.

2.2.1.2 *Mechanical stimulation*

A 512nM punctate probe was used to elicit mechanical pain. It was applied to normal skin, as well as to areas of hyperalgesia secondary to topical capsaicin application.

2.2.1.3 *Thermal stimulation*

Thermal pain was administered with a Medoc pathway system and a contact heat evoked potential stimulator (CHEPS) thermode. It was a circular thermode, with a 27mm diameter. A CHEPS thermode was chosen due to its rapid heating and cooling ability, meaning that the temperature could be quickly adjusted for a set duration, without long ramps in either direction.

2.2.2 Pain Thresholding

Standardisation of the painful stimulus that is applied can be achieved by two methods: nociception locking (also called fixed stimulus intensity), in which the same stimulus is applied regardless of the resulting pain perception; and perception locking, in which the stimulus is adapted to give a defined subjective pain measure. Neither method is perfect, but both have advantages, and both are used extensively in pain research (Adamczyk et al., 2022).

The main advantage of nociception locking is that since every participant receives the same stimulus, any differences seen are more easily interpreted as differences in the processing of that stimulus. However, a big disadvantage of this method is that it produces considerable between-subject variability in reported pain. This heterogeneity may be due to factors unrelated to the experiment, including natural variability in the sensory profile of the population; psychological factors, such as anxiety; and differences in the expectations and past experiences of individuals (Adamczyk et al., 2022). Not only does this make interpretation challenging, but it also makes stimulus selection difficult, since a nociception locked stimulus that is non-painful for one person may be unbearable to another. This may be particularly true of chronic pain

populations, in whom some have pre-existing hyperalgesia. There is also a risk that ratings might be affected by ceiling or floor effects if they are perceived to be at one extreme of the ratings scale (Adamczyk et al., 2022). Although a repeated measures design can be used to reduce the impact of between-subject variability, it does not address within-subject variability in pain ratings. This can occur across sessions due to the presence of different environmental factors, though it can also take place within a single session (Madden et al., 2021), and this is problematic for both types of thresholding.

For studies in which consistency of subjective responses across subjects and sessions is more important than that of the stimulus, perception locking provides a good alternative. Floor and ceiling effects are unlikely, and stimuli should remain tolerable to every individual (Adamczyk et al., 2022). Although between-subject (or between-session) variability in perception is reduced, within-session variability means that thresholding is not always totally accurate (Madden et al., 2021). A major disadvantage of this method is that it can make it difficult to determine whether measures such as neural activation are altered due to changes in stimulus intensity, due to the resulting changes in nociceptor recruitment, or due to subsequent stimulus processing. A further disadvantage is that pain input is required prior to the experimental stimulus in order to determine the stimulus required for each individual. This may be undesirable due to the risk of invoking modulatory mechanisms that affect subsequent perception. Although thresholding could be done in a separate session, environmental changes from one session to the next, including those that are disease-related, may alter perception of the stimulus, making the thresholding invalid.

2.2.3 Measures of evoked pain intensity and unpleasantness

A variety of pain rating scales were used to quantify pain intensity and unpleasantness during these studies. The most frequently used was the visual analogue scale (VAS). This is a line of a set length, with descriptive anchors at either end. For example, the two anchors may be “no pain” and “extreme pain”. Participants then select the point along the line which describes their current experience. This can be done on paper, using a pen or pencil to mark the line, or alternatively on a screen, using buttons to move a marker into position. The rating is calculated by measuring the distance from the left-hand end of the line to the participant’s marker. Advantages of this method are that it is possible to measure small increments, and that the anchors can be varied to suit the experiment. It shows good test-retest reliability, and correlates well with other pain measures, indicating validity (Haefeli & Elfering, 2006; Hawker, Mian, Kendzerska, & French, 2011). It is important that all participants have a good understanding of what the anchors mean and how to use the rating scale. In order to achieve this in these studies, a pre-written script was used to explain pain ratings to each participant.

The numerical rating scale (NRS) asks participants to select from a range of numbers that represent pain. For example, from 0-10, or from 0-100. Generally, the 11 point 0-10 scale is the easiest to administer. Due to the discrete nature of the possible responses, it is less able to discriminate between subtle changes than the VAS. Increasing the numerical range can help with this, but limited paper or screen space may make this challenging. The NRS gives good test-retest reliability and has been shown to be valid when compared with other pain measures (Hawker et al., 2011). A big advantage of this method is that it is possible to administer it verbally, making it a

useful measure when a participant might struggle to complete written responses, or when the participant is performing another task simultaneously and a quick indicator of current pain levels is required.

2.3 Validated Questionnaires

2.3.1 Symptom measurement

2.3.1.1 Brief pain inventory – short form

The brief pain inventory - short form (BPI) is recommended for the assessment of pain symptoms in clinical trials (Dworkin et al., 2005), and looks predominantly at the pain experienced over the preceding 24 hours (C. S. Cleeland & Ryan, 1994). It contains a pain severity subscale and a pain interference subscale. Pain interference measures the degree to which pain interferes with aspects of everyday life. The questionnaire has been shown to have good validity and reliability across a range of pain conditions (Jelsness-Jørgensen et al., 2016; Keller et al., 2004; Stanhope, 2016).

2.3.1.2 Gastrointestinal symptom rating scale – irritable bowel syndrome

This is a 13 item rating scale designed to assess gastrointestinal symptoms in irritable bowel syndrome (IBS; Wiklund et al., 2003). It assesses symptoms over the preceding week by obtaining ratings on a 7-point Likert scale. It provides a total score as well as scores for 5 symptom clusters (satiety, abdominal pain, diarrhoea, constipation and bloating). It has shown good validity and internal reliability in IBS.

2.3.2 Measures of depression and anxiety

Pain, anxiety and mood are closely interlinked. In a laboratory setting, experimentally-induced anxiety leads to increased pain perception (Ploghaus et al., 2001; Rhudy &

Meagher, 2000). This also applies in real-world situations, where those with increased anxiety are likely to experienced increased acute pain (Kain, Sevarino, Alexander, Pincus, & Mayes, 2000). In chronic pain patients, mood and anxiety disorders are often comorbid with pain disorders (Arnow et al., 2006; Bair, Robinson, Katon, & Kroenke, 2003; Demyttenaere et al., 2007; Gureje et al., 2008), and high levels of anxiety and depression are associated with increased chronic pain intensity and worsened disability (Bair et al., 2003; Martinez-Calderon et al., 2018).

2.3.2.1 State-trait anxiety inventory

The state-trait anxiety inventory (STAI) is a well validated 40 item self-reported questionnaire that consists of two 20 item subscales (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). The state subscale gives a measure of current anxiety, which may be situational and transient, while the trait subscale measures the more persistent underlying predisposition to anxiety. Higher subscale scores indicate greater anxiety. The state component is more sensitive to change than the trait component, and thus may be more useful in longitudinal research studies (Julian, 2011).

2.3.2.2 Hospital anxiety and depression scale

This is a self-reported questionnaire consisting of an anxiety scale and a depression scale (Zigmond & Snaith, 1983). Each subscale has 7 items. It is commonly used in the clinical environment due to its speed and simplicity. For each scale, the minimum score is 0 and the maximum is 21. A score of <8 is considered normal, 8-10 is borderline, and >10 is abnormal. At a cut-off score of 8/21, the questionnaire shows good sensitivity and specificity for detecting anxiety and depression in the general population (Bjelland, Dahl, Haug, & Neckelmann, 2002).

2.3.2.3 Visceral sensitivity index

The visceral sensitivity index (VSI) is a 15 item self-reported scale measuring gastrointestinal symptom-specific anxiety in IBS. It shows high levels of reliability and validity in an IBS population. Symptom-specific anxiety has been shown to have a strong link with symptom severity in IBS, and there is also evidence that the detrimental effect of high state anxiety on IBS symptoms may be mediated through symptom-specific anxiety (J. S. Labus et al., 2004).

2.3.3 Other psychological measures

2.3.3.1 Pain catastrophizing scale

Pain catastrophizing is an exaggerated negative cognitive and affective response to pain or anticipated pain (Leung, 2012). It is associated with increased pain perception in both experimental settings and patient groups. It is also associated with increased disability (Sullivan et al., 2001). fMRI studies have shown that catastrophizing is associated with increased activity in pain-related brain regions (Gracely et al., 2004; Seminowicz & Davis, 2006), and high levels of catastrophizing may be associated with reduced descending inhibitory control (Seminowicz & Davis, 2006). In IBS patients, catastrophizing may partially mediate the effect of depression on abdominal pain severity (Lackner, Quigley, & Blanchard, 2004).

The pain catastrophizing scale is a 13 item questionnaire that gives a total score of 0-52, and gives subscale scores for the catastrophizing dimensions of rumination, magnification and helplessness. A total score of ≥30 is thought to represent a clinically meaningful level of catastrophizing (Sullivan, 2009; Sullivan, Bishop, & Pivik, 1995).

2.3.3.2 BIS/BAS

The BIS/BAS scales are measures of sensitivity to two motivational systems (Carver & White, 1994). The behavioural inhibition system (BIS) regulates the motivation to avoid aversive outcomes, while the behavioural approach system (BAS) regulates the motivation to achieve desirable outcomes. The questionnaire has 24 items and gives 4 subscale scores. One subscale gives a BIS score, while 3 give scores for different components of BAS. BAS drive measures the motivation to achieve personal goals. BAS fun seeking measures the level of motivation for finding new rewards and the willingness to approach events that might be rewarding on the spur of the moment. BAS reward responsiveness measures sensitivity to positive reinforcement.

There is good evidence that motivational style has an important role in pain outcomes for both healthy individuals and chronic pain sufferers (Jensen, Tan, & Chua, 2015; Serrano-Ibáñez, López-Martínez, Ramírez-Maestre, Esteve, & Jensen, 2019; Vishvarani Wanigasekera et al., 2012). Pain relief is a rewarding experience (Leknes, Brooks, Wiech, & Tracey, 2008; Edita Navratilova & Porreca, 2014), and therefore BIS/BAS scores are highly relevant for studies of interventions aimed at producing analgesia.

2.3.3.3 Bond & Lader mood scale

The Bond-Lader scales are a set of 16 visual analogue scales that are used to assess changes in mood and alertness (Bond & Lader, 1974). They are commonly used in trials to determine drug effects, in particular with relation to sedative effects, and this was their purpose here. Each VAS is 100mm long, and is anchored at either end with words that describe opposing descriptors e.g. happy and sad. The score for each is

determined by the distance from the negative anchor, meaning that a higher score indicates a more positive mood or higher level of alertness.

2.3.4 Quality of life measures

It is generally accepted that chronic pain has an adverse effect on quality of life. From a patient's perspective, quality of life improvement is an important measure of an intervention's efficacy.

2.3.4.1 SF-36 version 2

The SF-36 is a well validated 36 item generic quality of life measure which yields 8 subscales as well as summary measures of mental and physical health (Ware, Kosinski, & Dewey, 2000). The subscales are bodily pain, general health, mental health, physical functioning, role-emotional, role-physical, social functional and vitality. Scores for each scale are transformed to a 0-100 scale where 0 is the worst possible health status and 100 is the best. A score of 50 is the norm for the general United States population.

2.3.4.2 Irritable bowel syndrome impact scale

This is a 26 item disease-specific questionnaire that gives a total score and scores for 5 subscales: fatigue, impact on daily activities, sleep disturbance, emotional distress and eating habits. It was designed to assess the degree to which IBS symptoms and treatment impacted the lives of patients (Longstreth et al., 2005).

2.4 Analysis of behavioural data

2.4.1 Dealing with multiplicity

A major limitation of the behavioural data analysis in this thesis is the issue of multiplicity, in which multiple comparisons are performed on the same set of data. This is particularly problematic when the sample size is small, as is the case for these studies since they are powered for the analysis of imaging rather than behavioural data.

The question of whether analyses should always be corrected for multiple comparisons is contentious (Perneger, 1998), and even experts who review papers on a regular basis lack confidence in stating whether certain techniques should be used or not (Cabin & Mitchell, 2000). The risk of performing multiple comparisons is that there is an increased likelihood of a type I error in at least one of the analyses, in which the null hypothesis is falsely rejected. The most common way of correcting for this is the Bonferroni correction, in which the p value used to determine whether or not the null hypothesis should be rejected is reduced by dividing the desired p value by the number or analyses performed on the data. There are a number of potential issues with this technique that made it less suitable for use on the data in this thesis. Firstly, those participants who showed improvement on one outcome measure are also likely to show improvement on other outcome measures, meaning that there is a high chance of correlation between the measures. In this situation a correction like the Bonferroni becomes much too conservative. A second issue with the Bonferroni correction is that it relies on the universal null hypothesis. This means that there is an assumption that the effect on all outcome measures will be similar, something that may or may not be true. It is clear that the Bonferroni, like other methods of multiple

comparisons correction, leads to an increase in type II errors, in which the null hypothesis is not rejected despite there being a real effect (Nakagawa, 2004; Perneger, 1998). This leads to studies being underpowered. In a small study such as these, this effect on power becomes very problematic, since power is already an issue. Any correction for multiplicity also suffers from the issue that the findings of one test will depend on how many other tests are performed, a factor which clearly does not influence the likelihood of that one outcome being true in real life (Perneger, 1998). Many statisticians are of the opinion that rather than correct data for multiple comparisons, it is better to limit the number of comparisons performed when possible, and to simply describe which statistical tests were done, and accept that multiplicity may be a limitation of some studies (Perneger, 1998).

In these studies, the chance of a type I error is higher than the 5% that is normally considered acceptable. However, a decision was made that to present uncorrected data was reasonable in order to avoid the effects of aggressive correction for multiple comparisons, which would have led to a very high rate of type II errors, with few, if any, significant findings.

In contrast, the imaging analysis does include correction for multiple comparisons. Hypothesis testing is performed on a voxel-by-voxel basis, meaning that thousands of comparisons are made during each statistical test. Without correction, the number of type I errors would be unacceptably high. The negative impact of correction for multiple comparisons is likely to be less for the imaging data than for the behavioural data, since the studies were appropriately powered for imaging in the first place.

2.5 Magnetic Resonance Imaging

2.5.1 MRI physics

Hydrogen ions in the blood spin on their axis at a characteristic frequency. When moved into a static magnetic field in the MRI scanner (the B_0 field), these axes align with the magnetic field, causing net magnetization in that direction. A radiofrequency pulse is then applied which oscillates at the same frequency as the hydrogen ions. This excites them, causing their direction of magnetization to tip away from the direction of the B_0 field, into the transverse plane. Magnetization in the transverse plane induces current in a wire coil which is proportional to the amount of magnetization. This is what creates MRI signal. When the radiofrequency pulse is removed, relaxation occurs, in which the magnetization leaves the transverse plane and returns to alignment with the B_0 field, causing signal decay. The speed of this relaxation varies between tissues, enabling an image to be built.

2.5.2 Blood oxygen level dependent effect

Haemoglobin in the blood combines with oxygen to create oxyhaemoglobin, and with carbon dioxide to create deoxyhaemoglobin. Oxyhaemoglobin is diamagnetic, while deoxyhaemoglobin is paramagnetic. The presence of deoxyhaemoglobin in vessels leads to local distortion of the magnetic field which results in dephasing of the oscillation frequencies of the hydrogen ions following excitation. This results in signal loss, and produces a difference in signal between oxygenated and deoxygenated blood. This is the blood oxygen level dependent (BOLD) contrast.

2.5.3 Haemodynamic response function

The proportion of oxygenated vs. deoxygenated blood is an indirect measure of neural activity. Neurotransmission requires energy, which is created through metabolic processes that use oxygen and produce carbon dioxide. When neuronal activity increases, the demand for oxygenated blood increases, and blood flow increases to meet this demand. Although more oxygen is extracted from the blood and more carbon dioxide is produced, the increased blood flow means that overall the blood in the area of neural activity contains more oxyhaemoglobin and less deoxyhaemoglobin, leading to an increase in MRI signal.

Due to the indirect nature of the relationship between neural activity and blood flow, the timecourse of the haemodynamic response does not map exactly onto the underlying change in neural activity, making the temporal resolution of the technique relatively low. However, the spatial resolution is high, making it a very useful tool for determining the location of changes in neural activity.

2.5.4 Task-based activity

Changes in regional BOLD activity can give an indication of the brain regions that are involved in processing a specific task of interest. In a task-based experiment, a task or block of tasks will be performed at particular timepoints. These timepoints are then compared to the timecourse of the MRI signal, and regions in which they track one another are identified to determine which brain regions show alterations in activity during the task. A subtraction technique may then be applied to determine whether there is a difference between task-related activity in two or more different conditions (e.g. task differences, drugs, disease states etc.).

2.5.5 Resting state functional connectivity

Functional MRI can be used to investigate the way in which different brain regions communicate with one another. To assess this connectivity, the BOLD signal between regions of interest can be compared. If two regions share a similar signal timecourse to one another, it is likely that they are communicating. This connectivity is usually assessed at rest (i.e. not while performing a specific task). As for task-based imaging, subtraction can be performed to compare resting connectivity across two or more groups.

2.5.6 Arterial spin labelling

Although BOLD imaging is a very useful tool, a disadvantage of the technique is that it is not quantitative, and is therefore unable to give an exact measure of perfusion. Instead, it provides information about changes that occur with relation to baseline. Baseline signal can be influenced by a wide range of factors, including blood pressure, disease states, altered ventilation, and drugs. Even within a scan, the baseline is prone to drift over time, meaning that BOLD is not an ideal technique for assessing longer, ongoing stimuli or tasks. Arterial spin labelling (ASL) is ideally placed for these kind of experiments as it is a quantitative technique, able to provide exact measures of perfusion over time.

ASL works by labelling blood as it passes through the main arteries of the neck, and then acquiring images of the brain to determine which brain tissues the labelled blood has been delivered to at a number of timepoints after labelling. These tag images are compared to a control image, prior to which no labelling took place. Labelling is

achieved by applying a radiofrequency pulse to invert the magnetization of hydrogen nuclei in the blood as it passes through the 'labelling plane'.

2.5.7 MRI acquisition

MRI safety screening was carried out at every visit prior to scanning. All imaging data were acquired on a 3T Siemens Prisma MRI scanner at the Oxford centre for functional MRI of the brain (FMRIB), Oxford, UK. A 32 channel receive-only head coil was used. Visual information for participants was displayed via software called Presentation (Neurobehavioural Systems). Presentation also recorded participant responses via a button box that was used in the scanner. Parameters for each scan are given in the relevant chapters.

2.5.8 BOLD task-based fMRI analysis

2.5.8.1 Pre-processing

2.5.8.1.1 Brain extraction

Brain extraction is performed in order to remove non-brain structures from the image. This was mostly achieved using the BET (brain extraction tool) function from FMRIB's software library (FSL; Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012; Smith, 2002).

2.5.8.1.2 Registration

In order to compare images of the same or different modalities, both within and between participants, those images must be aligned with one another. This is called registration. During BOLD pre-processing, the functional image must be registered to the structural image. In these studies, this was performed using FMRIB's Linear Image

Registration Tool (FLIRT; Jenkinson, Bannister, Brady, & Smith, 2002a; Jenkinson & Smith, 2001). Boundary based registration was used (Greve & Fischl, 2009), meaning that the anatomy was defined by the white-matter boundaries. Images were then registered to standard MNI space using nonlinear registration so that images from different participants could be compared with one another. This was done using FMRIB's Nonlinear Image Registration Tool (FNIRT; Andersson, Jenkinson, & Smith, 2007).

2.5.8.1.3 B0 unwarping

The presence of an object (including a person's head) within the B_0 field causes inhomogeneities that lead to distortions and signal loss. At the time of scanning, some of the inhomogeneity can be corrected by adjusting coils within the scanner to cancel out as much nonuniformity as possible. This is called shimming. However, it is not possible to eliminate inhomogeneity-related signal loss without also eliminating the BOLD effect. BOLD relies on the loss of signal associated with local field disruption in the presence of deoxyhaemoglobin. The skull contains air-filled sinuses, which also cause disturbance to the B_0 field, resulting in signal dropout and distortion around the frontal and temporal areas. While signal that has gone cannot be brought back, distortions can be corrected using a fieldmap that measures the field inhomogeneity and corrects for it in the analysis.

Fieldmaps were obtained for all scan sessions and were applied during BOLD image analysis using the MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) graphical user interface (GUI) in FSL (Jenkinson et al., 2012). Magnitude and phase images were acquired. The magnitude

images were brain extracted, then fieldmaps were prepared from both images using the FSL tool fsl_prepare_fieldmap. For some of the magnitude images, in order to remove all non-brain voxels during brain extraction, BET removed too much from other brain regions. In these cases, brain extraction was performed by registering the magnitude image to a structural image from the same individual, inverting the transformation, and then applying it to a mask of the brain extracted structural image. The mask was then used to generate a brain extracted magnitude image.

2.5.8.1.4 Noise correction

Noise can be introduced from a variety of sources. The MRI scanner produces thermal noise, the level of which depends on the strength of the magnetic field. There is little that can be done to correct this. Head movement also produces noise. A third type of noise is physiological noise. Physiological processes may cause noise through movement. This can be gross movement, for example head movement secondary to chest wall movement during breathing. Alternatively, it may be more subtle movement, for example cerebrospinal fluid (CSF) or arterial pulsation within the head. Some physiological noise is not movement related, for example altered vasculature and blood flow due to changes in carbon dioxide levels during breathing.

Motion during a scan is inevitable and problematic. Not only can it lead to obvious image distortion, but it can also induce changes in signal intensity due to changes in the contents of each voxel. In task-based experiments, there is a risk that motion may co-occur with a stimulus, making it difficult to determine whether signal changes are motion-related or stimulus-related. This is a particular risk in pain studies, in which pain may cause an involuntary motor reaction.

In order to reduce movement in the scanner, participants are instructed to lie as still as possible, and padding is placed around their head for support. However, head motion will still take place. Some of this can be corrected in pre-processing by obtaining motion parameters during the scan, and adding them to the general linear model (GLM) as confounds. This was performed in all studies using McFlirt (Jenkinson et al., 2002). This method is good for eliminating gross movement, but assumes that the whole brain is moving as one rigid body. This may not be the case if slices are not all acquired simultaneously, and may not be true of all motion that is physiological in origin. Other techniques for motion correction and noise reduction include physiological noise monitoring and independent component analysis (ICA). Both were used during the course of the studies in this thesis.

2.5.8.1.4.1 Physiological noise correction

Although this is not applied at the pre-processing stage, it is a technique for reducing physiological noise, so is dealt with in this section. Information about the timing of cardiac and respiratory cycles is obtained for the duration of each scan using a pulse meter and pneumatic belt. These measures are included as regressors in the GLM, and motion that is synchronous with the timing of these measures is removed. This is particularly important for regions that are most affected by physiological motion, such as the brainstem.

2.5.8.1.4.2 Independent component analysis

ICA works by splitting data into its spatial and temporal components. This outputs a spatial map and timecourse for each component. Each component can then be classified as either signal or noise according to its spatial and temporal pattern. This

can be performed by hand, by inspecting and labelling each component individually, or can be automated.

Automated denoising requires a set of training data in which the components are already classified. A training data set must be appropriate to the data requiring classification. This means that it must have been acquired from a similar population, using similar scanning parameters. No suitable training data was available for the studies in this thesis. Therefore, training data was created for each dataset by hand-classifying the components from a subset of the scans acquired. For each dataset, 18 scans were used to create the training data, and these scans sampled all types of participant within the dataset (e.g. patients and controls; pre-intervention and post-intervention). Hand-classification was performed using guidance from Griffanti and colleagues (Griffanti et al., 2017). This training data was then fed into the automated ICA denoising tool FIX (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014), and FIX was used to classify the entire dataset.

2.5.8.1.5 Slice timing correction

During the acquisition of multiple slices, different slices will be scanned at slightly different times, meaning that data from certain slices will be slightly offset with respect to the model. Slice timing correction moves each timeseries by an appropriate amount to counteract this. Slice timing correction was applied using FEAT (FMRI expert analysis tool; Woolrich, Ripley, Brady, & Smith, 2001).

2.5.8.1.6 Spatial smoothing

Spatial smoothing reduces the contrast between neighbouring voxels. Since most spatial noise is random and centred around zero, smoothing averages the spatial noise towards zero, improving the signal to noise ratio. A full width at half maximum (FWHM) of 5mm was used for all studies.

2.5.8.1.7 Temporal filtering

The timeseries from each voxel contains both signal and noise. Temporal filtering can be applied to remove a range of frequencies that is known not to include the signal of interest. All data in these studies was lowpass filtered using FEAT in order to remove high frequency noise (Woolrich et al., 2001). The cut-off for filtering was carefully set in each study using FEAT to ensure that it was greater than the frequencies of interest as specified in the GLM.

2.5.8.2 Single subject analysis

After pre-processing, each scan is analysed individually to determine which regions show task-related changes in BOLD activity using a GLM. The timecourse of the task is compared to the timecourse of activation changes for each voxel, and those that match are identified. Potential confounding factors can also be added to the model, and activation that has the same timecourse as a confounding factor can then be removed. In this thesis, all single-subject analysis was performed in FEAT (Woolrich et al., 2001), using FILM (FMRIB's Improved Linear Model).

2.5.8.3 Higher level analysis

Once task-related BOLD activity has been identified for each individual as above, the data can be combined in order to look for group effects. A wide range of higher level analyses can be performed, but the methods used most commonly in this thesis are outlined below. In most cases, higher level analysis was carried out in FEAT using FLAME (FMRIB's Local Analysis of Mixed Effects; Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004).

In some cases, higher level analysis was performed in two stages. The first stage was a within-subject analysis of multiple sessions. A fixed effects analysis was used for this. The results of this were then fed into a second stage analysis that combined the results from multiple participants using FLAME.

Some higher level analyses were performed using permutation testing. These analyses were performed using the FSL tool randomise (Winkler, Ridgway, Webster, Smith, & Nichols, 2014).

2.5.8.3.1 Group mean

This is the simplest type of higher level analysis, and involves averaging the activation across subjects to find the mean activation of the group. Figure 2.1 shows an example of the GLM setup and design matrix for this type of analysis.



Figure 2.1: Example of a general linear model setup and design matrix for a group mean analysis in FSL.

2.5.8.3.2 Independent samples t-test

This analysis determines the mean group difference between two unrelated groups. An example of its application would be when comparing a group of people with a specific disease with a group of healthy participants. Clusters of activation in which the group difference is above a specified Z threshold are considered significant. In this thesis, the Z threshold is 2.3.

Figure 2.2 shows an example of a GLM setup and design matrix for this analysis.

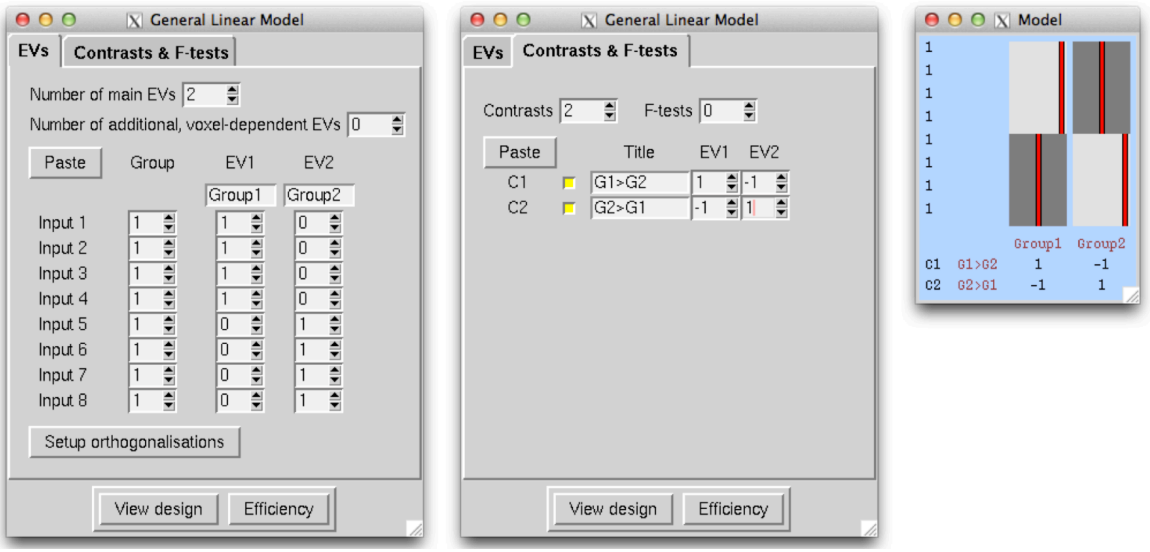


Figure 2.2: Example of a general linear model setup and design matrix for an independent samples t-test. Figure from www.fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT/UserGuide (FMRIB, 2018).

2.5.8.3.3 Paired t-test

This analysis determines the mean group difference between two related groups. For example, comparing data from the same participant on two different occasions. Figure 2.3 shows an example of a GLM setup and design matrix for this type of analysis.

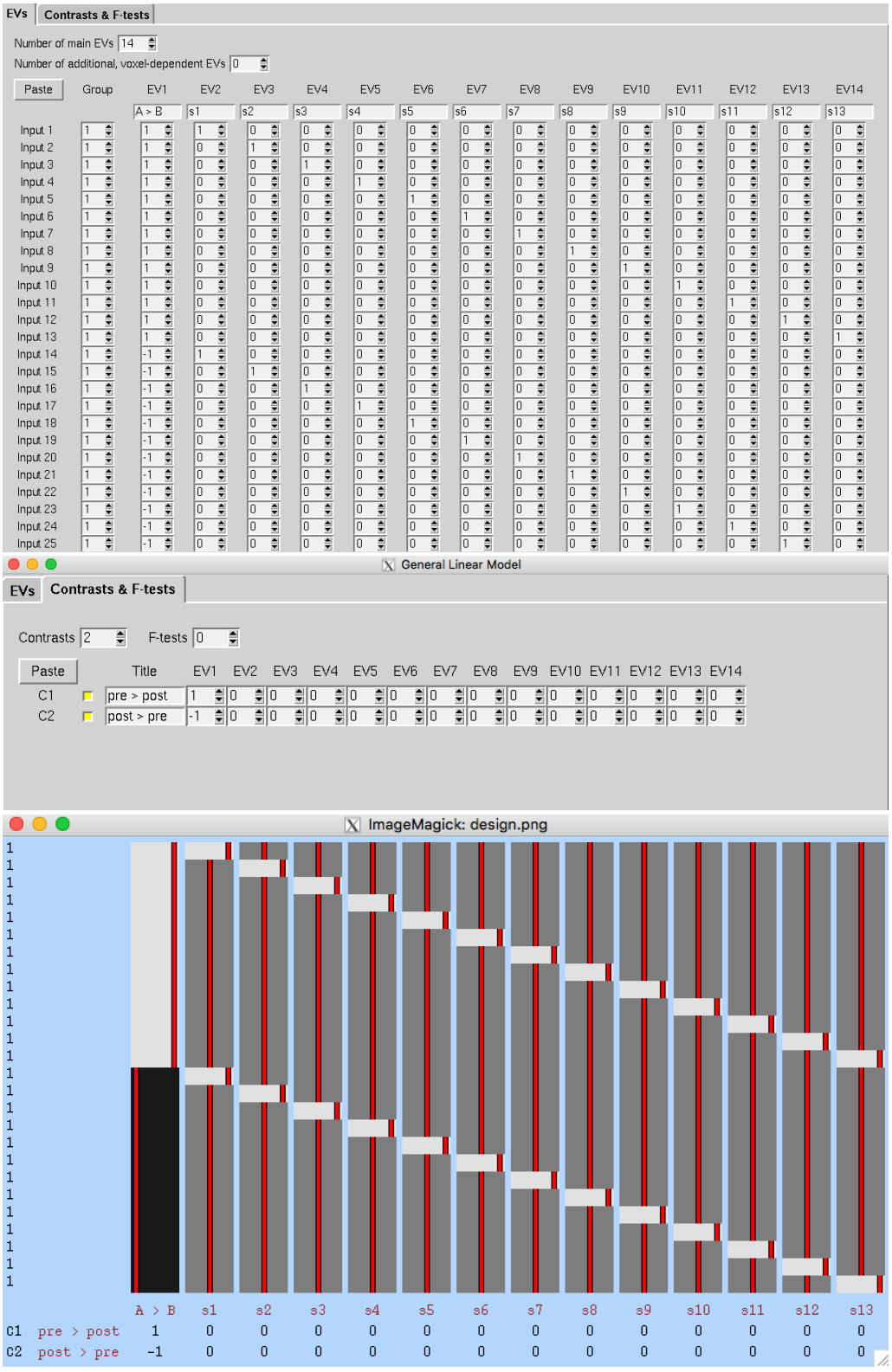


Figure 2.3: Example of a general linear model setup and design matrix for a paired t-test. There are 13 participants in this analysis, each with images at two timepoints.

2.5.8.3.4 Covariates

Covariates are variables that are not the main intervention under investigation, but that have the potential to alter the results of an analysis. By including a covariate in the design matrix, it is possible to control for its effect by removing any changes in brain activity that appear to vary as a function of the change in the covariate. Alternatively, the covariate may be a variable of interest, in which case identifying the brain regions in which activity correlates with the covariable can give insights into its neurological effect.

Figure 2.4 shows an example of a design matrix for an independent samples t-test with a covariate. The covariate is demeaned, meaning that the mean value becomes zero. This is important when controlling for a covariate, as it means that the resulting analysis will apply to a subject with an average value for the covariate, rather than to a subject with a covariate value of zero, which often makes no sense.

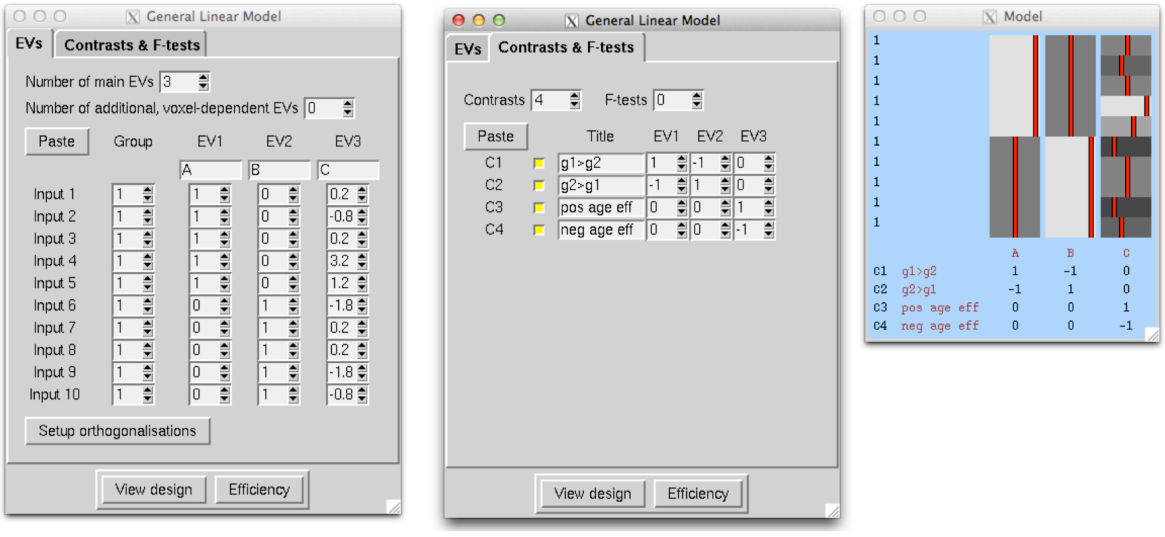


Figure 2.4: Example of a general linear model setup and design matrix for an independent samples t-test with a covariate. This design includes four contrasts. The first two contrasts compare the two groups with the effect of the covariate regressed out. The third and fourth contrast look for positive or negative effects caused specifically by the covariate. Figure from www.fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM (FMRIB, 2019).

2.5.8.4 Region of interest analysis

The above analyses all produce results for the whole brain. Due to the high number of voxels that are tested during these analyses, which are then corrected for multiple comparisons as part of the thresholding process, power to detect changes in individual regions can be low.

When there is a specific region of the brain that is of interest due to an a priori hypothesis, it is appropriate to do a region of interest (ROI) analysis. By investigating only the region of interest, the number of voxels is reduced and the power is increased. Two methods for ROI analysis were used here: featquery and pre-threshold masking.

2.5.8.4.1 Featquery

Featquery is a tool within FEAT that is used to extract statistics on the activation of voxels within a mask of the ROI. It is applied at the single subject level, so activity can be compared across individuals if desired. A range of statistics can be extracted, including the mean activation for the ROI, as well as the minimum, maximum, 10th percentile and 90th percentile. These values can also be converted into a percentage, enabling the determination of the % change in BOLD activity in response to a stimulus. This is particularly useful when comparing between participants or visits, since the baseline BOLD activity may be variable. Hypothesis testing can then be performed on these values or percentages using statistical analyses.

2.5.8.4.2 Pre-threshold masking

In pre-threshold masking, a mask of the ROI is applied during the higher-level analysis, prior to thresholding. The search for activation is then confined to that area alone. The

higher-level analysis is otherwise performed in the same way as the equivalent whole-brain analysis.

2.5.9 BOLD resting state analysis

2.5.9.1 Dual regression

Dual regression is used to investigate individual or group differences in whole-brain spatial networks.

2.5.9.1.1 Pre-processing

The pre-processing steps used were the same as those outlined above for task-based analysis, including the removal of noise components with FIX. The cleaned data for each participant was then registered to standard space.

2.5.9.1.2 Group independent component analysis

The purpose of this step is to identify patterns of connectivity that are common to the population of interest. The datasets from each participant/scan session were concatenated, and a group ICA was run using MELODIC. The output from this was a 4D image, with each volume corresponding to a separate component. The dimensionality can be specified at this stage. Restricting the number of components may cause the merging of components, but allowing too many components can cause reduced signal-to-noise and over-decomposition. In this thesis, group ICA was restricted to 25 components, as this gave more meaningful components than running it without restriction.

2.5.9.1.3 Dual regression

The output from the group ICA was used to generate individual spatial maps and timeseries for each participant/visit using dual regression (Beckmann, Mackay, Filippini, & Smith, 2009; Nickerson, Smith, Öngür, & Beckmann, 2017). First a multiple regression is used to regress the group average set of spatial maps into the individual 4D dataset. Multiple regression is then performed to regress the subject-specific timeseries that are outputted from this into the same individual 4D dataset. For each subject, this creates a series of individual spatial maps, corresponding to the group spatial maps identified in the group ICA.

2.5.9.1.4 Higher-level analyses

Group differences between spatial maps were investigated using t-tests with the same GLM setup and design matrices as described for the BOLD task-based analyses. The analysis was done using randomise, FSL's permutation-testing tool (Winkler et al., 2014).

2.5.9.2 Seed-based analysis

In seed-based analysis, a single brain region is chosen. Activity in that seed region is then compared to activity in the rest of the brain. Regions in which activity covaries with that of the seed region are identified as functionally connected with it.

2.5.9.2.1 Pre-processing and registration

The pre-processing steps used were the same as those outlined above for task-based analysis.

The cleaned data for each participant was then registered to standard space. A mask was made for each seed region, and the timecourse within that region was extracted using the FSL tool fslmeants. Timecourses were also extracted for the CSF and white matter.

2.5.9.2.2 First level analyses

For each subject and seed region, the timecourse was entered as a regressor of interest into the GLM, and voxels with a matching timecourse were identified. Timecourses for the white matter and CSF were included as regressors of no interest in the model. In this thesis, all of these single subject analyses were performed in FEAT (Woolrich et al., 2001).

2.5.9.2.3 Higher level analyses

Higher level analyses and region of interest analyses were performed in the same way as described above for task-based analysis.

2.5.10 Analysis of arterial spin labelling data

ASL analysis was performed using FSL.

2.5.10.1 Brain extraction and motion correction

As for the BOLD image analysis, brain-extraction was performed with BET (Smith, 2002), and images were motion corrected with McFlirt (Jenkinson et al., 2002).

2.5.10.2 Quantification of cerebral blood flow

Cerebral blood flow (CBF) was quantified as described in other studies (Mezue et al., 2014; Segerdahl et al., 2015). The period of time between the labelling of blood and the acquisition of a tag image is called the post-labelling delay. By acquiring images with a range of post-labelling delays it is possible to model the change in perfusion over a period of time, avoiding the risk that variability in the arrival time of the labelled blood may lead to an incorrect quantification calculation. For each post-labelling delay, the control image was subtracted from the tag image, creating a perfusion-weighted image. For each voxel, the concentration-time curves were then fitted to the general kinetic model, which describes the change in ASL signal over time (Buxton & Frank, 1997). This ensures that each concentration-time curve is temporally aligned. For each set of post-labelling delays, perfusion parameters were then estimated using BASIL (Bayesian Inference for Arterial Spin Labelling MRI; Chappell, Groves, Whitcher, & Woolrich, 2009), giving a timeseries of CBF for each set.

2.5.10.3 Registration

Using FLIRT (Jenkinson et al., 2002; Jenkinson & Smith, 2001), images were first registered to each participant's T1 structural image with boundary-based recognition (Greve & Fischl, 2009). Non-linear registration in FNIRT was then used to register images to standard MNI space (Andersson et al., 2007).

2.5.10.4 Higher level analyses

Higher statistical analyses were performed with FEAT, using the same methods as described above for BOLD imaging.

2.6 Summary

This chapter has outlined the general methods applied during this thesis. Specific details of the methodology used in individual studies will be given in subsequent chapters. The next chapter explains in detail the methods used to investigate a novel analgesic agent, and discusses how the requirements of a phase I clinical trial were met.

Chapter 3: Methods and requirements for a phase I randomised controlled trial of ABX-1431

3.1 Introduction

Chapter 2 outlined and discussed the general methods that were used across all studies in this thesis. This chapter describes the methods that were specifically used in a randomised controlled trial of the novel analgesic ABX-1431, which acts on the endocannabinoid system. This was a very early phase trial, and it was also the first imaging trial of a totally novel, first-in-class compound to be performed at the Oxford Centre for fMRI of the Brain (FMRIB). Therefore, this chapter also looks at the requirements of a phase I trial, and outlines how they were met.

3.1.1 Stages of clinical trials

Before a drug is brought to market, it must undergo extensive preclinical testing, followed by human clinical trials. Human trials take place in four main phases.

A phase I trial usually recruits a small number of healthy volunteers. The main aims of a trial at this early stage are to assess the safety of the Investigational Medicinal Product (IMP), to confirm that it has pharmacodynamic efficacy through the engagement of appropriate targets, and to look for evidence of possible therapeutic efficacy. The FDA estimate that approximately 70% of these drugs move to phase 2 trials (U.S. Food and Drug Administration, 2018). There are stringent requirements for the conduct of a phase I trial due to the fact that the IMP is in an early stage of

development, the risk profile is relatively unknown, and the participants are healthy volunteers who will not benefit from the drug.

During phase II trials, the goal is to confirm therapeutic efficacy by testing the IMP on patients with the target disease. For most interventions, the gold-standard trial design for this purpose is considered to be the randomised, placebo-controlled trial. Phase II trials may also aim to establish optimal dosing regimens. Around 33% of phase II drugs move to the next phase (U.S. Food and Drug Administration, 2018).

In a phase III trial, a larger group of patients (usually hundreds to thousands) is recruited in order to confirm the efficacy, dosing and safety of the IMP in the target population. At this stage the IMP may also be compared to existing treatments with the aim of establishing superiority. Approximately 25-30% of phase III trials are successful (U.S. Food and Drug Administration, 2018), meaning that an application can be made for approval of the IMP.

Phase IV trials take place once the IMP is approved for use. They enable long-term surveillance of a drug in the wider population. Overall, approximately 10% of drugs that enter phase I human trials reach the market. For analgesics the figure is around 2% (Houlton, 2019).

3.1.2 The use of cannabis as a treatment for pain

For many years, individuals have been using cannabis as a treatment for pain (Hand, Blake, Kerrigan, Samuel, & Friedberg, 2016). The literature on the efficacy of exocannabinoids is varied and of low quality, but overall they appear to have some

benefit for pain relief (Mücke, Phillips, Radbruch, Petzke, & Häuser, 2018; Stockings et al., 2018). However, their use is limited by a high incidence of adverse effects, which include altered perception, cognitive impairment, drowsiness, gastrointestinal symptoms and psychiatric disorders (Joint Formulary Committee, 2020). One meta-analysis estimated that the number needed to treat to achieve a 30% reduction in chronic non-cancer pain was 24, while the number needed to harm for one person to experience an adverse event was six (Stockings et al., 2018).

3.1.3 MGLL inhibition as a possible treatment for chronic pain

There is good evidence that intrinsic endocannabinoid tone has an important role in regulating descending pain modulation, and that altered endocannabinoid tone might influence both sensory and affective dimensions of pain perception (see section 1.2.5). Monoacylglycerol lipase (MGLL) is the enzyme predominantly responsible for the degradation of 2-arachidonoyl glycerol (2-AG), an endogenous ligand with high affinity for both of the main cannabinoid receptor types, CB1 and CB2. More details of the endocannabinoid system are given in section 1.2.5, where Figure 1.2 shows the mechanism by which MGLL inhibition leads to increased endocannabinoid signalling.

Preclinical studies in rodents show that MGLL inhibition has antinociceptive effects in models of neuropathic, inflammatory and chemical pain (Curry et al., 2018; Ghosh et al., 2013; B. M. Ignatowska-Jankowska et al., 2014; Kinsey et al., 2009; Long et al., 2009), and acts primarily through enhanced CB1 signalling in the brain (Kinsey et al., 2009). In rodents given appropriate doses, these effects do not appear to be accompanied by the cannabimimetic side effects seen following exocannabinoid administration (B. M. Ignatowska-Jankowska et al., 2014), although a degree of

hypomotility is seen with some compounds (B. Ignatowska-Jankowska et al., 2015; Long et al., 2009). The lack of side effects may relate to the fact that following injury, endocannabinoid activity is increased at pain-relevant sites (Sagar, Burston, Woodhams, & Chapman, 2012). The effect of inhibiting ligand degradation is greater in areas with higher endocannabinoid turnover, meaning that global effects are avoided (Woodhams et al., 2017).

ABX-1431 is a selective and irreversible inhibitor of MGLL. Preclinical studies have shown that it successfully penetrates the central nervous system (CNS) and increases 2-AG concentrations in rodent brains in vivo. It also reduces formalin-induced nociceptive behaviours. In vitro, ABX-1431 has been shown to be a potent and selective MGLL inhibitor in human post-mortem prefrontal cortex homogenates (Cisar et al., 2018). Positron emission tomography in rhesus monkeys using an MGLL tracer showed ABX-1431 to have good brain penetrance, and showed the regional distribution of the drug to be consistent with areas in which MGLL is expressed in CB1-containing neurons (Abide Therapeutics, 2017). First in man trials have shown ABX-1431 to be safe and well tolerated in a small number of healthy volunteers (Abide Therapeutics, 2017). Single ascending doses and multiple ascending doses of ABX-1431 were administered to participants in order to assess drug pharmacology as well as adverse effects. However, these phase I studies did not look at analgesic efficacy. Therefore, the next step is a further phase I study to look for evidence of efficacy in a group of healthy participants.

3.1.4 Study aims

The aim of this trial was primarily to use neuroimaging as a tool to assess the potential efficacy of ABX-1431 as an analgesic, and additionally to gain insight into its likely mechanisms of action. ABX-1431 is a novel drug with no previous efficacy data in human trials, and it is therefore at a very early phase of development. As outlined in chapter 1, the development of new analgesic agents is challenging, in part due to the lack of an objective biomarker for analgesia. Consequently, the decision about whether to pursue a novel pharmacological agent is based on subjective outcome measures that give little to no confirmation of target engagement or efficacy, nor of likely mechanisms of action. There is evidence from existing analgesics that neuroimaging can provide this objective biomarker, meaning that small imaging studies could enable crucial go/no-go decisions on whether to progress drugs to larger patient studies (Duff et al., 2015; Vishvarani Wanigasekera et al., 2016). Imaging can also give information about neural mechanisms, potentially enabling the better targeting of drugs to populations that will benefit.

The specific objectives identified in the protocol were as follows:

Primary objective:

- To evaluate the effect of ABX-1431 HCl on central pain processing and neural measures of experimentally-induced hyperalgesia.

Secondary objectives:

- To evaluate the effects of ABX-1431 HCl on experimentally-induced hyperalgesia in healthy volunteers.
- To evaluate the safety and tolerability of ABX-1431 after administration of single oral doses of ABX-1431 HCl to healthy adult subjects.

3.2 Study design

Participants completed three treatment visits. A three-way crossover design was used in order that participants acted as their own controls, with each participant taking ABX-1431 and placebo on different visits, as well as completing a visit with no drug. There was a minimum of seven days between each of the three visits to ensure drug washout as well as absence of capsaicin-induced hypersensitivity at subsequent visits. Participants were blinded to whether they were completing an ABX-1431/placebo or a no dose visit until shortly prior to dosing, and both participants and research staff were blinded to the order of ABX-1431/placebo dosing until the database was locked following completion of the study. The treatment order was randomised.

The inclusion of a no dose arm was to reduce the possibility that true drug effects were missed due to the assumption of additivity when comparing active drug with placebo. This issue is discussed in more detail in chapter 1.

At each visit, topical capsaicin was applied to the leg to induce an ongoing noxious stimulus. To produce an evoked noxious stimulus, a punctate probe was applied to an area of secondary hyperalgesia surrounding the capsaicin. Evoked pain ratings were then compared to pre-capsaicin ratings to determine the degree of subjective secondary hyperalgesia. Functional neuroimaging was completed during both ongoing and evoked pain stimuli.

3.3 Participants

Healthy, right-handed, male participants age 18-55 were recruited until 16 individuals had completed the study. Participants who withdrew prior to study completion were replaced. The full inclusion and exclusion criteria are listed in appendix B.

3.4 Administration of the interventions

Each participant received three interventions, one at each treatment visit. They were as follows:

- ABX-1431, 4x10mg capsules with 240ml water
- Placebo, 4x10mg capsules with 240ml water
- No dose, 240 ml water

All interventions were administered at 10am on the day of the visit. Participants were required to fast for at least 8 hours prior to dosing. This was due to the pronounced effect on pharmacokinetics that was seen when ABX-1431 was administered with food as opposed to in a fasted state during initial phase I trials (Abide Therapeutics, 2017).

3.5 Outcome measures

The primary outcome measures were brain activity (measured by fMRI) in response to ongoing and evoked noxious stimuli, recorded after sensitization with topical capsaicin. Activity following a single dose of IMP was compared to activity following placebo and no dose. Functional connectivity during tonic pain was also compared across the three interventions.

Secondary outcome measures were the increase in ongoing and evoked pain scores after sensitization with topical capsaicin. The effect of a single dose was compared to

that of placebo and no dose. In addition, the change in mood and sedation from pre-intervention to post-intervention was examined for ABX-1431 compared to placebo and no dose.

3.6 Sample size

A formal power calculation was not possible due to the absence of any suitable pilot data (Mumford, 2012). The sample size of 16 was based on previous experience of similar trials that used neuroimaging to investigate the effect of an analgesic on experimental pain in both patients and healthy controls (Harris et al., 2013; Iannetti et al., 2005; V. Wanigasekera et al., 2018). In particular, Lee and colleagues used a very similar experimental design to this study to investigate the effect of tetrahydrocannabinol (THC) on capsaicin-induced pain (Lee et al., 2013). In that study, a sample size of 15 was adequate to detect a significant effect using imaging.

A sample size of 16 is unlikely to have adequate power to detect an effect on self-reported behavioural data. However, the main aim of this study is to use imaging to enable the evaluation of a novel agent in a small group of participants in which behavioural data would not be sufficient. Therefore this is intentional.

3.7 Randomisation and allocation

The treatment given at each visit was block randomised using replicated 3x3 Latin squares, with participant and visit number as blocking factors. The randomisation schedule was devised by a statistician independent of the study team. Randomisation was performed at the point of formal enrolment into the study by the research team. This took place between the baseline visit and the first treatment visit, once eligibility

had been established. Participants were allocated to the randomisation schedule sequentially at the point of enrolment.

The advantage of this method of randomisation is that it is possible to control for the effects of both participant order and visit order independently. The disadvantage of using this method in a crossover design such as this is that it assumes that there is no interaction between the treatment allocated and the blocking factors (e.g. visit number). This may be an incorrect assumption. The allocations were not balanced for possible carryover effects from the preceding treatment due to the fact that there are only three possible treatment orders, and so during the course of the experiment, not every treatment directly follows every other treatment. One way around this would have been to use sequence order as a blocking factor.

3.8 Blinding and emergency unblinding

Both the research team and the participants were blinded to which of the two dosing visits were ABX-1431 and which were placebo. Only the independent statistician had knowledge of which visit was which for each participant. Drug and placebo were identical in appearance, and both were supplied in individual bottles containing 4 capsules. Participants were not told in advance which of the treatment visits were dosing visits and which were no dose visits, but they found this out at the time of dosing, and will have worked out whether or not they were being dosed in advance of the third treatment visit by a process of elimination. It is important that unblinding can take place in an emergency, and that this can be done by an investigator immediately, without having to contact any other parties (Association of the British Pharmaceutical Industry, 2018). For each dosing visit, a sealed envelope containing the treatment

allocation was kept in three locations in case of emergency. One copy was kept in pharmacy, another in FMRIB, and the third went with the participant to the ward. These were not to be opened unless required by a medical professional who felt that unblinding the visit would influence medical management. Blinding was maintained until after data collection was complete, the database was locked, and initial data analysis had been performed. There was no interim analysis.

3.9 Study procedures

The assessments performed during each of the study visits are outlined in Figure 3.1, and are described in more detail below. All assessments took place in FMRIB.

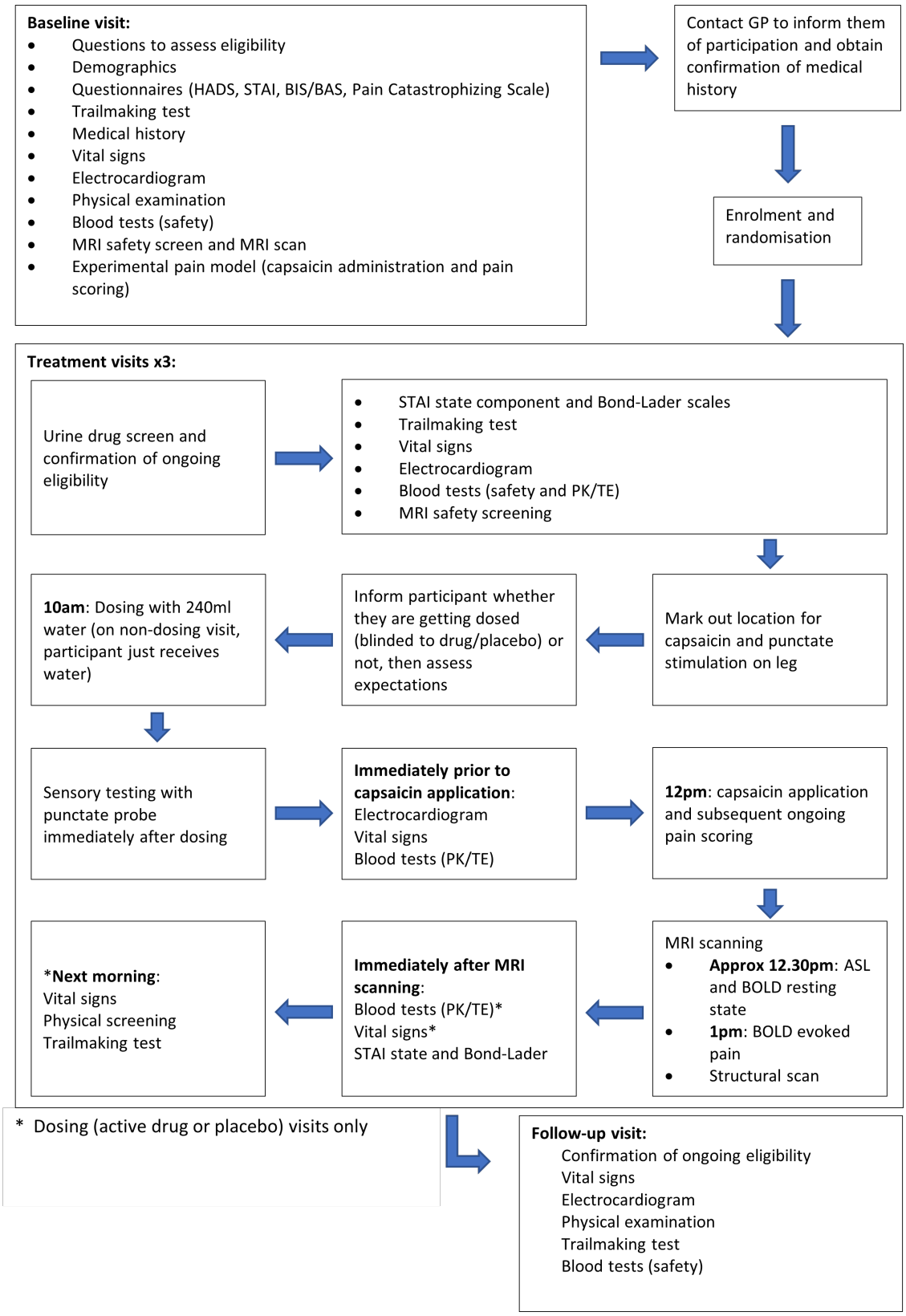


Figure 3.1: Schematic showing the sequence of study procedures for each participant. (HADS, hospital anxiety and depression scale; STAI, state-trait anxiety inventory; BIS/BAS, behavioural inhibition system/behavioural activation system; PK, pharmacokinetics; TE, target engagement; ASL, arterial spin labelling; BOLD, blood oxygen level dependent).

3.9.1 Experimental pain model and evoked pain task

A 4x4cm area was marked out on the anteromedial aspect of the right leg for the application of capsaicin cream. Both superior and inferior to this, a stimulation zone was marked out that was 2cm deep and 1cm from the border of the capsaicin area. This stimulation zone would be an area of secondary hyperalgesia after the application of capsaicin. Two hours after the time of dosing, 1 % capsaicin cream was applied to the central square (Figure 3.2).

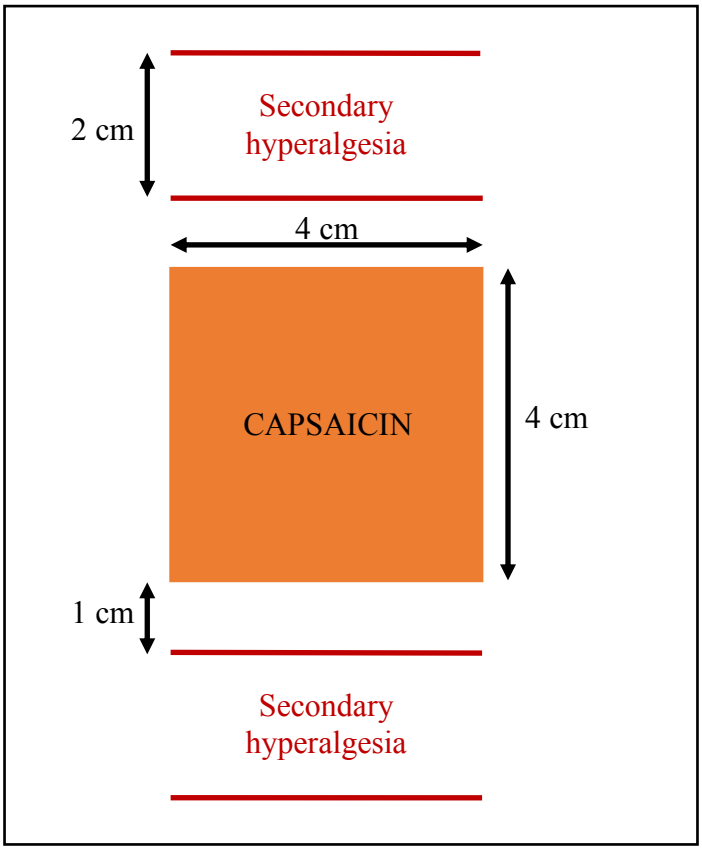


Figure 3.2: Capsaicin application and areas of secondary hyperalgesia. This diagram shows the relationship of the topical capsaicin to the areas of the skin that were tested for secondary hyperalgesia using punctate probes.

A 512 mN punctate probe was used to stimulate the surrounding area of secondary hyperalgesia before the application of the capsaicin, and one hour after capsaicin application. For the treatment visits, the pre-capsaicin testing occurred immediately

after dosing, and the testing one hour post-capsaicin took place three hours post-dose (Figure 3.1). The punctate stimulus was applied 20 times with an interstimulus interval of approximately 30 seconds (jittered). Following this, participants were asked to rate the average intensity and unpleasantness of the stimuli on a 100mm visual analogue scale (VAS) with anchors of “Not intense” and “Extremely intense”, and “Not unpleasant” and “Extremely Unpleasant” respectively.

Ongoing pain intensity ratings were taken on a paper-based VAS at five minute intervals for one hour (baseline visit) or until the participant entered the MRI scanner (treatment visits). During the three treatment visits, ratings were then taken using a 100mm computer-based VAS every five minutes, or as close to this as possible between scans. Ongoing pain unpleasantness ratings were taken immediately prior to and following the evoked pain task using a 100mm computer-based VAS. The ongoing pain intensity VAS had anchors of “No pain” and “Severe pain”, while the ongoing pain unpleasantness VAS had anchors of “Not unpleasant” and “Extremely Unpleasant”. Computer based ratings were adjusted using a button box.

The three hour time period between dosing and assessment of the primary outcome measure (evoked pain responses) was chosen to try and optimise the balance between ensuring a good level of target engagement but reducing the risk of the participant experiencing adverse effects while in the scanner. Pharmacokinetic data was not available for the 40mg dose used in this study, but existing clinical data showed that at a dose of 50mg, the peripheral Tmax was 1.8hours (+/- 0.44), and the $T_{½}$ was 3.44 hours (Abide Therapeutics, 2017). MRI scanning started approximately 2.5 hours post-dose and was complete by approximately four hours post-dose. Scans

were always completed in the same order to avoid large between-visit differences in timing with respect to dose. It was felt that during this time period, drug levels should be sufficiently high to be able to identify a drug effect, but participants would not be in the scanner as they approached maximal peripheral concentration, thereby reducing the risk that adverse effects would appear mid-scan.

The one hour gap between capsaicin application and assessment of the primary outcome measure was chosen based on pilot data, which showed that responders tended to report a good hyperalgesic effect by that time, and that most would already display a good response by around 30 minutes, when participants first entered the scanner (see appendix A). This period also allowed participants time to be transferred to the scanner and prepared for the scans without feeling rushed, which might have led to increased anxiety. The hyperalgesia in some responders started to reduce soon after 60 minutes so a longer gap was felt to be undesirable.

For a number of reasons, no dosing visits were conducted in the absence of capsaicin. Firstly, the capsaicin model of central sensitization is well established and has been used in multiple pain efficacy studies in the past (Iannetti et al., 2005; M. C. Lee et al., 2013; Vishvarani Wanigasekera et al., 2016). Additionally, the effect and neural correlates of capsaicin administration are already well known, so it was not felt necessary to investigate these further (M. C. Lee et al., 2008; Zambreanu et al., 2005). A previous study showed that when THC was compared to placebo during mechanical stimulation in both the presence and absence of topical capsaicin, THC was only found to be an effective analgesic in the presence of capsaicin (M. C. Lee et al., 2013). It was predicted that ABX-1431 might behave in the same way. With this in mind, it was

not felt to be appropriate to expose patients to additional study visits and drug exposure to test the effect of ABX-1431 in the absence of the capsaicin central sensitization model.

3.9.2 Assessment of mood, sedation and anxiety

As mentioned previously (see chapter 2, section 2.3.2), mood and anxiety are closely linked to pain perception. Therefore, it was important to assess whether these factors varied between participants or between visits, and whether there was any drug-induced variation following dosing. Due to the fact that some cannabinoids can be sedating (Campbell et al., 2001; Joint Formulary Committee, 2020), it was also important to assess participants for the presence of mental and physical sedation that may have influenced either their perception of pain or their ability to complete the study procedures.

At all three treatment visits, participants completed assessments of state anxiety and of mood and sedation both prior to dosing, and after the completion of the MRI scan at approximately four hours post-dose. The state component of the state-trait anxiety inventory (STAI) was used to measure anxiety (Spielberger et al., 1983). Mood and sedation was assessed using the Bond-Lader VAS scales (Bond & Lader, 1974).

At the baseline visit, the STAI trait and state components were used to measure both inherent tendency to anxiety and anxiety levels on the day. At the baseline visit, participants also completed the pain catastrophizing scale in order to assess this aspect of pain-related anxiety.

3.9.3 Assessment of expectation

Expectation can have a huge effect on the efficacy of an analgesic (Ulrike Bingel et al., 2011). At each treatment visit, participants were informed of whether or not they were on the no dosing arm immediately prior to dosing. They were then asked to rate how much pain relief they expected from the intervention using a 100mm computer-based VAS. The anchors were "No relief" and "Complete relief". The reason for informing them whether or not they were on the no dosing arm prior to the expectation rating was that depending on the order of the visits, some participants would have worked this out already by a process of elimination.

3.9.4 Cognitive function

Cognitive function was assessed at baseline, follow-up, and all treatment visits using the Trail Making Test. This is a neuropsychological test that was originally part of the Army Individual Test Battery (Tombaugh, 2004; US War Department, 1944). It consists of two parts. In part A, the numbers 1-25 are distributed on a page and the participant is required to join them up in numerical order as quickly as possible. This part assesses visual search ability and motor speed (Bowie & Harvey, 2006). In part B, both letters and numbers are displayed, and the participant is required to alternate between them (e.g. 1, A, 2, B etc.). This part assesses higher level cognition including executive function (Bowie & Harvey, 2006). Poor performance on the test is thought to be a good indicator of neurological impairment (Bowie & Harvey, 2006).

The decision to include this assessment in the protocol was made in line with recommendations that all phase I trials of drugs affecting the CNS should include neuropsychological assessment (Agence Nationale de Sécurité du Médicament et des

produits de santé, 2016; Casassus, 2016). These recommendations were made following a phase I trial of a fatty acid amide hydrolase (FAAH) inhibitor in which one participant died and others suffered severe neurological damage. This was thought to be due to the off-target effects of the drug rather than its inhibition of endocannabinoid breakdown (Van Esbroeck et al., 2017), making it highly unlikely for ABX-1431 to have the same issues, particularly given the high specificity of ABX-1431 in preclinical testing (Abide Therapeutics, 2017). Regardless of this, it was important to adhere to the guidelines, with the aim that at-risk subjects may be identified prior to dosing, and that the onset of behavioural or neuropsychological disorders may be detected early on during exposure (Agence Nationale de Sécurité du Médicament et des produits de santé, 2016). The protocol already included screening for pre-existing neurological conditions prior to enrolment, as well as examination of the neurological system and assessment of mood and sedation at each dosing visit.

3.9.5 Physiological assessments

It is important that the health of every participant is assessed throughout the trial. Guidelines state that this should include the measurement of vital signs, monitoring of the electrocardiogram (ECG), physical examination and safety tests of blood and urine (Association of the British Pharmaceutical Industry, 2018). This section summarises the physiological assessments that were performed.

3.9.5.1 Urine drug screen

A urine sample was screened for drug exposure at the beginning of every visit. The test kits were supplied by Alere toxicology (Oxfordshire, UK), and tested for methamphetamines, cocaine, THC, benzodiazepines, tricyclic antidepressants,

barbiturates, phencyclidine, amphetamines, morphine and methadone. This was done for safety reasons, due to the risk of drug interactions with the IMP, as well as for experimental reasons, as the concurrent administration of other drugs could have affected the study outcomes.

3.9.5.2 Vital signs

Heart rate, respiratory rate, blood pressure and temperature were recorded for health screening and to look for adverse effects.

Vital sign measurement took place once during each baseline and follow-up visit. During the treatment visits, vital signs were measured pre-dosing, at approximately two hours post-dose, and after the MRI scan (approximately four hours post-dose). On visits in which drug or placebo was received, they were also measured the following morning prior to discharge.

3.9.5.3 Electrocardiogram

An ECG was performed once during the baseline and follow-up visits. During the treatment visits, an ECG was done pre-dosing and at approximately two hours post-dose. The purpose of these ECGs was to check eligibility and to exclude adverse effects.

3.9.5.4 Blood tests

Safety bloods were performed once per visit as part of a general health screening to check ongoing eligibility and to exclude adverse effects. A list of the tests done is recorded in appendix C.

During the treatment visits, blood was also taken for the assessment of pharmacokinetics and target engagement. These tests were performed pre-dose, immediately pre-MRI (approximately two hours post-dose), and on the drug and placebo visits, immediately post MRI (approximately four hours post-dose).

3.9.5.5 Physical examination

At the baseline visit, a thorough physical examination of all main body systems was performed, including a complete neurological assessment. To confirm that there had been no physical side effects, a brief screening examination was performed the morning after dosing for the drug and placebo visits, as well as during the follow-up visit.

3.10 Risk Management

3.10.1 Participant identification and screening

At least seven days prior to commencing the treatment visits, participants attended a baseline visit to assess eligibility and familiarise them with the experimental tools. During this visit, participants underwent safety screening as outlined in section 3.9. It is good practice to inform each participant's general practitioner (GP) that they have volunteered for a trial, and to obtain confirmation of their medical history (Association of the British Pharmaceutical Industry, 2018). In this trial, permission to communicate personal medical information between the research team and the GP was a requirement for eligibility. Assuming each participant passed all of the baseline screening, a letter was sent to their GP to make them aware of the trial and to request a copy of the participant's medical history. Once this was received and eligibility was confirmed, participants were enrolled.

3.10.2 Preventing over-participation

It is important that trial participants do not volunteer for multiple trials simultaneously, and that there is an adequate gap between trials. This is due to the possibility of drug interactions, as well as the risks associated with exposure to a lot of IMPs and with excessive blood sampling. Participation in multiple trials would also make it difficult to establish the cause of adverse events.

Participants who had participated in another trial in the four months prior to the first visit were not eligible for this study, and they were asked about this at screening. However, there is a risk that volunteers may not disclose their participation in other clinical trials. The Health Research Authority (HRA) runs a database called The Over-volunteering Prevention System (TOPS; Health Research Authority, 2020) that is designed to help investigators identify these volunteers. Following their screening visit, all participants in this trial were registered on the TOPS database, and any previous entries were noted. Their records were subsequently updated to specify whether they had received a dose of the IMP/placebo, and on what date they had done so.

3.10.3 Site selection and staffing

Data were all collected at FMRIB, Oxford, UK. It is important that a phase I trial takes place in an environment in which unexpected emergencies can be dealt with quickly and effectively. The Medicines and Healthcare products Regulatory Agency (MHRA) advises that there should either be experienced Advanced Life Support (ALS) trained physicians available during and after dosing, or that the trial should take place within a hospital with critical care facilities and an emergency response team (Medicines & Healthcare products Regulatory Agency, 2015). FMRIB was selected as a suitable

site for dosing due to the fact that it is on the site of the John Radcliffe hospital, a tertiary referral hospital with extensive critical care provisions, and can be attended rapidly by the hospital resuscitation team. There is also a fully stocked resuscitation trolley within the building. A medical doctor was present within FMRIB from the time of dosing until the participant left FMRIB. A consultant anaesthetist was appointed as the study physician, and either he or another ALS trained doctor with anaesthetic training was available to attend during every dosing visit if required.

On dosing visits, participants were monitored for adverse events overnight. As FMRIB does not have the facilities to house participants at night, a side room was arranged for this purpose on one of the acute neurology wards in the main hospital. One of two experienced nurses with appropriate acute medical experience was hired to look after the participant during each overnight stay. At least one of the aforementioned trial doctors was available by telephone and could attend the hospital in the event of any concerns. In order to provide appropriate urgent care, a formal agreement was made with the hospital that NHS staff would attend to the participant in the event of an emergency.

3.10.4 Follow-up of participants

A follow-up visit took place at least 14 days after the last treatment visit. The purpose of this was to assess the participant for any ongoing adverse effects. Follow-up should be provided for participants even if they withdraw or are withdrawn from the trial (Association of the British Pharmaceutical Industry, 2018) . Participants who were unwilling to attend a follow-up visit were offered a telephone interview. If they did not

agree to any kind of follow-up, they were provided with contact details for the research team in case of any concerns.

3.10.5 Emergency contact

In a phase I trial, participants should have the means to contact a trial physician at any time (Association of the British Pharmaceutical Industry, 2018). To enable this, all participants were provided with a card with a 24-hour emergency number at their initial visit.

3.10.6 Handling of adverse events

An adverse event (AE) is defined as “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment” (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 1995). AEs are considered to be drug-related if a causal relationship is a possibility, even if not confirmed, and they are then defined as adverse drug reactions (ADRs). Some of these events may be deemed ‘expected’ on the basis of what has previously been observed and documented in the Investigator’s Brochure, while others will be ‘unexpected’.

An AE or ADR is labelled serious if it results in death; is life-threatening; requires inpatient hospitalization or prolongation of hospitalization; results in persistent or significant incapacity/disability; or is a congenital anomaly/birth defect (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 1995).

The sponsor has overall responsibility for monitoring the safety of the IMP. However, specific tasks can be delegated. Good clinical practice (GCP) guidelines state that all serious AEs should be reported immediately to the sponsor (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2016). ADRs that are both serious and unexpected should be subject to expedited reporting to appropriate regulatory authorities, alongside serious ADRs that are expected but occurring at an increased rate (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 1995). In this trial, the investigator completed a specific case report form for each adverse event that was identified at any time during the trial. The procedure for reporting serious adverse events was documented in detail in the protocol.

3.11 Handling of the IMP

During a phase I trial it is important that the IMP is handled safely and securely, and that blinding is maintained. For this trial, the IMP and placebo were both manufactured and packaged externally into single dose bottles. Each bottle was marked with the randomisation number of the participant, along with the visit number at which it was to be administered. This meant that all staff working on-site remained blinded.

Units that carry out phase I trials should have a designated, secure pharmacy area, with facilities for the storage and dispensing of the IMP, as well as procedures to keep records of its receipt, storage, use, disposal and retrieval (Association of the British Pharmaceutical Industry, 2018). The IMP for this study was handled by the clinical trials pharmacy at the John Radcliffe Hospital. The IMP and placebo were dispensed

by the pharmacy either on the morning of the visit, or on the preceding working day if the visit was during a weekend.

3.12 Pathology Laboratory

This trial required blood assays for safety purposes as well as for assessment of the pharmacokinetics and pharmacodynamics of the IMP.

Guidelines state that clinical trials units should have access to a pathology laboratory that is suitably accredited and has procedures in place for all aspects of specimen processing, including equipment maintenance and calibration; performance and validation of assays; record keeping; releasing results and notifying medical staff of abnormal results; and performing internal audit (Association of the British Pharmaceutical Industry, 2018). A contract was arranged with the Oxford University Hospitals (OUH) NHS Foundation Trust, enabling use of their haematology and clinical biochemistry laboratories for performing the assays required for physiological assessment. These laboratories have accreditation with the United Kingdom Accreditation Service.

Blood samples taken for the purpose of pharmacokinetic and pharmacodynamic assessment were initially processed by appropriately qualified research staff at one of the Oxford University clinical research laboratories within the John Radcliffe Hospital. Here the samples were processed to plasma and stored according to the study protocol. They were then shipped to Bioanalytical Systems, Inc. in the United States, who had the contract for analysing these samples.

3.13 Sponsorship

All clinical trials must have a sponsor, who has overall responsibility for the research. Their role includes overseeing all aspects of the study, including the protocol and research design, staffing, indemnity, reporting and monitoring, and compliance with relevant legislation (Health Research Authority, 2021b). The sponsor for this trial was Abide Therapeutics. The sponsor can delegate tasks to other individuals or organisations.

3.14 Monitoring

It is the duty of the sponsor to ensure that trials are monitored appropriately. The job of the monitor is to check that the trial is conducted and documented properly. This includes the assessment of all trial facilities; verifying adherence to the protocol; checking that informed consent was obtained for each participant; confirming that the IMP was handled appropriately; ensuring that all documentation is in place, and is complete and up-to-date; checking that reported data are accurate and complete; determining whether adverse events have been reported appropriately; and checking compliance with regulatory requirements (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2016).

For this trial, the sponsor employed an external contract research organisation, Covance, to provide monitoring. A Covance representative made regular visits to the trial site in order to review facilities, procedures and documentation.

3.15 Documentation

There are strict guidelines on the trial documentation that should be kept, and the way in which it should be stored. This includes the trial master file (TMF), which contains all essential documents, and enables regulators to assess the trial if required (Association of the British Pharmaceutical Industry, 2018; International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2016). GCP requires that both the sponsor and the investigator keep TMFs. The investigator's TMF was stored securely at FMRIB and updated throughout the planning and conduct of the trial by the research team.

3.16 Data Management

Data management refers to the process of inputting data, storing it, checking and correcting it, and retrieving it. Guidelines state that data managers should use validated, secure computer systems that contain an internal audit trail (Association of the British Pharmaceutical Industry, 2018). In this trial, data was initially recorded on paper case report forms (CRFs) to enable rapid data entry during trial visits. However, it was then transferred to an electronic database set up by Covance using Medidata Rave software. All data entry was performed by members of the research team. It is important that the data is either entered twice, or is checked fully for accuracy (Association of the British Pharmaceutical Industry, 2018). The monitor checked every entry against the source documentation/paper CRFs to identify any mistakes, and these were corrected by the research team. Following this, the database was locked so that no further changes could be made.

3.17 Trial registration

All clinical trials must be registered in a publicly accessible database prior to recruitment of any participants (Health Research Authority, 2021a; The World Medical Association, 2018). This trial was registered at clinicaltrials.gov, a global database maintained by the National Library of Medicine at the National Institutes of Health in the USA. The trial reference number is NCT02929264.

3.18 Regulatory approvals

3.18.1 Medicines and Healthcare products Regulatory Agency

The MHRA is part of the government's Department of Health and Social Care. It is responsible for ensuring the safety and efficacy of medicines in the UK, and grants approval for phase I trials via a Clinical Trials Authorisation (CTA), which the sponsor must apply for. It also reviews substantial protocol amendments, and can perform site inspections. Abide Therapeutics obtained the CTA for this trial and informed the MHRA of substantial amendments.

3.18.2 Research ethics committee

Every clinical trial must be approved by a research ethics committee (REC), run by the HRA. Each committee is made up of volunteers from a range of research and clinical backgrounds, as well as lay members. Substantial protocol amendments must also be approved by the REC. A REC application was made for this trial via the integrated Research Application System (IRAS), and the study was approved following a meeting with the South Central – Oxford A committee. Subsequent amendments were then approved through remote correspondence with the committee.

3.18.3 Health Research Authority approval

When research is NHS-based, approval is usually required from the HRA. However, this study involved no patients, and the only hospital facilities used were the pathology laboratories, the clinical trials pharmacy and the provisions for overnight accommodation. These were all purchased privately and were subject to contracts. Formal confirmation was provided by the HRA that their approval was not required in this situation.

3.18.4 NHS trust management approval

Studies that involve the use of NHS patients or resources require approval from the individual trust involved. This is to confirm that the trust has the capacity and capability for the research, and to ensure that the impact on the organisation is acceptable (Oxford University Hospitals NHS Foundation Trust, 2021). Approval was obtained from the Research and Development office at OUH NHS Foundation Trust. This application was made through IRAS.

3.19 Summary

A unit that is conducting a phase I clinical trial must meet stringent requirements to ensure the safety of participants as well as the scientific rigour of the trial. This is particularly important for a novel, first-in-class IMP such as ABX-1431. This chapter outlines the methodology for a phase I trial of ABX-1431. It describes how GCP requirements were met, despite the challenges associated with conducting this type of trial in a unit with minimal experience of such early phase studies. It does not summarise the data analysis, which is described in chapters 4 and 5. Nor does it cover acquisition details of the MRI scans, which are described in chapter 5.

The next chapter will address the behavioural outcomes of the study, while chapter 5 will investigate the imaging outcomes.

Chapter 4: Behavioural effects of an MGLL inhibitor in an experimental pain model in healthy participants

4.1 Introduction

As described in chapter 1, drug discovery is time-consuming and expensive. Analgesic discovery is particularly challenging due to the subjective nature of pain reporting. Pain-related outcome measures are highly variable, and are influenced by factors such as the emotional and cognitive context in which pain is experienced. An objective biomarker of pain and analgesia would enable early go/no-go decisions on the investigation of potential analgesics, thereby reducing wasted resources and preventing effective drugs from being inadvertently rejected. Data from existing analgesics suggests that functional neuroimaging could provide this biomarker (Duff et al., 2015; Vishvarani Wanigasekera et al., 2016), but it has yet to be used to test a novel agent.

In chapter 3, the monoacylglycerol lipase (MGLL) inhibitor ABX-1431 was introduced as a possible novel analgesic. Prior to assessing whether neuroimaging can assist with the development of ABX-1431, it is useful to establish whether subjective pain measures can provide any information on efficacy and on how the drug affects the perception of pain. Since a pain or analgesia biomarker has not yet been well established, industry still places high value on behavioural outcomes, with the FDA requiring pain intensity ratings as an efficacy output for pain trials (U.S. Food and Drug Administration, 2022). Therefore, the primary aim of this chapter is to look for behavioural evidence that ABX-1431 has analgesic efficacy when compared to

placebo or a non-dosing arm. However, since the study is powered for imaging rather than behavioural results, it may be that it will not be possible to conclude whether ABX-1431 is an effective analgesic from this data alone.

A secondary aim of this chapter is to assess whether it is possible to show that ABX-1431 has a pharmacodynamic effect on the brain using behavioural data alone. The effect of ABX-1431 on self-reported measures of mood, anxiety and sedation will be examined. A significant change in these measures compared to that seen in the placebo and non-dosing arms would indicate that ABX-1431 has crossed the blood-brain barrier and affected central neurological function.

The final aim of the chapter is to consider whether there are any specific confounding factors that may have influenced the ability to detect analgesic efficacy from this behavioural data. It is possible that factors such as expectation, visit order and drug level, in addition to the potential central neurological effects mentioned above, might introduce substantial variability into the pain rating data.

4.2 Methods

A randomised, double-blinded three-way crossover design was used. Sixteen participants completed three intervention visits, at which they received ABX-1431, placebo and no drug. Topical capsaicin was used at each visit to produce an experimental tonic pain model. A punctate probe was used to stimulate the area of secondary sensitization around the capsaicin in order to produce an experimental evoked pain model. Participants gave ratings of pain intensity and unpleasantness to determine the effect of the three interventions on behavioural pain measures. Mood,

sedation and anxiety were assessed both pre-dose and post-dose using Bond-Lader visual analogue scales (VAS) and the state component of the state-trait anxiety inventory (STAI). Expectation of pain relief was assessed prior to dosing. The methods are explained in detail in chapters 2 and 3.

4.2.1 Data analysis

Data were analysed using IBM SPSS and Microsoft Excel.

Analysis of variance (ANOVA) was used to determine the effect of the intervention on pain, mood and sedation. The Greenhouse-Geisser correction was applied when Mauchley's test of sphericity was significant. When relevant, posthoc paired t-tests were then performed to investigate specific differences between group means. Bootstrapping was applied to t-tests to minimise the effect of variables that might violate the assumption of normality.

Behavioural correlations were performed by calculating the Pearson correlation coefficient when the variables were deemed normally distributed, and the Spearman correlation coefficient when there was concern about the normality of the data.

4.3 Results

Sixteen men were included in this analysis, with a mean age of 23 (SD 3.55, range 19-31). Outliers were not excluded due to the risk of excluding a result that was due to natural variation, but they are discussed further in appendix D-1.1.

4.3.1 Section 1: effect on experimental pain

4.3.1.1 Evoked pain ratings

Punctate-induced pain was recorded at two timepoints. The first was immediately after dosing, and prior to capsaicin application. The second was approximately three hours after dosing, which was one hour after capsaicin application. The change from the first to the second timepoint was calculated. The group results are outlined in Figure 4.1.

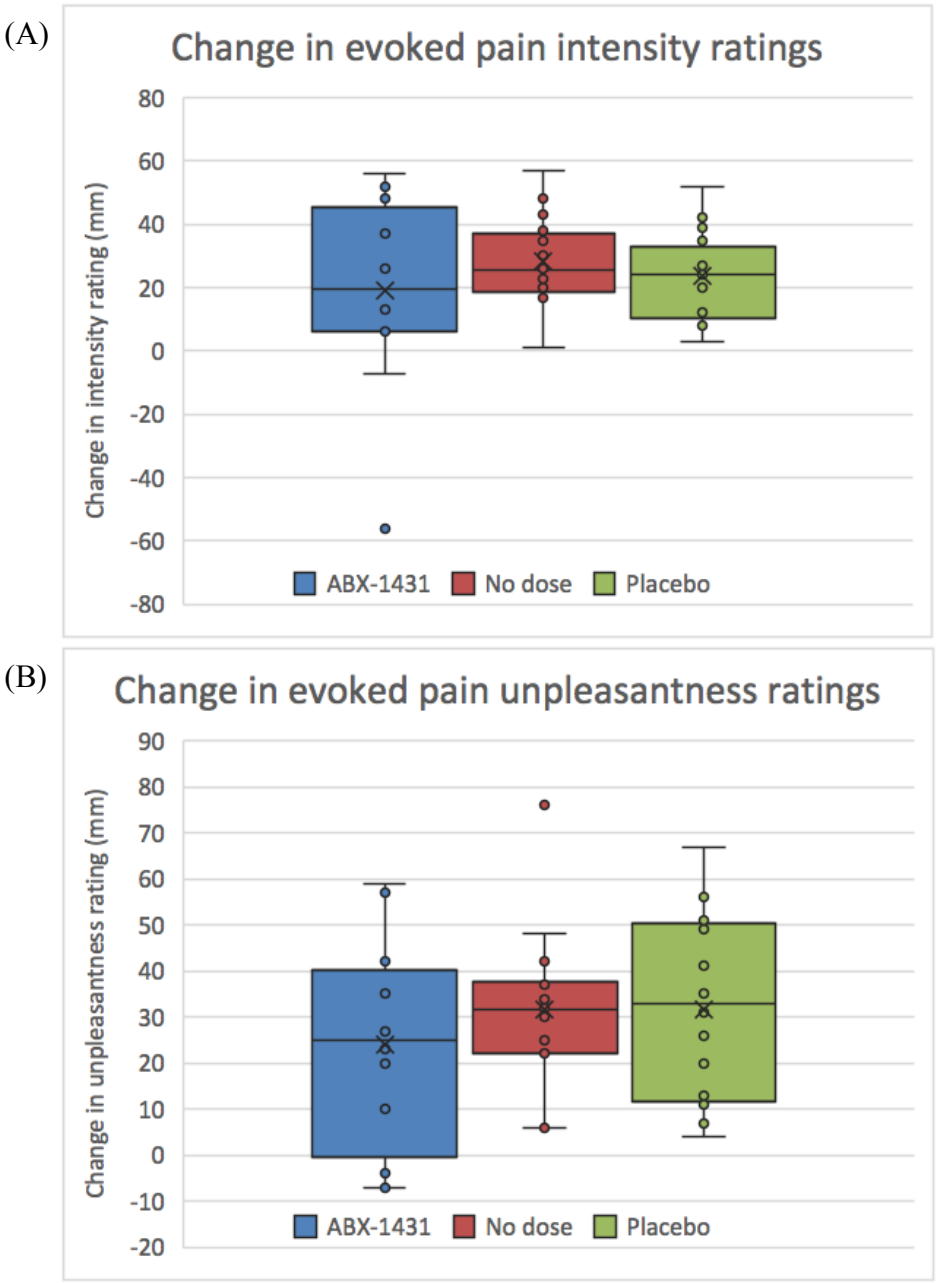


Figure 4.1: Effect of intervention on evoked pain ratings. For each of the three interventions, these boxplots show the distribution of the change in (A) punctate-induced pain intensity and (B) unpleasantness ratings for all participants from prior to capsaicin application to one hour after capsaicin application (n=16). Mean values are denoted by a cross and individual values are denoted by circles. A repeated measures analysis of variance showed no significant effect of intervention on evoked pain ratings (see section 4.3.1.3).

4.3.1.2 Ongoing pain ratings

An ongoing pain rating was recorded immediately prior to initiation of the evoked pain paradigm at both timepoints. The group results of this rating are outlined in Figure 4.2.

