Neuroimaging functional pain networks in health and disease



Jennifer Louise Brawn

Nuffield Department of Clinical Neurosciences
St. Hugh's College
University of Oxford

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Jennifer L Brawn
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St Hugh's College, University of Oxford

Abstract

Chronic pelvic pain (CPP) is considered a significant public health problem. In the United Kingdom, it has been estimated that 24% of women suffer from CPP. Using neuroimaging, this dissertation aims to characterise the pain experience by examining functional changes in critical pain networks.

I begin by examining the functional correlates of capsaicin-induced central sensitisation in healthy individuals. Most of the results did not reach significance, largely due to the use of early 7T data and small sample sizes. In an exploratory analysis, amygdala - anterior insula connectivity is positively correlated with pain intensity.

The remaining experimental chapters examine mechanisms of pain in women with CPP. I begin by attempting to better characterise this diverse patient population. I demonstrate correlations between measures of the pain experience and psychological scores. Following this, I examine clinically-relevant changes in functional connectivity. I demonstrate that there are potentially different underlying modulatory mechanisms in women with neuropathic components to their pelvic pain. Furthermore, women who experience pain for longer than 24 months have increased functional connectivity between the hippocampus and periaqueductal gray, suggesting pain-related alterations in central circuitry. The final experimental chapters explore surgical and pharmaceutical treatments for CPP and endometriosis; however, due to small sample sizes and non-significant findings, limited conclusions can be drawn from the results.

In this dissertation, I found changes in important pain networks that could either represent the maintenance of the pain state or are consequence of the pain itself. These findings may serve as an indicator of underlying central changes that relate to relevant components of the clinical pain experience.

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List of Abbreviations

ACC	Anterior cingulate cortex
Amyg	Amygdala
aINS	Anterior insula
ANOVA	Analysis of variance
ASL	Arterial Spin Labelling
AVIC	Acute Vascular Imaging Centre, University of Oxford
B_0	Static magnetic field
BET	Brain Extraction Tool (FSL)
BDI	Beck Depression Inventory
BLA	Basolateral nucleus (amygdala)
BOLD	Blood-oxygen-level dependent
CBF	Cerebral blood flow
CeA	Central nucleus (amygdala)
CNS	Central nervous system
COPE	Contrast of Parameter Estimates
CRIC	Clinical Research Imaging Centre, University of Edinburgh
EndoPain	Oxford chronic pelvic pain study
ERK	Extracellular signal-regulated kinase
FEAT	FMRI Expert Analysis Tool (FSL)
FIRST	Model-Based Segmentation/Registration Tool (FSL)
FIX	ICA-based Xnoiseifier (FSL Tool)
FLIRT	FMRIB's Linear Image Registration Tool (FSL)
FNIRT	FMRIB's Non-Linear Image Registration Tool (FSL)
fMRI	functional magnetic resonance imaging
FMRIB	Oxford Functional Magnetic Resonance Imaging of the Brain
FSL	FMRIB's Software Library
Fslmeants	FSL timeseries extraction tool (FSL)
FSLNets	FSL Network Modelling Tool (FSL)
FWE	Family-wise-error
Glx	Combination of glutamine and glutamate concentrations
Hz	Hertz
Hipp	Hippocampus
HPA	Hypothalamic-pituitary-adrenal
IC	Interstitial cystitis
ICA	Independent Component Analysis
IBS	Irritable bowel syndrome
LA	Lateral nucleus (amygdala)
MCC	Midcingulate cortex
MCFLIRT	Intra-Model Motion Correction Tool (FSL)
MD	Medial dorsal nucleus (thalamus)
MELODIC	Multivariate Exploratory Linear Decomposition into Independent Components (FSL Tool)
mPFC	Medial prefrontal cortex
MRI	Magnetic resonance imaging

Multi-TI	Multiple inversion times
NRS	Numeric rating scale
p(corr)	Corrected p-values
PAG	Periaqueductal gray
PBS	Painful bladder syndrome
pCASL	Pseudo-continuous arterial spin labelling
PET	Positron emission tomography
pINS	Posterior insula
PLD	Post-labelling delay
PNM	Physiological Noise Modelling (FSL Tool)
Puta	Putamen
PVAQ	Pain Vigilance and Awareness Questionnaire
Px	Pixel
rACC	Rostral anterior cingulate cortex
RF	Radio frequency
ROI	Region of interest
RSN	Resting state network
RVM	Rostral ventromedial nuclei
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
SNR	Signal-to-noise ratio
STAI	State-Trait Anxiety Inventory
STT	Spinothalamic tract
Thal	Thalamus
TE	Echo time
TR	Time of repetition
TRPV1	Transient receptor potential vanilloid type 1
tSNR	Temporal signal-to-noise ratio
VAS	Visual analogue scale
VP	Ventral posterior nucleus (thalamus)
VMpo	Ventromedial posterior nucleus (thalamus)
WDR	Wide dynamic range

Chapter 1

Introduction

Section 1

1.1 Review Publications and Conference Abstracts

Over the course of my D.Phil. I have co-written several review publications and presented abstracts at two international conferences.

Review Publications

Brawn J, Morotti M, Zondervan KT, Becker CM, and Vincent K. Central changes associated with chronic pelvic pain and endometriosis. *Hum Reprod Update* 2014: **20**; 737-747.

Morotti M, Vincent K, Brawn J, Zondervan KT, and Becker CM. Peripheral changes in endometriosis-associated pain. *Hum Reprod Update* 2014: **20**; 717-736.

Brawn J & Vincent K. Hormonal Influences on Magnetic Resonance Spectroscopy Measures. Magnetic Resonance Spectroscopy: Tools for Neuroscience Research and Emerging Clinical Applications 2014.

Brawn J & Vincent K. Female Sex Steroid Hormones and Pain. Encyclopaedia of Pain 2013, 2nd Edition: 1273-1277.

Conference Abstracts

Brawn J, Eugene D, Horne A, Tracey I, Vincent K. Imaging resting pain networks in women with chronic pelvic pain. World Congress on Pain 2014. Buenos Aires, Argentina.

Brawn J, Seretny M, Pernet C, Whitaker L, Horne A, Tracey I, Vincent K. Imaging central pain networks in women with chronic pelvic pain. World Congress of Endometriosis 2014. São Paulo, Brazil.

Part of this dissertation's introductory sections on chronic pelvic pain were derived from:

Brawn J, Morotti M, Zondervan KT, Becker CM, and Vincent K. Central changes associated with chronic pelvic pain and endometriosis. *Hum Reprod Update* 2014: **20**; 737-747.

Section 2

1.2 The Epidemic of Chronic Pain and its Impact on Women

1.2.1 Prevalence and Treatment of Chronic Pain

Chronic pain is a public health problem that impacts over 20% of the European population (Breivik et al. 2006) and, in the US, it has been estimated 31% of Americans suffer from the condition (Johannes et al. 2010). Despite its prevalence, effective treatments and management strategies are lacking. This has left patients with few options for relief, resulting in tremendous disability and diminished of quality of life. Even where treatment options are available, there are many hurdles that pain patients face as they navigate the clinical pipeline (Zondervan et al. 1999a; Upshur, Bacigalupe, and Luckmann 2010).

As the front line of medicine, primary care is where chronic pain sufferers will first present. Research has shown that many general practitioners (GPs) feel ill-equipped to treat these patients (Green et al. 2001). Indeed, those who suffer from chronic pain do not necessarily feel that their condition is considered legitimate by their GPs, which can be partly attributed to misconceptions about chronic pain itself (e.g. symptoms not being real (Upshur, Bacigalupe, and Luckmann 2010). Thus, referrals from primary care to secondary pain or other specialty services are not guaranteed, leaving many patients without the care they desperately need (Zondervan et al. 1999a). In for-profit health care structures, in the United States, even if a GP suspects a referral would be advantageous, patients may not have adequate coverage (or any coverage) for the specialist care. Even with insurance, payments for each medical visit are often required; this "paywall" can act as a significant financial barrier for less affluent Americans (Turner 2007).

1.2.2 Health Disparities in Women

Women are disproportionately affected by many chronic pain conditions including irritable bowel syndrome (IBS), migraine headache, and fibromyalgia (Fillingim and Ness 2000); however, the underlying mechanisms of this bias are not well-characterised.

Whilst there are many scientific challenges to understanding pain in women, it has been argued that one potential reason for this discrepancy is the trajectory of science priorities and funding over the past several decades (Beery and Zucker 2011). The National Institutes of Health (NIH) constitutes one of the largest medical research funding organization in the world. Until the NIH Revitalization Act was passed in 1993, female inclusion in NIH-funded medical research was not required (including human clinical research) (Beery and Zucker 2011). Indeed, Schmucker and colleagues (1993) observed that many studies did not include any women at all, which they argued was partially a consequence of avoiding menstrual cycle-related hormonal flux and the dangers of enrolling pregnant women. Across disciplines and decades, researchers have highlighted that this has undoubtedly left a significant void in understanding the impact of drugs on females but also even basic understanding of sex-specific physiology in many conditions including chronic pain (Schmucker and Vesell 1993; Fillingim and Ness 2000; Beery and Zucker 2011). In fact, it was determined that in the 1981 Physicians' Desk Reference, approximately 50% of the drugs had not been tested on women (Kinney et al. 1981; Schmucker and Vesell 1993). This has been consistently raised as a critical issue due to the potentially life-threatening consequences for women, since not all side-effects will have been observed in the trials (Kinney et al. 1981; Schmucker and Vesell 1993; Beery and

Zucker 2011). It also means that potentially effective mechanisms specific to females may have been side-lined early in the drug development pipeline.

A recent systematic review highlighted that neuroscience research in particular frequently fails to include females (Beery and Zucker 2011). Whilst it is unreasonable to expect the diversity of research subjects to perfectly align with population demographics, the observed biases have undoubtedly had consequences for women's access to effective treatments.

Additionally, there are gender differences in health seeking behaviour and pain experiences that also contribute to the poor understanding of pain in women, with the bulk relevant research having emerged within the past several decades (Fillingim et al. 2009). Fillingim and colleagues (2009) discuss these differences in their extensive literature review, highlighting that women with chronic pain tend to have higher disability (Keefe et al. 2000) and use a greater diversity of coping mechanisms (friends/family, positive affirmation, catastrophising) (Jensen et al. 1994; Unruh, Ritchie, and Merskey 1999; Keefe et al. 2000). However, some gender differences in measures, such as catastrophising, are not always observed (Unruh, Ritchie, and Merskey 1999; Fillingim et al. 2009). Compared to men, women present more frequently to primary care centres (Bertakis et al. 2000). Men are more likely than women to receive secondary health care treatment, particularly in pain management (Fillingim et al. 2009).

1.2.3 Chronic Pain: Female-Specific Conditions and Pathologies

Much of the following section comes directly from my 2014 review (Brawn et al. 2014).

Chronic pelvic pain (CPP) has recently been defined as 'chronic or persistent pain perceived in structures related to the pelvis of either men or women. It is often associated with negative cognitive, behavioural, sexual and emotional consequences as well as with symptoms suggestive of lower urinary tract, sexual, bowel, pelvic floor or gynaecological dysfunction' (Baranowski et al. 2012).

Only within the past decade and a half has there been an estimate of CPP within the population as a whole in the UK, with 24% of women reporting pain (Zondervan et al. 2001b). Recently it has been declared that CPP is a major public health problem throughout the developed world (Mathias et al. 1996; Zondervan et al. 2001b; Grace and Zondervan 2004; Garcia-Perez et al. 2010). CPP can present at all ages, with studies suggesting that the prevalence increases with age (18.2/1000 in 15–20 year olds; 27.6/1000 in women over 60) (Zondervan et al. 1999a, 1999b). Yet despite being as common as back pain and asthma (Zondervan et al. 1999a, 1999b), with a significant financial cost to both the individual and society (Mathias et al. 1996), there is frequently a significant delay in diagnosis and treatment. In fact, studies suggest that up to 50% of women have not received a CPP diagnosis after many years of follow-up (Grace 1995; Zondervan et al. 1999a, 1999b; Grace and Zondervan 2004).

A variety of pelvic pathologies are associated with CPP in women, including endometriosis, adenomyosis or chronic infection; it can be associated with functional disorders of the bowel (irritable bowel syndrome (IBS)) and the bladder (interstitial cystitis/painful bladder syndrome (IC/PBS)). In many cases, however, detailed investigation of the pelvis/pelvic viscera via both radiographic and surgical investigation

reveals no abnormality (chronic pelvic pain syndrome (CPPS)) (Baranowski et al. 2012). Furthermore, even when pathology is identified and treated the pain may still persist, suggesting that the pathology was either an incidental finding or that other mechanisms continue to generate pain without the need for a peripheral input (Baranowski 2009). There is little understanding of how female-specific pathologies, like endometriosis, relate to pain.

Section 3

1.3 Neurobiology of Pain: Nociception and Pain Perception

The International Association for the Study of Pain (IASP) describes pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey and Bogduk 1994). Pain can be further delineated into mechanistic sub-groups including: nociceptive (pain from stimulation of tissue) and neuropathic (pain from damage to the somatosensory system) (International Association for the Study of Pain 2012b). In the following section, I describe the basic neurobiology behind the pain experience.

1.3.1 Nociceptors: Peripheral Projections to the Spinal Cord

Much of what is known about nociception comes from animal research. These studies have characterised important pain pathways across multiple non-human species, which has helped direct human research.

1.3.1.1 Somatic Innervation

There are two key types of fibres involved in somatic peripheral nociception: unmyelinated C-fibres and myelinated Aδ-fibres. Aβ-fibres also exist but are generally responsible for the sensation of non-noxious mechanical stimulation (i.e. non-painful touch). Due to their myelination, A-fibres transmit information more quickly than C-fibres, and thus are responsible for the first sensation of pain, whilst C-fibres are responsible for slower but longer lasting pain sensation. Both C- and A-fibres respond to noxious thermal, mechanical, and chemical stimulation; however, A-fibres are thought to cover a wider range of these inputs (Ringkamp et al. 2013).