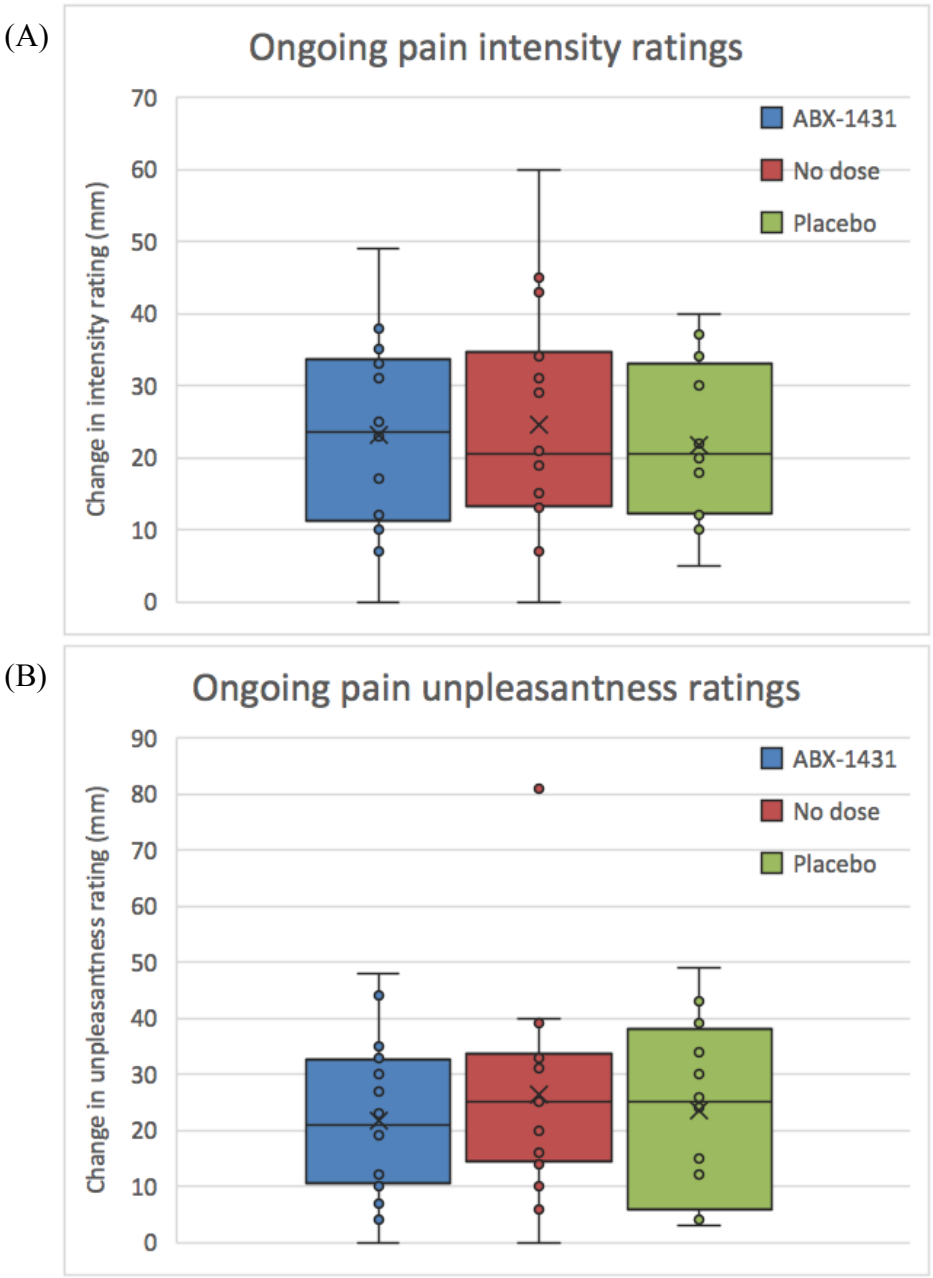


Figure 4.2: Effect of intervention on ongoing pain ratings. For each of the three interventions, these boxplots show the distribution of (A) ongoing pain intensity and (B) unpleasantness ratings for all participants an hour after capsaicin application (n=16). Mean values are denoted by a cross and individual values are denoted by circles. A repeated measures analysis of variance showed that there was no effect of intervention on ongoing pain ratings (see section 4.3.1.3).

4.3.1.3 Repeated-measures ANOVA to determine whether the intervention type had an effect on pain ratings.

A repeated measures ANOVA was performed with timepoint (before vs. after capsaicin application) and intervention (drug vs. placebo vs. no dose) as factors. The dependent variables were evoked pain intensity, evoked pain unpleasantness, ongoing pain intensity and ongoing pain unpleasantness. All values were the mean across all participants. The ongoing pain measures used for this analysis were those collected immediately prior to initiation of the evoked pain paradigm. This was to ensure that the capsaicin had been on sufficiently long to maximise tonic pain ratings, but that there was no risk of capturing the possible sensitization effect of the evoked stimulus in the ongoing rating. The pain scores were normally distributed.

There was a significant increase in all pain measures as a result of the capsaicin application, demonstrating that the model worked as expected. This did not vary according to group, indicating that the intervention did not significantly affect the perceived intensity or unpleasantness of the pain experienced (
Table 4.1).

Variation source	Measure	df, df_{error}	F	significance
Intervention	Evoked intensity	1.371, 20.572	0.319	0.649
	Evoked unpleasantness	2, 30	1.332	0.279
	Ongoing intensity	2, 30	0.383	0.685
	Ongoing unpleasantness	2, 30	0.876	0.427
Timepoint (pre- or post-capsaicin)	Evoked intensity	1, 15	39.359	0.000
	Evoked unpleasantness	1, 15	58.093	0.000
	Ongoing intensity	1, 15	68.198	0.000
	Ongoing unpleasantness	1, 15	45.885	0.000
Intervention * timepoint	Evoked intensity	2, 30	1.328	0.280
	Evoked unpleasantness	2, 30	1.300	0.287
	Ongoing intensity	2, 30	0.383	0.685
	Ongoing unpleasantness	2, 30	0.876	0.427

Table 4.1: Testing for a statistically significant effect of intervention on pain ratings. This table shows the results of a repeated measures analysis of variance with intervention (ABX-1431 vs. placebo vs. no treatment) and timepoint (pre-dose and post-dose) as factors, and pain ratings as outcome measures (n=16). Mauchley's test was significant for the effect of intervention on evoked intensity (p=0.014), so the Greenhouse-Geisser correction was applied (epsilon 0.686). Results are otherwise uncorrected. Significance levels in which $p \leq 0.05$ are shown in red. There is a significant change in all pain ratings from before capsaicin application to after capsaicin application, demonstrating efficacy of the pain model. There were no pain ratings for which the interaction between intervention type and timepoint was statistically significant, meaning that there is no evidence that the effect of capsaicin is dependent on the type of intervention administered.

4.3.1.4 Heterogeneity of responses between subjects

Widespread between-participant variability was seen in the degree to which pain responses were altered by the interventions (Figure 4.1; Figure 4.2). Initially it was thought that this might be explained by variability in the drug response of individuals. However, in some participants there was considerable within-subject variation in the effect of each intervention on the various pain outcome measures (e.g. participant 12). Therefore, there was no clear subpopulation for whom the drug was more effective at reducing pain (Figure 4.3).

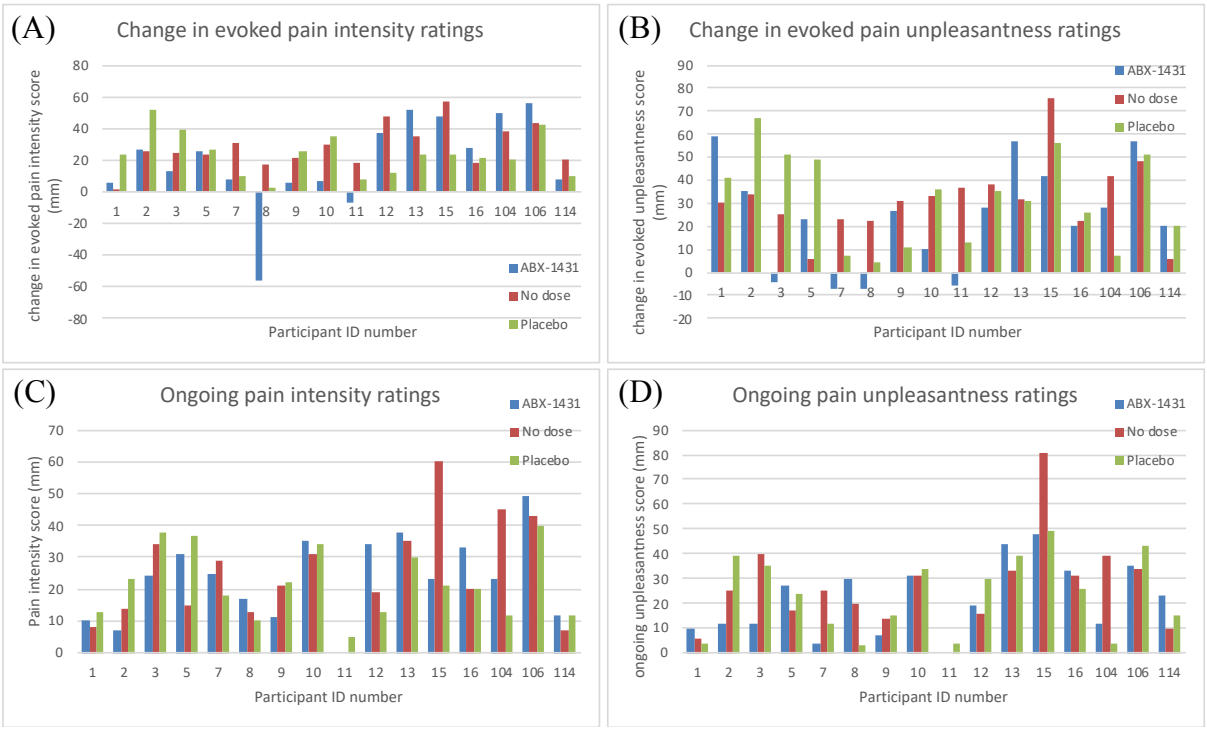


Figure 4.3: Variability in individual pain responses. These bar charts show individual pain ratings for each participant (n=16) and each intervention (ABX-1431, no dose and placebo). (A) Change in evoked pain intensity ratings from before capsaicin application to one hour after capsaicin application; (B) Change in evoked pain unpleasantness ratings from before capsaicin application to one hour after capsaicin application; (C) Ongoing pain intensity ratings one hour after capsaicin application; (D) Ongoing pain unpleasantness ratings one hour after capsaicin application. There is considerable between-subject heterogeneity across all four ratings. There is also within-subject variability across the four rating types with respect to which intervention sessions produced the biggest and smallest effects.

Peripheral drug levels, order effects, and the effect of expectation were also considered as possible sources of variability in pain ratings.

4.3.1.4.1 Effect of peripheral drug levels on pain ratings

A wide range of peripheral drug levels was seen between individuals (Figure 4.4; see appendix D-1.2 for raw data). To investigate the possibility that the variability in pain responses was linked to differences in drug concentration, a Pearson correlation coefficient was calculated to compare peripheral drug concentration with each of the pain measures. This showed no correlation between drug concentration and change in evoked or ongoing pain. Details of this analysis are shown in appendix D-1.3.

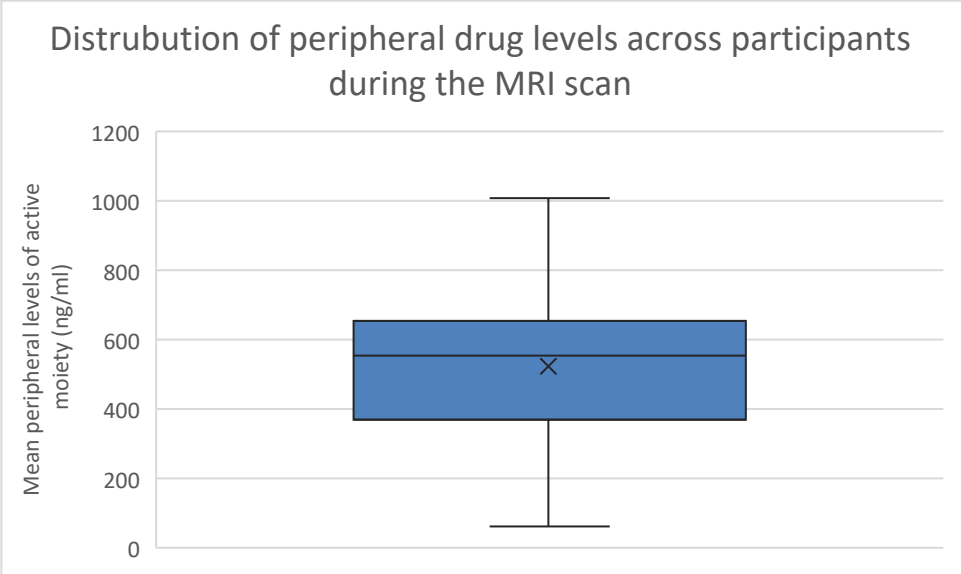


Figure 4.4: Boxplot showing the distribution of peripheral drug levels across participants following dosing with ABX-1431. Levels were tested immediately before and after the MRI scan, and the mean of the two results was used as an indicator of drug exposure during the scan. The concentrations given here are the sum of the parent compound and the main metabolite, since both are active and both cross the blood-brain barrier. The sample mean is denoted by a cross. There is wide variation in peripheral drug levels between individuals (n=16).

4.3.1.4.2 Effect of order on pain ratings

To determine whether variability may have been due to an order effect, visit order was added to the ANOVA as a between-subjects factor. Order dis have an effect on the change in evoked unpleasantness from pre- to post-capsaicin. Specifically, the participants who received no-dose first, followed by placebo and then ABX-1431 experienced a smaller increase in evoked unpleasantness than those who received interventions in another order (placebo, ABX-1431, no-dose; or ABX-1431, no-dose, placebo). However, this did not vary as a function of which intervention was received (Table 4.2). An equal number of participants was allocated to each of the three possible orders of intervention.

Variation source	Measure	df	F	significance
Intervention * order	Evoked intensity	4	1.241	0.320
	Evoked unpleasantness	4	2.070	0.116
	Ongoing intensity	4	0.590	0.673
	Ongoing unpleasantness	4	1.337	0.285
Timepoint (pre- or post-capsaicin) * order	Evoked intensity	2	1.157	0.347
	Evoked unpleasantness	2	5.458	0.021
	Ongoing intensity	2	1.077	0.372
	Ongoing unpleasantness	2	2.086	0.167
Intervention * timepoint * order	Evoked intensity	4, 26	0.302	0.874
	Evoked unpleasantness	4	0.915	0.471
	Ongoing intensity	4	0.590	0.673
	Ongoing unpleasantness	4	1.337	0.285

Table 4.2: Effect of intervention order on pain ratings. Table shows the results of a repeated measures analysis of variance with group (ABX-1431 vs. placebo vs. no treatment), timepoint (pre-dose and post-dose) and order (ABX-1431, no dose, placebo; no dose, placebo, ABX-1431; placebo, ABX-1431, no dose) as factors, and pain ratings as outcome measures (n=16). Significance levels in which $p \leq 0.05$ are shown in red. There was a statistically significant interaction between timepoint and order with respect to evoked unpleasantness. This indicates that the change in evoked unpleasantness from before capsaicin application to after capsaicin application was affected by the order in which the interventions took place. However this did not vary as a function of the intervention received.

4.3.1.4.3 Effect of expectation on pain relief

The effect of expectation on pain relief was assessed for both the ABX-1431 intervention and the placebo. The non-dosing arm was excluded from this analysis since participants were aware that no pain relief would be given prior to rating their expectations. For each intervention, the rating for expectation of pain relief was

compared to the change in evoked pain and unpleasantness from pre-capsaicin to post-capsaicin, as well as the ongoing pain ratings immediately prior to administration of the punctate stimuli. The distribution of expectation ratings was negatively skewed for both interventions (appendix D-1.4). For this reason, a Spearman correlation was used. Higher expectation of pain relief correlated with a smaller ongoing pain intensity response to capsaicin for ABX-1431 ($r_s(14)$=-0.427, p=0.05 1-tailed) but not for placebo ($r_s(14)$=-0.311, p=0.121 1-tailed). Figure 4.5 illustrates this further.

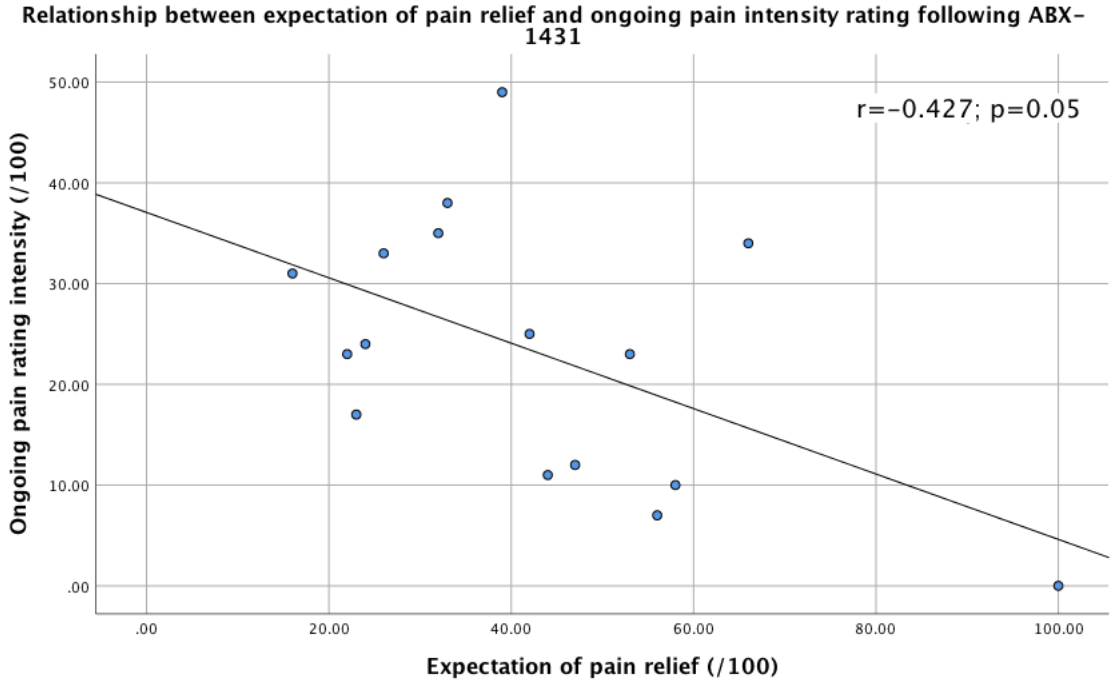


Figure 4.5: Scatterplot with line of best fit showing the relationship between expectation of pain relief and ongoing pain intensity rating. Higher expectation of pain relief is associated with reduced ongoing pain intensity ratings following ABX-1431 (n=16).

The expectation ratings for ABX-1431 (mean 42.563, SD 21.204) and placebo (mean 32.125, SD 24.317) were compared using a paired t-test with bootstrapping. The difference (mean 10.438, bCa 95% CI [-0.856, 20.230]) was not significant (t(15)=1.740, p=0.102), indicating that either participants did not guess which visit it

was prior to dosing or they expected no drug-related benefit. Although this does not rule out an effect of visit order on the ability of participants to guess what intervention they would receive, the fact that there was no interaction between intervention, timepoint and order (section 4.3.1.4.2) indicates that visit order does not have a differential effect on the outcome of each intervention. Therefore the relationship between expectation and order was not interrogated further.

4.3.2 Section 2: Impact of sedation, mood and anxiety

Sedation and mood were assessed with the Bond-Lader VAS scales prior to dosing and immediately after the end of the MRI scan (approximately four hours post-dose). For each participant, scores were calculated for four subscales: mental sedation, physical sedation, tranquillity and sociability. Mean scores for each of these domains were calculated across all participants. State anxiety was assessed at the same timepoints using the STAI state subscale and mean scores were calculated across all participants (Figure 4.6).

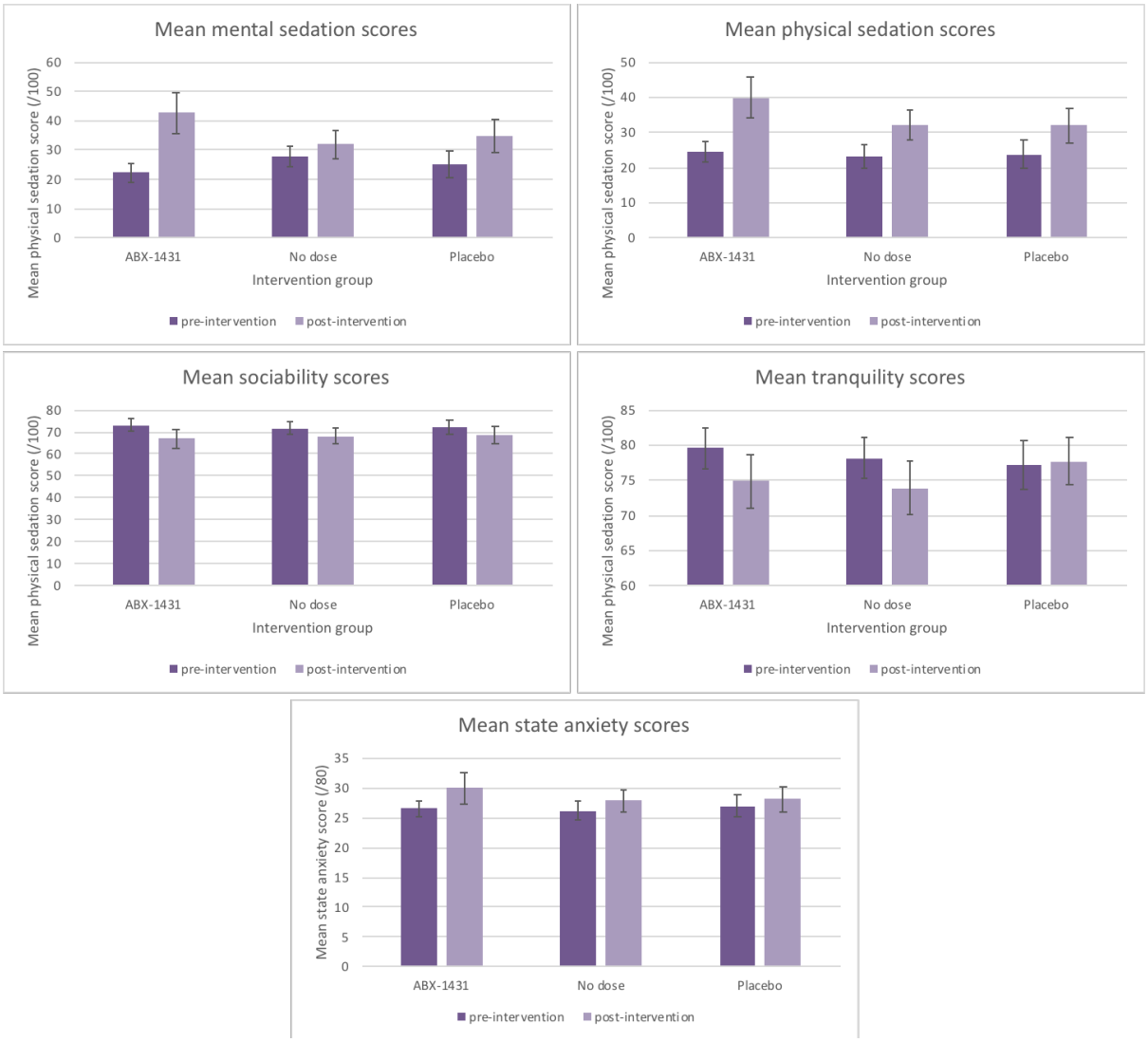


Figure 4.6: Bar charts to show the effect of intervention on mood/sedation and anxiety. These bar charts show the group mean scores for the Bond-Lader subscales and the state component of the state-trait anxiety inventory before and after the administration of each intervention (n=16). Error bars are calculated using standard error of the mean.

A repeated measures ANOVA was performed to determine whether these changes were statistically significant. There was a significant effect of intervention type (drug vs. placebo vs. no dosing) on physical sedation. With timepoint (pre- vs. post-treatment) alone as a factor, both physical sedation and mental sedation were significantly affected. There was also a significant intervention x timepoint interaction

for physical and mental sedation, indicating that the effect of the intervention on both measures was dependent on which intervention had been received (
Table 4.3). Based on the data, it was hypothesised that both mental and physical sedation would be increased more as a result of ABX-1431 than placebo or no dose (Figure 4.6). Post-hoc paired t-tests were performed to determine whether there was a significant change in mental or physical sedation pre-dose to post-dose for the interventions on an individual basis. These confirmed that mental sedation was increased following ABX-1431 but not following the other interventions (
Table 4.4). For physical sedation, there was a significant change pre-dose to post-dose for all interventions, though the mean change following ABX-1431 was nearly double that of the other interventions (
Table 4.5).

Variation source	Measure	df, df_{error}	F	significance
Intervention	Mental sedation	2,30	0.899	0.417
	Physical sedation	2,30	3.553	0.041
	Tranquillity	1.468, 22.022	0.416	0.603
	Sociability	2,30	0.048	0.953
	State anxiety	2,30	0.721	0.495
Timepoint (pre- or post-intervention)	Mental sedation	1,15	5.258	0.037
	Physical sedation	1,15	10.317	0.006
	Tranquillity	1,15	1.767	0.204
	Sociability	1,15	4.150	0.060
	State anxiety	1,15	2.181	0.160
Intervention * timepoint	Mental sedation	2,30	6.528	0.004
	Physical sedation	2,30	3.909	0.031
	Tranquillity	2,30	1.811	0.181
	Sociability	2,30	0.447	0.644
	State anxiety	2,30	0.647	0.531

Table 4.3: The effect of intervention on mood/sedation and anxiety. Table shows the results of a repeated measures analysis of variance with group (ABX-1431 vs. placebo vs. no dose) and timepoint (pre-dose and post-dose) as factors. n=16. Outcome measures are the four Bond-Lader subscales and the state component of the STAI. Mauchley's test was significant for the effect of intervention on tranquillity (p=0.043), so the Greenhouse-Geisser correction was applied (epsilon 0.734). Results are otherwise uncorrected. Significance levels in which $p \leq 0.05$ are shown in red. Physical sedation was significantly affected by which intervention was given, regardless of whether it was pre- or post-intervention. Physical sedation and mental sedation were significantly altered from pre-intervention to post-intervention. This varied as a function of which intervention was given, indicating that the effect of the intervention on mental and physical sedation was dependent on which intervention was given.

		95% Confidence interval of difference			
Intervention	mean change in mental sedation: post-dose minus pre-dose (SD)	lower	upper	t	p
ABX-1431	20.35 (27.33)	5.79	34.91	2.979	0.009
No dose	4.04 (19.08)	-6.13	14.20	0.847	0.410
Placebo	9.63 (20.00)	-1.03	20.28	1.926	0.073

Table 4.4: The effect of intervention on mental sedation. Table shows the results of post-hoc paired t-tests performed to determine whether there was a significant change in mental sedation associated with a specific intervention (n=16). P values ≤0.05 are considered significant and are shown in red. Mental sedation was significantly increased after ABX-1431 but not after either of the other two interventions.

		95% Confidence interval of difference			
Intervention	mean change in physical sedation: post-dose minus pre-dose (SD)	lower	upper	t	p
ABX-1431	15.30 (19.44)	4.94	25.65	3.148	0.007
No dose	8.77 (12.90)	1.89	15.64	2.718	0.016
Placebo	8.26 (11.05)	2.37	14.14	2.990	0.009

Table 4.5: The effect of intervention on physical sedation. Table shows the results of post-hoc paired t-tests performed to determine whether there was a significant change in physical sedation associated with a specific intervention (n=16). P values ≤0.05 are considered significant and are shown in red. Physical sedation was significantly increased after all interventions. Mean values show that the change following ABX-1431 was nearly double that following no dose or placebo.

A Pearson correlation was performed within the active drug arm to determine whether the degree of increased mental or physical sedation may have affected pain scores in

that group. There was no significant relationship found. Results are shown in appendix D-1.5.

There was huge individual variability in the degree to which all of these measures were affected, and also the direction of effect. To exclude visit order as a driver of this heterogeneity, it was added to the ANOVA as a between-subjects factor. There was no significant order effect on any of these outcome measures, nor on their interactions. Results are shown in appendix D-1.6.

Drug concentration was also investigated as a possible source of variability. Peripheral drug levels were compared with the change in Bond-Lader and STAI state scores using a Pearson correlation. No significant correlations were seen. Results are shown in appendix D-1.7.

4.3.3 Adverse events

Three quarters of participants reported at least one adverse event. Not all of these were deemed possibly treatment-related (see chapter 3, section 3.10.6 for definitions relating to adverse events). However, those that were occurred much more frequently in the active drug arm than in the placebo or non-dosing arms. The most common events were fatigue/tiredness, sleepiness, and dizziness/vasovagal episodes. See appendix **Error! Reference source not found.** for tables summarising the numbers and types of adverse events seen. Adverse events occurred at both high and low peripheral drug concentrations.

During the evoked pain task, four participants fell asleep following ABX-1431, while only two fell asleep after placebo and none during the non-dosing session. After ABX-1431 an additional participant was found to be asleep at the end of the BOLD resting state scan, and reported feeling increased tiredness compared to normal for the remainder of the day. They also experienced a period of reduced neurocognitive function and a feeling of pressure in the head. Another participant reported feeling fatigued and mildly euphoric after taking ABX-1431.

Dizziness or vasovagal episodes occurred after all three interventions, although more frequently after ABX-1431. These symptoms were often associated with standing up after lying down for a long period, and this sudden change in posture may have been causal. However, the fact that the symptoms were more common in the drug arm does indicate the possibility that the intervention may have exacerbated this effect.

4.4 Discussion

4.4.1 Pain

In this study, there was no statistically significant effect of ABX-1431 on evoked or ongoing pain when compared to placebo, nor compared to a non-dosing arm. Nor was there a significant effect of placebo compared to no dose.

In spite of this it is not possible to conclude definitively that the drug is ineffective as an analgesic. There was widespread variability in self-reported pain ratings, both within and between subjects. The perception of pain is a composite experience, with nociceptive input, central wind-up, cognition and emotion all forming important modulators of perceived analgesia. It is therefore inevitable that individuals will

experience differences in their behavioural response to an analgesic regardless of its mechanism of action. This variability means that for the small sample size used in this study, there is a high risk of type II errors, in which a true difference between groups is not detected. Despite this fact, the collection of subjective behavioural data such as this remains important. It is a requirement by industry partners, for whom the holy grail would be an in vivo marker of target engagement and drug action and efficacy that could be used as a biomarker in clinical trials to complement human behavioural data (Lipton & Nordstedt, 2016).

The same factors might explain why no significant placebo analgesic effect was seen. In particular, it is possible that the variation in the expectations that individuals had for pain relief was sufficient to mask any consistent placebo effect. It is well known that expectation contributes hugely to the degree of placebo effect achieved (Price et al., 1999; Tracey, 2010; Vase, Robinson, Verne, & Price, 2003). The effect of expectation as well as some of the other potential sources of variability are discussed in more detail below.

4.4.2 Sedation

Physical sedation was increased from pre-dose to post-dose across all sessions, indicating a large environmental component. There was an interval of several hours between these two sets of ratings, during which participants mainly sat or lay down in one room with minimal interaction with other individuals, other than for the completion of study procedures. The second set of ratings was also given shortly after completion of the MRI scan, during which participants were lying down for an extended period in a relatively dark space, and were asked to remain still. Participants also remained

fasted from at least 2am and were not able to eat until 2pm, which was around the time that they completed their post-MRI sedation assessments. It is likely that a combination of these factors was responsible for the global increase in physical sedation. However, there was a significant effect of interaction between intervention type and timepoint, indicating that the increase in physical sedation was dependent on the intervention. This is likely to be a drug effect given that the increase in the drug arm was nearly double that in the other two arms.

Mental sedation was significantly increased following dosing with ABX-1431, but was not affected by the other two interventions, indicating this was a pure drug effect with no environmental component. The presence of sedation which is specific to the drug arm indicates that ABX-1431 crossed the blood-brain barrier and had a pharmacodynamic effect.

Exogenous cannabinoids are well known to produce a sedative effect in humans (Campbell et al., 2001; Joint Formulary Committee, 2020). In mice, increased 2-arachidonoyl glycerol (2-AG) concentration through MGLL inhibition has been reported to cause reduced motility (Long et al., 2009). However, administration of 10mg/kg of ABX-1431 to rats did not alter locomotor activity (Cisar et al., 2018). It is difficult to tell to what extent MGLL inhibition would be expected to cause sedation in humans, both due to the difficulty of translating drug effects direct from animal models into humans, and due to the inability to assess cognition and mental sedation accurately in non-verbal animals. However, it might be expected that sedation would be a plausible side effect of the compound. This is supported by first in man data, which found fatigue to be a common adverse effect (Abide Therapeutics, 2017).

There was concern that drug-related sedation may have influenced pain perception or altered the ability of participants to record their pain levels accurately. However, there was no correlation between reported pain measures and the degree of increased sedation in the active drug arm, suggesting that this was unlikely. This is in keeping with the results of a previous study of tetrahydrocannabinol (THC) for analgesia, in which THC caused sedation across all sessions, but only caused analgesia when capsaicin-induced pain was present (M. C. Lee et al., 2013). A further risk of drug-induced sedation would be that it might cause inadvertent unblinding of either participant or researcher. No data was collected on this, which is a limitation of the study design.

4.4.3 Individual variability

Variability, whether within or between participants, leads to reduced study sensitivity (Gewandter et al., 2020). In this study the degree of variability between individual responses was extremely high, both for pain measures and for measures of mood, sedation and anxiety. The variability was not just a matter of scaling, where some people had a greater effect and others a smaller effect. The pattern of response also differed both within and between subjects, with variation in which intervention produced the bigger effect on each outcome measure. This lack of within-subject consistency across different measures suggests that the variability was not due to a simple division between those who responded to the drug and those who did not. A range of possible alternative reasons for the heterogeneity were considered, including differences in drug levels, expectation, visit order, age, rating ability, and the experimental model. Given the complex nature of pain perception, which can be

affected by past experience as well as by factors on the day of the visit, there may be additional sources of variation that were not accounted for.

4.4.3.1 Drug levels

Data from initial phase I trials showed that food intake, especially high-fat foods, had a major impact on ABX-1431 pharmacokinetics (Abide Therapeutics, 2017). For that reason all participants were fasted for a minimum of eight hours in order to remove this source of variability. Even so, a wide range of peripheral drug levels was seen between individuals following ABX-1431 administration, and it was thought that variability in drug exposure may have been a factor in between-subject heterogeneity.

In spite of this variability, there was no correlation between peripheral drug level and any behavioural measure. Peripheral drug concentration may not reflect target engagement, as the drug has to cross the blood-brain barrier, and the relationship between pharmacokinetics and pharmacodynamics is not always straightforward. However, should the variability have been due to differences in drug target engagement, it might be expected that there would have been less within-subject variability between the different behavioural measures. Individual variation in drug absorption does make it more difficult to interpret study outcomes. This is a limitation of this study, and should be addressed in future studies through manipulation of the drug formulation if possible.

4.4.3.2 Expectation

Expectation is known to be an important predictor of pain perception. A wide range of studies demonstrate that creating an expectation of pain relief leads to a reduction in

reported pain (Peerdeman et al., 2016). Expectation is a key component of placebo analgesia. Furthermore, the degree of expectation of pain relief has been found to override the effect of a powerful known analgesic (Ulrike Bingel et al., 2011).

It was therefore unsurprising to find that an expectation of greater pain relief was associated with a lower level of reported ongoing pain intensity in the active drug arm. Evoked pain ratings were not affected in the same way. This is consistent with literature that finds that expectancy and placebo appear to have a greater effect in studies that use long-duration stimuli as opposed to those examining short-duration stimuli (Vase, Petersen, Riley, & Price, 2009).

The absence of an expectation effect in the placebo arm was unexpected. Although this may be due to a type II error, other possibilities include the effect of visit order or a factor that has not been considered. Since the effect of visit order on pain ratings did not vary by intervention (see section 4.3.1.4.2), the relationship between order and expectation was not investigated further.

4.4.3.3 Visit order

There was a significant interaction between timepoint (pre- vs. post-capsaicin) and order for evoked unpleasantness. Those who completed the visit with the active drug first and placebo last showed the biggest mean increase in evoked unpleasantness, while those who completed the visit with the active drug last showed the smallest. It is not clear why this should have been the case given that there was no three-way interaction between timepoint, order and group, indicating that the effect on evoked unpleasantness was not significantly greater in one intervention than another. It may

be that an aspect of the participant's experience of their first visit in some way influenced all of their subsequent evoked unpleasantness ratings. Alternatively, this finding may simply be a type I error. Whichever is the case, the number of participants assigned to each visit order was balanced, so the presence of any order effect should have been negated to a large degree.

4.4.3.4 Age

Age was considered as a possible source of variation, given the known effect of age on descending pain modulation (R.R. Edwards, Fillingim, & Ness, 2003; Larivière, Goffaux, Marchand, & Julien, 2007), but the age range in this sample size was small. Had it been greater, age would have been considered as a covariate in the analysis.

4.4.3.5 Pain rating ability

The ability of raters to report their pain accurately is variable. In this trial, care was taken to give clear instructions on how to use the pain measures at every visit, using a script to ensure uniformity, but this does not remove the variability completely. Given that participants who lack consistency in subjective pain ratings produce smaller effect sizes in analgesic trials (Farrar et al., 2014), investigators are looking at other strategies to mitigate this. These include excluding participants with poor baseline consistency and training participants on how to perform ratings prior to experiments (Gewandter et al., 2020). The benefits of these measures are yet to be confirmed, and they were not applied in this study.

4.4.3.6 Experimental model

The capsaicin model of central sensitization is a source of between- and within-subject variability, with multiple factors affecting the resulting level of hyperalgesia and allodynia, including temperature, skin surface area and body part (Liu et al., 1998). In this study, the concentration and quantity of capsaicin were kept the same at each session, as well as the site and skin surface area to which it was applied and the temperature of the room. Participants were also screened prior to inclusion, and those in whom capsaicin induced ongoing pain ratings of less than three or who showed no measurable hyperalgesia were excluded. Despite these measures, a pilot study demonstrated a wide range of responses between individuals in terms of intensity and timecouse (see Appendix A:).

4.4.4 Limitations of the study

As discussed above, the variability of peripheral drug levels is a limitation of the study. Other limitations are: the generalisability of the participants to the population; the use of pre-emptive dosing; weaknesses associated with the experimental model; the adverse effects of the drug, particularly sedation; the potential for inadvertent unblinding; the lack of knowledge on optimal dosing; the timing of pain scores; and the presence of multiple comparisons . These limitations are discussed below, and apply to both this chapter and the subsequent imaging chapter.

4.4.4.1 Generalisability to the population

Due to the fact this was an early phase study, the research was conducted using an experimental pain model in healthy men, all of whom were a similar age. This does not reflect real life, and the outcomes may have been different had the population been

a group with pre-existing chronic pain, with other health conditions, or with a broader demographic.

4.4.4.2 Pre-emptive drug dosing

Due to the timecourse of the capsaicin model and the pharmacokinetics of ABX-1431, it was necessary to administer ABX-1431 prior to establishing the model. This pre-emptive dosing does not represent a real life scenario, in which pain would precede administration of an analgesic drug, and this should be addressed in future trials.

4.4.4.3 The capsaicin model

Capsaicin produces a model of a neuropathic pain mechanism (central sensitization). Different drugs are frequently used to treat neuropathic versus non-neuropathic pain (Fitzmaurice & Rayen, 2018; Moore, Chi, Wiffen, Derry, & Rice, 2015), and an alternative model, such as a model of inflammatory pain, could produce different results. Indeed the mode of action of ABX-1431 would support the possibility of efficacy in inflammatory conditions, since it blocks the hydrolysis of 2-AG into arachidonic acid. Arachidonic acid is a precursor for proinflammatory mediators such as prostaglandins and leukotrienes. The literature supports the idea that cannabinoids act as anti-inflammatory agents (Nagarkatti, Pandey, Rieder, Hegde, & Nagarkatti, 2009). These are all factors that should be considered when taking the compound forward into future studies.

4.4.4.4 Adverse events and inadvertent unblinding

A potential confound to the results of the study were the adverse events that occurred. In particular, the high incidence of sedation. Behavioural data (section 4.3.2) showed

that ABX-1431 was associated with significantly increased levels of mental sedation. It was common for participants to feel fatigued or sleepy, and five individuals fell asleep during the evoked pain paradigm. In four cases this was following dosing with the drug, and it happened twice after placebo dosing (one participant fell asleep during both dosing visits). Ratings were taken in retrospect once they were woken up, but it's impossible to know at what stage of the paradigm they fell asleep, and therefore how many stimulations they were aware of. It's also unclear how accurate their recall was on waking. One participant was also asleep when they were spoken to at the end of the BOLD resting state scan, and there may have been instances of participants sleeping at other scan timepoints without the research team being aware. Participant 008 failed to adjust the VAS pain ratings immediately after the evoked pain paradigm during one of the treatment visits. They denied falling asleep and reported that they intentionally recorded a score of 0, but this was not in keeping with any of their other pain scores around that time. There is a suspicion that this participant may have fallen asleep without realising. Two other participants who were not found to be asleep during the scan still reported feeling fatigued following administration of ABX-1431. One of these participants also reported mild euphoria and the other felt a pressure in their head and also experienced reduced neurocognitive function for a period of time.

All of these adverse events had the potential to affect self-reported pain, mood and anxiety ratings, as well as leading to possible altered functional neurology. The fact that adverse events occurred more frequently in the active drug session than the placebo or non-dosing session also raises the possibility that inadvertent unblinding may have occurred, due to participants or researchers assuming that the adverse

events were associated with the active drug. In future studies it would be useful to collect data on this from both participants and researchers at the end of each visit.

4.4.4.5 Drug dosing

The issue of variable drug plasma levels at a single drug dose is discussed above. In addition, the optimal dose for enabling the detection of analgesic efficacy is unknown. Due to the fact that this is an early phase study, there is no pre-existing efficacy data. Therefore, decisions on appropriate drug dosing were made on the basis of bioavailability data and tolerability data alone. It is also a single dose study. Since there is no efficacy data comparing this to multiple doses, the ideal regimen is unknown. There is some preclinical evidence that chronic use of an MGLL inhibitor may lead to tolerance (Schlosburg et al., 2010), and this is not something that could be determined from a single dose study.

4.4.4.6 The timing of pain scores

The timing of pre-capsaicin pain scores is a limitation of the protocol. The ideal time for these pain scores to be done would have been prior to drug dosing. However, due to the tight timing available for study procedures, they were done immediately post-dose. In practice, due to the time taken for drug absorption and metabolism (T_{max} for a single 40mg dose is approximately 3.33 hours (Abide Therapeutics, 2017), it is unlikely that this impacted the scores in any way.

Another protocol limitation is that despite participants receiving multiple pinprick stimuli, they were only asked for an average evoked pain score at the end rather than a score after each stimulus. It is possible that by the end of the run of stimuli, recall of

the initial stimuli may have been biased. However, asking for a score between each stimulus would have required a much longer interstimulus interval in order to avoid interfering with the haemodynamic response function associated with the stimulus. This would have reduced the number of stimulations possible, making the imaging paradigm less powerful.

4.4.4.7 Multiple comparisons

The collection of multiple behavioural outcomes meant that multiplicity of analyses was a potential issue with data analysis. This was kept to a minimum when possible by performing formal hypothesis testing only when necessary for each dataset/subset. There was no correction for multiple comparisons for reasons discussed in chapter 2.

4.5 Summary

It was not possible to determine whether ABX-1431 has analgesic efficacy from the self-reported pain scores alone. There was no significant reduction in pain scores following the active drug compared to placebo or no dose. However, this study had a small sample size and participants showed widespread within-subject and between-subject variability in their responses to the three interventions. Therefore the statistical tests applied are not likely to be powerful enough to detect an effect on pain ratings, and it is not justified to state that ABX-1431 is not effective based on this. The presence of mental and physical sedation following dosing with ABX-1431 indicates a pharmacodynamic effect. Although this gives no information about efficacy, it does indicate a central neurological effect.

The fact that subjective behavioural data often requires large sample sizes to reveal a true effect is problematic for a lot of early phase trials, and can make analgesic discovery very challenging. There is a clear need for an objective biomarker of analgesic effect to assist with this. Even if the behavioural data had revealed clear drug efficacy, mechanistic data is important for pharmaceutical companies who wish to understand the mechanisms that underlie changes in behavioural data. The next chapter will explore whether the use of fMRI as a biomarker improved the ability to detect efficacy, and provided mechanistic information in this study, and thus whether it might be an important tool in early analgesic discovery.

Chapter 5: Effect of ABX-1431 on the neural response to a noxious stimulus

5.1 Introduction

The previous chapter demonstrated that it was not possible to determine the analgesic efficacy of ABX-1431, a monoacylglycerol lipase (MGLL) inhibitor, from the behavioural data alone. The presence of sedation in the active drug arm indicates the probability of a pharmacodynamic effect, but this does not confirm target engagement, as the sedation may be due to off-target effects. The behavioural data gives minimal insight into the neural mechanisms of the drug, making its effect difficult to disambiguate from placebo. It is thought that imaging will assist with this, enabling a more powerful assessment of drug efficacy. An understanding of the mechanisms that underlie the drug's action could also be a useful tool in deciding what patients might benefit from its use. Not only is mechanistic data useful, but as outlined in chapter 1 (section 1.4) it is often a prerequisite for interest in a drug by partners in industry (Lipton & Nordstedt, 2016).

5.1.1 Neural correlates of cannabinoid-induced pain relief

There is plenty of preclinical data to show the importance of endocannabinoids and intrinsic endocannabinoid tone in endogenous pain modulation. In rodents, regions involved in this endocannabinoid-mediated modulation include the prefrontal cortex (PFC), anterior cingulate cortex (ACC), amygdala and brainstem (see chapter 1, section 1.2.5 for more details).

Multiple studies have looked at the neural correlates of cannabis administration in humans, but few have done this in the context of acute dosing for pain relief. In a randomised, placebo controlled trial of tetrahydrocannabinol (THC) on capsaicin-induced pain, THC reduced the unpleasantness but not the intensity of both tonic pain and capsaicin-induced punctate hyperalgesia (M. C. Lee et al., 2013). Hyperalgesia-related activity in the anterior midcingulate cortex (aMCC) was reduced with THC. THC also caused an increased stimulus-induced blood oxygen level dependent (BOLD) response in the right amygdala which correlated with the analgesic effect. Connectivity between the amygdala and primary somatosensory cortex (SI) was reduced during ongoing pain, and the greater the reduction in connectivity, the more the unpleasantness and intensity ratings were dissociated from one another.

Although this was a study of healthy participants, the effect of THC on functional connectivity has also been examined in a chronic pain population. A small randomised controlled study comparing acute THC administration with placebo in men with chronic neuropathic pain found that THC significantly reduced perceived ongoing pain (Weizman et al., 2018). In the THC group, the magnitude of pain relief correlated with the degree to which functional connectivity was reduced between the aMCC and sensorimotor cortex (secondary somatosensory cortex [SI] and primary motor cortex [MI]). Using graph theory, the aMCC was found to have reduced global connectivity with a network of regions implicated in chronic pain. There was also reduced connectivity between the right dorsolateral PFC (dlPFC) and the rest of the pain network, and this correlated with the degree of pain relief.

In both studies, THC caused reduced connectivity between sensory regions and regions processing affective aspects of the pain experience. The aMCC, dlPFC and amygdala were all regions that were implicated in THC-induced pain relief. They are components of the pain network that are important in the cognitive-affective aspects of pain processing, and all three are implicated in descending pain modulation (Lorenz et al., 2003; Rainville, Duncan, Price, Carrier, & Bushnell, 1997; Tracey & Mantyh, 2007). It is predicted that the use of ABX-1431 may produce similar findings.

5.1.2 Chapter objectives

This chapter has three objectives. The first is to use imaging to confirm that ABX-1431 has a pharmacodynamic effect on the brain and to determine whether there is appropriate central target engagement. The second is to assess whether ABX-1431 has efficacy as an analgesic. As discussed previously, it is predicted that neuroimaging can provide objective evidence of analgesic efficacy in small samples, even when behavioural data is not sufficient to show pain modulation. Attenuation of the central response to a painful stimulus would suggest efficacy. The final objective is to assess the potential mechanisms of ABX-1431 by examining its effect on the activity of key pain regions during pain and at rest, as well as the functional connectivity between them.

It is predicted that compared to placebo and no dose, ABX-1431 will cause attenuation of neural activity evoked by capsaicin-induced punctate hyperalgesia. Given that this is the first human neuroimaging study of ABX-1431, and that CB1 receptors and MGLL are distributed widely across the brain, no definite target region has been identified. However, based on previous literature on THC-induced analgesia (M. C. Lee et al.,

2013; Weizman et al., 2018), it is hypothesised that aside from the aforementioned attenuation of pain-evoked activity, the right amygdala and the aMCC are specific regions in which activity may be modulated by ABX-1431. Additionally, there may be altered resting state connectivity between the right amygdala, a region important for the affective-motivational component of pain, and sensory regions such as SI and the left dorsal posterior insula (dpINS; a region in which bloodflow has been shown to track pain intensity).

5.2 Methods

As described previously, a randomised, double-blinded three-way crossover design was used. Participants completed three intervention visits, at which they received ABX-1431, placebo and no drug. Topical capsaicin was used at each visit to produce an experimental tonic pain model. A punctate probe was used to stimulate the area of secondary sensitization around the capsaicin in order to produce an experimental evoked pain model. Brain imaging was performed during both tonic and evoked pain using fMRI to determine the differences in neural responses to pain in the three arms of the study. BOLD imaging was used to assess responses to evoked pain, responses to a control (visual) stimulus, and differences in functional connectivity during tonic pain; while arterial spin labelling (ASL) was used to compare activity during tonic pain. Participants also gave ratings of pain intensity and unpleasantness to determine the effect of the three interventions on behavioural pain measures (see chapter 4 for behavioural results). The methods are explained in detail in chapters 2 and 3.

5.2.1 Participants

Data from all 16 participants were included in this chapter. However, due to technical issues with one of the ASL scans and one of the BOLD checkerboard (visual stimulus) scans, the ASL and checkerboard results are based on scans from 15 individuals.

5.2.2 Brain imaging acquisition

At baseline, participants completed whole brain ASL scans, BOLD resting state scans and structural scans. A fieldmap was also obtained to correct for field inhomogeneities in all echo-planar imaging (EPI) acquisitions. A T1-weighted structural image with 2x2mm voxels was obtained for registration and voxel selection.

At treatment visits, participants completed the above, and also underwent BOLD scans while completing the evoked pain task (described above) and a visual checkerboard task.

5.2.2.1 BOLD imaging

5.2.2.1.1 Resting state

Whole brain resting state data were acquired using a whole-brain gradient echo EPI sequence (echo time (TE) 33.4ms; flip angle 60°; field of view (FOV) 192x192mm; matrix size 96x96 with 72 axial slices, angled to AC-PC). Slice acquisition was interleaved. Slice thickness was 2mm with an in-plane resolution of 2x2 mm^2. Parallel imaging was used with an acceleration factor of six. 644 volumes were acquired with a repetition time (TR) of 933ms, giving a scan time of 10 minutes.

During the resting state scan, participants were asked to fix their gaze on a cross in the centre of the screen, blink normally, and try not to fall asleep.

5.2.2.1.2 Evoked pain

BOLD data were also acquired throughout the evoked pain task using identical parameters to the resting state except that 864 volumes were acquired. Since the scan was slightly longer than the task, the final 16 volumes were removed prior to analysis, leaving 850 volumes (approximately 13 minutes).

5.2.2.1.3 Checkerboard task

Participants were asked to fixate on a cross in the centre of the screen. They were then repeatedly presented with a black and white checkerboard flickering at 2 Hz with a fixation cross in the centre. The checkerboard was presented five times for 30 seconds each time. This was interleaved with 30 second periods in which the screen was blank other than the fixation cross.

BOLD data were acquired throughout using identical parameters to the resting state except that 354 volumes were acquired, giving a scan time of approximately five minutes 30 seconds.

The purpose of the checkerboard task was to check for drug-related effects on blood flow which may cause altered brain activation findings for any sensory task rather than being specific to pain.

5.2.2.2 Arterial spin labelling

Whole brain ASL data was acquired using a pCASL sequence with background pre-saturation suppression, similar to that described previously in other studies performed at FMRIB (Mezue et al., 2014; Segerdahl et al., 2015). The labelling plane location was manually specified for each scan using a time of flight scan. An axial slice was chosen for this between the two curvatures in the vertebral arteries, parallel to the feeding arteries. Six different post-labelling delays were used (0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 seconds), following a labelling duration of 1.4 seconds. Other parameters were: FOV 220 x 220 mm; slice thickness 4.5mm; 24 slices; TR 4100ms; TE 14ms; flip angle 90°; angled to AC-PC; slice acquisition interleaved; bandwidth (BW) 2004 Hz/Px. Ninety-six volumes were acquired, giving a scan time of approximately six minutes.

A reference calibration image was acquired with no labelling or background suppression to enable the estimation of the equilibrium magnetization of blood. A second calibration image was collected using the body coil for signal detection. This was used to correct for the uneven sensitivity profile of the 32-channel head coil (Mezue et al., 2014).

During ASL acquisition, participants were asked to fix their gaze on a cross in the centre of the screen, blink normally, and try not to fall asleep.

5.2.3 Data analysis

5.2.3.1 BOLD imaging data

Processing of images was performed using FMRIB's Software Library (FSL; Smith et al., 2004).

5.2.3.1.1 Task-based imaging

This section describes the analysis methods for both the evoked pain imaging and for the visual checkerboard stimulus. Initial pre-processing of functional images was performed with the MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) GUI. BET (brain extraction tool) was used to remove non-brain tissue from the images (Smith, 2002). The images were motion corrected with McFlirt (Jenkinson et al., 2002). Slice timing correction was applied using Fourier-space time-series phase-shifting to correct for interleaved slice acquisition. Highpass temporal filtering was applied with a cutoff of 90 seconds (Gaussian-weighted least-squares straight line fitting, with sigma 45s). Spatial smoothing was applied using a Gaussian kernel of FWHM 5mm. B_0 unwarping was performed using fieldmaps acquired in the same scan session. Functional images were registered to brain-extracted structural images using boundary-based recognition (Greve & Fischl, 2009), and then nonlinear registration to MNI standard space was performed using FNIRT (Andersson et al., 2007).

MELODIC was then used to perform independent component analysis (ICA) in order to differentiate signal components from noise. The number of components was estimated automatically. Timecourses were variance-normalised. The independent component map threshold was set to 0.5. A training dataset was created by hand

classifying all components of a subset of the scans (Griffanti et al., 2017). This data was then fed into the automated ICA denoising tool FIX, and FIX was then used to classify the entire dataset (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014).

In addition to ICA denoising, physiological noise modelling (PNM) was performed (Brooks et al., 2008). Information about the timing of cardiac and respiratory cycles was collected using a pulse meter and respiratory bellows. These measures were then included as regressors in the general linear model (GLM) in order to remove any motion that was synchronous.

First level analysis of each scan was performed in FEAT (FMRI Expert Analysis Tool; Woolrich et al., 2001). Outputs from both PNM and ICA denoising were used in this analysis. For the evoked pain imaging, the timecourse of the punctate stimuli was entered as the regressor of interest. Timecourses were also created for the visual presentation of instructions to the participant, and for the completion of pain rating scales. These were included as regressors of no interest. For the visual stimulus imaging, the timecourse of the checkerboard presentation was entered as the regressor of interest.

For each participant, a second level analysis was performed in FEAT comparing their three sessions (drug, placebo and no dose). This was a fixed effects analysis. Contrasts were set up to find the mean activation for each session individually, and to compare each of the three sessions with each of the other sessions (Woolrich et al., 2004).

A third level analysis was then performed in which the results for each of these contrasts were pooled across all participants. Whole brain analyses with mixed effects were performed using FLAME 1 (Woolrich et al., 2004). Cluster-based familywise error correction was used for multiple comparisons ($Z > 2.3$; $p < 0.05$).

Region of interest (ROI) analyses were performed using randomise, a tool for nonparametric permutation testing (Winkler et al., 2014). For each ROI, 5000 permutations were performed on threshold-free cluster-enhanced values, yielding a familywise error correction rate of 0.05. The right amygdala was chosen as a ROI due to literature showing that the analgesic effect of THC correlates with its effect on the BOLD response to capsaicin-induced punctate hyperalgesia in this region (M. C. Lee et al., 2013). The mask used for this was an anatomical mask defined using the Harvard-Oxford probabilistic atlas and thresholded to 50%.

Peripheral drug concentration was deemed a possible confound due to its variability (appendix D-1.2). The expectation of pain relief was also considered a potential confounding factor, as expectation is known to have a strong effect on pain perception (Ulrike Bingel et al., 2011), and was shown to correlate with a behavioural outcome of ABX-1431 in chapter 4. The effects of these on evoked pain imaging outcomes were examined by adding them to the GLM as regressors.

5.2.3.1.2 Functional connectivity during tonic pain

An anatomical mask of the right amygdala was used as a seed for whole brain BOLD resting state analysis. The amygdala was chosen due to existing evidence for its role in cannabinoid-induced analgesia (M. C. Lee et al., 2013; B. H. Manning et al., 2003;

Barton H. Manning, Merin, Meng, & Amaral, 2001). The right amygdala in particular has been implicated in both cannabinoid analgesia and in pain processing more generally. Preclinical studies using models of visceral pain and somatic inflammatory and neuropathic pain indicate that pain-related amygdala function is lateralised to the right (Carrasquillo & Gereau IV, 2008; Gonçalves & Dickenson, 2012; G. Ji & Neugebauer, 2009; Sadler et al., 2017). In humans, there is also evidence of right hemispheric lateralization of amygdala function in pain (C.-L. Lu et al., 2004).

Pre-processing was performed as described above (section 5.2.3.1.1), except the highpass temporal filtering cutoff was 100 seconds (Gaussian-weighted least-squares straight line fitting with sigma 50s). Data were denoised in FEAT using both FIX and PNM (Brooks et al., 2008; Griffanti et al., 2014; Salimi-Khorshidi et al., 2014). The cleaned data was registered to standard space. An anatomical mask of the right amygdala was created (appendix G), and the timecourse within that region was extracted using the tool fslmeants from FSL. The mask was defined using the Harvard-Oxford probabilistic atlas, and was thresholded to 50%. Timecourses were also extracted for the cerebrospinal fluid (CSF) and white matter.

For each visit, a first level analysis was performed in FEAT with the timecourse of the seed region as the regressor of interest, and the CSF and white matter timecourses as regressors of no interest. Whole brain higher level analyses were performed as described above for task-based imaging.

ROI analyses were performed using randomise, a tool for nonparametric permutation testing (Winkler et al., 2014). For each ROI, 5000 permutations were performed on

threshold-free cluster-enhanced values, yielding a familywise error correction rate of 0.05.

The ROIs selected for analysis were the dpINS, the rostral ACC (rACC), the left SI, the right SI, and the aMCC. These regions were chosen based on existing literature. The administration of THC has previously been shown to reduce functional connectivity between the right amygdala and left SI during tonic capsaicin-induced pain (M. C. Lee et al., 2013), indicating a possible role for the uncoupling of the amygdala from regions involved in sensory aspects of pain processing. SI and the dpINS are both regions implicated in the sensory component of pain (Ploner et al., 1999; Segerdahl et al., 2015; Uhelski et al., 2012). The aMCC has been implicated in THC-induced analgesia (M. C. Lee et al., 2013; Weizman et al., 2018), and has a high density of CB1 receptors (Bloomfield et al., 2019). The rACC has a key role in placebo analgesia through its involvement in descending pain modulation (U. Bingel et al., 2006; Petrovic, Kalso, Petersson, & Ingvar, 2002), so its connectivity is likely to be altered by placebo administration. However, like the aMCC it also has a high density of CB1 receptors (Bloomfield et al., 2019), meaning that there is the potential for modulation by ABX-1431.

Left and right SI masks were anatomical masks, defined using the Juelich histological atlas, and thresholded to 50%. Anatomical masks of the rACC and aMCC were created from an ACC mask defined using the Harvard-Oxford probabilistic atlas and thresholded to 50%. The subdivisions were then delineated as described by Vogt and colleagues (Vogt et al., 2003).

The left dpINS mask was a functional mask based on the findings of Segerdahl and colleagues (Segerdahl et al., 2015). The mask was an 8mm sphere centred around the area of the dpINS which correlated most closely with perceived pain intensity.

Each of these masks is illustrated in appendix G.

5.2.3.2 Arterial spin labelling

Single subject analysis was performed as described in chapter 2. Higher statistical analysis was performed with FEAT (Woolrich et al., 2004, 2001). For each participant, a within-subject whole brain fixed effects analysis was performed, comparing each of their three sessions (drug, placebo and no dose) with each of the other sessions. The results for each of these contrasts were then pooled across all participants with a mixed effects analysis using FLAME 1 (Woolrich et al., 2004). Cluster-based familywise error correction was used for multiple comparisons ($Z > 2.3$; $p < 0.05$). Peripheral drug concentration was deemed a possible confound, and the effect of this on ASL outcomes was examined by adding it as a regressor to the GLM.

5.3 Results

5.3.1 Confirming the effectiveness of the experimental model

In order to check that the experimental pain model was effective, the group mean of the stimulus-induced activation was examined for each of the three intervention arms. All three groups showed activation in key pain regions in response to the stimulus (Figure 5.1), including the mesencephalic reticular formation, a region important for the maintenance of central sensitization (M. C. Lee et al., 2008). This demonstrates that the model was functioning as expected.

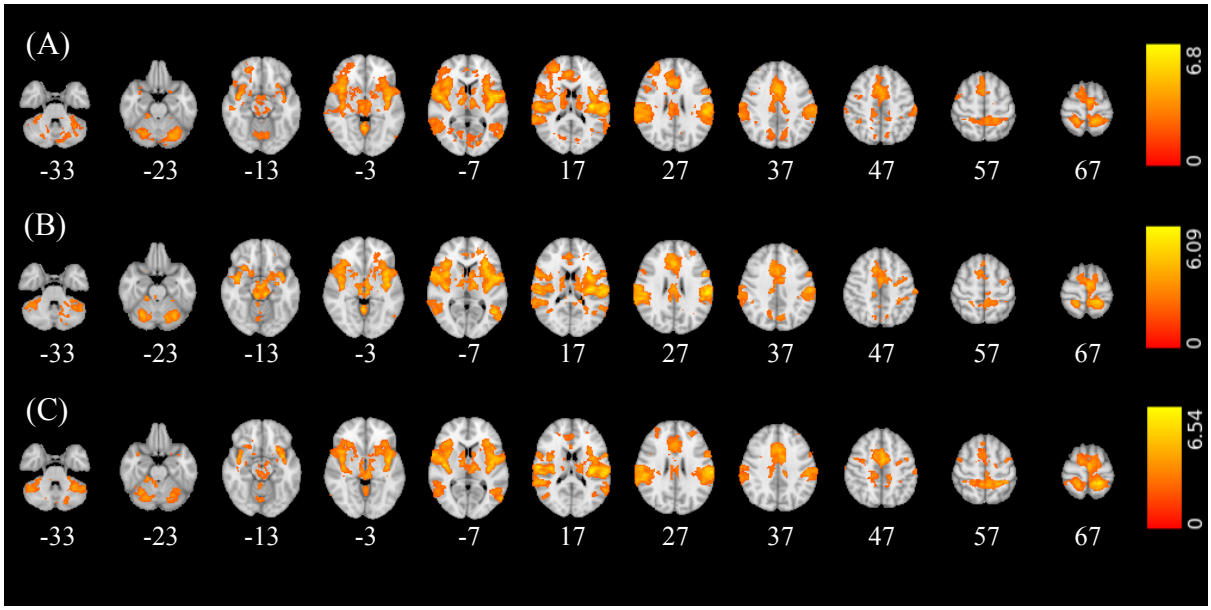


Figure 5.1: Group averaged activation maps showing the neural correlates of capsaicin-induced punctate hyperalgesia in the presence of (A) ABX-1431, (B) no dose and (C) placebo (n=16; mixed effects, Z>2.3, p<0.05). All images are in radiological convention. MNI Z-axis coordinates are shown underneath each slice. In all groups there was activation in key pain regions, including the insula, thalamus, basal ganglia, periaqueductal gray, rostral ventromedial medulla , anterior cingulate cortex, and primary and secondary somatosensory cortices.

5.3.2 The effect of the intervention on punctate-induced neural activation

Whole-brain paired t-tests were performed comparing the active drug and placebo with no treatment, as well as with each other.

5.3.2.1 ABX-1431 vs. no dose

There were no regions in which ABX-1431 suppressed stimulus-related BOLD activity significantly compared to no dose. However, when compared to no dose, ABX-1431 was associated with greater activity in the right dlPFC and the right lateral occipital cortex during a noxious stimulus (

Figure 5.2). See appendix F for the complete activation map for the ABX-1431>no dose contrast.

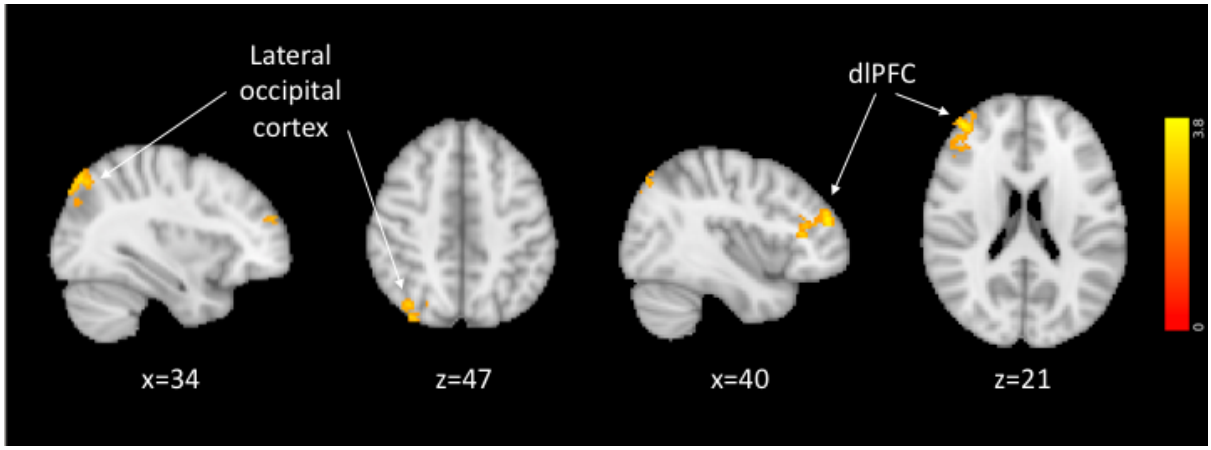


Figure 5.2: Paired t-test comparing punctate-related BOLD activation following dosing with ABX-1431 and no dose. Brain regions in which activation was greater after ABX-1431 are shown (n=16; mixed effects; Z>2.3, p<0.05; cluster corrected). Images are in radiological convention. Slices are identified by MNI coordinates. Activity in the right dorsolateral prefrontal cortex (dlPFC) and lateral occipital cortex was greater after ABX-1431 than after no dose.

5.3.2.2 Placebo vs. no dose

In the presence of placebo there was significantly reduced BOLD activity in the left angular gyrus during a noxious stimulus compared to no dose (Figure 5.3). There were no significant whole brain differences in the placebo>no dose contrast. See appendix F for the complete activation map for the no dose>placebo contrast.

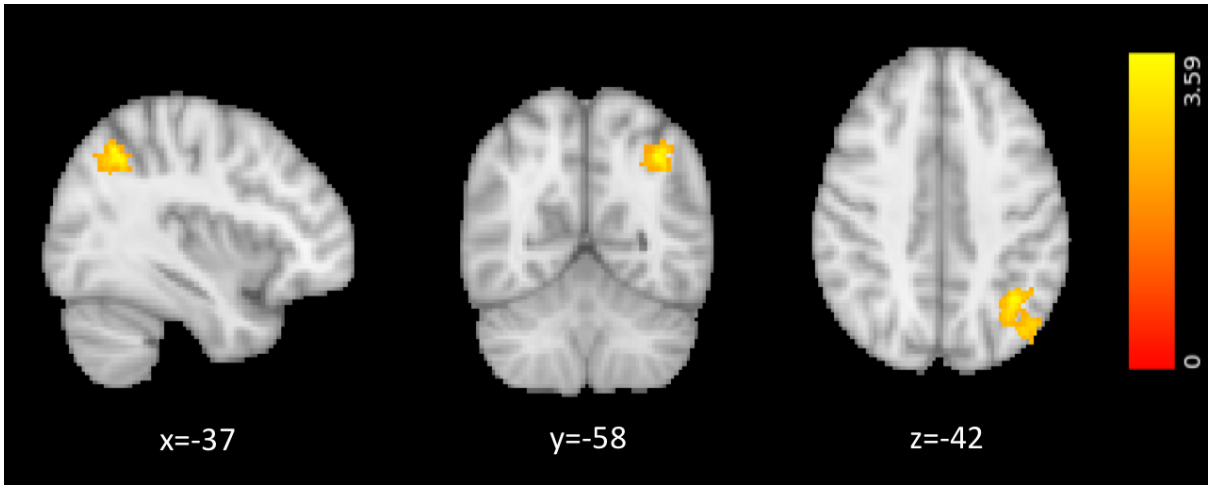


Figure 5.3: Results of the no dose>placebo contrast in a paired t-test comparing punctate-related BOLD activation following no dose and placebo (n=16; mixed effects; Z>2.3, 0<0.05; cluster corrected). Images are in radiological convention. Slices are identified by MNI coordinates. Activity in the left angular gyrus was suppressed after placebo.

5.3.2.3 ABX-1431 vs. placebo

ABX-1431 significantly increased stimulus-related BOLD activity in the right angular gyrus, right lateral occipital cortex and right frontal pole compared to placebo (Figure 5.4). Right frontal pole activity was predominantly lateral PFC (both dorsolateral and ventrolateral) activity. None of the suprathreshold regions in this contrast matched those in the ABX-1431>no dose contrast above. There were no significant whole brain differences in the placebo>ABX-1431 contrast. See appendix F for the complete activation map for the ABX-1431>placebo contrast.

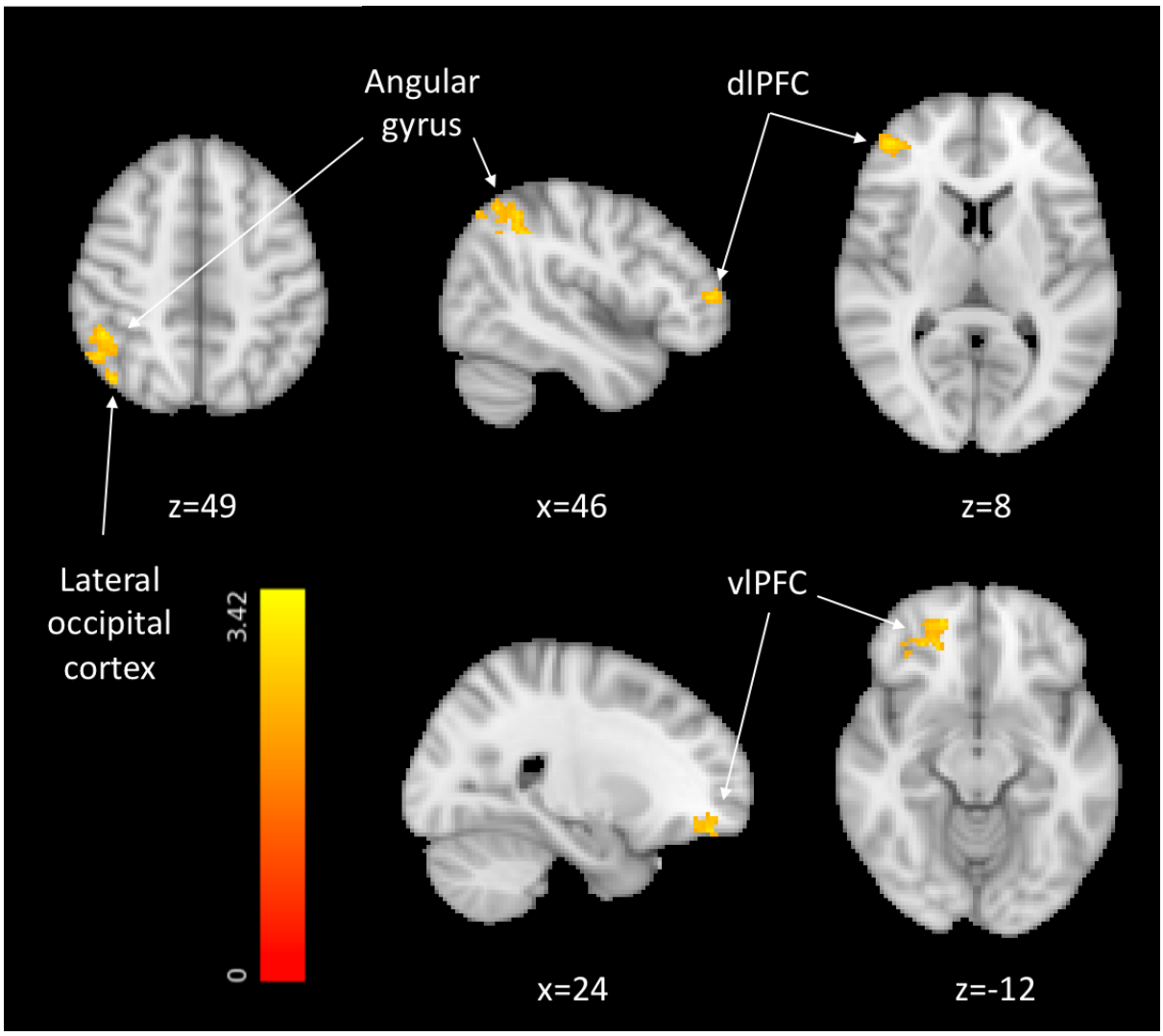


Figure 5.4: The ABX-1431>placebo contrast in a paired t-test comparing punctate-related BOLD activation following active drug and placebo (n=16; mixed effects; Z>2.3, p<0.05; cluster corrected). Images are in radiological convention. Slices are identified by MNI coordinates. ABX-1431 led to greater activity in the right dorsolateral prefrontal cortex (dlPFC), right ventrolateral prefrontal cortex (vlPFC), right angular gyrus and right lateral occipital cortex.

5.3.3 Examining the effect of ABX-1431 on neural responses to punctate hyperalgesia in the right amygdala

A region of interest analysis was performed for the right amygdala. As for the whole brain analysis, paired t-tests were performed comparing the active drug and placebo with no treatment, as well as with each other. No differences were seen in BOLD responses to punctate hyperalgesia across the three interventions.

5.3.4 Investigating the effect of peripheral drug levels on evoked responses.

For the ABX-1431 intervention, peripheral drug concentration was added as a regressor of interest to the group mean BOLD response to capsaicin-induced punctate hyperalgesia. (See appendix D-1.2 for an explanation of how peripheral drug concentration was calculated). A negative correlation was found between drug concentration and activity in parts of the precuneus, posterior cingulate cortex (PCC), posterior MCC, right dorsomedial PFC, right ventromedial PFC, right middle and inferior temporal gyri, right angular gyrus and right lateral occipital cortex (Figure 5.5A).

Despite this apparent dose-related suppression of stimulus-related activity, when the t-tests in section 0 were repeated with peripheral active moiety level as a regressor of no interest, there was no evidence that ABX-1431 reduced stimulus-related activity compared to either of the other interventions. ABX-1431 continued to cause increased stimulus-related activity in some regions compared to placebo and no dose, and controlling for drug concentration had minimal impact on this (Figure 5.5B&C). There was no overlap between the areas in which the ABX-1431>placebo or ABX-1431>no dose contrasts were significant and the areas in which activity in the ABX-1431 group was correlated with drug concentration.

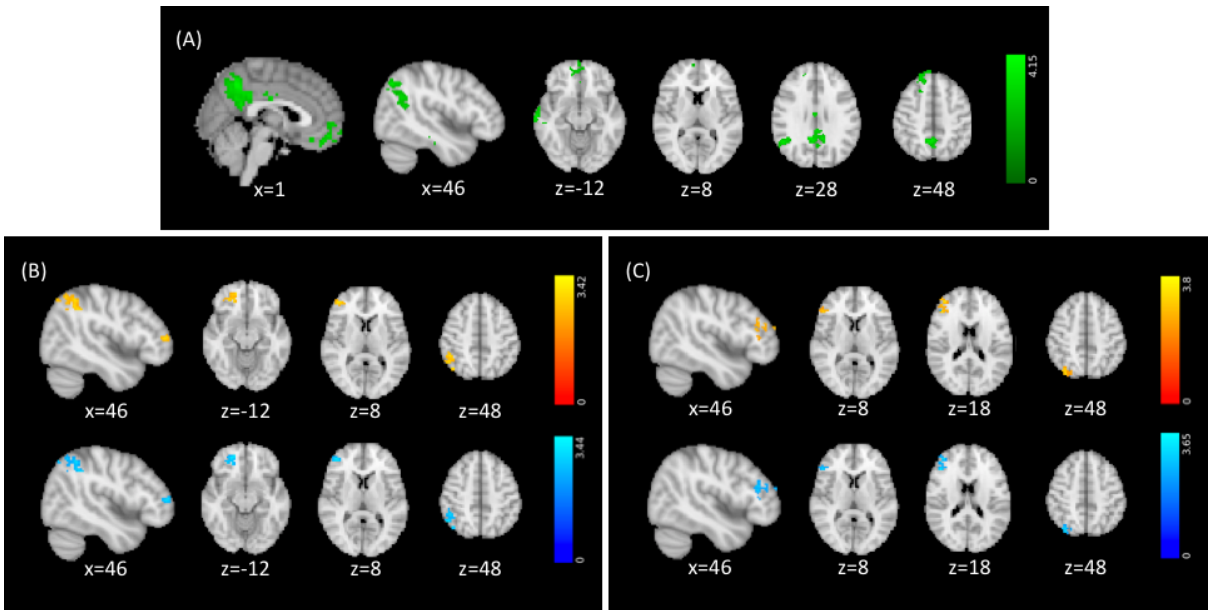


Figure 5.5: The effect of peripheral drug concentration on the neural response to a noxious stimulus. Panel A shows clusters in which there was a significant negative correlation between peripheral drug level and the BOLD response to capsaicin-induced punctate hyperalgesia. Panels B and C show regions in which a paired t-test shows that the stimulus-induced BOLD response is significantly greater after ABX-1431 dosing than after placebo (B) and no dose (C) when peripheral drug concentration is controlled for as a regressor of no interest (blue) and when it is not (red-yellow). Despite the apparent dose-related suppression seen in panel A, controlling for dose had minimal impact on the stimulus-related activity that is specific to ABX-1431. All group analyses used a mixed effects model ($Z>2.3$, $p<0.05$; cluster corrected; $n=16$). All images are in radiological convention. Slices are identified by MNI coordinates.

5.3.5 Investigating the effect of expectation on evoked responses

For the ABX-1431 and placebo interventions, expectation of pain relief was added as a regressor of interest to the group mean BOLD response to capsaicin-induced punctate hyperalgesia. For ABX-1431, a positive correlation was found between expectation and activity in the visual cortex (Figure 5.6A). For placebo, a positive correlation was found between expectation and the right angular gyrus, left lateral occipital cortex, right ventrolateral PFC (vlPFC) and right temporal pole (Figure 5.6B).

To ensure that the comparisons between interventions were not affected by participants' expectations, the t-tests in 1.3.2. were repeated with expectation of pain relief as a regressor of no interest. The ABX-1431 vs. no dose contrasts produced almost identical activation maps to those produced without the added regressor, with no additional regions showing intervention-related differences in stimulus-related activity. The same was true of the placebo>ABX-1431 contrast.

In the ABX-1431>placebo contrast, controlling for expectation removed activity in the right dlPFC (Figure 5.6C).

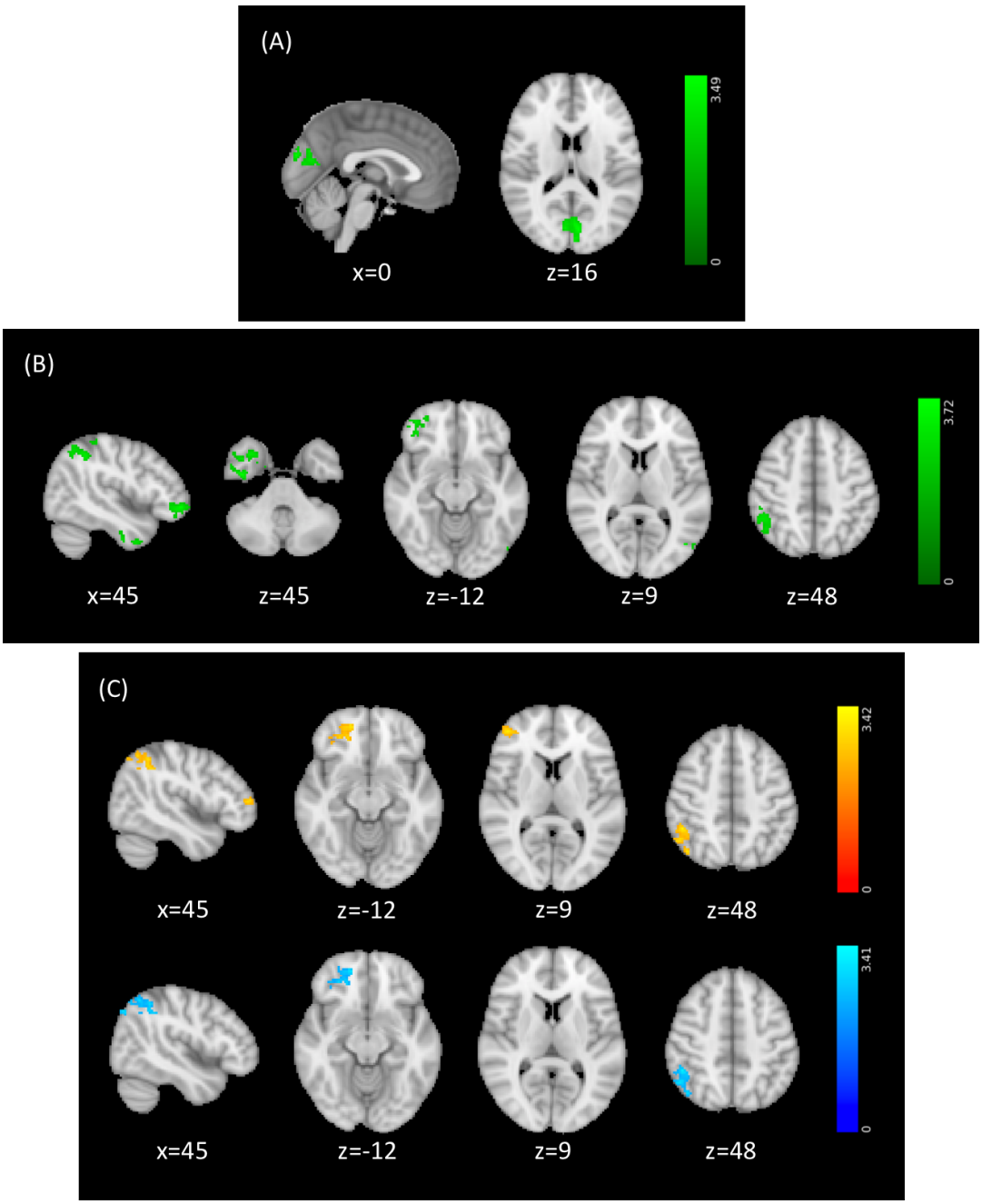


Figure 5.6: The effect of expectation on the neural response to a noxious stimulus. Panel A and B show clusters in which there was a significant positive correlation between expectation of pain relief and the BOLD response to capsaicin-induced punctate hyperalgesia after ABX-1431 (A) and placebo (B). Panel C shows regions in which a paired t-test shows that the stimulus-induced BOLD response is significantly greater after ABX-1431 dosing than after placebo when expectation is controlled for (blue) and when it is not (red-yellow). The neural activity associated with expectation of analgesia is different in the active drug group compared to the placebo group. Controlling for expectation eliminated the dorsolateral prefrontal cortex (dlPFC) activity seen in the ABX-1431>placebo contrast. Group analyses used a mixed effects model (Z>2.3, p<0.05; cluster corrected; n=16). All images are in radiological convention. Slices are identified by MNI coordinates.

5.3.6 The effect of the intervention on neural responses to a visual stimulus

Both the ABX-1431 and the placebo interventions were associated with modulation of the neural responses to a visual stimulus. However, there was no effect of ABX-1431 on the primary visual cortex compared to placebo or no dose, indicating that drug-related findings do not simply reflect a generalised drug-induced increase in blood flow. Furthermore, the effects of ABX-1431 on responses to the visual stimulus and the pain stimuli were dissociable. This indicates that the effects of ABX-1431 on pain-induced responses are modality specific. These results are described and discussed in full in appendix E.

5.3.7 The effect of the intervention on resting state connectivity during tonic pain

The right amygdala was used as a seed region for BOLD functional connectivity analysis during tonic capsaicin-induced pain.

5.3.7.1 Whole brain analyses

Three paired t-tests were performed in order to determine the effect of the three interventions on whole brain connectivity with the right amygdala. ABX-1431 was compared to both placebo and no dose, and placebo and no dose were compared with one another.

For the placebo>ABX-1431 contrast, there was increased connectivity between the right amygdala and parts of the right vlPFC (Figure 5.7). This may reflect suppression by the active drug or increased connectivity due to placebo. No differences were seen in the ABX-1431>placebo contrast, nor in the other t-tests. See appendix F for the complete activation map for the placebo>ABX-1431 contrast.

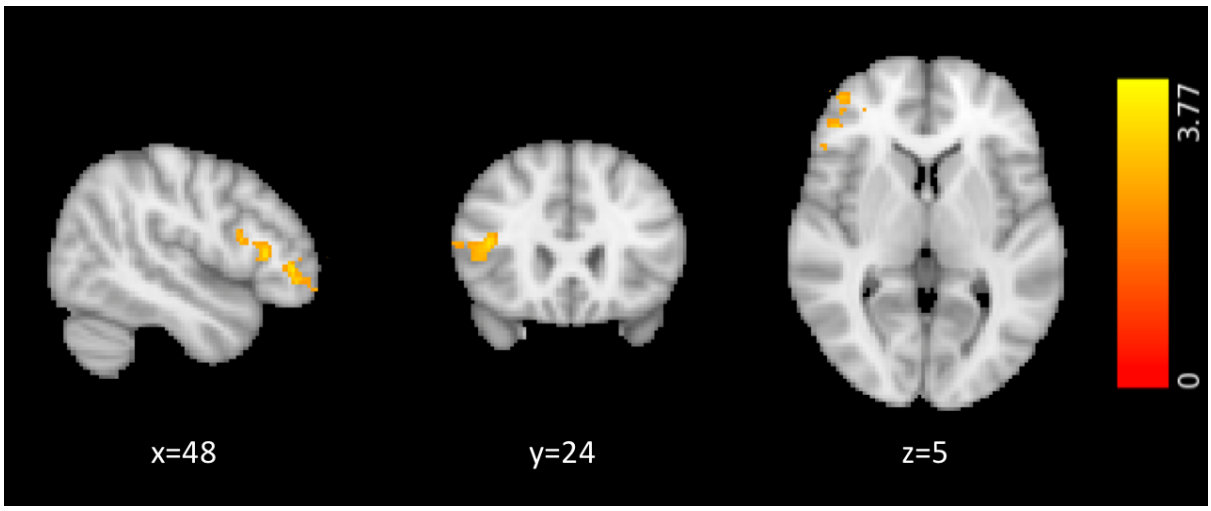


Figure 5.7: Comparing the effect of ABX-1431 and placebo on whole brain connectivity with the right amygdala. Activation map shows areas that survive the placebo>ABX-1431 contrast in a paired t-test (n=16; mixed effects; Z>2.3, p<0.05; cluster corrected). Activity in the right ventrolateral prefrontal cortex (vlPFC) may represent increased connectivity due to placebo or reduced connectivity due to active drug. Images are in radiological convention and slices are identified by MNI coordinates.

5.3.7.2 Region of interest analyses

For each of the t-tests above, ROI analyses were performed to investigate the connectivity of the right amygdala with the left dpINS, the rACC, the left and right SI, and the aMCC.

Compared to no dose, ABX-1431 led to significantly reduced connectivity between the right amygdala and the left SI, and between the right amygdala and the left dpINS (Figure 5.8). Connectivity between the right amygdala and right SI was not significantly affected by the intervention.

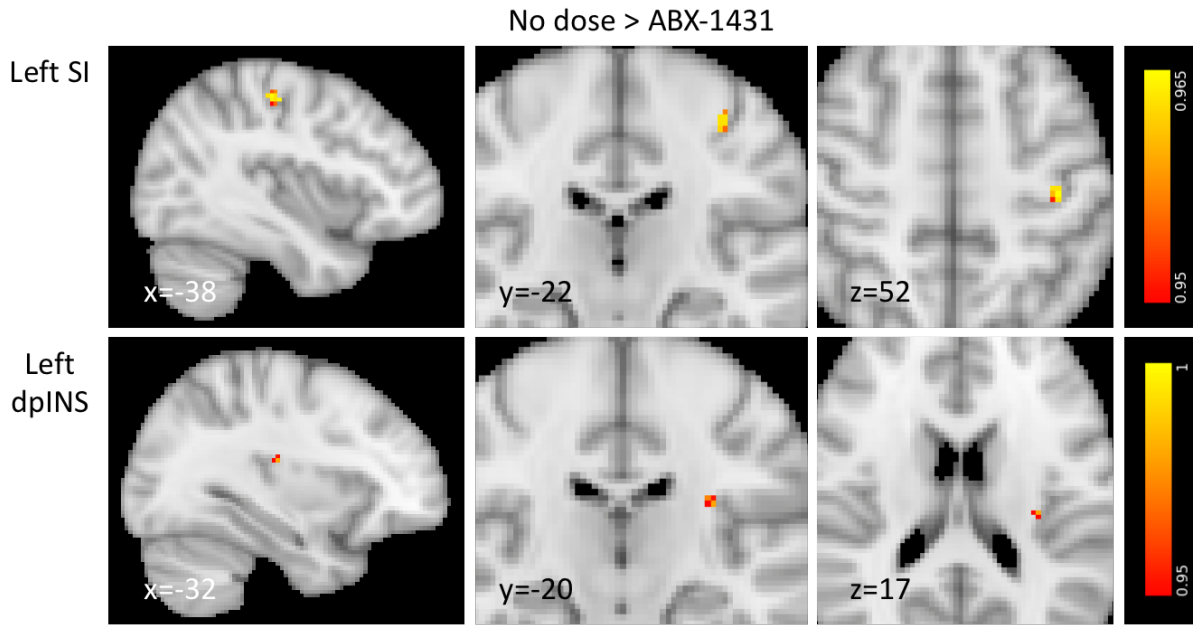


Figure 5.8: Region of interest analysis of functional connectivity in the no dose>ABX-1431 contrast of a paired t-test (n=16; 5000 permutations; TFCE, FWE-corrected $p<0.05$). Activation maps show the areas in which ABX-1431 caused reduced connectivity between the right amygdala and the left primary somatosensory cortex (SI; top row) and the left dorsal posterior insula (dpINS; bottom row). Images are in radiological convention and slices are identified with MNI coordinates.

In the ABX-1431>placebo contrast there was increased connectivity between the right amygdala and the aMCC (Figure 5.9). In the placebo arm there was significantly more connectivity between the right amygdala and the rACC than in both the ABX-1431 arm and the no dose arm, and the same part of the rACC was identified in both contrasts (Figure 5.9).

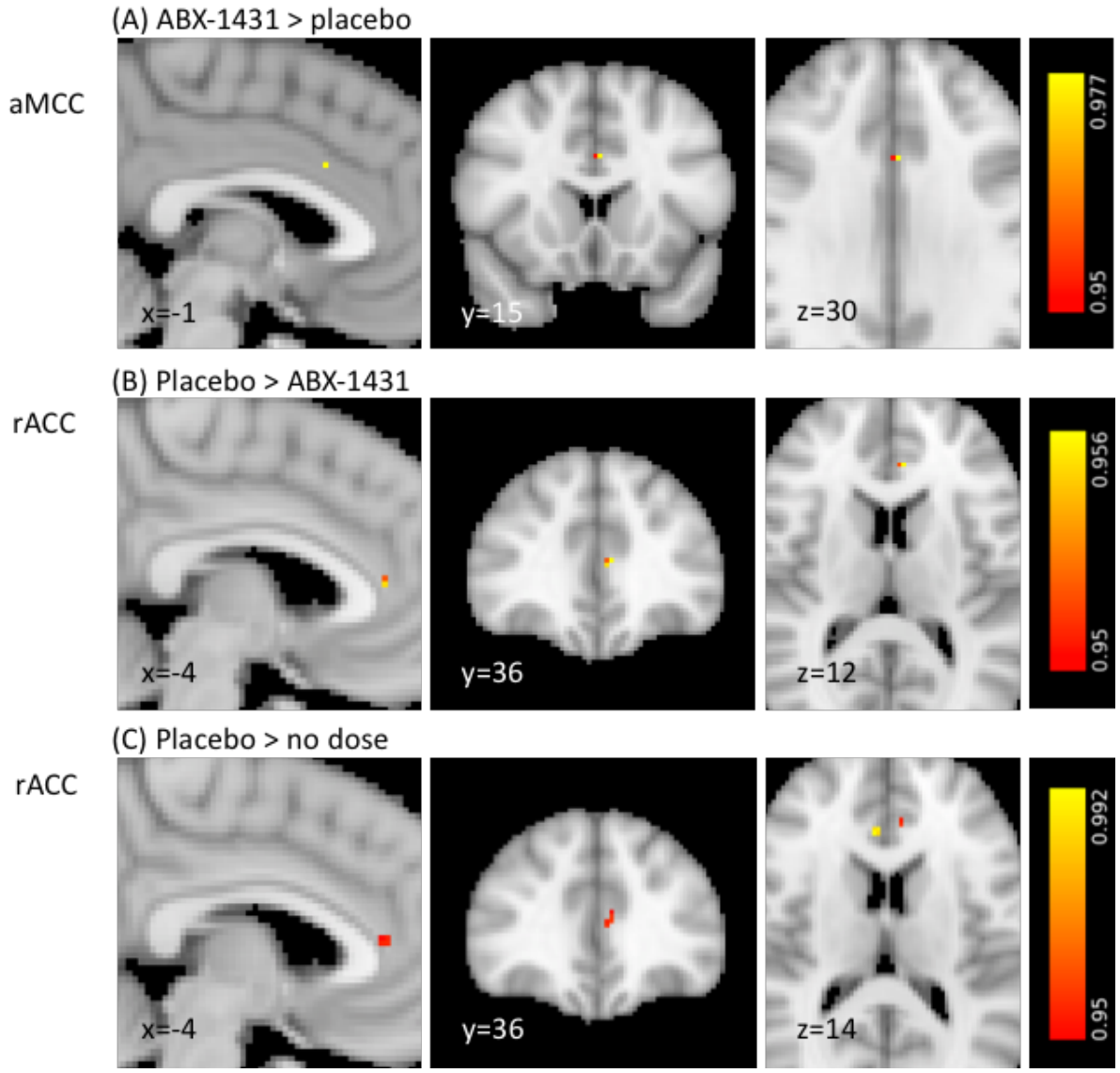


Figure 5.9: The effect of intervention on the functional connectivity between the right amygdala and the rostral anterior cingulate cortex (rACC) and anterior midcingulate cortex (aMCC) regions of interest. Activation maps show the statistically significant results from paired t-tests comparing ABX-1431 with placebo and no dose, and placebo with no dose (n=16; 5000 permutations; TFCE, familywise error corrected p<0.05). In the ABX-1431>placebo contrast (A), there was increased connectivity between the right amygdala and the aMCC. This may be due to either increased connectivity following the active drug, or reduced connectivity following placebo. Compared to both ABX-1431 (B) and no dose (C), placebo caused increased connectivity between the right amygdala and the rACC. Images are in radiological convention and slices are identified with MNI coordinates.

5.3.8 The effect of the intervention on blood flow during tonic pain

ASL data was acquired during tonic capsaicin-induced pain. Whole brain analysis was performed for 15 of the 16 participants. One participant was excluded from this analysis due to technical issues which led to the incomplete acquisition of one of their ASL scans.

There were no regions in which activity in the placebo arm differed significantly to activity in the no dose arm. Neither were there any regions in which activity in the ABX-1431 arm was suppressed compared to placebo or no dose. However, there were multiple pain-related regions in which there was more activity after ABX-1431 than the other interventions.

When active drug was compared to placebo, regions in which the active drug arm produced more activity than the placebo arm included the rACC, aMCC, PCC, precuneus, PFC, orbitofrontal cortex (OFC), thalamus, posterior insulae, basal ganglia and amygdalae (Figure 5.10). When active drug was compared to no dose, regions in which the active drug arm produced more activity than the no dose arm included the rACC, aMCC, PCC, precuneus, PFC, OFC, anterior and posterior insulae, thalamus and pontine reticular formation (Figure 5.11).

See appendix F for the complete activation maps for the ABX-1431>placebo and ABX-1431>no dose contrasts.

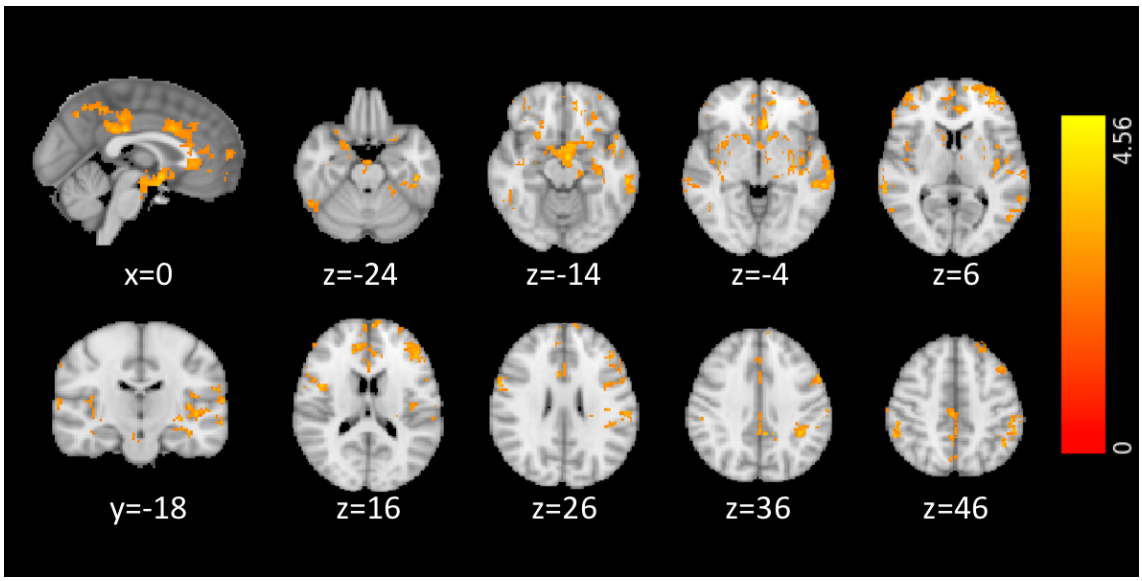


Figure 5.10: Whole brain ASL activity in the ABX-1431>placebo contrast of a paired t-test (n=15; Z>2.3, p<0.05; cluster corrected). Activation map shows the areas in which there was more activity for ABX-1431 than for placebo. ABX-1431 led to greater activity in the rostral anterior cingulate, anterior midcingulate, posterior cingulate, precuneus, thalamus, posterior insulae, basal ganglia, amygdalae and prefrontal cortices. Images are in radiological convention and slices are identified with MNI coordinates.

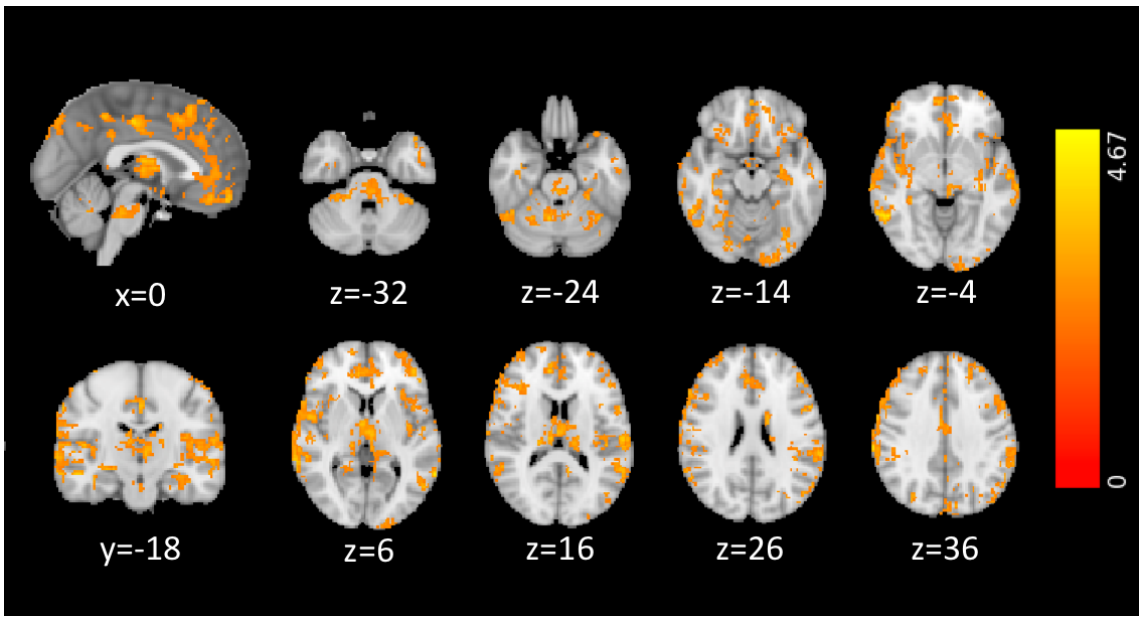


Figure 5.11: Whole brain ASL activity in the ABX-1431>no dose contrast of a paired t-test (n=15; Z>2.3, p<0.05; cluster corrected). Activation map shows the areas in which there was more activity for ABX-1431 than for no dose. ABX-1431 led to greater activation in the rostral anterior cingulate, anterior midcingulate, posterior cingulate, precuneus, thalamus, anterior insulae, posterior insulae, prefrontal cortices and pontine reticular formation. Images are in radiological convention and slices are identified with MNI coordinates.

5.3.9 Investigating the effect of peripheral drug levels on blood flow during tonic pain

For the ABX-1431 intervention, peripheral drug concentration was added as a regressor of interest to the group mean ASL activity. A positive correlation was found between peripheral drug concentration and parts of the precuneus, PCC, right middle temporal gyrus, right posterior insula (pINS), right SI and right MI (Figure 5.12). Despite this dose-response effect, minimal difference was seen in regions of altered blood flow when the contrasts in section 5.3.8 were repeated while controlling for peripheral concentration of the active moiety.

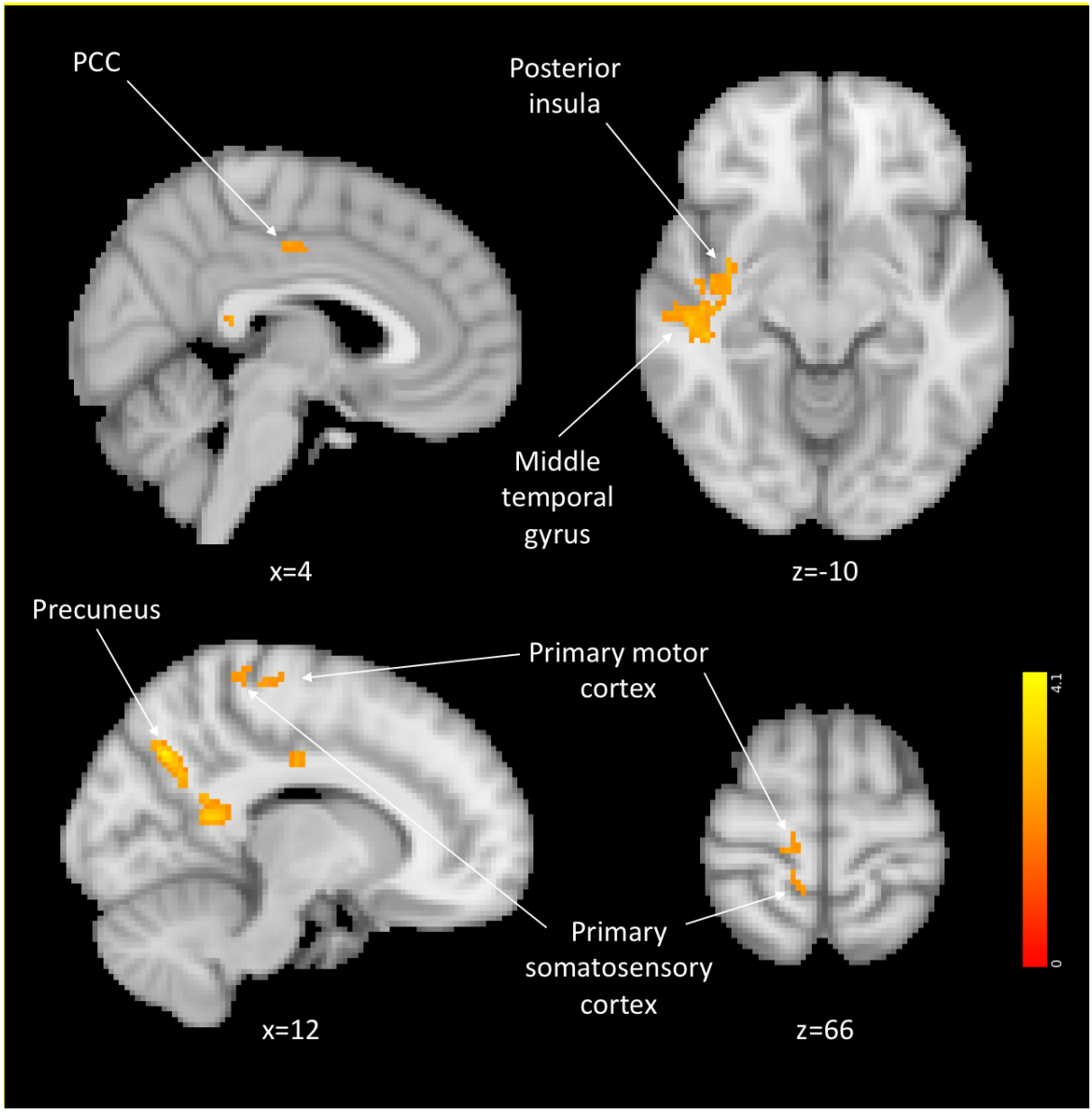


Figure 5.12: Activation maps show regions in which, following the administration of ABX-1431, mean whole brain ASL activity has a positive correlation with peripheral drug concentration (n=15; Z>2.3, p<0.05; cluster-corrected). Increased drug concentration was associated with increased blood flow in parts of the precuneus, posterior cingulate cortex (PCC), right middle temporal gyrus, right primary somatosensory cortex, right primary motor cortex and right posterior insula. Images are in radiological convention and slices are identified with MNI coordinates.

5.4 Discussion

The aims of this chapter were threefold. Firstly to determine whether ABX-1431 had a pharmacodynamic effect on neural activity, and whether there was suitable target engagement. Secondly to assess whether, using functional neuroimaging as a biomarker for pain relief, there was evidence of analgesic efficacy. The final aim was to use neuroimaging to give insight into the mechanisms of ABX-1431.

The presence of a dose-response effect on neural activation shows that ABX-1431 did produce a pharmacodynamic effect. Data has previously shown that a wide range of analgesic agents (including opioids, non-steroidal anti-inflammatories, pregabalin and gabapentin; reuptake inhibitors were not assessed) can be identified by functional neuroimaging as they produce attenuation of pain responses in pain-related regions. These regions include the insula, cingulate and sensory cortices (Duff et al., 2015). It was hypothesised that ABX-1431 would follow this pattern. However, ABX-1431 did not attenuate activity in classical pain regions during an evoked stimulus. Therefore it was not possible to prove analgesic efficacy. Instead, ABX-1431 led to increased frontal activity during both evoked and tonic pain, as well as altered resting functional connectivity between cortical and subcortical regions that are implicated in descending nociceptive control. It can be concluded from this that it is unlikely to function in the same way as the analgesic agents imaged previously, but may still manipulate the control of top-down modulatory processes. Altered frontal activity may lead to downstream modulation of the relationship between regions responsible for affective and sensory aspects of pain. These findings are discussed in further detail below.

Although cannabinoid receptors are present throughout the central nervous system, the effects of ABX-1431 are modality specific. This was demonstrated by the dissociable effects of noxious and visual stimuli. A proposed advantage of a drug such as ABX-1431 that inhibits endocannabinoid degradation is that it will have a greater impact on areas with high endocannabinoid turnover (Woodhams et al., 2017), a feature seen in pain-related regions following injury (Sagar et al., 2012). This may explain the discrete effects of ABX-1431 that were seen, despite the widespread presence of its target.

5.4.1 The presence of a dose-response effect during evoked and tonic pain

After dosing with ABX-1431, the peripheral concentration of active moiety was very variable between participants (see appendix D-1.2). This may be due to differences in drug absorption or metabolism. Increased peripheral drug concentration was associated with reduced BOLD activation in multiple regions during evoked pain, indicating the possibility of a dose-response effect. There was also a dose-response effect in the ASL data, in which increased peripheral drug concentration was associated with increased cerebral blood flow (CBF) in multiple regions during tonic pain. Importantly, these correlations confirm that at least some of the active moiety does cross the blood-brain barrier, producing a pharmacodynamic effect. The regions that were suppressed were not those that were identified in previous studies of effective analgesics from other classes (Duff et al., 2015), meaning that target engagement cannot be confirmed from this data. However, the mechanisms of this drug may differ from those of other drugs previously investigated in a similar way. Therefore this does not exclude the possibility of efficacy through CB1 engagement in other regions.

In order to determine whether variability in drug concentration led to reduced power to detect drug-induced changes in neural activation, peripheral concentration of the active moiety was added to both the evoked pain model and the ASL analysis as a regressor of no interest. The fact that this did not alter the findings in any meaningful way suggests that the dose-response relationship is relatively weak compared to other sources of variance. In a small sample, the presence of other sources of variance may have limited the power of neuroimaging to detect the group effects of ABX-1431. The findings are also consistent with the behavioural data (see chapter 4), in which peripheral drug concentration had no consistent effect on pain ratings, mood or sedation. The presence of a dose-response relationship in the neuroimaging data but not in the behavioural data may reflect the fact that the subjective nature of behavioural data introduces more additional variance. In future studies of ABX-1431 it will be crucial to refine the formulation in order to provide a more standardised drug concentration throughout the population.

5.4.2 The effect of ABX-1431 on neural activity in the thalamus and posterior insula

Compared to placebo and no dose, ABX-1431 was associated with increased blood flow in the thalamus and pINS during tonic pain. The thalamus is the main relay point between spinal input and cortical structures, and is therefore an important region for both ascending nociception and descending control. The pINS is also a key sensory region for nociception (Groh, Mease, & Krieger, 2017; Moayedi, 2014). Therefore the modulation of these regions by ABX-1431 is in keeping with the idea that it acts on nociceptive processing. It might be expected that an analgesic agent would attenuate activity in the pINS rather than increase it, particularly since the dorsal part of the pINS has been shown to track pain intensity (Segerdahl et al., 2015). However, preclinical

data has shown that CB1 receptor activation in the insula via exogenous agonists or via fatty acid amide hydrolase (FAAH) inhibition can produce analgesia in models of neuropathic pain, (Jee Kim, Tanioka, Woo Um, Hong, & Hwan Lee, 2018; N. Wang, Zhang, Wang, & Luo, 2021), as can electrical stimulation of the pINS at 60Hz (Dimov et al., 2018; Goncalves et al., 2022). This antinociception is reversed by either opioid receptor antagonism or by CB1 receptor antagonism (Dimov et al., 2018; Goncalves et al., 2022; N. Wang et al., 2021), indicating that the analgesia is mediated by both endogenous opioidergic and cannabinoid systems. In light of this evidence, the increased pINS activity seen after treatment with ABX-1431 is consistent with a CB1 mediated mechanism of analgesia.

5.4.3 The effect of ABX-1431 on neural activity in the frontal cortex

During a tonic painful stimulus, ABX-1431 produced increased ASL activity in multiple regions compared to both placebo and no dose. Although some of the regions seen in the ABX-1431>placebo contrast might relate to placebo-induced suppression, where activity also survives in the ABX-1431>no dose contrast this is likely to be a drug effect. Regions in which ABX-1431 produced increased CBF in both contrasts included the rACC, aMCC and multiple subdivisions of the PFC.

5.4.3.1 Modulation of activity in the lateral prefrontal cortex

ABX-1431 was associated with increased activity in the right dlPFC when compared with placebo or no-dose during tonic capsaicin-induced pain, and also in response to capsaicin-induced punctate hyperalgesia. During tonic pain, ABX-1431 also produced increased activity in the right vlPFC compared to both placebo and no-dose. Right vlPFC activity was greater for ABX-1431 than for placebo during punctate

hyperalgesia. There was no significant difference in vlPFC response when comparing ABX-1431 with no-dose. However, there was also no difference between placebo and no dose, making it unlikely that this is simply a result of placebo-induced suppression.

As outlined in chapter 1, the dlPFC and vlPFC are both involved in the top-down cognitive modulation of nociceptive input. They are well connected to one another, as well as to other parts of the descending modulatory system, and they are likely to interact with one another in order to exert descending control (Katja Wiech, Ploner, et al., 2008).

Lorenz et al. (2003) found that in humans, increased dlPFC activity was associated with reduced pain unpleasantness and reduced coupling between the thalamus and midbrain and between the midbrain and rACC, indicating that modulation of the functional connectivity between these regions may be a mechanism by which affective responses to pain are mediated. A pilot study showed that transcranial direct current stimulation of the dlPFC modulates the functional connectivity of the thalamus to both sensory and affective networks, and that this modulation is greater in individuals with high pain tolerance (Sankarasubramanian et al., 2017). ABX-1431 may act in a similar way, engaging the dlPFC and thereby altering downstream connectivity between other supraspinal regions involved in pain perception. Preclinical data support this hypothesis. In rodents, altered circuitry is seen between the forebrain and the amygdala in pain states, and this is mediated by 2-arachidonoyl glycerol (2-AG) acting on presynaptic CB1 receptors in the mPFC, a functional homologue of the human dlPFC (Huang et al., 2019; Woodhams et al., 2017). Increased cannabinoid activity in this system leads to increased descending inhibition via the rostral ventromedial

medulla (RVM) and locus coeruleus (LC), as well as inhibition of output from the amygdala (Huang et al., 2019; G. Ji & Neugebauer, 2014; Kiritoshi et al., 2016). More details of these preclinical findings are given in section 1.2.5.

The vlPFC shows increased engagement during pain modulation that is related to beliefs about the pain, such as expectations and perceived control, and is thought to have a role in the reappraisal of pain (Katja Wiech et al., 2014). This is in keeping with the fact that right vlPFC activity correlated with expectation of pain relief following ABX-1431. However, given that ABX-1431 was associated with increased right vlPFC activity compared to placebo, another high expectancy condition, but not compared to no dose, it is not clear that expectation explains all of the drug-related activity in this region. It is likely that as for the dlPFC, ABX-1431 generates increased activity in the vlPFC, and that these two regions work in parallel or in sequence to manipulate downstream pain regions, including the amygdala, which is discussed in more detail below (section 5.4.4).

5.4.3.2 Assessing the effect of expectation on dlPFC activity

As mentioned above, ABX-1431 produced more right dlPFC activity than both placebo and no dose during a noxious stimulus, though the activity was in a slightly different region of the dlPFC in the two contrasts.

The dlPFC is a region known to be associated with positive expectancy of analgesia (Ulrike Bingel et al., 2011), but it is also an area rich in CB1 receptors (Bloomfield et al., 2019). Controlling for expectation removed the effect of ABX-1431 on the dlPFC compared to placebo, but not compared to no dose. However, the effect of expectation

on neural responses during the two dosing visits was not equal. During the visit in which active drug was received, greater expectation of analgesia was associated with increased stimulus-related activity in the visual cortex, whereas for the placebo visit, increased expectation correlated with greater activity in the right angular gyrus, left lateral occipital cortex, right vlPFC and right temporal pole.

This differential activity suggests that the neural mechanisms engaged by expectation are different in the two dosing conditions, meaning that additivity cannot be assumed. Therefore, subtracting the placebo effect from the drug effect may remove activity inappropriately, and this could be a reason for the for the preservation of dlPFC activity in the ABX-1431>no dose contrast despite the absence of activity in the ABX-1431>placebo contrast once the effect of expectation was accounted for.

5.4.3.3 Modulation of activity in the anterior cingulate and midcingulate cortex

When compared to both placebo and no dose, ABX-1431 was associated with increased CBF in the rACC and aMCC in the presence of tonic pain.

The cingulate cortex is rich in CB1 receptors (Bloomfield et al., 2019). The aMCC has been shown to have an important role in the affective response to pain (Kulkarni et al., 2005; M. C. Lee et al., 2013; Vogt, 2005), and is consistently activated by experiments eliciting a combination of negative affect, pain and cognitive control (Shackman et al., 2011). It has been implicated in both THC-induced analgesia and placebo analgesia (M. C. Lee et al., 2013; Weizman et al., 2018), as well as in opioid analgesia (Watson et al., 2009), though pain relief in each has generally been associated with suppressed rather than increased stimulus-related activity (Ashar, Chang, & Wager, 2017; M. C.

Lee et al., 2013). However, these were all studies in which activity was measured during a transient noxious stimulus. Watson et al. (2009) examined activity in the aMCC during both the anticipatory and the nociceptive phase of placebo analgesia. They found that although aMCC activity was suppressed during nociception, it was increased during the anticipation of analgesia. They hypothesised that the role of the aMCC may be to act as the link between anticipation and nociception, maintaining the brain responses required to reduce the affective experience of pain. The increased aMCC activity with ABX-1431 was identified during a tonic, relatively low intensity noxious stimulus, after which participants knew that they would undergo a more unpleasant evoked pain task. Therefore it may be that tonic pain and transient evoked pain modulate aMCC function differently, or that this finding represents alterations during the anticipatory phase of analgesia.

The rACC is implicated in studies eliciting both pain and negative affect (Shackman et al., 2011), and it is thought to be an important region for affective aspects of pain processing (Vogt, 2005). Like the aMCC, increased rACC activity is linked to increased placebo analgesia, and it is thought that it may be responsible for recruiting subcortical structures in endogenous pain control, including the amygdala, LC and RVM (U. Bingel et al., 2006). Its role in placebo analgesia is discussed further in section 5.4.4.4. In rats, injection of an exogenous cannabinoid into the rACC leads to a reduction in behavioural proxies of both affective and sensory aspects of the acute pain experience (Genaro et al., 2017). Therefore the finding that ABX-1431 leads to increased rACC activity during tonic pain is in keeping with the theory that ABX-1431 may manipulate top-down processing of noxious stimuli.

5.4.4 Functional connectivity of the right amygdala during tonic pain

The amygdala has a high density of CB1 receptors (Bloomfield et al., 2019), and is known to be an important region for the descending control of pain, in which its role is thought to be related to determining the emotional significance of a stimulus and modulating its perception and cognitive effects accordingly (Veinante et al., 2013). During tonic pain, ABX-1431 was associated with altered connectivity between the right amygdala and subcortical regions implicated in the sensory aspects of pain. There was also altered connectivity between the right amygdala and the vlPFC and aMCC, both of which are regions in which neural activity was modulated by ABX-1431.

Altered connectivity between the right amygdala and the rACC was a placebo effect that was not present in the drug arm, indicating a dissociation between the mechanisms of ABX-1431 and placebo.

5.4.4.1 Connectivity with sensory regions

It was predicted that ABX-1431 would alter the resting state functional connectivity between the right amygdala and both SI and the dpINS, and this was shown to be true. Connectivity between the right amygdala and the left SI was suppressed by ABX-1431 compared to no dose. The fact that this was not seen in the placebo vs. no dose comparison indicates that it is a true drug effect. This effect is in keeping with the findings of Lee and colleagues (M. C. Lee et al., 2013), who found that THC administration led to reduced functional connectivity between the right amygdala and left SI during ongoing capsaicin-induced pain. Connectivity between the right amygdala and the left dpINS was also suppressed by ABX-1431. Again, this is likely to be a true drug effect, since it was not replicated in the placebo arm. Both SI and the

left dpINS are regions that are thought to be important for encoding sensory aspects of pain, such as intensity (Ploner et al., 1999; Segerdahl et al., 2015; Uhelski et al., 2012). The amygdala is an important region for the affective component of pain perception (Veinante et al., 2013). Therefore, it appears that like THC (M. C. Lee et al., 2013), ABX-1431 may lead to reduced communication between affective and sensory regions during a tonic painful stimulus.

5.4.4.2 Connectivity with the vlPFC

As discussed above, the vlPFC, like the amygdala, is an important region in descending pain control. The vlPFC and amygdala are well connected with one another anatomically (Murray & Fellows, 2021; Ray & Zald, 2012), and the finding of altered connectivity between the right vlPFC and amygdala during tonic pain is in accordance with the fact that ABX-1431 is associated with altered vlPFC activity during both punctate hyperalgesia and tonic pain. Like the lateral PFC, the amygdala is a region implicated in emotional reappraisal (Ochsner & Gross, 2008). There is evidence that a cortical-subcortical interaction between the vlPFC and the amygdala may be particularly important in negative appraisal (Tor D. Wager et al., 2008). Therefore, it is conceivable that reduced connectivity between the vlPFC and amygdala following ABX-1431 administration may represent a shift away from the negative appraisal of tonic pain. This is in keeping with the hypothesis that ABX-1431 modulates frontal activity, which in turn modulates the coupling between subcortical sensory and affective regions.

5.4.4.3 Connectivity with the aMCC

Altered connectivity between the right amygdala and aMCC may have been due to reduced connectivity in the placebo arm, increased connectivity following ABX-1431 or a combination of the two. As mentioned in section 5.1.1, existing literature implicates the aMCC in THC-induced analgesia. This is true of both experimental and clinical pain states (M. C. Lee et al., 2013; Weizman et al., 2018). In healthy individuals, the effectiveness of the analgesia is predicted by the reduction in aMCC activity elicited by a painful stimulus (M. C. Lee et al., 2013). A study with patients with chronic neuropathic pain showed that THC led to reduced integration between the aMCC and the rest of the pain network, and that reduced functional connectivity between the aMCC and sensorimotor cortex (SII and MI) correlated with the reduction in pain ratings (Weizman et al., 2018). However, the aMCC is also a key region for placebo analgesia. Like THC, nociception during placebo is associated with reduced pain-related activity in the aMCC (Ashar et al., 2017; Watson et al., 2009). Meanwhile placebo anticipation is associated with increased aMCC activity (Watson et al., 2009).

5.4.4.4 Placebo effects on connectivity with the rACC

In the placebo arm, there was increased connectivity between the right amygdala and the rACC compared to both the active drug arm and the no treatment arm. This effect was not seen in the active drug arm when compared to no treatment. Therefore this appears to be a placebo-specific effect. As outlined in section 5.4.3.3 above, the rACC is likely to have a role in cannabinoid-induced analgesia. However, the rACC is also known to be an important region for placebo analgesia, during which it shows increased activity. (U. Bingel et al., 2006; Ulrike Bingel et al., 2011; Petrovic et al., 2002). During placebo analgesia, rACC activity has been found to correlate with that

in the bilateral amygdalae, periaqueductal gray and RVM, indicating a possible role for the rACC in recruiting these subcortical structures in endogenous pain control (U. Bingel et al., 2006; Eippert et al., 2009; Frisaldi et al., 2020; Tracey, 2010; see sections 1.2.4.1.4.1 and 1.2.4.3 for more information on the rACC and endogenous pain control). The amygdala is an important component of the descending inhibitory system and is also known to be an important region in placebo analgesia (Tracey, 2010). The findings of altered rACC-amygdala connectivity during placebo are therefore in keeping with existing literature and may reflect cortical control over descending pain inhibition.

5.4.4.5 Implications for differences in drug and placebo effects on connectivity

The fact that the active drug and placebo have different and dissociable effects on resting state connectivity suggests that they act via different mechanisms, and therefore additivity should not be assumed. This translates to behavioural measures, in which subtraction of the placebo effect from the drug effect may lead to underestimation of the true drug effect. Should ABX-1431 proceed to phase II behavioural trials it will be important to take this into account.

5.4.5 Limitations of the study

Some of the limitations of this study have already been discussed in chapter 4. With respect to the imaging, the main limitations are the variability in peripheral drug concentration, which is discussed above, and the presence of adverse effects. Several participants reported side effects from ABX-1431 including fatigue and sleepiness. Some participants actually fell asleep during the imaging. Participants showed significantly more sedation as a result of ABX-1431 than following the placebo or no

dose. These factors may have affected brain imaging outcomes independently of the direct effect of the drug itself.

In the absence of a study arm in which participants received ABX-1431 and underwent imaging without a noxious stimulus, it is not possible to disentangle the effect of sedation on functional imaging from that of analgesia. Studies of other sedating drugs are of limited help in determining the impact of sedation on imaging in this study, as the imaging results vary across agents and protocols/stimulus types (DiFrancesco, Robertson, Karunanayaka, & Holland, 2013; Slupe & Kirsch, 2018). Additionally, imaging studies of sedation often involve deeper levels of sedation than seen in this study, including unconscious sedation, meaning that their results are not likely to generalise (e.g. Fontan et al., 2021; van Alst et al., 2019). Despite this ,the fact that the majority of anaesthetic agents lead to reduced CBF while ABX-1431 is associated with widespread increases in CBF (see section 5.3) makes it less likely that the BOLD changes identified are attributable to sedation (Slupe & Kirsch, 2018).

5.5 Summary

Using functional neuroimaging it was possible to show that ABX-1431 has a pharmacodynamic effect in the brain. Since ABX-1431 did not cause attenuation of hyperalgesia-related activity in a similar way to other analgesics, it was not possible to conclude that it has definite analgesic efficacy. However, since it acts on endogenous pathways, altering the general tone of the cannabinoid system across the whole brain, it would not necessarily be expected to have the same effect as exogenous ligands or drugs that act on more specific, discrete components of the pain system.

There is good evidence that ABX-1431 affects activity in regions that are key for the top-down modulation of nociception, including the lateral PFC and anterior cingulate and midcingulate cortex. It also disrupts the connectivity between these regions and the amygdala, a region involved in both top-down modulation and affective pain processing, that has been previously implicated in THC-induced analgesia (M. C. Lee et al., 2013). Additionally, it alters the connectivity between the amygdala and two key areas for sensory perception; the dpINS and SI. Given these findings, it may be that ABX-1431 is able to modulate pain responses through the manipulation of cortical control over descending pain modulatory systems. In particular, it seems likely that ABX-1431 acts through the modulation of frontal activity, which in turn alters the connectivity between subcortical regions implicated in sensory and affective processing. If this is the case, then ABX-1431 could be a candidate for the treatment of chronic pain, in which the mechanisms of top-down modulation are known to be deranged (Ossipov et al., 2014), rather than acute pain. This may also explain why there is no consistent trend towards self-reported analgesia when tested in the context of an acute noxious stimulus in a healthy population. Future studies of ABX-1431 should look at the effect of multiple doses on patients with chronic pain in order to determine whether the drug has a more robust analgesic effect under these circumstances.

Chapter 6: Assessing the behavioural effects of a cognitive intervention for irritable bowel syndrome

6.1 Introduction

In previous chapters, neuroimaging has been evaluated as a tool to investigate a novel pharmacological agent in healthy volunteers. Although it was not possible to prove analgesic efficacy using imaging, when compared to less objective measures imaging was able to give greater insight into the pharmacodynamic effects of ABX-1431, and the mechanisms by which it might function.

The remaining chapters focus on the evaluation of a cognitive intervention, mindfulness-based cognitive therapy (MBCT), for patients with chronic visceral pain due to irritable bowel syndrome (IBS). This chapter will focus specifically on how MBCT-IBS affects pain outcomes, and will examine whether behavioural data, including measures of anxiety and quality of life, can give insight into its therapeutic mechanisms. The chapter that follows will investigate whether, as for the pharmacological agent, neuroimaging could provide more information in this regard, and whether imaging has the potential to provide a biomarker for these types of therapies.

6.1.1 Characteristics of patients with IBS

IBS is a functional bowel disorder characterised by abdominal pain associated with a change in defaecation (Longstreth et al., 2006). Its aetiology is unclear, but it is very common, affecting approximately 11% of the world's population and accounting for

about 20% of gastroenterology outpatient work (Canavan, West, & Card, 2014). It falls into the relatively new classification of nociplastic pain, which is “pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors, or evidence for disease or lesion of the somatosensory system” (IASP, 2020). Although IBS occurs in both sexes, it is more prevalent in women (Canavan et al., 2014). It affects all age groups, but is less common in those over the age of 50 (Lovell & Ford, 2012). Individuals with IBS experience reduced quality of life (Mönnikes, 2011), with abdominal pain being the most problematic symptom (Cain et al., 2006).

The link between pain and anxiety is well established in both health and disease, with anxiety being a common feature of chronic and acute pain (Bener et al., 2013; Carleton et al., 2018; Kapoor, White, Thorn, & Block, 2016; McWilliams, Cox, & Enns, 2003; Taenzer, Melzack, & Jeans, 1986). Anxiety is particularly prevalent in visceral pain, in which interoceptive stimuli are more salient, and more likely to lead to anticipatory fear (Laura Ricarda Koenen et al., 2018). This is certainly true in IBS, in which anxiety has a key role in mediating expectations about the threat of pain (A Icenhour et al., 2015). When levels of the stress hormone cortisol are increased acutely, pain sensitivity is increased and pain-related emotional learning is reduced in visceral pain, but not in somatic pain, indicating that stress is likely to be of greater importance in visceral pain perception (Benson et al., 2019).

The co-occurrence of IBS and psychiatric disorders is well known. Multiple studies show that patients with IBS tend to have higher rates of anxiety and depression than the general population (Folks, 2004; Fond et al., 2014; Mykletun et al., 2010; Wu,

2012). While some authors have suggested that anxiety is involved in the maintenance of IBS symptoms (Hauser, Pletikosic, & Tkalcic, 2014), others have suggested that high levels of anxiety may predispose an individual to develop IBS (Sibelli et al., 2016). In reality, there is likely to be a bidirectional effect between physical and psychological symptoms in functional disorders. There is a strong bidirectional relationship between anxiety and chronic pain more generally (Bondesson et al., 2018). There have been a few prospective studies looking specifically at functional gastrointestinal disorders (FGIDs). One study showed that anxiety at baseline led to an increased risk of developing functional dyspepsia in the next 10 years (Aro, Talley, Johansson, Agréus, & Ronkainen, 2015). Koloski and colleagues (2012) looked at over 1000 participants who completed a questionnaire at baseline and then 12 years later. They found that in those with no FGID at baseline, the presence of anxiety was predictive of the development of a FGID at follow-up. However, the reverse was also true, with participants who had a FGID but no anxiety or depression at baseline reporting significantly higher levels of anxiety and depression at follow-up.

6.1.2 Hyperalgesia in patients with IBS

Widespread hyperalgesia is a common finding in chronic pain disorders. The presence of widespread hyperalgesia is indicative of central sensitization, which is likely to be a key factor in the establishment and persistence of chronic pain, particularly where it appears disproportionate to the underlying pathology (Harte et al., 2018).

The literature on hypersensitivity in FGIDs such as IBS is varied, with most studies reporting it in a subset of their participants (Azpiroz et al., 2007; Moshiree, Price, Robinson, Gaible, & Nicholas Verne, 2007; Rodrigues, Verne, Schmidt, & Mauderli,

2005; Verne, Robinson, & Price, 2001; Q. Zhou et al., 2009). Overall it appears that there is considerable heterogeneity between individuals. In one series of 86 patients with IBS, 90% were found to have visceral hypersensitivity to rectal dilatation. Other studies have reported as few as 50%. Somatic hypersensitivity is less common, affecting around 33% of patients with FGIDs (Azpiroz et al., 2007). However, the presence of widespread hyperalgesia in such a big group of patients, even if not a universal finding, indicates that mechanisms of central sensitization may be of importance in the pathophysiology of the disease.

6.1.3 The current treatment of IBS

The current mainstays of treatment for IBS are dietary and lifestyle adjustments, and pharmacological therapies that are targeted at individual symptoms rather than disease mechanisms. Antispasmodics are used to treat abdominal pain, and laxatives or antimotility agents are indicated for constipation or diarrhoea respectively. The number needed to treat for improvement of abdominal pain by antispasmodics compared to placebo is seven (Ruepert et al., 2011). In patients for whom these measures are insufficient, tricyclic antidepressants can be used as a second line therapy, with selective serotonin reuptake inhibitors being an option if these are ineffective (National Institute for Health and Care Excellence, 2008). The number needed to treat for improvement of abdominal pain with antidepressants is five (Ruepert et al., 2011), while the number needed to harm is 8.5 (Ford, Lacy, Harris, Quigley, & Moayyedi, 2019). It is clear that pharmacological treatments are far from universally effective, and antidepressants in particular can have serious adverse effect profiles and should be used with care.

The National Institute for Health and Care Excellence (NICE) currently recommends psychological therapies for patients who have refractory IBS, with no response to pharmacological therapies after 12 months (National Institute for Health and Care Excellence, 2008). Multiple meta-analyses have shown that psychological therapies are effective for reducing symptoms and improving daily functioning in IBS (Ford et al., 2014; Laird, Tanner-Smith, Russell, Hollon, & Walker, 2017), and are able to achieve sustained improvements (Everitt et al., 2019; Laird, Tanner-Smith, Russell, Hollon, & Walker, 2016). Cognitive behavioural therapy (CBT) is the most common psychological treatment in the literature. Other common treatments include interpersonal therapy, hypnotherapy and relaxation (Henrich et al., 2015). There is currently limited evidence on mindfulness-based therapies, though this is an area with therapeutic potential.

6.1.4 Mindfulness as a possible treatment for IBS

Mindfulness meditation involves the development of increased moment-to-moment awareness but without evaluation. Practitioners are taught to recognise mind-wandering and return their attention to the present moment, but to treat their current experiences in a non-judgemental way.

There is some evidence that mindfulness meditation can improve outcomes in patients with chronic pain conditions. A meta-analysis of randomised controlled trials (RCTs) in 2016 (Veehof, Trompetter, Bohlmeijer, & Schreurs, 2016) found that compared to controls, mindfulness had beneficial short and long-term effects on pain, depression, anxiety and quality of life in chronic pain patients. Two RCTs of mindfulness-based stress reduction (MBSR) have focussed specifically on patients with IBS (Gaylord et

al., 2011; Zernicke et al., 2013). Both found that immediately after the intervention, the MBSR group had reduced symptoms compared to the controls. One study showed that this improvement was maintained at six months, but at this stage the control group, who were treated as usual, showed similar levels of improvement. Quality of life and mood improved regardless of group assignment (Zernicke et al., 2013). The other study found that symptoms were reduced immediately and at three months compared to a group receiving social support. Quality of life, psychological distress and symptom-related anxiety were similar in both groups immediately after the intervention, but were improved in the MBSR group at three months (Gaylord et al., 2011). A longitudinal study of 68 patients with IBS found that an eight week MBSR intervention was associated with improvements in symptom severity, symptom-specific anxiety and quality of life (Naliboff et al., 2020). Conclusions on causality are hampered by the lack of a control group. However, the study did find that symptom improvement was predicted by the increased ability to stay in the present moment and act with awareness, indicating that this may be a key psychological mechanism for the mediation of symptoms using mindfulness techniques.

MBCT is an intervention that was developed from MBSR. It combines methods used in CBT with mindfulness techniques in order to promote non-judgemental interoceptive awareness and address maladaptive metacognitive processing of symptoms. The participants who completed the mindfulness intervention in this thesis are a subset of the 67 patients who completed a larger RCT that compared MBCT that was adapted for patients with IBS (MBCT-IBS) to a waitlist control group. In this RCT the mindfulness group reported greater improvements in symptoms and quality of life than the control group. These changes were mediated by changes in symptom-specific

anxiety and pain catastrophizing, as well as changes in self-referential processing combined with changes in non-judgmental awareness. This may indicate that MBCT-IBS works through reducing maladaptive cognitions about symptoms and reducing self-critical appraisal (Henrich, Gjelsvik, Surawy, Evans, & Martin, 2020).

The fact that symptom-specific anxiety and pain catastrophizing mediated changes in symptom severity is in keeping with the broader literature about IBS and anxiety as well as the findings of a pilot study of IBS (appendix H).

6.1.5 Chapter objectives

Although the larger study from which this cohort was derived found improvements in IBS symptoms, it did not focus specifically on pain outcomes and their relationship with other outcome measures. The majority of this chapter will focus on the effect of MBCT-IBS on self-reported pain measures. The larger study showed that symptom improvement was mediated by changes in-symptom specific anxiety (Henrich et al., 2020), and this chapter will determine whether there is a specific correlation between the effects of the intervention on anxiety and pain. Additionally, the SF-36 questionnaire will be used to differentiate between the effects of MBCT-IBS on the psychological and physical aspects of quality of life. It is hypothesised that MBCT-IBS will lead to improvement in pain outcomes, and that reductions in both general and symptom-specific anxiety may covary with the improvement in pain. It is also hypothesised that since MBCT-IBS may work through the alteration of cognitions and self-appraisal, improved pain outcomes may relate more to better coping mechanisms than to attenuated sensory input. Therefore, it may be that psychological aspects of quality of life will be affected to a greater extent than physical aspects.

The larger study relied on the subjective self-reporting of symptoms through questionnaires. The final objective of this chapter is to examine the effect of MBCT-IBS on somatic sensory function. Sensory testing relies on the self-reporting of a subjective experience. However, the fact that participants are asked to rate a known sensory input means that there is a slightly higher degree of objectivity compared to a questionnaire. Hyperalgesia is a common finding in chronic pain populations, including IBS (Moshiree et al., 2007; Rodrigues et al., 2005; Verne et al., 2001; Q. Zhou et al., 2009; Q. Q. Zhou, Fillingim, Riley, Malarkey, & Verne, 2010). A reduction in the temperature required to achieve a specific pain rating would indicate that MBCT-IBS had led to reduced pain sensitivity. However, as demonstrated in both pilot data (appendixAppendix H:) and the literature (section 6.1.2), hyperalgesia is not a universal finding, meaning that an absence of effect would not label MBCT-IBS as an ineffective treatment.

6.2 Methods

6.2.1 Participants

Female participants were eligible if they were 18-65 years old and were diagnosed with IBS by a general practitioner (GP) or gastroenterologist according to Rome III criteria. Participants were also required to speak fluent English and have normal or corrected to normal vision.

Participants were excluded if they had a severe psychiatric disorder, a gastrointestinal diagnosis other than IBS, insufficient manual dexterity for computerised tasks, no access to the internet, contraindications to MRI scanning, or if they were recently bereaved (within one year) or pregnant. They were also excluded if they had prior

experience of mindfulness of meditation, if they were unwilling to commit to practice in sessions and at home, if they were enrolled in another IBS treatment study, or if they were unwilling to allow contact with their GP. Full inclusion and exclusion criteria are given in appendix I.

6.2.2 Recruitment

Participants were recruited through gastroenterology clinics, as well as by word of mouth and local advertising.

6.2.3 Study design

This was the imaging component of a larger RCT of MBCT-IBS in which participants completing the mindfulness intervention were compared to those on a waitlist (Henrich et al., 2020). This extension to the main study had a within-subject design, with participants completing two visits. There was no control arm. The first visit took place prior to starting any mindfulness training. They then completed a six week course in MBCT-IBS. This consisted of six two-hour sessions over a period of six to seven weeks. Participants were expected to complete home-practice six times per week. Their second visit took place within three weeks of completing the MBCT-IBS course. Visits were not restricted to periods in which participants were symptomatic, as the nature of IBS means that the timing and duration of symptoms would have been too unpredictable. Both visits involved the same assessments, consisting of questionnaires and neuroimaging, with the addition of a question about expectations at the first visit. Participants were taken from both arms of the main study, with those who were originally randomised to the waitlist completing their visits when they

completed the MBCT-IBS course later on. This chapter looks at the behavioural data, while the next chapter analyses the imaging.

6.2.4 Questionnaires

The following questionnaires were administered at the scanning visits:

- Brief pain inventory – short form (BPI; C. S. Cleeland & Ryan, 1994)
- IBS-impact scale (IBS-IS; Longstreth et al., 2005)
- Hospital anxiety and depression scale (HADS; Zigmond & Snaith, 1983)
- Short Form-36 health survey v2 (SF-36; Ware et al., 2000)
- Behavioural inhibition system/behavioural activation system (BIS/BAS) scales (Carver & White, 1994)

Participants were also asked about their age, time since diagnosis and frequency of pain, as well as questions relating to the length and regularity of their menstrual cycle, the severity of their period pain, and their current use of hormonal contraception. At their first visit they were asked to rate their expectation of pain relief from the intervention using a 100mm VAS. The anchors for this were “No relief” and “Complete relief”.

Results were also collected from the following questionnaires, which were not collected at the same visits as the scans. They were administered by Julia Henrich. These were:

- Visceral sensitivity index (VSI; J. S. Labus et al., 2004)
- Gastrointestinal symptom rating–scale - IBS (GSRS-IBS; Wiklund et al., 2003)
- Pain catastrophizing scale (Sullivan et al., 1995)

These questionnaires were administered at multiple timepoints, but the data used here was that which was collected at the timepoints closest to the two scanning visits.

6.2.5 Sensory testing and brain imaging

Participants completed a series of MRI brain scans at both visits. These will be described in more detail in chapter 7, which will examine the results of the brain imaging in this cohort.

At both visits, an evoked pain paradigm was administered during scanning. The stimulus was perception locked in order to ensure that all participants found it painful but tolerable. This is particularly important in a chronic pain population, in whom pain pathways may be dysfunctional, making it difficult to predict perceived intensity from a specific stimulus. A thermal stimulus was chosen since it is common in the IBS literature, which shows that thermal hypersensitivity occurs in many patients, though not all (Moshiree et al., 2007; Rodrigues et al., 2005; Verne et al., 2001; Q. Zhou et al., 2009). In addition, a thermal stimulus is well tolerated, easy to perception lock, and easy to apply in an MRI scanner. A series of three second heat stimuli were applied to the left forearm using a 1.7cm diameter CHEPS thermode and Medoc pathway system. Temperature was perception locked to a numerical pain intensity rating of 5/10 immediately prior to the start of the evoked pain task. This was done with verbal responses from the participant.

Ten identical stimuli were applied with an interstimulus interval of approximately 60 seconds (jittered). After each stimulus, pain intensity and unpleasantness ratings were collected using computerised visual analogue scales which participants adjusted using

a button box. Scales were 100mm long and the anchors were “no pain/severe pain” and “not unpleasant/extremely unpleasant”. On-screen instructions and response measures were programmed using Neurobehavioral systems Presentation software.

6.2.6 Data analysis

Data were analysed using Microsoft Excel and IBM SPSS. All changes from pre-intervention to post-intervention were calculated by subtracting the pre-intervention score from the post-intervention score in order that a negative change represents a reduced score and a positive change represents an increase.

6.2.6.1 Comparing means between groups

Paired t-tests were used to compare questionnaire and sensory testing data between the pre-intervention and post-intervention visits. Not all of the changes were normally distributed and sample sizes were small, meaning that violating the assumption of normality was more likely to influence the outcome. For this reason, bootstrapping was applied to all t-tests in this section. This is a method that is robust to violations of assumptions (Field, 2018).

6.2.6.2 Determining the relationship between variables

A Spearman correlation coefficient was used to determine the presence of significant correlations between variables. This was chosen over a Pearson correlation coefficient as it does not require all variables to be normally distributed, and is less susceptible to outliers.

6.3 Results

Fifteen women aged 22-63 completed both the pre-intervention and post-intervention visits. The median age was 27 years.

6.3.1 Effect of mindfulness on symptoms of IBS

Gastrointestinal symptoms, as measured by the total GSRS-IBS, were significantly reduced following mindfulness ($t(13)=-7.357$, $p=0.024$; Figure 6.1A). Although not tested with a formal hypothesis test in order to reduce multiple comparisons, the GSRS subscales (including pain) all showed a downward trend, indicating that the change was universal rather than driven by one symptom type. BPI pain severity scores were reduced but not significantly. Pain interference scores were significantly reduced ($t(14)=-2.596$, $p=0.021$), indicating improvement (Figure 6.1B).

The BPI pain interference score is sometimes divided into two subscales. The affective dimension looks at the effect of pain on relations with others, enjoyment of life, and mood. Meanwhile, the activity dimension reflects the impact on walking, work, general activity and sleep (Charles S. Cleeland et al., 1996). When the two subscales were assessed separately, affective interference was significantly reduced following the intervention ($t(14)=-1.088$, $p=0.003$), while interference with activity was not (Figure 6.1B). The complete results for all t-tests performed in this section are provided in appendix J-1.

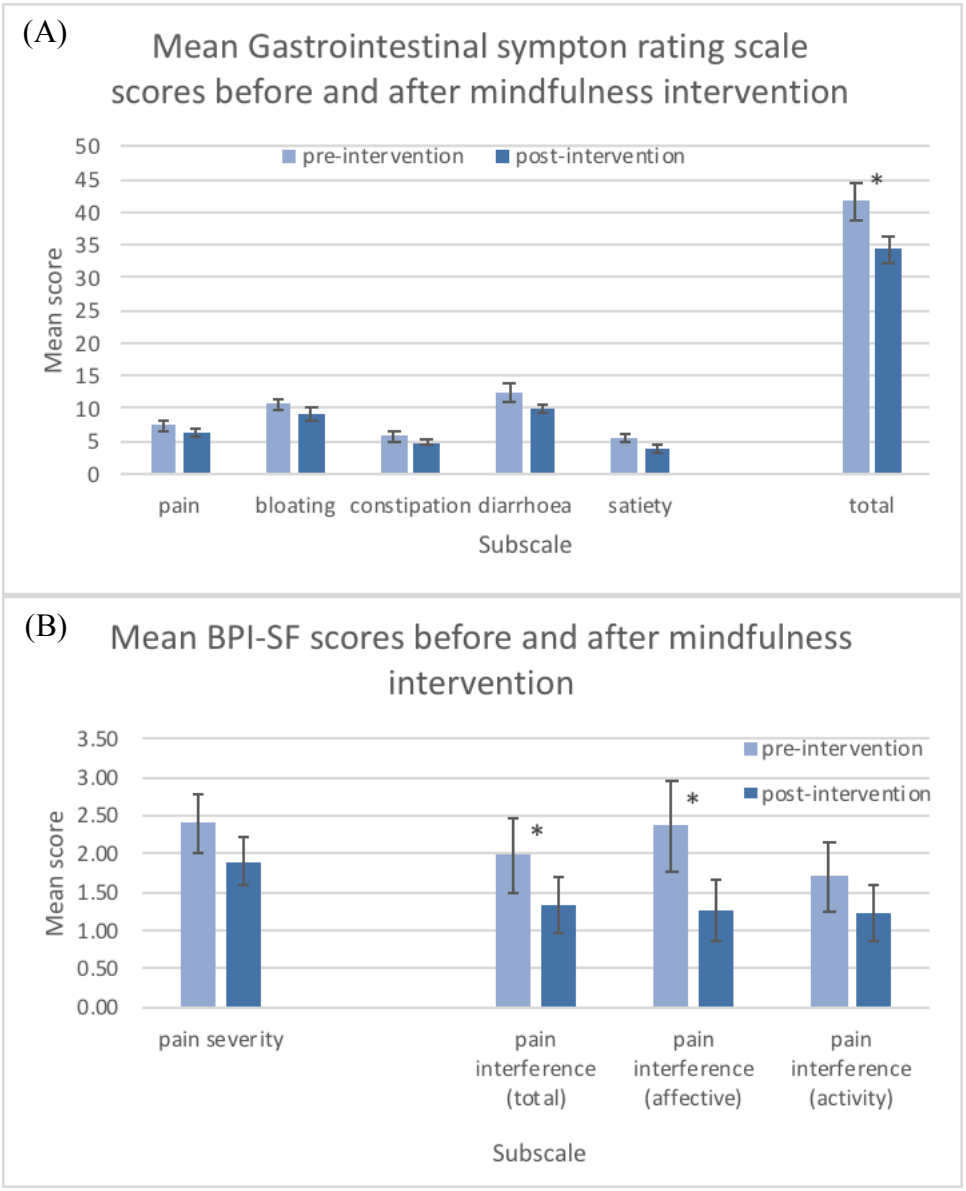


Figure 6.1: Effect of MBCT-IBS on symptoms of irritable bowel syndrome. Charts show the group mean scores for the gastrointestinal symptom rating scale (A) and the Brief pain inventory short form (B) before and after mindfulness intervention (gastrointestinal symptom rating scale [GSRS]: n=14; brief pain inventory short form (BPI-SF): n=15). Error bars show standard errors. A reduction in score equates to an improvement in symptoms. Paired 2-tailed t-tests were performed on the total GSRS score and all BPI-SF subscales to compare scores before and after the MBCT-IBS intervention. Differences were considered statistically significant if $p \leq 0.05$, and changes meeting this criterion are marked with an asterisk. There was a statistically significant reduction in GSRS following the intervention. The total pain interference score was significantly reduced, as was the affective subscale. The change in the activity subscale of the pain interference score was not statistically significant.

In order to check that changes in symptom severity were not related to the expectations of participants about whether the treatment would work, expectation of pain relief was compared to the change in total GSRS score and the BPI pain severity and pain interference scores. A Spearman correlation was used as the expectation score was not normally distributed. There was no correlation found between expectation of pain relief and any of the other measures. (GSRS: $r_s(12)=-0.154$, $p=0.598$; BPI severity: $r_s(13)=-0.395$, $p=0.145$; BPI interference: $r_s(13)=0.110$, $p=0.696$).

6.3.2 Effect of mindfulness on anxiety and depression scores

Both HADS anxiety (HADS-A) scores and VSI scores were significantly reduced following mindfulness, indicating improvement (HADS-A: $t(14)=-2.794$, $p=0.014$; VSI: $t(13)=-2.738$, $p=0.017$). Pain catastrophizing was also significantly reduced ($t(13)=-8.5$, $p=0.01$). Although the HADS depression scores were reduced, this did not reach significance (Figure 6.2). The complete results for all t-tests performed in this section are provided in appendix J-2.

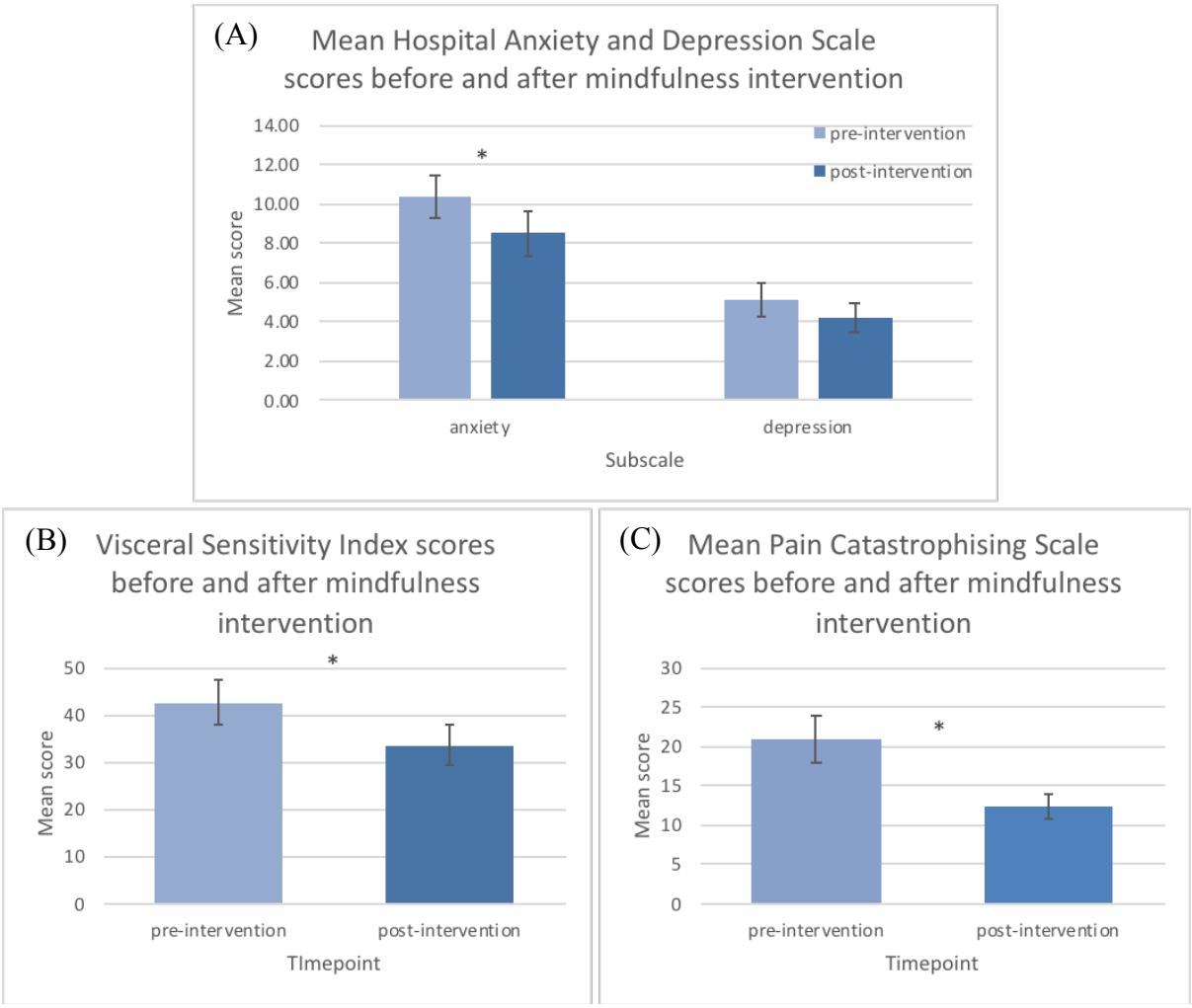


Figure 6.2: Effect of MBCT-IBS on anxiety and mood in irritable bowel syndrome. Charts show group mean scores both before and after mindfulness for (A) Hospital anxiety and depression scale (n=15); (B) Visceral sensitivity index (n=14); (C) Pain catastrophizing scale (n=14). Error bars show standard errors. A reduction in score equates to an improvement in anxiety/depression/catastrophizing. Paired 2-tailed t-tests were performed to compare scores before and after the MBCT-IBS intervention. Differences were considered significant if $p \leq 0.05$, and changes meeting this criterion are marked with an asterisk. There was a statistically significant reduction in scores from the anxiety subscale of the hospital anxiety and depression scale, the visceral sensitivity index and the pain catastrophizing scale following the intervention.

6.3.3 Relationship between anxiety and IBS symptoms

The changes in anxiety scores (both HADS and VSI) from before to after MBCT-IBS were compared to the changes in symptoms scores (GSRS and BPI) to determine the presence of any correlation. A Spearman correlation was used as some of the

changes were not normally distributed. Improvement in anxiety was found to covary with improvement in pain severity (Figure 6.3). This was true for both generalised anxiety, as measured by HADS, and symptom-related anxiety, as measured by VSI. The complete results for all correlations performed in this section are provided in appendix J-3.

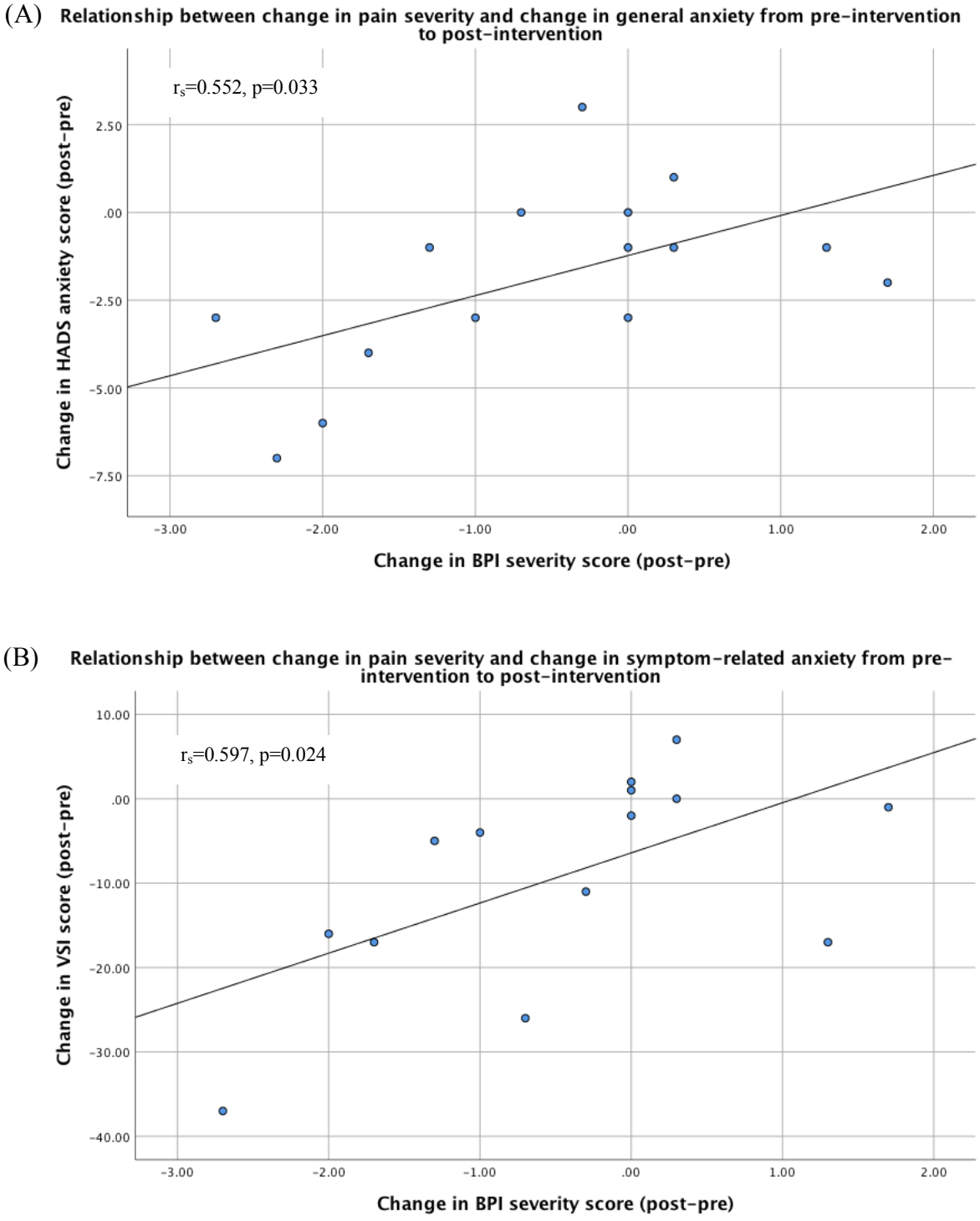


Figure 6.3: Scatterplot with line of best fit showing the relationship between change in brief pain inventory (BPI) pain severity score and change in (A) hospital anxiety and depression scale (HADS) anxiety score (n=15) and (B) visceral sensitivity index (VSI) score (n=14) from pre-intervention to post-intervention in patients with irritable bowel syndrome. A negative change in score indicates a reduction in anxiety. A Spearman correlation was calculated, and the correlation was considered significant if p≤0.05. Reduced pain severity was correlated with the reduction in both anxiety measures.

6.3.4 Effect of mindfulness on quality of life

The IBS-IS total score was significantly improved following mindfulness (t(14)=2.417, p=0.03). In the SF-36, the mental component summary (MCS) score was significantly improved (t(14)=2.595, p=0.021). Although the mean physical component summary (PCS) score improved, this was not statistically significant (Figure 6.4). The complete results for all t-tests performed in this section are provided in appendix J-4.

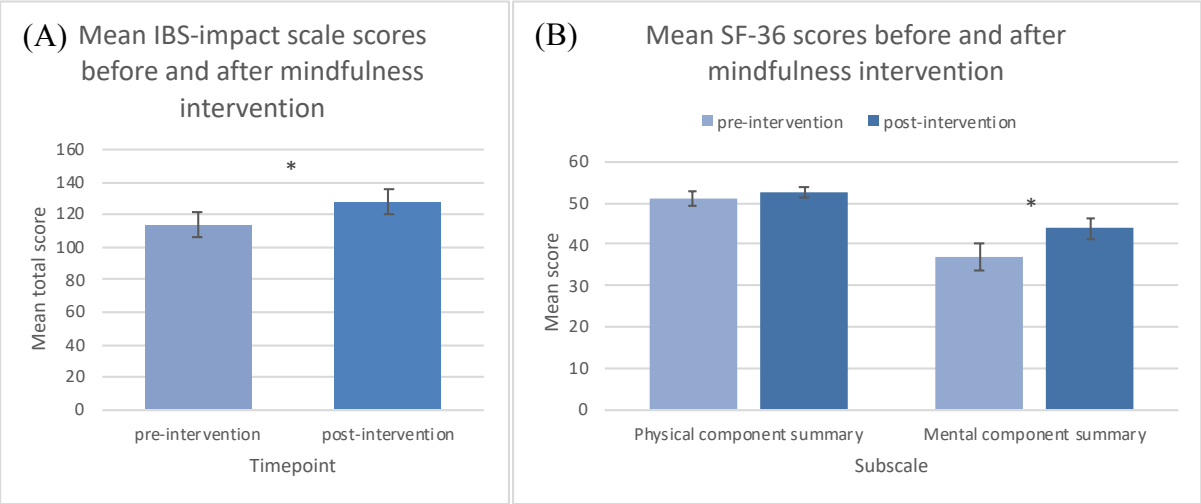


Figure 6.4: Effect of MBCT-IBS on quality of life in irritable bowel syndrome (IBS). Charts show group mean scores for the IBS-impact scale (A) and SF-36 (B) before and after the mindfulness intervention (n=15). Error bars show standard errors. An increased score equates to an improvement in quality of life. Paired 2-tailed t-tests were performed to compare scores before and after the MBCT-IBS intervention. Differences were considered statistically significant if p≤0.05, and changes meeting this criterion are marked with an asterisk. The improvement was statistically significant for the total IBS-impact scale and for the mental component summary of the SF-36, but not for the SF-36 physical component summary.

6.3.5 Effect of mindfulness on sensory testing

The thermode temperature required to give a 5/10 pain intensity rating increased following MBCT-IBS, and this was a consistent finding across participants (Figure 6.5). A paired t-test was performed, showing that the change in temperature was significant

(t(13)=4.757, p<0.001). There was no significant change in evoked pain intensity or evoked pain unpleasantness, indicating that perception locking was consistent across visits. The complete results for all t-tests performed in this section are provided in appendix J-5.

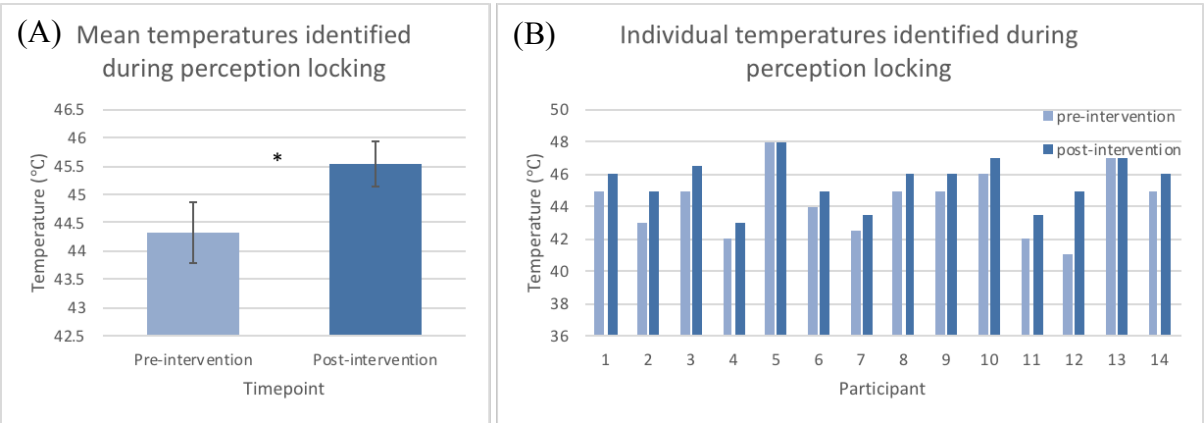


Figure 6.5: Bar charts showing the temperatures required to achieve perception locking to a pain intensity rating of 5/10. (A) Mean group values are shown pre-intervention and post-intervention for those participants with irritable bowel syndrome who completed the mindfulness intervention (n=14). Error bars show standard errors. A paired 2-tailed t-test was performed to compare scores before and after the MBCT. Temperature was significantly increased following the intervention ($p \leq 0.05$), and this significant result is marked with an asterisk. (B) Individual values are shown pre-intervention and post-intervention, demonstrating that the increase in temperature following the mindfulness intervention was a consistent finding.

Undergoing MRI scanning can make participants anxious, particularly on their first visit. To rule out the possibility that the reduction in perceived pain intensity for a given stimulus was related to greater anticipatory anxiety before the pre-intervention than the post-intervention visit, the change in temperature was compared to the change in HADS-A for each participant. No correlation was found between the two measures, making this unlikely ($r_s(12)=0.313$, $p=0.276$).

6.3.6 Relationship between motivational traits and effectiveness of mindfulness

Baseline BIS/BAS scores were compared with the change in symptom scores (GSRS and BPI) following the intervention. A positive correlation was found between BAS drive and fun-seeking subscales prior to intervention and the change in GSRS scores following MBCT-IBS, with higher BAS scores predicting greater symptom improvement. There was no relationship between BAS reward responsiveness scores at baseline and change in GSRS scores. Participants with a higher baseline score on the BAS fun-seeking subscale also showed greater improvement in the BPI pain severity score, but there was no effect on pain interference. There was no relationship between scores on the BAS reward responsiveness or drive subscales and change in BPI scores. No relationship was seen between BIS score at baseline and the effect of MBCT-IBS on general symptoms or pain. The complete results for all correlations performed in this section are provided in appendix J-6.

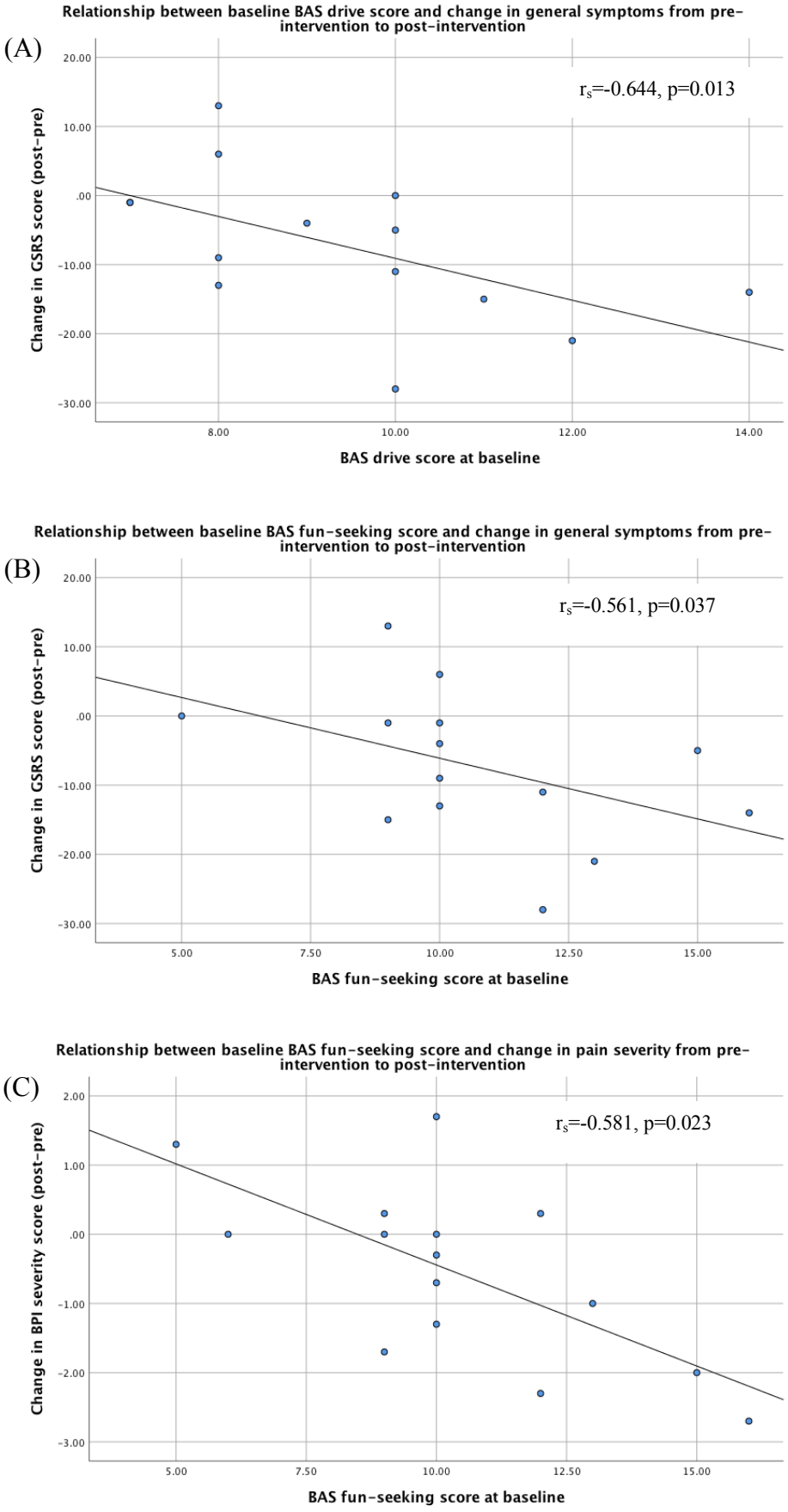


Figure 6.6: Scatterplots with lines of best fit showing the relationship between trait behavioural activation system (BAS) scores and the change in symptoms from pre-intervention to post-intervention. They show how the change in gastrointestinal symptom rating scale (GSRS) score is related to (A) BAS drive, and (B) BAS fun-seeking scores at baseline. Plot C shows the association between the BAS fun-seeking score at baseline and the change in the brief pain inventory (BPI) severity score. In all cases there was a correlation between higher BAS scores at baseline and greater reduction in symptoms.

6.4 Discussion

Based on the behavioural and sensory data above, MBCT-IBS may be an effective intervention for IBS. In this study it was associated with improvements in pain-related outcomes, anxiety and quality of life.

6.4.1 The effect of MBCT-IBS on symptoms and quality of life

Post-intervention there was a reduction in gastrointestinal symptoms as a whole. There was also a significant improvement in the IBS-IS, which reflects a reduction in the negative impact of IBS symptoms on daily life. This is in keeping with the findings of the larger study of which this data forms a subset (Henrich et al., 2020).

With reference to pain specifically, the effect of mindfulness on the pain severity subscale of the BPI was not statistically significant. The interpretation of negative behavioural findings is limited by the study's small sample size, meaning that from this data it is not possible to conclude with certainty whether MBCT-IBS has an effect on pain severity. In spite of this limitation, it was possible to detect a significant improvement in pain interference, indicating that the impact of pain on daily function was reduced. The fact that pain interference was affected with more certainty than pain severity suggests that the effects of mindfulness on pain are more complex than a simple reduction in perceived intensity. Instead, modulation of factors that influence coping ability may be key. It was the affective dimensions of the pain interference score that were impacted by MBCT-IBS. This is in keeping with the non-specific quality of life data, in which the MCS score of the SF-36 was significantly improved while the PCS score was not. The fact that mental aspects of quality of life are more affected then physical aspects might indicate that MBCT-IBS has more effect on the

psychological than the physical sequalae of the disease. Since pain has a huge psychological component, this does not detract from the potential of MBCT-IBS for analgesic efficacy.

6.4.2 The link between anxiety and IBS symptoms

Both generalised anxiety and symptom-related anxiety were reduced post-intervention, as was pain catastrophizing. Participants can feel anxious about taking part in a study, particularly prior to their first visit. Therefore, it is possible that some of the reduction in generalised anxiety, as measured by the HADS-A score, could be ascribed to reduced anticipatory anxiety in the week prior to the second visit. However, this is unlikely to influence symptom-specific anxiety, as measured by the VSI.

A bidirectional relationship between anxiety and functional gastrointestinal disorders is well established (Aro et al., 2015; Koloski et al., 2012), as is the relationship between anxiety and chronic pain more generally (McWilliams et al., 2003). In other pain conditions, high anxiety has been linked to high scores in both the severity and interference subscales of the BPI (Bair et al., 2003; Gore et al., 2005; Kempton, Buckner, Fridman, Iyer, & Cooper, 2018). In this study, the degree to which anxiety was reduced following mindfulness correlated with the reduction in pain severity, despite the fact that the change in pain severity did not reach statistical significance. This was true for more generalised anxiety (as measured by HADS-A scores) as well as symptom-related anxiety (from VSI scores). This was an effect which appeared to be specific to pain severity rather than pain-related functioning or gastrointestinal symptoms in general, as the improvements in the pain interference and GSRS scores did not covary with anxiety in the same way. While this could indicate that reduced

anxiety does not mediate the effect of mindfulness on these outcomes (or that they do not mediate the effect of mindfulness on anxiety), it could also reflect a non-linear relationship between them. The same is true of the changes in sensory function, which were also not linearly correlated with changes in anxiety.

6.4.3 The effect of trait motivational style on the effectiveness of MBCT-IBS

BIS/BAS is a questionnaire designed to assess motivational styles with respect to pursuit of reward (BAS) and avoidance of punishment (BIS). The scales are explained in more detail in chapter 2 (section 2.3.3.2).

In this study, patients with higher BAS scores (reflecting greater reward-related motivation and desire) had a better response to MBCT-IBS in terms of general symptom reduction. With respect to pain specifically, high scores in the fun-seeking BAS subscale at baseline were associated with a greater reduction in pain severity. This indicates that the desire to seek out new rewards is important for the effectiveness of MBCT-IBS for pain relief. Baseline BIS scores showed no association with the effect of MBCT-IBS on symptoms.

Motivational states are known to be important to the pain experience. Some literature has indicated that BIS scores may have more relevance than BAS scores for pain, on the basis that pain is an aversive state, and individuals with high BIS scores are more sensitive to punishment (Serrano-Ibáñez et al., 2019). In healthy individuals, BIS scores have been shown to correlate with pain intensity scores (Jensen et al., 2015), while chronic pain patients with higher BIS scores have been shown to have greater pain-related impairment than those with lower scores (Serrano-Ibáñez et al., 2019).

However, although pain relief can be viewed as the cessation of a negative emotional state, it actually produces positive emotions, and is thus highly rewarding. Therefore, pain produces a reward-seeking motivational state, leading to behavioural adaptations aimed at inducing pain relief (Leknes et al., 2008; Edita Navratilova & Porreca, 2014).

Since BAS scores are thought to reflect motivation for and sensitivity to reward, it is often assumed that those with chronic pain have lower BAS scores than the general population. There is some evidence that reward responsiveness is reduced in chronic pain, though drive may not be affected, indicating that patients do seek reward but that the reward that is experienced is less than that of their healthy counterparts (Elvemo, Landrø, Borchgrevink, & Håberg, 2015; Håberg, 2017).