C-fibres are responsible for the prolonged feeling of burning pain and can be further delineated into quick C-fibres and slow C-fibres. In general, these fibres transmit input at approximately < 2 m/s and respond to chemical, mechanical, and thermal stimulation (>39°C) (Churyukanov et al. 2012; Ringkamp et al. 2013). Aδ-fibres can be further delineated into Type I and II. Primate research has demonstrated that Type I fibres have conduction velocities of 25 - 55 m/s whilst Type II fibres transmit slightly slower at approximately 15 m/s. Type I fibres respond to mechanical, chemical, and thermal (>53°C) stimulation. Type II fibres do not generally respond to mechanical stimulation but do respond to chemical and thermal input at a lower threshold (47°C) (Treede, Meyer, and Campbell 1998; Ringkamp et al. 2013). Both C- and Aδ- fibres synapse at dorsal horn laminae I, II, V, and VI; however, they do project to other laminae (Millan 2002).

1.3.1.2 Visceral Innervation

The transmission of peripheral, visceral nociceptive input to the central nervous system (CNS) is not as straightforward or well-characterised as somatic transmission. However, much like the cutaneous innervation, the majority of nociceptors in the viscera comprise unmyelinated C- and myelinated Aδ-fibres (Gebhart 2000). There are low (< 5 mm Hg) and high threshold (> 30 mm Hg) myelinated mechanoreceptors, with the majority being of the former (Gebhart 2000; Todd and Koerber 2013). On the other hand, unmyelinated visceral afferents detect both chemical and mechanical stimulation (Kumazawa 1996; Todd and Koerber 2013). The myelinated mechanoreceptors are believed to synapse at laminae I, V, and VI (de Groat et al. 1981; Todd and Koerber 2013), whilst the unmyelinated afferents project to dorsal horn laminae I, II, V, and X (Todd and Koerber 2013).

1.3.2 Central Mechanisms of Nociception and Pain Perception

As previously described, somatic and visceral peripheral input converges at various laminae of the dorsal horn. From here, nociceptive information is transmitted to higher processing centres of the CNS.

1.3.2.1 Spinal Nociceptive Communication: Ascending Somatic and Visceral Afferents

The spinothalamic tract (STT) is one of the most important and best characterised of the nociceptive spinal pathways. It comprises both lateral and anterior components, with the former being especially critical in nociception. The lateral STT receives C- and Aδ-fibre input from the dorsal horn that terminate within the thalamus (Craig 2003b; Dostrovsky and Craig 2013). The input from lamina I is of particular importance as it includes two important nociceptive neuron types: nociceptive-specific (NS; most primary projections are from Aδ-fibres) and polymodal (most primary projections are from C-fibres) neurons (Craig, Krout, and Andrew 2001; Craig and Andrew 2002; Dostrovsky and Craig 2013). It has been postulated that polymodal neurons transmit visceral sensory information (Dostrovsky and Craig 2013). Laminae IV and V possess wide-dynamic-range (WDR) neurons (encode non-noxious and noxious stimulation) (Willis 1985; Craig 2003b; Dostrovsky and Craig 2013).

Animal research has demonstrated that thalamic nuclei (ventromedial posterior (VMpo), medial dorsal (MD), and ventral posterior (VP)) all receive lamina I STT afferents (Craig et al. 1994; Craig 2003b; Dostrovsky and Craig 2013). The VP also receives STT afferents from lamina V WDR neurons (Craig 2003b).

The spinobulbar tract is another defined pathway, which receives inputs from laminae I, V, and VII (Wiberg, Westman, and Blomqvist 1987; Dostrovsky and Craig 2013). The majority of secondary neurons originating from lamina I terminate in regions of brainstem (including at the PAG and parabrachial nucleus (PB)), whereas the reticular formation receives input from laminae V and VII (Dostrovsky and Craig 2013). Both the reticular formation and PAG have ascending connections to the thalamus, whilst the PAG also has connections to the hypothalamus (Mantyh 1983; Dostrovsky and Craig 2013).

Saper and colleagues (2002) have highlighted that, unlike the somatic ascending pathways, visceral sensory and nociceptive spinal communication is not as clearly defined. However, research has shown that visceral information ascends along the STT (lamina I), spinobulbar tract, and dorsal column (lamina X) (Willis et al. 1999; Saper 2002; Critchley and Harrison 2013; Dostrovsky and Craig 2013). The nucleus tractus solitarius (NTS), which has been characterised as fundamental and unique to visceral sensation and nociception, receives ascending projections from lamina I (Millan 2002; Craig 2003a; Critchley and Harrison 2013) and non-nociceptive visceral pathways (vagus nerve) (Millan 2002; Critchley and Harrison 2013) (see Figure 1.01). The NTS also receives input from the hypothalamus and PB (Millan 2002). From the NTS, there are connections with the PB, PAG, hypothalamus, and thalamus (VMpo) (Millan 2002; Saper 2002; Craig 2003a).

There is also spinohypothalamic tract, which receives input from multiple laminae (I, V, VII, and X) (Dado, Katter, and Giesler 1994); however, this pathway is not well-characterised (Dostrovsky and Craig 2013) and thus will not be discussed further. There is also the trigeminothalamic tract which is an ascending cranial pain system that directly

connects with the thalamus (Dostrovsky and Craig 2013). However, since this dissertation does not investigate cranial pain, it will not be described here.

Complexity of Visceral Input to the Spinal Cord and Ascending Pathways

As described above, viscero-sensory and somatosensory afferents share common pathways within the spinal cord (Bielefeldt and Gebhart 2013). This "convergence" is considered responsible for somatic referral pain. In women with CPP, this is well-characterised with referred somatic abdominal and/or lower back pain (Margaria, Gollo, and Castelletti 1993; Bielefeldt and Gebhart 2013). Viscera also share pathways into the spinal cord. For example, the colon, uterus, and urinary tract all have common projections to the spine (Giamberardino et al. 2010). However, Bielefeldt and Gebhart (2013) emphasise that one of the unique characteristics of visceral innervation is input spanning across spinal cord levels (see Figure 1.01) and that many of these common pathways will diverge in the CNS as part of the ascending spinal tracts. This results in "viscero-visceral hyperalgesia", a term first coined by Giamberardino, which describes pain originating in one viscera that is also perceived in another. This has important clinical implications: treatments that alleviate pain in one region appear to reduce pain associated with other visceral regions (Giamberardino et al. 2010).

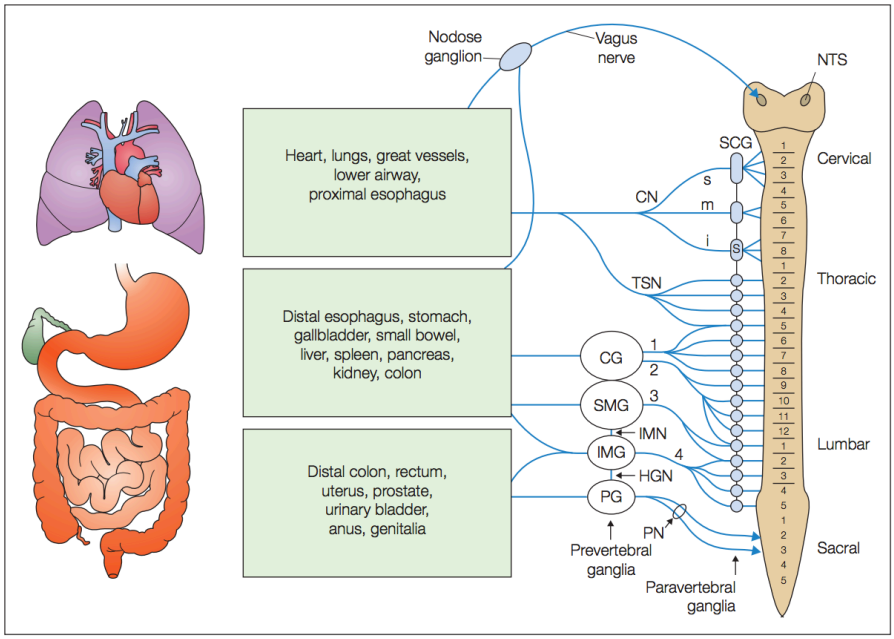


Figure 1.01 Visceral Innervation

Innervation of the viscera is uniquely complex with peripheral input spanning across multiple spinal cord levels. NTS - nucleus tractus solitarius; CG - celiac ganglion; IMG - inferior mesenteric ganglion; SMG - superior mesenteric ganglion; PG - pelvic ganglion; S - stellate ganglion; SCG - super and middle cervical ganglia; CN - cardiac nerves (s - superior; m - middle; i - inferior); HGN - hypogastric nerve; IMN - intermesenteric nerve; PN - pelvic nerve; TSN = thoracic splanchnic nerves (1 - greater; 2 - lesser; 3 - least; 4 - lumbar). Figure from Bielefeldt and Gebhart (2013).

1.3.2.2 Pain in the Brain

Pain Imaging

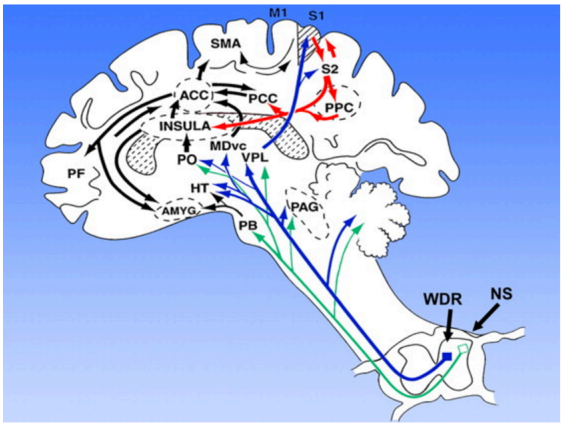
Functional magnetic resonance imaging (fMRI) research has been invaluable in characterising pain processing in the human CNS. Much of what is known about pain in both healthy individuals and those with chronic pain, is derived from event-related/response-driven paradigms (Tracey and Johns 2010). However, recently there has been increased interest in understanding central markers of the pain experience from resting neural activity (Cole, Smith, and Beckmann 2010; Baliki et al. 2014). Resting activity is thought to represent inherent neurobiological functioning (Damoiseaux et al. 2006; Fox and Raichle 2007) and could provide insights into underlying mechanisms associated with acute and chronic pain experiences (Baliki et al. 2014).

Central Pain Processing: Coordination of Cortical and Sub-Cortical Structures

Both non-imaging and imaging research have implicated many regions in nociceptive processing and pain perception including, but not limited to, the insular, cingulate, somatosensory and prefrontal cortices, as well as the thalamus, amygdalae, midbrain and brainstem regions. However, as described above, somatic and visceral pathways to these regions can differ (see Figure 1.02).

Ascending Nociceptive Pathways

1



Visceral Nociceptive Pathway via the NTS

2

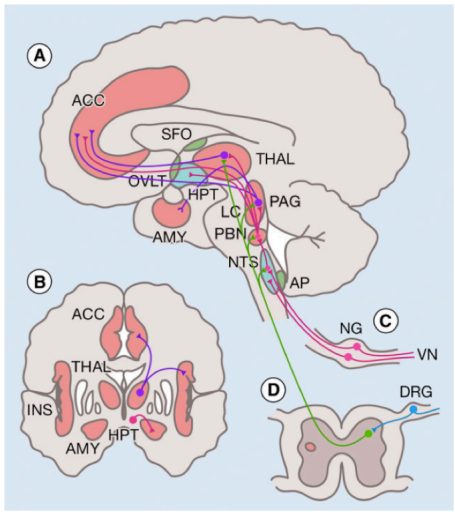


Figure 1.02 Ascending Nociceptive Pathways

Image 1 (left) illustrates an overview of ascending nociceptive spinal pathways to the brain. Image 2 (right) illustrates an important component of the ascending visceral system (lamina I projections in green and vagus nerve in pink). Whilst the ascending projections of viscero-sensation are not as well characterised as the somatic system, it is known that the nucleus tractus solitarius (NTS) is an important and unique component to viscero-sensation and visceral nociception (Millan 2002). WDR – wide-dynamic range neurons; NS – nociceptive-specific neurons; PAG – periaqueductal grey; PB/PBN – parabrachial nuclei; AMY/AMYG – amygdala; NTS – nucleus tractus solitarius; DRG – dorsal root ganglion; ACC – anterior cingulate cortex; VN – vagus nerve; Thal - thalamus; PF - prefrontal cortex; LC - locus coeruleus; PCC - posterior cingulate cortex; PPC - posterior parietal cortex; M1 - primary motor cortex; SMA - supplementary motor cortex; S1 - primary somatosensory cortex; S2 - secondary somatosensory cortex; INS - insula; HPT/HT - hypothalamus; AP - area postrema; OVLT - organum vasculosum of lamina terminalae; SFO - subfornical organ; VPL -ventral posterolateral nucleus (thalamus); MDvc - ventral medial nucleus (thalamus); PO - preoptic area; PF - prefrontal cortex. Images 1 and 2 were adapted from Price and colleagues (2006) and Critchley and Harrison (2013), respectively.

Broadly speaking, pain processing can be separated into the following components: sensory-discriminatory, cognitive-evaluative, and affective-motivational (Melzack and Casey 1968). Within these categories, there are many factors that can influence an individual's pain experience. Emotional (depressed, anxious, fearful) and cognitive states

(threat, expectation, attention or distraction), and physiological predisposition (hormones, biological sex, maladaptive structural changes/central sensitisation) are just some of the many factors that shape nociception and pain perception (Tracey and Mantyh 2007). This complexity has made it difficult to objectively characterise the cortical components of pain processing in the healthy population, let alone chronic pain patients.