In acute pain, higher reward responsiveness is associated with a greater response to opioid analgesia (Vishvarani Wanigasekera et al., 2012). The BAS score (and in particular the fun-seeking subscale) is known to correlate with opioid receptor availability in the frontal cortex, amygdala, ventral striatum, brainstem, cingulate cortex and insula (Karjalainen et al., 2016). These are regions involved in processing both reward and nociception. In particular the nucleus accumbens (NAc), a large part of the ventral striatum, is known to be a key region for relief. Not only does the BAS fun-seeking score correlate with opioid receptor availability in this region (Karjalainen et al., 2016), but blood oxygen level dependent (BOLD) activation in the NAc is linked to the offset of acute pain (Becerra & Borsook, 2008), and relief pleasantness covaries with accumbens activation (Leknes, Lee, Berna, Andersson, & Tracey, 2011). Meanwhile, chronic pain patients are less responsive to reward than healthy controls, and the reduction is associated with reduced accumbens volume (Elvemo et al., 2015).

Wanigasekera et al. (2012) found that the magnitude of opioid-induced pain relief in healthy volunteers is predicted by pre-treatment BOLD responses to noxious stimulation in multiple reward-related brain regions (specifically the orbitofrontal cortex, rostral anterior cingulate cortex, hippocampi, amygdalae, caudate, NAc and ventral tegmental area), and that these BOLD responses correlate with BAS scores. They concluded that BAS reward responsiveness scores provided a psychophysical measure of the ability of the endogenous opioid system to react to exogenous opioids.

It is possible that those with greater desire for new rewards and more willingness to approach potentially rewarding events are better able to engage reward circuitry and opioid-mediated endogenous analgesia, and that this contributes to the relationship seen between higher BAS scores and better treatment response to MBCT-IBS in this study.

6.4.4 Changes in sensory function following MBCT

Although sensory data relies on subjective ratings, it is more objective than the other data presented in this chapter. Following mindfulness, the temperature of the thermode required to give the same perceived heat pain intensity was increased, indicating reduced sensitivity to thermal pain.

Although repeated daily administration of a painful stimulus can lead to long-term habituation (U. Bingel, Schoell, Herken, Büchel, & May, 2007; A. May et al., 2012), this is not the case for a single series of painful stimuli, from which habituation should recover spontaneously within minutes of stimulus cessation (Ibinson et al., 2004). In

this study, there was a minimum of six weeks between the two stimulus sessions, indicating that changes in sensory function were not a result of habituation.

Evidence for the effect of mindfulness on sensory function in a pain population rather than in healthy individuals is lacking. However, there is literature from healthy volunteers indicating that mindfulness may have an impact on pain sensitivity. Bilevicius and colleagues performed a meta-analysis looking at the effect of mindfulness on neural activity during nociception (Bilevicius, Kolesar, & Kornelsen, 2016). Five studies were included, three of which compared very experienced meditators to controls, while two others looked at the effects of four days of mindfulness training. All studies used experimental pain models rather than individuals with clinical pain, and four out of the five used a thermal stimulus. The impact on sensory function was mixed. The two studies examining the effect of brief training in mindfulness found a reduction in pain intensity, while two of the studies looking at experienced meditators found no effect. The remaining study, which looked at experienced meditators compared to controls, found that a higher temperature was required to reach the same pain intensity level in the meditation group. The effect on pain unpleasantness was clearer, with four of the five studies finding reduced unpleasantness in the meditation/mindfulness conditions.

The fact that other studies have shown an effect of mindfulness on sensory processing supports the idea that MBCT-IBS reduces pain sensitivity in those with IBS. However, it indicates that this might be a universal effect of mindfulness rather than resulting from the reversal of IBS-related pathology.

6.4.5 Limitations of the study

IBS is a disease with episodic symptoms, the severity of which may change over short periods of time, and this makes it very challenging to study. This is particularly true due to the functional nature of the disease, which means that at the current time, disease status can only be determined by symptoms and not by underlying pathology. For this reason, there is no easy way to differentiate between intervention-induced changes and those that would have occurred spontaneously. The GSRS measures symptom severity over the preceding seven days rather than just the day of testing, meaning that the effect of short-term fluctuation is reduced, but this is not the case for the BPI short form, which measures pain over the preceding 24 hours. The best way of reducing this uncertainty would be with the presence of a control group.

The lack of a control group with whom to compare the group undergoing mindfulness was the biggest limitation of this study. This behavioural data was collected alongside the imaging component of a larger study which did include a control group. Since the data collection was opportunistic and had to be done quickly, there were insufficient time and resources to collect control group data. As mentioned above, this creates issues in a disease with episodic symptoms. It is also possible that some of the changes seen may have been due to other factors unrelated to the MBCT-IBS intervention. For example, participants attending for their second visit may well have been less worried about the visit than they were on the first occasion, and this could have affected their anxiety scores. Expectancy is also an important factor in determining pain outcomes, and has a central role in placebo analgesia (Price et al., 1999; Tracey, 2010; Vase et al., 2003). The fact that there was no correlation between expectation of pain relief and change in symptoms, as measured by GSRS and BPI,

provides some reassurance that symptom improvement was not entirely expectancy-related in the absence of a proper placebo arm. Although the RCT from which these participants were taken did not include a validated pain measure, it did look at both symptoms and anxiety, and found that both were improved in the MBCT-IBS arm compared to the control arm (Henrich et al., 2020). This provides support to the conclusion that the improvements found here are likely to be intervention-related.

The absence of a contemporaneous measure of generalised state anxiety on the day of each visit meant that generalised anxiety ratings were based on how participants had felt over the preceding week. While this was suitable for assessing the effect of the intervention on daily anxiety at home, it may not have given a good approximation to anxiety related to study procedures. Given the potential effect of such anxiety on other outcomes, future studies should consider including a measure such as the state component of the state-trait anxiety inventory for this purpose (Spielberger et al., 1983).

As outlined in chapter 2, a decision was made not to correct for multiple comparisons when analysing the behavioural data in this thesis. This could be considered a limitation here. However, since all effects in this chapter were in the same direction, which was consistent with what was hypothesised, and was biologically plausible, it is thought that the uncorrected data represents a genuine effect in the majority of cases.

Another limitation of the study was the fact that all of the participants were female. There is good evidence that sex hormones are important in pain perception (Vincent & Tracey, 2008), and differences in pain perception between men and women are

commonly reported (Bartley & Fillingim, 2013). Therefore, it is not possible to generalise the results of this chapter to the male population with IBS. Although IBS is more common in women than men, approximately one third of sufferers are male (National Institute for Health and Care Excellence, 2008). Further studies into cognitive/psychological therapies for IBS should consider recruiting a proportion of men that is representative of the disease population.

6.5 Summary

Despite a small sample of 15 individuals, behavioural data was sufficient to show that a six week course of MBCT-IBS was an effective treatment for this group of patients with IBS. Participants reported reduced gastrointestinal symptoms, including pain, as well as improved quality of life and reduced symptoms of anxiety. Sensory function was also affected by the intervention, with reduced pain sensitivity providing evidence for modulation of pain processing. However, improvement in function and quality of life was seen in mental rather than physical domains, indicating a possible improvement in coping ability.

A correlation between anxiety and pain severity identified anxiety as a possible mediator of some of the effects of MBCT-IBS. However, no linear relationship was found between anxiety and other improvements in outcome. From the behavioural data alone, the mechanisms by which mindfulness improves features of IBS remain unclear.

Neuroimaging has shown utility as a biomarker of analgesic effect in studies of pharmacological agents, providing evidence for both pharmacodynamic effect and

analgesic efficacy, as well as mechanistic information. Consequently, neuroimaging might also be a useful tool in elucidating the neurological mechanisms that underlie effective psychological interventions, as well as providing objective confirmation that the effects seen in trials are due to the mediation of brain function rather than the non-specific influence of other factors on subjective report.

Chapter 7: Central effects of mindfulness in chronic visceral pain

7.1 Introduction

In chapter 1, the need for a biomarker of pain or analgesia was discussed with respect to drug development, and neuroimaging was introduced as an objective way to make go/no-go decisions in early phase drug trials. Chapters 4 and 5 confirmed the utility of neuroimaging in this context by examining the behavioural and neural correlates of a novel pharmacological agent. This demonstrated that while behavioural data was inconclusive, imaging could give clear evidence that the drug had engaged key regions for pain modulation in the brain, and gave insight into the possible mechanisms of drug action, thus informing future clinical trials.

Unlike pharmacological agents, psychological and cognitive therapies do not have to go through a rigorous approval process prior to being used to treat patients. However, the issues around subjective, behavioural outcome measures remain the same. Proving efficacy often requires large trials, which can be costly and time-consuming. Therefore, there is a risk that therapies may be offered in which efficacy has not been robustly assessed, leading to the potential for suboptimal management of pain. The use of a biomarker of pain or analgesia could be of great benefit in determining which therapies should be recommended.

Additionally, behavioural trials give little insight into the neural mechanisms that underlie effective psychological treatments. Behavioural analgesia can be related to

contextual factors surrounding the administration of the intervention as opposed to being a direct result of the intervention itself. Imaging could help determine whether the reported effect on pain is accompanied by an intervention-mediated mechanism. Understanding the mechanistic basis of such interventions would also enable treatment to be targeted better at those who will benefit from it the most, and may also help to identify potential synergistic relationships between pharmacological and psychological therapies. (Goodwin et al., 2018).

The data presented in chapter 6 showed that mindfulness-based cognitive therapy (MBCT) may be an effective therapy for reducing the symptoms of irritable bowel syndrome (IBS), including the degree to which pain interferes with everyday activity. It also identified anxiety as a possible mediator of pain relief in mindfulness-based cognitive therapy for IBS (MBCT-IBS), and showed that MBCT-IBS may act on factors that influence the coping ability of an individual, thereby mediating the psychological sequelae of IBS. This chapter investigates the neural basis of these effects, and considers whether imaging could provide a useful biomarker for the efficacy of psychological/cognitive interventions for pain.

7.1.1 The central representation of pain in IBS

7.1.1.1 Structural imaging in IBS

Imaging studies have shown differences in the structure and organisation of the brain in IBS patients compared to healthy controls (Blankstein, Chen, Diamant, & Davis, 2010; J. Y. W. Chen, Blankstein, Diamant, & Davis, 2011; Ellingson et al., 2013; Jennifer S. Labus et al., 2014; Piché et al., 2013; Seminowicz et al., 2010). Although there is some variability in the regions that are implicated, a common finding is reduced

grey matter in parts of the prefrontal cortex (PFC; Jennifer S. Labus et al., 2014; Seminowicz et al., 2010). A study using diffusion tensor imaging to compare IBS patients and healthy controls found that the IBS patients had microstructural changes in regions involved in sensory integration and corticothalamic modulation, including the basal ganglia and thalamus. It also showed that there was increased structural connectivity between the thalamus and the anterior cingulate cortex (ACC) and PFC (Ellingson et al., 2013).

7.1.1.2 Imaging evoked pain in IBS

Imaging shows functional differences in the way that IBS patients and healthy controls respond to a noxious stimulus. As for acute somatic pain, the most commonly activated areas in visceral stimulation are the insulae and ACC, while the thalamus, primary somatosensory cortex (SI), PFC and posterior parietal cortex are also frequently affected. In patients with chronic abdominal pain, stimulus-related activity is seen in similar regions, but the amplitude of the modulation may differ (Mayer et al., 2009).

Although many patients with IBS experience both visceral and somatic hypersensitivity, imaging studies have focussed on neural responses to visceral stimuli. However, a pilot study comparing the effect of somatic thermal pain in patients with IBS and healthy controls showed differences in blood oxygen level dependent (BOLD) activity in the thalamus and in nodes of the default mode network, indicating that the function of attentional networks may be altered. Full details are given in appendix H.

A meta-analysis of 18 studies showed that in response to rectal stimulation, those with IBS showed more activity in regions implicated in both emotional arousal and endogenous pain modulation (amygdala, rostral anterior cingulate cortex [rACC], midbrain), while controls showed more activity in the medial PFC and dorsolateral PFC (dlPFC), possibly indicating engagement of mechanisms involved in the cognitive modulation of pain (Tillisch, Mayer, & Labus, 2011). Regions implicated in the sensory aspects of visceral pain, such as the thalamus, posterior insula, and SI, showed similar activation in both patients and controls. This raises the idea that it is the affective and cognitive aspects of pain processing that are affected in IBS.

The effect of IBS on the affective processing of pain is illustrated by a study investigating the impact of emotional context on neural responses to rectal distension. Compared to healthy controls, in the presence of psychological stress, subjects with IBS experienced increased stimulus-induced pain, as well as altered emotional modulation of activity in the insula, anterior midcingulate cortex (aMCC), ventrolateral PFC (vlPFC) and dlPFC (Elsenbruch et al., 2010).

Cognitive factors that influence pain perception in patients with IBS include attention and expectation. Patients with IBS show increased vigilance to visceral sensations (Posserud, Svedlund, Wallin, & Simrén, 2009). Subjects with IBS took part in a longitudinal study looking at the effect of rectal stimulation administered regularly over the course of a year. Repeated exposure led to the normalisation of subjective ratings, indicating reduced vigilance, and was accompanied by reduced activity in a central emotional arousal network incorporating limbic, paralimbic and pontine regions (Naliboff et al., 2006).

Patients with IBS differ from healthy controls with respect to their anticipation of a noxious stimulus. Berman et al. found that in IBS patients, the anticipation of rectal distension leads to less deactivation of the posterior insula and dorsal pons than in controls. The greater the anticipatory deactivation in the dorsal pons, the lower the negative affect reported during scanning, and the more activation that is seen in the right vlPFC and bilateral supragenual ACC during distension. This indicates that in IBS patients with low mood, the mechanisms of pain anticipation are abnormal, and that this may lead to reduced descending modulation from cortical regions (Berman et al., 2008; E. A. Mayer et al., 2009). Anticipatory anxiety is also altered in IBS, and the acquisition of pain-related fear in IBS patients is associated with increased activity in the vlPFC and amygdala (A Icenhour et al., 2015). Since the vlPFC is implicated in re-appraisal and attention (Katja Wiech, Ploner, et al., 2008), increased vlPFC activity might represent increased processing of possible threat and hypervigilance. The amygdala is a key part of the emotional network, and its increased activity is in keeping with enhanced fear-related learning (A Icenhour et al., 2015), which is known to be a feature of visceral pain (Benson et al., 2019; A Icenhour et al., 2015).

In healthy individuals, it has been shown that benign interoceptive sensations can become predictors of threat through context-dependent conditioning, and that this involves altered activity in fear-related structures including the amygdala, ventromedial PFC and hippocampus (Adriane Icenhour et al., 2021). Fear conditioning can generalise to other visceral stimuli, for which sensory thresholds may be altered (Zaman et al., 2016). These are mechanisms by which innocuous interoceptive sensations may invoke hypervigilance and symptom-related anxiety as well as hypersensitivity in patients with chronic visceral pain, such as those with IBS.

Placebo analgesia provides a means of demonstrating the effect of expectation on treatment outcomes. Placebo is known to act through manipulation of descending endogenous pain modulatory systems. Imaging studies of placebo in IBS show an impaired placebo response compared to healthy controls, with altered modulation of activity in regions associated with the affective or cognitive components of pain perception, such as the midcingulate cortex (MCC), vlPFC and insula (H. F. Lee et al., 2012; Schmid et al., 2015). These studies also indicate an association between psychiatric co-morbidity (specifically anxiety and depression) and an impaired placebo response. This supports the theory that descending pain modulation is disrupted in IBS in association with altered cognitive and emotional processing.

Further evidence that IBS is associated with less efficient endogenous pain modulation comes from studies of conditioned pain modulation (CPM), the phenomenon by which the perception of a noxious pain stimulus is reduced by the administration of a heterotopic noxious stimulus. CPM is widely used as a measure of the efficiency of descending pain inhibition. The response to a CPM paradigm can be very variable, and may depend on a range of factors relating to both the individual and the paradigm, including sex, age, features of the heterotopic stimulus, genetics and psychological factors (Firouzian et al., 2020; Graeff, Itter, Wach, & Ruscheweyh, 2021), making the determination of causal factors challenging. Compared to healthy controls, patients with IBS show reduced CPM (Albusoda et al., 2018; Marcuzzi et al., 2019), indicating reduced descending inhibitory control. Within this population, reduced CPM responses also correlate with measures of anxiety (Marcuzzi et al., 2019). Imaging during heterotopic stimulation shows that in IBS patients this difference is reflected by the presence of abnormal patterns of activation in multiple areas

implicated in endogenous pain modulation, including periaqueductal gray (PAG), anterior insula (aINS), amygdala and PFC (Song et al., 2006; Wilder-Smith, Schindler, Lovblad, Redmond, & Nirkko, 2004).

7.1.1.3 Resting state imaging in IBS

The imaging of responses to evoked pain gives useful information about the way in which acute noxious stimuli are processed. However, the imaging of patients during tonic pain, or at rest, may be more representative of their normal experiences. There is minimal data regarding the effect of IBS on resting brain activity.

One study used ALFF (amplitude of low-frequency fluctuation), a surrogate of spontaneous neural activity, to demonstrate that those with IBS had reduced resting activity in regions involved in the cognitive modulation of pain, including the dlPFC (Ma et al., 2015). ALFF has also been used to show that IBS patients display altered resting activity in several key nodes of the default mode network (the medial PFC, posterior cingulate cortex [PCC], and inferior parietal lobule), as well as parts of the PFC and ACC/MCC (Qi et al., 2015).

A limited number of studies have used resting state imaging to investigate resting functional connectivity in IBS. Using seed-based analysis, Qi et al. showed that in those with IBS, compared to healthy controls, the amygdalae had greater functional connectivity with the insula and midbrain, and this connectivity correlated with the symptom severity reported by patients. There was also greater functional connectivity with sensorimotor regions, which correlated with pain intensity (R. Qi et al., 2016). In another study using seed-based analysis, women with IBS were found to have more

negative functional connectivity than healthy controls between the dorsal aINS and the PFC and left precuneus, the latter correlating with their symptom intensity. Meanwhile, in men with IBS there was a correlation between symptom-related anxiety and the connectivity between the dorsal aINS and the dorsomedial PFC (dmPFC; Hong et al., 2014). Another study that used seed-based analysis showed that IBS patients had increased functional connectivity between the MCC and parts of the PFC (Ma et al., 2015).

Icenhour et al. used independent component analysis (ICA) and dual regression to compare the functional connectivity of the default mode network (DMN), salience network (SN) and sensorimotor network in hypersensitive and normosensitive IBS patients as well as healthy controls (Adriane Icenhour et al., 2017). They found that compared to normosensitive patients, patients with visceral hypersensitivity had increased connectivity within the SN and sensorimotor network. Meanwhile normosensitive patients showed altered DMN connectivity compared to both other groups.

7.1.1.4 The role of brain networks in IBS

It is increasingly understood that functions such as sensory processing, emotional processing and cognition rely on the interaction of multiple regions operating in networks. Networks implicated in the processing and modulation of visceral pain include the SN, central executive network (CEN), sensorimotor network, emotional arousal network, DMN, and central autonomic network, and these networks interact with one another (Mayer et al., 2019; figure 7.1). The evidence outlined above shows that patients with IBS may show disturbances in brain regions associated with each of

these networks, and this may contribute to symptoms. For example, abnormalities in salience detection could make gastrointestinal sensations appear more prominent. In turn this may lead to increased engagement of the CEN, resulting in hypervigilance and catastrophizing. All of these changes may lead to alterations in endogenous pain modulation and in autonomic output to the gastrointestinal tract (Mayer, Labus, Tillisch, Cole, & Baldi, 2015).

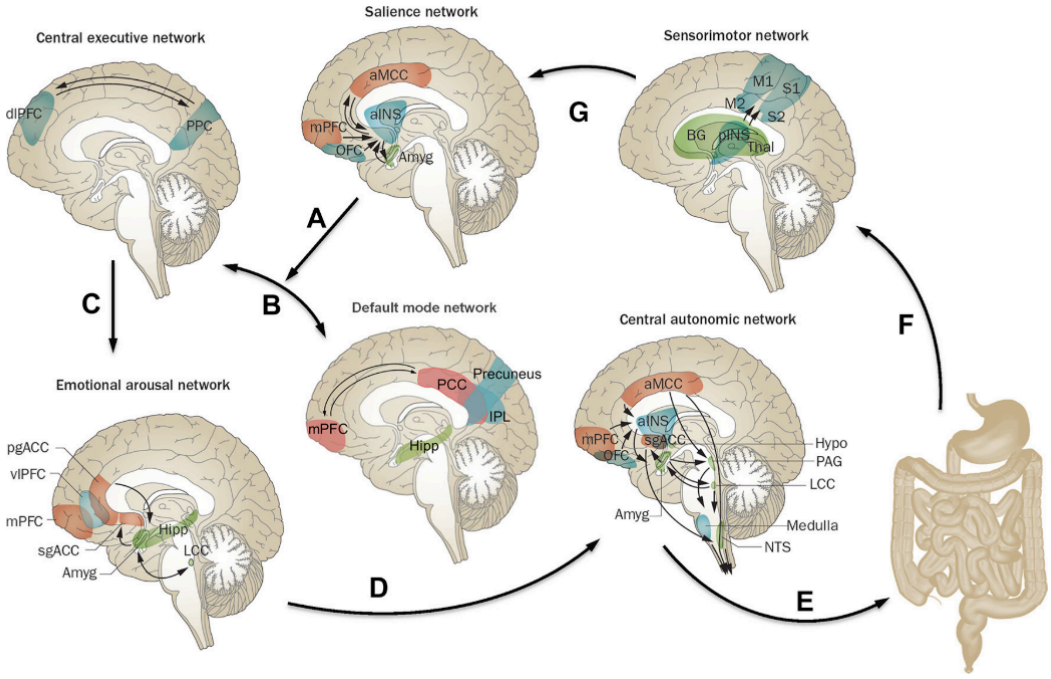


Figure 7.1: Brain networks involved in central processing and modulation of visceral pain. Arrows indicate: (A) shift of activity from the default mode network to the task-related networks in response to input from the salience network; (B) switching between default mode network and central executive network depending on input from the salience network; (C) engagement of emotional arousal network in response to central executive network activation; (D) engagement of central autonomic network in response to emotional arousal network activation; (E) central autonomic network activation with output in the form of descending pain modulation and autonomic nervous system activity to gastrointestinal tract; (F) ascending viscerosensory signals from gut to sensorimotor network; and (G) assessment of information from sensorimotor network by salience network. From Mayer et al. (2019).

7.1.2 The neural mechanisms of mindfulness meditation

Multiple studies have investigated the effect of mindfulness meditation on neural activity as measured by functional MRI. The results are not always consistent, and this is likely to be partly due to the diversity in study protocols (Marchand, 2014). The populations assessed are very variable, as are the definitions of mindfulness and the level of experience of the meditators.

Across studies, the regions in which activation is most consistently altered following a mindfulness intervention are the insula cortex, the ACC and the PFC (Tang, Hölzel, & Posner, 2015; Young et al., 2018). The insula and ACC are key regions of the SN and are also implicated in the emotional and cognitive aspects of pain. They are important regions for interoceptive attention (Craig, 2002; X. Wang et al., 2019). The PFC has a role in executive function and attention, and some sub-regions have been shown to be heavily implicated in the top-down cognitive control of pain processing (Katja Wiech, Ploner, et al., 2008). A meta-analysis done in 2018 showed that in novice meditators, mindfulness produced increased activity in the insula, while in experienced meditators activity was seen in the medial frontal gyrus/rACC and the putamen/globus pallidus (Falcone & Jerram, 2018). Meanwhile a systematic review looking purely at individuals undergoing eight-session mindfulness interventions showed the most consistent longitudinal effect to be increased activity in the insular cortex. Altered ACC and PFC activity were also noted to be common, although the PFC sub-region affected was not consistent (Young et al., 2018). Other regions that have been identified in multiple studies of mindfulness include the precuneus and PCC, which also have a role in self-referential processing; the caudate, a key region for reward; and the amygdala, an important region for emotional regulation and learning and for

descending pain modulation (Gotink, Meijboom, Vernooij, Smits, & Hunink, 2016; Marchand, 2014; Young et al., 2018; Zeidan, Baumgartner, & Coghill, 2019).

Resting state studies have focussed on the effect of mindfulness on attentional networks. There is evidence that trait mindfulness, which is associated with higher pain thresholds, is associated with reduced connectivity between nodes of the DMN (Harrison, Zeidan, Kitsaras, Ozcelik, & Salomons, 2019). Reduced connectivity within the DMN is also seen in those who have better mindfulness ability after a short period of training (Doll, Hölzel, Boucard, Wohlschläger, & Sorg, 2015).

Bilevicius et al. (2018) correlated trait mindfulness scores with connectivity within the DMN, SN, and left and right CENs. They found that scores correlated with connectivity both within and between networks. This is not the only study to show that attentional networks are altered in mindfulness. Doll et al. (2015) showed that increased mindfulness ability correlated with reduced connectivity between anterior and posterior components of the DMN, and reduced connectivity between the DMN and parts of the SN. Overall, the effects of mindfulness on attentional network connectivity have produced variable results, and in reality there's probably increased connectivity between some regions and decreased connectivity between others. The key message appears to be that connectivity both within and between networks is significantly altered as a function of mindfulness.

7.1.3 The neural mechanisms of mindfulness in pain

Fewer studies have focussed specifically on the neuroimaging of mindfulness as an intervention for pain. The majority of these have used thermal or electrical pain as an

experimental pain model in healthy individuals. Consistent with the other mindfulness literature, these studies found that post-mindfulness there was altered activity in the ACC/aMCC and insula during evoked pain (Gard et al., 2012; Grant, Courtemanche, & Rainville, 2011; Lutz, McFarlin, Perlman, Salomons, & Davidson, 2013; Zeidan et al., 2015, 2011). Zeiden et al. (2011) found that increased stimulus-induced activity in the ACC and aINS was correlated with the reduction in pain intensity that was achieved, while an increase in orbitofrontal activity correlated with reduction in pain unpleasantness. They also found that meditation led to reduced pain-induced SI activity. Although not consistent, some studies also reported altered activity in the PFC (particularly the dlPFC), thalamus, amygdala, SI and SII (Gard et al., 2012; Grant et al., 2011; Zeidan et al., 2015, 2011).

There is evidence that mindfulness-induced analgesia may be reduced by the administration of naloxone, indicating that it is at least partially mediated by opioidergic mechanisms (Sharon et al., 2016). This supports the hypothesis that it acts through the descending modulatory system, the components of which include many of the regions mentioned above, including the ACC, PFC and amygdala. However, these findings have been disputed by one research group, who found that naloxone did not prevent mindfulness-induced analgesia in either experienced meditators (L. M. May, Kosek, Zeidan, & Berkman, 2018), or meditation-naïve participants (Zeidan et al., 2016).

A criticism of evoked pain studies in healthy individuals is that they may not reflect real-life outcomes in chronic pain patients due to a lack of underlying pathology and a model that is not reflective of the tonic nature of chronic pain. One study used BOLD

fMRI to examine the effect of mindfulness on chronic pain patients. Su et al. (2016) found that a six week mindfulness intervention was effective at reducing self-reported pain scores, and that increased connectivity between the aINS and the dorsal aMCC was correlated with the change in pain scores. However, the nature of the chronic pain in this cohort is not clear and the main inclusion criterion of a present pain score of at least 1/10 does not appear to be a very robust indicator of chronic pain. In view of this, a study of mindfulness in patients with chronic pain and/or a tonic pain model would be a useful next step.

7.1.4 Chapter objectives

The aims of this chapter are twofold. Firstly, to determine the neural mechanisms of mindfulness-induced analgesia in a group of patients with chronic pain, and secondly, to assess whether neuroimaging could be a useful biomarker for the analgesic efficacy of a psychological/cognitive intervention.

Based on the existing literature, it is hypothesised that MBCT-IBS will be associated with altered activity in and connectivity between regions involved in the affective aspects of pain perception, as well as those implicated in the cognitive control of descending pain modulation. These regions could include the amygdalae, PFC, ACC and insulae, as well as brainstem structures such as the PAG and rostral ventromedial medulla (RVM). It is also predicted that there will be altered functional connectivity in networks implicated in attentional control, such as the DMN and SN.

Given the link seen between reported anxiety and pain in chapter 6, it is also hypothesised that controlling for anxiety will have a significant impact on the changes

that are seen in neural activation as a result of MBCT-IBS. In particular, it is predicted that it will impact the effect of MBCT-IBS on activity in components of the descending modulatory system, since anxiety is known to influence pain through top-down pathways (Wiech et al., 2014). Finally, it is predicted that altered neural activity in the descending modulatory system will relate closely to behavioural measures of symptom improvement. Given the fact that MBCT-IBS was shown to be an effective therapy for IBS, not only in chapter 6, but also in the larger randomised controlled trial from which these patients were recruited (Henrich et al., 2020), this would indicate that neuroimaging has the potential to provide an objective biomarker of efficacy in cognitive therapies.

7.2 Methods

7.2.1 Study design

This was a within-subject interventional study, with imaging visits taking place before and after MBCT-IBS. Full details of the study design are given in chapter 6.

7.2.2 Participants

This chapter refers to the imaging data from the 15 participants whose behavioural data was presented in chapter 6. However, due to technical issues with the operating of the thermode, the BOLD thermal pain results are based on scans from 13 individuals.

7.2.3 Brain imaging acquisition

Participants completed a whole brain arterial spin labelling (ASL) scan and a BOLD resting state scan. They also underwent BOLD imaging while completing an evoked

thermal pain task. A T1-weighted structural image with 2x2mm voxels was obtained for registration and voxel selection. A fieldmap was obtained to correct for field inhomogeneities in EPI acquisitions. All imaging data were acquired on a Siemens 3T Prisma using a 32-channel head coil.

7.2.3.1 BOLD imaging

7.2.3.1.1 Resting state

Whole brain resting state data were acquired as described in chapter 5 (section 5.2.2.1.1), except for the flip angle which was 64°.

7.2.3.1.2 Evoked pain

BOLD data were acquired throughout an evoked pain task. 654 volumes were acquired using identical parameters to the BOLD resting state scan. The evoked pain task is described in chapter 6, section 6.2.5.

7.2.3.2 Arterial spin labelling

Whole brain ASL data was acquired as described in chapter 5 (section 5.2.2.2), using the same parameters.

7.3 Analysis of brain imaging data

7.3.1 BOLD imaging of evoked pain

Processing of images from both studies was performed using FSL (FMRIB's Software Library; www.fmrib.ox.ac.uk/fsl). Initial pre-processing of functional images was performed with the Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) GUI using the same protocol as described in

chapter 5 (section 5.2.3.1.1), except that the highpass temporal filtering cutoff was 100 seconds. ICA denoising was also performed using the same protocol as chapter 5 (section 5.2.3.1.1), and was applied during first-level analysis. Physiological noise monitoring was not applied as use of the thermode interfered with the cardiac and respiratory trace.

First level analysis of each scan was performed in FEAT (FMRI Expert Analysis Tool; Woolrich, Ripley, Brady, & Smith, 2001). The timecourse of the evoked pain stimuli was entered as the regressor of interest. Timecourses were also created for the visual presentation of instructions to the participant, and for the completion of pain rating scales. These were included as regressors of no interest.

Higher level analyses were then completed using a design matrix appropriate to each research question. Whole brain analyses with mixed effects were performed using FLAME 1 (Woolrich et al., 2004). Cluster-based familywise error correction was used for multiple comparisons ($Z>2.3$; $p<0.05$). Possible confounds were examined by adding them as regressors.

Region of interest (ROI) analyses were performed using randomise, a tool for nonparametric permutation testing (Winkler et al., 2014). For each ROI, 5000 permutations were performed on threshold-free cluster-enhanced values, yielding a familywise error correction rate of 0.05. The temperature of the thermode was added to the model as a regressor of no interest.

The ROIs selected for analysis were the left and right amygdalae, the PAG and the RVM. These regions were chosen based on their role in descending pain modulation. The mask for each amygdala was an anatomical mask defined using the Harvard-Oxford probabilistic atlas and thresholded to 50%. The RVM and PAG were masked using hand-drawn anatomical masks, with Duvernoy's Brainstem Atlas as a guide. The RVM mask was originally utilised in Soni et al. (2019).

7.3.2 BOLD imaging of resting functional connectivity

7.3.2.1 Dual regression

Pre-processing was performed as described above (section 7.3.1). A group-average ICA was performed using MELODIC. Then subject-specific versions of the spatial maps and associated timecourses were generated by regressing the group-average spatial maps and timecourses, using multiple regression, into each subject's dataset (Beckmann et al., 2009; Nickerson et al., 2017). The permutation-testing tool randomise was then used to look for group differences (Winkler et al., 2014).

7.3.2.2 Seed-based analysis

Whole brain BOLD resting state analysis was performed with the right amygdala, precuneus, left and right dlPFC and left and right dmPFC as seed regions. The right amygdala was chosen as it showed altered stimulus-induced activation following MBCT-IBS, and is known to have a role in descending pain modulation and the affective component of pain. An anatomical mask was defined using the Harvard-Oxford subcortical structural atlas, and was thresholded to 50%. A functional mask was also created from the pattern of activation seen during the task-based analysis.

The precuneus also showed changes in stimulus-related activity after MBCT-IBS, as did the left and right dmPFC once thermode temperature was controlled for. Both are key nodes of the DMN, a network thought to be affected by mindfulness (Marchand, 2014). Functional masks for these areas were created based on the regions identified in the task-based analysis. Functional masks were also created for the left and right dlPFC. Both regions showed altered stimulus-related activity post-mindfulness, and are thought to be involved in the cognitive control of descending pain modulation (Katja Wiech, Ploner, et al., 2008).

Pre-processing was performed as described above (section 7.3.1). Data were denoised using the automated ICA denoising tool FIX (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014). The cleaned data was registered to standard space. A mask of each seed region was created, and the timecourse within that region was extracted using the tool fslmeants from FSL. Timecourses were also extracted for the cerebrospinal fluid (CSF) and white matter.

Whole brain paired t-tests comparing pre-intervention to post-intervention were performed within FEAT, with the timecourse of each seed region as the regressor of interest, and the CSF and white matter timecourses as regressors of no interest.

The masks for all seed regions are illustrated in appendixAppendix K:

7.3.3 Imaging cerebral blood flow with ASL

Single subject analysis was performed as described in chapter 2 (section **Error! Reference source not found.**).

Higher statistical analysis was performed with FEAT (Woolrich et al., 2004, 2001). A paired t-test was performed with a mixed effects analysis using FLAME 1 (Woolrich et al., 2004). Cluster-based familywise error correction was used for multiple comparisons ($Z > 2.3$; $p < 0.05$).

7.4 Results

7.4.1 Examining the effects of mindfulness-based cognitive therapy on neural correlates of experimental pain

7.4.1.1 Checking the effectiveness of the experimental pain model

In a pilot study (appendix H), the experimental pain model was shown to be effective in activating pain-related regions as expected in healthy volunteers and in IBS patients who had not undergone any mindfulness therapy. Assessment of the group mean activation during thermal pain for the participants completing MBCT-IBS confirms that the model is effective in this group. As seen in the pilot data, there is activation in key pain regions, including the somatosensory cortices, insulae, thalamus, PAG, basal ganglia, ACC and PFC (Figure 7.2). There appears to be greater activation in pain-related areas following MBCT-IBS. However, it is not clear whether this is the effect of the intervention or of differences in peripheral input. The fact that a higher temperature was required to induce the same pain intensity rating at the second visit indicates that MBCT-IBS did have an effect on sensory processing (see section 6.3.5).

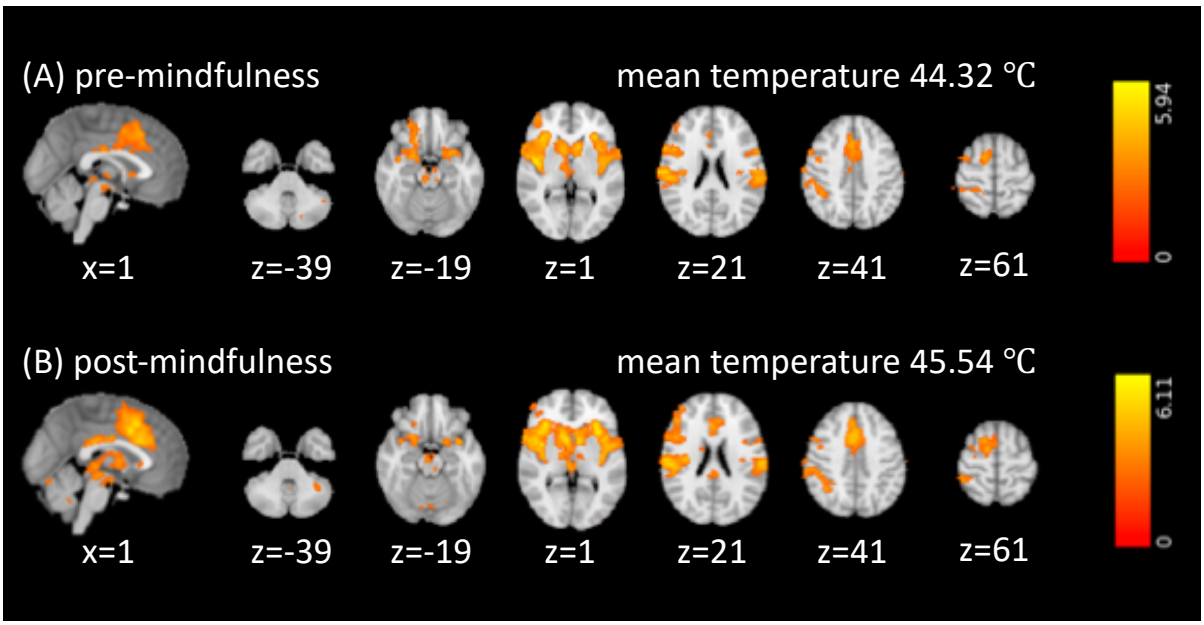


Figure 7.2: Group averaged activation maps showing the neural correlates of a thermal pain stimulus prior to MBCT-IBS (A) and after completion of MBCT-IBS (B). The stimulus was perception locked to a pain intensity rating of 5/10, and the mean temperature of the stimulus for each timepoint is shown. All images are in radiological convention. Slices are identified by MNI coordinates. In both groups there was activation in key pain regions including the primary and secondary somatosensory cortices, thalamus, periaqueductal gray, basal ganglia, insula, anterior cingulate cortex and prefrontal cortex (n=13; mixed effect; Z>2.3, p<0.05; cluster corrected).

7.4.1.2 Comparing neural responses to pain before and after mindfulness

A whole brain paired t-test was performed comparing the neural activation evoked by a painful thermal stimulus before and after MBCT-IBS. In the pre-intervention>post-intervention contrast, no differences in activity were seen. In the post-intervention>pre-intervention contrast significant activity was seen in the precuneus, right angular gyrus, right lateral occipital cortex and parts of the visual cortex (Figure 7.3).

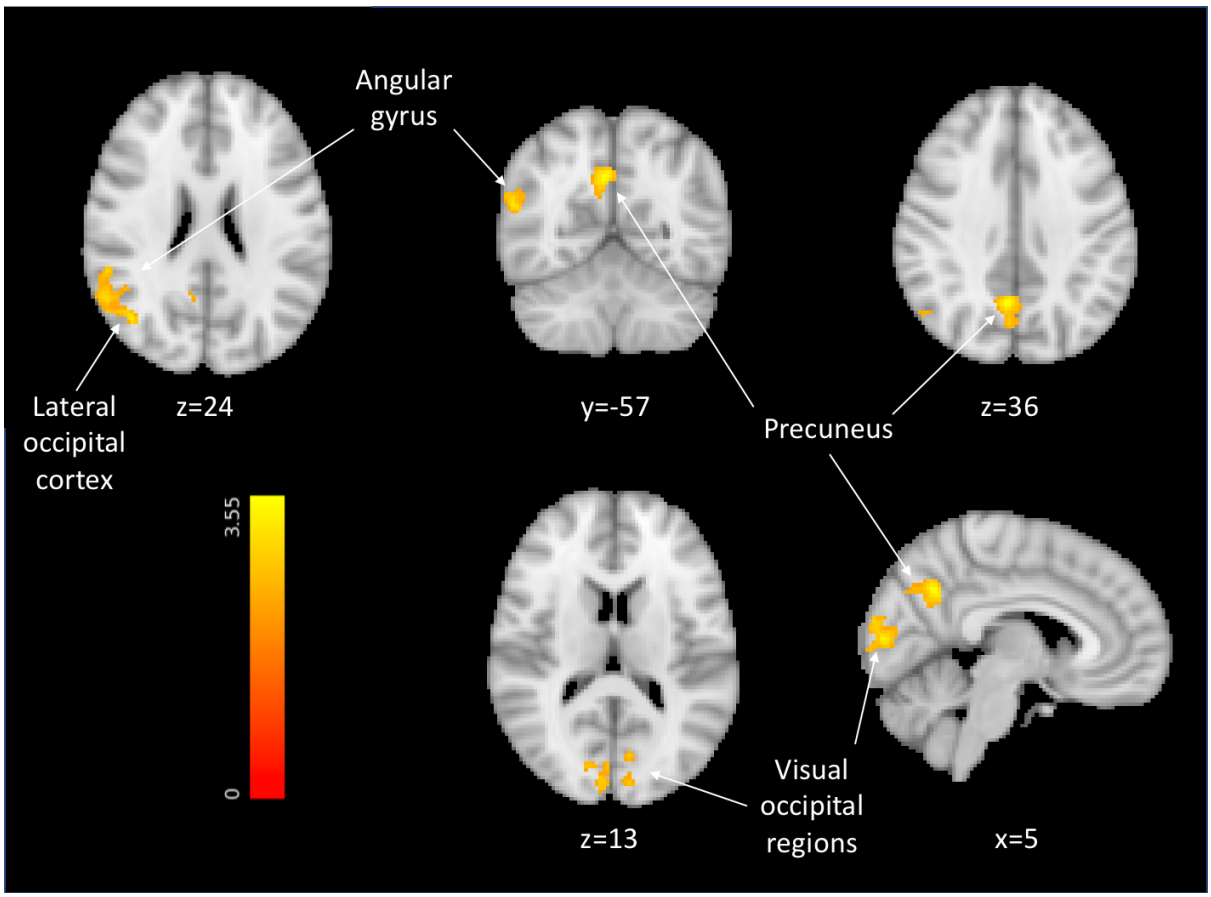


Figure 7.3: Effect of MBCT-IBS on brain activity during a noxious stimulus. Activation map shows the clusters in which there was a significant increase in stimulus-related activity from pre-intervention to post-intervention in a whole brain paired t-test (n=13; mixed effects; Z>2.3, p<0.05; cluster corrected). Images are in radiological convention and slices are identified by MNI coordinates. Following the intervention, there was increased activity in the precuneus, right angular gyrus, right lateral occipital cortex and bilateral visual occipital regions.

Within the areas of the precuneus in which stimulus-induced activity was altered following the intervention, mean change in BOLD activity was extracted for each participant using featquery. The apparent increased activation was found to represent a reduction in deactivation in this region following MBCT-IBS (Figure 7.4).

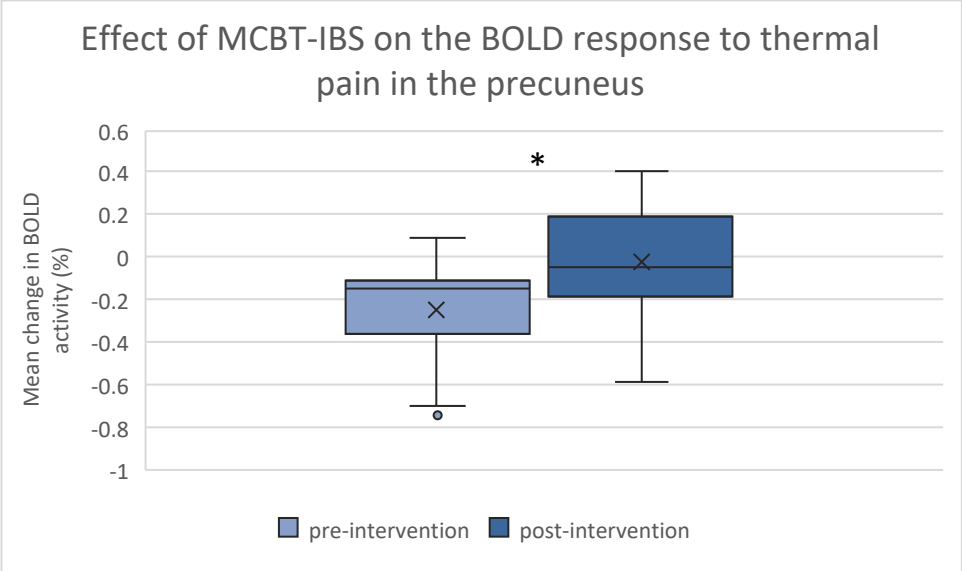


Figure 7.4: Effect of MBCT-IBS on stimulus-induced activity in the precuneus. Boxplots show the distribution of mean BOLD responses to noxious thermal stimulation in the precuneus in participants with irritable bowel syndrome both pre-intervention and post-intervention (n=13). The group mean is denoted by a cross. Prior to MBCT-IBS, the majority of participants showed stimulus-induced deactivation, demonstrated by a negative change in BOLD activity. On average there was reduced deactivation post-intervention, and an increased number of participants showed stimulus-induced activation instead. The change is marked with an asterisk as it was statistically significant (p≤0.05),

7.4.1.3 Removing the effect of variation in peripheral input

As the thermal stimulus was perception locked, the temperature administered varied between individuals and between visits. In order to determine whether variation in peripheral nociceptive input was affecting the outcome of the pre-intervention to post-intervention comparison, the whole brain paired t-test was repeated with temperature as a regressor of no interest (Figure 7.5). There remained no regions in which activity was reduced following MBCT-IBS. However, the post-intervention>pre-intervention contrast revealed extensive increased stimulus-related activity in multiple sub-regions of the bilateral PFC as well as a small area of the rACC. Increased activity was still present in the precuneus, and was also now present in other areas of the superior

parietal lobule. There was no longer increased activity in the right angular gyrus and lateral occipital cortex, and although there was still increased activity in the visual cortex, it was in a slightly different region.

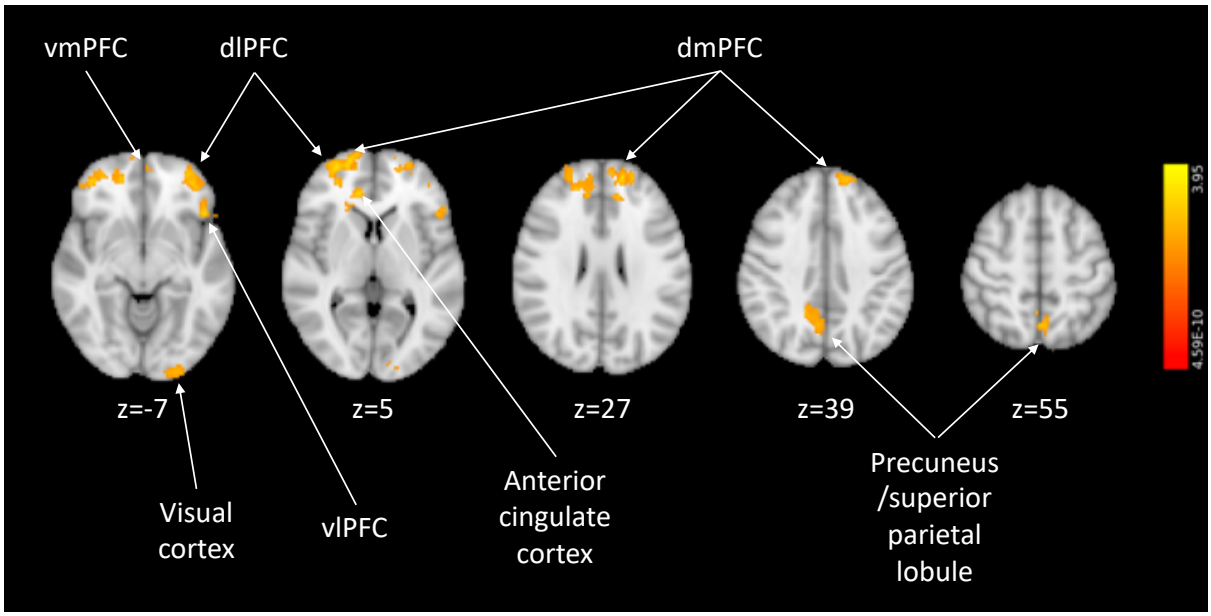


Figure 7.5: Effect of MBCT-IBS on brain activity during a noxious thermal stimulus when controlling for temperature of stimulus. Activation map shows the clusters in which there was a significant increase in stimulus-related activity in IBS patients from pre-intervention to post-intervention in a whole brain paired t-test (n=13; mixed effects; Z>2.3, p<0.05; cluster corrected). Images are in radiological convention and slices are identified by MNI coordinates. Following the intervention, there was increased activity in the prefrontal cortex bilaterally, as well as in the rostral anterior cingulate cortex, precuneus/superior parietal lobule and visual cortex. *dorsolateral (dlPFC); dorsomedial (dmPFC); ventrolateral (vlPFC); ventromedial (vmPFC).*

Within the regions of the PFC in which stimulus-induced activity was altered following MBCT-IBS, the mean % change in activity was extracted with featquery. On average, this change was found to represent reduced deactivation rather than increased activation (Figure 7.6).

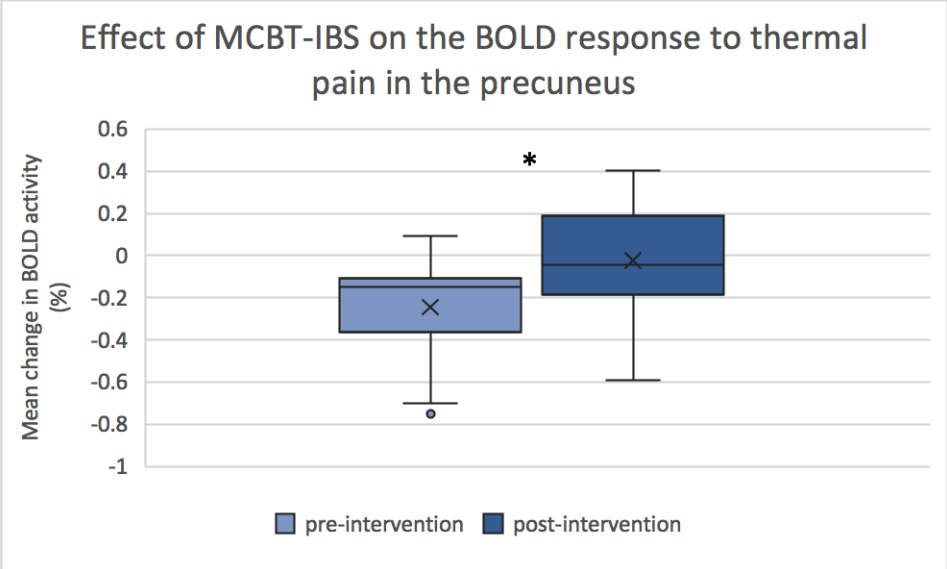


Figure 7.6: Effect of MBCT-IBS on stimulus-induced activity in the left prefrontal cortex (PFC). Boxplots show the distribution of mean BOLD responses to noxious thermal stimulation in the left PFC in participants with IBS both pre-intervention and post-intervention (n=13). The group mean is denoted by a cross. Prior to MBCT-IBS, the majority of participants showed stimulus-induced deactivation, demonstrated by a negative change in BOLD activity. On average there was reduced deactivation post-intervention. The change is marked with an asterisk as it was statistically significant ($p \leq 0.05$),

To determine whether the change in frontal activation correlated with the change in symptoms or anxiety, change in left and right PFC activation was compared with change in brief pain inventory – short form (BPI) pain severity, gastrointestinal symptom rating scale (GSRS) and visceral sensitivity index (VSI) using Spearman correlation coefficients. Functional masks were used to ensure that the analysis was restricted to voxels in which there was a significant change in activity. A significant negative correlation was found between the change in BPI pain severity and the change in left PFC activity. In order to determine whether this was due to changes in a specific sub-region of interest in the left PFC, change in BPI severity was compared to change in left dmPFC activation, left dlPFC activation and left vlPFC activation separately (

Table 7.1). Only the left dlPFC activation correlated significantly with BPI severity. This is illustrated in Figure 7.7.

(A)

Outcome measure	Region of activation	Correlation coefficient (r_s)	N	Significance
BPI severity	L PFC	-0.579	13	0.038
	R PFC	-0.160	13	0.602
GSRS	L PFC	-0.182	12	0.571
	R PFC	-0.214	12	0.505
VSI	L PFC	-0.081	12	0.803
	R PFC	0.039	12	0.905

(B)

Outcome measure	Region of activation	Correlation coefficient (r_s)	N	Significance
BPI severity	L dlPFC	-0.614	13	0.025
	L dmPFC	-0.322	13	0.283
	L vlPFC	-0.004	13	0.990

Table 7.1: Table showing Spearman correlation coefficients for (A) comparison of change in pain severity (BPI), symptoms (GSRS) and symptom-specific anxiety (VSI) with change in left and right PFC activation from pre-intervention to post-intervention; (B) comparison of change in pain severity (BPI) with change in left dorsolateral (dlPFC), left dorsomedial (dmPFC) and left ventrolateral (vlPFC) PFC activation from pre-intervention to post-intervention. P values of ≤0.05 were considered significant and are coloured red.

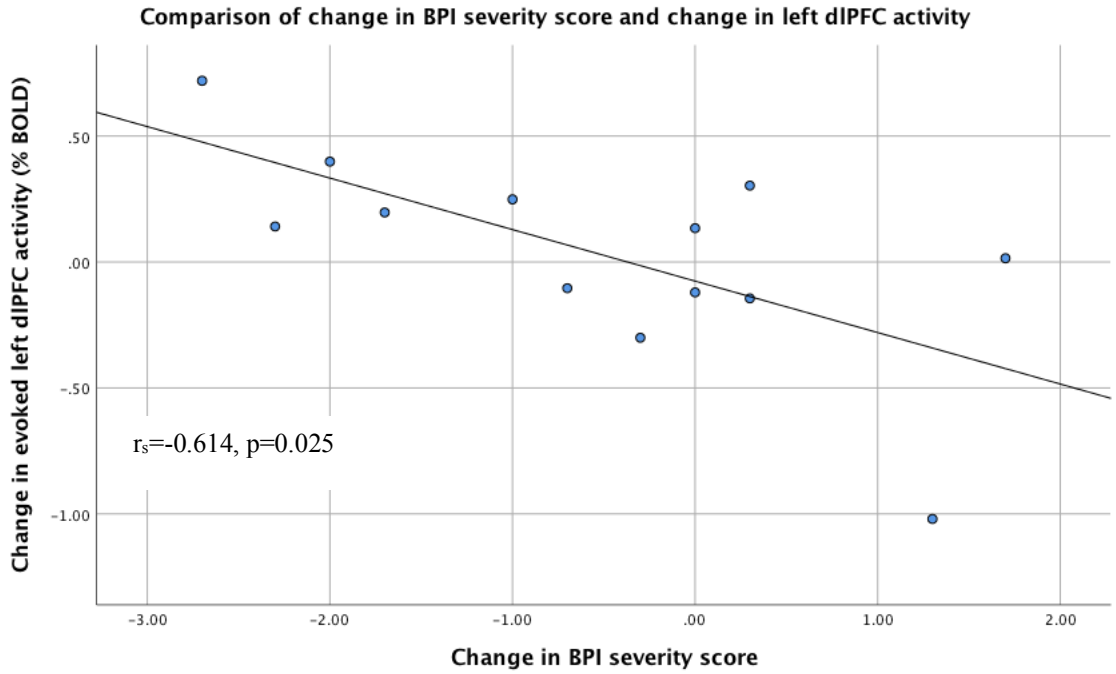


Figure 7.7: Scatterplot with line of best fit showing the correlation between the change in pain severity (brief pain inventory [BPI], pain severity score) and the change in evoked left dorsolateral prefrontal cortex (dlPFC) activity in participants with irritable bowel syndrome from pre-intervention to post-intervention (n=13). A negative change in the BPI severity score indicates reduced pain severity, while a positive change indicates increased pain severity. A bigger reduction in pain severity was associated with increased left dlPFC activation (or reduced deactivation).

7.4.1.4 Considering background pain as a possible confound

Pilot data (see appendix H-3.4.3) showed that in IBS, current background pain was positively correlated with reduced stimulus-induced deactivation in a range of regions, most notably several components of the DMN (precuenus, PCC, bilateral angular gyri). Therefore, background pain was considered a possible confound to the imaging results in this chapter. However, the decision of whether or not to correct for background pain is complicated by the fact that variation may be disease related, since episodic pain is characteristic of IBS, but may also represent the effect of the intervention. Therefore correcting for background pain may reduce the ability of imaging to detect the true effects of MBCT-IBS.

Pain intensity on the day of imaging was variable (pre-intervention mean 1.80, SD -1.74; post-intervention mean 1.33, SD -1.50), and the change from pre-intervention to post-intervention was not consistent (mean -0.33, SD -2.02). However, the mean change was small, and a paired t-test showed that it was not statistically significant (mean 0.467, BCa 95% CI [-0.533, 1.467]; t(14)=0.875, p=0.396). Therefore the decision was made not to correct for background pain intensity in this chapter.

7.4.1.5 Determining the effect of anxiety

Scores from the anxiety subscale of the hospital anxiety and depression scale (HADS-A) and VSI were added as regressors in order to investigate the effect of controlling for either general anxiety or symptom-specific anxiety respectively. When VSI score was regressed out, all changes in stimulus-induced activity from pre-intervention to post-intervention were eliminated regardless of whether or not temperature was also controlled for. Controlling for HADS-A led to removal of the effects on activation when temperature was not controlled for. When temperature was also controlled for, the majority of changes in frontal activation were abolished. However, a minimal increase in right rostral middle frontal gyrus activity remained, as did the small area of increased rACC activity (Figure 7.8).

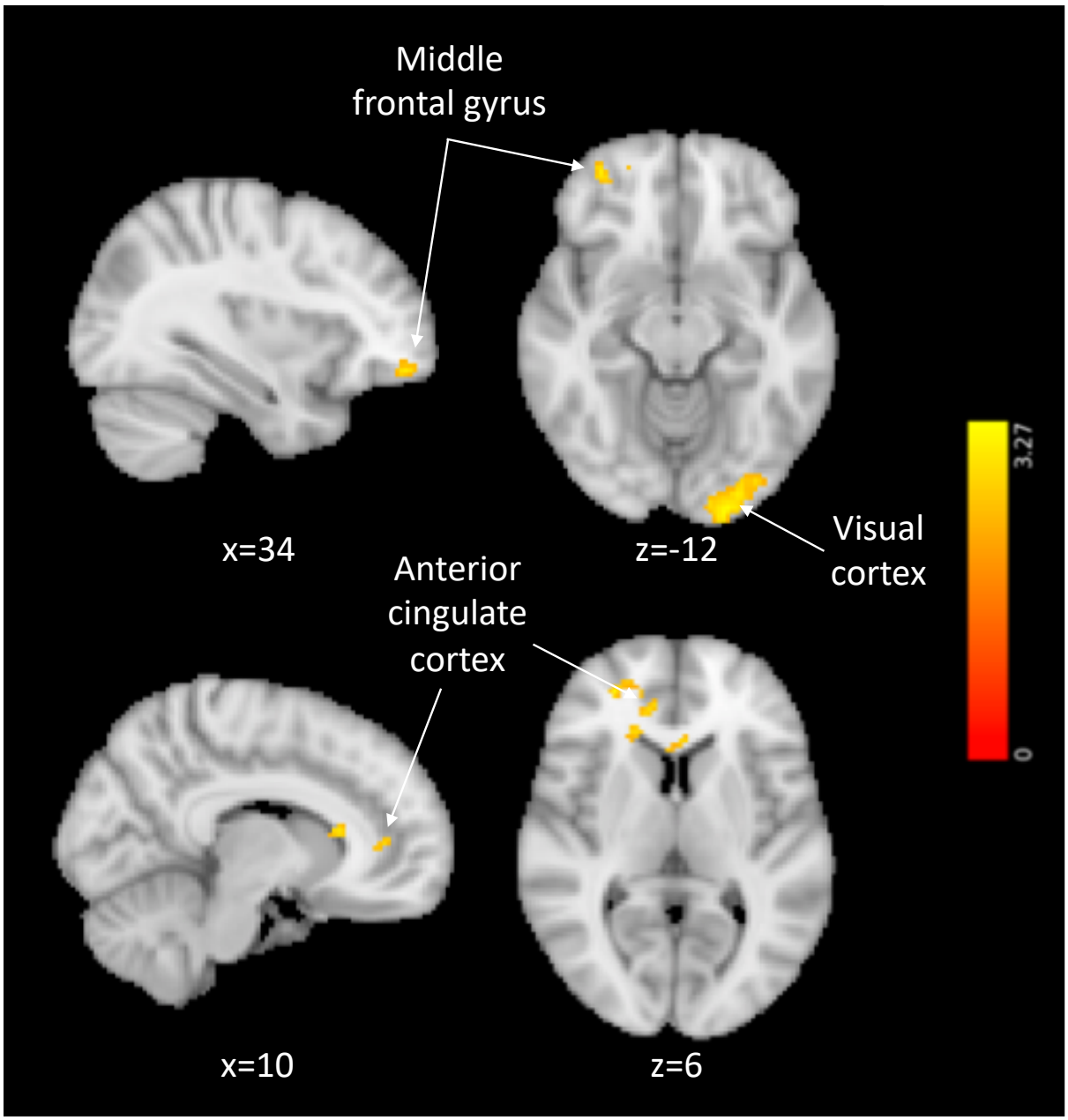


Figure 7.8: Determining the effect of MBCT-IBS on brain activity during a noxious thermal stimulus when controlling for generalised anxiety (hospital anxiety and depression scale – anxiety subscale) and thermode temperature. Activation map shows the clusters in which there was a significant increase in stimulus-related activity in participants with irritable bowel syndrome from pre-intervention to post-intervention in a whole brain paired t-test (n=13; mixed effects; Z>2.3, p<0.05; cluster corrected). Images are in radiological convention and slices are identified by MNI coordinates. Following the intervention, there was increased activity in the rostral anterior cingulate cortex and the right middle frontal gyrus.

7.4.1.6 Determining the effect of trait motivational style on left dlPFC activity

In chapter 6, it was shown that those with higher baseline scores in the BAS fun-seeking subscale of the behavioural inhibition system/behavioural activation system (BIS/BAS) scale had a greater reduction in the BPI pain severity score following MBCT-IBS. In this chapter, the effect of MBCT-IBS on BPI pain severity scores has been shown to correlate with changes in left dlPFC activity. This, in addition to the known role of the dlPFC in motivational processing (Spielberg et al., 2012), led to the hypothesis that motivational style at baseline might impact the change seen in left dlPFC activity following MBCT-IBS.

To look for an effect of trait motivational style on changes in left dlPFC activity, baseline scores on the BIS/BAS scale were compared with the change in stimulus-induced left dlPFC activity from pre-intervention to post-intervention using a Spearman correlation coefficient. This showed a significant positive correlation for BAS fun-seeking, indicating that those with higher BAS fun-seeking scores at baseline had a greater increase (or smaller decrease) in the left dlPFC response to a thermal stimulus as a result of MBCT-IBS (Figure 7.9). The BAS drive and reward responsiveness subscales showed no correlation with change in left dlPFC activity, and nor did the BIS scale. Full results are shown in appendix J-7.

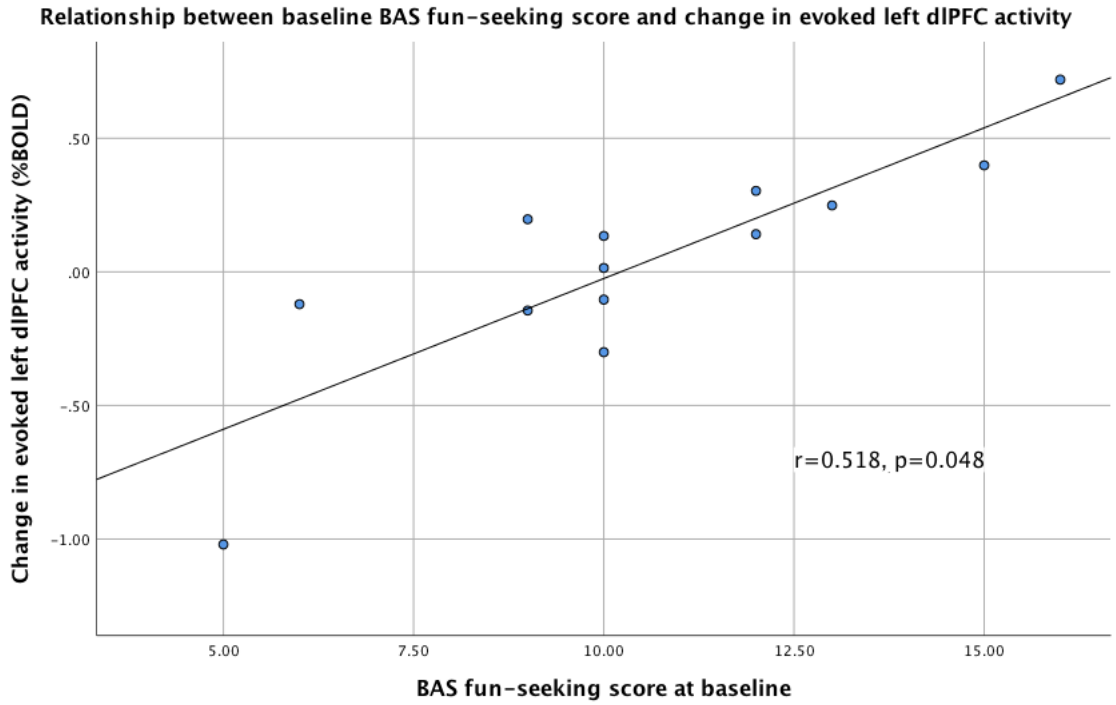


Figure 7.9: Scatterplot with line of best fit showing the correlation between trait motivational style (behavioural activation system [BAS] fun-seeking score) and the change in evoked left dorsolateral prefrontal cortex (dlPFC) activity in participants with irritable bowel syndrome from pre-intervention to post-intervention (n=13). A higher baseline fun-seeking score was associated with increased left dlPFC activation (or reduced deactivation).

7.4.1.7 Examining the effect of mindfulness on regions of interest

Due to their role in the descending modulatory system, randomise was used to perform region of interest analyses on the right amygdala, left amygdala, PAG and RVM. Pre-intervention and post-intervention activity was compared for each region using a paired t-test. The temperature administered was added as a regressor of no interest. In the right amygdala there was a significant reduction in BOLD activity following the mindfulness intervention (Figure 7.10). No significant differences were seen in the other regions.

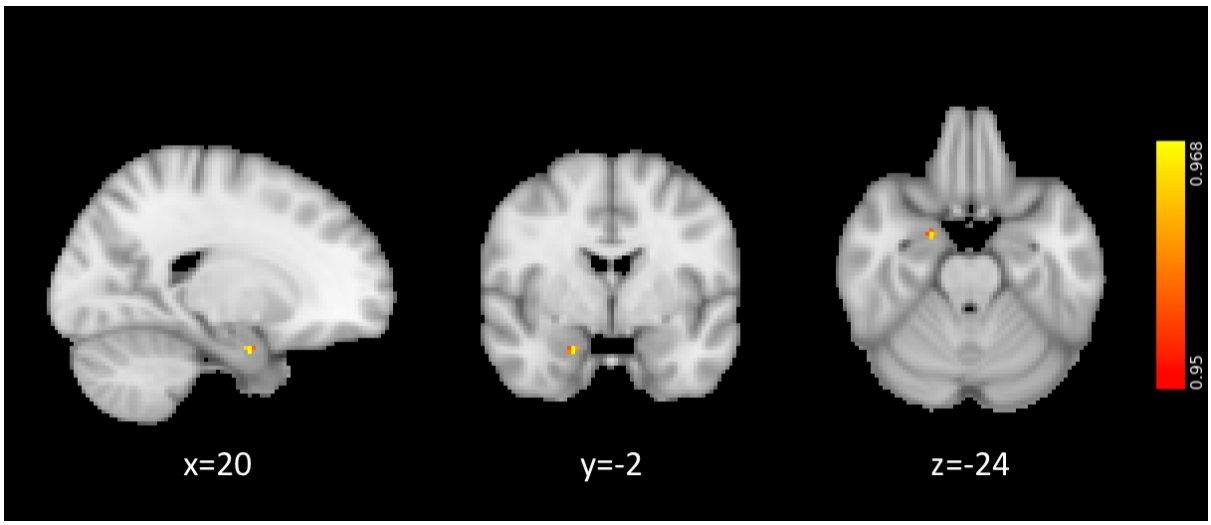


Figure 7.10: Effect of MBCT-IBS on right amygdala activity in participants with irritable bowel syndrome during a noxious stimulus. A region of interest analysis was done using an anatomical mask of the right amygdala. Activation map shows the cluster in which a paired t-test showed a significant reduction in stimulus-related activity following the intervention when the temperature of the stimulus was controlled for (n=13; nonparametric permutation testing, TFCE, familywise error correction rate 0.05). Images are in radiological convention and slices are identified by MNI coordinates.

7.4.2 Examining the effects of mindfulness on the resting brain in IBS

7.4.2.1 Using ASL to examine cerebral blood flow at rest

Whole brain ASL was used to look for changes in blood flow following MBCT-IBS. No differences were seen from pre-intervention to post-intervention.

7.4.2.2 Using dual regression to compare spatial networks

Dual regression was performed to compare whole brain spatial networks pre-intervention with those post-intervention. No significant change was identified.

7.4.2.3 Using seed-based analysis to look at the connectivity of individual regions

7.4.2.3.1 Connectivity between the precuneus and the rest of the brain

A mask was made of the part of the precuneus that showed altered activity following MBCT-IBS during a noxious stimulus when temperature was controlled for. This was used as a seed for whole brain resting state analysis. A paired t-test was performed comparing pre-intervention and post-intervention connectivity between this region and other parts of the brain. There were no regions with which connectivity was significantly reduced following MBCT-IBS. However, increased connectivity was seen with the PCC, bilateral ventromedial PFC, left dmPFC and visual cortex (Figure 7.11).

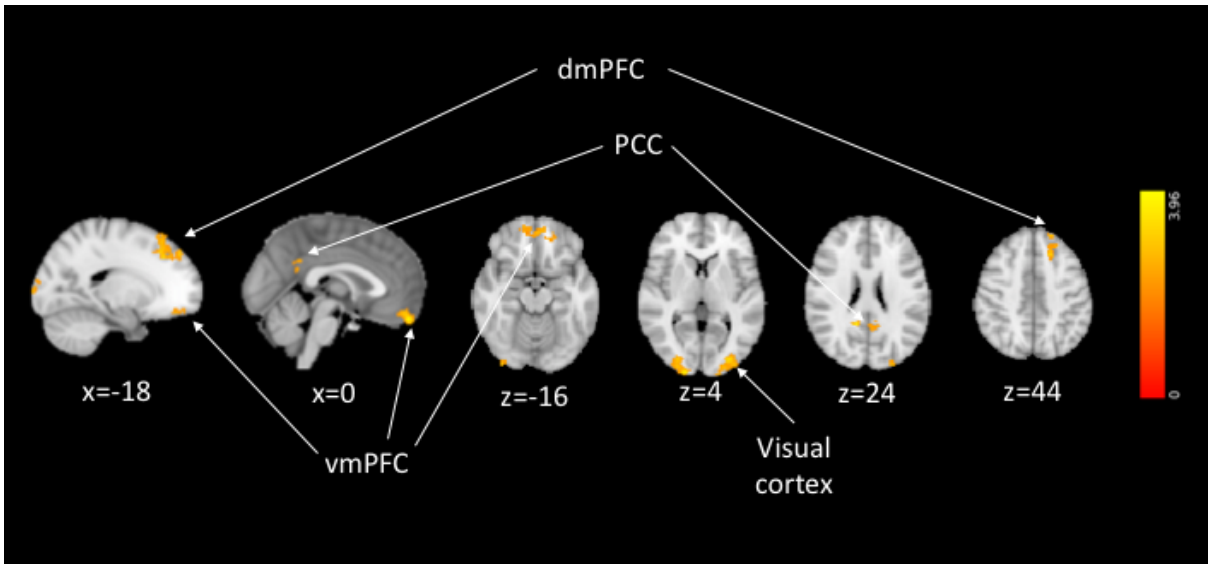


Figure 7.11: Determining the effect MBCT-IBS on whole brain resting functional connectivity with the precuneus. The seed was those parts of the precuneus in which the intervention led to altered stimulus-induced activity when differences in peripheral nociceptive input were controlled for. Activation map shows clusters that that survived the post-intervention>pre-intervention contrast in a paired t-test (n=15; mixed effects; Z>2.3, p<0.05; cluster-corrected). Images are in radiological convention. Slices are identified by MNI coordinates. Following MBCT-IBS, the precuneus had increased functional connectivity with the posterior cingulate cortex (PCC), dorsomedial prefrontal cortex (dmPFC), ventromedial prefrontal cortex (vmPFC) and visual cortex.

7.4.2.3.2 Connectivity between the dorsal PFC and the rest of the brain

Functional masks were created for regions of the dorsal PFC that showed altered activity during a noxious stimulus following MBCT-IBS when temperature was controlled for. As for the precuneus, paired t-tests were performed comparing the connectivity of each masked region with the rest of the brain before and after MBCT-IBS.

Both the right and left dmPFC showed increased connectivity with the precuneus following MBCT-IBS. The right dmPFC also showed increased connectivity with the PCC (Figure 7.12A). The left dmPFC showed increased connectivity with SI bilaterally, and the right inferior parietal lobule/SII (Figure 7.12B).

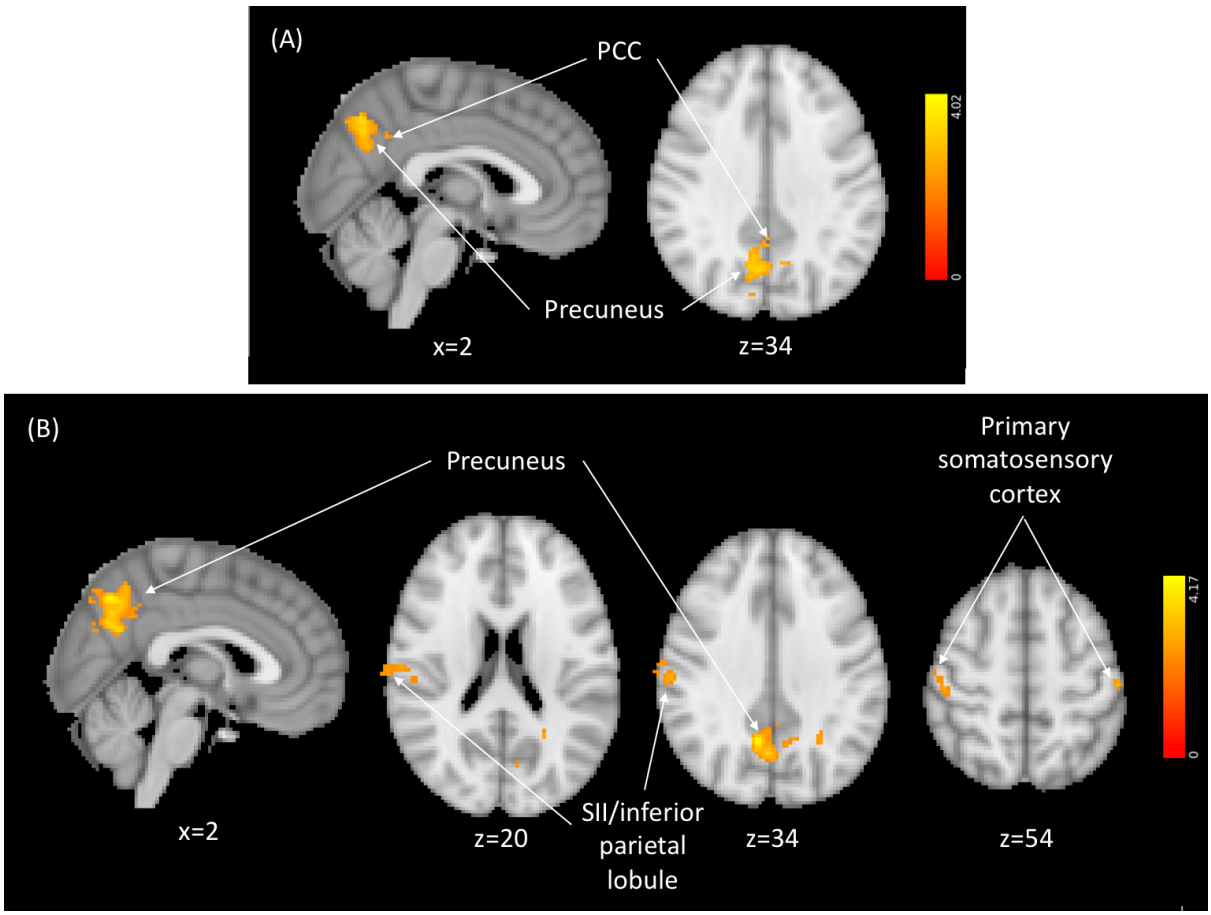


Figure 7.12: Determining the effect of MBCT-IBS on whole brain resting functional connectivity with the dorsomedial prefrontal cortex (dmPFC) in participants with irritable bowel syndrome. The seeds were those parts of the dmPFC in which MBCT-IBS led to altered activity related to a noxious stimulus when differences in peripheral nociceptive input were controlled for. Activation map shows clusters that that survived the post-intervention>pre-intervention contrast for the right dmPFC (A) and the left dmPFC (B) in a paired t-test (n=15; mixed effects; Z>2.3, p<0.05; cluster-corrected). Images are in radiological convention. Slices are identified by MNI coordinates. Following MBCT-IBS, the right dmPFC had increased functional connectivity with the precuneus and the posterior cingulate cortex (PCC). The left dmPFC had increased functional connectivity with the primary somatosensory cortex, the secondary somatosensory cortex (SII), and other parts of the inferior parietal lobule.

The left dlPFC showed no significant change in connectivity with any other brain regions from pre-intervention to post-intervention. However, the right dlPFC showed decreased connectivity with the right dorsal striatum (caudate and putamen), right thalamus, right hippocampus and right amygdala post MBCT-IBS (Figure 7.13). The

region of altered connectivity in the amygdala was not the same region as that identified above as having altered activity during a noxious stimulus.

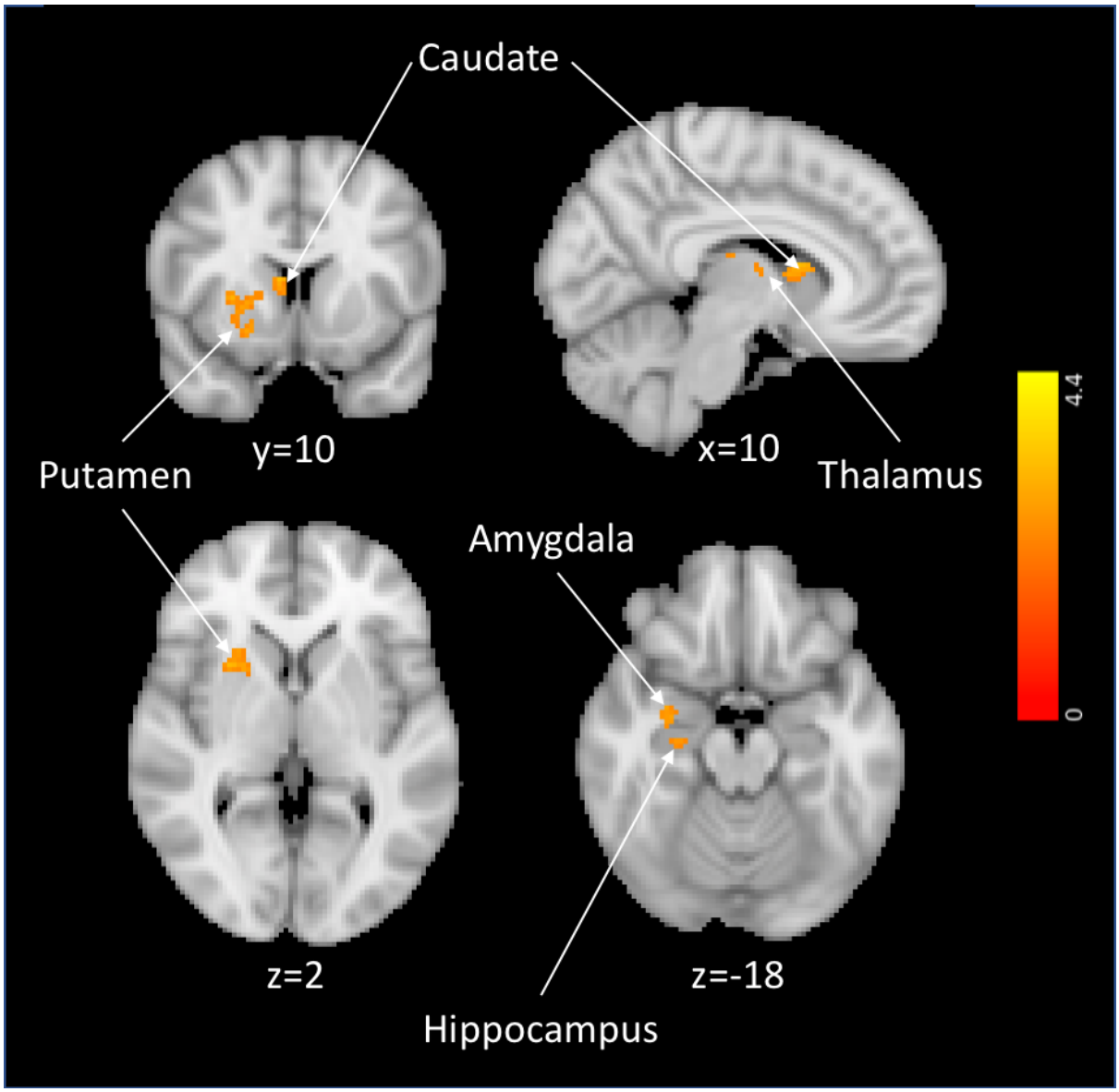
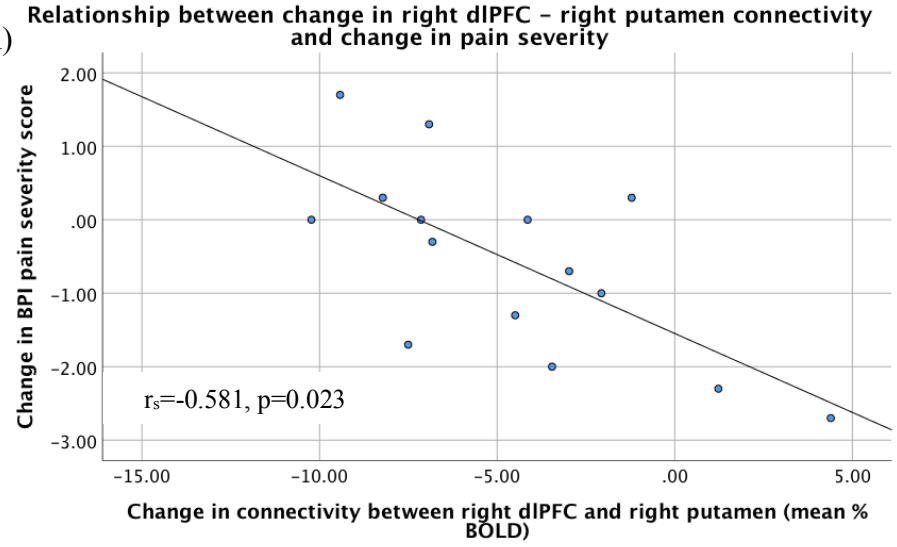


Figure 7.13: Determining the effect of MBCT-IBS on whole brain resting functional connectivity with the right dorsolateral prefrontal cortex (dlPFC) in participants with IBS. The seed was those parts of the right dlPFC in which MBCT-IBS led to altered activity related to a noxious stimulus when differences in peripheral nociceptive input were controlled for. Activation map shows clusters that that survived the pre-intervention>post-intervention contrast in a paired t-test (n=15; mixed effects; Z>2.3, p<0.05; cluster-corrected). Images are in radiological convention. Slices are identified by MNI coordinates. Following mindfulness, the dlPFC had decreased functional connectivity with the right caudate, putamen, thalamus, amygdala and hippocampus.

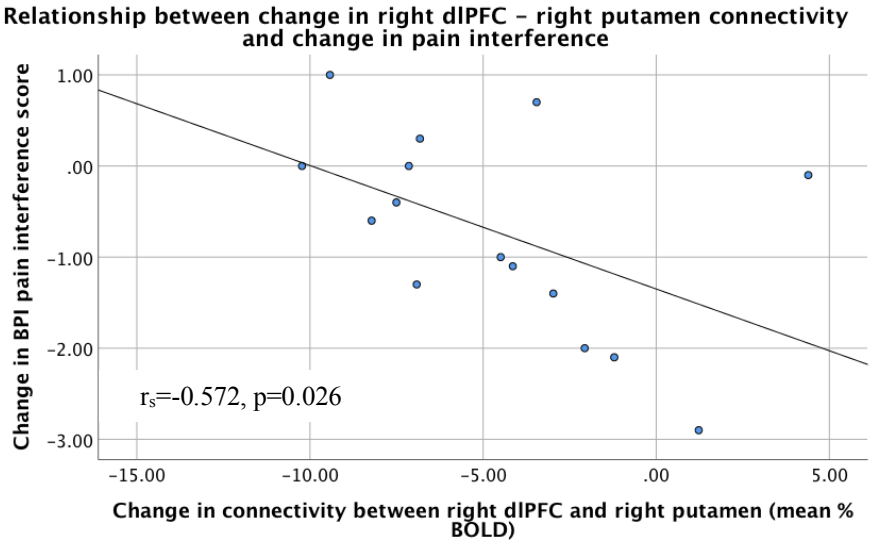
7.4.2.3.2.1 Relationship between right dlPFC connectivity and symptoms/anxiety

To determine whether there was a relationship between altered right dlPFC connectivity and the effect of MBCT-IBS on symptoms or anxiety, change in connectivity for each of these regions was compared with change in BPI, GSRS and VSI scores using Spearman correlation coefficients. Following MBCT-IBS, the improvement in both BPI pain severity and pain interference scores correlated with increased/less reduced connectivity between the right dlPFC and the right putamen. The reduction in pain interference scores also correlated with increased/less reduced connectivity between the right dlPFC and the right caudate (Figure 7.14). Full results are shown in appendix J-8.

(A)



(B)



(C)

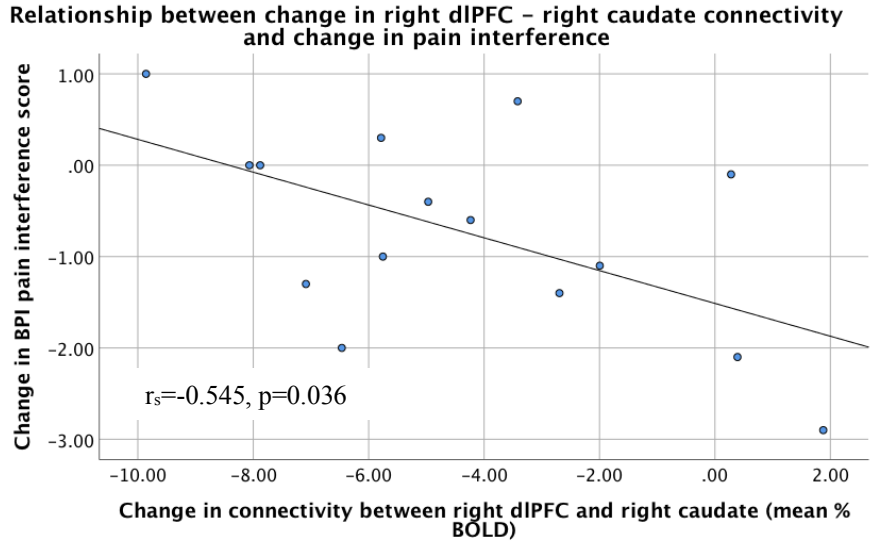


Figure 7.14: Scatterplots with lines of best fit showing statistically significant correlations between changes in right dorsolateral prefrontal cortex (dlPFC) functional connectivity and changes in pain scores from pre- to post-mindfulness. They show that there is a negative correlation between (A) change in functional connectivity with the right putamen and change in brief pain inventory (BPI) pain severity score; (B) change in functional connectivity with the right putamen and change in BPI pain interference score; and (C) change in functional connectivity with the right caudate and change in BPI pain interference score. Overall, participants who showed a larger increase or smaller decrease in connectivity between the right dlPFC and the right dorsal striatum also reported a larger reduction in pain severity or interference.

7.4.2.3.2.2 Relationship between trait motivational style and right dlPFC connectivity changes

To determine whether there was a relationship between trait motivational style and the effect of MBCT-IBS on right dlPFC connectivity, BAS scores at baseline were compared with the change in connectivity for each of the regions identified above (see Figure 7.13) using Spearman correlation coefficients. Higher BAS fun-seeking and BAS drive scores at baseline were both correlated with increased/less reduced connectivity between the right dlPFC and the right putamen after MBCT-IBS. Higher BAS drive scores also correlated with increased/less reduced connectivity between the right dlPFC and the right thalamus (Figure 7.15). Full results are shown in appendix J-9.

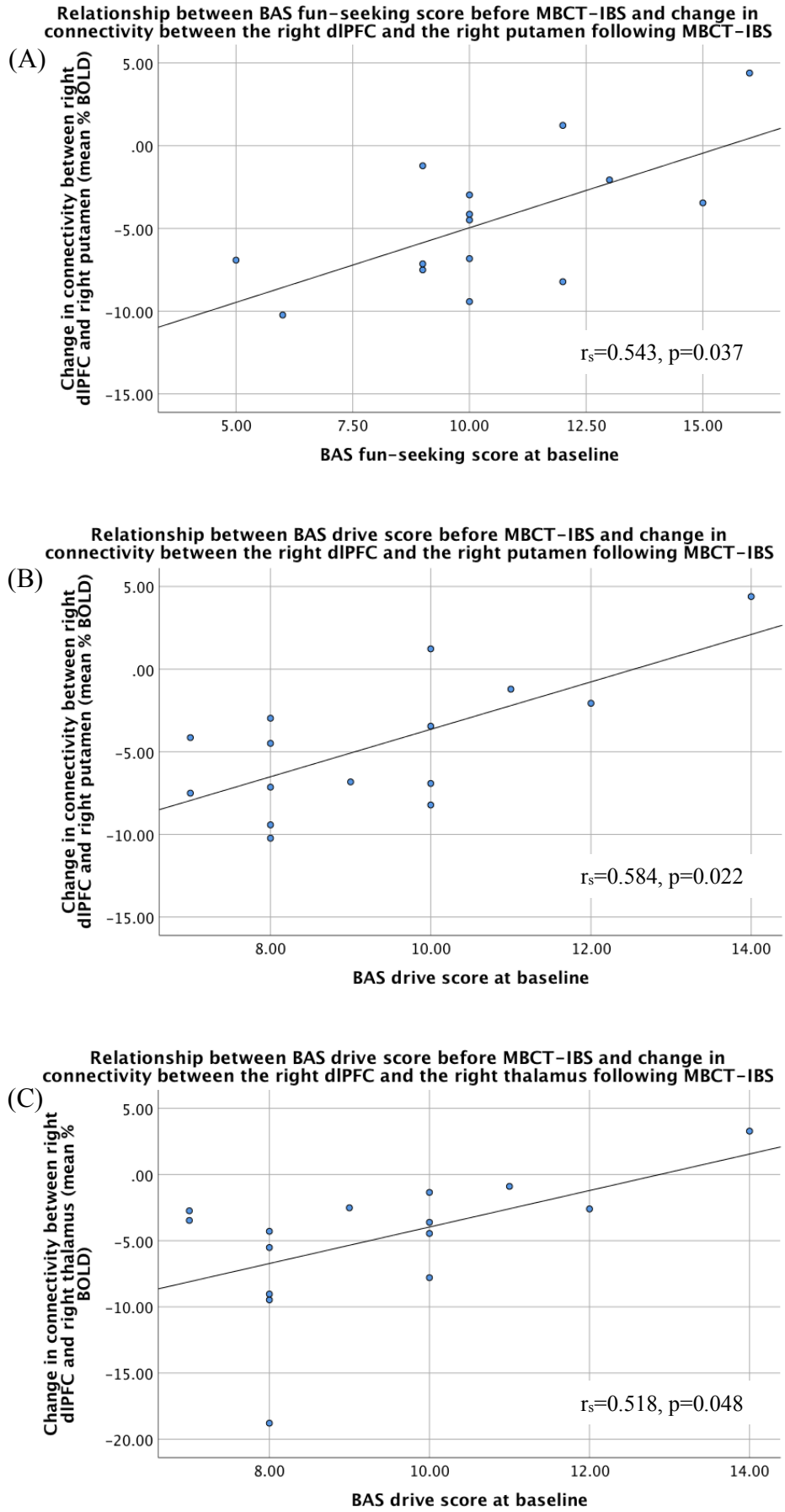


Figure 7.15: Scatterplots with lines of best fit showing statistically significant relationships between trait motivational style prior to MBCT-IBS and changes in right dorsolateral prefrontal cortex (dlPFC) connectivity after MBCT-IBS. They show that there is a positive correlation between (A) Behavioural activation system (BAS) fun-seeking score and change in functional connectivity with the right putamen; (B) BAS drive score and change in functional connectivity with the right putamen; and (C) BAS drive score and change in functional connectivity with the right thalamus. Overall, participants with higher BAS scores, reflecting greater reward-related motivation and desire, showed a larger increase or smaller decrease in connectivity between the right dlPFC and the right putamen/thalamus.

7.4.2.3.3 Connectivity between right amygdala and the rest of the brain

As mentioned above, following MBCT-IBS, areas of the right amygdala showed altered activation during a noxious stimulus (section 7.4.1.7), as well as altered connectivity with the right dlPFC at rest (section 7.4.2.3.2).

In order to determine the effect of MBCT-IBS on the resting connectivity of the right amygdala as a whole, an anatomical mask of the right amygdala was used as a seed for whole brain resting state analysis. This showed that from pre- to post-intervention there was reduced connectivity between the right amygdala and the bilateral thalamus (Figure 7.16A). There was also increased connectivity between the right amygdala and SI bilaterally, as well as the secondary somatosensory cortex (SII), MI and the supplementary motor area/premotor cortex (Figure 7.16B).

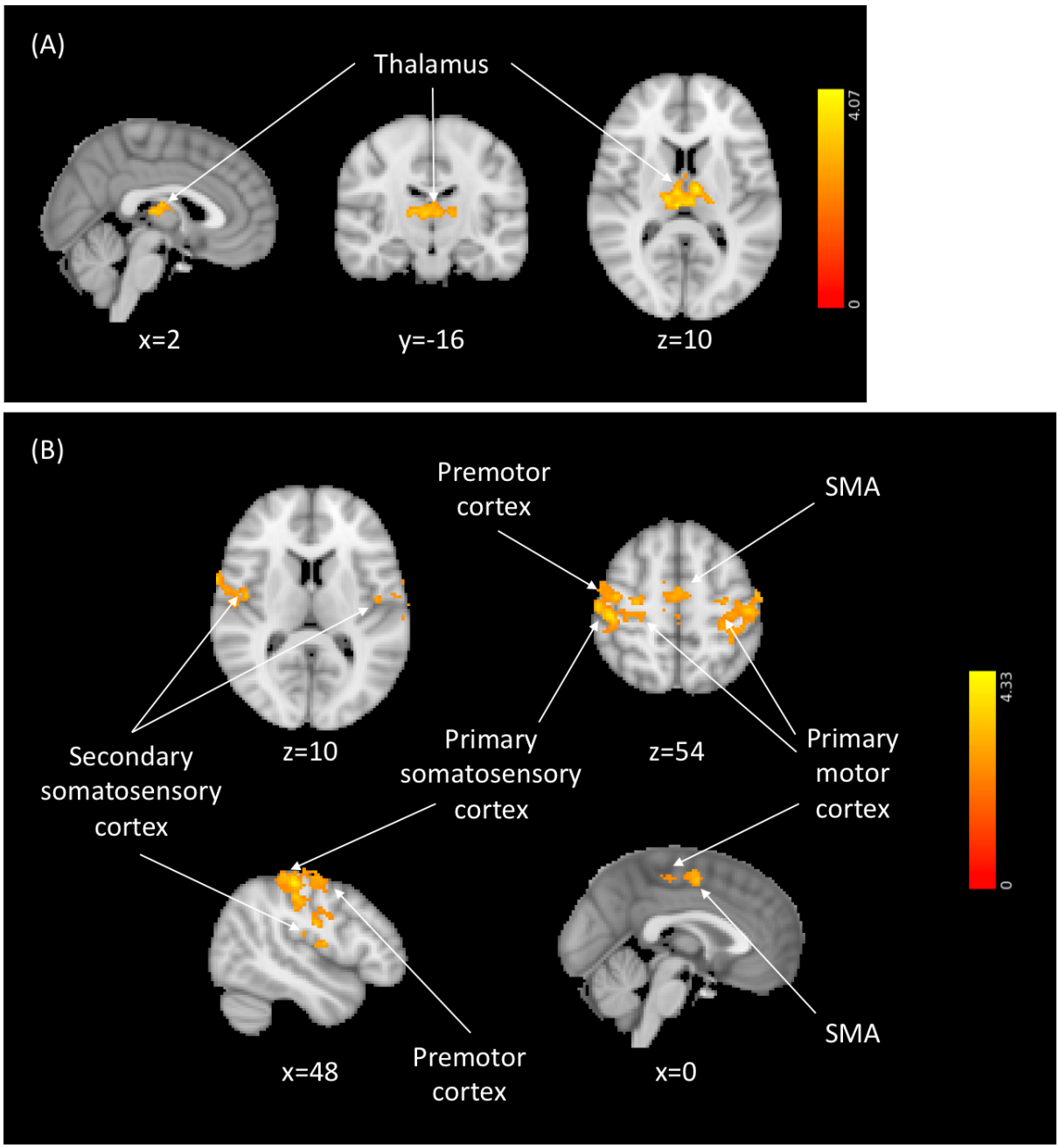


Figure 7.16: Determining the effect of MBCT-IBS on whole brain resting connectivity with the right amygdala in participants with irritable bowel syndrome. Activation maps show areas that survived the pre-intervention>post intervention (A) and post-intervention>pre-intervention (B) contrasts in a paired t-test (n=15; mixed effects; Z>2.3, p<0.05; cluster corrected). Images are in radiological convention. Slices are identified by MNI coordinates. Following MBCT-IBS, the right amygdala had reduced functional connectivity with the thalamus, and increased functional connectivity with the sensorimotor cortex. SMA = supplementary motor area.

The seed region was then restricted to the part of the right amygdala that showed altered BOLD responses to a thermal stimulus following MBCT-IBS, and the analysis was repeated. There was no change in resting connectivity between this region and the rest of the brain following the intervention.

7.5 Discussion

Following a six-week mindfulness-based intervention, a number of regions showed altered responses to painful stimuli. These included the ACC, amygdala, precuneus, and all divisions of the PFC. There were also changes in resting functional connectivity. In particular, there was increased connectivity between several nodes of the DMN, and between the DMN and both SI and SII. Additionally, there was reduced connectivity between the dlPFC and a number of subcortical regions known to influence pain perception, including the amygdala. Lastly, there was increased connectivity between the right amygdala and the sensorimotor cortex, and reduced connectivity between the right amygdala and the thalamus.

Although the absence of a control group means that caution must be exercised when interpreting these findings, the results are consistent with the likely effects of a cognitive intervention that targets the perception of pain and other visceral symptoms. This will be discussed in greater detail below.

7.5.1 Controlling for peripheral input

There are pros and cons to controlling for peripheral input as part of the analysis, and neither method is more correct than the other.

The pain intensity in this study was perception locked. As a result, the imaging gives an indication of how different individuals process stimuli of equal perceived intensity, but does not give information about differences in how the perceived intensity is derived from the input. The main advantage of controlling for temperature is that differences in results should then reflect differences in processing of the input rather than differences in the input itself. However, this does assume that differences in input do not cause differences in neural activity other than that which varies as a linear function of input level.

7.5.2 The effect of MBCT-IBS on descending modulatory pathways

7.5.2.1 The link between altered dlPFC activity and perceived pain after MBCT-IBS

There was a reduction in deactivation of the left and right dlPFC during a noxious stimulus following MBCT-IBS when peripheral nociceptive input was controlled for. In the left dlPFC, this correlated with the degree to which the BPI pain severity score was reduced.

As discussed in chapter 1 and chapter 5, the dlPFC has an important role in descending pain modulation and the cognitive control of pain perception. The left dlPFC in particular has been implicated in mediating the affective responses to pain, and has been said to be responsible for 'keeping pain out of mind' (Lorenz et al., 2003). The dlPFC is thought to act by influencing activity in other components of the pain network (Sankarasubramanian et al., 2017).

Following MBCT-IBS, there was decreased functional connectivity between the regions of the right dlPFC in which deactivation was reduced, and the right thalamus,

amygdala, hippocampus and dorsal striatum. These are all important regions in pain perception. In particular the thalamus is implicated in sensory aspects of pain (though it also has a role in the affective dimension of pain through its interaction with other supraspinal regions; Jalon et al., 2023), while the amygdala, hippocampus and dorsal striatum are implicated in affective and cognitive aspects of pain (Martucci & MacKey, 2018). Changes in the connectivity between the right dlPFC and subregions of the right dorsal striatum were found to correlate with changes in pain severity and interference scores. However, the direction of the correlation was unexpected, showing an association between increased connectivity and a reduction in both pain severity and the degree to which pain interferes with daily life. This contrasts with the overall reduction in connectivity between these regions that is seen following MBCT-IBS, and indicates that there may be an additional mediating factor influencing the relationship between functional connectivity and pain outcomes.

Seminowicz & Davis (2006) showed that pain-induced activation in the dlPFC correlated negatively with pain catastrophizing scores. Given that catastrophizing and anxiety about pain are well-linked to increased pain and chronicity of pain (Bondesson et al., 2018; Robert R. Edwards, Dworkin, Sullivan, Turk, & Wasan, 2016; Sullivan et al., 1995), this provides more support to the role of the dlPFC in the cognitive and emotional mechanisms of pain control.

The idea that the dlPFC is dysfunctional in patients with chronic pain conditions such as IBS is supported by the literature. Chronic pain patients, including those with visceral pain, have commonly been found to have reduced dlPFC grey matter volume (Karen D. Davis & Moayedi, 2013; Ong et al., 2019), while patients with hip

osteoarthritis who undergo successful hip replacement surgery have a subsequent increase in grey matter volume in this region (Rodriguez-Raecke, Niemeier, Ihle, Ruether, & May, 2009). Functional imaging has demonstrated alteration in function as well as structure. Patients with chronic facial pain were found to have higher resting cerebral blood flow in the dlPFC compared to healthy controls (Youssef et al., 2014). A few studies also show altered connectivity between the dlPFC and other brain regions in patients with chronic pain (Seminowicz & Moayedi, 2017).

There is good evidence that meditation affects prefrontal activity in response to aversive stimuli. A review of neural activity associated with mindfulness found dlPFC deactivation in mindfulness compared to healthy controls during noxious stimulation (Bilevicius et al., 2016). This contradicts the findings in this study, which show that a noxious stimulus produces reduced deactivation in the left dlPFC following mindfulness. However, the review included only healthy controls rather than chronic pain patients, who, as outlined above, may show differences in dlPFC structure and function compared to healthy controls. Therefore, direct comparisons to the IBS population in this study are difficult.

The trait motivational style of an individual was predictive of many of the changes in dlPFC activity and connectivity seen following MBCT-IBS, as well as the improvement in IBS symptoms, including pain severity scores. Specifically, this applied to the reward-related aspects of motivation (BAS) rather than the avoidance of aversive outcomes (BIS).

The regions with which changes in right dlPFC connectivity correlated with baseline BAS scores were the thalamus, a key region for enabling descending feedback, and the dorsal striatum, a region implicated in reward processing and the integration of emotional, cognitive and sensorimotor input (Balleine, Delgado, & Hikosaka, 2007). These findings add further weight to the importance of endogenous modulatory systems in the mechanisms of MBCT-IBS.

Similar to the relationship between altered right dlPFC connectivity and pain outcomes, the direction of the correlation between trait motivational style and altered right dlPFC connectivity was unexpected. Higher BAS scores were associated with increased connectivity, despite the fact that higher BAS scores were predictive of better outcomes, and that overall, connectivity was reduced following MBCT-IBS. As before, this could indicate the presence of an additional mediating factor that has not yet been identified.

7.5.2.2 Anxiety as a mediating factor

In chapter 6 it was shown that both symptom-specific and more generalised anxiety were reduced following MBCT-IBS. There was a significant linear correlation between the change in anxiety scores and the change in the BPI pain severity score, with increased anxiety covarying with increased pain severity. As mentioned above, left dlPFC activity in these participants was also linearly correlated with the BPI pain severity score, although not with anxiety. However, controlling for the change in anxiety led to the abolition of changes in evoked PFC activity. In the case of symptom-specific anxiety, it led to the abolition of all changes in evoked brain activity. These findings indicate that changes in anxiety, particularly anxiety that is specific to the

symptoms being experienced, are likely to be of great importance in the mechanisms that underlie the effectiveness of MBCT-IBS in this population. It is possible that changes in anxiety may mediate the effect of MBCT-IBS on pain perception, and that the brain mechanisms that underlie this may be related to changes in top-down modulation from frontal regions. However, the lack of correlation between change in frontal activation and change in anxiety suggests that this may not be a simple linear relationship.

7.5.2.3 The role of the right amygdala

The amygdala is an important region for pain, and is regularly cited as forming part of the 'pain matrix' that contributes to the experience of pain (Ossipov et al., 2010). It is a region that is well-connected to the descending modulatory system, and is likely to be important for driving descending inhibition. Amygdala volume has been found to be reduced in individuals with persistent pain compared to those who have recovered from pain (Vachon-Presseau et al., 2016), and the same study showed persistent pain sufferers to have stronger connectivity in the medial PFC-amygdala-nucleus accumbens pathway. It is likely that in chronic pain sufferers, the role of the amygdala in descending inhibition is impaired (Tracey, 2016). The amygdala is also an important region in fear and anxiety, and is likely to be important for the integration of these emotions with the pain experience, and the subsequent effects on pain perception (Ossipov et al., 2010). The right amygdala in particular appears to be most important for nociception (G. Ji & Neugebauer, 2009).

Hölzel et al. (2013) looked at the effect of a mindfulness intervention on the amygdala in patients with generalised anxiety disorder. They found that patients with anxiety had

greater amygdala activation than healthy controls in response to neutral (emotionally ambiguous) faces. Post-intervention there was reduced amygdala activation in both the mindfulness group and in an active control group. However, in the mindfulness group there was a greater increase in vlPFC activity, as well as increased resting functional connectivity between the amygdala and PFC. Both the altered vlPFC activity and the change in connectivity correlated with changes in anxiety scores, indicating that this change in fronto-limbic connectivity may be important for the effect of mindfulness on emotion regulation. This is supported further by a study of healthy participants showing that an aversive visual stimulus leads to down-regulation of amygdala activity as well as to increased amygdala-PFC activity, the amount of which is linked to each individual's trait mindfulness (Doll et al., 2016).

Preclinical data confirms the presence of altered forebrain-amygdala circuitry in nociception, and shows that connectivity between the two regions has an important role in both sensory and affective aspects of pain (Huang et al., 2019; G. Ji & Neugebauer, 2014; Woodhams et al., 2017). This is discussed in more detail in chapter 1 (section 1.2.5).

In the current study, following MBCT-IBS, the right amygdala showed reduced activation in response to a noxious stimulus as well as altered resting connectivity with the thalamus and other key sensorimotor regions (SI, SII, MI, premotor cortex, SMA), as well as the right dlPFC,. Given the clear behavioural link seen between anxiety and pain outcomes, it is very likely that changes in the ability of the right amygdala to regulate the emotional aspects of pain and to impact the descending modulatory system may have a role in the effectiveness of MBCT-IBS in this group.

7.5.2.4 The absence of changes to brainstem activity

Multiple brainstem nuclei are involved in descending pain modulation, and the PAG and RVM are of particular importance (see section 1.2.3.1). Despite this, no changes in pain-induced activity were seen in these two regions following MBCT-IBS. The absence of altered BOLD activity does not prove the absence of a biological effect, and there are a number of other possible explanations for the fact that no effect was seen. Small regions in the brainstem are notoriously difficult to image due to their size and position (Ezra et al., 2015; Satpute et al., 2013). In addition, the PAG and RVM are both heterogenous regions, in which different areas or cell types have different functions. Therefore, differing changes across a single nucleus may cancel each other out. To unpick this in more detail would require the use of ultra-high field MRI to give increased spatial resolution.

7.5.3 The effect of MBCT-IBS on the default mode network

During a noxious stimulus, there was reduced deactivation in components of the DMN following MBCT-IBS. Specifically, in the precuneus and angular gyrus. These findings are in keeping with those of pilot data (appendix H-3.4.5), in which healthy controls were found to have less deactivation in the DMN compared to participants with IBS during a noxious stimulus. It suggests that mindfulness may help to normalise the functioning of the DMN during experimental pain in IBS.

Seed-based resting state analysis demonstrated increased resting connectivity between components of the DMN following MBCT-IBS, specifically the PCC, precuneus and dmPFC. There was also increased connectivity between the left dmPFC and the bilateral SI and right SII, indicating possible increased communication

between the DMN and sensory regions. These findings indicate that MBCT-IBS not only alters the neural response to an isolated painful stimulus, but also alters the way in which the brain's intrinsic networks function, even at rest.

Multiple studies have implicated key nodes of the DMN, such as the PCC and precuneus, in mindfulness (A. P. King et al., 2016; Marchand, 2014; Scheibner, Bogler, Gleich, Haynes, & Bermpohl, 2017). This may be related to improved attentional control and an altered balance between interoceptive and externally focussed attention. Components of the DMN are known to be activated during self-referential processing (Davey & Harrison, 2018), and so the fact that this study found reduced task-based deactivation and increased within-network connectivity is in keeping with the idea that mindfulness is able to influence the appraisal and conscious awareness of visceral sensations.

7.5.4 Limitations of the study

As discussed in chapter 6, the biggest limitation of this study is the lack of a control group, and this is something that should be addressed in future studies. Other limitations also outlined in chapter 6 include participant heterogeneity and the lack of a contemporaneous measure of state anxiety.

A shortcoming of the imaging component of this study is that participants were not told whether or not they should actively practise mindfulness during the post-intervention scan. Therefore, some participants may have done so and others not. This may be more reflective of what would happen in real-life, but it adds heterogeneity to the study,

particularly given that no data was collected on whether they had done so in retrospect.

As for chapter 6, the participants in this chapter were all female. Sex is known to impact neural pain processing, and it has been shown that there are sex differences in neuroimaging findings in IBS patients (Gupta et al., 2017; Hong et al., 2014; Jennifer S. Labus et al., 2013; Naliboff et al., 2003). Therefore, the findings in this chapter may not be generalisable to men with IBS.

7.6 Summary

A key mechanism of MBCT-IBS is likely to be the manipulation of top-down pain processing. MBCT-IBS is associated with altered activity in prefrontal regions that are known to be involved in the cognitive control of top-down pain modulation. In the left dlPFC, this change in activity correlates with the degree to which perceived pain severity is reduced. MBCT-IBS also modulates the communication that takes place between these prefrontal regions and other parts of the pain network implicated in sensory, affective and cognitive aspects of pain perception. These regions include the amygdala, which itself is a key region for descending pain modulation as well as the affective components of pain, and which demonstrates reduced activity post-mindfulness, as well as altered connectivity with sensory regions. It is possible that, similar to the pharmacological agent investigated in chapter 5, MBCT-IBS acts through the modulation of frontal activity, and that the resulting changes in cortico-subcortical connectivity cause alterations in the connectivity between regions important for affective and sensory processing. It is likely that anxiety is an important mediator of some of these effects.

MBCT-IBS also changes the way in which intrinsic brain networks function. Following the intervention, there was reduced stimulus-related deactivation in the precuneus, a key component of the DMN. There was also increased connectivity within the DMN and between the DMN and sensory regions. It is likely that the intervention modulates the function of intrinsic brain networks and their interaction with pain-related regions, thereby influencing the attentional and interoceptive processing associated with visceral sensations, and their subsequent perception.

The absence of a control group is problematic for this study, and means that a properly controlled study should be undertaken to confirm these findings. However, assuming that the changes seen are confirmed to be specific to those receiving MBCT-IBS, the fact that functional imaging is able to show clear changes in neural activity and connectivity following MBCT-IBS indicates that neuroimaging has the potential to provide a biomarker for psychological/cognitive interventions. Although the correlation between dlPFC activity and pain severity provides some evidence that it might be possible to determine efficacy in this way, further work would be required to confirm and validate this, and to determine the most robust markers of clinical improvement, as well as the generalisability to other non-pharmacological interventions for pain.

Chapter 8: Conclusions

The existence of a safe, in vivo, objective biomarker of analgesic efficacy would transform the development of pain therapies, enabling decisions to be taken at an early stage on which drugs should progress to larger trials (U.S. Food and Drug Administration, 2017). There are indications that neuroimaging could provide such a biomarker.

Neuroimaging has previously been used to assess the efficacy of existing pharmacological compounds, and it is known that patterns of neural response can differentiate effective from ineffective analgesics (Duff et al., 2015; Vishvarani Wanigasekera et al., 2016). It has been hypothesised that these techniques could be translated to the investigation of novel analgesics, but prior to this thesis they had never been used to assess a completely new, first-in-class pharmacological agent.

A suitable biomarker also has the potential to change the way in which cognitive therapies are assessed and targeted, avoiding the use of ineffective treatments, and leading to the vastly improved management of chronic pain conditions. Cognitive treatments are relatively unregulated, and evidence for their use is often sparse.

The aim of this thesis was to assess whether neuroimaging could be a useful tool for the assessment of two interventions for pain. The first was ABX-1431, a novel pharmacological agent with analgesic potential; and the second was MBCT-IBS, a mindfulness-based cognitive intervention for patients with irritable bowel syndrome

(IBS; Henrich et al., 2020). In both cases, neuroimaging was assessed as a potential biomarker for therapeutic efficacy.

8.1 Overview of thesis

A double-blind randomised controlled trial was performed comparing ABX-1431 (a monoacylglycerol lipase inhibitor) with placebo and no dose using a three-way crossover design. This was the first time that a phase I clinical trial of a novel analgesic agent had been conducted in this laboratory, and chapter 3 outlines the methods used and the substantial work that went into ensuring that all the requirements of an early phase trial were met.

In chapter 4, it was shown that the subjective self-reported pain measures gathered during this trial were insufficient to determine whether ABX-1431 had analgesic efficacy. The absence of mechanistic information made it difficult to disambiguate the effects of the drug from placebo. Additionally, pain outcomes showed huge variability both within and between participants, reducing the power of the data to provide information on efficacy. However, the presence of mental and physical sedation indicated the likelihood of a pharmacodynamic effect in the brain.

Existing literature shows that effective analgesics lead to the attenuation of hyperalgesia-related activity in the brain (Duff et al., 2015). Since this was not a finding replicated by ABX-1431, it was not possible to discern whether this novel agent has analgesic efficacy. However, the imaging data presented in chapter 5 did confirm that there was a clear pharmacodynamic effect in the brain, and that many of the targets engaged were regions associated with the top-down modulation of pain and the

affective response to pain. It was therefore concluded that although ABX-1431 may not act exactly as hypothesised, it has the potential to act on chronic pain through the manipulation of dysfunctional pain control pathways. In particular, ABX-1431 was associated with altered activity in the lateral prefrontal cortex (PFC) and anterior cingulate and midcingulate cortices, as well as modified connectivity between these frontal regions and the amygdala, a key region for affective pain processing.

Having demonstrated the benefits of neuroimaging for the assessment of a novel pharmacological agent in healthy volunteers, in chapters 6 and 7 the same techniques were applied to the evaluation of a cognitive intervention in patients with chronic visceral pain. Mindfulness-based therapies show promise for the treatment of chronic pain conditions (Veehof et al., 2016), but are not routinely used in the management of IBS. Unlike ABX-1431, there was evidence for the possible efficacy of MBCT-IBS from the self-reported behavioural data despite its subjectivity. In chapter 6, questionnaire responses showed that MBCT-IBS reduced the effect of pain on daily life, while sensory data provided evidence that pain perception was modulated. It was hypothesised that improvement in the ability to cope with symptoms may be important, and anxiety was identified as a possible mediator of the effect of MBCT-IBS. However, it was not possible to establish the mechanisms that drove these effects from the behavioural data alone.

The imaging data presented in chapter 7 showed that, like ABX-1431, MBCT-IBS may act through the manipulation of top-down sensory and affective processing. Using both task-based and resting state data, it was possible to show that MBCT-IBS was associated with altered activity in frontal regions that are key areas for the cognitive

control of descending pain modulation, and that in the left dorsolateral PFC this change in activity correlated with changes to perceived pain. These findings were eliminated by controlling for anxiety levels, supporting the idea that anxiety is involved in mediating the effect of MBCT-IBS on the frontal cortex. MBCT-IBS was also associated with altered connectivity between the PFC and other components of the pain network, including the amygdala, in which activity was also modulated post-intervention, and which showed altered connectivity with sensory regions.

There was also good evidence that MBCT-IBS modulates the function of the default mode network and the way in which it interacts with the pain network. This may be a mechanism by which MBCT-IBS is able to alter the attentional processing of visceral sensations, leading to altered perception.

8.2 Summary

In this thesis, functional neuroimaging has been shown to have utility in the development and assessment of novel interventions for pain, both pharmacological and cognitive. Using a range of imaging modalities, it was possible to confirm that both ABX-1431 and MBCT-IBS had a pharmacodynamic effect in the brain. It was anticipated that imaging would provide an objective biomarker of efficacy for the interventions. Although it was unable to do so for ABX-1431, this was due to the presence of novel mechanisms of action, which show it to be a possible candidate for the treatment of chronic pain. For MBCT-IBS, efficacy was indicated using behavioural outcomes, but like ABX-1431, the imaging was key for identifying its mechanisms of action and providing objective confirmation that the behavioural results reflected the mediation of neural function.

The mechanisms of the two interventions showed remarkable similarities, with the neuroimaging data providing evidence that they both act through the manipulation of central mechanisms of pain control. In particular they were both shown to induce altered frontal activity, which may in turn modulate the activity in and connectivity between regions responsible for both sensory and affective aspects of pain processing, including the amygdala.

The data from both studies demonstrates that following an intervention for pain, whether it be pharmacological or cognitive, functional neuroimaging is able to show clear changes in neural activity and connectivity. This indicates that it has the potential to act as an objective biomarker for a wide range of interventions, including those that don't act acutely and produce the suppression of pain-induced neural activity. However, since a biomarker for the effective treatment of chronic pain may look different to that seen in acute pain, further work will be required to characterise it and validate its use.

8.3 Future work

Although the work in this thesis indicates that ABX-1431 has potential as a treatment for chronic pain, it has not been possible to prove analgesic efficacy. ABX-1431 is now undergoing phase II trials as a treatment for Tourette's syndrome, but a phase II trial of longer-term dosing in a chronic pain cohort would be of huge value to confirm whether it has analgesic efficacy in this group. It will be important to modify the drug formulation prior to this in order to reduce the between-subject variability in drug concentration. Further work will also be done with the data from this thesis to

determine whether machine learning and data from existing analgesics could help to identify whether ABX-1431 has analgesic efficacy.

MBCT-IBS shows huge potential as a therapy for chronic pain patients with dysfunctional descending control systems. However, the conclusions that can be drawn from this work are hampered by the absence of a suitable control group. Therefore, consideration should be given to the addition of a healthy volunteer group to this study. Background pain was considered as a possible confound, but was not corrected for in this thesis. Further work will be done to explore this further, and to determine whether correcting for background pain would influence the findings of the study. Due to the exploratory nature of much of the analysis done in the study, large numbers of hypothesis tests are performed. Future studies of MBCT-IBS should ask more targeted questions, based on the findings of this study, in order to reduce multiple comparisons

References

Abboud, C., Duveau, A., Bouali-Benazzouz, R., Masse, K., Mattar, J., Brochoire, L., … Landry, M. (2021). Animal models of pain: Diversity and benefits. *Journal of Neuroscience Methods*, *348*. https://doi.org/10.1016/j.jneumeth.2020.108997

Abide Therapeutics. (2017). *Investigator's Brochure ABX-1431*.

Adamczyk, W. M., Szikszay, T. M., Nahman-Averbuch, H., Skalski, J., Nastaj, J., Gouverneur, P., & Luedtke, K. (2022). To Calibrate or not to Calibrate? A Methodological Dilemma in Experimental Pain Research. *The Journal of Pain*, *23*(11), 1823–1832. https://doi.org/10.1016/j.jpain.2022.07.007

Agence Nationale de Sécurité du Médicament et des produits de santé. (2016). Report by the Temporary Specialist Scientific Committee (TSSC), "FAAH (Fatty Acid Amide Hydrolase)", on the causes of the accident during a Phase 1 clinical trial in Rennes in January 2016. Retrieved December 15, 2019, from https://ansm.sante.fr/var/ansm_site/storage/original/application/744c7c6daf96b141bc9509e2f85c227e.pdf

Albusoda, A., Friss, K., Gysan, M., Ruffle, J., Aziz, Q., & Farmer, A. (2018). OWE-026 Conditioned pain modulation in functional gastrointestinal disorders: systematic review & meta-analysis. *Gut*, *67*(Suppl 1), A207–A207. https://doi.org/10.1136/GUTJNL-2018-BSGABSTRACTS.414

Amanzio, M., Pollo, A., Maggi, G., & Benedetti, F. (2001). Response variability to analgesics: a role for non-specific activation of endogenous opioids. *Pain*, *90*(3), 205–215. https://doi.org/10.1016/S0304-3959(00)00486-3

Ammitzbøll, N., Arendt-Nielsen, L., Bertoli, D., Brock, C., Olesen, A. E., Kappel, A., … Petersen, K. K. (2021). A mechanism-based proof of concept study on the

effects of duloxetine in patients with painful knee osteoarthritis. *Trials*, *22*(1), 1–11. https://doi.org/10.1186/S13063-021-05941-Y/FIGURES/2

Andersson, J. L. R., Jenkinson, M., & Smith, S. (2007). *Non-linear registration aka Spatial normalisation FMRIB Technial Report TR07JA2*. *fmrib.ox.ac.uk*. Retrieved from https://www.fmrib.ox.ac.uk/datasets/techrep/tr07ja2/tr07ja2.pdf

Andrzejewski, J. A., Greenberg, T., & Carlson, J. M. (2019). Neural correlates of aversive anticipation: An activation likelihood estimate meta-analysis across multiple sensory modalities. *Cognitive, Affective and Behavioral Neuroscience*, *19*(6), 1379–1390. https://doi.org/10.3758/s13415-019-00747-7

Apkarian, A. V., Bushnell, M. C., Treede, R.-D., & Zubieta, J.-K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain*, *9*(4), 463–463. https://doi.org/10.1016/j.ejpain.2004.11.001

Apkarian, A. V., Bushnell, M. C., & Schweinhardt, P. (2013). Representation of pain in the brain. In S.B. McMahon, M. Koltzenburg, I. Tracey, & D. C. Turk (Eds.), *Textbook of Pain (6th edition)*. Elsevier Saunders.

Arnow, B. A., Hunkeler, E. M., Blasey, C. M., Lee, J., Constantino, M. J., Fireman, B., … Hayward, C. (2006). Comorbid depression, chronic pain, and disability in primary care. *Psychosomatic Medicine*, *68*(2), 262–268. https://doi.org/10.1097/01.psy.0000204851.15499.fc

Aro, P., Talley, N. J., Johansson, S. E., Agréus, L., & Ronkainen, J. (2015). Anxiety is linked to new-onset dyspepsia in the Swedish population: A 10-year follow-up study. *Gastroenterology*, *148*(5), 928–937. https://doi.org/10.1053/j.gastro.2015.01.039

Ashar, Y. K., Chang, L. J., & Wager, T. D. (2017). Brain Mechanisms of the Placebo Effect: An Affective Appraisal Account. *Annual Review of Clinical Psychology*,

13(1), 73–98. https://doi.org/10.1146/annurev-clinpsy-021815-093015

Association of the British Pharmaceutical Industry. (2018). *Guidelines for Phase I clinical trials*.

Azpiroz, F., Bouin, M., Camilleri, M., Mayer, e. a., Poitras, P., Serra, J., & Spiller, r. c. (2007). Mechanisms of hypersensitivity in IBS and functional disorders. *Neurogastroenterology & Motility*, *19*(s1), 62–88. https://doi.org/10.1111/j.1365-2982.2006.00875.x

Baba, H., Goldstein, P. A., Okamoto, M., Kohno, T., Ataka, T., Yoshimura, M., & Shimoji, K. (2000). Norepinephrine Facilitates Inhibitory Transmission in Substantia Gelatinosa of Adult Rat Spinal Cord (Part 2): Effects on Somatodendritic Sites of GABAergic Neurons. *Anesthesiology*, *92*, 485. https://doi.org/10.1097/00000542-200002000-00031

Bach, D. R., Hurlemann, R., & Dolan, R. J. (2015). Impaired threat prioritisation after selective bilateral amygdala lesions. *Cortex*, *63*, 206–213. https://doi.org/10.1016/j.cortex.2014.08.017

Bair, M. J., Robinson, R. L., Katon, W., & Kroenke, K. (2003, November 10). Depression and Pain Comorbidity: A Literature Review. *Archives of Internal Medicine*. American Medical Association. https://doi.org/10.1001/archinte.163.20.2433

Balleine, B. W., Delgado, M. R., & Hikosaka, O. (2007). The Role of the Dorsal Striatum in Reward and Decision-Making. *Journal of Neuroscience*, *27*(31), 8161–8165. https://doi.org/10.1523/JNEUROSCI.1554-07.2007

Bandler, R., & Shipley, M. T. (1994). Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? *Trends in Neurosciences*, *17*(9), 379–389.

Banks, S. J., Eddy, K. T., Angstadt, M., Nathan, P. J., & Phan, K. L. (2007). Amygdala–frontal connectivity during emotion regulation. *Social Cognitive and Affective Neuroscience*, *2*(4), 303–312. https://doi.org/10.1093/scan/nsm029

Bannister, K., & Dickenson, A. H. (2016). What do monoamines do in pain modulation? *Current Opinion in Supportive and Palliative Care*, *10*(2), 143–148.

Bannister, K., Lockwood, S., Goncalves, L., Patel, R., & Dickenson, A. H. (2016). An investigation into the inhibitory function of serotonin in diffuse noxious inhibitory controls in the neuropathic rat. https://doi.org/10.1002/ejp.979

Baron, R., Baron, Y., Disbrow, E., & Roberts, T. P. L. (1999). Brain processing of capsaicin-induced secondary hyperalgesia: a functional MRI study. *Neurology*, *53*(3), 548–557. https://doi.org/10.1212/WNL.53.3.548

Bartley, E. J., & Fillingim, R. B. (2013). Sex differences in pain: a brief review of clinical and experimental findings. *British Journal of Anaesthesia*, *111*(1), 52–60. https://doi.org/10.1093/bja/aet127

Baxter, M. G., & Murray, E. A. (2002, July). The amygdala and reward. *Nature Reviews Neuroscience*. Nature Publishing Group. https://doi.org/10.1038/nrn875

Becerra, L., & Borsook, D. (2008). Signal Valence in the Nucleus Accumbens to Pain Onset and Offset. *European Journal of Pain (London, England)*, *12*(7), 866. https://doi.org/10.1016/J.EJPAIN.2007.12.007

Becker, S., Gandhi, W., Pomares, F., Wager, T., & Schweinhardt, P. (2017). Orbitofrontal cortex mediates pain inhibition by monetary reward. *Social Cognitive and Affective Neuroscience*, *12*(4), 651–661.

Beckmann, C. F., Mackay, C. E., Filippini, N., & Smith, S. M. (2009). *Group comparison of resting-state FMRI data using multi-subject ICA and dual regression.*

Bednarska, O., Icenhour, A., Tapper, S., Witt, S. T., Tisell, A., Lundberg, P., … Walter, S. (2019). Reduced excitatory neurotransmitter levels in anterior insulae are associated with abdominal pain in irritable bowel syndrome. *Pain*, *160*(9), 2004–2012. https://doi.org/10.1097/j.pain.0000000000001589

Benarroch, E. E. (2012). Periaqueductal gray: An interface for behavioral control. *Neurology*, *78*(3). https://doi.org/https://doi.org/10.1212/WNL.0b013e31823fcdee

Benedetti, F., Amanzio, M., Rosato, R., & Blanchard, C. (2011). Nonopioid placebo analgesia is mediated by CB1 cannabinoid receptors. *Nature Medicine*, *17*(10), 1228–1230. https://doi.org/10.1038/nm.2435

Benedetti, F., Arduino, C., Costa, S., Vighetti, S., Tarenzi, L., Rainero, I., & Asteggiano, G. (2006). Loss of expectation-related mechanisms in Alzheimer's disease makes analgesic therapies less effective. *Pain*, *121*(1–2), 133–144. https://doi.org/10.1016/J.PAIN.2005.12.016

Bener, A., Verjee, M., Dafeeah, E. E., Falah, O., Al-Juhaishi, T., Schlogl, J., … Khan, S. (2013). Psychological factors: Anxiety, depression, and somatization symptoms in low back pain patients. *Journal of Pain Research*, *6*, 95–101. https://doi.org/10.2147/JPR.S40740

Bennett, D. L. H., & Woods, C. G. (2014, June 1). Painful and painless channelopathies. *The Lancet Neurology*. Lancet Publishing Group. https://doi.org/10.1016/S1474-4422(14)70024-9

Benson, S., Siebert, C., Koenen, L. R., Engler, H., Kleine-Borgmann, J., Bingel, U., … Elsenbruch, S. (2019). Cortisol affects pain sensitivity and pain-related emotional learning in experimental visceral but not somatic pain: A randomized controlled study in healthy men and women. *Pain*, *160*(8), 1719–1728.

https://doi.org/10.1097/J.PAIN.0000000000001579

Berge, O. G. (2011, October). Predictive validity of behavioural animal models for chronic pain. *British Journal of Pharmacology*. Wiley-Blackwell. https://doi.org/10.1111/j.1476-5381.2011.01300.x

Berman, S. M., Naliboff, B. D., Suyenobu, B., Labus, J. S., Stains, J., Ohning, G., … Mayer, E. A. (2008). Reduced brainstem inhibition during anticipated pelvic visceral pain correlates with enhanced brain response to the visceral stimulus in women with irritable bowel syndrome. *Journal of Neuroscience*, *28*(2), 349–359. https://doi.org/10.1523/JNEUROSCI.2500-07.2008

Bhave, G., & Gereau, R. W. (2004). Posttranslational mechanisms of peripheral sensitization. *Neurobiology*, *61*(1), 88–106.

Bielefeldt, K., & Gebhart, G. F. (2013). Visceral pain: basic mechanisms. In Stephen B. McMahon, M. Koltzenburg, I. Tracey, & D. C. Turk (Eds.), *Textbook of Pain (6th edition)*. Elsevier Saunders.

Bilevicius, E., Kolesar, T. A., & Kornelsen, J. (2016, April 19). Altered neural activity associated with mindfulness during nociception: A systematic review of functional MRI. *Brain Sciences*. MDPI AG. https://doi.org/10.3390/brainsci6020014

Bilevicius, E., Smith, S. D., & Kornelsen, J. (2018). Resting-State Network Functional Connectivity Patterns Associated with the Mindful Attention Awareness Scale. *Brain Connectivity*, *8*(1), 40–48. https://doi.org/10.1089/BRAIN.2017.0520

Bingel, U., Lorenz, J., Schoell, E., Weiller, C., & Büchel, C. (2006). Mechanisms of placebo analgesia: rACC recruitment of a subcortical antinociceptive network. *Pain*, *120*(1–2), 8–15. https://doi.org/10.1016/j.pain.2005.08.027

Bingel, U., Schoell, E., Herken, W., Büchel, C., & May, A. (2007). Habituation to

painful stimulation involves the antinociceptive system. *Pain*, *131*(1–2), 21–30. https://doi.org/10.1016/J.PAIN.2006.12.005

Bingel, Ulrike, Wanigasekera, V., Wiech, K., Mhuircheartaigh, R. N., Lee, M. C., Ploner, M., & Tracey, I. (2011). The effect of treatment expectation on drug efficacy: Imaging the analgesic benefit of the opioid remifentanil. *Science Translational Medicine*, *3*(70). https://doi.org/10.1126/scitranslmed.3001244

Bjelland, I., Dahl, A. A., Haug, T. T., & Neckelmann, D. (2002). The validity of the Hospital Anxiety and Depression Scale: An updated literature review. *Journal of Psychosomatic Research*, *52*(2), 69–77. https://doi.org/10.1016/S0022-3999(01)00296-3

Bjørkedal, E., & Flaten, M. A. (2011). Interaction between expectancies and drug effects: An experimental investigation of placebo analgesia with caffeine as an active placebo. *Psychopharmacology*, *215*(3), 537–548. https://doi.org/10.1007/s00213-011-2233-4

Blankman, J. L., Simon, G. M., & Cravatt, B. F. (2007). A Comprehensive Profile of Brain Enzymes that Hydrolyze the Endocannabinoid 2-Arachidonoylglycerol. *Chemistry and Biology*, *14*(12), 1347–1356. https://doi.org/10.1016/j.chembiol.2007.11.006

Blankstein, U., Chen, J., Diamant, N. E., & Davis, K. D. (2010). Altered Brain Structure in Irritable Bowel Syndrome: Potential Contributions of Pre-Existing and Disease-Driven Factors. *Gastroenterology*, *138*(5), 1783–1789. https://doi.org/10.1053/j.gastro.2009.12.043

Bliss, T. V. P., Collingridge, G. L., Kaang, B. K., & Zhuo, M. (2016). Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain. *Nature Reviews Neuroscience 2016 17:8*, *17*(8), 485–496.

https://doi.org/10.1038/nrn.2016.68

Bloomfield, M. A. P., Hindocha, C., Green, S. F., Wall, M. B., Lees, R., Petrilli, K., … Freeman, T. P. (2019, March 1). The neuropsychopharmacology of cannabis: A review of human imaging studies. *Pharmacology and Therapeutics*. Elsevier Inc. https://doi.org/10.1016/j.pharmthera.2018.10.006

Boadas-Vaello, P., Castany, S., Homs, J., Alvarez-Perez, B., Deulofeu, M., & Verdu, E. (2016). Neuroplasticity of ascending and descending pathways after somatosensory system injury: reviewing knowledge to identify neuropathic pain therapeutic targets. *Spinal Cord*, *54*, 330–340.

Boccard, S. G. J., Prangnell, S. J., Pycroft, L., Cheeran, B., Moir, L., Pereira, E. A. C., … Aziz, T. Z. (2017). Long-Term Results of Deep Brain Stimulation of the Anterior Cingulate Cortex for Neuropathic Pain. *World Neurosurgery*, *106*, 625–637. https://doi.org/10.1016/J.WNEU.2017.06.173

Bond, A., & Lader, M. (1974). The use of analogue scales in rating subjective feelings. *British Journal of Medical Psychology*, *47*(3), 211–218. https://doi.org/10.1111/j.2044-8341.1974.tb02285.x

Bondesson, E., Larrosa Pardo, F., Stigmar, K., Ringqvist, Å., Petersson, I. F., Jöud, A., & Schelin, M. E. C. (2018). Comorbidity between pain and mental illness - Evidence of a bidirectional relationship. *European Journal of Pain*, *22*(7), 1304–1311. https://doi.org/10.1002/ejp.1218

Bowie, C. R., & Harvey, P. D. (2006). Administration and interpretation of the Trail Making Test. *Nature Protocols*, *1*(5), 2277–2281. https://doi.org/10.1038/nprot.2006.390

Bräscher, A. K., Becker, S., Hoeppli, M. E., & Schweinhardt, P. (2016). Different brain circuitries mediating controllable and uncontrollable pain. *Journal of*

Neuroscience, *36*(18), 5013–5025. https://doi.org/10.1523/JNEUROSCI.1954-15.2016

Bravo, L., Llorca-Torralba, M., Berrocoso, E., & Mico, J. A. (2019). Monoamines as drug targets in chronic pain: Focusing on neuropathic pain. *Frontiers in Neuroscience*. https://doi.org/10.3389/fnins.2019.01268

Braz, J. M., Nassar, M. A., Wood, J. N., & Basbaum, A. I. (2005). Parallel "pain" pathways arise from subpopulations of primary afferent nociceptor. *Neuron*, *47*(6), 787–793. https://doi.org/10.1016/j.neuron.2005.08.015

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2006). Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *European Journal of Pain*, *10*(4), 287–287. https://doi.org/10.1016/j.ejpain.2005.06.009

Brodersen, K. H., Wiech, K., Lomakina, E. I., Lin, C. shu, Buhmann, J. M., Bingel, U., … Tracey, I. (2012). Decoding the perception of pain from fMRI using multivariate pattern analysis. *NeuroImage*, *63*(3), 1162–1170. https://doi.org/10.1016/j.neuroimage.2012.08.035

Brooks, J. C. W., Beckmann, C. F., Miller, K. L., Wise, R. G., Porro, C. A., Tracey, I., & Jenkinson, M. (2008). Physiological noise modelling for spinal functional magnetic resonance imaging studies. *NeuroImage*, *39*(2), 680–692. https://doi.org/10.1016/j.neuroimage.2007.09.018

Budai, D., Harasawa, I., & Fields, H. L. (1998). Midbrain Periaqueductal Gray (PAG) Inhibits Nociceptive Inputs to Sacral Dorsal Horn Nociceptive Neurons Through α_2 -Adrenergic Receptors. *Journal of Neurophysiology*, *80*(5), 2244–2254. https://doi.org/10.1152/jn.1998.80.5.2244

Bushnell, M. C., Ceko, M., & Low, L. A. (2013). Cognitive and emotional control of

pain and its disruption in chronic pain. *Nature Reviews Neuroscience*, *14*, 502–511.

Butler, R.K., & Finn, D. P. (2009). Stress-induced analgesia. *Progress in Neurobiology*, *88*, 184–202.

Butler, Ryan K., Rea, K., Lang, Y., Gavin, A. M., & Finn, D. P. (2008). Endocannabinoid-mediated enhancement of fear-conditioned analgesia in rats: Opioid receptor dependency and molecular correlates. *Pain*, *140*(3), 491–500. https://doi.org/10.1016/J.PAIN.2008.10.002

Buxton, R. B., & Frank, L. R. (1997). A model for the coupling between cerebral blood flow and oxygen metabolism during neural stimulation. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *17*(1), 64–72. https://doi.org/10.1097/00004647-199701000-00009

Cabin, R., & Mitchell, R. (2000). To Bonferroni or Not to Bonferroni: When and How Are the Questions. *Bulletin of the Ecological Society of America*, *81*, 246–248.

Cain, K. C., Headstrom, P., Jarrett, M. E., Motzer, S. A., Park, H., Burr, R. L., … Heitkemper, M. M. (2006). Abdominal pain impacts quality of life in women with irritable bowel syndrome. *American Journal of Gastroenterology*, *101*(1), 124–132. https://doi.org/10.1111/j.1572-0241.2006.00404.x

Calejesan, A. A., Kim, S. J., & Zhou, M. (2000). Descending facilitatory modulation of a behavioral nociceptive response by stimulation in the adult rat anterior cingulate cortex. *European Journal of Pain*, *4*, 83–96. https://doi.org/10.1053/eujp.1999.0158

Campbell, F. A., Tramèr, M. R., Carroll, D., Reynolds, D. J. M., Moore, r. A., & Mcquay, H. J. (2001). Are cannabinoids an effective and safe treatment option

in the management of pain? A qualitative systematic review. *BMJ*, *323*(7303), 13. https://doi.org/10.1136/bmj.323.7303.13

Canavan, C., West, J., & Card, T. (2014, February 4). The epidemiology of irritable bowel syndrome. *Clinical Epidemiology*. https://doi.org/10.2147/CLEP.S40245

Carleton, R. N., Afifi, T. O., Taillieu, T., Turner, S., El-Gabalawy, R., Sareen, J., & Asmundson, G. J. G. (2018). Anxiety-related psychopathology and chronic pain comorbidity among public safety personnel. *Journal of Anxiety Disorders*, *55*, 48–55. https://doi.org/10.1016/j.janxdis.2018.03.006

Carrasquillo, Y., & Gereau IV, R. W. (2008). Hemispheric lateralization of a molecular signal for pain modulation in the amygdala. *Molecular Pain*, *4*, 24. https://doi.org/10.1186/1744-8069-4-24

Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales. *Journal of Personality and Social Psychology*, *67*(2), 319–333. https://doi.org/10.1037/0022-3514.67.2.319

Casassus, B. (2016, May 28). New report on drug trial disaster in France. *The Lancet*. Lancet Publishing Group. https://doi.org/10.1016/S0140-6736(16)30661-4

Case, L. K., Laubacher, C. M., Olausson, H., Wang, B., Spagnolo, P. A., & Bushnell, M. C. (2016). Encoding of touch intensity but not pleasantness in human primary somatosensory cortex. *Journal of Neuroscience*, *36*(21), 5850–5860. https://doi.org/10.1523/JNEUROSCI.1130-15.2016

Chapman, S., & Martin, M. (2011). Attention to pain words in irritable bowel syndrome: Increased orienting and speeded engagement. *British Journal of Health Psychology*, *16*(1), 47–60. https://doi.org/10.1348/135910710X505887

Chapman, V. (1999). The cannabinoid CB1 receptor antagonist, SR141716A, selectively facilitates nociceptive responses of dorsal horn neurones in the rat. *British Journal of Pharmacology*, *127*(8), 1765–1767. https://doi.org/10.1038/SJ.BJP.0702758

Chappell, M. A., Groves, A. R., Whitcher, B., & Woolrich, M. W. (2009). Variational Bayesian inference for a nonlinear forward model. *IEEE Transactions on Signal Processing*, *57*(1), 223–236. https://doi.org/10.1109/TSP.2008.2005752

Chen, J., Matias, I., Dinh, T., Lu, T., Venezia, S., Nieves, A., … Di Marzo, V. (2005). Finding of endocannabinoids in human eye tissues: Implications for glaucoma. *Biochemical and Biophysical Research Communications*, *330*(4), 1062–1067. https://doi.org/10.1016/j.bbrc.2005.03.095

Chen, J. Y. W., Blankstein, U., Diamant, N. E., & Davis, K. D. (2011). White matter abnormalities in irritable bowel syndrome and relation to individual factors. *Brain Research*, *1392*, 121–131. https://doi.org/10.1016/j.brainres.2011.03.069

Chen, Q., & Heinricher, M. M. (2019). Descending Control Mechanisms and Chronic Pain. *Current Rheumatology Reports*, *21*. https://doi.org/10.1007/s11926-019-0813-1

Chen, S. L., Wu, X. Y., Cao, Z. J., Fan, J., Wang, M., Owyang, C., & Li, Y. (2008). Subdiaphragmatic vagal afferent nerves modulate visceral pain. *Am J Physiol Gastrointest Liver Physiol*, *294*, 1441–1449. https://doi.org/10.1152/ajpgi.00588.2007.-Activation

Chen, T., Taniguchi, W., Chen, Q.-Y., Tozaki-Saitoh, H., Dong, Q., Liu, R.-H., … Zhou, M. (2018). Top-down descending facilitation of spinal sensory excitatory transmission from the anterior cingulate cortex. *Nature Communications*, *9*, 1886. https://doi.org/10.1038/s41467-018-04309-2

Chiang, M. C., Nguyen, E. K., Canto-Bustos, M., Papale, A. E., Oswald, A.-M., & Ross, S. E. (2020). Divergent neural pathways emanating from the lateral parabrachial nucleus mediate distinct components of the pain response. *Neuron*, *106*(6), 927–939.

Chincholkar, M. (2018). Analgesic mechanisms of gabapentinoids and effects in experimental pain models: a narrative review. *British Journal of Anaesthesia*, *120*(6), 1315–1334.

Chong, W.-S., & Johnson, D. S. (2012). Update on opioid pharmacology. *Update in Anaesthesia*, 28. Retrieved from www.wfsahq.org/resources/update-in-anaesthesia

Cisar, J. S., Weber, O. D., Clapper, J. R., Blankman, J. L., Henry, C. L., Simon, G. M., … Grice, C. A. (2018). Identification of ABX-1431, a Selective Inhibitor of Monoacylglycerol Lipase and Clinical Candidate for Treatment of Neurological Disorders. *Journal of Medicinal Chemistry*, *61*(20), 9062–9084. https://doi.org/10.1021/acs.jmedchem.8b00951

Clark, J. D. (2016). Preclinical Pain Research: Can we do Better? *Anesthesiology*, *125*(5), 846. https://doi.org/10.1097/ALN.0000000000001340

Cleeland, C. S., & Ryan, K. M. (1994). Pain assessment: global use of the Brief Pain Inventory. *Annals of the Academy of Medicine, Singapore*.

Cleeland, Charles S., Nakamura, Y., Mendoza, T. R., Edwards, K. R., Douglas, J., & Serlin, R. C. (1996). Dimensions of the impact of cancer pain in a four country sample: New information from multidimensional scaling. *Pain*, *67*(2–3), 267–273. https://doi.org/10.1016/0304-3959(96)03131-4

Coghill, R. C., Sang, C. N., Maisog, J. M., & Iadarola, M. J. (1999). Pain intensity processing within the human brain: A bilateral, distributed mechanism. *Journal*

of Neurophysiology, *82*(4), 1934–1943. https://doi.org/10.1152/JN.1999.82.4.1934/ASSET/IMAGES/LARGE/9K1090509005.JPEG

Connell, K., Bolton, N., Olsen, D., Piomelli, D., & Hohmann, A. G. (2006). Role of the basolateral nucleus of the amygdala in endocannabinoid-mediated stress-induced analgesia. *Neuroscience Letters*, *397*(3), 180–184. https://doi.org/10.1016/J.NEULET.2005.12.008

Corcoran, L., Mattimoe, D., Roche, M., & Finn, D. P. (2020). Attenuation of fear-conditioned analgesia in rats by monoacylglycerol lipase inhibition in the anterior cingulate cortex: Potential role for CB2 receptors. *British Journal of Pharmacology*, *177*(10), 2240–2255. https://doi.org/10.1111/BPH.14976

Cottam, W. J., Condon, L., Alshuft, H., Reckziegel, D., & Auer, D. P. (2016). Associations of limbic-affective brain activity and severity of ongoing chronic arthritis pain are explained by trait anxiety. *NeuroImage: Clinical*, *12*, 269–276. https://doi.org/10.1016/j.nicl.2016.06.022

Coulombe, M.-A., Erpelding, N., Kucyi, A., & Davis, K. D. (2016). Intrinsic functional connectivity of periaqueductal gray subregions in humans. *Human Brain Mapping*, *37*, 1514–1530.

Craig, A. D. (2002). How do you feel? Interoception: The sense of the physiological condition of the body. *Nature Reviews Neuroscience*, *3*(8), 655–666. https://doi.org/10.1038/nrn894

Craig, A. D. (2009, June). A rat is not a monkey is not a human: Comment on Mogil (Nature Rev. Neurosci. 10, 283-294 (2009)). *Nature Reviews Neuroscience*. Nature Publishing Group. https://doi.org/10.1038/nrn2606-c1

Craig, A. D., Chen, K., Bandy, D., & Reiman, E. M. (2000). Thermosensory activation

of insular cortex. *Nature Neuroscience*, *3*(2), 184–190. https://doi.org/10.1038/72131

Cravatt, B. F., Demarest, K., Patricelli, M. P., Bracey, M. H., Giang, D. K., Martin, B. R., & Lichtman, A. H. (2001). Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(16), 9371–9376. https://doi.org/10.1073/pnas.161191698

Crawford, L. S., Mills, E. P., Hanson, T., Macey, P. M., Glarin, R., Macefield, V. G., … Henderson, L. A. (2021). Brainstem mechanisms of pain modulation: A within-subjects 7T fMRI study of placebo analgesic and nocebo hyperalgesic responses. *The Journal of Neuroscience*, *41*(47), 9794–9806.

Critchley, H. D., & Harrison, N. A. (2013). Visceral Influences on Brain and Behavior. *Neuron*, *77*(4), 624–638. https://doi.org/10.1016/J.NEURON.2013.02.008

Cui, M., Feng, Y., McAdoo, D. J., & Willis, W. D. (1999). Periacqueductal gray stimulation-induced inhibition of nociceptive dorsal horn neurons in rats is associated with the release of norepinephrine, serotonin, and amino acids. *The Journal of Pharmacology and Experimental Therapeutics*, *289*(2), 868–876.

Curry, Z. A., Wilkerson, J. L., Bagdas, D., Kyte, S. L., Patel, N., Donvito, G., … Lichtman, A. H. (2018). Monoacylglycerol Lipase Inhibitors Reverse Paclitaxel-Induced Nociceptive Behavior and Proinflammatory Markers in a Mouse Model of Chemotherapy-Induced Neuropathy s. *THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS J Pharmacol Exp Ther*, *366*, 169–183. https://doi.org/10.1124/jpet.117.245704

Davey, C. G., & Harrison, B. J. (2018, October 1). The brain's center of gravity: how the default mode network helps us to understand the self. *World Psychiatry*.

Blackwell Publishing Ltd. https://doi.org/10.1002/wps.20553

Davis, K. D., Pope, G., Chen, J., Kwan, C. L., Crawley, A. P., & Diamant, N. E. (2008). Cortical thinning in IBS: Implications for homeostatic, attention, and pain processing. *Neurology*, *70*(2), 153–154. https://doi.org/10.1212/01.wnl.0000295509.30630.10

Davis, Karen D., & Moayedi, M. (2013, June 24). Central mechanisms of pain revealed through functional and structural MRI. *Journal of Neuroimmune Pharmacology*. Springer. https://doi.org/10.1007/s11481-012-9386-8

Demyttenaere, K., Bruffaerts, R., Lee, S., Posada-Villa, J., Kovess, V., Angermeyer, M. C., … Von Korff, M. (2007). Mental disorders among persons with chronic back or neck pain: Results from the world mental health surveys. *Pain*, *129*(3), 332–342. https://doi.org/10.1016/j.pain.2007.01.022

Deuis, J. R., Dvorakova, L. S., & Vetter, I. (2017). Methods used to evaluate pain behaviors in rodents. *Frontiers in Molecular Neuroscience*. https://doi.org/10.3389/fnmol.2017.00284

DiFrancesco, M. W., Robertson, S. A., Karunanayaka, P., & Holland, S. K. (2013). BOLD fMRI in infants under sedation: Comparing the impact of pentobarbital and propofol on auditory and language activation. *J Magn Reson Imaging*, *38*(5). https://doi.org/10.1002/jmri.24082

Dimov, L. F., Toniolo, E. F., Alonso-Matielo, H., de Andrade, D. C., Garcia-Larrea, L., Ballester, G., … Dale, C. S. (2018). Electrical stimulation of the insular cortex as a novel target for the relief of refractory pain: An experimental approach in rodents. *Behavioural Brain Research*, *346*, 86–95. https://doi.org/10.1016/J.BBR.2017.11.036

Dogrul, A., Ossipov, M. H., & Porreca, F. (2009). Differential mediation of

descending pain facilitation and inhibition by spinal 5HT-3 and 5HT-7 receptors. *Brain Research*, *1280*, 52–59. https://doi.org/10.1016/j.brainres.2009.05.001

Doll, A., Hölzel, B. K., Boucard, C. C., Wohlschläger, A. M., & Sorg, C. (2015). Mindfulness is associated with intrinsic functional connectivity between default mode and salience networks. *Frontiers in Human Neuroscience*, *9*(AUGUST), 461. https://doi.org/10.3389/fnhum.2015.00461

Doll, A., Hölzel, B. K., Mulej Bratec, S., Boucard, C. C., Xie, X., Wohlschläger, A. M., & Sorg, C. (2016). Mindful attention to breath regulates emotions via increased amygdala-prefrontal cortex connectivity. *NeuroImage*, *134*, 305–313. https://doi.org/10.1016/j.neuroimage.2016.03.041

Dostrovsky, J. O., & Craig, A. D. (2013). Ascending projection systems. In S. McMahon, M. Koltzenburg, I. Tracey, & D. Turk (Eds.), *Textbook of Pain (6th edition)*. Elsevier.

Duff, E. P., Vennart, W., Wise, R. G., Howard, M. A., Harris, R. E., Lee, M., … Smith, S. M. (2015). Learning to identify CNS drug action and efficacy using multistudy fMRI data, *7*(274).

Dum, R. P., Levinthal, D. J., & Strick, P. L. (2009). The spinothalamic system targets motor and sensory areas in the cerebral cortex of monkeys. *Journal of Neuroscience*, *29*(45), 14223–14235. https://doi.org/10.1523/JNEUROSCI.3398-09.2009

Duncan, B., Lascelles, X., & Flecknell, P. A. (2010). Do Animal Models Tell Us about Human Pain? *Pain: Clinical Updates*, *18*(5). Retrieved from www.iasp-pain.org

Dunckley, P., Wise, R. G., Aziz, Q., Painter, D., Brooks, J., Tracey, I., & Chang, L. (2005). Cortical processing of visceral and somatic stimulation: Differentiating pain intensity from unpleasantness. *Neuroscience*, *133*(2), 533–542.

https://doi.org/10.1016/J.NEUROSCIENCE.2005.02.041

Dunckley, Paul, Wise, R. G., Fairhurst, M., Hobden, P., Aziz, Q., Chang, L., & Tracey, I. (2005). Behavioral/Systems/Cognitive A Comparison of Visceral and Somatic Pain Processing in the Human Brainstem Using Functional Magnetic Resonance Imaging. https://doi.org/10.1523/JNEUROSCI.1100-05.2005

Dworkin, R. H., Turk, D. C., Farrar, J. T., Haythornthwaite, J. A., Jensen, M. P., Katz, N. P., … Witter, J. (2005). Topical Review and Recommendations Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. *Pain*, *113*, 9–19. https://doi.org/10.1016/j.pain.2004.09.012

Eccleston, C., Morley, S. J., De, A. C., & Williams, C. (2013). Psychological approaches to chronic pain management: evidence and challenges. *British Journal of Anaesthesia*, *111*(1), 59–63. https://doi.org/10.1093/bja/aet207

Edwards, R.R., Fillingim, R. B., & Ness, T. J. (2003). Age-related differences in endogenous pain modulation: a comparison of diffuse noxious inhibitory controls in healthy older and younger adults. *Pain*, *101*, 155–165.

Edwards, Robert R., Dworkin, R. H., Sullivan, M. D., Turk, D., & Wasan, A. D. (2016). The role of psychosocial processes in the development and maintenance of chronic pain disorders. *The Journal of Pain : Official Journal of the American Pain Society*, *17*(9 Suppl), T70. https://doi.org/10.1016/J.JPAIN.2016.01.001

Eippert, F., Bingel, U., Schoell, E. D., Yacubian, J., Klinger, R., Lorenz, J., & Buchel, C. (2009). Activation of the opioidergic descending pain control system underlies placebo analgesia. *Neuron*, *63*(4), 533–543.

El-Serag, H. B., Pilgrim, P., & Schoenfeld, P. (2004). Natural history of irritable bowel syndrome. *Alimentary Pharmacology and Therapeutics*, *19*(8), 861–870.

https://doi.org/10.1111/j.1365-2036.2004.01929.x

Ellingson, B. M., Mayer, E., Harris, R. J., Ashe-Mcnally, C., Naliboff, B. D., Labus, J. S., & Tillisch, K. (2013). Diffusion tensor imaging detects microstructural reorganization in the brain associated with chronic irritable bowel syndrome. *Pain*, *154*(9), 1528–1541. https://doi.org/10.1016/j.pain.2013.04.010

Elsenbruch, S., Rosenberger, C., Bingel, U., Forsting, M., Schedlowski, M., & Gizewski, E. R. (2010). Patients with irritable bowel syndrome have altered emotional modulation of neural responses to visceral stimuli. *Gastroenterology*, *139*(4), 1310-1319.e4. https://doi.org/10.1053/j.gastro.2010.06.054

Elvemo, N. A., Landrø, N. I., Borchgrevink, P. C., & Håberg, A. K. (2015). Reward responsiveness in patients with chronic pain. *European Journal of Pain*, *19*(10), 1537–1543. https://doi.org/10.1002/EJP.687

Evans, V., Koh, R. G. L., Duarte, F. C. K., Linde, L., Amiri, M., & Kumbhare, D. (2021). A randomized double blinded placebo controlled study to evaluate motor unit abnormalities after experimentally induced sensitization using capsaicin. *Scientific Reports 2021 11:1*, *11*(1), 1–13. https://doi.org/10.1038/s41598-021-93188-7

Everitt, H. A., Landau, S., O'Reilly, G., Sibelli, A., Hughes, S., Windgassen, S., … Moss-Morris, R. (2019). Cognitive behavioural therapy for irritable bowel syndrome: 24-month follow-up of participants in the ACTIB randomised trial. *The Lancet Gastroenterology and Hepatology*, *4*(11), 863–872. https://doi.org/10.1016/S2468-1253(19)30243-2

Ezra, M., Faull, O. K., Jbabdi, S., & Pattison, K. T. (2015). Connectivity-based segmentation of the periaqueductal gray matter in human with brainstem optimized diffusion MRI. *Human Brain Mapping*, *36*(9), 3459–3471.

Falcone, G., & Jerram, M. (2018). Brain Activity in Mindfulness Depends on Experience: a Meta-Analysis of fMRI Studies. *Mindfulness*, *9*(5), 1319–1329. https://doi.org/10.1007/s12671-018-0884-5

Farrar, J. T., Troxel, A. B., Haynes, K., Gilron, I., Kerns, R. D., Katz, N. P., … Dworkin, R. H. (2014). Effect of variability in the 7-day baseline pain diary on the assay sensitivity of neuropathic pain randomized clinical trials: An ACTTION study. *Pain*, *155*(8), 1622–1631. https://doi.org/10.1016/J.PAIN.2014.05.009

Fayaz, A., Croft, P., Langford, R. M., Donaldson, L. J., & Jones, G. T. (2016). Prevalence of chronic pain in the UK: A systematic review and meta-analysis of population studies. *BMJ Open*, *6*(6). https://doi.org/10.1136/bmjopen-2015-010364

FDA. (2014). Analgesic Indications: Developing Drug and Biological Products. Retrieved May 16, 2020, from c

Feng, B., La, J., Schwartz, E. S., Tanaka, T., McMurray, T. P., & Gebhart, G. F. (2012). Long-term sensitization of mechanosensitive and -insensitive afferents in mice with persistent colorectal hypersensitivity. *Am J Physiol Gastrointest Liver Physiol.*, *302*(7), G676–G683.

Field, A. (2018). *Discovering statistics using IBM SPSS statistics* (5th ed.). SAGE Publications.

Fields, H., & Heinricher, M. (2013). Central nervous system mechanisms of pain modulation. In S. McMahon, M. Koltzenburg, I. Tracey, & D. Turk (Eds.), *Textbook of Pain (6th edition)*. Elsevier Saunders.

Fields, H. L., Vanegas, H., Hentall, I. D., & Zorman, G. (1983). Evidence that disinhibition of brain stem neurones contributes to morphine analgesia. *Nature*, *306*(5944), 684–686. https://doi.org/10.1038/306684a0

Finn, D. P., Beckett, S. R. G., Richardson, D., Kendall, D. A., Marsden, C. A., & Chapman, V. (2004). Evidence for differential modulation of conditioned aversion and fear-conditioned analgesia by CB1 receptors. *European Journal of Neuroscience*, *20*(3), 848–852. https://doi.org/10.1111/J.1460-9568.2004.03509.X

Finn, D. P., Haroutounian, S., Hohmann, A. G., Krane, E., Soliman, N., & Rice, A. S. C. (2021). Cannabinoids, the endocannabinoid system, and pain: a review of preclinical studies. *Pain*, *162*(7), S5–S25.

Finn, D. P., Jhaveri, M. D., Beckett, S. R. G., Roe, C. H., Kendall, D. A., Marsden, C. A., & Chapman, V. (2003). Effects of direct periaqueductal grey administration of a cannabinoid receptor agonist on nociceptive and aversive responses in rats. *Neuropharmacology*, *45*(5), 594–604. https://doi.org/10.1016/S0028-3908(03)00235-1

Finnerup, N. B., Kuner, R., & Jensen, T. S. (2020). Neuropathic pain: From mechanisms to treatment. *Physiological Reviews*, *101*(1), 259–301.

Firouzian, S., Osborne, N. R., Cheng, J. C., Kim, J. A., Bosma, R. L., Hemington, K. S., … Davis, K. D. (2020). Individual variability and sex differences in conditioned pain modulation and the impact of resilience, and conditioning stimulus pain unpleasantness and salience. *Pain*, *161*(8), 1847–1860.

Fischer-Tenhagen, C., Meier, J., & Pohl, A. (2022). “Do not look at me like that”: Is the facial expression score reliable and accurate to evaluate pain in large domestic animals? A systematic review. *Frontiers in Veterinary Science*, *9*. https://doi.org/10.3389/FVETS.2022.1002681

Fitzmaurice, B. C., & Rayen, A. T. A. (2018). Treatments for neuropathic pain: up-to-date evidence and recommendations. *BJA Education*, *18*(9), 277–283.

https://doi.org/10.1016/j.bjae.2018.06.002

FMRIB. (2018). FEAT/UserGuide - FslWiki. Retrieved December 31, 2019, from https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT/UserGuide

FMRIB. (2019). GLM - FslWiki. Retrieved December 31, 2019, from https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM

Folks, D. G. (2004). The interface of psychiatry and irritable bowel syndrome. *Current Psychiatry Reports*. Current Medicine Group LLC 1. https://doi.org/10.1007/s11920-004-0066-0

Foltz, E. L., & White, L. E. (1962). Pain “relief” by frontal cingulumotomy. *Journal of Neurosurgery*, *19*(2), 89–100. https://doi.org/10.3171/jns.1962.19.2.0089

Fond, G., Loundou, A., Hamdani, N., Boukouaci, W., Dargel, A., Oliveira, J., … Boyer, L. (2014). Anxiety and depression comorbidities in irritable bowel syndrome (IBS): a systematic review and meta-analysis. *European Archives of Psychiatry and Clinical Neuroscience*, *264*(8), 651–660. https://doi.org/10.1007/s00406-014-0502-z

Fontan, A., Lindgren, L., Pedale, T., Brorsson, C., Bergstrom, F., & Eriksson, J. (2021). A reduced level of conciousness affects non-concious processes. *Neuroimage*, *244*. https://doi.org/10.1016/j.neuroimage.2021.118571

Ford, A. C., Lacy, B. E., Harris, L. A., Quigley, E. M. M., & Moayyedi, P. (2019). Effect of Antidepressants and Psychological Therapies in Irritable Bowel Syndrome: An Updated Systematic Review and Meta-Analysis. *American Journal of Gastroenterology*, *114*(1), 21–39. https://doi.org/10.1038/S41395-018-0222-5

Ford, A. C., Quigley, E. M. M., Lacy, B. E., Lembo, A. J., Saito, Y. A., Schiller, L. R., … Moayyedi, P. (2014). Effect of antidepressants and psychological therapies,

including hypnotherapy, in irritable bowel syndrome: systematic review and meta-analysis. *The American Journal of Gastroenterology*. https://doi.org/10.1038/ajg.2014.148

Foreman, R. D. (2004, December 1). Mechanisms of visceral pain: From nociception to targets. *Drug Discovery Today: Disease Mechanisms*. Elsevier Ltd. https://doi.org/10.1016/j.ddmec.2004.11.022

Foster, E., Wildner, H., Tudeau, L., Haueter, S., Ralvenius, W. T., Jegen, M., … Zeilhofer, H. U. (2015). Targeted ablation, silencing, and activation establish glycinergic dorsal horn neurons as key components of a spinal gate for pain and itch. *Neuron*, *85*(6), 1289–1304.

Freynhagen, R., Baron, R., Gockel, U., & Tölle, T. R. (2006). painDETECT: A new screening questionnaire to identify neuropathic components in patients with back pain. *Current Medical Research and Opinion*, *22*(10), 1911–1920. https://doi.org/10.1185/030079906X132488

Frisaldi, E., Shaibani, A., & Benedetti, F. (2020). Understanding the mechanisms of placebo and nocebo effects. *Swiss Medical Weekly*, *150*, w20340. https://doi.org/10.4414/smw.2020.20340

Gard, T., Hölzel, B. K., Sack, A. T., Hempel, H., Lazar, S. W., Vaitl, D., & Ott, U. (2012). Pain attenuation through mindfulness is associated with decreased cognitive control and increased sensory processing in the brain. *Cerebral Cortex*, *22*(11), 2692–2702. https://doi.org/10.1093/cercor/bhr352

Gaylord, S. A., Palsson, O. S., Garland, E. L., Faurot, K. R., Coble, R. S., Mann, J. D., … Whitehead, W. E. (2011). Mindfulness training reduces the severity of irritable bowel syndrome in women: Results of a randomized controlled trial. *American Journal of Gastroenterology*, *106*(9), 1678–1688.

https://doi.org/10.1038/ajg.2011.184

Genaro, K., Fabris, D., Arantes, A. L. F., Zuardi, A. W., Crippa, J. A. S., & Prado, W. A. (2017). Cannabidiol is a potential therapeutic for the affective-motivational dimension of incision pain in rats. *Frontiers in Pharmacology*, *8*(JUN). https://doi.org/10.3389/fphar.2017.00391

Gewandter, J. S., Dworkin, R. H., Turk, D. C., Devine, E. G., Hewitt, D., Jensen, M. P., … Witter, J. (2020). Improving Study Conduct and Data Quality in Clinical Trials of Chronic Pain Treatments: IMMPACT Recommendations. *Journal of Pain*, *21*(9–10), 931–942. https://doi.org/10.1016/J.JPAIN.2019.12.003

Ghosh, S., Wise, L. E., Chen, Y., Gujjar, R., Mahadevan, A., Cravatt, B. F., & Lichtman, A. H. (2013). The monoacylglycerol lipase inhibitor JZL184 suppresses inflammatory pain in the mouse carrageenan model. In *Life Sciences* (Vol. 92, pp. 498–505). https://doi.org/10.1016/j.lfs.2012.06.020

Giamberardino, M. A., Affaitati, G., Lerza, R., & De Laurentis, S. (2002). Neurophysiological basis of visceral pain. In *Journal of Musculoskeletal Pain* (Vol. 10, pp. 151–163). Taylor & Francis. https://doi.org/10.1300/J094v10n01_12

Gomtsian, L., Bannister, K., Eyde, N., RObles, D., Dickenson, A. H., Porreca, F., & Navratilova, E. (2018). Morphine effects within the rodent anterior cingilate cortex and rostral ventromedial medulla reveal separable modulation of affective and sensory qualities of acute or chronic pain. *Pain*, *159*(12), 2512–2521.

Goncalves, E. S., Matielo, H. A., Teixeira, M. J., Ciampi de Andrade, D., Hamani, C., & Dale, C. S. (2022). Electrical stimulation of the posterior insular cortex induces opioid and cannabinoid-dependent antinociception and regulates glial cells in the spinal cord. *Brazilian Journal of Pain*, *5*(3), 239–247.

Gonçalves, L., & Dickenson, A. H. (2012). Asymmetric time-dependent activation of right central amygdala neurones in rats with peripheral neuropathy and pregabalin modulation. *European Journal of Neuroscience*, *36*(9), 3204–3213. https://doi.org/10.1111/j.1460-9568.2012.08235.x

Goodwin, G. M., Holmes, E. A., Andersson, E., Browning, M., Jones, A., Lass-Hennemann, J., … Visser, R. M. (2018). From neuroscience to evidence based psychological treatments – The promise and the challenge, ECNP March 2016, Nice, France. *European Neuropsychopharmacology*, *28*(2), 317–333. https://doi.org/10.1016/j.euroneuro.2017.10.036

Gore, M., Brandenburg, N. A., Dukes, E., Hoffman, D. L., Tai, K. S., & Stacey, B. (2005). Pain severity in diabetic peripheral neuropathy is associated with patient functioning, symptom levels of anxiety and depression, and sleep. *Journal of Pain and Symptom Management*, *30*(4), 374–385. https://doi.org/10.1016/j.jpainsymman.2005.04.009

Gotink, R. A., Meijboom, R., Vernooij, M. W., Smits, M., & Hunink, M. G. M. (2016). 8-week Mindfulness Based Stress Reduction induces brain changes similar to traditional long-term meditation practice – A systematic review. *Brain and Cognition*, *108*, 32–41. https://doi.org/10.1016/j.bandc.2016.07.001

Gracely, R., Geisser, M., Giesecke, T., Grant, M., Petzke, F., Williams, D., & Clauw, D. (2004). Pain catastrophizing and neural responses to pain among persons with fibromyalgia. *Brain*, *127*(4), 835–843.

Graeff, P., Itter, A., Wach, K., & Ruscheweyh, R. (2021). Inter-individual differences explain more varaince in conditioned pain modulation than age, sex and conditioning stimulus intensity combined. *Brain Sciences*, *11*(9), 1186.

Grant, J. A., Courtemanche, J., & Rainville, P. (2011). A non-elaborative mental

stance and decoupling of executive and pain-related cortices predicts low pain sensitivity in Zen meditators. *Pain*, *152*(1), 150–156. https://doi.org/10.1016/j.pain.2010.10.006

Green, A. L., Wang, S., Bittar, R. G., Owen, S. L. F., Paterson, D. J., Stein, J. F., … Aziz, T. Z. (2007). Deep brain stimulation: A new treatment for hypertension? *Journal of Clinical Neuroscience*, *14*(6), 592–595.

Greve, D. N., & Fischl, B. (2009). Accurate and robust brain image alignment using boundary-based registration. *NeuroImage*, *48*(1), 63–72. https://doi.org/10.1016/j.neuroimage.2009.06.060

Griffanti, L., Douaud, G., Bijsterbosch, J., Evangelisti, S., Alfaro-Almagro, F., Glasser, M. F., … Smith, S. M. (2017). Hand classification of fMRI ICA noise components. *NeuroImage*, *154*, 188–205. https://doi.org/10.1016/j.neuroimage.2016.12.036

Griffanti, L., Salimi-Khorshidi, G., Beckmann, C. F., Auerbach, E. J., Douaud, G., Sexton, C. E., … Smith, S. M. (2014). ICA-based artefact removal and accelerated fMRI acquisition for improved resting state network imaging. *NeuroImage*, *95*, 232–247. https://doi.org/10.1016/j.neuroimage.2014.03.034

Groh, A., Mease, R. A., & Krieger, P. (2017, August 1). Pain processing in the thalamocortical system. *Neuroforum*. Spektrum Akademischer Verlag. https://doi.org/10.1515/nf-2017-A019

Grundy, D. (2002). Neuroanatomy of visceral nociception: vagal and splanchnic afferent. *Gut*, *51*(Suppl I), i2–i5.

Gupta, A., Mayer, E. A., Fling, C., Labus, J. S., Naliboff, B. D., Hong, J. Y., & Kilpatrick, L. A. (2017, January 1). Sex-based differences in brain alterations across chronic pain conditions. *Journal of Neuroscience Research*. John Wiley

and Sons Inc. https://doi.org/10.1002/jnr.23856

Gureje, O., Von Korff, M., Kola, L., Demyttenaere, K., He, Y., Posada-Villa, J., … Alonso, J. (2008). The relation between multiple pains and mental disorders: Results from the World Mental Health Surveys. *Pain*, *135*(1), 82–91. https://doi.org/10.1016/j.pain.2007.05.005

Håberg, A. K. (2017). Authors' reply to the comment by Dalton. *European Journal of Pain*, *21*(5), 950–951. https://doi.org/10.1002/EJP.1038

Hadjipavlou, G., Dunckley, P., Behrens, T. E., & Tracey, I. (2006). Determining anatomical connectivities between cortical and brainstem pain processing regions in humans: A diffusion tensor imaging study in healthy controls. *Pain*, *123*, 169–178.

Haefeli, M., & Elfering, A. (2006, January). Pain assessment. *European Spine Journal*. Springer. https://doi.org/10.1007/s00586-005-1044-x

Hand, A., Blake, A., Kerrigan, P., Samuel, P., & Friedberg, J. (2016). History of medical cannabis. *J Pain Manage*, *9*(4), 387–394.

Hardy, P. S. G., & Haigler, H. J. (1985). Prefrontal influences upon the midbrain: A possible route for pain modulation. *Brain Research*, *339*(2), 285–293. https://doi.org/10.1016/0006-8993(85)90094-0

Harris, R. E., Napadow, V., Huggins, J. P., Pauer, L., Kim, J., Hampson, J., … Clauw, D. J. (2013). Pregabalin rectifies aberrant brain chemistry, connectivity, and functional response in chronic pain patients. *Anesthesiology*, *119*(6), 1453–1464. https://doi.org/10.1097/aln.0000000000000017

Harrison, R., Zeidan, F., Kitsaras, G., Ozcelik, D., & Salomons, T. V. (2019). Trait Mindfulness Is Associated With Lower Pain Reactivity and Connectivity of the Default Mode Network. *Journal of Pain*, *20*(6), 645–654.

https://doi.org/10.1016/j.jpain.2018.10.011

Harte, S. E., Harris, R. E., & Clauw, D. J. (2018). The neurobiology of central sensitization. *Journal of Applied Biobehavioral Research*, *23*(2). https://doi.org/10.1111/jabr.12137

Hashmi, J. A., Baria, A. T., Baliki, M. N., Huang, L., Schnitzer, T. J., & Apkarian, A. V. (2012). Brain networks predicting placebo analgesia in a clinical trial for chronic back pain. *Pain*, *153*(12), 2393–2402. https://doi.org/10.1016/j.pain.2012.08.008

Hauser, G., Pletikosic, S., & Tkalcic, M. (2014). Cognitive behavioral approach to understanding irritable bowel syndrome. *World Journal of Gastroenterology*, *20*(22), 6744–6758. https://doi.org/10.3748/wjg.v20.i22.6744

Hawker, G. A., Mian, S., Kendzerska, T., & French, M. (2011). Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). *Arthritis Care and Research*, *63*(SUPPL. 11), S240–S252. https://doi.org/10.1002/acr.20543

Health Research Authority. (2020). The Over-Volunteering Prevention System. Retrieved April 8, 2021, from https://www.hra.nhs.uk/about-us/committees-and-services/the-over-volunteering-prevention-system/

Health Research Authority. (2021a). Research registration and research project identifiers. Retrieved May 27, 2021, from https://www.hra.nhs.uk/planning-and-improving-research/research-planning/research-registration-research-project-identifiers/

Health Research Authority. (2021b). UK Policy Framework for Health and Social Care Research. Retrieved May 10, 2021, from https://www.hra.nhs.uk/planning-and-improving-research/policies-standards-legislation/uk-policy-framework-health-social-care-research/uk-policy-framework-health-and-social-care-research/

Heinricher, M. M., Morgan, M. M., & Fields, H. L. (1992). Direct and indirect actions of morphine on medullary neurons that modulate nociception. *Neuroscience*, *48*(3), 533–543. https://doi.org/10.1016/0306-4522(92)90400-V

Heinricher, M. M., Tavares, I., Leith, J. L., & Lumb, B. M. (2009). Descending control of nociception: Specificity, recruitment and plasticity. *Brain Research Reviews*, *60*(1), 214–225. https://doi.org/10.1016/J.BRAINRESREV.2008.12.009

Heinsinger, N. M., Spagnuolo, G., Allahyari, R. V., Galer, S., Fox, T., Jaffe, D. A., … Lepore, A. C. (2020). Facial grimace testing as an assay of neuropathic pain-related behavior in a mouse model of cervical spinal cord injury. *Experimental Neurology*, *334*. https://doi.org/10.1016/J.EXPNEUROL.2020.113468

Helmstetter, F. J., Tershner, S. A., Poore, L. H., & Bellgowan, P. S. F. (1998). Antinociception following opioid stimulation of the basolateral amygdala is expressed through the periaqueductal gray and rostral ventromedial medulla. *Brain Research*, *779*(1–2), 104–118. https://doi.org/10.1016/S0006-8993(97)01104-9

Henrich, J. F., Gjelsvik, B., Surawy, C., Evans, E., & Martin, M. (2020). A randomized clinical trial of mindfulness-based cognitive therapy for women with irritable bowel syndrome-effects and mechanisms. *Journal of Consulting and Clinical Psychology*, *88*(4), 295–310. https://doi.org/10.1037/ccp0000483

Henrich, J. F., Knittle, K., De Gucht, V., Warren, S., Dombrowski, S. U., & Maes, S.