Below, I begin by summarising research on regions that will be examined and discussed in the experimental chapters. I also describe regions that are not directly examined, but are structurally and/or functionally relevant to the regions of interest and pain modulatory processes in general.

Thalamus

Historically, the thalamus was considered the centre of pain perception; however, further research helped demonstrate that, whilst important, the thalamus is not the sole player in pain and that it acts in conjunction with many other brain regions (Treede et al. 1999). As previously described, the thalamus comprises multiple nuclei that are critical in the transmission of nociceptive information to higher cortical regions.

The VMpo, VP, and MD nuclei are some of the most important thalamic nuclei in nociception and have ascending afferents to several well-characterised pain regions. The VP has ascending projections to both the primary (SI) and secondary somatosensory (SII) cortices (Dostrovsky and Craig 2013). The anterior cingulate cortex (ACC) receives ascending input from the MD (Treede et al. 1999). The VMpo, a region much larger in humans than in non-human primates, connects with the insula (Craig et al. 1994; Treede et al. 1999; Dostrovsky and Craig 2013).

Amygdalae

Through animal research, the amygdalae's internal and external structural connectivity has been well characterised. The central (CeA) nucleus is an important pain microstructure within the amygdala. It is largely responsible for nociceptive processing (Bernard, Huang, and Besson 1992) as it receives ascending input from the thalamus (Neugebauer et al. 2004), PAG (Rizvi et al. 1991), and spinal cord (Burstein and Potrebic 1993). Additionally, it receives cortical top-down projections (for example, ACC, mPFC, and insula) which descend to the PAG (via the lateral (LA) and basolateral (BLA) nuclei of the amygdalae) (Rizvi et al. 1991; Neugebauer et al. 2004; Likhtik et al. 2005; Heinricher and Fields 2013) (see Figure 1.03). fMRI research has demonstrated that the amygdalae contribute to both the affective and sensory components of pain processing. Studies have demonstrated the amygdalae's emotional modulatory impact on pain perception (Berna et al. 2010) in addition to their ability to encode pain intensity (Bornhovd et al. 2002).

Hippocampal Formation

In pain, the hippocampal formation is another important sub-cortical region which comprises regions including the hippocampus and entorhinal cortex. Much of what is known about the hippocampus involves memory (Squire 1992); however, it too plays a fundamental role in pain perception largely through its emotional and cognitive modulatory mechanisms. Expectation paradigms have been shown to impact pain-associated hippocampal activity (Ploghaus et al. 2003; Kong et al. 2008). By inducing a depressed state, researchers were able to increase both response to pain and activity in the hippocampus in healthy subjects (Berna et al. 2010). Similarly, heightened anxiety has increased both pain ratings and pain-associated activation in the entorhinal cortex (Ploghaus et al. 2001). In women with dysmenorrhoea (pain with menstruation), activity

in the entorhinal cortex was amplified in response to noxious heat, when compared to healthy controls (Vincent et al. 2011).

Insular Cortices

The insula is one of the most frequently cited regions in pain imaging research (Duerden and Albanese 2013). It has distinct cytoarchitectural anterior, middle, and posterior divisions (Augustine 1985). These divisions have been implicated in the different dimensions of pain processing (Craig 2009).

The anterior insula is thought to be more centred on the affective-motivational and cognitive-evaluative processes. Studies of resting brain activity have demonstrated that the anterior insula has increased structural and resting functional connections to the frontal cortices, including the anterior cingulate cortex (Cauda et al. 2011; Wiech et al. 2014a). Indeed, this fits with what is known about the region in pain. Pain-related activity in the anterior insula is amplified by increased attention and negative affect (Ploner et al. 2011).

It has been postulated that the anterior insula is key in viscero-sensation with observed activation in association with noxious distension (Craig 2009). Further, it has been suggested that the anterior insula is more heavily involved in chronic pain than experimental pain processing (Schweinhardt and Bushnell 2010).

The posterior insular division is thought to be more sensory-discriminatory oriented. Compared to the other divisions, the posterior insula has increased structural and resting functional connections with the somatosensory cortices (Wiech et al. 2014b). Many

imaging studies have demonstrated the sensory role it plays. It processes stimulus intensity changes (Oertel et al. 2012) and is less influenced by attention modulation paradigms than the anterior division (Brooks et al. 2002). Recently, in healthy subjects, Segerdahl and colleagues (2015) demonstrated that the dorsal posterior insula (dpINS) encodes pain intensity over prolonged pain experiences.

Primary and Secondary Somatosensory Cortices

As previously described, the posterior insula is connected to the primary (SI) and secondary (SII) somatosensory cortices. SI and SII play critical roles in the sensory-discriminatory component of pain processing. SI in particular shows somatotopy, with distinct regions for bodily representation that can be distinguished with relative precision using fMRI (Bingel et al. 2004). Research has also demonstrated that SI and SII are sensitive to discrete changes in noxious input. Altered activation has been observed in response to noxious heat, with input changes as small as 2°C (Coghill et al. 1999). Magnetoencephalography (MEG) research has demonstrated that SI can differentiate between a wider range of sensory information, including non-painful stimulation, whilst SII tends to be most responsive to noxious stimulation (Timmermann et al. 2001).

Prefrontal Cortex

The prefrontal cortex (PFC) has proven important in the affective-motivational and cognitive-evaluative components of pain processing. Distraction paradigms have increased activation in response to pain in the orbitofrontal cortex (Bantick et al. 2002; Valet et al. 2004), whilst they have decreased activation in the medial PFC (mPFC) (Wiech et al. 2005). Berna and colleagues (2010) demonstrated pain-induced amplification of orbitofrontal and dorsolateral PFC (dlPFC) activity during depression paradigms. Anxiety and depression measures in women with CPP have been positively correlated with resting

mPFC - anterior insula coupling (As-Sanie et al. 2016).

Cingulate Cortex

The PFC and cingulate cortex are proximal to each other and share connections as well. The cingulate cortex comprises anterior, middle, and posterior divisions. Early positron emission tomography (PET) research implicated the anterior cingulate cortex (ACC) in the processing of the unpleasantness of noxious input; changes in unpleasantness were accompanied by corresponding activation changes (Rainville et al. 1997). Indeed, attention/distraction fMRI studies have demonstrated that the ACC is very much a part of cognitive-evaluative dimension of pain processing (Longe et al. 2001; Bantick et al. 2002). The ACC can be further divided with the midcingulate (MCC) being more cognitively-oriented and the perigenual ACC being responsible for emotional processes (Devinsky, Morrell, and Vogt 1995). Distraction paradigms have increased activation in the perigenual ACC (Bantick et al. 2002; Valet et al. 2004) and decreased in activation in the MCC in response to painful stimulation (Bantick et al. 2002). Dunckley and colleagues (2005) demonstrated that the perigenual ACC had reduced activity in association with visceral but not somatic noxious stimulation.

1.3.3 Endogenous Modulation: Descending Pain Mechanisms

The research described above not only illustrates the many factors involved in nociception and pain perception but that simple external manipulations can alter the pain experience. Over history there has been ample evidence pointing to the existence of endogenous descending pain modulatory systems that alter how individuals perceive pain. A commonly cited example is Beecher's observation that soldiers with extensive injuries did not have seemingly appropriate pain responses (Beecher 1959; Ossipov, Dussor, and

Porreca 2010). Less extreme examples also exist: Melzack & Wall (1982) demonstrated that even with day-to-day injuries, there was delay in the onset of pain.

Since these critical observations, researchers have determined that the ability for an individual to engage descending modulatory mechanisms depends on a variety of factors including genetics (Mogil 1999; Zubieta et al. 2003), psychological state, life history (past pain experiences), and other cognitive factors, including context (Tracey and Mantyh 2007; Ossipov, Dussor, and Porreca 2010).

From an evolutionary perspective, there is a clear advantage to having these systems in place. Individuals are more likely to survive perilous situations if they can escape despite sustaining what could be considered extraordinarily painful injuries (Bingel and Tracey 2008). Since survival is key to fitness (procreation to pass on one's DNA), it is plausible that this physiological mechanism would be a highly selected trait.

There are multiple supraspinal regions that are known modulators of incoming nociceptive input at the dorsal horn including the ACC, amygdalae, hypothalamus, periaqueductal gray (PAG), and rostral ventromedial medulla (RVM) (the latter two will be discussed in more detail below). Coordinated activity between these regions can either inhibit or facilitate incoming nociceptive input at the site of the dorsal horn (Heinricher and Fields 2013) (see Figure 1.04).

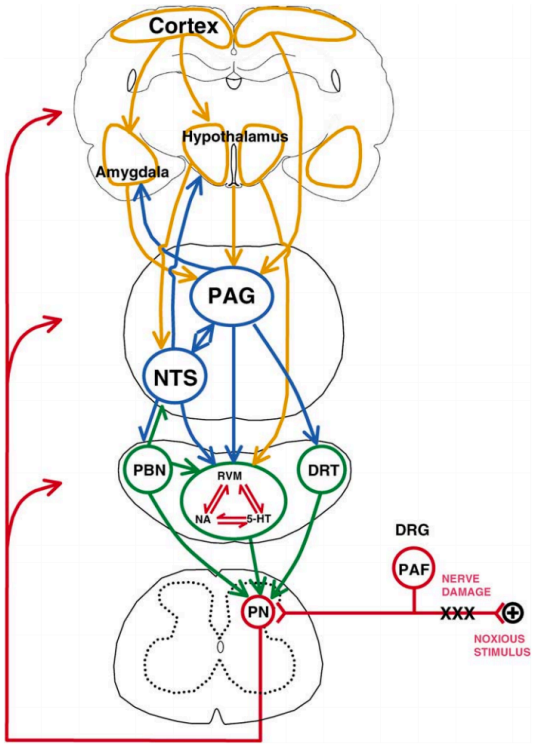


Figure 1.03 Descending Modulatory System

Schematic of pathways of descending modulatory system. PAG - periaqueductal gray; NTS - nucleus tractus solitarius; RVM - rostral ventromedial medulla; NA - noradrenaline; PBN - parabrachial nucleus; DRT - dorsoreticular nucleus; PAF - primary afferent fibre; DRG - dorsal root ganglion, PN - projection neurones; 5-HT - seratonin. Figure from *Millan (2002).*

1.3.3.1 Periaqueductal Gray

Reynolds was the first to observe the anti-nociceptive role of the PAG. By stimulating the PAG in awake rats, a laparotomy could be performed without any associated behavioural distress (Reynolds 1969). Subsequent research demonstrated that PAG stimulation reduces activity in STT neurons (Gerhart et al. 1984). In humans, the outcome is similar; implanting stimulation devices in the PAG have resolved patients' chronic pain (Baskin et al. 1986; Fields 2000).

Indeed, early fMRI research in humans has demonstrated the PAG's modulatory role. Increased activity in the PAG has been associated with decreased pain perception (Tracey et al. 2002). In Linnman and colleagues' (2012) review, the authors eloquently summarise

the many roles of the PAG within and beyond pain. Expectation and attention paradigms have implicated the PAG in the cognitive-evaluative dimension of pain processing. Further, the authors highlight the PAG's role in visceral sensory processing in those with chronic stress (Rosenberger et al. 2009) and the predictive nature of its coupling with the anterior insula on pain perception (Ploner et al. 2010).

Beyond pain, resting functional connectivity has been observed between the PAG and hippocampus, anterior insula, ACC, and RVM (Kong et al. 2010). Animal studies have shown that the PAG has direct structural connections with higher cortical and sub-cortical structures including the anterior insula, amygdalae, ACC, hypothalamus (Fields 2004) (for white matter connectivity, see Figure 1.04). It also receives direct input from the NTS and the dorsal horn (laminae I and V) (Keay et al. 1997; Millan 2002; Heinricher and Fields 2013). Its descending projections extend to regions including the RVM, which extend to the dorsal horn (Fields 2000).

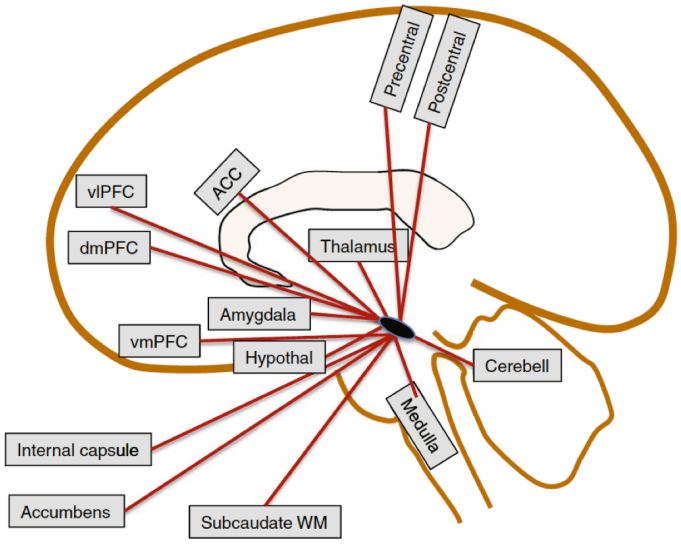


Figure 1.04 White Matter Connectivity of the Periaqueductal Gray

Human white matter connectivity of the periaqueductal gray (PAG) as measured by diffusion tensor imaging (DTI). The PAG has connections to important cortical and sub-cortical regions. WM - white matter; vlPFC - ventrolateral prefrontal cortex; dmPFC - dorsomedial prefrontal cortex; vmPFC - ventromedial prefrontal cortex; ACC - anterior cingulate cortex. Figure from Linnman and colleagues (2012).

1.3.3.2 Rostral Ventromedial Medulla

Research has demonstrated that stimulation of the RVM can either increase or decrease nociceptor activity (Fields and Heinricher 1989). The RVM comprises multiple types of neurons including opiate-sensitive ON- and OFF-cells. Due to their direct connection with the dorsal horn, they are largely responsible for modulating nociceptive input. ON- and OFF-cells are thought to be pro- and anti-nociceptive, respectively (Fields et al. 1983; Fields, Heinricher, and Mason 1991).

The RVM receives direct input from the amygdalae, frontal cortex, hypothalamus, and PAG (Fields, Heinricher, and Mason 1991). Its descending connections extend to the well-characterised nociceptive laminae I and V in addition to II, VI, and VII (Fields and Heinricher 1989; Fields, Heinricher, and Mason 1991).

1.3.3.3 PAG-RVM Circuitry

PAG and RVM coupling is a well-characterised component of descending modulatory circuitry. Administering anaesthetic to the RVM prevents stimulation-induced analgesia by the PAG, indicating the importance of the RVM in descending inhibition (Fields, Heinricher, and Mason 1991). Additionally, together, the PAG and RVM are thought to be responsible for maintaining states of increased excitation in the central nervous system (central sensitisation) (Pertovaara, Wei, and Hamalainen 1996; Urban and Gebhart 1999; Woolf 2011).

As previously mentioned, there are cortical and sub-cortical structures that feed into the PAG-RVM circuitry that can either increase or decrease incoming nociceptive input. The ACC has descending connections to the amygdala and PAG (Rainville 2002) and has demonstrated nociceptive facilitation through the dorsal reticular nucleus and RVM (Calejesan, Kim, and Zhuo 2000; Zhang, Zhang, and Zhao 2005). As previously described, the CeA nucleus of the amygdala, which plays a role in nociception, has connections with PAG as part of descending modulatory circuitry (Rizvi et al. 1991; Carrasquillo and Gereau 2007). Researchers have hypothesised that the inability to activate these inhibitory mechanisms and/or over-activity of facilitatory mechanisms are some of the underlying drivers behind chronic pain (De Felice et al. 2011).

1.3.4 Patterns of Brain Activity in the Context of Experimental Pain

1.3.4.1 Neuroimaging Pain Experiments in Healthy Individuals

There is a vast literature of neuroimaging research evaluating coordinated changes in brain activity as they relate to pain. As discussed above, there are sensory-discriminatory, affective-motivational, and cognitive-evaluative components of the pain experience

(Melzack and Casey 1968). Below, I briefly summarise how these dimensions have been evaluated using neuroimaging and relevant networks that have emerged from this literature. It is important to note that these dimensions are complex and are often intertwined.

Treede and colleagues (1999) describe the sensory-discriminatory processes as comprising "stimulus localisation, intensity discrimination and quality discrimination". Neuroimaging work has demonstrated that changes in pain intensity are reflected in the thalamus, putamen, SI, SII, and insula (Coghill et al. 1999; Bornhovd et al. 2002; Kong et al. 2006; Oertel et al. 2012), with SI showing somatotopy (Bingel et al. 2004). There is evidence suggesting that the posterior insula appears to be more involved in encoding intensity changes than the anterior division (Oertel et al. 2012). Within the posterior insula, the dorsal segment has demonstrated pain intensity encoding over prolonged pain paradigms (Segerdahl et al. 2015).

Regions such as the ACC, insula, hippocampus, thalamus, and amygdalae have been implicated in the affective-motivational domain of pain (Lee and Tracey 2010; Apkarian, Bushnell, and Schweinhardt 2013; Wiech and Tracey 2013). Affective-motivational processes include the perception of pain unpleasantness. Further, fear and anxiety (Wiech and Tracey 2013) as well as depression and stress can alter pain perception, demonstrating their modulatory role. Measures of pain unpleasantness have been positively correlated with activity in the ACC and thalamus (Rainville et al. 1997; Villemure and Bushnell 2009). Suspecting harm from experimental stimulation heightens MCC and anterior insula connectivity as well as reported pain (Wiech et al. 2010). Ploghaus and

colleagues (2001) demonstrated that pain-induced activation in the hippocampal formation is increased by anxiety. Further, this activity was functionally connected with that of the perigenual ACC and insula. Pain unpleasantness and activity in the amygdala, prefrontal cortex, ACC, basal ganglia, and hippocampus are increased by acute depression (Berna et al. 2010). In otherwise healthy females, chronic stress can amplify activity in the thalamus, posterior cingulate cortex, PAG, and posterior insula during noxious visceral distension (Rosenberger et al. 2009).

Within the cognitive-evaluative dimension, attention and expectation paradigms have altered pain perception. Generally, the insular and prefrontal cortices as well as the brainstem appear to be critical regions in these processes (Wiech, Ploner, and Tracey 2008). Distraction has decreased pain-induced ratings and activation in the MCC, insula, and thalamus (Bantick et al. 2002) whilst increasing ACC, orbitofrontal cortex, and PAG activity (Bantick et al. 2002; Valet et al. 2004). Subjects who were tasked with assessing pain intensity demonstrated increased activation in regions including the ACC/mPFC and anterior insula (Kong et al. 2006). Further, negative expectation (negative placebo/nocebo) has heightened intensity ratings and pain-induced activation in the ACC and orbitofrontal cortex as well as the hippocampus, putamen, and insula (Kong et al. 2008).

1.3.4.2 Neuroimaging of Chronic Pain Patients

The methodology in the aforementioned studies (i.e. use of acute experimental pain stimulation) has been utilised to examine many chronic pain conditions including headache, chronic regional pain syndrome, arthritis and lower-back pain (Apkarian et al. 2005). Apkarian and colleagues (2005) underscore that, in those with chronic pain,

evoked-pain elicits more consistent activation in affective regions, including the PFC, whilst healthy individuals demonstrate more engagement in certain sensory regions including the thalamus, SI, and SII. Within the anterior insula, the location of pain-induced activation differs between those with and without chronic pain (Schweinhardt et al. 2006).

Tillisch and colleagues (2011) conducted a meta-analysis investigating differences in the brain response to rectal distension between male and female IBS patients and healthy individuals. There were some similarities to the results reported by Apkarian and colleagues (2005) but also certain differences. Those with IBS had more activations in the amygdala, midbrain and less in the putamen and SII. Location of activation within the thalamus and insular cortex differed between IBS patients and healthy controls.

When narrowing down the literature to CPP in females, it is more difficult to characterise relevant network changes because, as has been previously highlighted in a literature review, there are few neuroimaging studies the exclusively examine this population (Brawn et al. 2014). CPP has several important associated factors that potentially make it unique relative to other chronic pain conditions. Firstly, CPP engages of both somatic and visceral sensory systems (Morotti et al. 2014). Women with CPP may have concerns about their fertility and/or have difficulty conceiving. Further, some woman may experience pain during intercourse, which can be a source of relationship stress (Culley et al. 2013). Importantly, this population experiences significant diagnosis and treatment delays that can last many years (Grace and Zondervan 2004). Finally, women with CPP comprise a uniquely diverse patient population, characterised by numerous pain symptoms and underlying pelvic pathologies (Brawn et al. 2014).

Section 4

1.4 Chronic Pelvic Pain in Women

Pain is considered chronic when it continues beyond typical tissue healing, which is estimated to be 3-months. In chronic pelvic pain, the threshold is 6-months (Treede et al. 2015).

1.4.1 Peripheral Mechanisms of Endometriosis-Associated Pain

Endometriosis is a pathology where endometrial tissue exists outside the uterus. One proposed mechanism for the migration of this tissue is retrograde menstruation (Sampson 1927); one study reported that potentially 90% of their participants experienced this (Halme et al. 1984). However, it is suspected that there are more women who experience retrograde menstruation than develop endometriosis and that differences in the immune system are responsible for this divergence (Kyama et al. 2003).

Traditionally, it was believed that the principal mechanisms driving endometriosis-associated pain were compression and infiltration, thus surgical treatment was the ideal intervention. However, researchers are beginning to understand that there are other factors, including inflammation (Morotti et al. 2014), which cannot necessarily be resolved with surgery. There are inconsistent research findings with respect to inflammatory markers and pain. One study found that within a cohort of women with endometriosis, interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), platelet-derived growth factor (PDGF) were not correlated with the extent of disease or pain ratings (Overton et al. 1996). However, in a more recent study, Scholl and colleagues (2009) observed that in women with endometriosis, glycodelin and TNF-α were positively correlated with the pain severity of dysmenorrhoea. More research needs to be done to

determine the exact nature of the relationship between inflammation and pain in these women.

Important nociceptive receptors have also been examined in these patients. Studies have observed that transient receptor potential vanilloid type 1 (TRPV1) expression is higher in women with endometriosis-associated pain than in healthy women (Poli-Neto et al. 2009) and those with endometriosis but without pelvic pain (Rocha et al. 2011). However, endometriosis severity and receptor expression were not correlated (Rocha et al. 2011).

Shifting towards the patient experience, the relationship between endometriosis severity and pain is tenuous at best. Vercellini and colleagues (2007) observed that the severity of endometriosis and dyspareunia (pain with sexual intercourse) were not correlated. Dysmenorrhoea and non-cyclical pain did reach statistical significance; however, the authors were not convinced that this was a valid result. However, as always, there is one exception: the presence of deep infiltrating endometriosis (DIE). It is a more severe disease and is positively correlated with increased pain severity (Fauconnier and Chapron 2005).

1.4.2 Altered Nociception and Pain Perception in Women with Chronic Pelvic Pain

Much of the following section comes directly from my 2014 review (Brawn et al. 2014).

1.4.2.1 Experimental Pain at Sites of Pain Symptoms

Several studies have evaluated differences in the behavioural response to both somatic and visceral noxious experimental stimuli in women with CPP and healthy pain-free women (see Appendix, Tables 1 and 2 for complete summaries). Compared to healthy women, women with CPP demonstrate hyperalgesia when noxious stimulation is

delivered to their lower abdomen (Giamberardino et al. 1997; Bajaj et al. 2002; Vincent et al. 2011) and vulva (Pukall et al. 2002; Giesecke et al. 2004; Lowenstein et al. 2004; Pukall et al. 2005; Song et al. 2006; Schweinhardt et al. 2008; Sutton, Pukall, and Chamberlain 2009; Hampson et al. 2013) but not always the back (Bajaj et al. 2002, 2003). Similarly, women with CPP have shown increased behavioural response to both noxious and innocuous stimulation of their bladder (Ness et al. 2005; Powell-Boone et al. 2005) and bowel (Song et al. 2006; Brinkert et al. 2007; Wilder-Smith and Robert-Yap 2007; Elsenbruch et al. 2010; Piche et al. 2010). However, these findings are not consistent, as not all studies observed an increase in all measures of the pain experience (Giamberardino et al. 1997; Bohm-Starke et al. 2001; Bajaj et al. 2002; Pukall et al. 2002; Bajaj et al. 2003; Song et al. 2006; Brinkert et al. 2007; Wilder-Smith and Robert-Yap 2007; Sutton, Pukall, and Chamberlain 2009; Elsenbruch et al. 2010), whilst some studies did not observe any heightened response in women with CPP (Wilder-Smith et al. 2004; Ringel et al. 2008). Since many factors were different between the studies (i.e. inclusion criteria, differing experimental pain techniques, methods for determining pain thresholds, pain rating protocols, number of study visits, and sample sizes), it is likely that methodology contributed to some of the inconsistency.

1.4.2.2 Experimental Pain at Sites Distal to Pain Symptoms

Since testing the pain response at the site of the chronic pain complaint will not necessarily reflect alterations in the CNS (but might just reflect local changes), many studies have also evaluated the pain response at sites distal to the locations of their pain complaints. In the majority of studies in women with CPP, the hand or arm is the distal site, however, the leg has also been used. As above, the findings are not consistent, but increased behavioural responses to noxious stimulation at a distant site have been

demonstrated in women with CPP including in association with dysmenorrhoea (Giamberardino et al. 1997; Granot et al. 2001; Bajaj et al. 2002; Vincent et al. 2011), endometriosis (Bajaj et al. 2003; Laursen et al. 2005; He et al. 2010; Neziri et al. 2010; As-Sanie et al. 2012), IBS (Wilder-Smith and Robert-Yap 2007; Piche et al. 2010), IC (Clauw et al. 1997; Ness et al. 2005; Powell-Boone et al. 2005) and vulvodynia/provoked vestibulodynia (Granot et al. 2002; Pukall et al. 2002; Giesecke et al. 2004; Foster, Dworkin, and Wood 2005; Granot 2005; Powell-Boone et al. 2005; Pukall et al. 2006; Johannesson et al. 2007; Sutton, Pukall, and Chamberlain 2012; Hampson et al. 2013). Such observations are likely to reflect plastic changes in the CNS, leading to increased sensitivity to noxious stimuli (central sensitisation) (Latremoliere and Woolf 2009). Whilst there is some evidence that this increased sensitivity may in part be reversible after successful treatment of the underlying pathology (Bajaj et al. 2003; He et al. 2010), it does not appear to be purely a reflection of the presence of ongoing pain, as women with dysmenorrhoea demonstrated enhanced behavioural responses to noxious stimulation of both the abdomen and arm throughout the menstrual cycle and not just at the time they were experiencing pain (Vincent et al. 2011).

1.4.3 Central Mechanisms of Chronic Pelvic Pain

Much of the following section comes directly from my 2014 review (Brawn et al. 2014).

Using fMRI, it has been well documented that chronic pain patients process acute, experimental pain differently than healthy individuals both during pain episodes and in their pain-free states (Apkarian, Baliki, and Geha 2009); however, objective characterisation of the chronic pain state remains elusive. As previously described, to date, most of the research has focused on stimulation based responses in patient groups.

There has been a recent surge in imaging studies that measure resting brain activity in patients with chronic pain (Baliki et al. 2014; Kilpatrick et al. 2014; As-Sanie et al. 2016). While the research is far from conclusive in identifying objective markers of chronic pain in these patients, it has begun to highlight inherent central changes that are likely reflective of the chronic pain state.