(2015, March 1). Identifying effective techniques within psychological treatments for irritable bowel syndrome: A meta-analysis. *Journal of Psychosomatic Research*. Elsevier Inc. https://doi.org/10.1016/j.jpsychores.2014.12.009

Hodkinson, D. J., Khawaja, N., O'Daly, O., Thacker, M. A., Zelaya, F. O., Wooldridge, C. L., … Howard, M. A. (2015). Cerebral analgesic response to nonsteroidal anti-inflammatory drug ibuprofen. *Pain*, *156*(7), 1301–1310. https://doi.org/10.1097/J.PAIN.0000000000000176

Hohmann, A. G., Martin, W. J., Tsou, K., & Walker, J. M. (1995). Inhibition of noxious stimulus-evoked activity of spinal cord dorsal horn neurons by the cannabinoid WIN 55,212-2. *Life Sciences*, *56*(23–24), 2111–2118. https://doi.org/10.1016/0024-3205(95)00196-D

Hohmann, A. G., Suplita, R. L., Bolton, N. M., Neely, M. H., Fegley, D., Mangieri, R., … Piomelli, D. (2005). An endocannabinoid mechanism for stress-induced analgesia. *Nature*, *435*(7045), 1108–1112. https://doi.org/10.1038/nature03658

Hölzel, B. K., Hoge, E. A., Greve, D. N., Gard, T., Creswell, J. D., Brown, K. W., … Lazar, S. W. (2013). Neural mechanisms of symptom improvements in generalized anxiety disorder following mindfulness training. *NeuroImage: Clinical*, *2*(1), 448–458. https://doi.org/10.1016/j.nicl.2013.03.011

Hong, J. Y., Kilpatrick, L. A., Labus, J. S., Gupta, A., Katibian, D., Ashe-McNalley, C., … Hong, J. Y. (2014). Sex and disease-related alterations of anterior insula functional connectivity in chronic abdominal pain. *Journal of Neuroscience*, *34*(43), 14252–14259. https://doi.org/10.1523/JNEUROSCI.1683-14.2014

Houlton, S. (2019). Developing new medicines for the treatment of pain. *Prescriber*, *30*(2), 25–27. https://doi.org/10.1002/PSB.1740

Hsieh, J. C., Belfrage, M., Stone-Elander, S., Hansson, P., & Ingvar, M. (1995).

Central representation of chronic ongoing neuropathic pain studied by positron emission tomography. *Pain*, *63*(2), 225–236. https://doi.org/10.1016/0304-3959(95)00048-W

Huang, J., Gadotti, V. M., Chen, L., Souza, I. A., Huang, S., Wang, D., … Zamponi, G. W. (2019). A neuronal circuit for activating descending modulation of neuropathic pain. *Nature Neuroscience*, *22*, 1659–1668.

Hubbard, C. S., Khan, S. A., Keaser, M. L., Seminowicz, D. A., Mathur, V. A., & Goyal, M. (2014). Altered brain structure and function correlate with disease severity and pain catastrophizing in migraine patients. *ENeuro*, *1*(1). https://doi.org/10.1523/ENEURO.0006-14.2014

Hughes, D. I., & Todd, A. J. (2020). Central nervous system targets: Inhibitory interneurons in the spinal cord. *Neurotherapeutics*, *17*(3), 874–885.

Iacovides, S., Avidon, I., & Baker, F. C. (2015). What we know about primary dysmenorrhea today: A critical review. *Human Reproduction Update*, *21*(6), 762–778. https://doi.org/10.1093/humupd/dmv039

Iannetti, G. D., Zambreanu, L., Wise, R. G., Buchanan, T. J., Huggins, J. P., Smart, T. S., … Tracey, I. (2005). *Pharmacological modulation of pain-related brain activity during normal and central sensitization states in humans*. Retrieved from www.pnas.orgcgidoi10.1073pnas.0506624102

IASP. (2020). IASP Terminology. Retrieved August 22, 2020, from https://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698&navItemNumber=576#Pain

Ibinson, J. W., Small, R. H., Algaze, A., Roberts, C. J., Clark, D. L., & Schmalbrock, P. (2004). Functional Magnetic Resonance Imaging Studies of Pain An Investigation of Signal Decay during and across Sessions. *Anesthesiology*, *101*,

960–969. Retrieved from http://pubs.asahq.org/anesthesiology/article-pdf/101/4/960/356799/0000542-200410000-00022.pdf

Icenhour, A, Langhorst, J., Benson, S., Schlamann, M., Hampel, S., Engler, H., … Elsenbruch, S. (2015). Neural circuitry of abdominal pain-related fear learning and reinstatement in irritable bowel syndrome. *Neurogastroenterol Motil*, *27*, 114–127. https://doi.org/10.1111/nmo.12489

Icenhour, Adriane, Petrakova, L., Hazzan, N., Theysohn, N., Merz, C. J., & Elsenbruch, S. (2021). When gut feelings teach the brain to fear pain: Context-dependent activation of the central fear network in a novel interoceptive conditioning paradigm. *NeuroImage*, *238*. https://doi.org/10.1016/J.NEUROIMAGE.2021.118229

Icenhour, Adriane, Witt, S. T., Elsenbruch, S., Lowén, M., Engström, M., Tillisch, K., … Walter, S. (2017). Brain functional connectivity is associated with visceral sensitivity in women with Irritable Bowel Syndrome. *NeuroImage: Clinical*, *15*, 449–457. https://doi.org/10.1016/j.nicl.2017.06.001

Ignatowska-Jankowska, B. M., Ghosh, S., Crowe, M. S., Kinsey, S. G., Niphakis, M. J., Abdullah, R. A., … Lichtman, A. H. (2014). In vivo characterization of the highly selective monoacylglycerol lipase inhibitor KML29: Antinociceptive activity without cannabimimetic side effects. *British Journal of Pharmacology*, *171*(6), 1392–1407. https://doi.org/10.1111/bph.12298

Ignatowska-Jankowska, B., Wilkerson, J. L., Mustafa, M., Abdullah, R., Niphakis, M., Wiley, J. L., … Lichtman, A. H. (2015). Selective monoacylglycerol lipase inhibitors: Antinociceptive versus cannabimimetic effects in Mices. *Journal of Pharmacology and Experimental Therapeutics*, *353*(2), 424–432. https://doi.org/10.1124/jpet.114.222315

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (1995). *ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*. Retrieved from http://www.emea.eu.int

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (2016). *Harmonised guideline integrated addendum to ICH E6(R1): Guideline for good clinical practice E6(R2)*.

Jalon, I., Berger, A., Shofty, B., Goldway, N., Artzi, M., Gurevitch, G., … Strauss, I. (2023). Lesions to both somatic and affective pain pathways lead to decreased salience network connectivity. *Brain*, *00*, 1–10. https://doi.org/10.1093/brain/awac403

Jantsch, H. H. F., Gawlitza, M., Geber, C., Baumgärtner, U., Krämer, H. H., Magerl, W., … Birklein, F. (2009). Explicit episodic memory for sensory-discriminative components of capsaicin-induced pain: Immediate and delayed ratings. *Pain*, *143*(1–2), 97–105. https://doi.org/10.1016/j.pain.2009.02.004

Jee Kim, M., Tanioka, M., Woo Um, S., Hong, S.-K., & Hwan Lee, B. (2018). Analgesic effects of FAAH inhibitor in the insular cortex of nerve-injured rats. *Molecular Pain*, *14*, 1744806918814345. https://doi.org/10.1177/1744806918814345

Jelsness-Jørgensen, L. P., Moum, B., Grimstad, T., Jahnsen, J., Opheim, R., Prytz Berset, I., … Bernklev, T. (2016). Validity, reliability, and responsiveness of the brief pain inventory in inflammatory bowel disease. *Canadian Journal of Gastroenterology and Hepatology*, *2016*. https://doi.org/10.1155/2016/5624261

Jenkinson, M., Bannister, P., Brady, M., & Smith, S. (2002). Improved optimization for the robust and accurate linear registration and motion correction of brain

images. *NeuroImage*, *17*(2), 825–841. https://doi.org/10.1016/S1053-8119(02)91132-8

Jenkinson, M., Beckmann, C. F., Behrens, T. E. J., Woolrich, M. W., & Smith, S. M. (2012). Review FSL. *NeuroImage*, *62*, 782–790. https://doi.org/10.1016/j.neuroimage.2011.09.015

Jenkinson, M., & Smith, S. (2001). A global optimisation method for robust affine registration of brain images. *Medical Image Analysis*, *5*(2), 143–156. https://doi.org/10.1016/S1361-8415(01)00036-6

Jensen, M. P., Tan, G., & Chua, S. M. (2015). Pain intensity, headache frequency, and the behavioral activation and inhibition systems. *Clinical Journal of Pain*, *31*(12), 1068–1074. https://doi.org/10.1097/AJP.0000000000000215

Ji, G., & Neugebauer, V. (2009). Hemispheric lateralization of pain processing by amygdala neurons. *Journal of Neurophysiology*, *102*(4), 2253–2264. https://doi.org/10.1152/jn.00166.2009

Ji, G., & Neugebauer, V. (2014). CB1 augments mGluR5 function in medial prefrontal cortical neurons to inhibit amygdala hyperactivity in an arthritis pain model. *European Journal of Neuroscience*, *39*(3), 455–466. https://doi.org/10.1111/EJN.12432

Ji, R.-R., Nackley, A., Huh, Y., Terrando, N., & Maixner, W. (2018). Neuroinflammation and central sensitization in chronic and widespread pain. *Anesthesiology*, *129*(2), 343–366.

Johansen, J. P., & Fields, H. L. (2004). Glutamatergic activation of anterior cingulate cortex produces an aversive teaching signal. *Nature Neuroscience*, *7*(4), 398–403. https://doi.org/10.1038/nn1207

Johansen, J. P., Fields, H. L., & Manning, B. H. (2001). The affective component of

pain in rodents: Direct evidence for a contribution of the anterior cingulate cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(14), 8077–8082. https://doi.org/10.1073/pnas.141218998

Joint Formulary Committee. (2020). CANNABIS EXTRACT. Retrieved January 11, 2020, from https://bnf.nice.org.uk/drug/cannabis-extract.html

Julian, L. J. (2011). Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). *Arthritis Care and Research*, *63*(SUPPL. 11). https://doi.org/10.1002/acr.20561

Kahnt, T., Heinzle, J., Park, S. Q., & Haynes, J. D. (2010). The neural code of reward anticipation in human orbitofrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(13), 6010–6015. https://doi.org/10.1073/pnas.0912838107

Kain, Z. N., Sevarino, F., Alexander, G. M., Pincus, S., & Mayes, L. C. (2000). Preoperative anxiety and postoperative pain in women undergoing hysterectomy: A repeated-measures design. *Journal of Psychosomatic Research*, *49*(6), 417–422. https://doi.org/10.1016/S0022-3999(00)00189-6

Kam-Hansen, S., Jakubowski, M., Kelley, J. M., Kirsch, I., Hoaglin, D. C., Kaptchuk, T. J., & Burstein, R. (2014). Altered placebo and drug labeling changes the outcome of episodic migraine attacks. *Science Translational Medicine*, *6*(218), 218ra5. https://doi.org/10.1126/scitranslmed.3006175

Kapoor, S., White, J., Thorn, B. E., & Block, P. (2016). Patients Presenting to the Emergency Department with Acute Pain: The Significant Role of Pain Catastrophizing and State Anxiety. *Pain Medicine*, *17*(6), 1069–1078. https://doi.org/10.1093/pm/pnv034

Karjalainen, T., Tuominen, L., Manninen, S., Kalliokoski, K. K., Nuutila, P., Jääskeläinen, I. P., … Nummenmaa, L. (2016). Behavioural activation system sensitivity is associated with cerebral μ-opioid receptor availability. *Social Cognitive and Affective Neuroscience*, *11*(8), 1310. https://doi.org/10.1093/SCAN/NSW044

Keller, S., Bann, C. M., Dodd, S. L., Schein, J., Mendoza, T. R., & Cleeland, C. S. (2004). Validity of the brief pain inventory for use in documenting the outcomes of patients with noncancer pain. *Clinical Journal of Pain*, *20*(5), 309–318. https://doi.org/10.1097/00002508-200409000-00005

Kempton, C. L., Buckner, T. W., Fridman, M., Iyer, N. N., & Cooper, D. L. (2018). Factors associated with pain severity, pain interference, and perception of functional abilities independent of joint status in US adults with hemophilia: Multivariable analysis of the Pain, Functional Impairment, and Quality of Life (P-FiQ) study. *European Journal of Haematology*, *100*, 25–33. https://doi.org/10.1111/ejh.13025

Kendroud, S., Fitzgerald, L. A., Murray, I., & Hanna, A. (2022). *Physiology, Nociceptive pathways*. Treasure Island, Florida: StatPearls Publishing.

King, A. P., Block, S. R., Sripada, R. K., Rauch, S., Giardino, N., Favorite, T., … Liberzon, I. (2016). Altered default mode network (DMN) resting state functional connectivity following a mindfulness-based exposure therapy for posttraumatic stress disorder (PTSD) in combat veterans of Afghanistan and Iraq. *Depression and Anxiety*, *33*(4), 289–299. https://doi.org/10.1002/da.22481

King, T., Vera-Portocarrero, L., Gutierrez, T., Vanderah, T. W., Dussor, G., Lai, J., … Porreca, F. (2009). Unmasking the tonic-aversive state in neuropathic pain. *Nature Neuroscience*, *12*(11), 1364–1366.

Kinsey, S. G., Long, J. Z., O'Neal, S. T., Abdullah, R. A., Poklis, J. L., Boger, D. L., … Lichtman, A. H. (2009). Blockade of endocannabinoid-degrading enzymes attenuates neuropathic pain. *Journal of Pharmacology and Experimental Therapeutics*, *330*(3), 902–910. https://doi.org/10.1124/jpet.109.155465

Kiritoshi, T., Ji, G., & Neugebauer, V. (2016). Rescue of Impaired mGluR5-Driven Endocannabinoid Signaling Restores Prefrontal Cortical Output to Inhibit Pain in Arthritic Rats. https://doi.org/10.1523/JNEUROSCI.4047-15.2016

Kissin, I. (2010). The development of new analgesics over the past 50 years: A lack of real breakthrough drugs. *Anesthesia and Analgesia*, *110*(3), 780–789. https://doi.org/10.1213/ANE.0b013e3181cde882

Kleggetveit, I. P., Namer, B., Schmidt, R., Tormod, H., Ruckel, M., Orstavik, K., … Jorum, E. (2012). High spontaneous activity of C-nociceptors in painful polyneuropathy. *Pain*, *153*(10), 2040–2047.

Kleine-Borgmann, J., Schmidt, K., Scharmach, K., Zunhammer, M., Elsenbruch, S., Bingel, U., & Forkmann, K. (2022). Does pain modality play a role in the interruptive function of acute visceral compared with somatic pain? *Pain*, *163*(4), 735–744.

Knotkova, H., Hamani, C., Sivansesan, E., Elgueta Le Beuffe, M. F., Moon, J. Y., Cohen, S. P., & Huntoon, M. A. (2021). Neuromodulation for chronic pain. *The Lancet*, *397*(10289), 2111–2124.

Koenen, L.R., Icenhour, A., Forkmann, K., Pasler, A., Theysohn, N., Forsting, M., … Elsenbruch, S. (2017). Greater fear of visceral pain contributes to differences between visceral and somatic pain in healthy women. *Pain*, *158*(8), 1599–1608.

Koenen, Laura Ricarda, Icenhour, A., Forkmann, K., Theysohn, N., Forsting, M., Bingel, U., & Elsenbruch, S. (2018). From Anticipation to the Experience of Pain:

The Importance of Visceral Versus Somatic Pain Modality in Neural and Behavioral Responses to Pain-Predictive Cues. *Psychosomatic Medicine*, *80*(9), 826–835. https://doi.org/10.1097/PSY.0000000000000612

Koga, K., Shimoyama, S., Yamada, A., Furukawa, T., Nikaido, Y., Furue, H., … Ueno, S. (2018). Chronic inflammatory pain induced GABAergic synaptic plasticity in the adult mouse anterior cingulate cortex. *Molecular Pain*, *14*. https://doi.org/10.1177/1744806918783478

Koga, Kohei, Descalzi, G., Chen, T., Ko, H. G., Lu, J., Li, S., … Zhuo, M. (2015). Coexistence of Two Forms of LTP in ACC Provides a Synaptic Mechanism for the Interactions between Anxiety and Chronic Pain. *Neuron*, *85*(2), 377–389. https://doi.org/10.1016/J.NEURON.2014.12.021

Koloski, N. A., Jones, M., Kalantar, J., Weltman, M., Zaguirre, J., & Talley, N. J. (2012). The brain - Gut pathway in functional gastrointestinal disorders is bidirectional: A 12-year prospective population-based study. *Gut*, *61*(9), 1284–1290. https://doi.org/10.1136/gutjnl-2011-300474

Kremer, M., Becker, L. J., Barrot, M., & Yalcin, I. (2020). How to study anxiety and depression in rodent models of chronic pain? *European Journal of Neuroscience*, *53*, 236–270.

Kropf, E., Syan, S. K., Minuzzi, L., & Frey, B. N. (2019). From anatomy to function: the role of the somatosensory cortex in emotional regulation. *Revista Brasileira de Psiquiatria (Sao Paulo, Brazil : 1999)*, *41*(3), 261–269. https://doi.org/10.1590/1516-4446-2018-0183

Krummenacher, P., Candia, V., Folkers, G., Schedlowski, M., & Schönbächler, G. (2010). Prefrontal cortex modulates placebo analgesia. *Pain*, *148*(3), 368–374. https://doi.org/10.1016/J.PAIN.2009.09.033

Kulkarni, B., Bentley, D. E., Elliott, R., Youell, P., Watson, A., Derbyshire, S. W. G., … Jones, A. K. P. (2005). Attention to pain localization and unpleasantness discriminates the functions of the medial and lateral pain systems. *European Journal of Neuroscience*, *21*(11), 3133–3142. https://doi.org/10.1111/j.1460-9568.2005.04098.x

LaBar, K. S., Gatenby, J. C., Gore, J. C., Ledoux, J. E., & Phelps, E. A. (1998). *Human Amygdala Activation during Conditioned Fear Acquisition and Extinction: a Mixed-Trial fMRI Study*. *Neuron* (Vol. 20).

Labus, J. S., Bolus, R., Chang, L., Wiklund, I., Naesdal, J., Mayer, E. A., & Naliboff, B. D. (2004). The Visceral Sensitivity Index: Development and validation of a gastrointestinal symptom-specific anxiety scale. *Alimentary Pharmacology and Therapeutics*, *20*(1), 89–97. https://doi.org/10.1111/j.1365-2036.2004.02007.x

Labus, Jennifer S., Dinov, I. D., Jiang, Z., Ashe-Mcnalley, C., Zamanyan, A., Shi, Y., … Mayer, E. A. (2014). Irritable bowel syndrome in female patients is associated with alterations in structural brain networks. *Pain*, *155*(1), 137–149. https://doi.org/10.1016/j.pain.2013.09.020

Labus, Jennifer S., Gupta, A., Coveleskie, K., Tillisch, K., Kilpatrick, L., Jarcho, J., … Mayer, E. A. (2013). Sex differences in emotion-related cognitive processes in irritable bowel syndrome and healthy control subjects. *Pain*, *154*(10), 2088–2099. https://doi.org/10.1016/j.pain.2013.06.024

Lackner, J. M., Quigley, B. M., & Blanchard, E. B. (2004). Depression and abdominal pain in IBS patients: The mediating role of catastrophizing. *Psychosomatic Medicine*, *66*(3), 435–441. https://doi.org/10.1097/01.psy.0000126195.82317.46

Laird, K. T., Tanner-Smith, E. E., Russell, A. C., Hollon, S. D., & Walker, L. S. (2016, July 1). Short-term and Long-term Efficacy of Psychological Therapies for

Irritable Bowel Syndrome: A Systematic Review and Meta-analysis. *Clinical Gastroenterology and Hepatology*. W.B. Saunders. https://doi.org/10.1016/j.cgh.2015.11.020

Laird, K. T., Tanner-Smith, E. E., Russell, A. C., Hollon, S. D., & Walker, L. S. (2017, February 1). Comparative efficacy of psychological therapies for improving mental health and daily functioning in irritable bowel syndrome: A systematic review and meta-analysis. *Clinical Psychology Review*. Elsevier Inc. https://doi.org/10.1016/j.cpr.2016.11.001

Lamotte, R. H., Thalhammer, J. G., Torebjork, H. E., & Robinson, C. J. (1982). Peripheral neural mechanisms of cutaneous hyperalgesia following mild injury by heat. *Journal of Neuroscience*, *2*(6), 765–781. https://doi.org/10.1523/jneurosci.02-06-00765.1982

Larivière, M., Goffaux, P., Marchand, S., & Julien, N. (2007). Changes in pain perception and descending inhibitory controls start at middle age in healthy adults. *Clinical Journal of Pain*, *23*(6), 506–510. https://doi.org/10.1097/AJP.0B013E31806A23E8

Lascelles, B. D. X., Brown, D. C., Maixner, W., & Mogil, J. S. (2018). Spontaneous painful disease in companion animals can facilitate the development of chronic pain therapies for humans. *Osteoarthritis and Cartilage*, *26*(2), 175–183.

Laumet, G., Ma, J., Robison, A. J., Kumari, S., Heijnen, C. J., & Kavelaars, A. (2019). T cells as an emerging target for chronic pain therapy. *Frontiers in Molecular Neuroscience*, *12*, 216. https://doi.org/10.3389/fnmol.2019.00216

Lawson, K. (2017). A Brief Review of the Pharmacology of Amitriptyline and Clinical Outcomes in Treating Fibromyalgia. *Biomedicines*, *5*(24). https://doi.org/10.3390/biomedicines5020024

Lee, H. F., Hsieh, J. C., Lu, C. L., Yeh, T. C., Tu, C. H., Cheng, C. M., … Chang, F. Y. (2012). Enhanced affect/cognition-related brain responses during visceral placebo analgesia in irritable bowel syndrome patients. *Pain*, *153*(6), 1301–1310. https://doi.org/10.1016/j.pain.2012.03.018

Lee, J.-H. A., Chen, Q., & Zhou, M. (2022). Synaptic plasticity in the pain-related cingulate and insular cortex. *Biomedicines*, *10*, 2745. https://doi.org/10.3390/biomedicines10112745

Lee, M. C., Ploner, M., Wiech, K., Bingel, U., Wanigasekera, V., Brooks, J., … Tracey, I. (2013). Amygdala activity contributes to the dissociative effect of cannabis on pain perception. *Pain*, *154*(1), 124–134. https://doi.org/10.1016/j.pain.2012.09.017

Lee, M. C., Zambreanu, L., Menon, D. K., & Tracey, I. (2008). Identifying brain activity specifically related to the maintenance and perceptual consequence of central sensitization in humans. *Journal of Neuroscience*, *28*(45), 11642–11649. https://doi.org/10.1523/JNEUROSCI.2638-08.2008

Leichsenring, A., Andriske, M., Bäcker, I., Stichel, C. C., & Lübbert, H. (n.d.). Analgesic and antiinflammatory effects of cannabinoid receptor agonists in a rat model of neuropathic pain. https://doi.org/10.1007/s00210-008-0386-4

Leknes, S., Brooks, J. C. W., Wiech, K., & Tracey, I. (2008). Pain relief as an opponent process: a psychophysical investigation. *European Journal of Neuroscience*, *28*(4), 794–801. https://doi.org/10.1111/J.1460-9568.2008.06380.X

Leknes, S., Lee, M., Berna, C., Andersson, J., & Tracey, I. (2011). Relief as a Reward: Hedonic and Neural Responses to Safety from Pain. *PLOS ONE*, *6*(4), e17870. https://doi.org/10.1371/JOURNAL.PONE.0017870

Leung, L. (2012, July). Pain catastrophizing: An updated review. *Indian Journal of Psychological Medicine*. Wolters Kluwer -- Medknow Publications. https://doi.org/10.4103/0253-7176.106012

Lieberman, M. D., Jarcho, J. M., Berman, S., Naliboff, B. D., Suyenobu, B. Y., Mandelkern, M., & Mayer, E. A. (2004). The neural correlates of placebo effects: A disruption account. *NeuroImage*, *22*(1), 447–455. https://doi.org/10.1016/j.neuroimage.2004.01.037

Lin, R. L., Douaud, G., Filippini, N., Okell, T. W., Stagg, C. J., & Tracey, I. (2017). Structural Connectivity Variances Underlie Functional and Behavioral Changes during Pain Relief Induced by Neuromodulation. *Scientific Reports*, *7*. https://doi.org/10.1038/srep41603

Lipton, S. A., & Nordstedt, C. (2016). Partnering with Big Pharma - What Academics Need to Know. *Cell*, *165*, 512–515.

Liu, M., Max, M. B., Robinovitz, E., Gracely, R. H., & Bennett, G. J. (1998). The human capsaicin model of allodynia and hyperalgesia: Sources of variability and methods for reduction. *Journal of Pain and Symptom Management*, *16*(1), 10–20. https://doi.org/10.1016/S0885-3924(98)00026-8

Lockwood, S., & Dickenson, A. H. (2020, April 1). What goes up must come down: insights from studies on descending controls acting on spinal pain processing. *Journal of Neural Transmission*. Springer. https://doi.org/10.1007/s00702-019-02077-x

Long, J. Z., Li, W., Booker, L., Burston, J. J., Kinsey, S. G., Schlosburg, J. E., … Cravatt, B. F. (2009). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nature Chemical Biology*, *5*(1), 37–44. https://doi.org/10.1038/nchembio.129

Longstreth, G. F., Bolus, R., Naliboff, B., Chang, L., Kulich, K. R., Carlsson, J., … Wiklund, I. K. (2005). Impact of irritable bowel syndrome on patients' lives: Development and psychometric documentation of a disease-specific measure for use in clinical trials. *European Journal of Gastroenterology and Hepatology*, *17*(4), 411–420. https://doi.org/10.1097/00042737-200504000-00004

Longstreth, G. F., Thompson, W. G., Chey, W. D., Houghton, L. A., Mearin, F., & Spiller, R. C. (2006). Functional Bowel Disorders. *Gastroenterology*, *130*(5), 1480–1491. https://doi.org/10.1053/j.gastro.2005.11.061

Lorenz, J., Minoshima, S., & Casey, K. L. (2003). Keeping pain out of mind: The role of the dorsolateral prefrontal cortex in pain modulation. *Brain*, *126*(5), 1079–1091. https://doi.org/10.1093/brain/awg102

Lovell, R. M., & Ford, A. C. (2012, July 1). Global Prevalence of and Risk Factors for Irritable Bowel Syndrome: A Meta-analysis. *Clinical Gastroenterology and Hepatology*. W.B. Saunders. https://doi.org/10.1016/j.cgh.2012.02.029

Lu, C.-L., Wu, Y.-T., Yeh, T.-C., Chen, L.-F., Chang, F.-Y., Lee, S.-D., … Hsieh, J.-C. (2004). Neuronal correlates of gastric pain induced by fundus distension: a 3T-fMRI study. *Neurogastroenterology and Motility*, *16*(5), 575–587. https://doi.org/10.1111/j.1365-2982.2004.00562.x

Lu, C., Yang, T., Zhao, H., Zhang, M., Meng, F., Fu, • Hao, … Xu, • Hui. (2016). Insular Cortex is Critical for the Perception, Modulation, and Chronification of Pain. *Neuroscience Bulletin*, *32*(2), 191–201. https://doi.org/10.1007/s12264-016-0016-y

Lu, H. C., & MacKie, K. (2016). An introduction to the endogenous cannabinoid system. *Biological Psychiatry*. Elsevier USA. https://doi.org/10.1016/j.biopsych.2015.07.028

Lutz, A., McFarlin, D. R., Perlman, D. M., Salomons, T. V., & Davidson, R. J. (2013). Altered anterior insula activation during anticipation and experience of painful stimuli in expert meditators. *NeuroImage*, *64*(1), 538–546. https://doi.org/10.1016/j.neuroimage.2012.09.030

Ma, X., Li, S., Tian, J., Jiang, G., Wen, H., Wang, T., … Xu, Y. (2015). Altered brain spontaneous activity and connectivity network in irritable bowel syndrome patients: A resting-state fMRI study. *Clinical Neurophysiology*, *126*(6), 1190–1197. https://doi.org/10.1016/j.clinph.2014.10.004

Mackie, K. (2005). Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handbook of Experimental Pharmacology*, *168*, 299–325. https://doi.org/10.1007/3-540-26573-2-10

Madden, V. J., Kamerman, P. R., Catley, M. J., Bellan, V., Russek, L. N., Camfferman, D., & Lorimer Moseley, G. (2021). Variability in experimental pain studies: nuisance or opportunity? *British Journal of Anaesthesia*, *126*, e61–e64. https://doi.org/10.1016/j.bja.2020.10.033

Manning, B. H., Martin, W. J., & Meng, I. D. (2003). The rodent amygdala contributes to the production of cannabinoid-induced antinociception. *Neuroscience*, *120*(4), 1157–1170. https://doi.org/10.1016/S0306-4522(03)00356-7

Manning, Barton H., & Mayer, D. J. (1995). The central nucleus of the amygdala contributes to the production of morphine antinociception in the formalin test. *Pain*, *63*(2), 141–152. https://doi.org/10.1016/0304-3959(95)00027-P

Manning, Barton H., Merin, N. M., Meng, I. D., & Amaral, D. G. (2001). Reduction in opioid- and cannabinoid-induced antinociception in rhesus monkeys after bilateral lesions of the amygdaloid complex. *Journal of Neuroscience*, *21*(20), 8238–8246. https://doi.org/10.1523/jneurosci.21-20-08238.2001

Marchand, W. R. (2014). Neural mechanisms of mindfulness and meditation: Evidence from neuroimaging studies. *World Journal of Radiology*, *6*(7), 471. https://doi.org/10.4329/wjr.v6.i7.471

Marcuzzi, A., Chakiath, R. J., Siddall, P. J., Kellow, J. E., Hush, J. M., Jones, M. P., … Wrigley, P. J. (2019). Conditioned Pain Modulation (CPM) is Reduced in Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis of CPM and the Role of Psychological Factors. *Journal of Clinical Gastroenterology*, *53*(6), 399–408. https://doi.org/10.1097/MCG.0000000000001181

Martin, L. J., Acland, E. L., Cho, C., Gandhi, W., Chen, D., Corley, E., … Mogil, J. S. (2019). Male-Specific Conditioned Pain Hypersensitivity in Mice and Humans. *Current Biology : CB*, *29*(2), 192-201.e4. https://doi.org/10.1016/J.CUB.2018.11.030

Martin, W. J., Tsou, K., & Walker, J. M. (1998). Cannabinoid receptor-mediated inhibition of the rat tail-flick reflex after microinjection into the rostral ventromedial medulla. *Neuroscience Letters*, *242*(1), 33–36. https://doi.org/10.1016/S0304-3940(98)00044-5

Martinez-Calderon, J., Meeus, M., Struyf, F., Miguel Morales-Asencio, J., Gijon-Nogueron, G., & Luque-Suarez, A. (2018, April 1). The role of psychological factors in the perpetuation of pain intensity and disability in people with chronic shoulder pain: A systematic review. *BMJ Open*. BMJ Publishing Group. https://doi.org/10.1136/bmjopen-2017-020703

Martins, I., & Tavares, I. (2017). Reticular formation and pain: The past and the future. *Frontiers in Neuroanatomy*, *11*. https://doi.org/10.3389/fnana.2017.00051

Martucci, K. T., & MacKey, S. C. (2018, June 1). Neuroimaging of Pain: Human Evidence and Clinical Relevance of Central Nervous System Processes and

Modulation. *Anesthesiology*. Lippincott Williams and Wilkins. https://doi.org/10.1097/ALN.0000000000002137

May, A., Rodriguez-Raecke, R., Schulte, A., Ihle, K., Breimhorst, M., Birklein, F., & Jürgens, T. P. (2012). Within-session sensitization and between-session habituation: a robust physiological response to repetitive painful heat stimulation. *European Journal of Pain (London, England)*, *16*(3), 401–409. https://doi.org/10.1002/J.1532-2149.2011.00023.X

May, L. M., Kosek, P., Zeidan, F., & Berkman, E. T. (2018). Enhancement of Meditation Analgesia by Opioid Antagonist in Experienced Meditators. *Psychosomatic Medicine*, *80*(9), 807–813. https://doi.org/10.1097/PSY.0000000000000580

Mayer, E. A., Aziz, Q., Coen, S., Kern, M., Labus, J. S., Lane, R., … Tracey, I. (2009). Brain imaging approaches to the study of functional GI disorders: A Rome Working Team Report. *Neurogastroenterology & Motility*, *21*(6), 579–596. https://doi.org/10.1111/j.1365-2982.2009.01304.x

Mayer, E.A., Labus, J. S., Tillisch, K., Cole, S. W., & Baldi, P. (2015). Towards a systems view of IBS. *Nature Publishing Group*, *12*, 592–605. https://doi.org/10.1038/nrgastro.2015.121

Mayer, Emeran A, Labus, J., Aziz, Q., Tracey, I., Kilpatrick, L., Elsenbruch, S., … Borsook, D. (2019). Role of brain imaging in disorders of brain-gut interaction: a Rome Working Team Report. *Gut*, *0*, 1–15. https://doi.org/10.1136/gutjnl-2019-318308

McGaraughty, S., & Heinricher, M. M. (2002). Microinjection of morphine into various amygdaloid nuclei differentially affects nociceptive responsiveness and RVM neuronal activity. *Pain*, *96*(1–2), 153–162. https://doi.org/10.1016/S0304-

3959(01)00440-7

McGarry, L. M., & Carter, A. G. (2017). Prefrontal Cortex Drives Distinct Projection Neurons in the Basolateral Amygdala. *Cell Reports*, *21*(6), 1426–1433. https://doi.org/10.1016/J.CELREP.2017.10.046

McWilliams, L. A., Cox, B. J., & Enns, M. W. (2003). Mood and anxiety disorders associated with chronic pain: An examination in a nationally representative sample. *Pain*, *106*(1–2), 127–133. https://doi.org/10.1016/S0304-3959(03)00301-4

Medicines & Healthcare products Regulatory Agency. (2015). *MHRA PHASE I ACCREDITATION SCHEME REQUIREMENTS*.

Mehta, A., Halder, S., Khanna, N., Tandon, O., & Sharma, K. (2012). Antagonism of stimulation-produced analgesia by naloxone and N-methyl-D-aspartate: role of opioid and N-methyl-D-aspartate receptors. *Human & Experimental Toxicology*, *31*(1), 51–56. https://doi.org/10.1177/0960327111417908

Melzack, R., & Casey, K. (1968). Sensory, motivational, and central control determinants of pain: A new conceptual model. In D. R. Kenshalo (Ed.), *The Skin Senses* (pp. 423–443). Springfield, IL: Thomas.

Mena, N. B., Marthur, R., & Nayar, U. (1995). Amygdalar involvement in pain. *Indian J Physiol Pharmacol.*, *39*(4), 339–346.

Meng, I. D., Manning, B. H., Martin, W. J., & Fields, H. L. (1998). An analgesia circuit activated by cannabinoids. *Nature*, *395*(6700), 381–383. https://doi.org/10.1038/26481

Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Structure & Function*. https://doi.org/10.1007/s00429-010-0262-0

Mezue, M., Segerdahl, A. R., Okell, T. W., Chappell, M. A., Kelly, M. E., & Tracey, I. (2014). Optimization and reliability of multiple postlabeling delay pseudo-continuous arterial spin labeling during rest and stimulus-induced functional task activation. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *34*(12), 1919–1927. https://doi.org/10.1038/jcbfm.2014.163

Middleton, S. J., Barry, A. M., Comini, M., Li, Y., Ray, P. R., Shiers, S., … Bennett, D. L. (2021). Studying human nociceptros: from fundamentals to clinic. *Brain*, *144*(5), 1312–1335.

Mills, E. P., Di Pietro, F. D., Alshelh, Z., Peck, C. C., Murray, G. M., Russell Vickers, E., & Henderson, L. A. (2018). Brainstem pain-control circuitry connectivity in chronic neuropathic pain. *The Journal of Neuroscience*, *38*(2), 465–473.

Mills, E. P., Keay, K. A., & Henderson, L. A. (2021). Brainstem pain-modulation circuitry and its plasticity in neuropathic pain: Insights from human brain imaging investigations. *Frontiers in Pain Research*. https://doi.org/10.3389/fpain.2021.705345

Moayedi, M. (2014). All roads lead to the insula. https://doi.org/10.1016/j.pain.2014.07.023

Moehring, F., Halder, P., Seal, R. P., & Stucky, C. L. (2018). Uncovering the cells and circuits of touch in normal and pathological settings. *Neuron*, *100*(2), 349–360.

Mogil, J. S. (2009). Animal models of pain: progress and challenges. *Nature Reviews Neuroscience*, *10*, 283–294.

Mogil, J. S. (2019). The translatability of pain across species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*,

374. https://doi.org/10.1098/rstb.2019.0286

Mönnikes, H. (2011, July). Quality of life in patients with irritable bowel syndrome. *Journal of Clinical Gastroenterology*. https://doi.org/10.1097/MCG.0b013e31821fbf44

Moore, R. A., Chi, C. C., Wiffen, P. J., Derry, S., & Rice, A. S. C. (2015, October 5). Oral nonsteroidal anti-inflammatory drugs for neuropathic pain. *Cochrane Database of Systematic Reviews*. John Wiley and Sons Ltd. https://doi.org/10.1002/14651858.CD010902.pub2

Morgan, P., Van Der Graaf, P. H., Arrowsmith, J., Feltner, D. E., Drummond, K. S., Wegner, C. D., & Street, S. D. A. (2012). Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discovery Today*, *17*(9–10), 419–424. https://doi.org/10.1016/J.DRUDIS.2011.12.020

Moshiree, B., Price, D. D., Robinson, M. E., Gaible, R., & Nicholas Verne, G. (2007). Thermal and Visceral Hypersensitivity in Irritable Bowel Syndrome Patients With and Without Fibromyalgia. *The Clinical Journal of Pain*, *23*(4), 323–330. https://doi.org/10.1097/AJP.0b013e318032e496

Mountz, J. M., Bradley, L. A., Modell, J. G., Alexander, R. W., Triana-Alexander, M., Aaron, L. A., … Mountz, J. D. (1995). Fibromyalgia in women. *Arthritis & Rheumatism*, *38*(7), 926–938. https://doi.org/10.1002/art.1780380708

Mücke, M., Phillips, T., Radbruch, L., Petzke, F., & Häuser, W. (2018, March 7). Cannabis-based medicines for chronic neuropathic pain in adults. *Cochrane Database of Systematic Reviews*. John Wiley and Sons Ltd. https://doi.org/10.1002/14651858.CD012182.pub2

Mumford, J. A. (2012). A power calculation guide for FMRI studies. *Social Cognitive*

and Affective Neuroscience, *7*(6), 738–742. https://doi.org/10.1093/scan/nss059

Murray, E. A., & Fellows, L. K. (2021). Prefrontal cortex interactions with the amygdala in primates. *Neuropsychopharmacology 2021*, 1–17. https://doi.org/10.1038/s41386-021-01128-w

Mykletun, A., Jacka, F., Williams, L., Pasco, J., Henry, M., Nicholson, G. C., … Berk, M. (2010). Prevalence of mood and anxiety disorder in self reported irritable bowel syndrome (IBS). An epidemiological population based study of women. *BMC Gastroenterology*, *10*. https://doi.org/10.1186/1471-230X-10-88

Nagarkatti, P., Pandey, R., Rieder, S. A., Hegde, V. L., & Nagarkatti, M. (2009, October). Cannabinoids as novel anti-inflammatory drugs. *Future Medicinal Chemistry*. https://doi.org/10.4155/fmc.09.93

Nakagawa, S. (2004). A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behavioral Ecology*, *15*(6), 1044–1045. https://doi.org/10.1093/beheco/arh107

Naliboff, B. D., Berman, S., Chang, L., Derbyshire, S. W. G., Suyenobu, B., Vogt, B. A., … Mayer, E. A. (2003). Sex-related differences in IBS patients: Central processing of visceral stimuli. *Gastroenterology*, *124*(7), 1738–1747. https://doi.org/10.1016/S0016-5085(03)00400-1

Naliboff, B. D., Berman, S., Suyenobu, B., Labus, J. S., Chang, L., Stains, J., … Mayer, E. A. (2006). Longitudinal Change in Perceptual and Brain Activation Response to Visceral Stimuli in Irritable Bowel Syndrome Patients. *Gastroenterology*, *131*(2), 352–365. https://doi.org/10.1053/j.gastro.2006.05.014

Naliboff, B. D., Smith, S. R., Serpa, J. G., Laird, K. T., Stains, J., Connolly, L. S., … Tillisch, K. (2020). Mindfulness-based stress reduction improves irritable bowel syndrome (IBS) symptoms via specific aspects of mindfulness.

Neurogastroenterology and Motility, *32*(9), e13828. https://doi.org/10.1111/nmo.13828

Namer, B., Schick, M., Kleggetveit, I. P., Orstavik, K., Schmidt, R., Jorum, E., … Schmelz, M. (2015). Differential sensitization of silent nociceptors to low pH stimulation by prostaglandin E2 in human volunteers. *European Journal of Pain*, *19*(2), 159–166. https://doi.org/10.1002/EJP.532

Napadow, V., Sclocco, R., & Henderson, L. A. (2019). Brainstem neuroimaging of nociception and pain circuitries. *PAIN Reports*, *4*(4), e745.

National Institute for Health and Care Excellence. (2008). Irritable bowel syndrome in adults: diagnosis and management (clinical guideline CG61). Retrieved April 6, 2020, from https://www.nice.org.uk/guidance/cg61

National Institute for Health and Care Excellence. (2013). Neuropathic pain in adults: pharmacological management in non-specialist settings (clinical guideline 173). Retrieved May 19, 2020, from https://www.nice.org.uk/guidance/cg173

National Institute for Health and Care Excellence. (2021). Chronic pain (primary and secondary) in over 16s: assessment of all chronic pain and management of chronic primary pain (clinical guideline 193). Retrieved November 7, 2022, from https://www.nice.org.uk/guidance/ng193

Navratilova, E., Nation, K., Remeniuk, B., Neugebauer, V., Bannister, K., Dickenson, A. H., & Porreca, F. (2020). Selective modulation of tonic aversive qualities of neuropathic pain by morphine in the central nucleus of the amygdala requires endogenous opioid signaling in the anterior cingulate cortex. *Pain*, *161*(3), 609–618.

Navratilova, E., Xie, J. Y., Meske, D., Qu, C., Morimura, K., Okun, A., … Porreca, F. (2015). Endogenous opioid activity in the anterior cingulate cortex is required for

the relief of pain. *The Journal of Neuroscience*, *35*(18), 7264–7271.

Navratilova, Edita, & Porreca, F. (2014). Reward and motivation in pain and pain relief. *Nature Neuroscience 2014 17:10*, *17*(10), 1304–1312. https://doi.org/10.1038/nn.3811

Nees, T., Wand, N., Adamek, P., Verkest, C., La Porta, C. L., Schaefer, I., … Lachner, S. G. (2022). The molecular mechanism and physiological role of silent nociceptor activation. *BioRxiv*.

Nicholas, M., Vlaeyen, J. W. S., Rief, W., Barke, A., Aziz, Q., Benoliel, R., … Treede, R.-D. (2019). The IASP classification of chronic pain for ICD-11. *PAIN*, *160*(1), 28–37. https://doi.org/10.1097/j.pain.0000000000001390

Nickerson, L. D., Smith, S. M., Öngür, D., & Beckmann, C. F. (2017). Using dual regression to investigate network shape and amplitude in functional connectivity analyses. *Frontiers in Neuroscience*, *11*(MAR), 115. https://doi.org/10.3389/fnins.2017.00115

Nomura, D. K., Morrison, B. E., Blankman, J. L., Long, J. Z., Kinsey, S. G., Marcondes, M. C. G., … Cravatt, B. F. (2011). Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science*, *334*(6057), 809–813. https://doi.org/10.1126/science.1209200

O'Neill, J., Brock, C., Olesen, A. E., Andresen, T., Nilsson, M., & Dickenson, A. H. (2012). Unravelling the Mystery of Capsaicin: A Tool to Understand and Treat Pain. *Pharmacological Reviews*, *64*(4), 939. https://doi.org/10.1124/PR.112.006163

Ochsner, K. N., & Gross, J. J. (2008). Cognitive Emotion Regulation: Insights from Social Cognitive and Affective Neuroscience. *Current Directions in Psychological Science*, *17*(2), 153–158. https://doi.org/10.1111/j.1467-

8721.2008.00566.x

Ohashi, N., & Kohno, T. (2020). Analgesic Effect of Acetaminophen: A Review of Known and Novel Mechanisms of Action. *Frontiers in Pharmacology*, *11*, 1916. https://doi.org/10.3389/FPHAR.2020.580289/BIBTEX

Ohno-Shosaku, T., Maejima, T., & Kano, M. (2001). Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron*, *29*(3), 729–738. https://doi.org/10.1016/S0896-6273(01)00247-1

Ong, W.-Y., Stohler, C. S., & Herr, D. R. (2019). Role of the Prefrontal Cortex in Pain Processing. *Molecular Neurobiology*, *56*(2), 1137. https://doi.org/10.1007/S12035-018-1130-9

Ossipov, M. H., Dussor, G. O., & Porreca, F. (2010, November 1). Central modulation of pain. *Journal of Clinical Investigation*. American Society for Clinical Investigation. https://doi.org/10.1172/JCI43766

Ossipov, M. H., Morimura, K., & Porreca, F. (2014). Descending pain modulation and chronification of pain. *Current Opinion in Supportive and Palliative Care*. Lippincott Williams and Wilkins. https://doi.org/10.1097/SPC.0000000000000055

Oxford University Hospitals NHS Foundation Trust. (2021). Local Trust Management Approval or Confirmation of Capacity and Capability and Ongoing Study Oversight - Research and Development. Retrieved May 15, 2021, from https://www.ouh.nhs.uk/researchers/approvals/capacity-capability/

Payne, L. A., Rapkin, A. J., Seidman, L. C., Zeltzer, L. K., & Tsao, J. C. I. (2017, September 12). Experimental and procedural pain responses in primary dysmenorrhea: A systematic review. *Journal of Pain Research*. Dove Medical

Press Ltd. https://doi.org/10.2147/JPR.S143512

Peerdeman, K. J., Van Laarhoven, A. I. M., Keij, S. M., Vase, L., Rovers, M. M., Peters, M. L., & Evers, A. W. M. (2016, June 1). Relieving patients' pain with expectation interventions: A meta-analysis. *Pain*. Lippincott Williams and Wilkins. https://doi.org/10.1097/j.pain.0000000000000540

Percie Du Sert, N., & Rice, A. S. C. (2014). Improving the translation of analgesic drugs to the clinic: Animal models of neuropathic pain. *British Journal of Pharmacology*. John Wiley and Sons Inc. https://doi.org/10.1111/bph.12645

Pereira, E. A. C., Lu, G., Wang, S., Schweder, P. M., Hyam, J. A., Stein, J. F., … Green, A. L. (2010). Ventral periaqueductal grey stimulation alters heart rate variability in humans with chronic pain. *Experimental Neurology*, *223*(2), 574–581.

Pereira, E. A., Wang, S., Peachey, T., Lu, G., Shlugman, D., Stein, J. F., … Green, A. L. (2013). Elevated gamma band power in humans receiving naloxone suggests dorsal periaqueductal and periventricular gray deep brain stimulation produced analgesia is opioid mediated. *Experimental Neurology*, *239*, 248–255.

Perneger, T. V. (1998, April 18). What's wrong with Bonferroni adjustments. *British Medical Journal*. British Medical Journal Publishing Group. https://doi.org/10.1136/bmj.316.7139.1236

Pertovaara, A. (2006). Noradrenergic pain modulation. *Progress in Neurobiology*, *80*(2), 53–83.

Petitjean, H., Pawlowski, S. A., Fraine, S. L., Sharif, B., Hamad, D., Fatima, T., … Sharif-Naeini, R. (2015). Dorsal horn parvalbumin neurons are gate-keepers of touch-evoked pain after nerve injury. *Cell Reports*, *13*(6), 1246–1257.

Petrovic, P., Kalso, E., Petersson, K. M., Andersson, J., Fransson, P., & Ingvar, M.

(2010). A prefrontal non-opioid mechanism in placebo analgesia. *Pain*, *150*(1), 59–65. https://doi.org/10.1016/j.pain.2010.03.011

Petrovic, P., Kalso, E., Petersson, K. M., & Ingvar, M. (2002). Placebo and opioid analgesia - Imaging a shared neuronal network. *Science*, *295*(5560), 1737–1740. https://doi.org/10.1126/science.1067176

Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002, June 1). Functional neuroanatomy of emotion: A meta-analysis of emotion activation studies in PET and fMRI. *NeuroImage*. Academic Press. https://doi.org/10.1006/nimg.2002.1087

Piché, M., Chen, J. I., Roy, M., Poitras, P., Bouin, M., & Rainville, P. (2013). Thicker posterior insula is associated with disease duration in women with irritable bowel syndrome (IBS) whereas thicker orbitofrontal cortex predicts reduced pain inhibition in both IBS patients and controls. *Journal of Pain*, *14*(10), 1217–1226. https://doi.org/10.1016/j.jpain.2013.05.009

Pimental, M. (2018). Evidence-Based Management of Irritable Bowel Syndrome With Diarrhea. *Am. J. Manag. Care*, *24*, S0. Retrieved from https://www.ajmc.com/journals/supplement/2018/evidence-based-management-of-ibsd/evidencebased-management-of-irritable-bowel-syndrome-with-diarrhea

Ploghaus, A., Narain, C., Beckmann, C. F., Clare, S., Bantick, S., Wise, R., … Tracey, I. (2001). Exacerbation of pain by anxiety is associated with activity in a hippocampal network. *Journal of Neuroscience*, *21*(24), 9896–9903. https://doi.org/10.1523/jneurosci.21-24-09896.2001

Ploner, M., Freund, H.-J., & Schnitzler, A. (1999). Pain affect without pain sensation in a patient with a postcentral lesion. *Pain*, *81*(1), 211–214. https://doi.org/10.1016/S0304-3959(99)00012-3

Posserud, I., Svedlund, J., Wallin, J., & Simrén, M. (2009). Hypervigilance in irritable bowel syndrome compared with organic gastrointestinal disease. *Journal of Psychosomatic Research*, *66*(5), 399–405. https://doi.org/10.1016/j.jpsychores.2008.09.020

Prato, V., Taberner, F. J., Hockley, J. R. F., Callejo, G., Arcourt, A., Tazir, B., … Lechner, S. G. (2017). Functional and Molecular Characterization of Mechanoinsensitive "Silent" Nociceptors. *Cell Reports*, *21*(11), 3102–3115. https://doi.org/10.1016/J.CELREP.2017.11.066

Price, D. D., Milling, L. S., Kirsch, I., Duff, A., Montgomery, G. H., & Nicholls, S. S. (1999). An analysis of factors that contribute to the magnitude of placebo analgesia in an experimental paradigm. *Pain*, *83*(2), 147–156. https://doi.org/10.1016/S0304-3959(99)00081-0

Qi, R., Liu, C., Ke, J., Xu, Q., Ye, Y., Jia, L., … Lu, G. M. (2016). Abnormal amygdala resting-state functional connectivity in irritable bowel syndrome. *American Journal of Neuroradiology*, *37*(6), 1139–1145. https://doi.org/10.3174/ajnr.A4655

Qi, Rongfeng, Liu, C., Ke, J., Xu, Q., Zhong, J., Wang, F., … Lu, M. (2015). Intrinsic brain abnormalities in irritable bowel syndrome and effect of anxiety and depression. *Brain Imaging and Behavior*, *10*(4), 1127–1134. https://doi.org/10.1007/s11682-015-9478-1

Racz, I., Nadal, X., Alferink, J., Baños, J. E., Rehnelt, J., Martín, M., … Maldonado, R. (2008). Crucial Role of CB 2 Cannabinoid Receptor in the Regulation of Central Immune Responses during Neuropathic Pain. *Neurobiology of Disease*, *28*(46), 12125–12135. https://doi.org/10.1523/JNEUROSCI.3400-08.2008

Rademacher, D. J., Meier, S. E., Shi, L., Vanessa Ho, W. S., Jarrahian, A., & Hillard, C. J. (2008). Effects of acute and repeated restraint stress on endocannabinoid

content in the amygdala, ventral striatum, and medial prefrontal cortex in mice. *Neuropharmacology*, *54*(1), 108–116. https://doi.org/10.1016/J.NEUROPHARM.2007.06.012

Rahman, W., Bannister, K., Bee, L. A., & Dickenson, A. H. (2011). A pronociceptive role for the 5-HT2 receptor on spinal nociceptive transmission: An in vivo electrophysiological study in the rat. *Brain Research*, *1382*, 29–36. https://doi.org/10.1016/J.BRAINRES.2011.01.057

Rainville, P., Duncan, G. H., Price, D. D., Carrier, B., & Bushnell, M. C. (1997). Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science*, *277*(5328), 968–971. https://doi.org/10.1126/science.277.5328.968

Ray, R. D., & Zald, D. H. (2012, January). Anatomical insights into the interaction of emotion and cognition in the prefrontal cortex. *Neuroscience and Biobehavioral Reviews*. NIH Public Access. https://doi.org/10.1016/j.neubiorev.2011.08.005

Reilly, S., & Trifunovic, R. (2000). Lateral parabrachial nucleus lesions in the rat: aversive and appetitive gustatory conditioning. *Brain Research Bulletin*, *52*(4), 269–278.

Reynolds, D. V. (1969). Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science*, *164*(3878), 444–445. https://doi.org/10.1126/science.164.3878.444

Rhudy, J. L., & Meagher, M. W. (2000). Fear and anxiety: Divergent effects on human pain thresholds. *Pain*, *84*(1), 65–75. https://doi.org/10.1016/S0304-3959(99)00183-9

Ringkamp, M., Raja, S. N., CAmpbell, J. N., & Meyer, R. A. (2013). Peripheral mechanisms of cutaneous nociception. In S.B. McMahon, M. Koltzenburg, I. Tracey, & D. C. Turk (Eds.), *Textbook of Pain (6th edition)*. Elsevier Saunders.

Rivat, C., & Ballantyne, J. (2016). The dark side of opioids in pain management. *PAIN Reports*, *1*(2), e570. https://doi.org/10.1097/PR9.0000000000000570

Rodrigues, A. C., Verne, N. G., Schmidt, S., & Mauderli, A. P. (2005). Hypersensitivity to cutaneous thermal nociceptive stimuli in irritable bowel syndrome. *Pain*, *115*(1), 5–11. https://doi.org/10.1016/j.pain.2005.01.023

Rodriguez-Raecke, R., Niemeier, A., Ihle, K., Ruether, W., & May, A. (2009). Brain gray matter decrease in chronic pain is the consequence and not the cause of pain. *Journal of Neuroscience*, *29*(44), 13746–13750. https://doi.org/10.1523/JNEUROSCI.3687-09.2009

Ruepert, L., Quartero, A. O., de Wit, N. J., van der Heijden, G. J., Rubin, G., & Muris, J. W. (2011, August 10). Bulking agents, antispasmodics and antidepressants for the treatment of irritable bowel syndrome. *Cochrane Database of Systematic Reviews*. John Wiley and Sons Ltd. https://doi.org/10.1002/14651858.CD003460.pub3

Ruffle, J. K., Coen, S. J., Giampietro, V., Williams, S. C. R., Aziz, Q., & Farmer, A. D. (2018). Preliminary report: parasympathetic tone links to functional brain networks during the anticipation and experience of visceral pain. *Scientific Reports*, *8*, 13410. https://doi.org/10.1038/s41598-018-31522-2

Sadler, K. E., McQuaid, N. A., Cox, A. C., Behun, M. N., Trouten, A. M., & Kolber, B. J. (2017). Divergent functions of the left and right central amygdala in visceral nociception. *PAIN*, *158*(4), 747–759. https://doi.org/10.1097/j.pain.0000000000000830

Sagar, D. R., Burston, J. J., Woodhams, S. G., & Chapman, V. (2012). Dynamic changes to the endocannabinoid system in models of chronic pain. *Philosophical Transactions of the Royal Society of London. Series B, Biological*

Sciences, *367*, 3300–3311. https://doi.org/10.1098/rstb.2011.0390

Salimi-Khorshidi, G., Douaud, G., Beckmann, C. F., Glasser, M. F., Griffanti, L., & Smith, S. M. (2014). Automatic denoising of functional MRI data: Combining independent component analysis and hierarchical fusion of classifiers. *NeuroImage*, *90*, 449–468. https://doi.org/10.1016/j.neuroimage.2013.11.046

Sankarasubramanian, V., Cunningham, D. A., Potter-Baker, K. A., Beall, E. B., Roelle, S. M., Varnerin, N. M., … Plow, E. B. (2017). Transcranial Direct Current Stimulation Targeting Primary Motor Versus Dorsolateral Prefrontal Cortices: Proof-of-Concept Study Investigating Functional Connectivity of Thalamocortical Networks Specific to Sensory-Affective Information Processing. *Brain Connectivity*, *7*(3), 182–196. https://doi.org/10.1089/brain.2016.0440

Satpute, A. B., Wager, T. D., Cohen-Adad, J., Bianciardi, M., Choi, J.-K., Buhle, J. T., … Feldman Barrett, L. (2013). Identification of discrete functional subregions of the human periaqueductal gray. *Proceedings of the National Academy of Sciences*, *110*(42), 17101–17106.

Schafer, S., Geuter, S., & Wager, T. D. (2018). Mechanisms of placebo analgesia: A dual-process model informed by insights from cross-species comparisons. *Progress in Neurobiology*, *160*, 101–122.

Scheibner, H. J., Bogler, C., Gleich, T., Haynes, J. D., & Bermpohl, F. (2017). Internal and external attention and the default mode network. *NeuroImage*, *148*, 381–389. https://doi.org/10.1016/j.neuroimage.2017.01.044

Schenk, L. A., Sprenger, C., Geuter, S., & Büchel, C. (2014). Expectation requires treatment to boost pain relief: An fMRI study. *Pain*, *155*(1), 150–157. https://doi.org/10.1016/j.pain.2013.09.024

Schlosburg, J. E., Blankman, J. L., Long, J. Z., Nomura, D. K., Kinsey, S. G.,

Nguyen, P. T., … Cravatt, B. F. (2010). Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system, *13*(9). https://doi.org/10.1038/nn.2616

Schmelz, M., Schmid, R., Handwerker, H. O., & Torebjörk, H. E. (2000). Encoding of burning pain from capsaicin-treated human skin in two categories of unmyelinated nerve fibres. *Brain*, *123*(3), 560–571. https://doi.org/10.1093/BRAIN/123.3.560

Schmid, J., Langhorst, J., Gaß, F., Theysohn, N., Benson, S., Engler, H., … Elsenbruch, S. (2015). Placebo analgesia in patients with functional and organic abdominal pain: A fMRI study in IBS, UC and healthy volunteers. *Gut*, *64*(3), 418–427. https://doi.org/10.1136/gutjnl-2013-306648

Schmidt-Wilcke, T., Leinisch, E., Straube, A., Kämpfe, N., Draganski, B., Diener, H. C., … May, A. (2005). Gray matter decrease in patients with chronic tension type headache. *Neurology*, *65*(9), 1483–1486. https://doi.org/10.1212/01.wnl.0000183067.94400.80

Schmidt, R., Schmelz, M., Torebjörk, H. E., & Handwerker, H. O. (2000). Mechano-insensitive nociceptors encode pain evoked by tonic pressure to human skin. *Neuroscience*, *98*(4), 793–800. https://doi.org/10.1016/S0306-4522(00)00189-5

Schwitzer, T., Schwan, R., Angioi-Duprez, K., Ingster-Moati, I., Lalanne, L., Giersch, A., & Laprevote, V. (2015, January 1). The cannabinoid system and visual processing: A review on experimental findings and clinical presumptions. *European Neuropsychopharmacology*. Elsevier. https://doi.org/10.1016/j.euroneuro.2014.11.002

Scottish Intercollegiate Guidelines Network. (2013). *SIGN 136 Management of chronic pain*. *Edinburgh: SIGN*. https://doi.org/10.1080/00224490409552240

Segerdahl, A. R., Mezue, M., Okell, T. W., Farrar, J. T., & Tracey, I. (2015). The dorsal posterior insula subserves a fundamental role in human pain. *Nature Neuroscience*, *18*(4), 499–500. https://doi.org/10.1038/nn.3969

Seminowicz, D. A., & Davis, K. D. (2006). Cortical responses to pain in healthy individuals depends on pain catastrophizing. *Pain*, *120*(3), 297–306. https://doi.org/10.1016/j.pain.2005.11.008

Seminowicz, D. A., Labus, J. S., Bueller, J. A., Tillisch, K., Naliboff, B. D., Bushnell, M. C., & Mayer, E. A. (2010). Regional Gray Matter Density Changes in Brains of Patients With Irritable Bowel Syndrome. *Gastroenterology*, *139*(1), 48. https://doi.org/10.1053/j.gastro.2010.03.049

Seminowicz, D. A., & Moayedi, M. (2017). The Dorsolateral Prefrontal Cortex in Acute and Chronic Pain. *Journal of Pain*, *18*(9), 1027–1035. https://doi.org/10.1016/j.jpain.2017.03.008

Serra, J., Bostock, H., Sola, R., Aleu, J., Garcia, E., Cokic, B., … Quiles, C. (2012). Microneurographic identification of spontaneous activity in C-nociceptors in neuropathic pain states in humans and rats. *Pain*, *153*(1), 42–55.

Serra, J., Collado, A., Sola, R., Antonelli, F., Torres, X., Salgueiro, M., … Bostock, H. (2013). Hyperexcitable C nociceptors in fibromyalgia. *Annals of Neurology*, *75*(2), 196–208.

Serrano-Ibáñez, E. R., López-Martínez, A. E., Ramírez-Maestre, C., Esteve, R., & Jensen, M. P. (2019). The behavioral inhibition and activation systems and function in patients with chronic pain. *Personality and Individual Differences*, *138*, 56–62. https://doi.org/10.1016/J.PAID.2018.09.021

Shackman, A. J., Salomons, T. V, Slagter, H. A., Fox, A. S., Winter, J. J., & Davidson, R. J. (2011). The integration of negative affect, pain and cognitive

control in the cingulate cortex. https://doi.org/10.1038/nrn2994

Sharon, H., Maron-Katz, A., Ben Simon, E., Flusser, Y., Hendler, T., Tarrasch, R., & Brill, S. (2016). Mindfulness Meditation Modulates Pain Through Endogenous Opioids. *American Journal of Medicine*, *129*(7), 755–758. https://doi.org/10.1016/j.amjmed.2016.03.002

Shih, D. Q., & Kwan, L. Y. (2007). All Roads Lead to Rome: Update on Rome III Criteria and New Treatment Options. *The Gastroenterology Report*, *1*(2), 56–65. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21544252

Sibelli, A., Chalder, T., Everitt, H., Workman, P., Windgassen, S., & Moss-Morris, R. (2016). A systematic review with meta-analysis of the role of anxiety and depression in irritable bowel syndrome onset. *Psychological Medicine*, *46*(15), 3065–3080. https://doi.org/10.1017/S0033291716001987

Sikandar, S., & Dickenson, A. H. (2012, March). Visceral pain: The ins and outs, the ups and downs. *Current Opinion in Supportive and Palliative Care*. https://doi.org/10.1097/SPC.0b013e32834f6ec9

Sikes, R. W., & Vogt, B. A. (1992). Nociceptive neurons in area 24 of rabbit cingulate cortex. *Journal of Neurophysiology*, *68*(5), 1720–1732. https://doi.org/10.1152/jn.1992.68.5.1720

Simons, L. E., Moulton, E. A., Linnman, C., Carpino, E., Becerra, L., & Borsook, D. (2014). The human amygdala and pain: Evidence from neuroimaging. *Human Brain Mapping*, *35*(2), 527–538. https://doi.org/10.1002/hbm.22199

Sims-Williams, H., Matthews, J. C., Talbot, P. S., Love-Jones, S., Brooks, J. C. W., Patel, N. K., & Pickering, A, E. (2017). Deep brain stimulation of the periaqueductal gray releases endogenous opioids in humans. *NeuroImage*, *146*, 833–842.

Slupe, A. M., & Kirsch, J. R. (2018). Effects of anesthesia on cerebral blood flow, metabolism, and neuroprotection. *Journal of Cerebral Blood Flow & Metabolism*, *38*(12), 2192. https://doi.org/10.1177/0271678X18789273

Smith, S. M. (2002). Fast robust automated brain extraction. *Human Brain Mapping*, *17*(3), 143–155. https://doi.org/10.1002/hbm.10062

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., … Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. In *NeuroImage* (Vol. 23). https://doi.org/10.1016/j.neuroimage.2004.07.051

Soliman, N., Haroutounian, S., Hohman, A. G., Krane, E., Liao, J., MacLeod, M., … Rice, A. S. C. (2021). Systemic review and meta-analusis of cannabinoids, cannabis-based medicines, and endocannabinoid system modulators tested for antinociceptive effects in animal models of injury-related or pathological persistent pain. *Pain*, *162*, S26–S44.

Song, G. H., Venkatraman, V., Ho, K. Y., Chee, M. W. L., Yeoh, K. G., & Wilder-Smith, C. H. (2006). Cortical effects of anticipation and endogenous modulation of visceral pain assessed by functional brain MRI in irritable bowel syndrome patients and healthy controls. *Pain*, *126*(1–3), 79–90. https://doi.org/10.1016/j.pain.2006.06.017

Soni, A., Wanigasekera, V., Mezue, M., Cooper, C., Javaid, M. K., Price, A. J., & Tracey, I. (2019). Central Sensitization in Knee Osteoarthritis: Relating Presurgical Brainstem Neuroimaging and PainDETECT-Based Patient Stratification to Arthroplasty Outcome. *Arthritis & Rheumatology*, *71*(4), 550–560. https://doi.org/10.1002/ART.40749

Spielberg, J. M., Miller, G. A., Warren, S. L., Engels, A. S., Crocker, L. D., Sutton, B.

P., & Heller, W. (2012). Trait motivation moderates neural activation associated with goal pursuit. *Cognitive, Affective & Behavioral Neuroscience*, *12*(2), 308. https://doi.org/10.3758/S13415-012-0088-8

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Sridharan, D., Levitin, D. J., & Menon, V. (2008). A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(34), 12569–12574. https://doi.org/10.1073/pnas.0800005105

Stanhope, J. (2016). Brief Pain Inventory review. *Occupational Medicine*, *66*(6), 496–497.

Stockings, E., Campbell, G., Hall, W. D., Nielsen, S., Zagic, D., Rahman, R., … Degenhardt, L. (2018). Cannabis and cannabinoids for the treatment of people with chronic noncancer pain conditions: A systematic review and meta-analysis of controlled and observational studies. *Pain*. Lippincott Williams and Wilkins. https://doi.org/10.1097/j.pain.0000000000001293

Sturgeon, J. A. (2014, April 10). Psychological therapies for the management of chronic pain. *Psychology Research and Behavior Management*. DOVE Medical Press Ltd. https://doi.org/10.2147/PRBM.S44762

Su, I. W., Wu, F. W., Liang, K. C., Cheng, K. Y., Hsieh, S. T., Sun, W. Z., & Chou, T. L. (2016). Pain perception can be modulated by mindfulness training: A resting-state fMRI study. *Frontiers in Human Neuroscience*, *10*(NOV2016). https://doi.org/10.3389/fnhum.2016.00570

Sullivan, M. J. L. (2009). *The pain catastrophizing scale user manual*.

Sullivan, M. J. L., Bishop, S. R., & Pivik, J. (1995). The Pain Catastrophizing Scale: Development and Validation. *Psychological Assessment*, *7*(4), 524–532. https://doi.org/10.1037/1040-3590.7.4.524

Sullivan, M. J. L., Thorn, B., Haythornthwaite, J. A., Keefe, F., Martin, M., Bradley, L. A., & Lefebvre, J. C. (2001). Theoretical perspectives on the relation between catastrophizing and pain. *Clinical Journal of Pain*, *17*(1), 52–64. https://doi.org/10.1097/00002508-200103000-00008

Taenzer, P., Melzack, R., & Jeans, M. E. (1986). Influence of psychological factors on postoperative pain, mood and analgesic requirements. *Pain*, *24*(3), 331–342. https://doi.org/10.1016/0304-3959(86)90119-3

Tan, L. L., Pelzer, P., Heinl, C., Tang, W., Gangadharan, V., Flor, H., … Kuner, R. (2017). A pathway from midcingulate cortex to posterior insula gates nociceptive hypersensitivity. *Nature Neuroscience*, *20*(11), 1591–1601. https://doi.org/10.1038/nn.4645

Tang, Y. Y., Hölzel, B. K., & Posner, M. I. (2015, April 21). The neuroscience of mindfulness meditation. *Nature Reviews Neuroscience*. Nature Publishing Group. https://doi.org/10.1038/nrn3916

Tavares, I., Costa-Pereira, J. T., & Martins, I. (2021). Monoaminergic and opioidergic modulation of brainstem circuits: New insights into the clinical challenges of pain treatment? *Frontiers in Pain Research*, *2*, 696515. https://doi.org/10.3389/fpain.2021.696515

Tershner, S. A., & Helmstetter, F. J. (2000). *Antinociception produced by mu opioid receptor activation in the amygdala is partly dependent on activation of mu opioid and neurotensin receptors in the ventral periaqueductal gray*. *Brain*

Research (Vol. 865). Retrieved from www.elsevier.com/locate/bres

The World Medical Association. (2018). WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. Retrieved May 27, 2021, from https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

Thompson, J. M., & Neugebauer, V. (2017). Amygdala Plasticity and Pain. https://doi.org/10.1155/2017/8296501

Tillisch, K., Mayer, E. A., & Labus, J. S. (2011). Quantitative meta-analysis identifies brain regions activated during rectal distension in irritable bowel syndrome. *Gastroenterology*, *140*(1), 91–100. https://doi.org/10.1053/j.gastro.2010.07.053

Timmermann, L., Ploner, M., Haucke, K., Schmitz, F., Baltissen, R., & Schnitzler, A. (2001). Differential Coding of Pain Intensity in the Human Primary and Secondary Somatosensory Cortex. *Journal of Neurophysiology*, *86*(3), 1499–1503. https://doi.org/10.1152/jn.2001.86.3.1499

Tombaugh, T. N. (2004). Trail Making Test A and B: Normative data stratified by age and education. *Archives of Clinical Neuropsychology*, *19*(2), 203–214. https://doi.org/10.1016/S0887-6177(03)00039-8

Tracey, I. (2010, November). Getting the pain you expect: Mechanisms of placebo, nocebo and reappraisal effects in humans. *Nature Medicine*. https://doi.org/10.1038/nm.2229

Tracey, I. (2016, July 1). A vulnerability to chronic pain and its interrelationship with resistance to analgesia. *Brain*. Oxford University Press. https://doi.org/10.1093/brain/aww147

Tracey, I., & Mantyh, P. W. (2007, August 2). The Cerebral Signature for Pain Perception and Its Modulation. *Neuron*. Cell Press.

https://doi.org/10.1016/j.neuron.2007.07.012

Tracey, I., Woolf, C. J., & Andrews, N. A. (2019, March 6). Composite Pain Biomarker Signatures for Objective Assessment and Effective Treatment. *Neuron*. Cell Press. https://doi.org/10.1016/j.neuron.2019.02.019

U.S. Food and Drug Administration. (2017). What Are Biomarkers and Why Are They Important? Retrieved June 7, 2021, from https://www.fda.gov/drugs/biomarker-qualification-program/what-are-biomarkers-and-why-are-they-important-transcript

U.S. Food and Drug Administration. (2018). Step 3: Clinical Research. Retrieved May 24, 2021, from https://www.fda.gov/patients/drug-development-process/step-3-clinical-research

U.S. Food and Drug Administration. (2022). *Guidance: Development of Non-Opioid Analgesics for Acute Pain Guidance for Industry Development of Non- Opioid Analgesics for Acute Pain*. Retrieved from https://www.fda.gov/media/156063/download

Uddin, L. Q. (2015, December 19). Salience processing and insular cortical function and dysfunction. *Nature Reviews Neuroscience*. Nature Publishing Group. https://doi.org/10.1038/nrn3857

Uddin, L. Q., Nomi, J. S., Hébert-Seropian, B., Ghaziri, J., & Boucher, O. (2017, July 1). Structure and Function of the Human Insula. *Journal of Clinical Neurophysiology*. Lippincott Williams and Wilkins. https://doi.org/10.1097/WNP.0000000000000377

Uhelski, M. L., Davis, M. A., & Fuchs, P. N. (2012). Pain affect in the absence of pain sensation: Evidence of asomaesthesia after somatosensory cortex lesions in the rat. *Pain*, *153*(4), 885–892. https://doi.org/10.1016/j.pain.2012.01.018

US War Department. (1944). *Army individual test battery. Manual of directions and scoring*. Washington, DC.

Vachon-Presseau, E., Tétreault, P., Petre, B., Huang, L., Berger, S. E., Torbey, S., … Apkarian, A. V. (2016). Corticolimbic anatomical characteristics predetermine risk for chronic pain. *Brain*, *139*(7), 1958–1970. https://doi.org/10.1093/brain/aww100

Valverde, O., Ledent, C., Beslot, F., Parmentier, M., & Roques, B. P. (2000). Reduction of stress-induced analgesia but not of exogenous opioid effects in mice lacking CB1 receptors. *European Journal of Neuroscience*, *12*(2), 533–539. https://doi.org/10.1046/J.1460-9568.2000.00929.X

van Alst, T. M., Wachsmuth, L., Datunashvili, M., Albers, F., Just, N., Budde, T., & Faber, C. (2019). Anesthesia differentially modulates neuronal and vascular contributions to the BOLD signal. *Neuroimage*, *195*, 89–103.

Van Esbroeck, A. C. M., Janssen, A. P. A., Cognetta, A. B., Ogasawara, D., Shpak, G., Van Der Kroeg, M., … Van Der Stelt, M. (2017). Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. *Science*, *356*(6342), 1084–1087. https://doi.org/10.1126/science.aaf7497

Van Norman, G. A. (2016). Drugs, Devices, and the FDA: Part 1: An Overview of Approval Processes for Drugs. *JACC: Basic to Translational Science*, *1*(3), 170–179. https://doi.org/10.1016/j.jacbts.2016.03.002

Van Oudenhove, L., Kragel, P. A., Dupont, P., Giao Ly, H., Pazmany, E., Enzlin, P., … Wager, T. D. (2020). Common and distinct neural representations of aversive somatic and visceral stimulation in healthy individuals. *Nature Comm*. https://doi.org/10.1038/s41467-020-19688-8

Vardeh, D., & Naranjo, J. F. (2017). Peripheral and central sensitization. In R. Yong,

M. Nguyen, E. Nelson, & R. Urman (Eds.), *Pain Medicine*. Cham: Springer.

Vase, L., Petersen, G. L., Riley, J. L., & Price, D. D. (2009). Factors contributing to large analgesic effects in placebo mechanism studies conducted between 2002 and 2007. *Pain*, *145*(1–2), 36–44. https://doi.org/10.1016/j.pain.2009.04.008

Vase, L., Robinson, M. E., Verne, G. N., & Price, D. D. (2003). The contributions of suggestion, desire, and expectation to placebo effects in irritable bowel syndrome patients: An empirical investigation. *Pain*, *105*(1–2), 17–25. https://doi.org/10.1016/S0304-3959(03)00073-3

Veehof, M. M., Trompetter, H. R., Bohlmeijer, E. T., & Schreurs, K. M. G. (2016, January 2). Acceptance- and mindfulness-based interventions for the treatment of chronic pain: a meta-analytic review. *Cognitive Behaviour Therapy*. Routledge. https://doi.org/10.1080/16506073.2015.1098724

Veinante, P., Yalcin, I., & Barrot, M. (2013). The amygdala between sensation and affect: a role in pain. *Journal of Molecular Psychiatry*, *1*(1), 9. https://doi.org/10.1186/2049-9256-1-9

Verne, G. N., Robinson, M. E., & Price, D. D. (2001). Hypersensitivity to visceral and cutaneous pain in the irritable bowel syndrome. *Pain*, *93*(1), 7–14. https://doi.org/10.1016/S0304-3959(01)00285-8

Vincent, K., & Tracey, I. (2008). Hormones and Their Interaction with the Pain Experience. *Reviews in Pain*, *2*(2), 20–24. https://doi.org/10.1177/204946370800200206

Vogt, B. A. (2005, July). Pain and emotion interactions in subregions of the cingulate gyrus. *Nature Reviews Neuroscience*. NIH Public Access. https://doi.org/10.1038/nrn1704

Vogt, B. A. (2016, July 1). Midcingulate cortex: Structure, connections, homologies,

functions and diseases. *Journal of Chemical Neuroanatomy*. Elsevier B.V. https://doi.org/10.1016/j.jchemneu.2016.01.010

Vogt, B. A. (2019a). Cingulate cortex in the three limbic subsystems. *Handbook of Clinical Neurology*, *166*, 39–51. https://doi.org/10.1016/B978-0-444-64196-0.00003-0

Vogt, B. A. (2019b). The cingulate cortex in neurologic diseases: History, Structure, Overview. *Handbook of Clinical Neurology*, *166*, 3–21. https://doi.org/10.1016/B978-0-444-64196-0.00001-7

Vogt, B. A., Berger, G. R., & Derbyshire, S. W. G. (2003). Structural and functional dichotomy of human midcingulate cortex. *European Journal of Neuroscience*, *18*(11), 3134–3144. https://doi.org/10.1111/j.1460-9568.2003.03034.x

Vogt, B. A., Watanabe, H., Grootoonk, S., & Jones, A. K. P. (1995). Topography of diprenorphine binding in human cingulate gyrus and adjacent cortex derived from coregistered PET and MR images. *Human Brain Mapping*, *3*(1), 1–12. https://doi.org/10.1002/hbm.460030102

Vos, T., Barber, R. M., Bell, B., Bertozzi-Villa, A., Biryukov, S., Bolliger, I., … Murray, C. J. L. (2015). Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*, *386*(9995), 743–800. https://doi.org/10.1016/S0140-6736(15)60692-4

Wager, T. D., & Roy, M. (2010). Separate mechanisms for placebo and opiate analgesia? *Pain*, *150*(1), 8–9. https://doi.org/10.1016/j.pain.2010.03.010

Wager, T.D., Rilling, J. K., Smith, E. E., Sokolik, A., Casey, K., Davidson, R. J., … Cohen, J. D. (2004). Placebo-induced changes in fMRI in the anticipation and

experience of pain. *Science*, *303*(5661), 1162–1167.

Wager, Tor D., Davidson, M. L., Hughes, B. L., Lindquist, M. A., & Ochsner, K. N. (2008). Prefrontal-Subcortical Pathways Mediating Successful Emotion Regulation. *Neuron*, *59*(6), 1037–1050. https://doi.org/10.1016/j.neuron.2008.09.006

Wang, N., Zhang, Y.-H., Wang, J.-Y., & Luo, F. (2021). Current Understanding of the Involvement of the Insular Cortex in Neuropathic Pain: A Narrative Review. *Int. J. Mol. Sci*, *22*, 2648. https://doi.org/10.3390/ijms22052648

Wang, X., Wu, Q., Egan, L., Gu, X., Liu, P., Gu, H., … Fan, J. (2019). Anterior insular cortex plays a critical role in interoceptive attention. *ELife*, *8*. https://doi.org/10.7554/eLife.42265

Wanigasekera, V., Wartolowska, K., Huggins, J. P., Duff, E. P., Vennart, W., Whitlock, M., … Tracey, I. (2018). Disambiguating pharmacological mechanisms from placebo in neuropathic pain using functional neuroimaging. *British Journal of Anaesthesia*, *120*(2), 299–307. https://doi.org/10.1016/j.bja.2017.11.064

Wanigasekera, Vishvarani, Lee, M. C., Rogers, R., Kong, Y., Leknes, S., Andersson, J., & Tracey, I. (2012). Baseline reward circuitry activity and trait reward responsiveness predict expression of opioid analgesia in healthy subjects. *Proceedings of the National Academy of Sciences*, *109*(43), 17705–17710. https://doi.org/10.1073/PNAS.1120201109

Wanigasekera, Vishvarani, Mezue, M., Andersson, J., Kong, Y., & Tracey, I. (2016). Disambiguating Pharmacodynamic Efficacy from Behavior with Neuroimaging: Implications for Analgesic Drug Development. In *Anesthesiology* (Vol. 124, pp. 159–168). Lippincott Williams and Wilkins. https://doi.org/10.1097/ALN.0000000000000924

Ward, L. (2013). The thalamus: gateway to the mind. *Wiley Interdisciplinary Reviews. Cognitive Science*, *4*(6), 609–622. https://doi.org/10.1002/WCS.1256

Ware, J., Kosinski, M., & Dewey, J. (2000). *How to Score Version 2 od the SF-36 Health Survey.* Lincoln: QualityMetric Incorporated.

Watson, A., El-Deredy, W., Iannetti, G. D., Lloyd, D., Tracey, I., Vogt, B. A., … Jones, A. K. P. (2009). Placebo conditioning and placebo analgesia modulate a common brain network during pain anticipation and perception. *Pain*, *145*(1–2), 24–30. https://doi.org/10.1016/j.pain.2009.04.003

Weizman, L., Dayan, L., Brill, S., Nahman-Averbuch, H., Hendler, T., Jacob, G., & Sharon, H. (2018). Cannabis analgesia in chronic neuropathic pain is associated with altered brain connectivity. *Neurology*, *91*(14), E1285–E1294. https://doi.org/10.1212/WNL.0000000000006293

Whalen, P. J., Shin, L. M., McInerney, S. C., Fischer, H., Wright, C. I., & Rauch, S. L. (2001). A Functional MRI Study of Human Amygdala Responses to Facial Expressions of Fear Versus Anger. *Emotion*, *1*(1), 70–83. https://doi.org/10.1037/1528-3542.1.1.70

Wiech, K., Jbabdi, S., Lin, C. S., Andersson, J., & Tracey, I. (2014). Differential structural and resting state connectivity between insular subdivisions and other pain-related brain regions. *Pain*, *155*(10), 2047–2055. https://doi.org/10.1016/j.pain.2014.07.009

Wiech, Katja, Edwards, R., Moseley, G. L., Berna, C., Ploner, M., & Tracey, I. (2014). Dissociable Neural Mechanisms Underlying the Modulation of Pain and Anxiety? An fMRI Pilot Study. *PLoS ONE*, *9*(12), e110654. https://doi.org/10.1371/journal.pone.0110654

Wiech, Katja, Farias, M., Kahane, G., Shackel, N., Tiede, W., & Tracey, I. (2008). An

fMRI study measuring analgesia enhanced by religion as a belief system. *Pain*, *139*(2), 467–476. https://doi.org/10.1016/j.pain.2008.07.030

Wiech, Katja, Kalisch, R., Weiskopf, N., Pleger, B., Stephan, K. E., & Dolan, R. J. (2006). Anterolateral prefrontal cortex mediates the analgesic effect of expected and perceived control over pain. *Journal of Neuroscience*, *26*(44), 11501–11509. https://doi.org/10.1523/JNEUROSCI.2568-06.2006

Wiech, Katja, Lin, C. S., Brodersen, K. H., Bingel, U., Ploner, M., & Tracey, I. (2010). Anterior insula integrates information about salience into perceptual decisions about pain. *Journal of Neuroscience*, *30*(48), 16324–16331. https://doi.org/10.1523/JNEUROSCI.2087-10.2010

Wiech, Katja, Ploner, M., & Tracey, I. (2008, August 1). Neurocognitive aspects of pain perception. *Trends in Cognitive Sciences*. Elsevier Current Trends. https://doi.org/10.1016/j.tics.2008.05.005

Wiklund, I. K., Fullerton, S., Hawkey, C. J., Jones, R. H., Longstreth, G. F., Mayer, E. A., … Naesdal, J. (2003). An irritable bowel syndrome-specific symptom questionnaire: Development and validation. *Scandinavian Journal of Gastroenterology*, *38*(9), 947–954. https://doi.org/10.1080/00365520310004209

Wilder-Smith, C. H., Schindler, D., Lovblad, K., Redmond, S. M., & Nirkko, A. (2004). Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. *Gut*, *53*(11), 1595–1601. https://doi.org/10.1136/gut.2003.028514

Winkler, A. M., Ridgway, G. R., Webster, M. A., Smith, S. M., & Nichols, T. E. (2014). Permutation inference for the general linear model. *NeuroImage*, *92*, 381–397. https://doi.org/10.1016/j.neuroimage.2014.01.060

Woodhams, S. G., Chapman, V., Finn, D. P., Hohmann, A. G., & Neugebauer, V. (2017). The cannabinoid system and pain. *Neuropharmacology*, *124*, 105–120. https://doi.org/10.1016/J.NEUROPHARM.2017.06.015

Woolf, C. J. (2007). Central sensitization: uncovering the relation between pain and plasticity. *Anesthesiology*, *106*(4), 864–867. https://doi.org/10.1097/01.anes.0000264769.87038.55

Woolrich, M. W., Behrens, T. E. J., Beckmann, C. F., Jenkinson, M., & Smith, S. M. (2004). Multilevel linear modelling for FMRI group analysis using Bayesian inference. *NeuroImage*, *21*(4), 1732–1747. https://doi.org/10.1016/j.neuroimage.2003.12.023

Woolrich, M. W., Ripley, B. D., Brady, M., & Smith, S. M. (2001). Temporal autocorrelation in univariate linear modeling of FMRI data. *NeuroImage*, *14*(6), 1370–1386. https://doi.org/10.1006/nimg.2001.0931

Wu, J. C. Y. (2012, January). Psychological co-morbidity in functional gastrointestinal disorders: Epidemiology, mechanisms and management. *Journal of Neurogastroenterology and Motility*. The Korean Society of Neurogastroenterology and Motility. https://doi.org/10.5056/jnm.2012.18.1.13

Xu, H., Wu, L.-J., Wang, H., Zhang, X., Vadakkan, K. I., Kim, S. S., … Zhou, M. (2008). Presynaptic and postsynaptic amplifications of neuropathic pain in the anterior cingulate cortex. *Journal of Neuroscience*, *28*(29), 7445–7453.

Yan, N., Cao, B., Xu, J., Hao, C., Zhang, X., & Li, Y. (2012). Glutamatergic activation of anterior cingulate cortex mediates the affective component of visceral pain memory in rats. *Neurobiology of Learning and Memory*, *97*(1), 156–164. https://doi.org/10.1016/j.nlm.2011.11.003

Yanes, J. A., & Akintola, T. (2022). Parabrachial projections to PAG-RVM axis may

promote placebo hypoalgesia and nocebo hyperalgesia. *Journal of Neuroscience*, *42*(16), 3302–3304.

Yarnitsky, D., Granot, M., Nahman-Averbuch, H., Khamaisi, M., & Granovsky, Y. (2012). Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. *Pain*, *153*(6), 1193–1198. https://doi.org/10.1016/j.pain.2012.02.021

Yekkirala, A. S., Roberson, D. P., Bean, B. P., & Woolf, C. J. (2017). Breaking barriers to novel analgesic drug development. *Nature Reviews Drug Discovery*, *16*, 545–564.

Young, K. S., Maj Van Der Velden, A., Craske, M. G., Pallesen, K. J., Fjorback, L., Roepstorff, A., & Parsons, C. E. (2018). The impact of mindfulness-based interventions on brain activity: A systematic review of functional magnetic resonance imaging studies. https://doi.org/10.1016/j.neubiorev.2017.08.003

Youssef, A. M., Gustin, S. M., Nash, P. G., Reeves, J. M., Petersen, E. T., Peck, C. C., … Henderson, L. A. (2014). Differential brain activity in subjects with painful trigeminal neuropathy and painful temporomandibular disorder. *Pain*, *155*(3), 467–475. https://doi.org/10.1016/j.pain.2013.11.008

Zaman, J., Weltens, N., Ly, H. G., Struyf, D., Vlaeyen, J. W. S., Van den Vergh, O., … Van Diest, I. (2016). Influence of Interoceptive Fear Learning on Visceral Perception. *Psychosomatic Medicine*, *78*(2), 248–258.