1.4.3.1 Imaging Central Changes in Pain Processing

fMRI research has demonstrated that women with CPP have different central activation (in some cases, amplified) in response to noxious stimulation when compared to healthy women. The concept of a 'brain-gut axis' has been widely accepted for many years (Tillisch and Labus 2011), initially focusing on the conscious processes controlling defecation, but subsequently extended to functional bowel syndromes. It is therefore not surprising that IBS is the pelvic pain condition most investigated with neuroimaging (see Appendix, Table 3). A recent meta-analysis of the studies investigating differences in the brain response to rectal distension between individuals with IBS and controls found differences in activation of the amygdala, thalamus, prefrontal, anterior cingulate, and insular cortices (Tillisch, Mayer, and Labus 2011). When studies are limited to those only including female subjects, the findings are regionally similar, with rectal distension eliciting decreased activation in the amygdala and hippocampus (Wilder-Smith et al. 2004) and increased activation in the insular and prefrontal cortices (Elsenbruch et al. 2010) in women with IBS compared with healthy women. However, in the latter study, significance was lost when anxiety and depression measures were included, which fits with what is known about those regions' modulatory mechanisms. As before, however, these findings are not consistent in all reported studies (see Appendix, Table 3) including one study that found no differences; however, it only investigated regions within the cingulate cortex (Ringel et al. 2008). Two

studies have investigated central pain processing in women with vulvodynia/provoked vestibulodynia (Pukall et al. 2005; Hampson et al. 2013). In the first, pressure stimulation of the vulva elicited activation of similar pain regions (middle/posterior insula) between women with provoked vestibulodynia and healthy controls; however, the women with provoked vestibulodynia demonstrated significantly stronger activation in several of these regions (Pukall et al. 2005). Similar findings were reported by the second study (Hampson et al. 2013), with patients showing increased activation in the insula, cingulate cortices, and thalamus in response to both vulval and thumb stimulation compared with controls. Of note, this study also identified subtle differences in the central response to stimulation between vulvodynia patients with different clinical presentations (primary versus secondary and provoked versus unprovoked pain). This observation suggests that the mechanisms underlying the symptoms in vulvodynia may differ depending on the clinical phenotype and warrants further investigation. There has recently been an increased interest in dysmenorrhoea within the pain field (Berkley 2013), including the publication of two neuroimaging studies investigating pain processing in women with dysmenorrhoea (Tu et al. 2009; Vincent et al. 2011). Perhaps not surprisingly, the first of these (Tu et al. 2009), showed that brain metabolism during menstruation was different in women experiencing dysmenorrhoea compared with those whose periods were not painful. More strikingly, the second study showed that even when women with dysmenorrhoea were not experiencing pain (Days 10–12 and 20–22 of the menstrual cycle), their brain response to noxious stimulation was different from that of control women (Vincent et al. 2011). Thus despite requiring a lower temperature for stimulation to produce the same perception of pain intensity, there was increased activity in two important pain-processing regions, the inferior/middle temporal gyrus and left entorhinal

cortex (ERC), when compared with controls. Moreover, the strength of activation in the ERC during these non-menstrual phases was positively correlated with reported pain severity at menstruation. Whilst it is important to remember that these studies use a variety of noxious stimuli and utilize relatively small subject cohorts (although with sophisticated statistical methods), it is noteworthy that the mechanisms amplifying the experience appear to vary between pelvic pain conditions (see Appendix, Table 3).

1.4.3.2 Resting Functional Connectivity Changes

More recently, resting fMRI studies have demonstrated that women with CPP have altered spontaneous/resting activity when compared to healthy women. In patients with IC/PBS, both the sensory motor area and anterior paracentral lobule had increased connectivity with the cerebellum and midbrain (Kilpatrick et al. 2014). In women with endometriosis-associated CPP, increased glutamate-glutamine in the anterior insula guided a functional connectivity analysis that found that there was increased connectivity between the mPFC and anterior insula (As-Sanie et al. 2016).

1.4.3.3 Changes in Endogenous Pain Modulation

As previously outlined, the descending pain modulatory system is key in nociception and pain perception. Furthermore, it has even been suggested that the inability to activate these systems may predispose individuals to the development of chronic pain after an acute insult (De Felice et al. 2011). Dysfunctional pain inhibition has been demonstrated in a variety of chronic pain states (Tracey and Mantyh 2007; Tracey and Bushnell 2009). Some of the findings described above may represent abnormal pain inhibition, as many of the studies identify changes in the prefrontal cortices, amygdala and insula, which are key regions in endogenous modulation. Experimentally, there are techniques available to specifically probe the ability of an individual to engage these systems. One such strategy

that has been used to investigate these mechanisms in women with CPP is diffuse noxious inhibitory control (DNIC)/conditioned pain modulation (CPM). Maintained by nociceptive inhibitory processes, DNIC is a mechanism by which painful stimulation to one location (conditioning stimulus) decreases perceived pain elicited by a separate noxious stimulus (test stimulus) at a distant part of the body (Heinricher and Fields 2013). DNIC has been investigated in women with IBS and provoked vestibulodynia. In women with IBS, four studies found that DNIC/CPM paradigms resulted in decreased pain from the test stimuli in healthy controls, while not significantly decreasing pain from the test stimuli in in patients (Wilder-Smith et al. 2004; Song et al. 2006; Wilder-Smith and Robert-Yap 2007; Piche et al. 2010). The increased activity in the midbrain of individuals with IBS identified in the recent fMRI meta-analysis may well be a reflection of increased activity/dysfunction of the endogenous modulatory systems (Tillisch, Mayer, and Labus 2011). However, women with provoked vestibulodynia were shown to have similar endogenous pain modulation to healthy controls (Johannesson et al. 2007; Sutton, Pukall, and Chamberlain 2012). Thus, while studies are conflicting, the literature suggests that women with CPP likely have altered endogenous pain mechanisms; however, more research is needed to better characterise the nature of these changes.

1.4.3.4 Structural Changes in the Context of Chronic Pain

The previously discussed results all relate to functional changes seen using fMRI or PET; however, structural changes in the brain have also been observed when comparing many types of chronic pain patients to healthy individuals and, more importantly, when correlating results to clinical measures. The mechanisms behind these alterations in brain volume are not understood. It has been suggested that these changes could represent direct neurotoxic effect of repeated episodes of pain leading to neuronal atrophy or

death, an effect of altered metabolic activity or neurotransmitter concentration, neurotoxic effects of drugs, and an effect related to psychological correlates such as anxiety and depression; however, further research is needed to elucidate the true meaning of these types of measures (May 2008, 2011).

Perhaps surprisingly, many chronic pain conditions have been associated with a reduction in brain volume. Of particular note, these changes are not global across the brain but occur in specific regions, which are functionally involved in the processing of pain (May 2011). Morphological studies exist for a variety of non-pelvic chronic pain conditions including chronic lower back pain, fibromyalgia and headache. Grey matter volume in the anterior cingulate cortex is reduced in almost all these conditions; however, reductions are also commonly seen in the volume of other regions particularly the thalamus, insula and prefrontal cortices (May 2011). In many cases the volume of specific brain regions correlated with the duration of the symptom suggesting that these volumetric changes are a consequence rather than a cause of the pain. Volumetric studies have also been undertaken in women with dysmenorrhoea, provoked vestibulodynia, and CPP, both without an obvious cause, and related to endometriosis and IBS (see Appendix, Table 4). Interestingly, although decreases in grey matter were observed in many pain processing regions (SII, prefrontal cortex, thalamus, insula, and putamen) areas with an increased volume were also observed in the midbrain/brainstem (including PAG), amygdala, caudate, hippocampal gyrus, hypothalamus, and ACC (Schweinhardt et al. 2008; Blankstein et al. 2010; Seminowicz et al. 2010; Tu et al. 2010; As-Sanie et al. 2012). Although these increases were observed in different brain regions from those where reductions in volume have been observed in other conditions, these regions are also

known to be involved in the processing of noxious stimuli. A number of reasons have been proposed to explain why increases in brain volume are seen with these specific pain conditions in contrast to the other conditions investigated, including the fact that the patients are generally younger, pain is intermittent/cyclical/provoked as opposed to continuous and that most women with these conditions do not use large doses of pain modifying drugs for a prolonged time period (Schweinhardt et al. 2008). Of particular interest is the recent elegantly designed study by As-Sanie and colleagues (2012). They investigated twenty-three women with CPP with and without laparoscopically proven endometriosis, in addition to women in whom endometriosis had been found but who did not suffer with CPP and compared each group to healthy pain-free controls. As expected, the women with CPP (whether or not there was endometriosis present) had reduced brain volume in the thalamus, and moreover there were also reductions in the cingulate, insula and putamen in those with endometriosis-related CPP. However, the more interesting finding was that women with endometriosis but without CPP had an increase in the volume of their periaqueductal gray (PAG). Furthermore, the volume of the PAG in these women correlated positively with the amount of pressure required to induce pain (i.e. the bigger their PAG the more pressure they could tolerate before reporting pain). The authors suggest that this may be due to the PAG's role in descending pain inhibition and potentially may explain why some women with endometriosis have little or no pain despite a significant burden of disease.

1.5 Conclusions

I have outlined the neurobiological mechanisms behind the transmission of nociceptive input from the periphery to the CNS. Further, I described the dynamic nature higher-level processing that shapes pain perception. Within CPP, there is evidence suggesting that alterations in both peripheral and central mechanisms contribute to the presence of chronic pain in these patients. This dissertation aims to characterise these changes by first exploring functional connectivity analyses methodology to examine the nature of potential changes in healthy individuals with experimentally-induced central sensitisation. Subsequently, I apply what is learned to resting fMRI data acquired from women with CPP. Given the unique challenges associated with CPP, I hypothesise that characteristics of the pain experience, such as delayed pain management and neuropathic components, will be reflected in relevant central networks.

In the following chapter, the experimental methods used in this dissertation are described. Following this methodological overview, there are six experimental chapters. Chapter 3 presents some of my early functional connectivity analyses examining experimentally-induced central sensitisation in healthy individuals. Chapter 4 introduces the patients that will be included in the subsequent imaging analyses with particular focus on the heterogeneity of symptoms in CPP. In Chapter 5, the neuropathic components of CPP (originating from peripheral or central nerve damage) are examined. Specifically, a functional network analysis is conducted to determine whether neuropathic-associated centrally sensitised states are modulated differently within this population. In Chapter 6, due to the delay in pain treatment experienced by women with CPP, the central impact of longer pelvic pain duration is measured in resting functional networks. Given effective

treatments for CPP (both with and without endometriosis) are lacking, Chapter 7 includes a preliminary examination of the impact of surgery on clinical measures (including pain and psychological scores) and descending modulation. Finally, in Chapter 8, pilot data from the University of Edinburgh's gabapentin trial is analysed to explore whether pharmaceutical intervention with gabapentin is effective in altering the relationship between central pain networks and the pain experience.

Chapter 2

Methods

2.1 Introduction

This dissertation comprises data from three studies: central sensitisation in healthy individuals, the Oxford chronic pelvic pain study (EndoPain), and the Edinburgh gabapentin trial. I begin this section by describing the questionnaires and tools used to acquire behavioural and psychological measures. Following this, I describe the methods for magnetic resonance imaging (MRI) acquisition, background on resting fMRI, and analysis of imaging and non-imaging data.

All the studies included in this dissertation received ethical approval. All participants provided informed consent before research procedures commenced. The imaging research involving healthy participants was approved by the National Research Ethics Service (NRES) South Central Hampshire - B Committee (Reference: 11/SC/0249). The Oxford pelvic pain clinical study was approved by the NRES South Central - Oxford B Committee (Reference: 12/SC0371) and the Edinburgh gabapentin trial was approved by the Scotland- A Committee (Reference 12/SS/0005).

2.2 Behavioural Measures

2.2.1 The Numerical Rating and Visual Analogue Scales to Quantify Pain

In the following experiments, both the visual analogue scale (VAS) and the numerical rating scale (NRS) were used to capture pain ratings. VAS scales do not contain quantitative information (i.e. no numbers) but rather descriptive anchors at the ends for reference. The anchors used in the healthy imaging and Edinburgh gabapentin

experiments were "No Pain" and "Worst Pain Imaginable". The NRS is slightly different in that it has both quantitative and qualitative anchors at each end. The scales used in EndoPain were anchored by "0 - no pain" and "10 - worst pain imaginable", with each tick mark between indicating the value from 0 - 10 (increasing by 1). In those with chronic pain, ratings on the NRS have proven to be the most consistent across multiple sessions when compared to the VAS or verbal ratings (Ferraz et al. 1990).

2.2.2 Validated Questionnaires

2.2.2.1 PainDETECT

PainDETECT is a questionnaire that quantifies neuropathic components of pain. It was created by Freynhagen and colleagues (2006) and validated on 392 lower back pain patients. PainDETECT scores can be separated into three categories: nociceptive (0 - 12), unclear (13 - 18), and neuropathic (19 - 38). The nociceptive category indicates that there is less than 15% probability that neuropathic components are present. The unclear category indicates that there could be neuropathic components but no conclusions can be made. In the neuropathic category, there is over a 90% chance that neuropathic components exist (Freynhagen et al. 2006). The questionnaire has been used in conjunction with fMRI research, with scores correlated with pain-related signal changes (Gwilym et al. 2009).

Freynhagen and colleagues (2006) reported that PainDETECT had 85% sensitivity (correctly diagnosing those with neuropathic pain) and 80% specificity (correctly excluding those without neuropathic pain from the neuropathic category). In a review of neuropathic pain questionnaires, Bennett and colleagues (2007) indicate that PainDETECT has comparable specificity and sensitivity to these other clinical tools. Specifically,

PainDETECT has higher sensitivity and specificity than the Neurophysiology of Pain (NPQ) and is equal in both measures to the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS). However, the Douleur Neuropathique 4 (DN4) has demonstrated higher specificity than PainDETECT (90% but sensitivity was lower at 83%). However, the authors highlight that an important advantage of certain questionnaires, including PainDETECT, is the ability for it to be completed by patients without the need for a physical exam as required by the Douleur Neuropathique 4 (DN4) and LANSS (Bennett et al. 2007).

2.2.2.2 Pain Catastrophising Scale

The Pain Catastrophising Scale quantifies rumination, magnification, and helplessness with respect to pain (Sullivan, Bishop, and Pivik 1995). It was developed using a cohort of Nova Scotian employees (both men and women) who were injured at work. Scores range from 0 - 52, with 30 being the threshold for what the authors concluded to be "clinically relevant" catastrophising (75th percentile). Sullivan and colleagues (1995) found that of those who scored at or above 30, over 60% had moderate depression, were unemployed, and reported pain-related disability.

2.2.2.3 State-Trait Anxiety Inventory

The State-Trait Anxiety Inventory (STAI) is a validated questionnaire that allows for the quantification of anxiety at the moment of the questionnaire (State) and inherent/static levels of anxiety (Trait) (Spielberger 1983). Scores range from 20 - 60.

2.2.2.4 Beck Depression Inventory

The Beck Depression Inventory (BDI) quantifies the severity of depression (Beck et al. 1961; Beck 1967; Beck, Steer, and Brown 1996). In this dissertation, the second version of the BDI is used. It is also known as the BDI-II but, for simplicity in this thesis, it is referred to as the BDI or Beck Depression Inventory) (Beck, Steer, and Brown 1996). It is scored on

a scale from 0 - 63 and score categories can be broken down into minimal (0 - 9), mild (10 - 18), moderate (19 - 29), and severe (30 - 63) depression.

2.2.2.5 Pain Vigilance and Awareness Questionnaire

The Pain Vigilance and Awareness Questionnaire (PVAQ) measures how attentive an individual is to pain by evaluating characteristics of awareness, vigilance, and preoccupation (McCracken 1997). The score can be broken down into sub-scores that evaluate "attention to pain" and "attention to changes in pain".

2.2.3 Research Studies

2.2.3.1 Healthy Pain Imaging (3T and 7T)

Data from this study was collected by a former PAIN Group student (Melvin Mezue); however, all the analyses are my work. This study involved three pseudo-randomised MRI visits: two 7T fMRI scans involving two conditions (sham or capsaicin cream) and one 3T fMRI involving capsaicin. State-Trait Anxiety Inventory scores were collected, with State Anxiety being measured at every visit.

2.2.3.2 EndoPain

This study comprised four visits: pre-surgical baseline and post-surgical follow-ups (6-weeks, 3-months, and 6-months). Two of the visits involved MRIs (baseline and 6-month follow-up). Our research midwife, Lisa Buck, was instrumental in recruiting and coordinating these visits. She also conducted the 6-week and 3-month follow-ups independently since they involved visiting the patients. At each of the four study visits, patients were asked to rate their different pain symptoms (dysmenorrhoea, dyspareunia, non-cyclical pain, dyschezia (pain when opening bowels), dysuria (pain with urination)) on an NRS. They were also asked to rate their pain intensity to quantify their overall pain experience (referred to as "average overall pain intensity") by rating "how strong was the

pain over the past 4 weeks on average". Space was also available where patients could write freely. Answers were evaluated from the prompt: type(s) of pain "bothers you most".

Additionally, patients completed the State-Trait Anxiety Inventory, Beck Depression Inventory, Pain Catastrophising Scale, PVAQ, and PainDETECT questionnaire at each visit. The women also completed a surgical expectation questionnaire at the baseline visit where they were asked about how they thought their pain symptoms (dysmenorrhoea, dyspareunia, and non-cyclical pain) would improve. Additionally, it asked how long they believed the surgery would improve their overall health and how long the pain changes would last.

2.2.3.3 Edinburgh Gabapentin Trial

This data was acquired by our collaborators at the University of Edinburgh. I assisted in setting up the scan sequences and pain paradigms remotely. The trial comprised four visits: pre-medication baseline and post-medication follow-ups (6-week MRI, 3-months, and 6-months). Patients in this trial recorded their pain intensity on a VAS at every visit with the exception of the 6-week MRI. Other behavioural measures were collected; however, due to the complex nature of trial databases, I did not gain access to this data in time for inclusion in this dissertation.

2.3 Magnetic Resonance Imaging

2.3.1 Functional Magnetic Resonance Imaging with Blood-Oxygen-Level Dependence

fMRI with blood-oxygen-level dependent (BOLD) signal largely relies on two components: water-based hydrogen and the changing magnetic properties of haemoglobin. The static

magnetic field (B_0) aligns the hydrogens along one axis. When they are excited by a radio-frequency (RF) pulse, their spin can be measured by the scanner hardware.

Deoxyhaemoglobin is paramagnetic and thus strongly interacts with B_0 whilst oxyhaemoglobin is weakly diamagnetic and does not interact with the magnetic field as strongly. Thus, deoxyhaemoglobin induces stronger distortions in the magnetic field compared to oxyhaemoglobin.

Increasing neuronal activity results in an increase in oxyhaemoglobin to the region. Therefore, an increase in the ratio of oxygenated to de-oxygenated blood decreases the relative distortions, which results in increased signal, due to slower relaxation of the hydrogens in the surrounding tissues. This increased signal is thought to be an indirect measure of increased neuronal activity in those regions (see Figure 2.01).

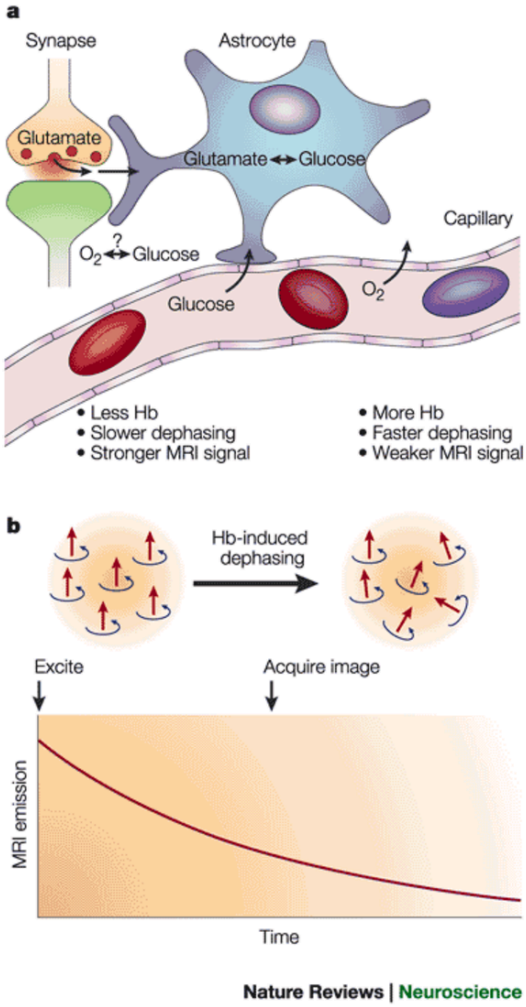


Figure 2.01 fMRI-BOLD Signal and Neurovascular Coupling

Changes in fMRI-BOLD signal rely on haemoglobin-driven changes in field homogeneity. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Neuroscience Heeger and Ress (2002), copyright 2002.

2.3.2 Resting fMRI-BOLD: Measuring Spontaneous Brain Activity

The majority of fMRI research utilises repetitive task or stimulation paradigms to understand the brain's functional dynamics in specific contexts. However, over the past two decades there has been growing interest in spontaneous brain activity (or brain activity unrelated to experimental stimuli): resting or resting-state fMRI (Cole et al. 2010). This activity is acquired when a subject is not attending to a specific task in the scanner (i.e. just lying there and ideally not asleep) and slow oscillations in the haemodynamic response are measured (Biswal et al. 1995).

Networks of resting activity are a commonly referred to as resting state networks (RSNs). RSNs include regions that, over the course of fMRI acquisition, have correlated activity. Importantly, regions in these networks can also be in separate areas of the brain (De Luca et al. 2006) (see Figures 2.02 & 2.03). Many of these networks are consistent between individuals and thus were thought to reflect biologically-relevant inherent neural activity (Damoiseaux et al. 2006). Indeed, changes in RSNs have been correlated with disease (Filippini et al. 2009) including chronic pain (Baliki et al. 2014).

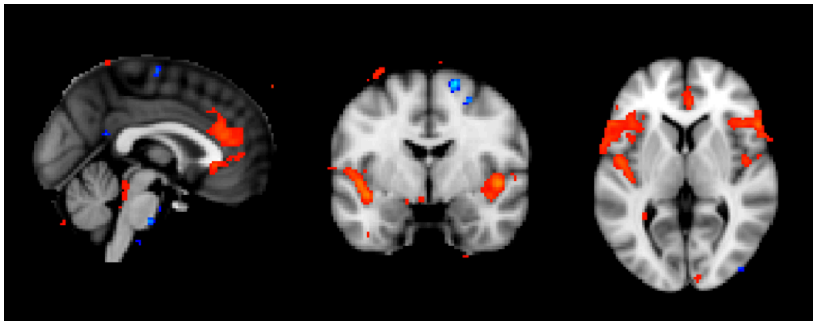


Figure 2.02 Resting State Network at 3T

Example of a resting state network from a small cohort of subjects at 3T. This network represents BOLD-derived brain activity when the individuals are not attending to a specific task and no experimental stimulation taking place.

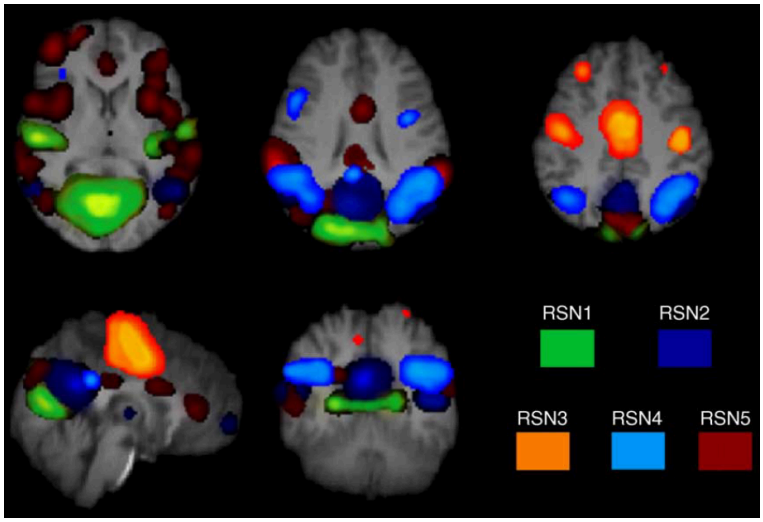


Figure 2.03 Resting State Networks

fMRI research has demonstrated that there are multiple resting state networks in the brain. Individual networks can comprise many regions — even those that are not proximal to each other. Figure from De Luca and colleagues (2006).

Cole and colleagues (2010) highlight that these RSNs can be generated from various analyses pipelines, including "data-driven" methods, such as independent component analysis (ICA). However, some studies examining RSNs do not necessarily utilise networks generated by ICA but instead from correlations with defined regions of interest (ROI) — ones that are known to be important to the neurobiological question at hand (Biswal et al. 1995; Cole, Smith, and Beckmann 2010). For example, the BOLD timeseries can be extracted from a specific ROI and then correlated with activity across the entire brain (seed-based global connectivity). This seed-based method is utilised to analyse the data in Chapter 3.

The majority of data in this dissertation is analysed by defining a specific network of ROIs. These self-defined ROIs are then examined for potential correlations in resting activity, but only within this specific network (similar methodology can be found in work by Wiech and colleagues (2014)). All the ROIs in this dissertation are structurally-defined (i.e. creation of a 3-dimensional mask of a structure). Further technical detail relevant to analysis of resting data will be discussed later in this chapter.

In the neuroimaging community, there is debate over whether certain types of neural activity can be defined as actual "resting" activity. Cole and colleagues (2010) highlight that researchers occasionally use the term "resting" in contexts where it may not typically be deemed appropriate. For example, a chronic pain patient might always be experiencing pain, thus, within the context of pain, his/her brain activity might not ever be at "rest". In this dissertation, there are cases where the subject or patient may not be truly at "rest": a healthy subject has capsaicin his/her leg (Chapter 3), a patient is/could be experiencing

pelvic pain (Chapters 6 - 8) or is undergoing pharmaceutical treatment (Chapter 8). However, Cole and colleagues (2010) argue that there is diminished importance in distinguishing between these quasi-resting and actual resting states. Furthermore, there is no difference in analysis methodology since 1) the haemodynamic response is not modelled on repeated stimulation during the scan and 2) the oscillating neural activity is akin to what is reported by the early resting studies (Biswal et al. 1995; De Luca et al. 2006; Cole, Smith, and Beckmann 2010). As such, for the sake of simplicity and to distinguish from event-related fMRI paradigms, I use the term "resting" for the fMRI data presented in the following experimental chapters.

2.3.2.1 General Linear Model and Threshold for Significance

FSL (FMRIB's Software Library) utilises the general linear model (GLM) as its statistical framework (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM). There are many methods of interrogating data using the GLM. In this dissertation, the following models have been employed for fMRI analysis: single-group paired difference (paired t-test), two-group difference (unpaired t-test), and single group average with a covariate of interest. Paired t-tests allow for examination of a group of subjects during two conditions (capsaicin vs. sham cream) or in response to an intervention (pre- vs. post-surgery). Unpaired t-tests compare two groups of different subjects (short vs. long duration of pain). Using a model for a covariate of interest allows for the assessment as to whether a continuous measure (pain intensity, for example) is related to relevant fMRI signal.

Depending on the statistical tools used, either the reported p-value threshold (alpha, $p < 0.05$) or the p-values are corrected for multiple comparisons. In this dissertation, they will be denoted by p(corr) (for corrected p-value or threshold). The details for the corrections

are described later in this chapter and in the following experimental chapters. Correcting for multiple comparisons is an important statistical principle because it adjusts the alpha or p-value when multiple statistical tests are run, which reduces the likelihood of false positives (null hypothesis is incorrectly rejected).