Zambreanu, L., Wise, R. G., Brooks, J. C. W., Iannetti, G. D., & Tracey, I. (2005). A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging. *Pain*, *114*(3), 397–407.

Zeidan, F., Adler-Neal, A. L., Wells, R. E., Stagnaro, E., May, L. M., Eisenach, J. C., … Coghill, R. C. (2016). Mindfulness-meditation-based pain relief is not

mediated by endogenous opioids. *Journal of Neuroscience*, *36*(11), 3391–3397. https://doi.org/10.1523/JNEUROSCI.4328-15.2016

Zeidan, F., Baumgartner, J. N., & Coghill, R. C. (2019). The neural mechanisms of mindfulness-based pain relief. *PAIN Reports*, *4*(4), e759. https://doi.org/10.1097/pr9.0000000000000759

Zeidan, F., Emerson, N. M., Farris, S. R., Ray, J. N., Jung, Y., McHaffie, J. G., & Coghill, R. C. (2015). Mindfulness meditation-based pain relief employs different neural mechanisms than placebo and sham mindfulness meditation-induced analgesia. *Journal of Neuroscience*, *35*(46), 15307–15325. https://doi.org/10.1523/JNEUROSCI.2542-15.2015

Zeidan, F., Martucci, K. T., Kraft, R. A., Gordon, N. S., Mchaffie, J. G., & Coghill, R. C. (2011). Brain mechanisms supporting the modulation of pain by mindfulness meditation. *Journal of Neuroscience*, *31*(14), 5540–5548. https://doi.org/10.1523/JNEUROSCI.5791-10.2011

Zernicke, K. A., Campbell, T. S., Blustein, P. K., Fung, T. S., Johnson, J. A., Bacon, S. L., & Carlson, L. E. (2013). Mindfulness-based stress reduction for the treatment of irritable bowel syndrome symptoms: A randomized wait-list controlled trial. *International Journal of Behavioral Medicine*, *20*(3), 385–396. https://doi.org/10.1007/s12529-012-9241-6

Zhang, X., Huang, J., & McNaughton, P. A. (2005). NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. *The EMBO Journal*, *24*(24), 4211–4223.

Zhang, Y. Q., Tang, J. S., Yuan, B., & Jia, H. (1997). Inhibitory effects of electrically evoked activation of ventrolateral orbital cortex on the tail-flick reflex are mediated by periaqueductal gray in rats. *Pain*, *72*(1–2), 127–135.

https://doi.org/10.1016/S0304-3959(97)00025-0

Zhou, M. (2016). Neural mechanisms underlying anxiety-chronic pain interactions. *Trends in Neurosciences*, *39*(3), 136–145.

Zhou, M. (2017). Descending facilitation. *Molecular Pain*, *13*, 1–12.

Zhou, Q., Fillingim, R. B., Riley III, J. L., Nicholas Verne Online Submissions, G., Nicholas Verne, G., Florida, N., … Guslandi, M. (2009). Thermal hypersensitivity in a subset of irritable bowel syndrome patients. *Wjg@wjgnet.Com World Journal of Gastroenterology*, *15*(26), 3254–3260. https://doi.org/10.3748/wjg.15.3254

Zhou, Q. Q., Fillingim, R. B., Riley, J. L., Malarkey, W. B., & Verne, G. N. (2010). Central and peripheral hypersensitivity in the irritable bowel syndrome. *Pain*, *148*(3), 454–461. https://doi.org/10.1016/j.pain.2009.12.005

Zigmond, A. S., & Snaith, R. P. (1983). The Hospital Anxiety and Depression Scale. *Acta Psychiatrica Scandinavica*, *67*(6), 361–370. https://doi.org/10.1111/j.1600-0447.1983.tb09716.x

Zubieta, J.-K., Bueller, J. A., Jackson, L. R., Scott, D. J., Xu, Y., Koeppe, R. A., … Stohler, C. S. (2005). Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *Journal of Neuroscience*, *25*(34), 7754–7762.

Appendix A: Pilot data for response to topical 1% capsaicin cream

Topical 1% capsaicin cream was sourced from The Specials Laboratory (www.specialslab.co.uk), an MHRA-licensed site for the manufacture of unlicensed pharmaceuticals. Unguentum M was used as a base for the active ingredient. This base cream has not previously been used for experiments at FMRIB. Absorption may have differed from other preparations, causing the cream to behave differently in terms of pain intensity and timecourse. Therefore a pilot study was performed to assess this.

A-1 Methods

Eight volunteers had 1% capsaicin cream applied to the anteromedial part of their leg as per the protocol for the main ABX-1431 trial (see chapter 3). Six of the volunteers were male, four were female. Every two minutes, verbal pain intensity ratings were obtained on a 0-10 scale for ongoing pain (0 being no pain and 10 being the maximum pain imaginable). This continued for 120 minutes.

Four participants repeated the experiment and completed evoked pain intensity ratings at three timepoints. The first was prior to capsaicin application, the second was at 60 minutes and the third was at 120 minutes. At each timepoint, the 512nM punctate probe was applied ten times to an area between 1cm and 3cm from the capsaicin, with 30 seconds between each one. Verbal pain intensity ratings were obtained after each one, again on a 0-10 scale.

A-2 Results/Conclusions

As would be expected from the literature on capsaicin-induced sensitization (O'Neill et al., 2012), there was substantial variability between individuals in the magnitude and timing of the pain response. For the purposes of the study of ABX-1431, a capsaicin responder was defined as someone who obtained an ongoing pain intensity rating of at least 3/10 with measurable hyperalgesia at their screening visit. Of the eight participants in the ongoing pain pilot, seven achieved this within the first hour. The participant who did not had very few ratings of 3/10 or over even after this time period, suggesting that they would have been a poor responder even if given longer to respond. Of the seven who achieved ratings of ≥3/10 within the first hour, only one reported that their ratings had reduced to less than 3/10 before the hour had passed. This indicated that 60 minutes after dosing would be a good time to perform the primary outcome measure (BOLD response to evoked pain). The majority of pilot participants reported a pain rating of 3/10 or more by around 30 minutes, indicating that it was reasonable to start other MRI scans at this stage.

Figure A.1 demonstrates the pain scores of each participant over time. Of the four participants completing evoked pain ratings, two demonstrated hyperalgesia and two did not (Figure A.2). The two that did reported increased evoked pain at both 60 minutes and 120 minutes, confirming that 60 minutes post-dose was a reasonable time to perform the evoked pain paradigm. In addition, from this data it was determined that 60 minutes should be the duration of capsaicin application used to determine whether participants were responders at the screening visit.

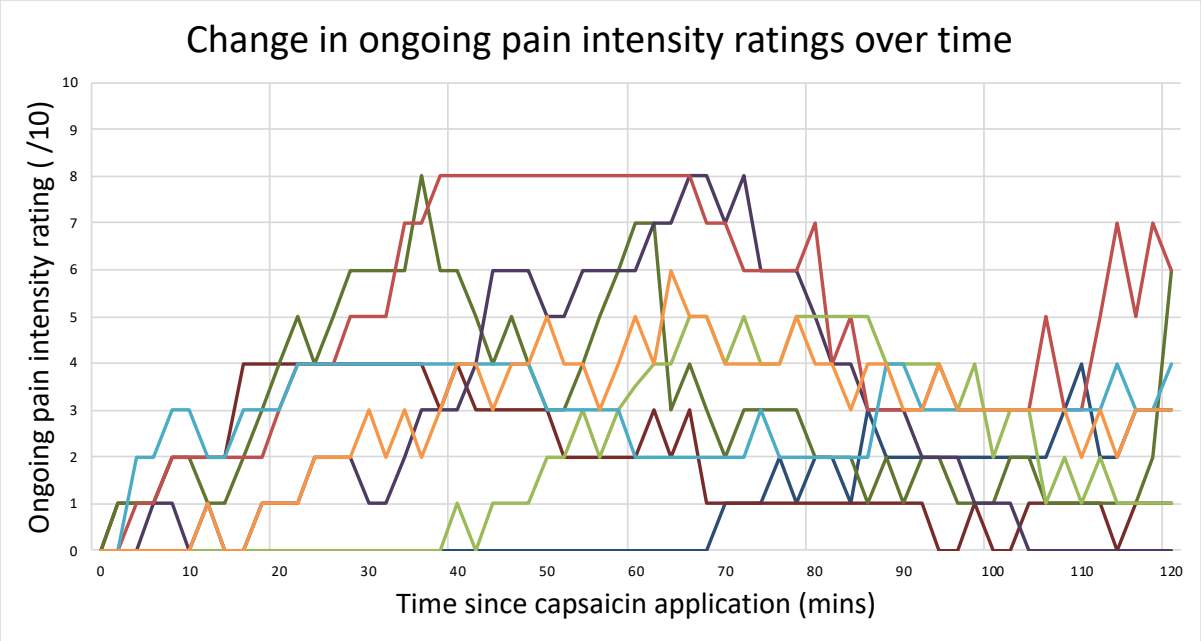


Figure A.1: Effect of topical capsaicin on ongoing pain intensity ratings. Graph shows the change in individual ongoing pain intensity ratings over a 2 hour period following 1% capsaicin cream application (n=8). Each line represents an individual participant.

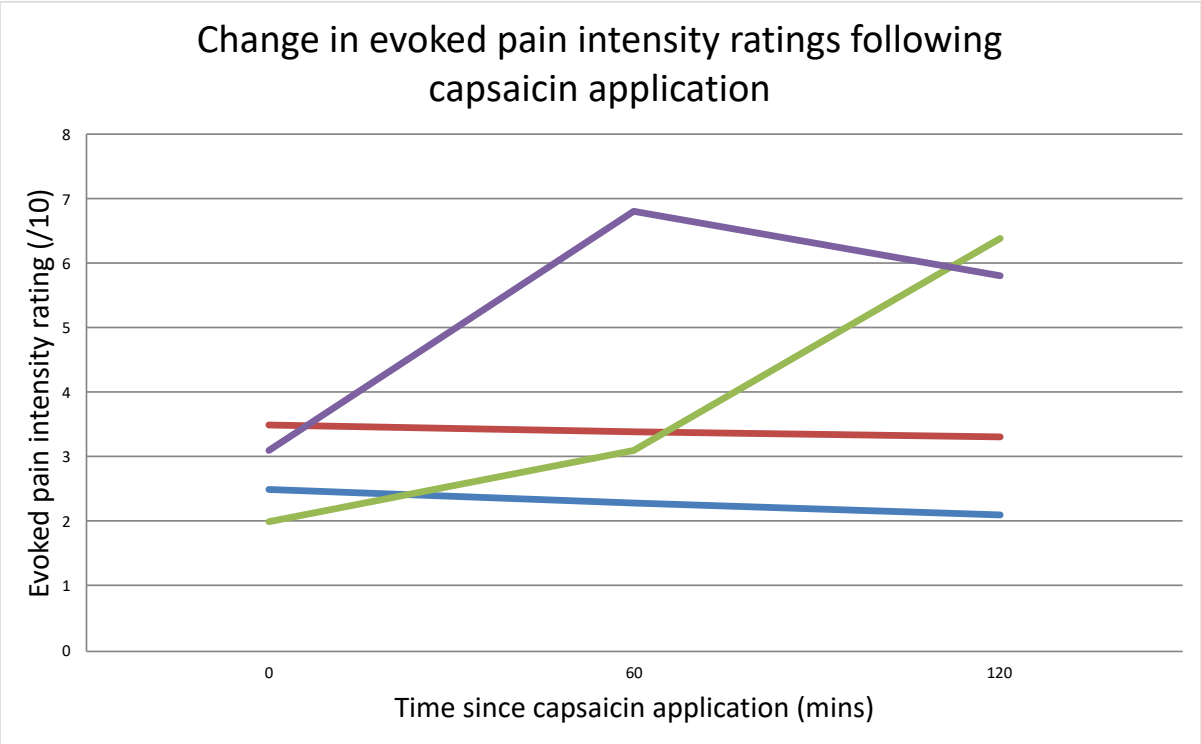


Figure A.2: Effect of topical capsaicin on pain intensity ratings in response to a noxious stimulus. Graph shows the change in individual punctate pain intensity ratings from before 1% capsaicin cream application to 1 hour and 2 hours following capsaicin cream application (n=4). Each line represents an individual participant.

Appendix B: Inclusion/exclusion criteria to accompany chapter 3

B-1 Inclusion Criteria

In order to participate, participants must meet ALL the following criteria:

1. Understands the study procedures and is willing and able to give informed consent for participation in the trial.
2. Male, right-handed, aged 18 to 55 years of age at the first visit.
3. Body mass index >18 and ≤30 kg/m^2 at the first visit.
4. Judged to be in good health based on medical history, physical examination, vital sign measurements, and laboratory safety tests obtained at the first visit.
5. No clinically significant abnormality of ECG performed on the first visit.
6. Participant is a non-smoker, or is a social smoker using no more than 4 cigarettes per day, and is willing to abstain from smoking and nicotine containing products for all study visits (from immediately before each visit until discharge at the completion of study procedures for that visit).
7. In the Investigator's opinion, participant is able and willing to comply with all trial requirements.
8. Willing to allow his or her General Practitioner to be notified of participation in the trial and to confirm the eligibility of the participant.
9. Able to tolerate MRI scanning at the first visit.
10. Responds to topical capsaicin by attaining an ongoing pain score of at least 3/10, with measurable hyperalgesia, assessed on the first visit.

B-2 Exclusion Criteria

The participant may not enter the trial if ANY of the following apply:

1. A current acute pain condition.
2. A personal history of a chronic pain condition.
3. Contraindications to MRI scanning.
4. Scheduled elective surgery or other procedures requiring general anaesthesia during the trial.
5. A personal history of a clinically significant psychiatric disorder (including severe affective disorder, anxiety disorders, psychotic tendencies and drug-induced psychoses). Participants who have had situational depression in the past may be enrolled at the discretion of the Investigator.
6. A first-degree family history of schizophrenia, major affective disorder, or other psychosis.
7. Participant is mentally or legally incapacitated, has significant emotional problems at the time of the first visit, or is expected to have potential for mental incapacitation during the conduct of the study.
8. A history of any illness that, in the opinion of the investigator, might confound the results of the study or pose an additional risk to the participant by virtue of their participation in the study.
9. An estimated creatinine clearance of ≤80 mL/min based on the Cockcroft-Gault equation. An actual creatinine clearance, as measured using a 24-hour urine collection, may be used in place of, or in conjunction with the Cockcraft-Gault calculation. Participants with an actual or calculated creatinine clearance that is in the range of 72-79 mL/min (i.e., within 10% of 80 mL/min) may be enrolled in the study at the discretion of the Investigator.

a. Cockcraft-Gault Equation:

b. CrCl = (140-age [yr]) * (body weight [kg])/(72)serum creatinine [mg/dL])

10. A history of stroke, chronic seizures, or major neurological disorder.
11. A history of clinically significant (in the opinion of the Investigator) endocrine, gastrointestinal, cardiovascular, peripheral vascular, haematological, hepatic, immunological, renal, respiratory, or genitourinary abnormalities or diseases. Participants with a history of uncomplicated kidney stones (defined as spontaneous passage and no recurrence in the last 5 years) or childhood asthma may be enrolled in the trial at the discretion of the Investigator.
12. A history of clinically significant neoplastic disease, with the exception of adequately treated localised or in situ non-melanoma carcinoma of the skin.
13. A history of significant multiple and/or severe allergies (e.g. food, drug, latex allergy), or has had an anaphylactic reaction or significant intolerability to prescription or non-prescription drugs (including capsaicin and marijuana or other cannabis-containing drugs) or food.
14. Participants who have had major surgery within three months, or have donated or lost 1 unit (approximately 500 mL) of blood within 4 weeks prior to the first visit.
15. Participants who have participated in another investigational trial within 4 months prior to the first visit. The 4-month window will be derived from the date of the last trial medication and/or blood collection in a previous trial, and/or AE related to trial drug to the first visit of the current trial. For participants who have participated in an investigational trial where no trial drug was administered or invasive procedure performed, the interval may be revised to include no interval requirement.

16. Participants who have had (within 8 weeks of the first visit) or plan to have a preventative vaccination during the course of the study (i.e. up to the post-study visit).
17. Participant is unable to refrain from or anticipates the use of any medication, including prescription and non-prescription drugs or herbal remedies (such as St. John's Wort [*Hypericum perforatum*]) beginning approximately 2 weeks (or 5 half-lives) prior to administration of the initial dose of study drug, throughout the study (including washout intervals between treatment periods), until the post-study visit.
18. Alcohol consumption exceeding an average of >4 units per day (averaged over one week).
19. Caffeine consumption exceeding >8 servings of coffee, tea, or other caffeinated beverages per day (1 serving is approximately 120mg of caffeine).
20. Participant is currently (defined as within approximately 3 months of the pre-study/screening visit) a regular user (including "recreational use") of any illicit drugs (including marijuana) or has a history of drug (including alcohol) abuse within approximately 6 months of the pre-study/screening visit. Use of any illicit drugs (including marijuana) during the study is prohibited.
21. Participant is unwilling to refrain from sperm donation and is unwilling to use a condom with female sexual partners from the time of first administration of study drug through three months after the final dose of study drug.
22. A family history of long QT syndrome.
23. A QTc interval of >450 msec.

24. The Investigator has concerns regarding the safe participation of the participant in the study or for any other reason the Investigator considers the participant inappropriate for participation in the study

Appendix C: List of laboratory safety blood tests to accompany chapter 3.

The following blood tests were performed at each timepoint at which safety blood tests were required, as specified in chapter 3.

- Biochemistry: urea, potassium, sodium, creatinine, bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, albumin, calcium.
- Haematology: haemoglobin, haematocrit, red blood cell count, white blood cell count (total and differential), platelet count.
- Glucose
- Total cholesterol, low-density lipoproteins, high-density lipoproteins, triglycerides

Appendix D: Supplementary information and results to accompany chapter 4.

D-1.1 Dealing with outliers in evoked and ongoing pain ratings

In the analysis of pain ratings in chapter 4, outliers were not excluded due to the fact that these could relate to natural variation. Participant 015 is an outlier for both evoked and ongoing pain unpleasantness in the non-dosing arm. This indicates that he found capsaicin-induced hyperalgesia very unpleasant, but there was no reason to suspect that this was due to an error.

Participant 008 is an outlier for the change in punctate-induced pain intensity following ABX-1431. This participant did not adjust the evoked pain visual analogue scale at the end of the experimental pain paradigm while in the MRI scanner, thus returning a rating of 0 post-capsaicin. The rating is not in keeping with any of their other pain ratings done at a similar time, raising the possibility that this may be erroneous, but they deny falling asleep and report this being intentional. Therefore their ratings have not been removed from the analysis. A sensitivity analysis was performed to determine whether this rating might have influenced the overall effect of intervention on evoked pain ratings. The analysis of variance (ANOVA) in section 4.3.1.3 was repeated with participant 008 excluded. There was no change in which of the outcomes had a significance of $p \leq 0.05$.

D-1.2 Calculation and distribution of peripheral drug concentration during the MRI scan.

Peripheral drug levels were measured pre-dose, pre-MRI and post-MRI. The drug concentration used for analysis was the sum of the parent compound and the main metabolite, since both are active and both cross the blood-brain barrier. As concentration was measured both before and after the MRI scan, the mean of the values at the two timepoints was taken to give an idea of exposure during the scan (Table D.1).

Participant	Peripheral drug level (ng/ml)		
	Pre-MRI	Post-MRI	mean
001	625	614	619.5
002	338	523	430.5
003	355	372	363.5
005	355	730	542.5
007	485	637	561
008	636	472	554
009	31	91.1	61.05
010	654	885	769.5
011	539	526	532.5
012	307	433	370
013	1080	546	813
015	671	658	664.5
016	1040	971	1005.5
104	683	554	618.5
106	343	246	294.5
114	46.1	64.3	55.2

Table D.1: Table showing peripheral drug levels for each participant following dosing with ABX-1431. Levels were tested immediately before and after the MRI scan, and the mean of the two results was used as an indicator of drug exposure during the scan. The concentrations given in this table are the sum of the parent compound and the main metabolite, since both are active and both cross the blood-brain barrier.

D-1.3 Table showing lack of correlation between peripheral drug concentration and change in pain ratings following ABX-1431 administration

Outcome measure	Correlation coefficient (Pearson)	N	Significance
Change in evoked pain intensity	0.132	16	0.625
Change in evoked pain unpleasantness	0.044	16	0.870
Ongoing pain intensity	0.303	16	0.254
Ongoing pain unpleasantness	0.391	16	0.134

Table D.2: Table showing Pearson correlation coefficients for the relationship between peripheral drug levels and change in pain ratings following capsaicin application. No statistically significant correlations were identified.

D-1.4 Distribution of expectation ratings for dosing visits.

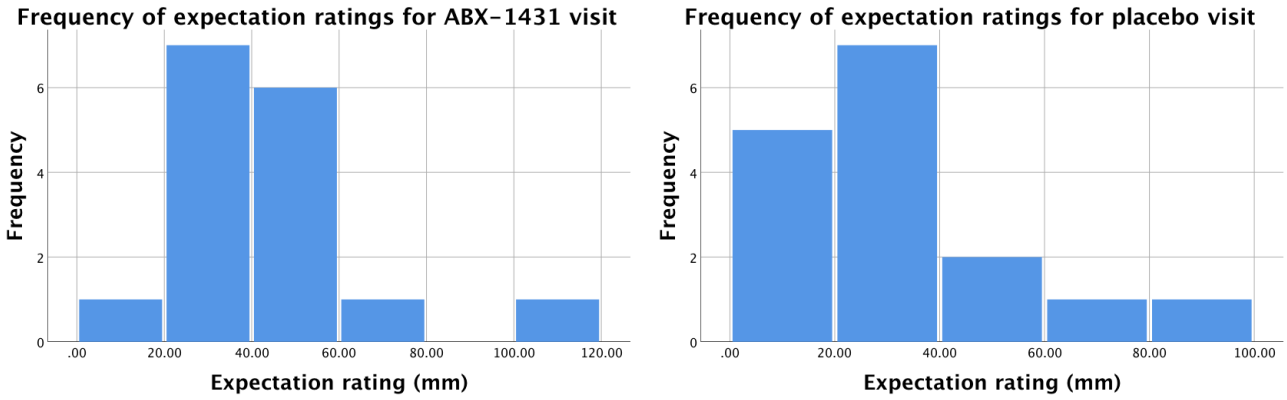


Figure D.1: Histograms to show the distribution of expectation ratings for all dosing visits (ABX-1431 and placebo). Participants were asked to rate their expectation of pain relief on a 100mm visual analogue scale. The ratings are not normally distributed. For this reason a Spearman correlation was used to investigate the relationship between expectations and pain ratings.

D-1.5 Table showing lack of correlation between the effect of ABX-1431 on sedation and on pain scores

		Correlation coefficient	N	Significance (p)
Mental sedation	Change in evoked pain intensity	-0.315	16	0.234
	Change in evoked pain unpleasantness	-0.207	16	0.441
	Ongoing pain intensity	0.068	16	0.803
	Ongoing pain unpleasantness	0.080	16	0.768
Physical sedation	Change in evoked pain intensity	-0.292	16	0.273
	Change in evoked pain unpleasantness	-0.261	16	0.330
	Ongoing pain intensity	-0.018	16	0.946
	Ongoing pain unpleasantness	-0.004	16	0.998

Table D.3: Results from a Pearson correlation coefficient calculation comparing the change in mental and physical sedation from pre-dose to post-dose to the change in pain scores during the visit in which ABX-1431 was administered. No significant correlations were found at a significance level of $p \leq 0.05$.

D-1.6 Repeated measures ANOVA showing no impact of intervention order on the change in mood and anxiety scores following the intervention.

Variation Source	Measure	df, df_{error}	F	significance
Intervention * order	Mental sedation	0.823	4, 26	0.523
	Physical sedation	1.332	4, 26	0.285
	Tranquillity	0.351	2.819, 18.327	0.777
	Sociability	1.878	4, 26	0.144
	State anxiety	0.476	4, 26	0.753
Timepoint (pre- or post-capsaicin) * order	Mental sedation	1.184	2, 13	0.337
	Physical sedation	0.468	2, 13	0.636
	Tranquillity	0.058	2, 13	0.943
	Sociability	0.576	2, 13	0.576
	State anxiety	0.416	2, 13	0.668
Intervention * timepoint * order	Mental sedation	0.195	4, 26	0.939
	Physical sedation	0.059	4, 26	0.993
	Tranquillity	0.815	4, 26	0.527
	Sociability	2.001	4, 26	0.124
	State anxiety	0.708	4, 26	0.594

*Table D.4: Effect of intervention order on mood/sedation and anxiety following each intervention. Table shows results of a repeated measures analysis of variance with group (ABX-1431 vs. placebo vs. no treatment), timepoint (pre-dose and post-dose) and order (ABX-1431, no dose, placebo; no dose, placebo, ABX-1431; placebo, ABX-1431, no dose) as factors. n=16. Outcome measures are the four Bond-Lader subscales and the state component of the state-trait anxiety inventory. Mauchley's test was significant for the effect of intervention on tranquillity (p=0.043), so the Greenhouse-Geisser correction was applied to the intervention * order interaction (epsilon 0.705). Results are otherwise uncorrected. Results were considered statistically significant if $p \leq 0.05$. There was no interaction between order and the other factors, indicating that intervention order had no impact on the effect of the intervention or the effect of the pain model on the outcome measures.*

D-1.7 Table showing lack of correlation between peripheral drug concentration and change in mood and anxiety scores following ABX-1431 administration

Outcome measure	Correlation coefficient	N	Significance
Change in mental sedation	-0.102	16	0.706
Change in physical sedation	-0.087	16	0747
Change in tranquillity	-0.017	16	0.949
Change in sociability	0.019	16	0.944
Change in STAI state	0.135	16	0.618

Table D.5: Results from a Pearson correlation coefficient calculation comparing peripheral drug levels to the change in Bond-Lader and state-trait anxiety inventory (STAI) scores during the visit in which ABX-1431 was administered. No significant correlations were found at a significance level of $p \leq 0.05$.

D-1.8 Summary of adverse events occurring during the trial

Table D.6 outlines the frequency of adverse events that took place during the study. Those adverse events that were treatment-related or possibly treatment related are broken down further in
Table D.7.

Type of adverse event	Number of participants (%)	Number of events
Any adverse event	12 (75)	35
Treatment-related adverse event	11 (69)	19
serious adverse event	0 (0)	0

Table D.6: Summary of the number of participants experiencing adverse events and the total number of adverse events reported. Treatment-related adverse events include those events that were possibly related to treatment as well as those with a definite or probable relationship.

Treatment-related adverse events	ABX-1431	No dose	Placebo
fatigue/tiredness	2	0	0
sleepiness	4	0	2
dizziness/vasovagal	4	1	1
mild euphoria	1	0	0
reduced neurocognitive function	1	0	0
pressure in head	1	0	0
headache	0	0	1

Table D.7: Table showing the frequency of treatment-related adverse events that occurred during each of the three visits. Treatment-related adverse events include those events that were possibly related to treatment as well as those with a definite or probable relationship.

Appendix E: The effect of ABX-1431 on neural responses to a visual stimulus – supplementary results to accompany chapter 5.

E-1 Group means for each intervention

To check that the visual stimulus engaged the visual system as expected, the group mean of the stimulus-induced activation was examined for each of the three intervention arms. All three groups showed activation in visual cortical regions in response to the stimulus (Figure E.1).

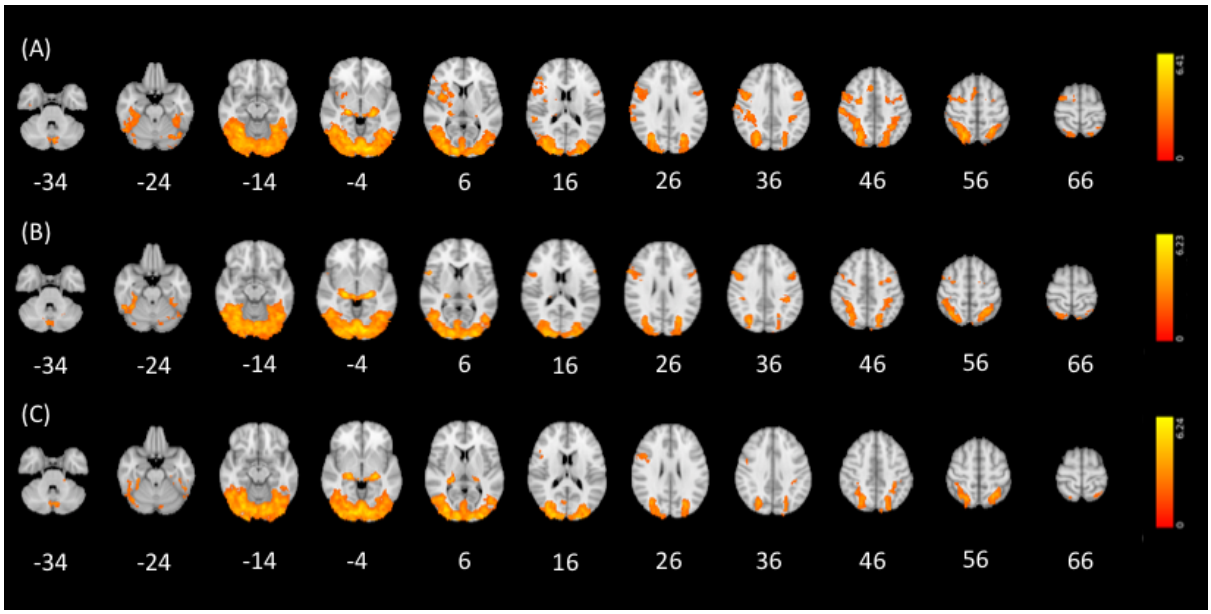


Figure E.1: Group averaged activation maps showing the neural correlates of a visual stimulus in the presence of (A) ABX-1431, (B) no dose and (C) placebo (n=16; mixed effects, Z>2.3, p<0.05). All images are in radiological convention. MNI Z-axis coordinates are shown underneath each slice. In all groups there was activation in the primary visual cortex and visual association regions, as well as the thalamus and bilateral ventrolateral prefrontal cortex.

E-2 Paired t-tests to compare interventions

As for the noxious stimulus, whole brain paired t-tests were performed comparing ABX-1431 and placebo with no dose, and with each other.

E-2.1 ABX-1431 vs. no dose

Consistent with the noxious stimulus, there were no regions in which ABX-1431 suppressed visual stimulus-related activity compared to no dose. Compared to no dose, ABX-1431 led to greater activity in the right middle temporal gyrus and right supramarginal gyrus (Figure E.2). These were different regions to those that were identified in response to the noxious stimulus.

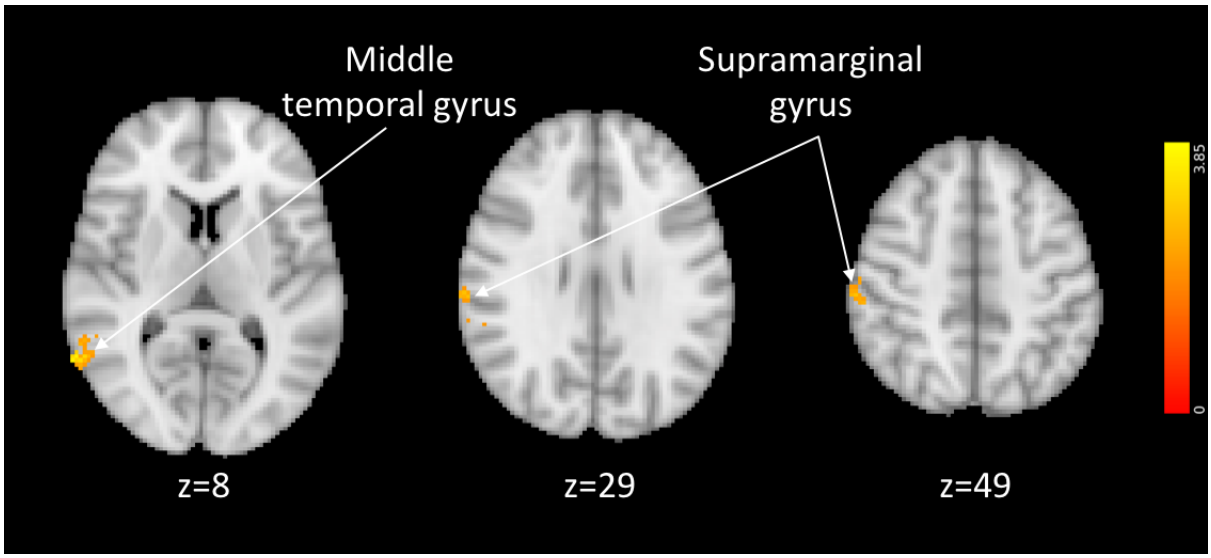


Figure E.2: Paired t-test comparing the effect of ABX-1431 and no dose on BOLD activation during a visual stimulus. Brain regions in which activation was greater after ABX-1431 are shown (n=16; mixed effects; Z>2.3, p<0.05; cluster corrected). ABX-1431 led to greater activity in the right middle temporal gyrus and supramarginal gyrus. Images are in radiological convention. Slices are identified by MNI coordinates.

E-2.2 Placebo vs. no dose

In the presence of placebo there was significantly increased BOLD activity in the left lateral occipital cortex and left angular gyrus during the visual stimulus compared to no dose (Figure E.3). There was no significant difference found in the no dose>placebo contrast. This follows the opposite pattern to the noxious stimulus, in which there was reduced left angular gyrus activity following placebo.

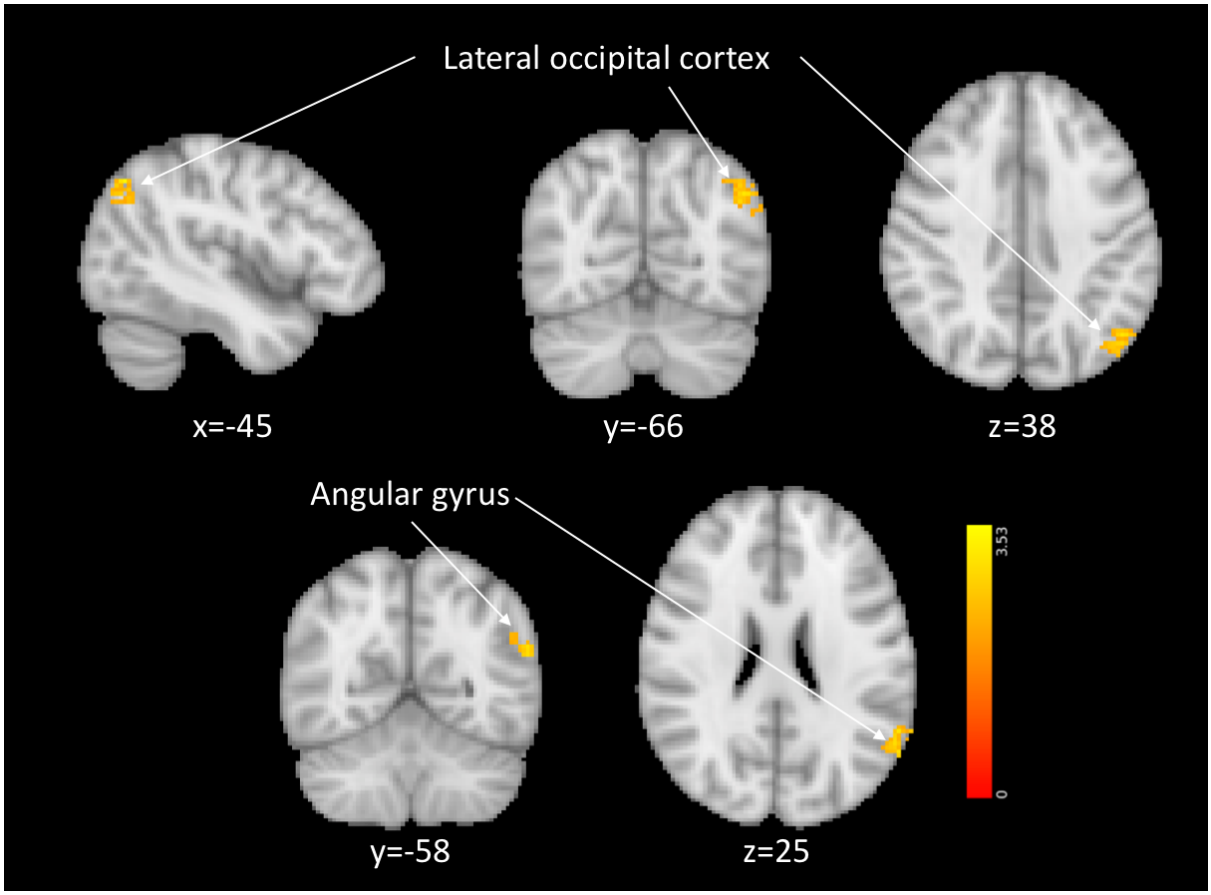


Figure E.3: Paired t-test comparing the effect of placebo and no dose on BOLD activation during a visual stimulus. Brain regions in which activation was greater after placebo are shown (n=16; mixed effects; Z>2.3, p<0.05; cluster corrected). Placebo led to greater activity in the left lateral occipital cortex and left angular gyrus. Images are in radiological convention. Slices are identified by MNI coordinates.

E-2.3 ABX-1431 vs. placebo

Unlike the noxious stimulus, for which there were no significant findings in the placebo>ABX-1431 contrast, the visual stimulus produced more left angular gyrus and left lateral occipital cortex activity after placebo than after ABX-1431.

Compared to placebo, ABX-1431 led to significantly increased activation in multiple regions in response to the visual stimulus. These regions were the left anterior insula, left ventrolateral PFC (vlPFC), right middle temporal gyrus, right temporal occipital fusiform cortex, right supramarginal gyrus, bilateral lateral occipital cortex, and a very small region in the right angular gyrus (Figure E.4). There was no overlap between the clusters identified in this analysis and those identified in the ABX-1431>placebo contrast for the noxious stimulus.

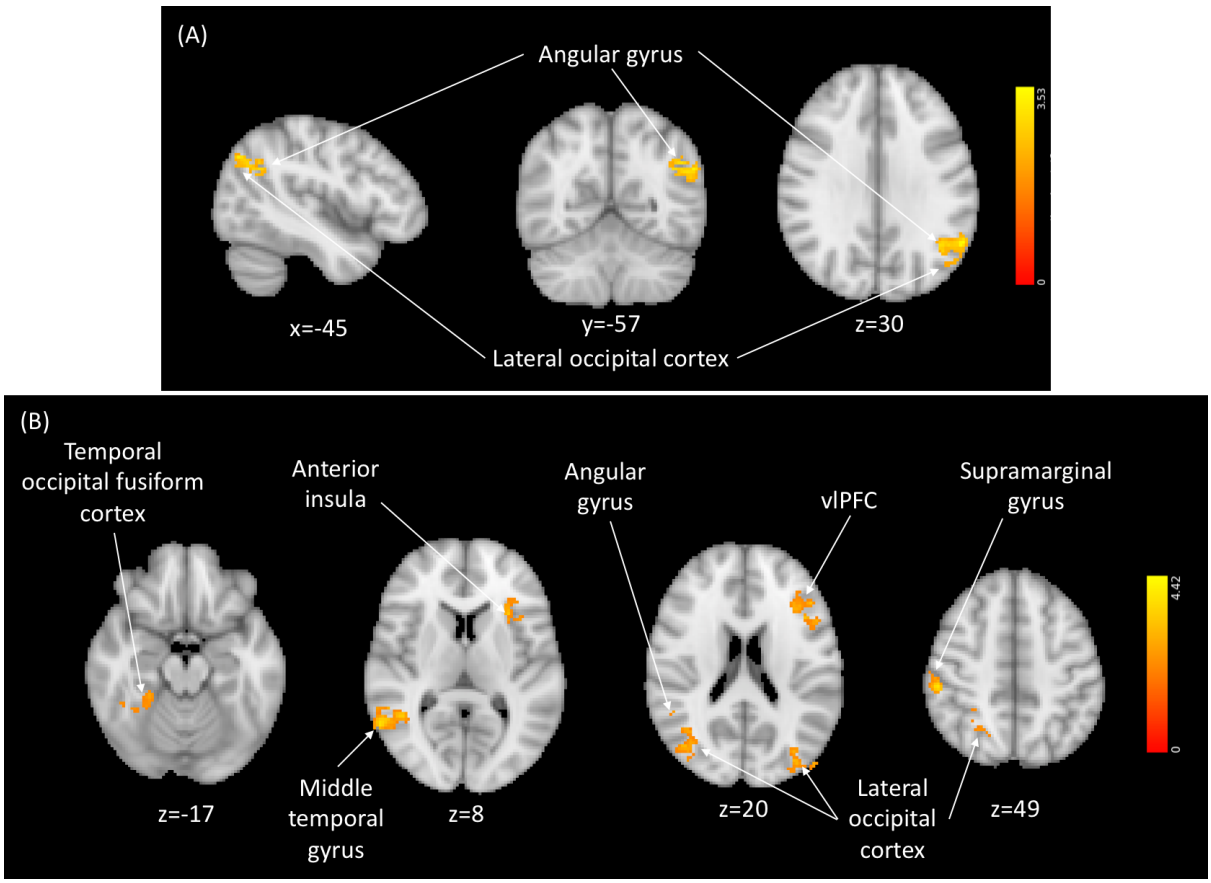


Figure E.4: Paired t-test comparing the effect of ABX-1431 and placebo on BOLD activation during a visual stimulus (n=16; mixed effects; Z>2.3, p<0.05; cluster corrected). Brain regions in which there was significantly more activity in placebo than ABX-1431 (A) and in which there was significantly more activity in ABX-1431 than placebo (B) are labelled on the images. Images are in radiological convention. Slices are identified by MNI coordinates. vlPFC = ventrolateral prefrontal cortex.

E-3 Discussion of the effect of ABX-1431 on neural responses to a visual stimulus

The purpose of the visual stimulus was as a control stimulus, to check that responses to evoked pain stimuli were pain-specific, and not universal responses to the presence of any stimulus, nor due to generalised changes in blood flow.

There were group differences present in the response to the visual stimulus between interventions. ABX-1431 led to increased activity in the right middle temporal gyrus

and the right supramarginal gyrus compared to both placebo and no dose, indicating a genuine drug effect. However, compared to placebo, ABX-1431 was also associated with increased activity in multiple other regions, namely the left anterior insula, the right temporal occipital fusiform gyrus, the left vlPFC, the right angular gyrus and parts of both lateral occipital cortices. Given the fact that these differences were not present compared to no dose, it is not clear whether they are drug-induced, placebo-induced, or a combination of the two. Placebo certainly had an effect on neural responses to the visual stimulus, despite the fact that participants believed the drug to be an analgesic. In the placebo arm there was increased activity in the left angular gyrus and left lateral occipital cortex compared to both ABX-1431 and no dose. Compared to no dose, placebo had opposite effects during the visual stimulus and the noxious stimulus, leading to increased activation and suppression of activity respectively. The clear presence of a placebo effect indicates that for the visual stimulus, like the noxious stimulus, there were sources of variance present that were not drug-related.

The presence of some drug-related modulation of the neural response to a visual stimulus shows that ABX-1431 does impact the visual system. This is to be expected given that CB1 receptors are present in the retina, the parts of the thalamus that project to the primary visual cortex and the visual cortex itself, and that endocannabinoids are expressed in all three locations (J. Chen et al., 2005; Schwitzer et al., 2015). CB1 agonists are thought to modulate the processing of visual information (Schwitzer et al., 2015), and a common adverse effect of cannabis use is blurred vision (Joint Formulary Committee, 2020).

The fact that there was no effect of ABX-1431 on the primary visual cortex compared to placebo or no dose indicates that the drug-related findings do not simply reflect a generalised increase in blood flow to all regions activated by the stimulus. Furthermore, there was no overlap between the clusters that showed significant intervention-related differences during the noxious stimulus versus the visual stimulus. The fact that the effects of the two stimuli were dissociable indicates that the pain-induced responses were modality specific.

Appendix F: Whole brain activation maps of key contrasts to accompany chapter 5

F-1 Punctate-induced BOLD activity

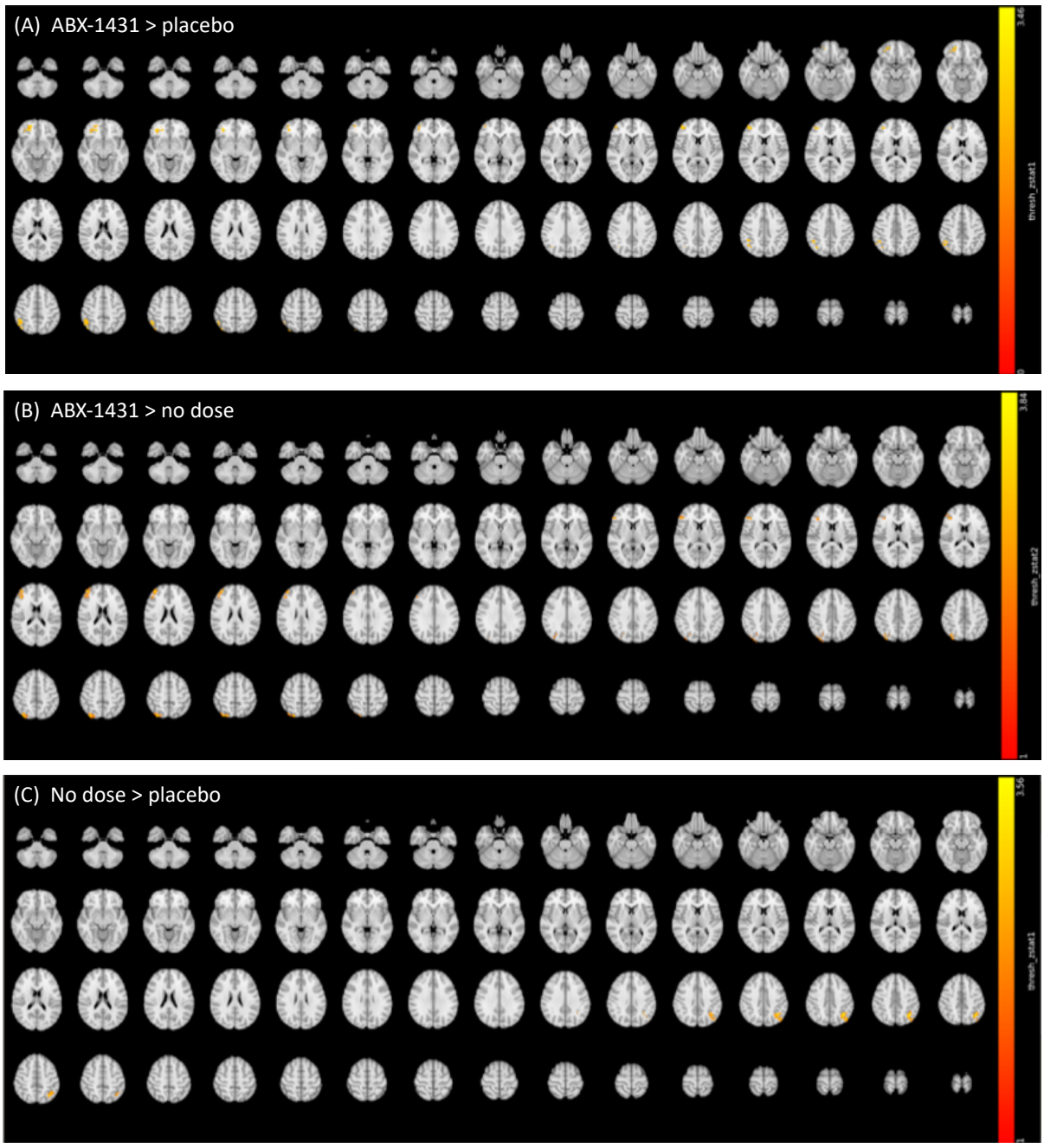


Figure F.1: Activation maps to show full results of whole brain paired t-tests for the contrasts in which significant differences in punctate-induced BOLD activity were found. The contrasts shown are (A) ABX-1431>placebo; (B) ABX-1431>no dose; and (C) no dose>placebo (n=16; mixed effects; Z>2.3, 0<0.05; cluster corrected). Images are in radiological convention. Slices are identified by MNI coordinates.

F-2 BOLD resting state connectivity with the right amygdala

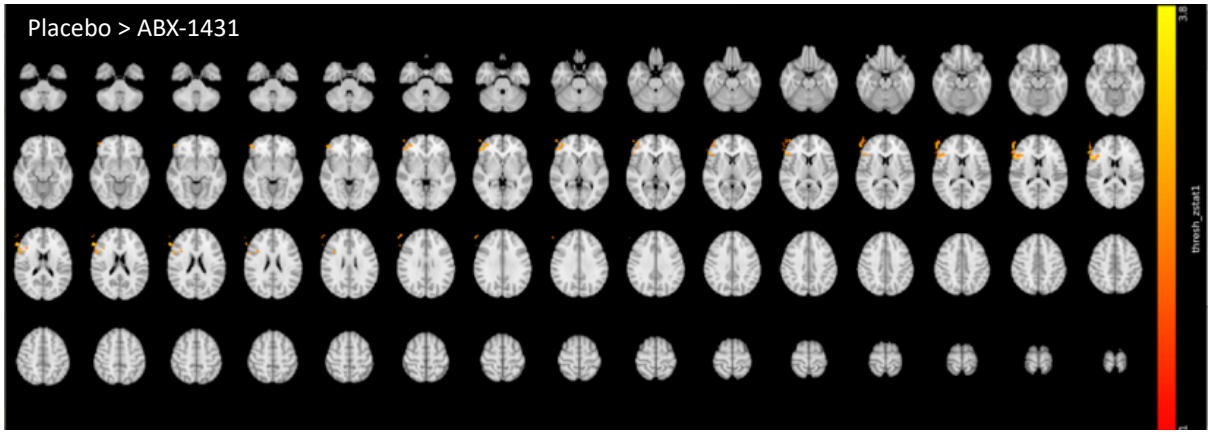


Figure F.2: Activation map to show full results of the placebo>ABX-1431 contrast in a paired t-test comparing differences in whole brain functional connectivity with the right amygdala (n=16; mixed effects; Z>2.3, p<0.05; cluster corrected) . This was the only whole brain contrast in which significant differences in functional connectivity were found. Images are in radiological convention. Slices are identified by MNI coordinates.

F-3 Cerebral blood flow at rest (ASL)

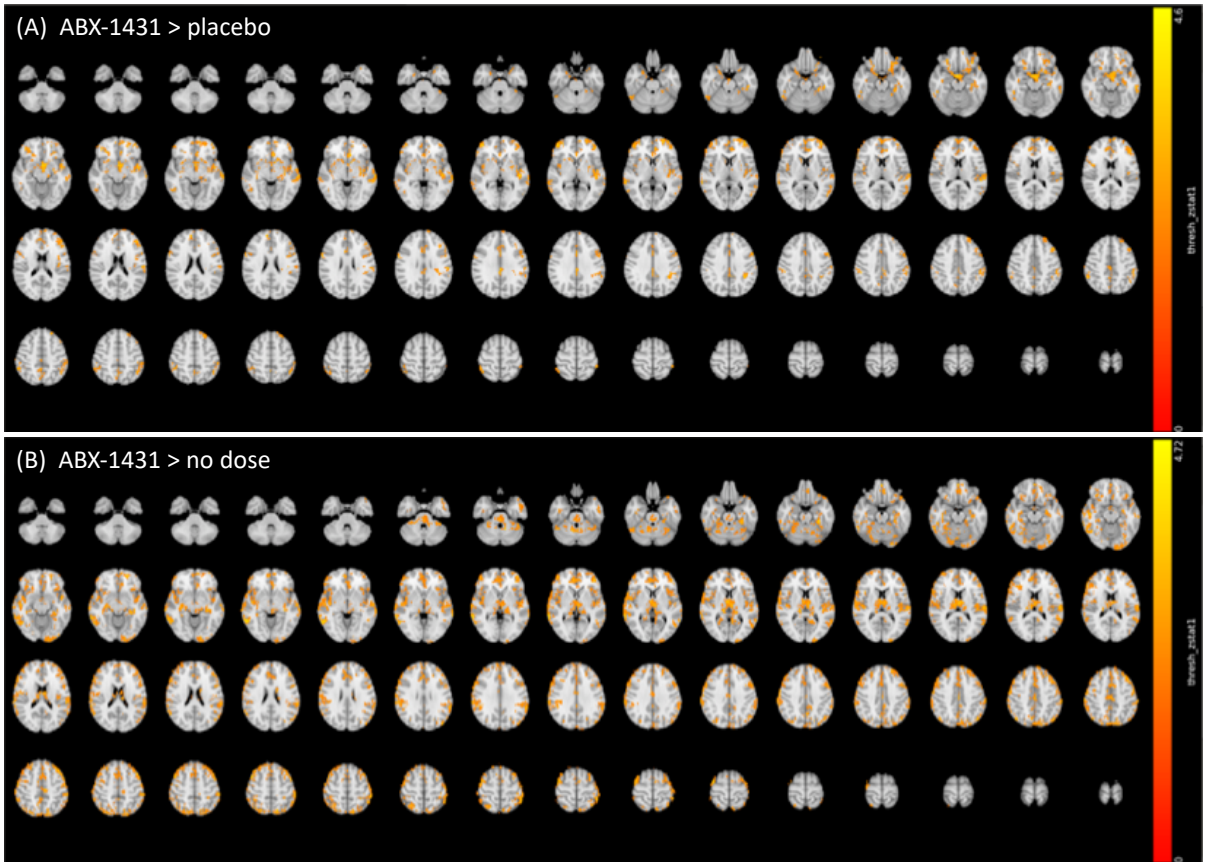


Figure F.3: Activation maps to show full results of whole brain paired t-test for the contrasts in which significant differences in blood flow were found, as assessed by ASL. The contrasts shown are (A) ABX-1431>placebo; and (B) ABX-1431>no dose (n=15; Z>2.3, p<0.05; cluster corrected). Images are in radiological convention and slices are identified with MNI coordinates.

Appendix G: Masks to accompany chapter 5

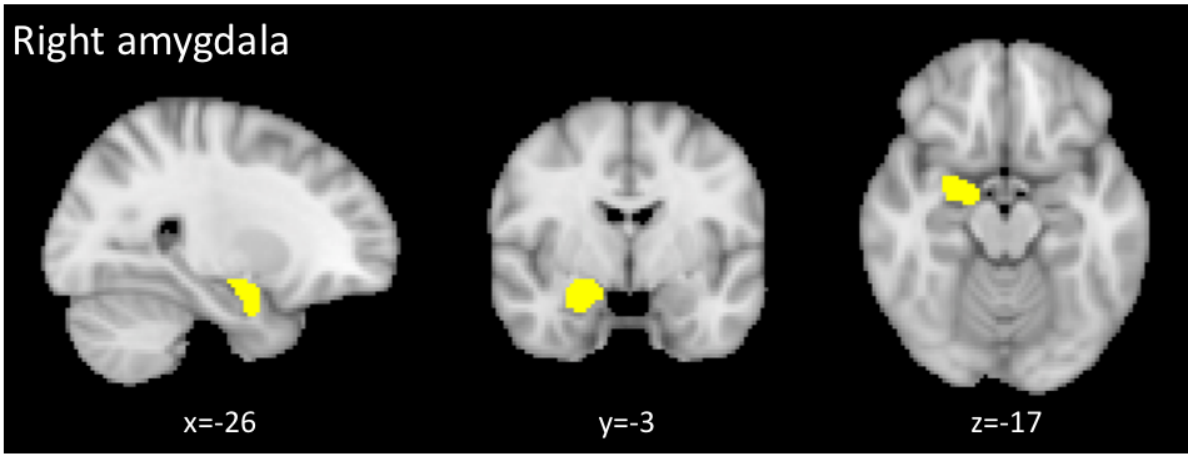


Figure G.1: Anatomical mask of the right amygdala. Defined using the Harvard-Oxford probabilistic atlas and thresholded to 50%. Images are in radiological convention and slices are identified with MNI coordinates.

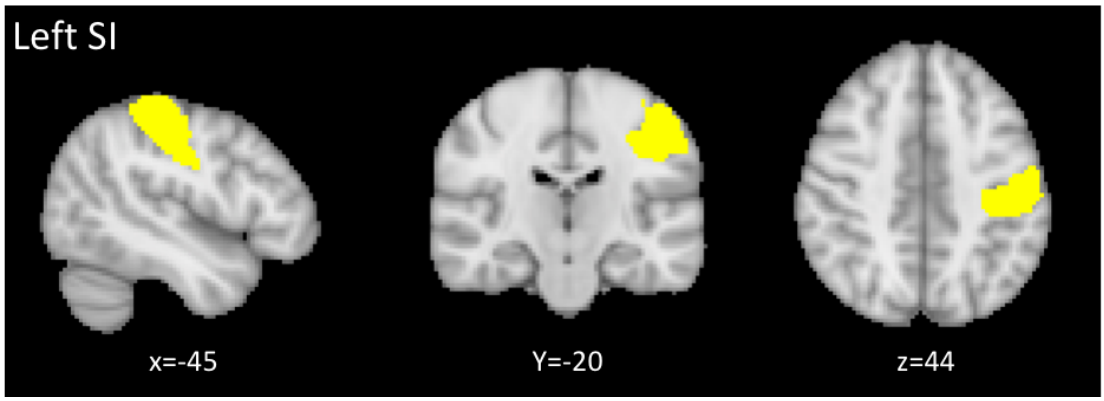


Figure G.2: Anatomical mask of the left primary somatosensory cortex (SI). Defined using the Juelich histological atlas and thresholded to 50%. Images are in radiological convention and slices are identified with MNI coordinates.

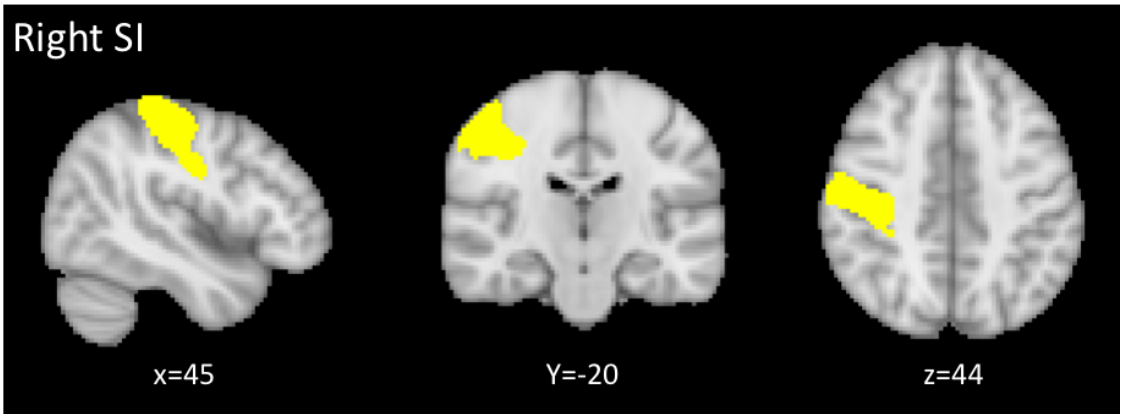


Figure G.3: Anatomical mask of the right primary somatosensory cortex (SI). Defined using the Juelich histological atlas and thresholded to 50%. Images are in radiological convention and slices are identified with MNI coordinates.

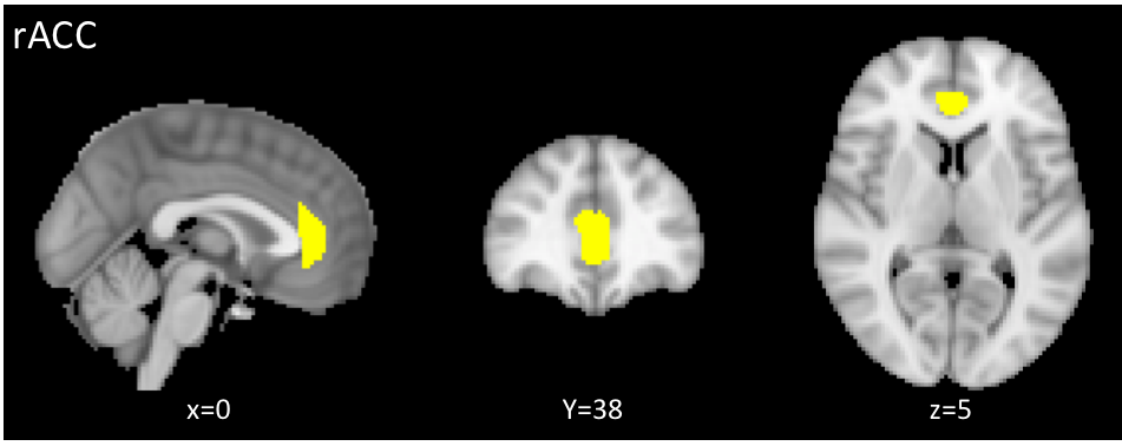


Figure G.4: Anatomical mask of the rostral anterior cingulate cortex (rACC). Created from an anterior cingulate cortex mask defined using the Harvard-Oxford probabilistic atlas and thresholded to 50%. rACC then delineated as described by Vogt et al. (2003). Images are in radiological convention and slices are identified with MNI coordinates.

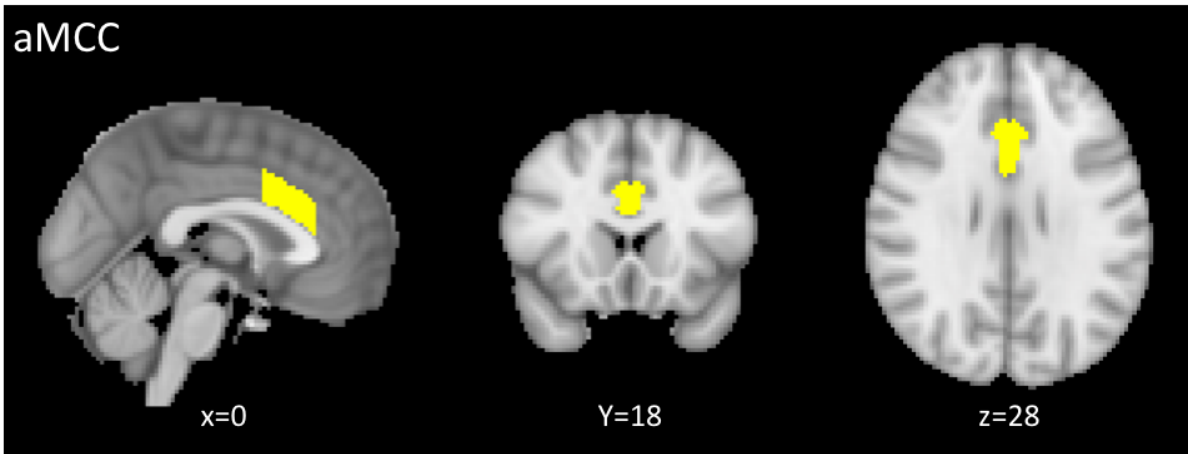


Figure G.5: Anatomical mask of the anterior midcingulate cortex (aMCC). Created from an anterior cingulate cortex mask defined using the Harvard-Oxford probabilistic atlas and threshold to 50 %. aMCC then delineated as described by Vogt et al. (2003). Images are in radiological convention and slices are identified with MNI coordinates.

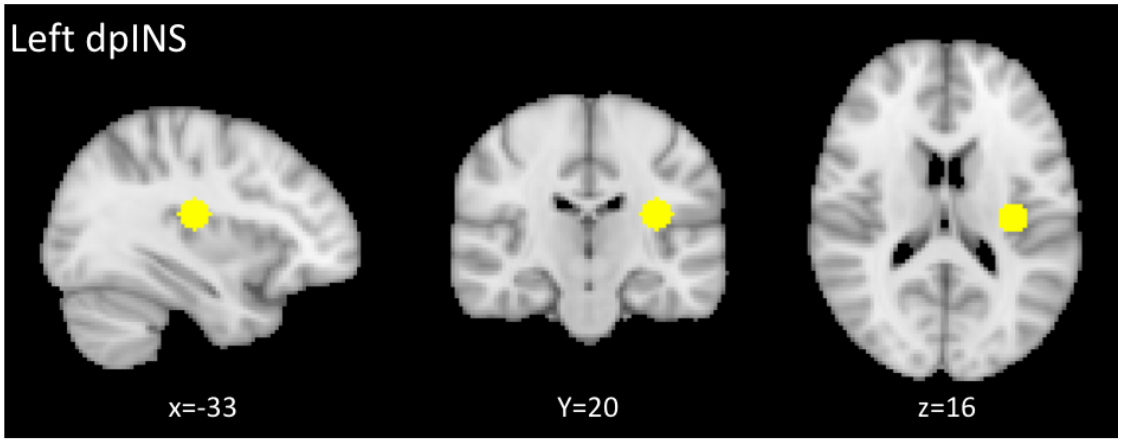


Figure G.6: Functional mask of the left dorsal posterior insula, based on the findings of Segerdahl et al. (2015). The mask was an 8mm sphere centred around the area of the dorsal posterior insula (dpINS) that correlated most closely with perceived pain intensity. Images are in radiological convention and slices are identified with MNI coordinates.

Appendix H: A pilot study to examine the baseline characteristics of individuals with irritable bowel syndrome

H-1 Introduction

The aim of this pilot study is to characterise the baseline characteristics of a cohort of individuals with irritable bowel syndrome (IBS), most of whom went on to take part in the mindfulness-based cognitive therapy (MBCT) intervention investigated in chapters 6 and 7, and to look for any differences in the way that they perceive and process pain compared to healthy controls.

The pilot will look first at the symptoms of IBS, the associated psychological symptoms, and the effect of these on participants' quality of life. These are all highly subjective, self-reported measures. The pilot then goes on to examine somatic sensory function. The measurement of pain ratings in response to known and quantified stimuli is a more objective tool than questionnaires. However, the self-reported nature of pain ratings confers a large subjective element to sensory testing. Brain imaging is an objective measure, and the pilot will look at whether it is a useful tool for characterising the differences between healthy controls and the patients in this cohort. It will also consider some of the pitfalls that may be associated with imaging a heterogeneous population of participants with chronic pain by assessing some of the factors that might explain within-group and between-group variation in those with IBS. These are factors that may be considered possible confounds in other studies of patients with IBS.

It was hypothesised that all three types of outcome measure will demonstrate differences between patients and controls, but that in addition to being more objective, imaging will provide more mechanistic information than the other methods of assessment. If the imaging shows reliable differences in pain processing between patients and controls in this cohort, then it is also likely to be a useful tool in assessing how pain processing in this group of patients is affected by treatment.

Based on existing literature, it was also hypothesised that participants with IBS would have more gastrointestinal symptoms than healthy controls, including pain, and that they would report higher levels of anxiety and depression and a reduced quality of life (Folks, 2004; Longstreth et al., 2006; Mönnikes, 2011; Wu, 2012). Additionally, it was predicted that compared to healthy controls, participants with IBS would be more likely to exhibit somatic hyperalgesia, demonstrated by lower thermode temperatures or higher evoked pain scores. Finally, it was thought that duration of disease and the presence of dysmenorrhoea might be confounding sources of between-participant variance. Meanwhile, background pain levels and perceived stimulus intensity might lead to between-participant variance in this pilot, indicating a possibility that they would introduce within-participant variance in a study with a repeated measures design.

H-2 Methods

Data from two studies were combined. Study A was a cross-sectional study that recruited participants with IBS and healthy controls for a single visit. Study B was a prospective interventional study that recruited participants with IBS for multiple visits, but only a single pre-intervention visit was analysed in this chapter. The study

procedures for the IBS group at this visit were identical in both studies. All participants completed a set of questionnaires, followed by an evoked pain task, carried out during a functional MRI brain scan. The evoked pain task is the same as that described in chapter 6 (section 6.2.5), while the MRI methodology matches that described in chapter 7 (section 7.2.3). The healthy controls followed the same procedures as the participants with IBS, but with a reduced set of questionnaires.

H-2.1 Questionnaires

In both studies, participants completed the following questionnaires. (Those in bold were only administered to the IBS group and not the healthy controls.):

- **Brief pain inventory – short form (BPI; Cleeland & Ryan, 1994)**
- **PainDetect** (Freynhagen et al., 2006)
- Visceral sensitivity index (VSI; Labus et al., 2004)
- Gastrointestinal symptom rating scale (GSRS) - IBS (Wiklund et al., 2003)
- **IBS-impact scale (IBS-IS; Longstreth et al., 2005)**
- Hospital anxiety and depression scale (HADS; Zigmond & Snaith, 1983)
- Short Form-36 health survey version 2 (SF-36; Ware, Kosinski, & Dewey, 2000)

For the controls and three of the IBS patients, all questionnaires were completed on the day of scanning. The remaining IBS patients were a subset of those taking part in a larger study, in which the VSI and GSRS-IBS were administered at multiple timepoints by Julia Henrich (Henrich et al., 2020). The data used in this pilot were that which was collected at the timepoint closest to the scanning visit. These participants completed the remainder of the questionnaires on the day of scanning.

The GSRS-IBS is not validated for use in healthy participants, but since gastrointestinal symptoms are common in the general population, it was administered to controls as well as IBS patients to enable an assessment of how symptom severity in this IBS cohort compared to individuals with no gastrointestinal diagnosis.

Participants were also asked about their age, time since diagnosis and frequency of pain, and women were asked about the severity of their menstrual pain.

H-2.2 Participants

The analysis incorporated data from 25 participants with IBS and 16 healthy controls. The participants with IBS were recruited from two studies, as outlined above (section H-2). All participants were over 18 and met Rome III criteria for IBS. Full inclusion and exclusion criteria are given for both studies in appendix I.

Technical issues with the thermode meant that two of the healthy controls were unable to complete the evoked pain paradigm. Therefore, the pain ratings and BOLD imaging were taken from 25 participants with IBS and 14 healthy controls. However, all participants were able to complete the perception locking, so the temperature required to achieve a pain intensity rating of 5/10 is available for all 16 healthy controls.

H-2.3 Recruitment

Healthy controls were recruited through advertisements. Participants with IBS were recruited through gastroenterology clinics at Oxford University Hospitals NHS Trust, as well as by word of mouth and local advertising.

H-2.4 Analysis of behavioural and sensory data

Data were analysed using Microsoft excel and IBM SPSS.

H-2.4.1 Comparing means between groups

Independent t-tests were used to compare questionnaire data between the group of participants with IBS and the control group. For each outcome measure, normality was assessed using histograms and Q-Q plots. In the IBS group, results were close to a normal distribution across all measures. However, in the control group the distributions were very variable, with most showing either positive or negative skew. The sample sizes were small, meaning that violating the assumption of normality was more likely to influence the outcome. For this reason, bootstrapping was applied to all t-tests in this appendix. The assumption of heteroscedasticity was also not met across all outcome measures. Therefore the Welch-Satterwaite method was used to correct for heterogeneity of variance.

H-2.4.2 Determining the relationship between variables

A Spearman correlation coefficient was used to determine the presence of significant correlations between variables. This was chosen over a Pearson correlation coefficient as it does not require all variables to be normally distributed, and is less susceptible to outliers.

H-3 Results

The results of this pilot study are divided into four sections. Section 1 provides data on age and sex matching between the participants with IBS and the healthy controls.

Section 2 focusses on the behavioural data, consisting of the symptoms of IBS, the associated psychological symptoms, and the effect of these on participants' quality of life. Section 3 gives the results of the somatic sensory data collected during the evoked pain task. Finally, section 4 outlines the neuroimaging results.

H-3.1 Section 1: Age and sex matching

Due to better recruitment to the IBS arm than the control arm, the groups were not of equal size. Both groups contained one male with the remainder of participants being female. The average age of the groups was fairly well matched, but the range was greater in the group with IBS due to the presence of some older patients (
Table H.1; Figure H.1). The group difference (0.800, BCa 95% CI [-3.848, 5.051]) was not statistically significant (t(37.826)=0.324, p=0.113).

		Group with IBS (N=25)	Healthy controls (N=16)
Age (years)	Mean (SD)	30.92 (10.27)	30.13 (5.37)
	median	28	28.5
	range	21-63	24-44
Sex	Number of males (%)	1 (4)	1 (6.25)
	Number of females (%)	24 (96)	15 (93.75)

Table H.1: Comparison of age and sex distribution in group with irritable bowel syndrome (IBS) versus healthy controls. Groups were generally well matched for age and sex, though there were some older outliers in the group with IBS. The majority of participants were female.

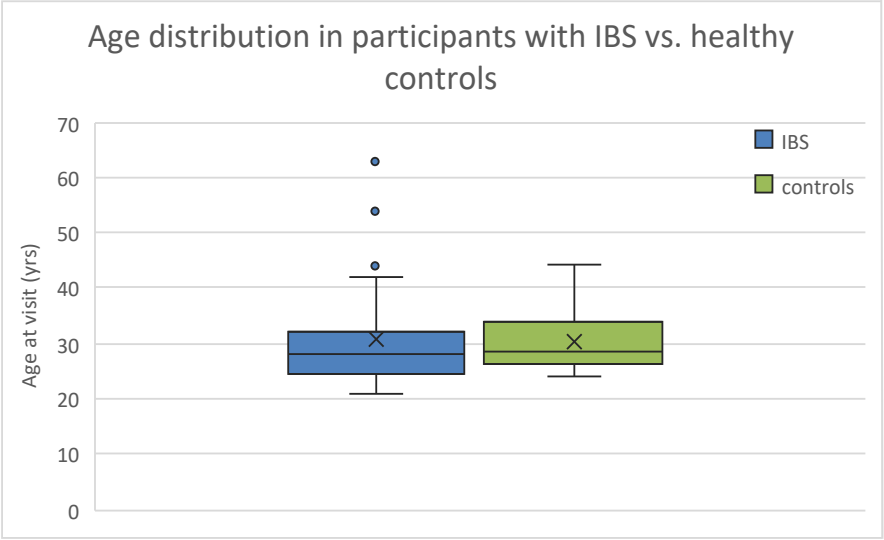


Figure H.1: Boxplots showing the age distribution in participants with irritable bowel syndrome (IBS; n=25) versus healthy controls (n=16). The mean value in each group is denoted by a cross. The distribution was similar in the two groups, barring some older outliers in the group with IBS. An independent samples t-test showed that there was no statistically significant difference between the group means.

H-3.2 Section 2: Behavioural data

H-3.2.1 Symptom severity

An independent samples t-test comparing total GSRS scores showed that on average the IBS group (mean=43.750, SD=12.553) reported more gastrointestinal symptoms than healthy controls (mean=15.688, SD=2.938). This difference (28.063, BCa 95% CI [22.951, 33.454]) was significant (t(26.658)= 10.528, p=<0.001). Hypothesis testing was carried out for the total score only in order to avoid excessive multiple comparisons, but it is clear from the data that this applies across all symptom domains (Figure H.2).

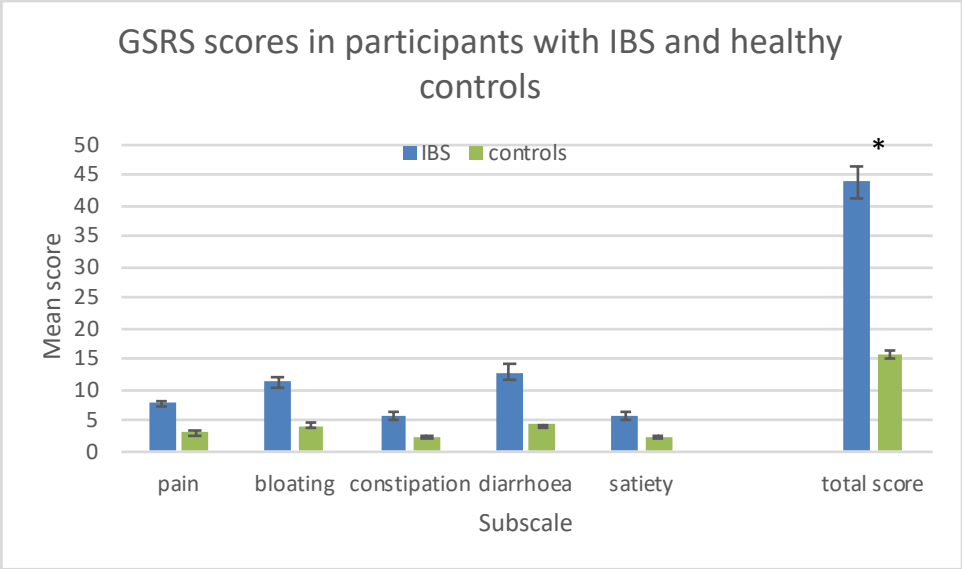


Figure H.2: Chart showing mean gastrointestinal symptom rating scale (GSRS) scores for irritable bowel syndrome (IBS) patients (n=23) and for healthy controls (n=16). Error bars show standard errors. Severity scores were higher in the group with IBS than in the healthy controls for all symptoms. Formal hypothesis testing was only performed for the total score, which showed the difference to be highly significant (p=0.001). This result is marked with an asterisk.

H-3.2.2 Anxiety and depression

Statistically, the IBS cohort had significantly higher scores than the healthy control cohort for symptoms of both anxiety and depression according to the HADS questionnaire and the VSI (

Table H.2, Figure H.3).

Outcome measure	N (IBS, control)	t	df	Mean difference	Std. error difference	P value (2-tailed)	BCa 95% confidence interval	
							lower	upper
HADS-A	25, 16	5.045	38.098	5.863	1.162	<0.001	3.674	8.209
HADS-D	25, 16	4.410	35.715	4.365	0.990	<0.001	2.520	6.292
VSI	24, 16	14.295	24.103	46.521	3.254	<0.001	40.285	52.602

Table H.2: Results of an independent samples t-test comparing mean group differences in hospital anxiety and depression scale (HADS) scores and visceral sensitivity index (VSI) scores. Differences were considered statistically significant if $p \leq 0.05$, and p values reaching this criterion are shown in red. Scores were significantly higher in the irritable bowel syndrome (IBS) group than the control group for both anxiety (HADS-A and VSI) and depression (HADS-D).

At a group level, the clinical significance of the HADS scores may appear minimal, since the mean depression score for both groups was within the normal range of 0-7. Meanwhile, the mean anxiety score for controls was within the normal range, and for IBS patients was at the lower end of the abnormal range (>10) (Figure H.3). However, as demonstrated by the error bars, wide individual variability means that the mean was a poor reflection of the experience of the cohort as a whole. In reality, 76% of participants with IBS reported abnormal or borderline abnormal levels of

anxiety, in contrast to only 25% of controls. For symptoms of depression, 36% of IBS participants fell within the abnormal or borderline abnormal range compared to 0% of controls (

Table H.3).

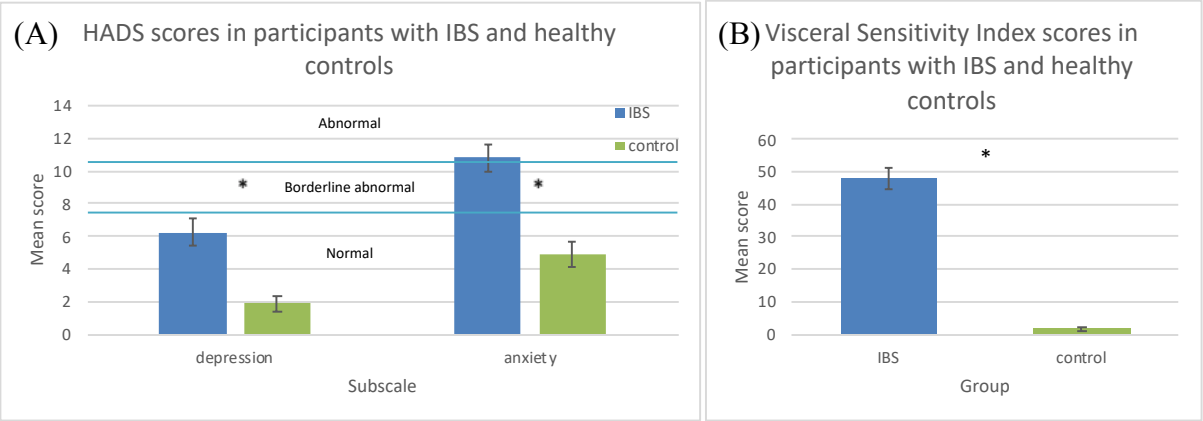


Figure H.3: Chart showing mean hospital anxiety and depression scale (HADS) scores (A) and visceral sensitivity index scores (B) for irritable bowel syndrome (IBS) patients (n=25) and for healthy controls (n=16). Error bars show standard errors. In panel A, horizontal blue lines show thresholds between normal, borderline abnormal and abnormal scores. Differences were considered significant if $p \leq 0.05$, and changes meeting this criterion are marked with an asterisk. Participants with IBS had significantly higher scores for all measures of anxiety/depression.

(A)

Score category (anxiety)	Number of IBS participants (%)	Number of control participants (%)
Normal (0-7)	6 (24)	12 (75)
Borderline abnormal (8-10)	5 (20)	2 (12.5)
Abnormal (>10)	14 (56)	2 (12.5)

(B)

Score category (depression)	Number of IBS participants (%)	Number of control participants (%)
Normal (0-7)	16 (64)	16 (100)
Borderline abnormal (8-10)	6 (24)	0 (0)
Abnormal (>10)	3 (12)	0 (0)

Table H.3: Table showing the percentage of participants reporting normal and abnormal scores for (A) anxiety and (B) depression in each group (IBS: n=25; healthy controls: n=16). For anxiety, the majority of healthy controls scored within the normal range, while the majority of participants with irritable bowel syndrome (IBS) reported scores in the abnormal range. For depression, all healthy controls scored within the normal range while 36% of participants with IBS reported scores in the abnormal or borderline abnormal range.

H-3.2.3 Quality of life

Control participants reported better quality of life than IBS participants across all domains of the SF-36 (Figure H.4). The domain with the biggest group difference was bodily pain, but overall, mental factors were affected more than physical factors, as demonstrated by the fact that the difference seen between the two groups in the mental component summary score (MCS) was more than treble the difference seen in the physical component summary score (PCS) (

Table H.4).

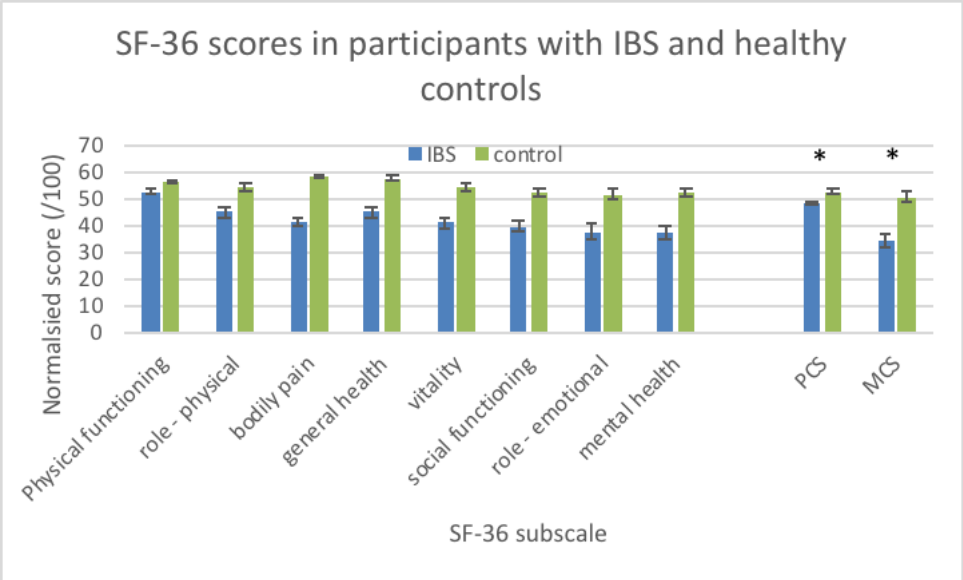


Figure H.4: Chart showing mean normalised SF-36 scores for irritable bowel syndrome (IBS) patients (n=25) and for healthy controls (n=16) across all domains as well as mental and physical component summary scores. Error bars show standard errors. An increased score equates to improved quality of life. Differences were considered significant if $p \leq 0.05$, and changes meeting this criterion are marked with an asterisk. Formal hypothesis testing was only performed for the physical component summary (PCS) and mental component summary (MCS) scores and not for individual subscales. Both scores showed quality of life to be significantly worse in those with IBS than in the control group.

A t-test was performed for both the MCS score and PCS score, showing that the group difference for both of these composite scores was strongly significant (Table H.4). As for the GSRS subscales, t-tests were not done across all subscales due to the issue of multiple comparisons.

Outcome measure	N (IBS, control)	t	df	Mean difference	Std. error difference	P value (2-tailed)	BCa 95% confidence interval

							lower	upper
SF-36 PCS	25, 16	-3.488	37.486	-4.456	1.277	0.001	-7.012	-1.894
SF-36 MCS	25, 16	-4.931	38.948	-16.171	3.279	<0.001	-22.963	-8.961

Table H.4: Results of an independent samples t-test comparing mean group differences in SF-36 physical component summary (PCS) and mental component summary (MCS) scores (IBS: n=25; healthy controls: n=16). Differences were considered statistically significant if p≤0.05, and p values reaching this criterion are shown in red. Scores were significantly lower in the irritable bowel syndrome (IBS) group than the control group for both categories, indicating lower quality of life.

H-3.2.4 Relationship between symptoms, quality of life, and anxiety and depression

The relationship between gastrointestinal symptoms and anxiety and SF-36 scores in both groups was examined using Spearman correlation coefficients. There was no correlation found between gastrointestinal symptoms (GSRS scores) and generalised anxiety (HADS scores). In the IBS group, there was a negative correlation between GSRS scores and symptom-related anxiety (VSI). There was also a correlation between higher GSRS scores and lower SF-36 MCS scores, indicating worse quality of life in the mental domain. In the control group, there was a positive correlation between GSRS scores and VSI scores (
Table H.5). Statistically significant correlations are illustrated in scatterplots (Figure H.5).

(A) IBS group (correlation with GSRS)

Outcome measure	Correlation coefficient (r_s)	N	Significance (2 tailed)
HADS-A	0.313	24	0.136
HADS-D	0.304	24	0.148
VSI	-0.434	24	0.034
SF-36 PCS	-0.174	23	0.417
SF-36 MCS	-0.421	23	0.040

(B) Control group (correlation with GSRS)

Outcome measure	Correlation coefficient (r_s)	N	Significance (2 tailed)
HADS-A	0.017	16	0.951
HADS-D	0.247	16	0.357
VSI	0.795	16	<0.001
SF-36 PCS	-0.070	16	0.796
SF-36 MCS	0.063	16	0.817

Table H.5: Tables showing Spearman correlation coefficients between gastrointestinal symptom rating scale (GSRS) scores and hospital anxiety and depression scale (HADS)/visceral sensitivity index (VSI)/SF-36 scores for (A) participants with irritable bowel syndrome (IBS), and (B) healthy controls. Correlations were considered significant if $p \leq 0.05$ and p values reaching this criterion are shown in red. There was a positive correlation between GSRS score and VSI in the control group, but a negative correlation in the IBS group. In those with IBS there was also a correlation between higher GSRS scores and lower SF-36 mental component summary (MCS) scores. HADS-A, HADS anxiety subscale; HADS-D, HADS depression subscale; PCS, physical component summary.

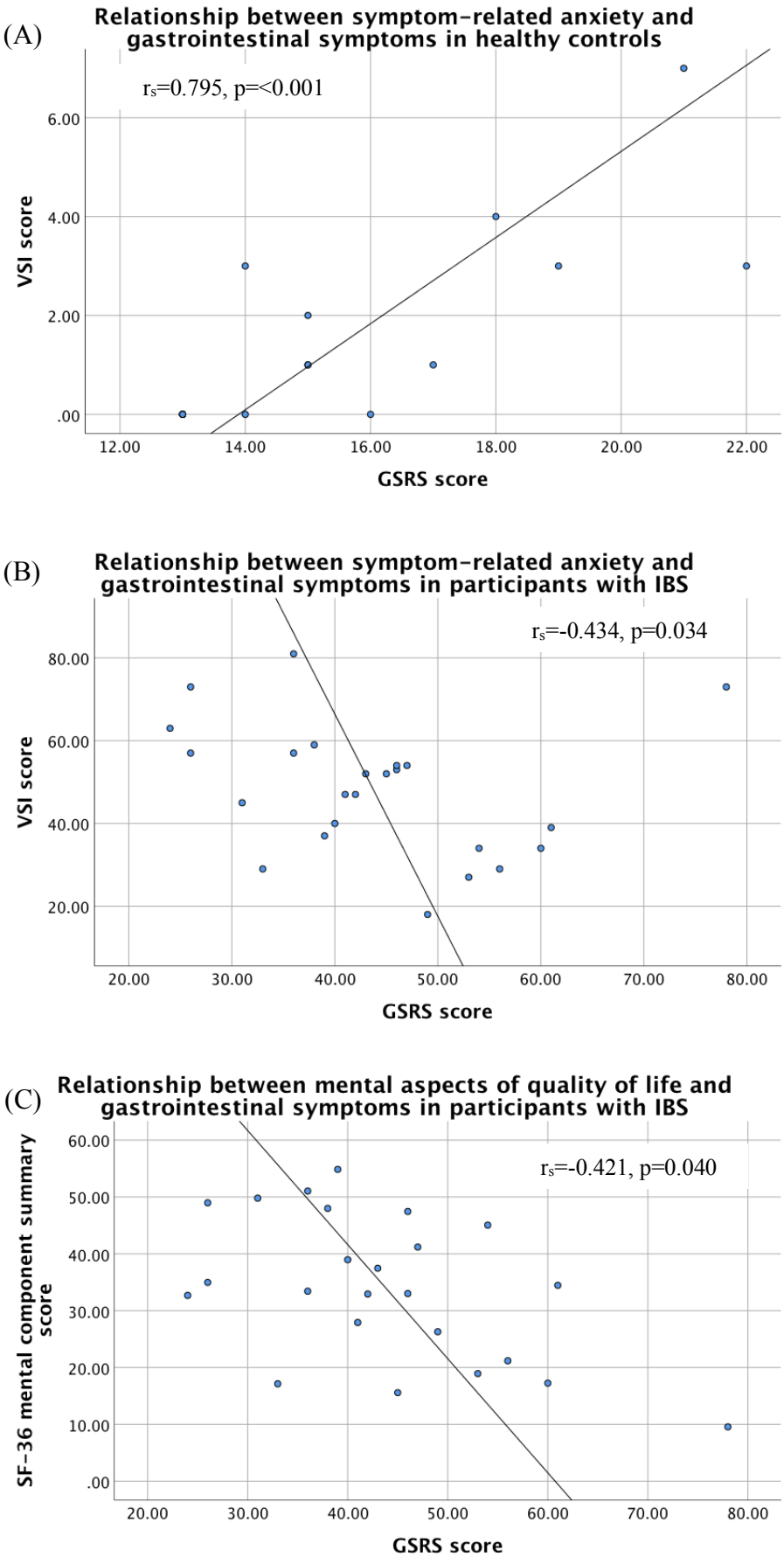


Figure H.5: Scatterplots with lines of best fit showing statistically significant correlations between gastrointestinal symptoms and anxiety/quality of life measures. They show how the gastrointestinal symptom rating scale (GSRS) score is related to (A) symptom related anxiety (visceral sensitivity index [VSI] score) in healthy controls; (B) symptom-related anxiety (VSI score) in participants with irritable bowel syndrome (IBS); and (C) mental aspects of quality of life (SF-36 mental component summary score) in participants with IBS. In healthy controls, a higher symptom score was associated with more symptom-related anxiety, while in participants with IBS the reverse was seen. In participants with IBS there was also an association between a higher symptom score and a lower quality of life score.

H-3.2.5 Effect of duration on behaviour

Within the group of IBS participants, the effect of self-reported duration since diagnosis on behavioural outcome measures (specifically the GSRS, BPI pain severity and BPI pain interference scores) was examined using Spearman correlation coefficients.

Duration was found to correlate negatively with pain interference scores ($r_s(22)$=-0.420, p=0.041), meaning that longer disease duration was associated with less pain interference (Figure H.6). None of the other outcome measures were found to correlate with duration.

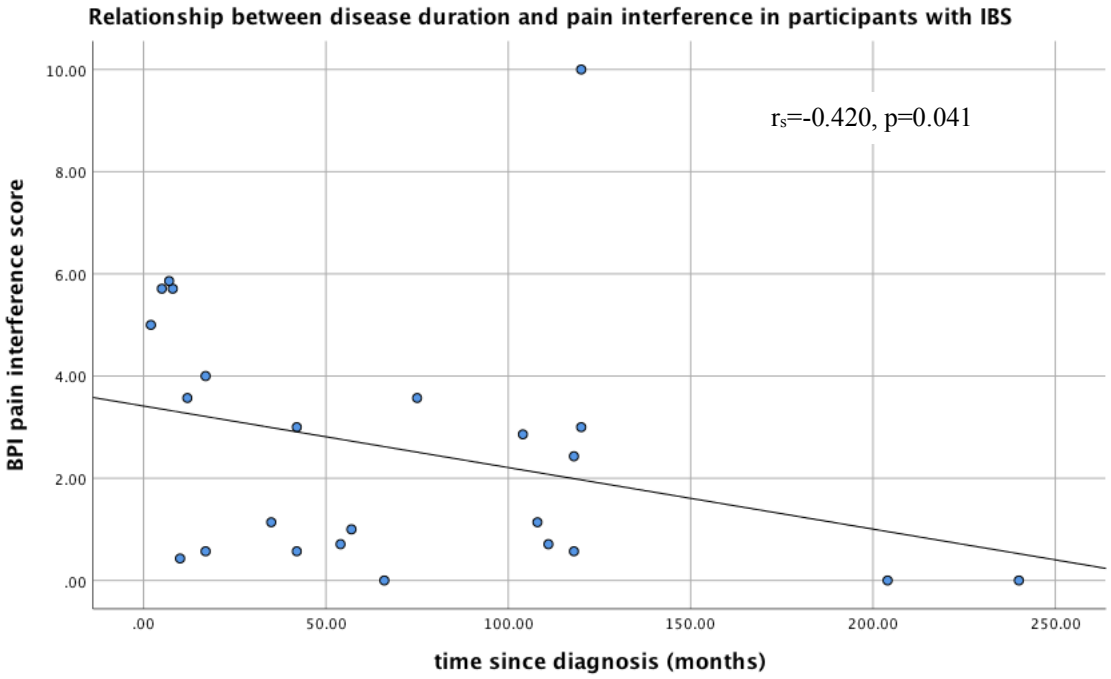


Figure H.6: Scatterplot showing the relationship between disease duration and pain interference in participants with irritable bowel syndrome (IBS; n=24). Patients with a longer disease duration reported that pain interfered less with their daily functioning.

H-3.2.6 Relationship between dysmenorrhoea and IBS-related pain

To determine whether differences in menstrual pain severity may have influenced pain scores in participants with IBS, maximum levels of menstrual pain were compared with BPI severity scores. A significant positive relationship was seen between maximum menstrual pain and BPI pain severity ($r_s(19)=0.477$, p=0.029; Figure H.7). An independent samples t-test was performed to compare the maximum menstrual pain between the group with IBS (mean=4.905, SD=2.468) and the healthy controls (mean=3.667, SD=2.795). The difference (1.238, BCa 95% CI [-0.352, 2.797]) was not significant, t(27.885)=1.375, p=0.180.

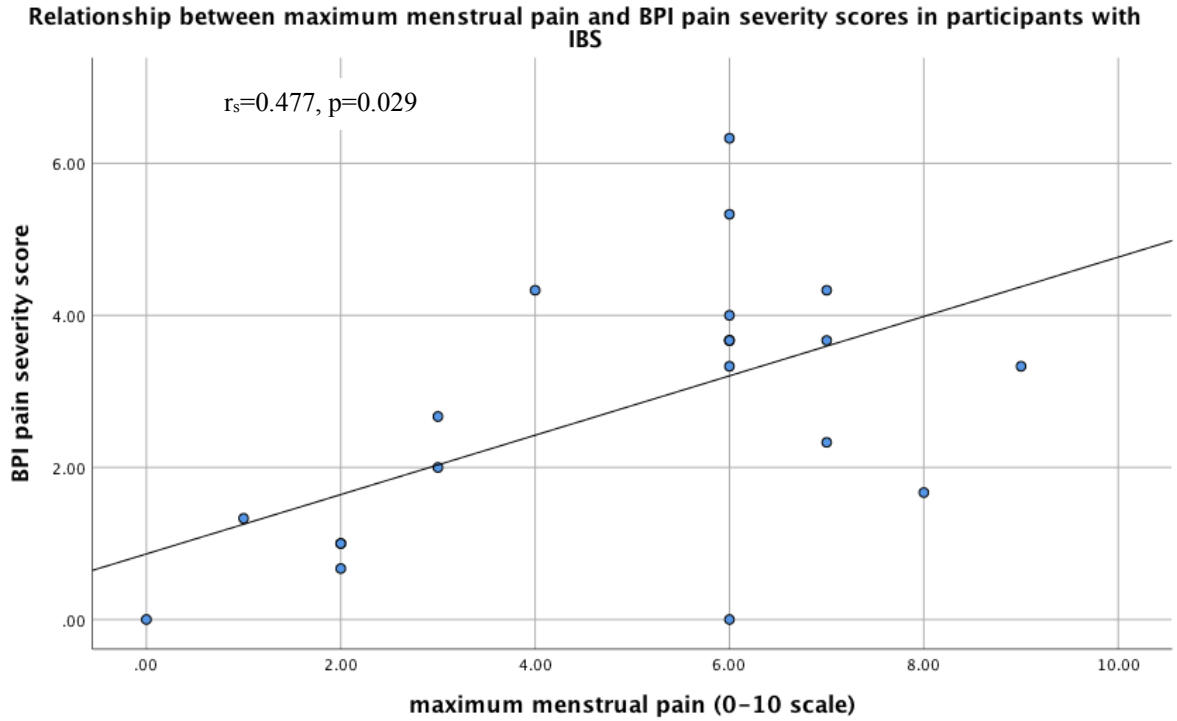


Figure H.7: Relationship between maximum period pain and brief pain inventory (BPI) severity scores in participants with irritable bowel syndrome (IBS; n=21). Increased severity of period pain was associated with an increased BPI pain severity score. The correlation was significant ($r_s(19)=0.477$, p=0.029).

H-3.3 Section 3: Sensory data from a noxious thermal stimulus

Neither the temperature nor the pain scores were significantly different between the group with IBS and the healthy control group (

Table H.6). Group results are illustrated in Figure H.8. There was no correlation between temperature and maximum menstrual pain ($r_s(19)=-0.153$, $p=0.509$), or temperature and duration since diagnosis ($r_s(22)=-0.232$, $p=0.276$). Nor was there any correlation between temperature and current background pain ($r_s(23)=-0.061$, $p=0.772$).

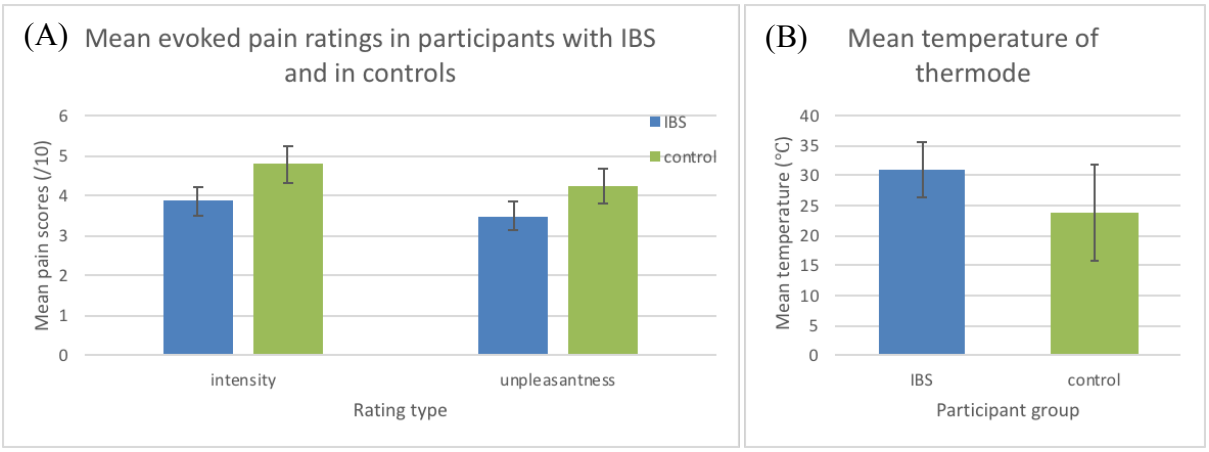


Figure H.8: Charts showing mean evoked pain ratings (A) and thermode temperatures (B) for patients with irritable bowel syndrome (IBS; n=25) and for healthy controls (temperature: n=16; pain ratings: n=14). Error bars show standard errors. The thermode temperature was perception locked to a pain intensity rating of 5/10. None of the differences between the two groups were statistically significant.

Outcome measure	N (IBS, control)	t	df	Mean difference	Std. error difference	P value (2-tailed)	BCa 95% confidence interval	
							lower	upper
Mean evoked pain intensity	25, 16	-1.554	26.306	-0.909	0.585	0.132	-2.117	0.306
Mean evoked pain unpleasantness	25, 14	-1.293	28.351	-0.766	0.592	0.206	-1.863	0.355
Mean temperature	25, 14	-1.743	34.004	-1.241	0.712	0.090	-2.813	0.172

Table H.6: Results of an independent samples t-test comparing mean group differences in evoked pain intensity, evoked pain unpleasantness and thermode temperature. There were no statistically significant differences between the irritable bowel syndrome (IBS) group and the control group.

H-3.4 Section 4: BOLD imaging during a noxious thermal stimulus

H-3.4.1 Evaluation of the evoked pain model

First, the IBS group and the control group were analysed separately to determine whether the experimental pain model was functioning well. Whole brain analysis was performed for each group to show areas in which the mean activation during a stimulus was significantly greater than baseline. Both groups showed strong activation of key pain regions during the evoked heat pain stimulus, demonstrating that the model was effective (Figure H.9).

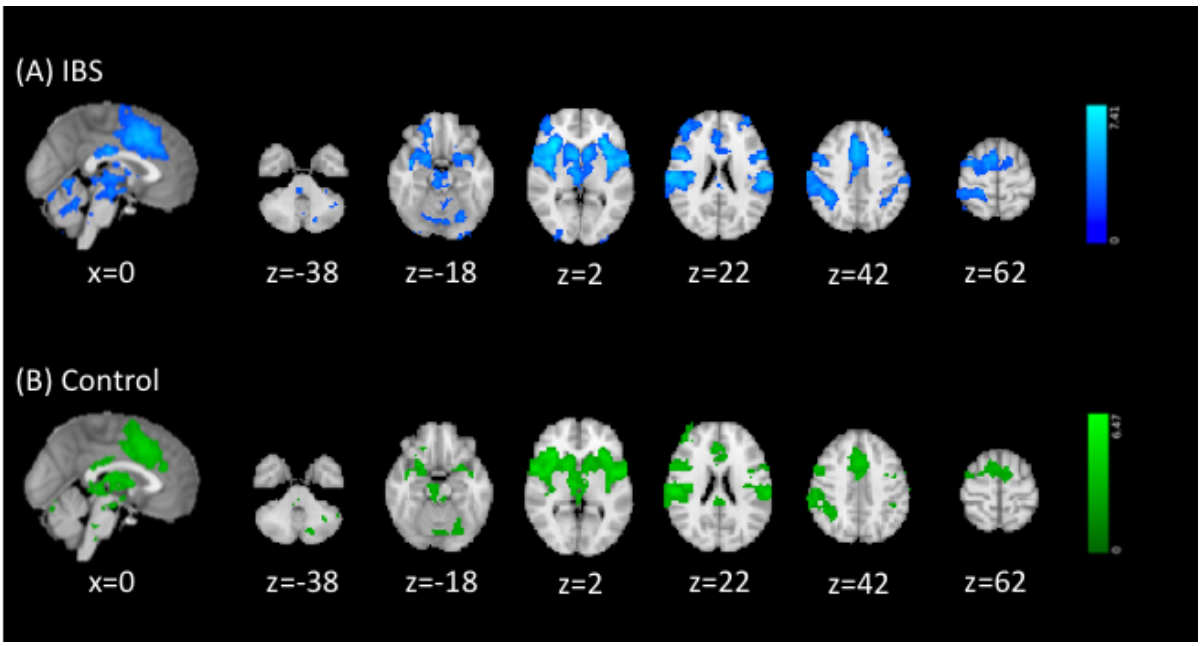


Figure H.9: Group mean whole brain activation during a noxious stimulus for (A) participants with irritable bowel syndrome (IBS; n=25) and (B) healthy controls (n=14). A mixed effects analysis was used (Z>2.3, p<0.05; cluster corrected). Slices are identified by MNI coordinates. Images are in radiological convention. Both groups showed strong activation of pain regions, indicating that the experimental pain model was effective.

H-3.4.2 Effect of evoked pain intensity on central pain processing

Evoked pain intensity was examined as a covariate of interest. For participants with IBS there was a positive correlation between evoked pain intensity and stimulus-induced activation in the bilateral primary somatosensory cortex (SI) and primary motor cortex (Figure H.10). In the control group there were no regions in which activity correlated with the perceived intensity of the noxious stimulus. Featquery was used to extract the mean % BOLD changes for those parts of SI that correlated with evoked pain intensity in the IBS group. This revealed that for the majority of participants, the apparent increase in SI activation with increased stimulus intensity was actually a reduction in stimulus-induced deactivation (Figure H.11).

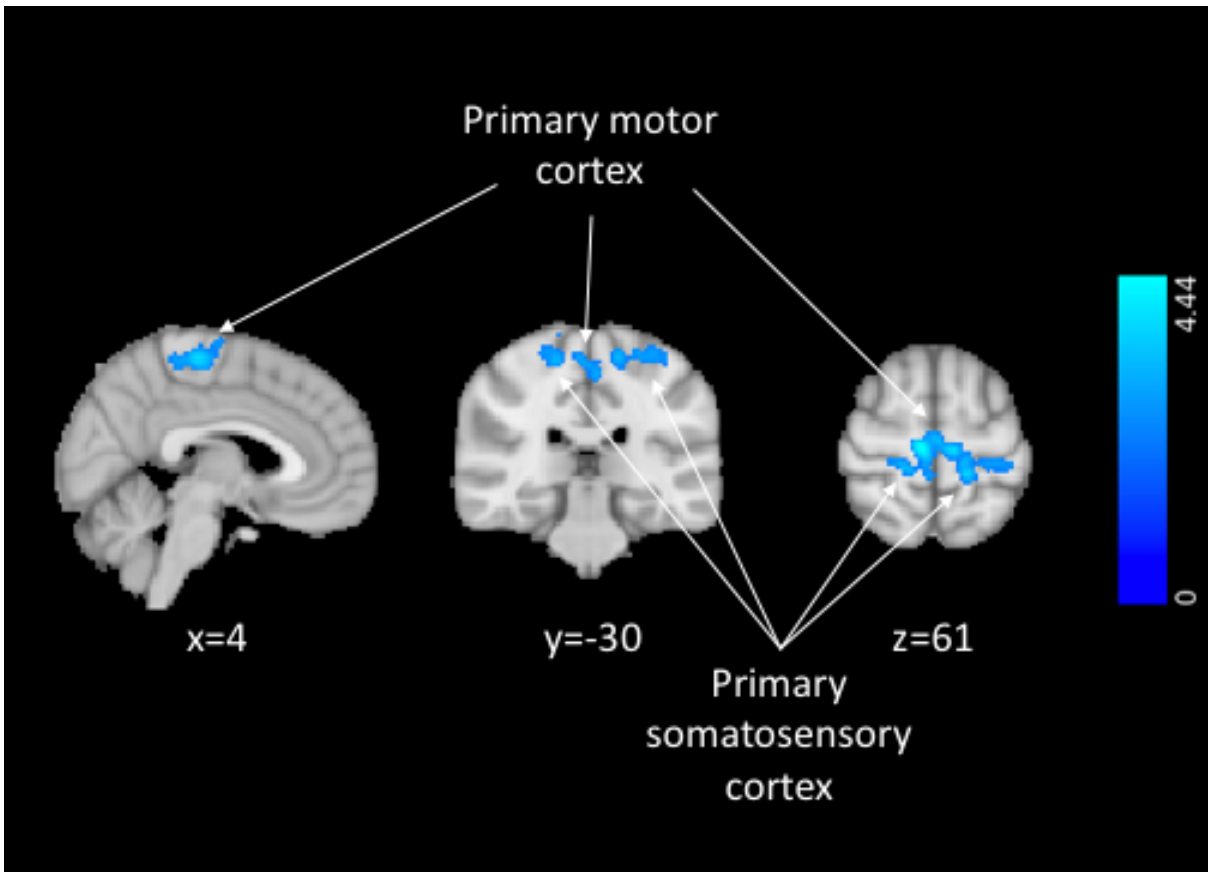


Figure H.10: Activation map showing regions in which pain-related BOLD activation showed a positive correlation with the intensity of the evoked pain in participants with irritable bowel syndrome (n=25; mixed effects; Z>2.3, p<0.05; cluster corrected). Slices are identified by MNI coordinates. Images are in radiological convention. Increased perceived stimulus intensity was associated with increased activation/decreased deactivation of the primary somatosensory and primary motor cortices.

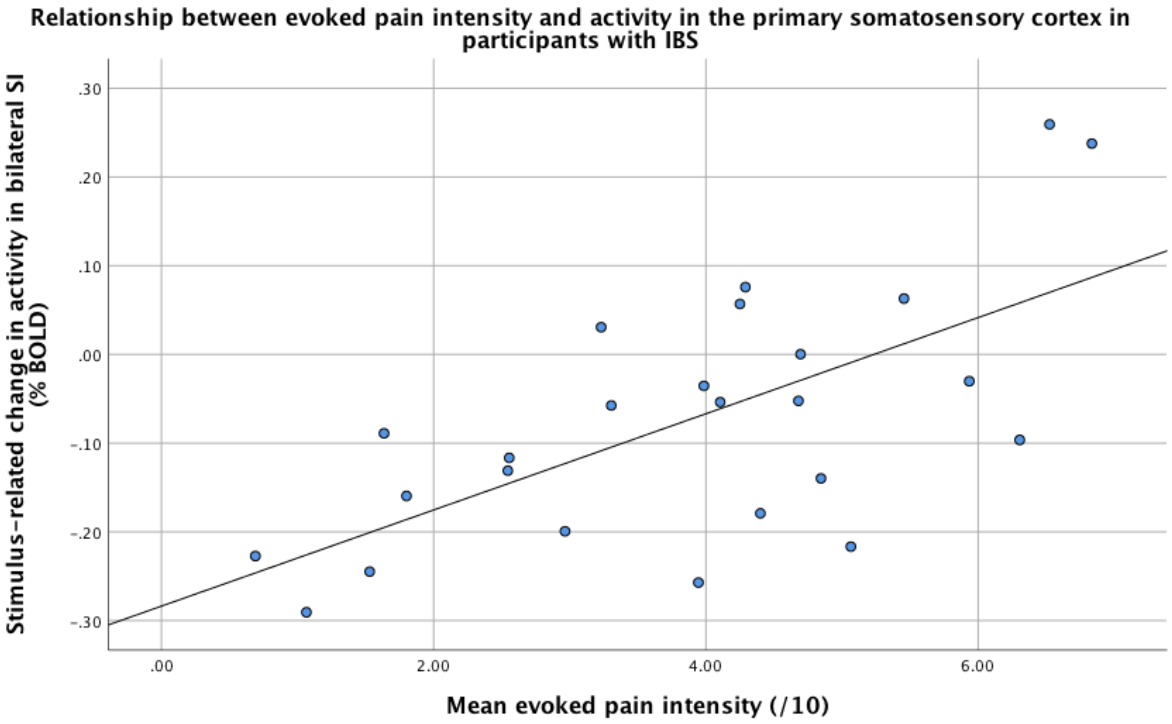


Figure H.11: Scatterplot with a line of best fit showing the relationship between the mean evoked pain intensity and the stimulus-induced change in BOLD activity in the primary somatosensory cortex (SI) for participants with irritable bowel syndrome (IBS; n=25). There was a correlation between increased pain intensity and increased SI activation/reduced SI deactivation.

H-3.4.3 Effect of background clinical pain on central pain processing in the IBS group

Participants in the IBS group reported a wide range of current background pain on the day of imaging (Figure H.12).

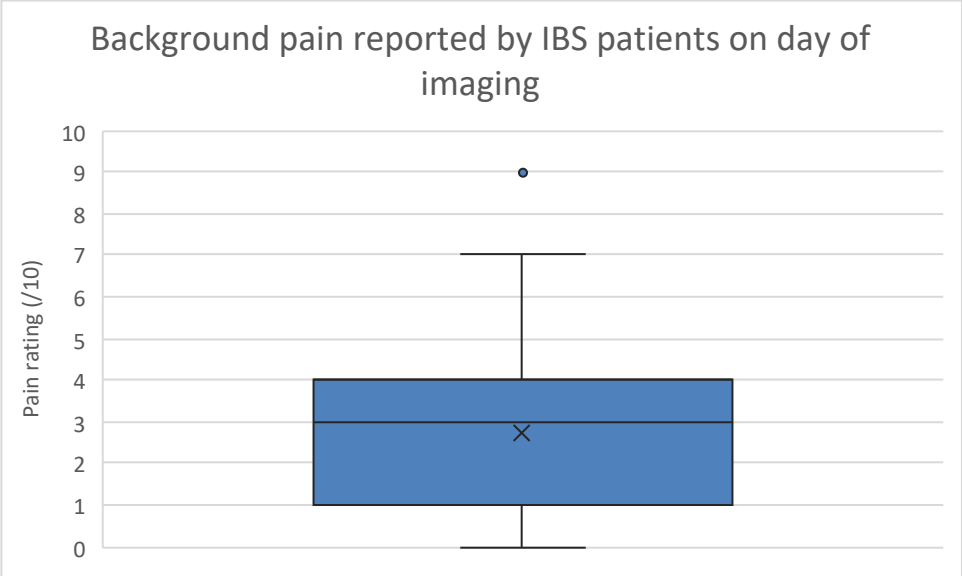


Figure H.12: Boxplot showing the current pain reported by irritable bowel syndrome (IBS) patients on the day of imaging (n=25). The group mean is denoted by a cross. There was widespread variation between participants.

When this was added as a covariate, a positive correlation was seen between background pain and activation in the precuneus, posterior cingulate cortex (PCC), bilateral angular gyri, bilateral lateral occipital cortex, bilateral supramarginal gyri, and multiple posterior temporal regions (bilateral middle and superior temporal gyri, right inferior temporal gyrus, left planum temporale). This is illustrated in Figure H.13.

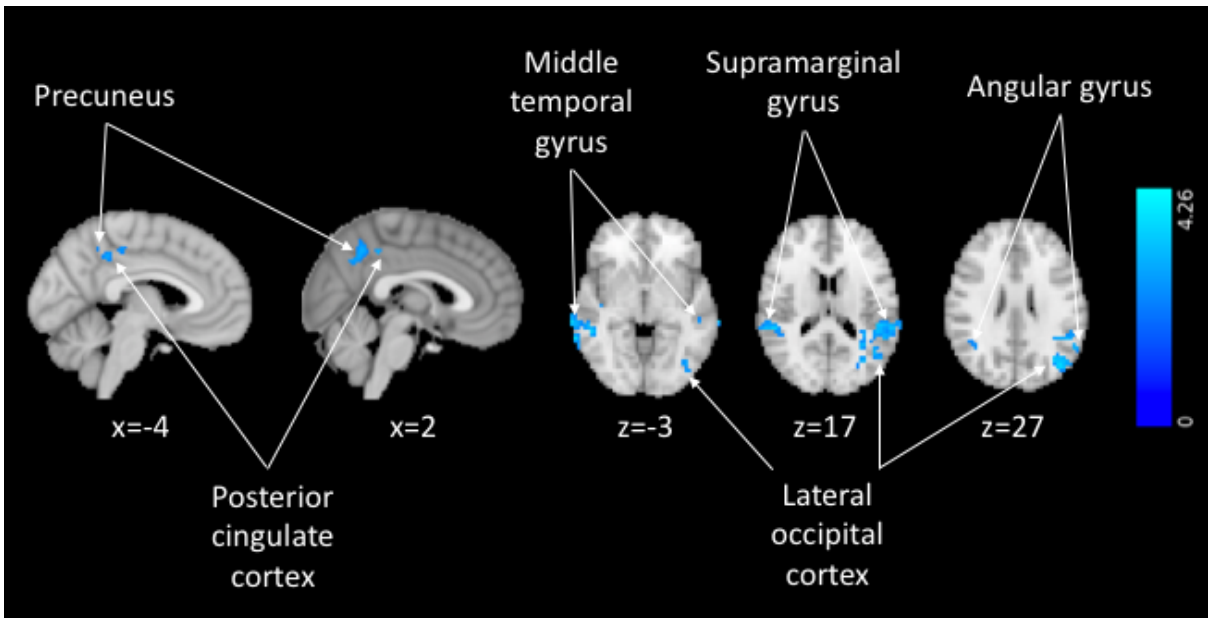


Figure H.13: Activation map showing regions in which pain-related BOLD activation showed a positive correlation with background pain in participants with irritable bowel syndrome (n=25; mixed effects; Z>2.3, p<0.05; cluster corrected). Slices are identified by MNI coordinates. Images are in radiological convention. Increased background pain was associated with increased activity in the precuneus, posterior cingulate cortex, angular gyri, lateral occipital cortices, supramarginal gyri and multiple posterior temporal regions.

Within the regions of the PCC and precuneus in which activation was correlated with background pain, mean BOLD activation during a stimulus was extracted using featquery. The fact that the values were predominantly negative shows that the activation seen was actually reduced deactivation. Therefore, there is a correlation between increased background pain and reduced deactivation in these regions (Figure H.14).

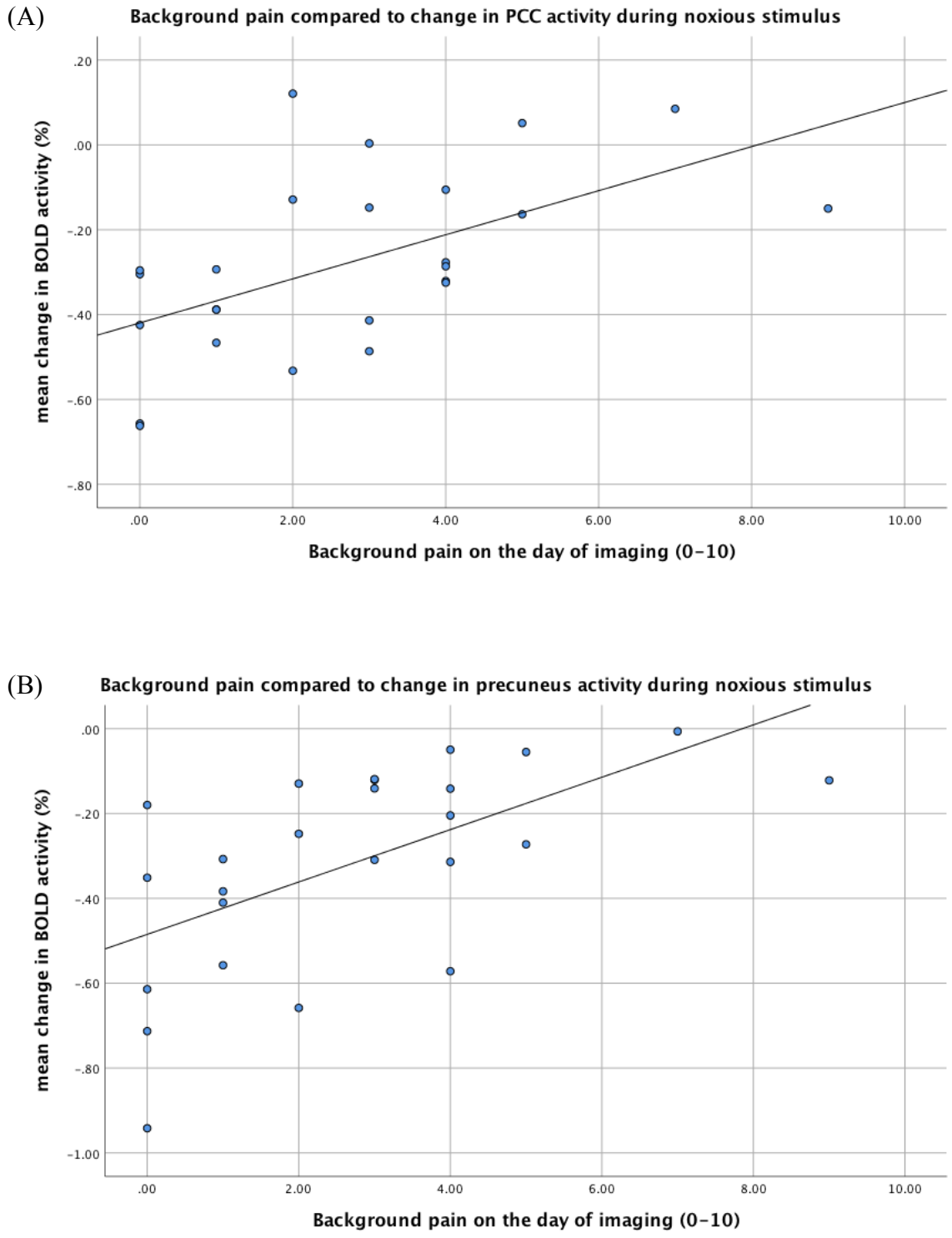


Figure H.14: Scatterplots showing the relationship between self-reported verbal pain ratings on the day of imaging and stimulus-induced BOLD activity in (A) the posterior cingulate cortex (PCC) and (B) the precuneus in participants with irritable bowel syndrome (n=25). A positive mean change in BOLD activity indicates activation, while a negative change indicates deactivation. Increased background pain was associated with reduced deactivation in both the PCC and the precuneus.

H-3.4.4 Effect of duration on central processing in the IBS group

Duration since diagnosis was negatively correlated with the amount of activation in the left lateral occipital cortex during a noxious stimulus. However, correction for differences in evoked pain intensity and current background pain revealed a negative correlation between duration since diagnosis and neural activity in the right pallidum and all segments of the right insula during a noxious stimulus (Figure H.15).

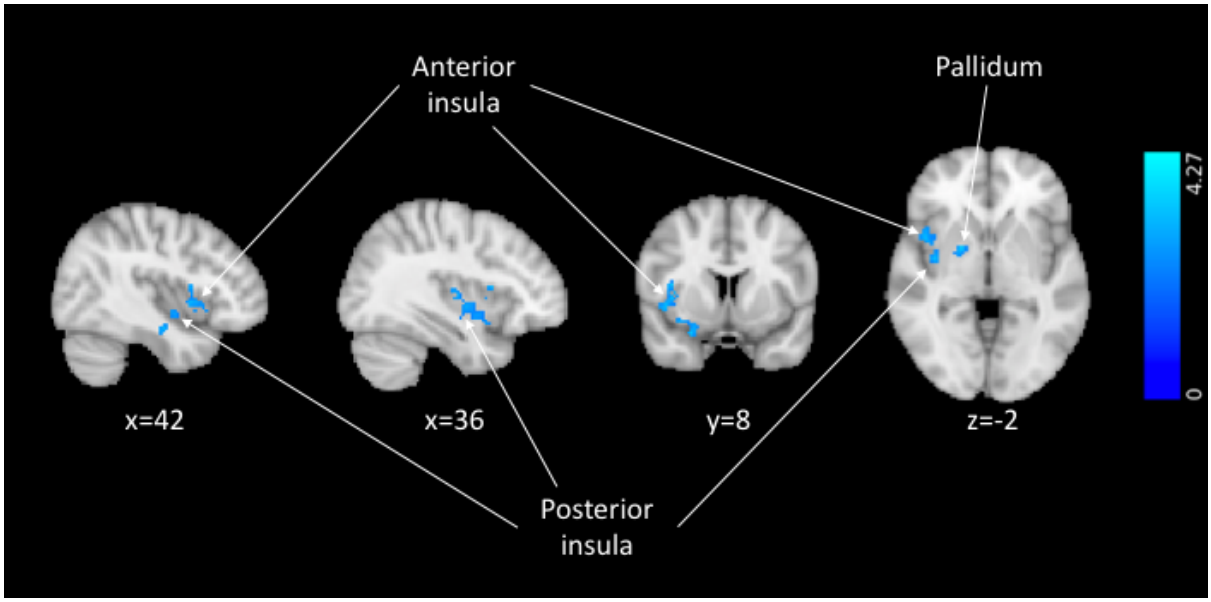


Figure H.15: Activation map showing regions in which pain-related BOLD activation showed a negative correlation with disease duration in participants with irritable bowel syndrome when differences in stimulus intensity and background pain were corrected for (n=24; mixed effects; Z>2.3, p<0.05; cluster corrected). Slices are identified by MNI coordinates. Images are in radiological convention. Increased symptom duration was associated with reduced evoked activity in the right insula and pallidum.

H-3.4.5 Central responses to a noxious stimulus in participants with IBS compared to control participants

An independent samples t-test comparing whole brain BOLD responses to a noxious forearm stimulus in the IBS group with those in the control group revealed no significant group differences. However, when corrected for individual differences in current background pain and intensity of the evoked stimulus, the control group showed greater neural activity in the precuneus, PCC and bilateral thalamus (Figure H.16).

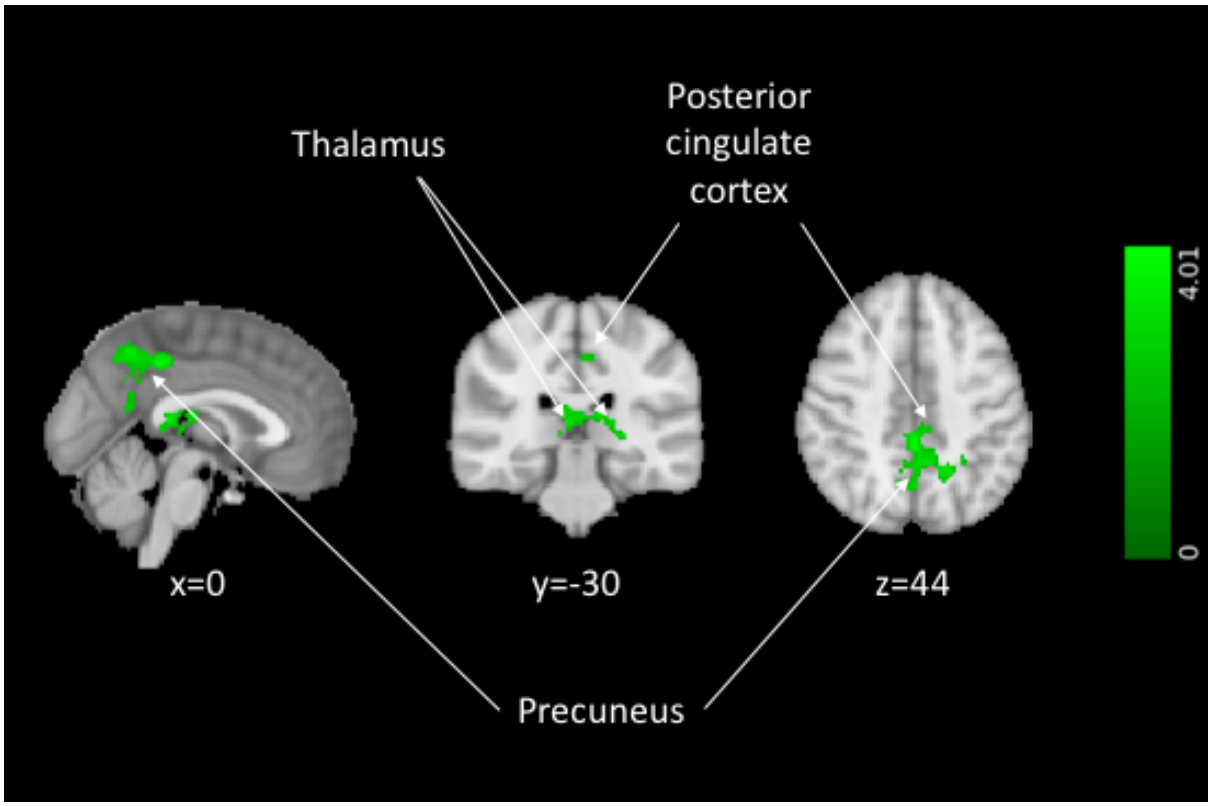


Figure H.16: Activation map showing regions in which evoked pain-related activity was greater in the control group (n=14) than the irritable bowel syndrome group (n=25) when controlling for differences in current background pain and perceived intensity of the painful stimulus. This was an independent samples t-test (mixed effects; Z>2.3, p<0.05; cluster corrected). Slices are identified by MNI coordinates. In the control group there was more activity in the precuneus, posterior cingulate cortex and thalamus.

Featquery was used to extract the mean % BOLD changes for those parts of the above regions that showed significantly different activation between groups. In the precuneus and PCC, BOLD changes were predominantly negative, indicating deactivation during the stimulus. The IBS group had greater deactivation than the controls.

In the bilateral thalamus, BOLD changes were predominantly positive, meaning that the IBS group showed less activation than the control group in this region.

H-4 Discussion

H-4.1 Symptoms, psychology and quality of life in IBS

As expected, participants with IBS experienced vastly more gastrointestinal symptoms than healthy controls. The presence of positive GSRS scores in the control group shows that healthy people do experience gastrointestinal symptoms but to a lesser extent.

The SF-36 subscale scores show that pain is a big issue for the IBS patients in this study, but also that there is impaired quality of life across all aspects of life, both physical and mental. The MCS score is affected to a greater degree than the PCS score, and higher MCS scores are associated with greater symptom scores in IBS, indicating that mental aspects of quality of life are affected the most. The HADS and VSI scores in this population certainly support the close relationship between physical and psychological symptoms, indicating increased symptoms of anxiety and depression in the participants with IBS compared to healthy controls. However, the lack of correlation between GSRS scores and HADS scores in either group, and the

differences in pattern of correlation between GSRS and VSI in the two groups, imply that this is not a simple linear relationship.

In a cross-sectional observational study such as described in this chapter, it is impossible to determine whether the physical or psychological symptoms were causal, or whether there is another factor which predisposed to both. To unpick this further would require a long-term prospective cohort.

H-4.2 The effect of IBS on somatic sensory function

In this study there was no evidence of increased somatic sensitivity to evoked pain in the IBS group compared to controls. Nor was there evidence of hypersensitive and normosensitive subgroups, since perception locked temperatures and pain scores in the IBS group were not bimodally distributed.

As mentioned in chapter 6, there is heterogeneity in the degree of hyperalgesia seen in functional gastrointestinal disorders (see section 6.1.2). This, combined with a small sample size may be the reason for the lack of statistically significant hypersensitivity seen in this study. Amongst those who do have somatic hypersensitivity, some studies have reported a greater effect in the feet than the hands (Verne et al., 2001; Q. Q. Zhou et al., 2010). Although not a universal finding, it is a possible additional factor in the lack of hypersensitivity seen in this study, which looked at somatic sensation in the forearm.

The lack of thermal hyperalgesia seen in these participants means that it is not possible to conclude that central sensitization is an important factor in their disease from the psychophysical data alone.

H-4.3 Evaluating the link between dysmenorrhea and pain or sensory function in IBS

A possible weak linear relationship was seen between severity of period pain and BPI pain severity scores. This raises the possibility that dysmenorrhoea may be a confounding factor in the study. The fact that there was no group difference in the maximum period pain reported by individual women suggests that this should not have had a big impact on group comparisons between those with IBS and healthy controls.

There is evidence that dysmenorrhoea is associated with somatic and visceral hypersensitivity (Iacovides, Avidon, & Baker, 2015; Payne, Rapkin, Seidman, Zeltzer, & Tsao, 2017), and there was wide between-subject variability in reported maximum menstrual pain intensity. However, the lack of correlation between maximum menstrual pain and temperature in the patients with IBS indicates that this is unlikely to have been a big confound.

Primary dysmenorrhea is now considered to be a chronic pain condition. However, many of the participants in this study did not report their menstrual pain when asked about medical conditions that give them pain. This is a limitation of the study, and future studies should exclude participants who experience moderate or severe cyclical pain.

H-4.4 Neuroimaging and factors that may confound it

This section will consider the brain imaging data to determine whether the symptoms of individuals with IBS are characterised by differences in their central representation of pain, and to look for factors that may confound brain imaging results in chronic pain patients. In patients with IBS, the level of perceived noxious input was correlated with stimulus-induced activity in sensorimotor regions, while the level of background pain was correlated with stimulus-induced activity in regions associated with the DMN. Controlling for these factors revealed that compared to healthy controls, patients with IBS had more stimulus-related deactivation in regions associated with the DMN, and more stimulus-related activation in the thalamus.

H-4.4.1 Imaging in participants with varying levels of perceived noxious input

Despite perception locking all participants to the same perceived pain intensity prior to testing, there was considerable heterogeneity in perceived pain intensity during testing of both IBS patients and healthy controls. In the IBS group, increased perceived noxious input was associated with reduced deactivation in the sensorimotor cortex. This was not true of the controls, for whom there was no correlation between perceived intensity and stimulus-induced activation. This indicates that IBS patients track acute pain in a different way to controls.

Since perceived pain intensity had clear correlates on imaging in IBS patients, it is important to consider evoked intensity as a possible confounding factor for brain imaging analyses in this group of patients. This is also likely to apply to other chronic pain conditions.

H-4.4.2 Imaging in patients with varying levels of background pain

IBS is a disease that produces episodic symptoms. Performing neuroimaging studies in these circumstances can be challenging due to fact that neural responses are also likely to vary according to the concurrent pain levels. In this group of patients, background pain levels prior to the MRI scan varied from 0/10 to 9/10. Increasing background pain on the day of imaging was associated with reduced deactivation in multiple components of the DMN during a noxious stimulus. This indicates that the processing of evoked pain may differ according to the background pain that is present.

One explanation for the correlation between background pain and reduced DMN deactivation during a noxious stimulus would be that the presence of existing background pain makes the additional stimulus less salient. Deactivation of the DMN may already have taken place due to the salience of the background pain, to which participants are already attending, meaning that less additional deactivation occurs.

Given the high level of heterogeneity in background pain reported, and the fact that background pain had clear stimulus-induced neural correlates, it is important to consider background pain as a possible confounding factor for brain imaging analyses in this group of patients as well as other chronic pain conditions.

H-4.4.3 Differences in central processing of noxious stimuli between IBS patients and healthy controls

Participants with IBS had greater deactivation of the PCC and precuneus, components of the DMN, than healthy controls during a noxious stimulus. This might seem contrary to the above finding of an association between reduced deactivation and increased

background pain in the IBS group. However, individuals with IBS display hypervigilance to gastrointestinal symptoms and pain (S. Chapman & Martin, 2011; Posserud et al., 2009). It would not be surprising that as a group, participants with IBS find a noxious stimulus more salient and attention-grabbing than the general population, even taking into account the fact that this effect may have been reduced slightly by concurrent background pain. The greater DMN deactivation may reflect this.

The thalamus was activated less in participants with IBS than in healthy controls during a noxious stimulus. This is in keeping with the fact that thalamic blood flow has often been shown to be reduced in chronic pain (Hsieh, Belfrage, Stone-Elander, Hansson, & Ingvar, 1995; Mountz et al., 1995; Tracey & Mantyh, 2007). Due to the fact that the thalamus acts as a bidirectional relay between the cortex and subcortical regions for multiple sensory modalities, and its function is therefore complex, it is difficult to interpret the precise mechanisms that underlie this difference. However, it is very likely to reflect differences in sensory processing between the two groups.

H-4.5 The effect of differences in disease duration on behaviour and imaging

The natural history of IBS is variable. While up to 50% of patients report that their symptoms remain stable over time, the remaining patients experience either deterioration or improvement (El-Serag, Pilgrim, & Schoenfeld, 2004).

In this study there was a weak negative correlation between disease duration and pain interference score, but no relationship between duration and pain severity score. This suggests that those who have had IBS for longer may find that their pain impacts less

on their everyday functioning. It is plausible that over time, patients find that they are able to adapt to their symptoms and find ways of coping with them better.

The imaging shows a clear negative correlation between the time since diagnosis and neural activity in response to a noxious stimulus. Increased duration was associated with reduced activity in the anterior and posterior right insula, as well as in the right pallidum.

The literature indicates that a link between duration and insula function is plausible. The insula cortex is an area that is commonly reported to have altered morphology in IBS patients, and this appears to be related to the duration of symptoms. Piché et al. compared women with IBS to healthy controls and found that those with IBS had a thicker right posterior insula cortex, and that thickness was positively correlated with IBS duration but not with severity (Piché et al., 2013). In contrast, Davis and colleagues reported thinning of the bilateral anterior insula cortex in IBS (K. D. Davis et al., 2008). Another study from the same group found that anterior insula cortical thickness increased with pain duration, but that patients with IBS started off with a thinner insula cortex than healthy controls, and that the thickness returned to normal after around 15 years of symptoms (Blankstein et al., 2010). The variation in findings may reflect differences in the symptomatology of the IBS population sampled in each study, especially since all of these studies used small samples. However, there is agreement that the morphology of the insula cortex is abnormal in IBS, and that it changes as a function of disease duration. Although differences in morphology do not prove functional differences, there is good evidence that insula function is altered in IBS compared to healthy controls. This is illustrated by studies showing differences in

pain-evoked activity as well as resting functional connectivity (Hong et al., 2014; R. Qi et al., 2016; Tillisch et al., 2011). None of these have assessed the impact of disease duration. However, a spectroscopy study found evidence of dysfunctional excitatory neurotransmission in the anterior insulae of women with IBS, where there were reduced concentrations of glutamine compared to that seen in healthy controls. In the right anterior insula, duration of abdominal pain predicted the magnitude of this effect (Bednarska et al., 2019). This supports the idea that the function of the right anterior insula may be impacted by the duration of disease.

The posterior insula is involved in sensory processing, and the fact that it is affected by symptom duration in this cohort indicates that sensory perception may be affected by chronicity of disease. The anterior insula is an important region for interoceptive attention and emotional processing (Uddin et al., 2017; X. Wang et al., 2019), and it is plausible that over time, the salience and emotional impact of symptoms may change. This is in keeping with the fact that those with a longer duration of symptoms found that pain interfered less with their daily functioning.

The globus pallidus, in conjunction with other components of the basal ganglia, is known to be involved in nociception (Braz, Nassar, Wood, & Basbaum, 2005). It receives projections from multiple cortical regions as well as nociceptive afferents from the spinal cord, making it well placed to influence pain processing. In spite of this there is minimal literature on its role in IBS. Patients with IBS have been shown to have microstructural changes in the globus pallidus compared to healthy controls, as well as showing reduced connectivity between the globus pallidus and thalamus (Ellingson et al., 2013). However, the effect of duration on such changes is not known.

A major limitation of this analysis is that participants were asked for the duration since their diagnosis rather than since the onset of their symptoms. The Rome III diagnostic criteria for IBS require that the onset of symptoms occurs at least 6 months prior to diagnosis. This means that by the time IBS is diagnosed, all patients will already meet chronic pain criteria (Shih & Kwan, 2007). Some patients experience symptoms for much longer prior to being diagnosed with IBS, either due to a failure of healthcare providers to recognise the pattern of symptoms, or due to delayed presentation to the healthcare system (Pimental, 2018). Even after receiving a diagnosis of IBS, patients often continue to have symptoms for many years. Future studies that assess disease duration should consider recording the timing of symptom onset rather than the stage at which those symptoms received the label of IBS.

H-5 Summary

Individuals with IBS experience more gastrointestinal symptoms, more symptoms of anxiety and depression, and reduced quality of life compared to healthy controls. Neural responses to experimental pain reveal differences in central pain processing between the two groups.

Investigating pain in patients with episodic symptoms can be challenging. Background pain level and evoked pain intensity correlated with stimulus-induced activity in important pain-related brain regions in this study, and correcting for them revealed differences between patients and controls that would not otherwise have been identified. These factors should be considered as potential confounds when imaging chronic pain patients. Duration of disease may make a difference to the central representation of pain and should be considered in between-subject designs.

Appendix I: Inclusion/exclusion criteria to accompany chapters 6 and 7 and appendix H

I-1.1 Study A: A case-controlled study to determine the functional neural correlates of chronic pain in patients with irritable bowel syndrome.

Data from this study were used in appendix H only.

I-1.1.1 Inclusion Criteria

I-1.1.1.1 Group 1: Participants with IBS

- Diagnosed with IBS according to Rome III criteria
- Judged to be in good health barring the diagnosis of IBS, based on their self-reported medical history and the opinion of the clinical team responsible for their recruitment.

I-1.1.1.2 Group 2: Healthy volunteers

- Judged to be in good health based on their self-reported medical history

I-1.1.1.3 Both groups:

- Participant is willing and able to give informed consent for participation in the study.
- Male or female, aged 18 to 70.
- Fluent in English

I-1.1.2 Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

I-1.1.2.1 Group 1: Irritable bowel syndrome

- The participant has a history of or current chronic pain condition other than IBS, or a current acute pain condition
- The participant has another current gastrointestinal disease
- The participant is taking regular medications other than those prescribed for IBS

I-1.1.2.2 Group 2: healthy volunteers

- The participant has a history of or current chronic pain condition, or current acute pain condition
- The participant has a current gastrointestinal disease
- The participant is taking regular medications other than the oral contraceptive pill.

I-1.1.2.3 Both groups:

- The participant has a personal history of a clinically significant psychiatric disorder (including severe affective disorder, psychotic tendencies and drug-induced psychoses). Subjects who have had situational depression or anxiety may be enrolled at the discretion of the investigator.
- The participant has a history of stroke, chronic seizures, or major neurological disorder.

- The participant has any of the commonly accepted contraindications to MRI scanning, for example, severe claustrophobia, presence of certain metallic implants, a pacemaker etc.
- The participant is pregnant. The risk to foetus of radiofrequency energy of the MRI scan is unknown.
- The participant does not have adequate understanding of verbal and written information in English, sufficient to complete an MRI safety screening.

I-1.2 Study B: Mindfulness for Irritable Bowel Syndrome.

Data from this study were used in chapters 6 and 7 and appendix H.

I-1.2.1 Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study.
- Female, aged 18-65 years.
- Diagnosed with IBS by Gastroenterologist or GP, meeting Rome III criteria for IBS, symptom onset at least 6 months prior to inclusion, discomfort or pain at least 2-3 days per month
- Participants speaks English fluently or at a native level
- Participant has normal or corrected to normal vision

I-1.2.2 Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- Participant has insufficient manual dexterity for the computerized tasks
- Severe DSM Axis I or II psychiatric disorder (diagnosis of dementia, amnesia, delirium, a dissociative disorder, a personality disorder, schizophrenia or any other psychotic disorder, a substance-related or induced disorder (such as alcoholism), ADHD, ADD, primary diagnosis of OCD, current suicidality, regular non-suicidal self-injury.
- Recent bereavement (within one year)
- Gastrointestinal diagnosis other than IBS (IBD, coeliac disease, another FGID)
- Pregnancy
- No access to the internet
- Prior participation in an 8–week mindfulness course (MBSR, MBCT) or any other structured mindfulness training. Prior participation in Vipassana meditation courses. Regular meditation practice (meditating more than once per month).
- Not able or unwilling to commit to amount of practice (sessions and home practice).
- Participant is currently enrolled in another IBS treatment study (psychological or pharmacological).
- For those participants completing the imaging component: Participant has any of the commonly accepted contraindications to MRI scanning, for example, severe claustrophobia, presence of certain metallic implants, a pacemaker etc.

- For those participants completing the imaging component: Participant not willing to allow contact with GP in the event of clinically relevant incidental findings

Appendix J: Results to accompany chapters 6 and 7.

J-1 Table showing the results of paired t-tests comparing symptoms of irritable bowel syndrome before and after MBCT-IBS.

Outcome measure		N	t	df	Mean difference	Std. error difference	P value (2-tailed)	BCa 95% confidence interval	
								lower	upper
GSRS		14	-2.557	13	-7.357	2.878	0.024	-12.427	-2.000
BPI severity		15	-1.675	14	-0.560	0.334	0.116	-1.217	0.031
BPI interference	Total	15	-2.596	14	-0.733	0.282	0.021	-1.227	-0.233
	Activity subscale	15	-1.537	14	-0.467	0.304	0.147	-1.017	0.083
	Affective subscale	15	-3.539	14	-1.088	0.308	0.003	-1.689	-0.511

Table J.1: Effect of MBCT-IBS on symptoms in irritable bowel syndrome. Table shows results of paired 2-tailed t-tests comparing results before and after MBCT-IBS for the total gastrointestinal symptom rating scale (GSRS) score and the brief pain inventory (BPI) pain severity and pain interference scores. A negative mean difference indicates a reduction in score, which equates to an improvement in symptoms. Differences were considered significant if $p \leq 0.05$, and p values reaching this criterion are shown in red. Total GSRS score and total BPI interference score were statistically significantly reduced following the intervention. When the pain interference subscales were analysed independently, the affective subscale was significantly reduced while the activity subscale was not.

J-2 Table showing the results of paired t-tests comparing measures of anxiety and depression before and after MBCT-IBS.

Outcome measure	N (IBS, control)	t	df	Mean difference	Std. error difference	P value (2-tailed)	BCa 95% confidence interval	
							lower	upper
VSI	14	-2.738	13	-9.000	3.287	0.017	-15.694	-3.286
HADS-A	15	-2.794	14	-1.867	0.668	0.014	-3.333	-0.400
HADS-D	15	-0.937	14	-0.867	0.925	0.365	-2.333	0.933
PCS	14	-2.993	13	-8.500	2.840	0.010	-15.151	-1.799

Table J.2: Effect of MBCT-IBS on anxiety and depression in irritable bowel syndrome (IBS). Table shows results of paired 2-tailed t-tests comparing results before and after MBCT-IBS for the visceral sensitivity index (VSI), hospital anxiety and depression scale (HADS) and total Pain Catastrophizing Scale scores. A negative mean difference indicates a reduction in score, which equates to an improvement in anxiety/depression. Differences were considered significant if $p \leq 0.05$, and p values reaching this criterion are shown in red. There was a statistically significant reduction in HADS-anxiety (HADS-A), VSI and pain catastrophizing following the intervention.

J-3 Table showing results of correlations between change in symptoms and change in anxiety following MBCT-IBS.

		Change in HADS anxiety score	Change in VSI
Change in BPI pain severity score	r_s	0.552	0.597
	Significance (2-tailed)	0.033	0.024
	N	15	14
Change in BPI pain interference score	r_s	0.141	-0.004
	Significance (2-tailed)	0.615	0.988
	N	15	14
Change in GSRS	r_s	0.387	-0.337
	Significance (2-tailed)	0.172	0.455
	N	14	13

Table J.3: Table showing results of Spearman correlations between changes in symptoms (gastrointestinal symptom ratings scale [GSRS] and brief pain inventory [BPI]) and changes in anxiety (hospital anxiety and depression scale [HADS] anxiety subscale and visceral sensitivity index [VSI]) after MBCT-IBS. Correlations were considered statistically significant if $p \leq 0.05$, and p values reaching this criterion are shown in red. There was a positive correlation between the change in pain severity and change in both anxiety scores, meaning that reduced pain severity was associated with reduced anxiety.

J-4 Table showing the results of paired t-tests comparing quality of life before and after MBCT-IBS.

Outcome measure	N (IBS, control)	t	df	Mean difference	Std. error difference	P value (2-tailed)	BCa 95% confidence interval	
							lower	upper
IBS-IS	15	2.417	14	14.267	5.902	0.030	4.755	24.379
SF-36 PCS	15	1.367	14	1.762	1.289	0.193	-0.702	4.071
SF-36 MCS	15	2.595	14	7.003	2.699	0.021	2.053	12.031

Table J.4: Effect of MBCT-IBS on quality of life in irritable bowel syndrome (IBS). Table shows results of paired 2-tailed t-tests comparing results before and after MBCT-IBS for the total IBS impact scale (IBS-IS) score and the SF-36 physical component summary (PCS) and mental component summary (MCS). A positive mean difference indicates an increase in score, which equates to an improvement in quality of life. Differences were considered significant if $p \leq 0.05$, and p values reaching this criterion are shown in red. There was a statistically significant increase in IBS-IS and SF-36 MCS following the intervention.

J-5 Table showing the results of paired t-tests comparing thermode temperature and pain ratings before and after MBCT-IBS.

Outcome measure	N	t	df	Mean difference	Std. error difference	P value (2-tailed)	BCa 95% confidence interval	
							lower	upper
Temperature	14	4.757	13	1.214	0.255	<0.001	0.857	1.607
Evoked pain intensity	14	0.086	13	0.034	0.393	0.933	-0.864	1.039
Evoked pain unpleasantness	14	0.634	13	0.226	0.357	0.537	-0.524	1.080

Table J.5 Effect of MBCT-IBS on somatic sensory testing in irritable bowel syndrome. Table shows results of paired 2-tailed t-tests comparing results before and after MBCT-IBS for perception locked temperature and for thermal pain intensity and unpleasantness. Differences were considered significant if $p \leq 0.05$, and p values reaching this criterion are shown in red. There was no change in thermal pain ratings, demonstrating that perception locking was consistent between visits. There was a statistically significant increase in temperature following the intervention, indicating reduced heat pain sensitivity.

J-6 Table showing the effect of trait motivational style on the change in symptoms following MBCT-IBS

		Change in GSRS score	Change in BPI severity score	Change in BPI interference score
BAS drive score at baseline	r_s	-0.644	-0.176	-0.281
	Significance (2-tailed)	0.013	0.530	0.310
	N	14	15	15
BAS reward responsiveness score at baseline	r_s	-0.494	-0.361	-0.486
	Significance (2-tailed)	0.073	0.187	0.066
	N	14	15	15
BAS fun-seeking score at baseline	r_s	-0.561	-0.581	0.024
	Significance (2-tailed)	0.037	0.023	0.933
	N	14	15	15
BIS score at baseline	r_s	-0.046	0.107	-0.395
	Significance (2-tailed)	0.876	0.704	0.145
	N	14	15	15

Table J.6: Impact of motivational style on efficacy of MBCT-IBS in IBS. Table shows results of Spearman correlations between behavioural inhibition system/behavioural activation system (BIS/BAS) scores at baseline and the change in symptoms following MBCT-IBS (gastrointestinal symptom rating scale [GSRS] and brief pain inventory [BPI]). Correlations were considered statistically significant if $p \leq 0.05$, and p values reaching this criterion are shown in red. For all three BAS subscales, there was a significant correlation between baseline BAS scores and change in GSRS scores, meaning individuals with higher BAS scores experienced a greater reduction in symptoms. The baseline BAS fun-seeking score also correlated with the change in BPI pain severity, indicating that those with higher trait motivation to seek out new rewards experienced a greater reduction in pain severity.

J-7 Table showing the effect of trait motivational style on the change in left dlPFC activity in response to thermal pain following MBCT-IBS.

	BAS drive score at baseline	BAS reward responsiveness score at baseline	BAS fun-seeking score at baseline	BIS score at baseline
r_s	0.297	0.458	0.824	-0.059
Significance (2-tailed)	0.324	0.127	0.001	0.849
N	13	13	13	13

Table J.7: Table showing results of Spearman correlations between behavioural inhibition system/behavioural activation system (BIS/BAS) scores at baseline and the change in stimulus-induced dorsolateral prefrontal cortex (dlPFC) activity after MBCT-IBS. Correlations were considered statistically significant if $p \leq 0.05$, and p values reaching this criterion are shown in red. There was a significant correlation between baseline BAS fun-seeking scores and left dlPFC activity, meaning individuals with higher BAS fun-seeking scores experienced a greater increase in stimulus-induced left dlPFC activity.

J-8 Table showing the relationship between changes in right dlPFC connectivity and changes in symptoms/anxiety following MBCT-IBS

	Change in functional connectivity between the right dlPFC and regions below					
		Right amygdala	Right caudate	Right putamen	Right hippocampus	Right thalamus
Change in GSRS score	r_s	-0.077	-0.314	-0.426	0.129	-0.184
	Significance (2-tailed)	0.802	0.297	0.146	0.674	0.547
	N	13	13	13	13	13
Change in BPI pain severity score	r_s	0.178	-0.490	-0.581	-0.113	-0.099
	Significance (2-tailed)	0.527	0.064	0.023	0.688	0.726
	N	15	15	15	15	15
Change in BPI pain interference score	r_s	-0.163	-0.545	-0.572	-0.134	-0.290
	Significance (2-tailed)	0.562	0.036	0.026	0.634	0.295
	N	15	15	15	15	15
Change in VSI score	r_s	0.464	-0.169	-0.411	0.095	-0.101
	Significance (2-tailed)	0.094	0.563	0.411	0.748	0.731
	N	14	14	14	14	14

Table J.8: Table showing results of Spearman correlations between the change in symptoms/anxiety and the change in functional connectivity between the right dorsolateral prefrontal cortex (dlPFC) and five subcortical regions of interest following MBCT-IBS. Correlations were considered statistically significant if $p \leq 0.05$, and p values reaching this criterion are shown in red. There was a significant correlation between the change in the brief pain inventory (BPI) pain severity score and connectivity between the right dlPFC and the right putamen, meaning that individuals with a greater reduction in pain severity experienced a greater increase/smaller decrease in connectivity. There was also a significant correlation between the change in BPI pain interference score and the change in connectivity between the right dlPFC and both the right putamen and right caudate, meaning that individuals in whom pain interference was reduced more experienced a greater increase/smaller decrease in connectivity. GSRS, gastrointestinal symptom rating scale; VSI, visceral sensitivity index.

J-9 Table showing the effect of trait motivational style on changes in right dlPFC connectivity following MBCT-IBS

	Change in functional connectivity between the right dlPFC and regions below					
		Right amygdala	Right caudate	Right putamen	Right hippocampus	Right thalamus
BAS drive score at baseline	r_s	-0.125	0.309	0.584	-0.340	0.518
	Significance (2-tailed)	0.657	0.263	0.022	0.215	0.048
	N	15	15	15	15	15
BAS reward responsiveness score at baseline	r_s	0.124	0.363	0.357	0.137	0.155
	Significance (2-tailed)	0.659	0.184	0.191	0.626	0.581
	N	15	15	15	15	15
BAS fun-seeking score at baseline	r_s	-0.124	0.438	0.543	0.002	0.327
	Significance (2-tailed)	0.659	0.102	0.037	0.995	0.234
	N	15	15	15	15	15

Table J.9: Table showing results of Spearman correlations between behavioural activation system (BAS) scores at baseline and the change in functional connectivity between the right dorsolateral prefrontal cortex (dlPFC) and five subcortical regions of interest after MBCT-IBS. Correlations were considered statistically significant if $p \leq 0.05$, and p values reaching this criterion are shown in red. There was a significant correlation between baseline BAS fun-seeking scores and the change in connectivity between the right dlPFC and the right putamen, meaning individuals with higher BAS fun-seeking scores experienced a greater increase/smaller decrease in connectivity. There was also a significant correlation between baseline BAS drive scores and the change in connectivity between the right dlPFC and both the right putamen and right thalamus, meaning individuals with higher BAS drive scores experienced a greater increase/smaller decrease in connectivity.

Appendix K: Masks to accompany chapter 7

K-1 Anatomical masks

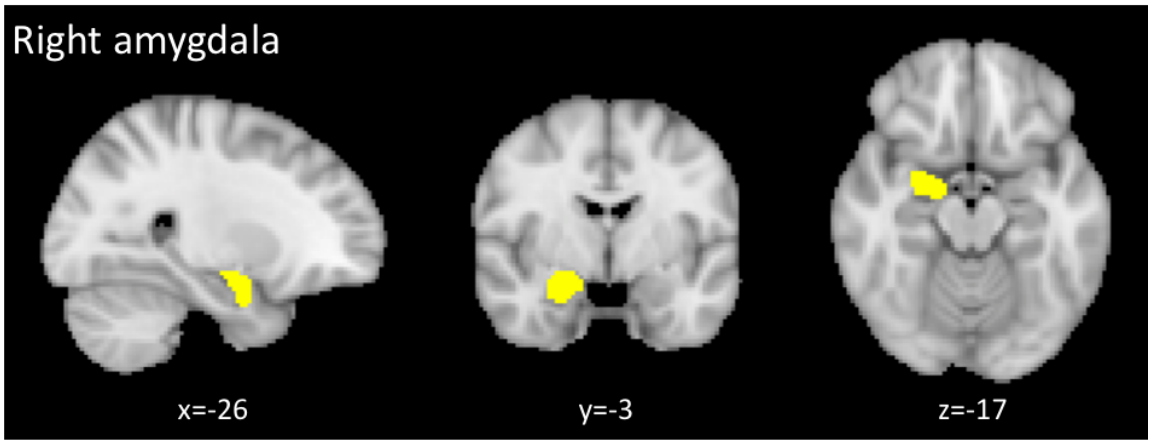


Figure K.1: Anatomical mask of the right amygdala. Defined using the Harvard-Oxford probabilistic atlas and thresholded to 50%. Images are in radiological convention and slices are identified with MNI coordinates.

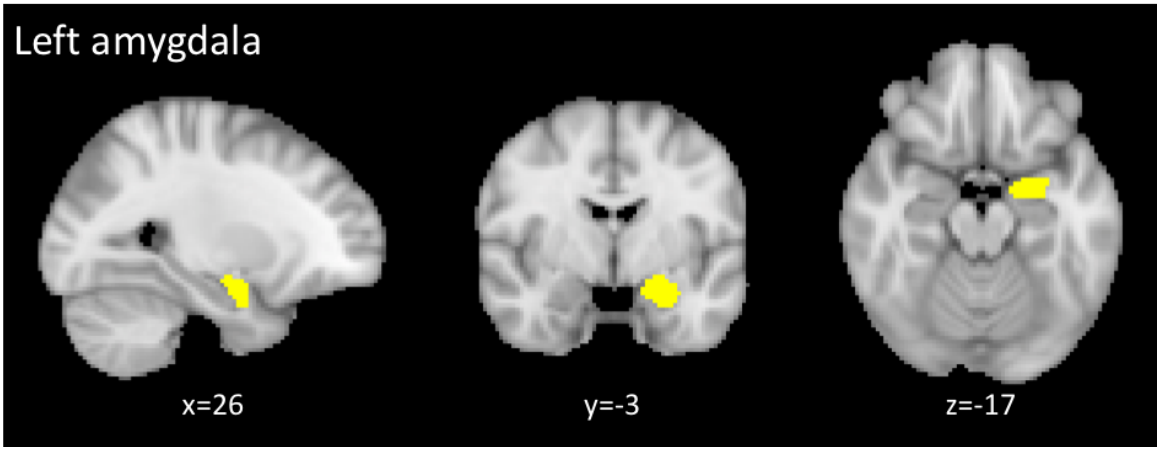


Figure K.2: Anatomical mask of the left amygdala. Defined using the Harvard-Oxford probabilistic atlas and thresholded to 50%. Images are in radiological convention and slices are identified with MNI coordinates.

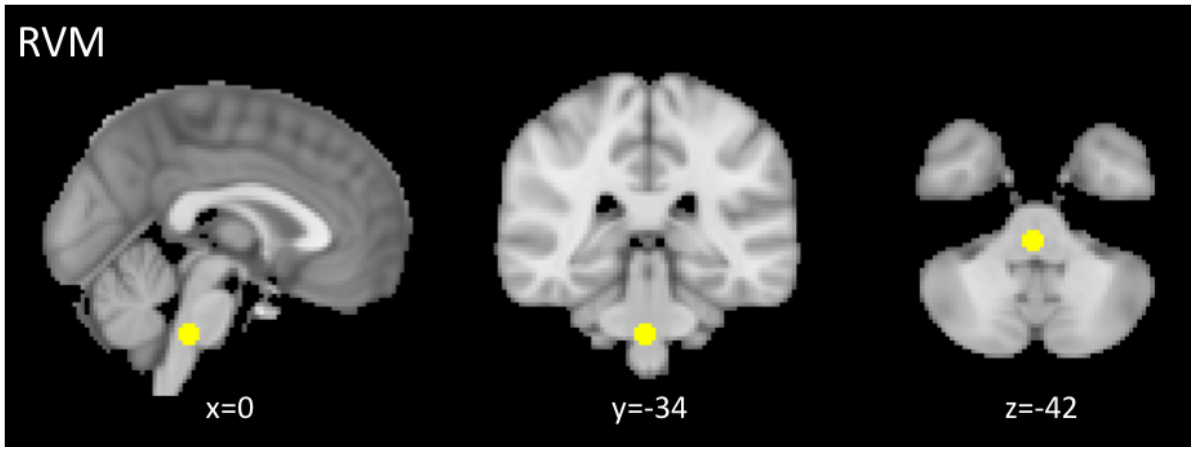


Figure K.3: Anatomical mask of the rostral ventromedial medulla (RVM), created using Duvernoy's Brainstem Atlas as a guide, and originally utilised by Soni et al., 2019. Images are in radiological convention and slices are identified with MNI coordinates.

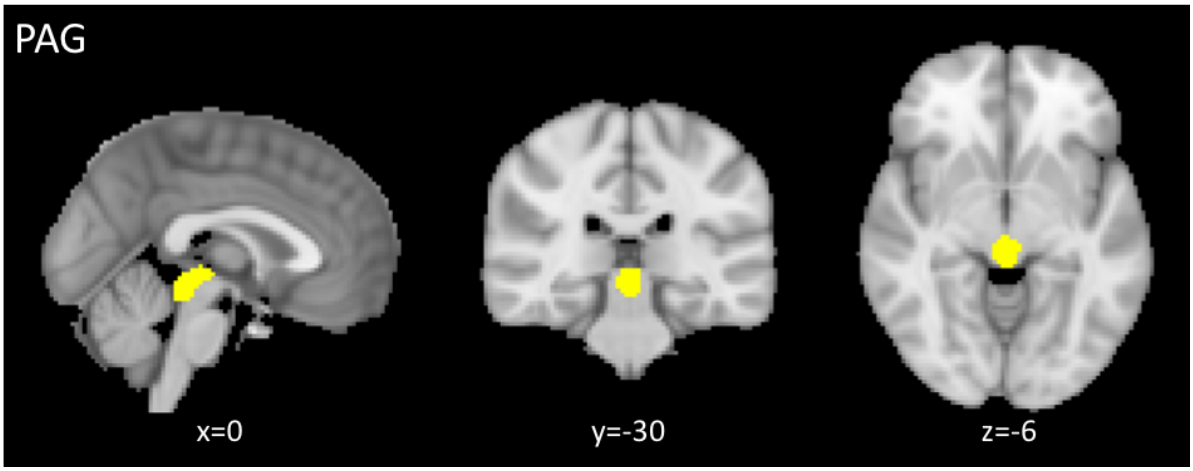


Figure K.4: Anatomical mask of the periaqueductal gray (PAG), created using Duvernoy's Brainstem Atlas as a guide. Images are in radiological convention and slices are identified with MNI coordinates.

K-2 Functional masks

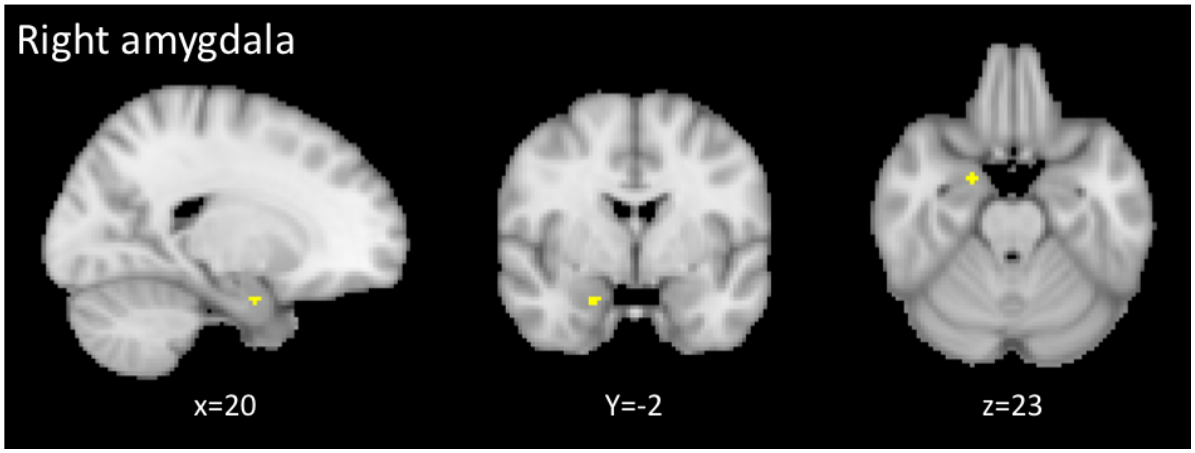


Figure K.5: Functional mask of the right amygdala. The mask was taken from those areas of the right amygdala in which there was a significant reduction in punctate hyperalgesia-induced activity after MBCT-IBS once thermode temperature was controlled for. Images are in radiological convention and slices are identified with MNI coordinates.

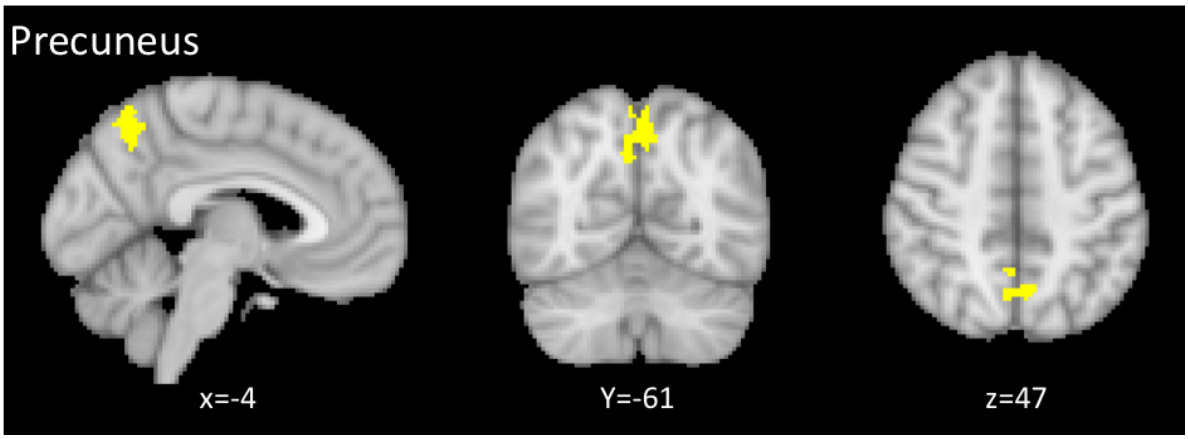


Figure K.6: Functional mask of the precuneus. The mask was taken from those areas of the precuneus in which there was a significant reduction in punctate hyperalgesia-induced activity after MBCT-IBS once thermode temperature was controlled for. Images are in radiological convention and slices are identified with MNI coordinates.

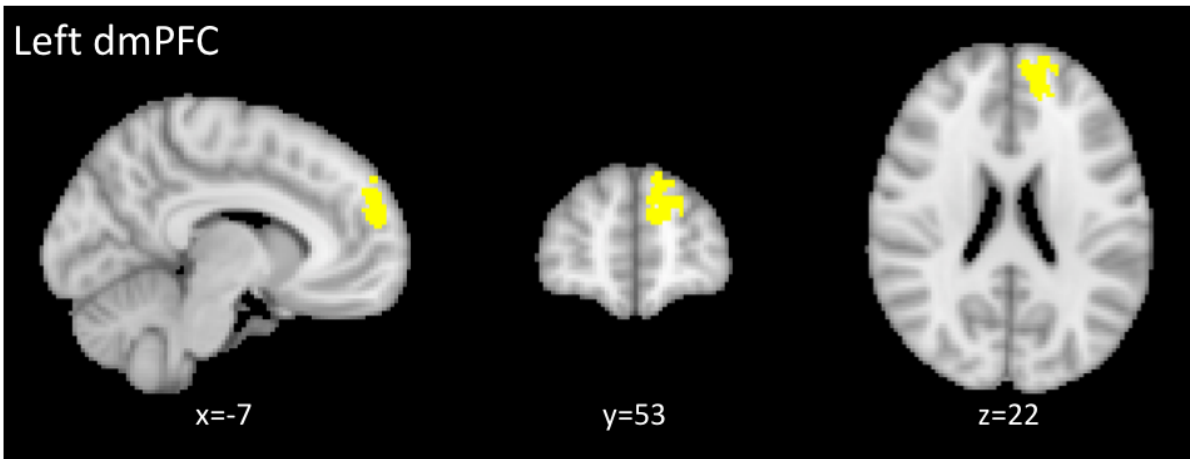


Figure K.7: Functional mask of the left dorsomedial prefrontal cortex (dmPFC). The mask was taken from those areas of the left dmPFC in which there was a significant increase in punctate hyperalgesia-induced activity after MBCT-IBS once thermode temperature was controlled for. Images are in radiological convention and slices are identified with MNI coordinates.

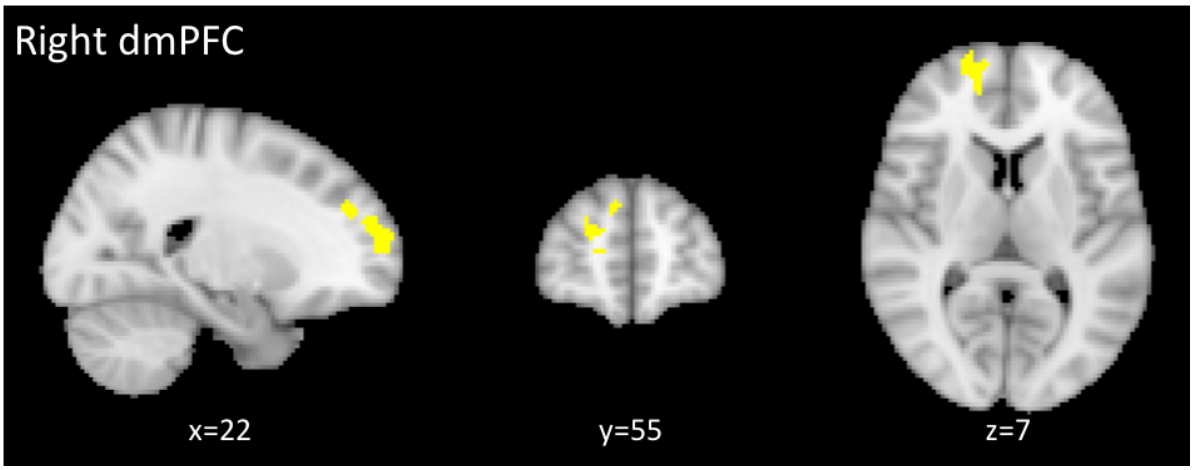


Figure K.8: Functional mask of the right dorsomedial prefrontal cortex (dmPFC). The mask was taken from those areas of the right dmPFC in which there was a significant increase in punctate-hyperalgesia induced activity after MBCT-IBS once thermode temperature was controlled for. Images are in radiological convention and slices are identified with MNI coordinates.

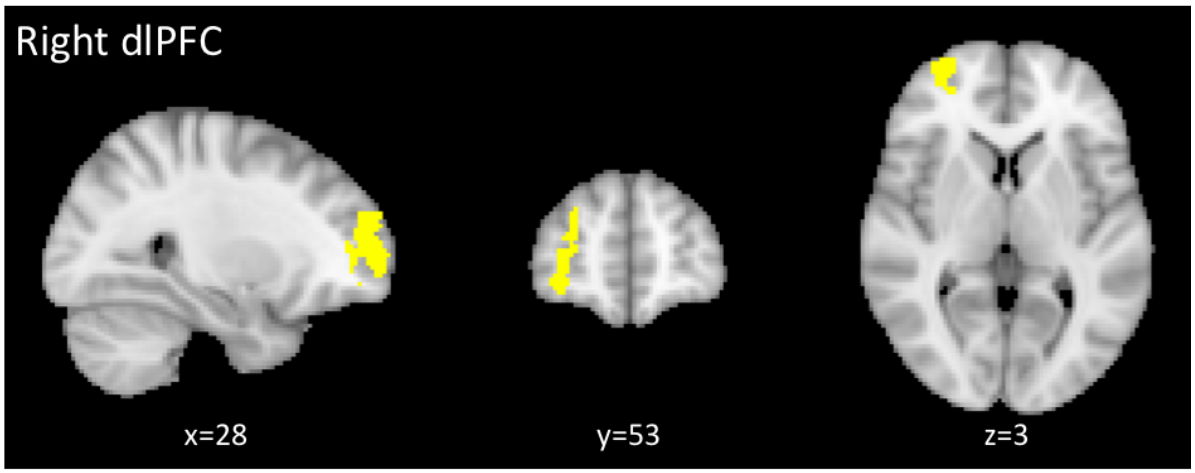


Figure K.9: Functional mask of the right dorsolateral prefrontal cortex (dlPFC). The mask was taken from those areas of the right dlPFC in which there was a significant increase in punctate hyperalgesia-induced activity after MBCT-IBS once thermode temperature was controlled for. Images are in radiological convention and slices are identified with MNI coordinates.

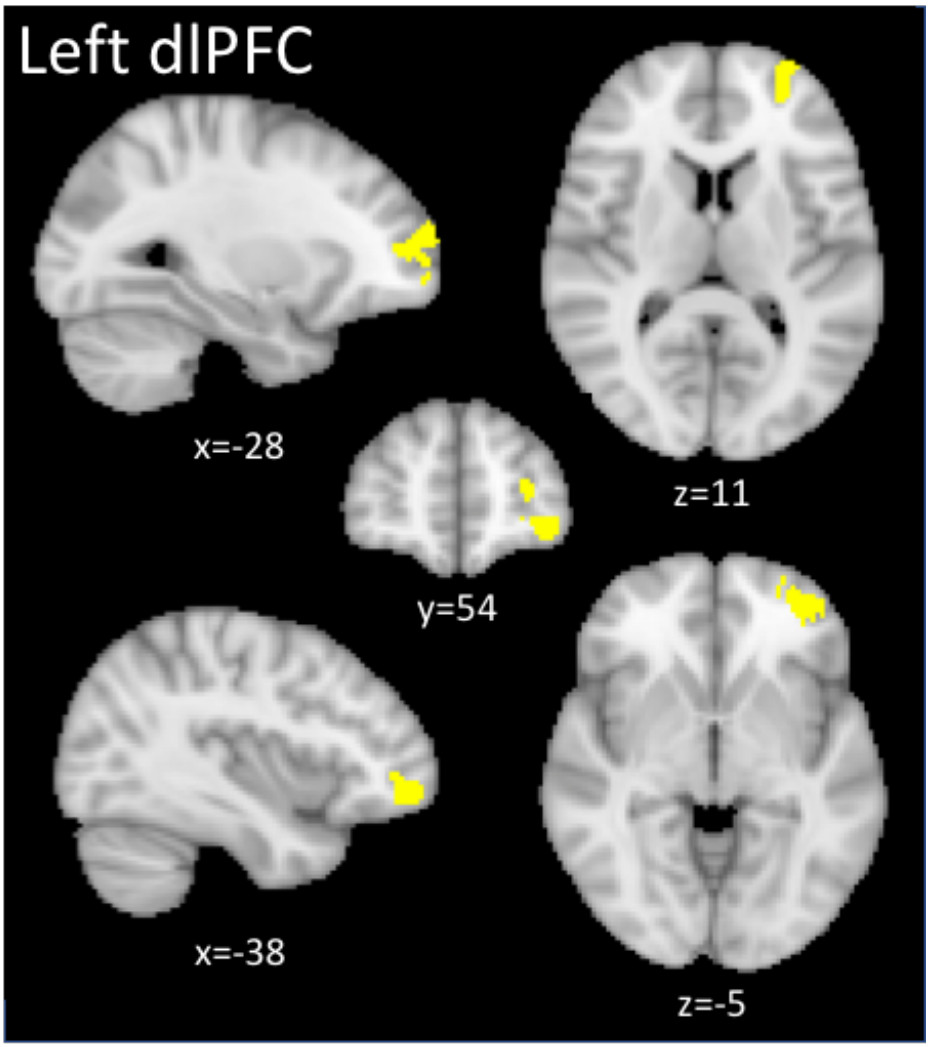


Figure K.10: Functional mask of the left dorsolateral prefrontal cortex (dlPFC). The mask was taken from those areas of the left dlPFC in which there was a significant increase in punctate hyperalgesia-induced activity after MBCT-IBS once thermode temperature was controlled for. Images are in radiological convention and slices are identified with MNI coordinates.