2.4 MRI Acquisition

All imaging data, with the exception of the gabapentin trial, were acquired at the Oxford Functional Magnetic Resonance Imaging of the Brain (FMRIB) Centre and Acute Vascular Imaging Centre (AVIC), Oxford, UK. The imaging data from the gabapentin trial were acquired at the Clinical Research Imaging Centre (CRIC) at the University of Edinburgh, Edinburgh, UK.

2.4.1 Healthy Subject Imaging (University of Oxford)

For the healthy subject imaging experiments, data were acquired by Dr. Melvin Mezue at the FMRIB 7T Siemens MRI and 3T Siemens Verio MRI scanners (Siemens Medical Systems, Erlangen, Germany). This data includes some of the very first to be collected on the 7T at FMRIB. FMRIB has two scanners and for comparison purposes, a similar dataset was collected on the 3T. The temporal signal-to-noise ratio (tSNR) of the 3T data was significantly higher than that of the 7T data, which is discussed in Chapter 3. The original study by Dr. Mezue had specific interest in the brainstem. Indeed, there was more distortion in the brainstem at 7T than 3T but this was successfully corrected using fieldmaps to allow for proper evaluation of activity in the PAG.

Data collection at 7T employed a multi-element receive and transmit head coil, whilst at 3T, a 32-channel receive-only head coil was used. The 7T MRI acquisition consisted of the

following parameters: 91 whole-brain volumes, voxel size 2x2x2mm, 61 slices, time of repetition (TR) 4000ms, echo time (TE) 25ms, flip angle 90°, bandwidth 1568 hertz per pixel (Hz/Px), matrix size 110x110 and 61 slices, parallel imaging (acceleration factor of 2). The 3T MRI acquisition consisted of the following parameters: 91 whole-brain volumes, voxel size 2x2x2mm, 61 slices, TR 4000ms, TE 30ms, flip angle 90°, bandwidth 1628 Hz/Px, 96x96 and 61 slices, parallel imaging (acceleration factor of 2). Field maps were acquired to correct field inhomogeneity.

2.4.2 EndoPain (University of Oxford)

For the Oxford chronic pelvic pain imaging study, data were acquired on 3T Siemens Verio MRI scanners at both FMRIB and AVIC (Siemens Medical Systems, Erlangen, Germany). All imaging data was collected using a 32-channel receive-only head coil.

2.4.2.1 fMRI-BOLD Data

Each acquisition consisted of 250 whole-brain volumes, 3x3x3mm voxel size, TR 2410ms, TE 30ms. Field maps were acquired to correct field inhomogeneity. All subjects were instructed to keep their eyes open to stay awake during the scan.

2.4.3 Chronic Pelvic Pain Gabapentin Trial (University of Edinburgh)

For the gabapentin imaging trial, data were acquired at the Clinical Research Imaging Centre (CRIC) 3T Siemens Verio MRI scanners (Siemens Medical Systems, Erlangen, Germany). Each acquisition was 200 whole-brain volumes, 3x3x3mm voxel size, 45 slices, TR 2500ms, TE 30ms. Field maps were acquired to correct field inhomogeneity. Subjects were instructed to keep their eyes open for the scan.

2.5 Analysis of MRI Datasets

fMRI-BOLD analysis was carried out using FSL (www.fmrib.ox.ac.uk/fsl). I investigated conducting power calculations with the FMRIB analysis team; however, it was decided that they would be of limited benefit. Specifically, it was highlighted that there is debate and uncertainty on how to appropriately conduct power calculations for analyses using tools employed in this dissertation, such as FSLNets, which is relatively new. Indeed, further exploration in the literature revealed that few functional connectivity studies conduct power calculations. Unlike task-based fMRI studies, there is no neuroimaging-specific toolbox, such as fmripower (Mumford and Nichols 2008), that conducts accurate calculations for resting functional connectivity fMRI data. Thus, when these experiments were designed, power calculations were not conducted for the resting connectivity analyses. This is a methodological limitation that is linked with the use of new analysis tools in this dissertation.

2.5.1 Pre-Processing

Functional images were motion corrected using MCFLIRT (Jenkinson et al. 2002) and slice-timing corrected. Brain Extraction Tool (BET) removed non-brain tissue (Smith 2002) and images underwent spatial smoothing (Gaussian kernel of FWHM 5mm). Registration of functional images (fMRI data) was performed using FMRIB's Linear Image Registration Tool (FLIRT) (with boundary-based registration (BBR)) transforming fMRI data into structural and standard space (Jenkinson and Smith 2001; Jenkinson et al. 2002). Additionally, nonlinear registration (FNIRT) was employed to improve standard space registration (Andersson, Jenkinson, and Smith 2007). Following this, MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) Version

3.14 was run to perform Probabilistic Independent Component Analysis (ICA) on the resting fMRI data (Beckmann and Smith 2004).

2.5.2 Removal of Physiological and Other Confounding Noise

All fMRI data had confounding noise (cardiac, respiratory, movement) removed using one of two methods. In most cases, noise was removed using ICA-based Xnoiseifier Version 1.06 (FIX) (Griffanti et al. 2014; Salimi-Khorshidi et al. 2014). FIX is an automated independent component based noise removal tool. Using pre-trained data, FIX examines independent components generated by MELODIC and removes networks classified as noise (movement in the scanner, signal in white matter, cardiac and respiratory rhythms). In experiments where cardiac and respiratory traces where collected during the scan (and were useable), de-noising was performed using Physiological Noise Modelling (PNM) (Brooks et al. 2008).

2.5.2.1 7T Data (Healthy Subjects)

For the 7T datasets physiological traces were collected; however, several were corrupted and inconsistent inclusion would potentially confound higher-level analyses. Given that the 7T fMRI is particularly vulnerable to physiological noise, I wanted to avoid these biases and opted for the consistent de-noising across all subjects using using ICA-based Xnoiseifier (FIX).

However, given that 7T data is still not ubiquitous, no training data was available. As such, the independent components were classified by examining the spatial patterns and time series (shape and frequency) for each component (see Figure. 2.04). The pipelines for the automated and hand-classification of noise components are similar, with the latter being more labour intensive. The hand-classification requires each component to be classified

for each subject, which is where these two methods differ (for the program interface to conduct this step, see Figure 2.05). Components are either identified as "signal" or one of the many specific classifications of "noise" (for example, white matter or movement). Components that are labelled "signal" are kept and anything with a "noise" classification is removed.

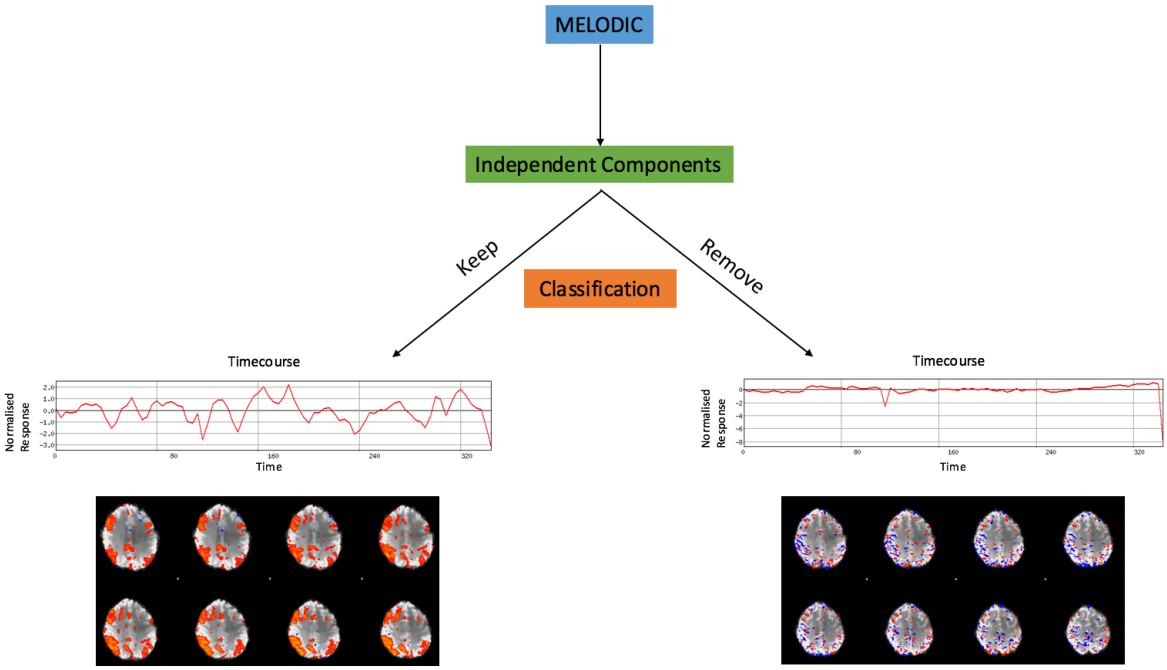


Figure 2.04 Pipeline for De-Noising Using FIX

Flow chart of de-noising using FIX. The data presented in this flow chart is from the hand-classification at 7T. MELODIC generates independent components that can be classified manually or by an automated system. Using either method, components can either be kept as "signal" or removed as "noise". In the schematic above, the independent component under "Keep" was classified as signal since it appears to be a potentially biologically-relevant component. The time series is fluctuating continuously and there is a coherence to the activation patterns. The independent component under "Remove" is an example of a component that was considered noise from movement of the subject in the scanner. There is one sharp dip in the time course and the "activation" patterns align with the sulci.

Some noise is easily identifiable by simply looking at spatial activation patterns. For example, rings of activation, particularly at the front or back of the head or around sulci, are very likely to be head movement (see Figures 2.04 & 2.05). However, noise can also be identified by examining the time series. A sudden, sharp spike would be suggestive of movement as well (see Figure 2.04). Further, there can be artefacts related to blood vessels (sagittal sinus) (Griffanti et al. 2014; Salimi-Khorshidi et al. 2014) (see Figure 2.06).

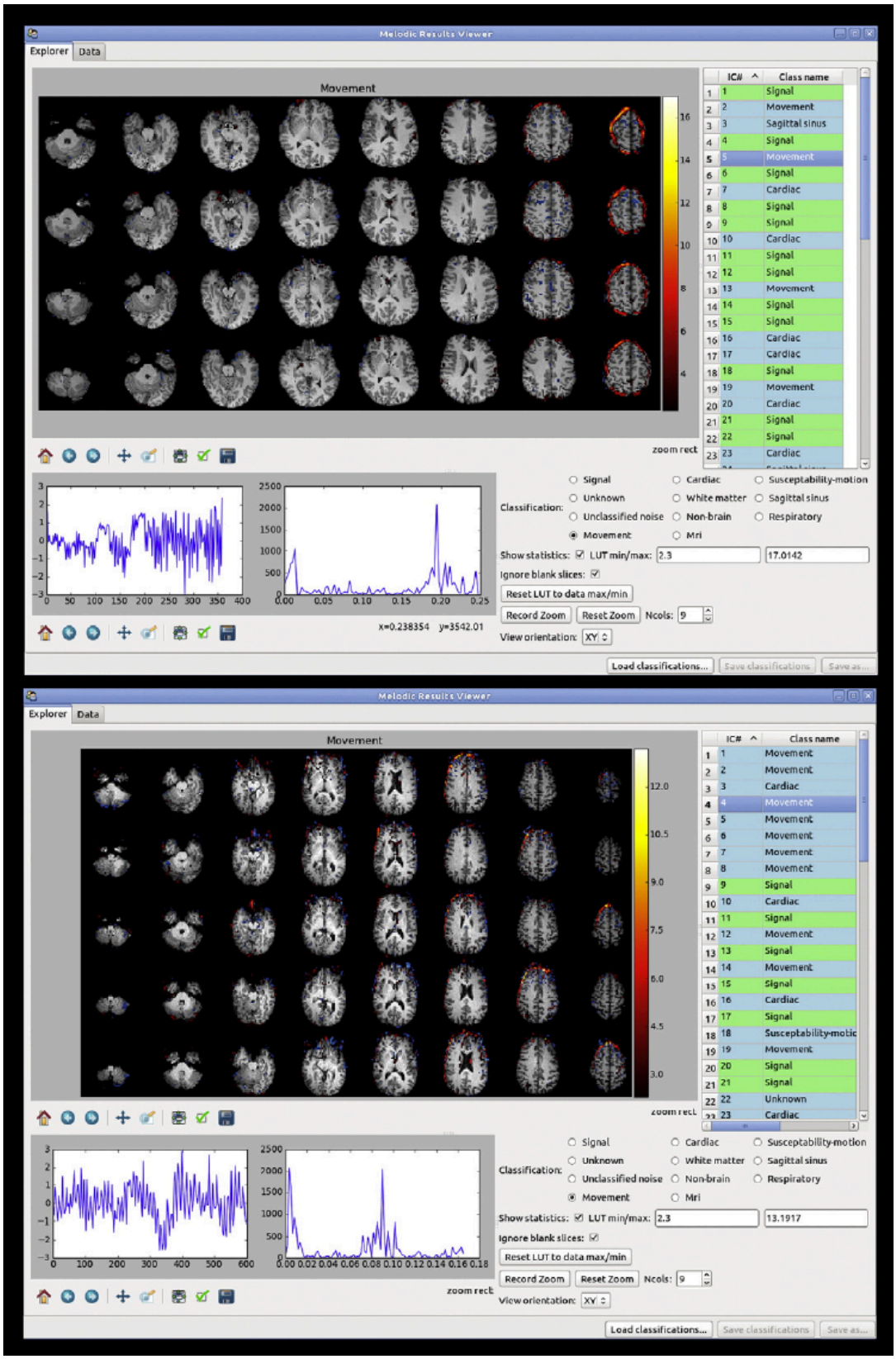


Figure 2.05 Hand-Classification of Noise using FIX - Movement

This figure includes hand-classifications of noise from the publication detailing the use of FIX. The bottom left graph is an illustration of the timeseries. This component is thought to be related to sagittal sinus. Figure from Salimi-Khorshidi and colleagues (2014).

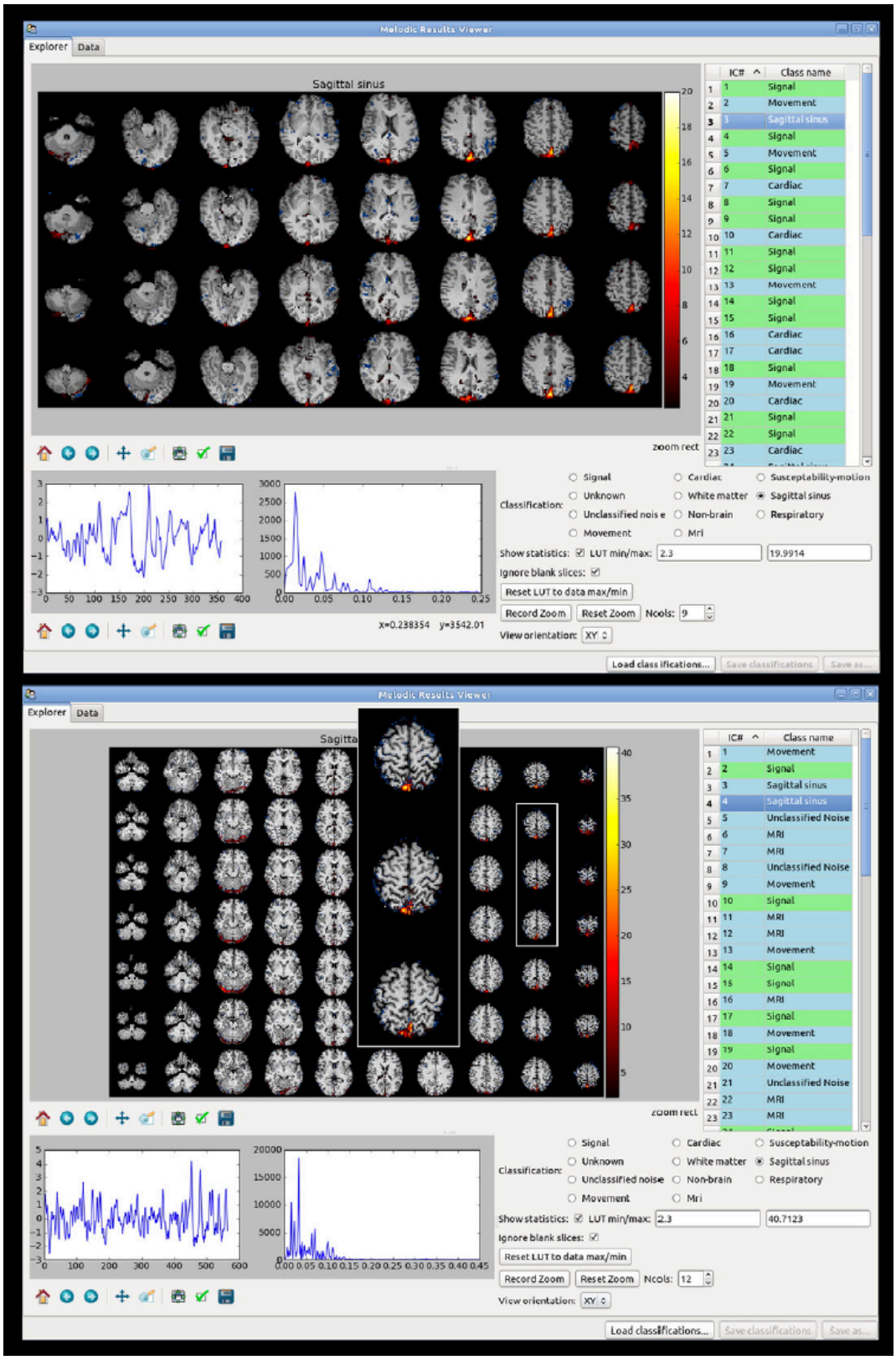


Figure 2.06 Hand-Classification of Noise Using FIX - Sagittal Sinus

This figure includes hand-classifications of noise from the publication detailing the use of FIX. The bottom left graph is an illustration of the timeseries. This component is thought to be related to sagittal sinus. Figure from Salimi-Khorshidi and colleagues (2014).

Classifications were performed at the guidance of the published paper describing FIX (Griffanti et al. 2014; Salimi-Khorshidi et al. 2014). As I was learning, some were verified with a member of the FMRIB analysis team to confirm their accuracy. Importantly, pre- and post-cleaning data quality changes were not assessed quantitatively in this dataset. However, care was taken to follow protocol for FIX, which demonstrated effective de-noising capabilities during its creation and validation (Griffanti et al. 2014; Salimi-Khorshidi et al. 2014). Again, to highlight the process, examples of components deemed "noise" can be found in Figures 2.05 and 2.06 (Salimi-Khorshidi et al. 2014).

2.5.2.2 3T Data (Healthy Subjects)

For the 3T healthy subject data, since all physiological traces were intact, noise was removed using PNM (Brooks et al. 2008). The physiological traces were prepared and entered into FEAT (FMRI Expert Analysis Tool, Version 6.00, part of FSL) as covariates of no interest (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT).

2.5.2.3 3T Data (All Patient Datasets)

Components identified as noise were systematically removed using FIX (threshold 20). When using FIX's automated process (i.e. not manually), the creators suggest using noise removal thresholds between 5 and 20, with lower thresholds resulting in less noise removal. There is no threshold that is ideal for all datasets and the user guide emphasises that the data should be inspected to ensure the threshold is correct and all obvious noise is removed whilst biologically-relevant components remain (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIX/UserGuide). In the patient datasets, a threshold of 20 was employed to ensure thorough noise removal. The data was inspected after running FIX. Again, pre- and post- data cleaning was not quantitatively assessed but the

data were inspected to ensure all obvious noise was removed whilst biologically-relevant components remained (see Figure 2.04).

2.5.3 Functional Connectivity Analysis

Two analysis pipelines were used for the fMRI-BOLD data. The first involved examining global connectivity changes with respect to a region of interest (ROI) (or seed-based connectivity changes). This initial approach allowed for the exploration of connectivity changes with the whole brain. The second method involved choosing multiple ROIs and examining the connectivity changes within this defined network. Given the a priori ROI inclusion, this second approach allowed for more specific questions to be answered. Further details of the scientific justification behind the choice of each analysis pipeline is described in the relevant experimental chapters. The methodology for each pipeline is described in detail below.

2.5.3.1 Analysis 1: Global Connectivity Changes with Respect to ROI (Seed-Based Connectivity)

Three healthy subject imaging analyses were performed using this pipeline. One involved the amygdalae as seeds whilst the other used the anterior and posterior insula as seeds of interest.

Amygdala Seed Mask Creation

Individual amygdala masks were created from each subject's structural image using FSL's sub-cortical segmentation tool (FIRST) (Patenaude et al. 2011). These masks were transformed into functional space. Each mask was restricted to a threshold of 50% and binarised (making the entire mask homogenous). Since dropout in the amygdala is common, the masks were modified to not include regions with constant time series values of zero. If any dropout occurred within the masks, it was only by a few voxels. I worked

with members of the analysis team to ensure the fieldmaps corrected the distortions appropriately. Each mask was examined to ensure it encompassed the amygdala. Further, masks were visually compared to those referenced in other published 7T fMRI studies (van der Zwaag et al. 2012; Sladky et al. 2013). Boubela and colleagues (2015) have argued that fMRI studies examining the amygdalae are potentially capturing signal changes associated with the basal vein of rosenthal (BVR). In this dissertation, the necessary images were not acquired to identify the vessels of interest (high spatial resolution T2* weighted images). However, with reference to the work of Boubela and colleagues (2015) (see Figure 2.07) and assistance from Professor Stuart Clare (FMRIB), I examined whether the fieldmaps successfully corrected image distortion, which would allow for the successful avoidance of the BVR. T1-weighted structural images were compared to the T2*-weighted functional images to examine both placement of the amygdalae masks and the BVR, as located on the high resolution structural images (see Figure 2.08).

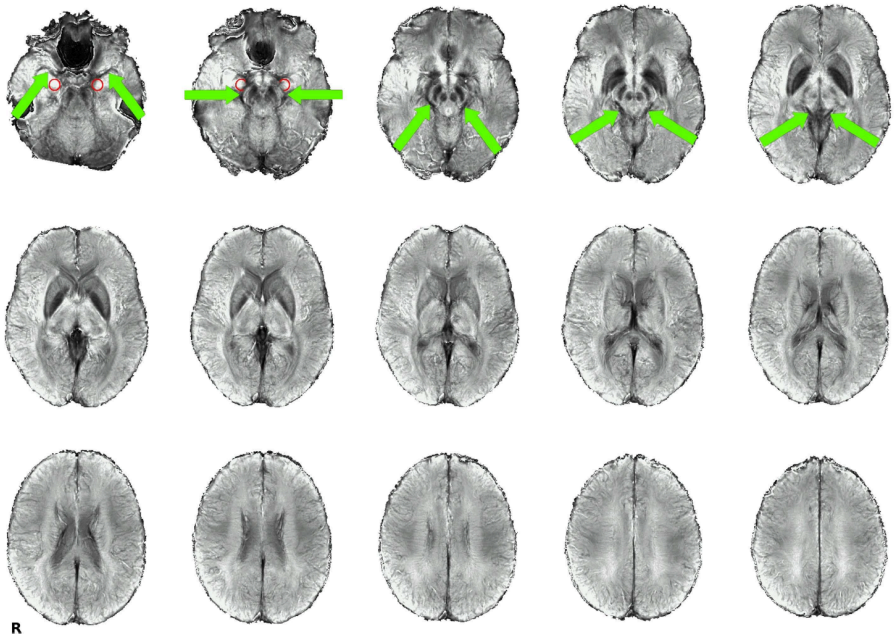


Figure 2.07 Locating the Basal Vein of Rosenthal

High-resolution T2 weighted structural images allow for confident identification of the basal vein of rosenthal (BVR). The green arrows point to the (BVR). The red circles are referencing the amygdalae. Figure from Boubela and colleagues (2015).*

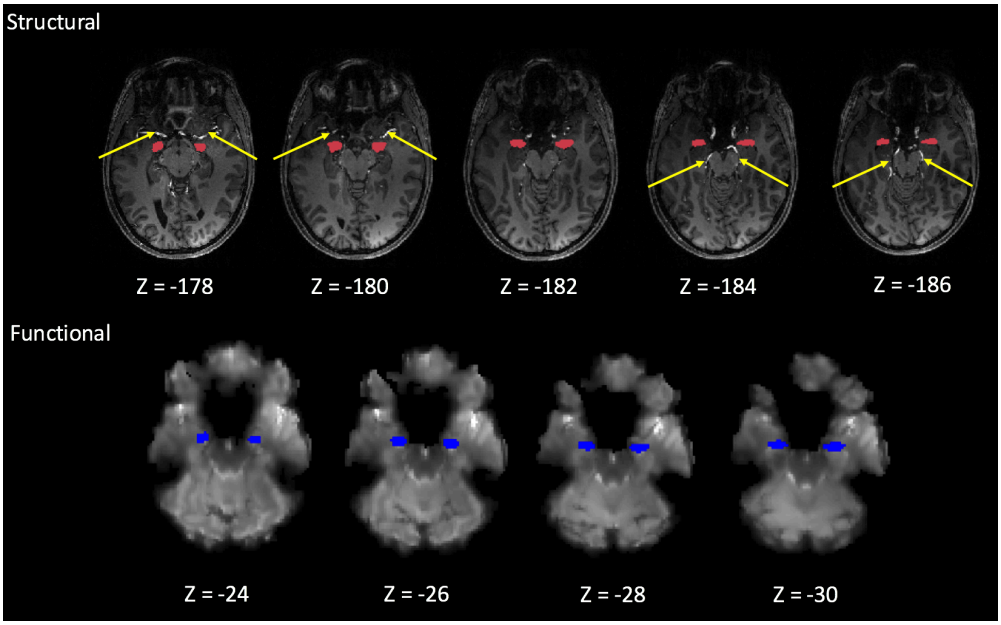


Figure 2.08 Demonstration of Fieldmap Correction

Data from a single subject at 7T. The top series of structural images (T1 weighted) highlight the amygdalae in red. The basal vein of rosenthal (BVR) is indicated by yellow arrows. The bottom series of images include the functional images (T2 weighted) with the amygdalae in blue. Due to their low spatial resolution, the BVR cannot be located with certainty. However, they comparison of the two image sets suggests that fieldmap correction assisted in avoiding the inclusion of these blood vessels in the amygdalae masks.*

Anterior and Posterior Insula Seed Mask Creation

The PAIN Group has an extensive library of masks generated in standard space. The anterior and posterior masks came from this library. The masks were transformed into the functional space of each subject, restricted to a threshold of 50%, and binarised.

Extraction of Time Series and Use in FEAT

Using an FSL analysis tool that extracts timeseries (fslmeants), the mean timeseries was extracted from the de-noised fMRI data within relevant masks (amygdala, anterior and posterior divisions of insula). For each subject, FEAT was run with the ROI timeseries as a covariate.

Concatenation of Statistical Images and RANDOMISE

For each subject, FEAT produced z-statistic images for whole-brain connectivity with the seeds. These z-statistic images were then fed into an FSL tool called RANDOMISE. RANDOMISE was used to perform nonparametric two-sample t-tests or examine changes

in connectivity with respect to a covariate of interest. Threshold-Free Cluster Enhancement and permutation testing were employed. RANDOMISE reports p-values that are family-wise-error (FWE) corrected. Further correction was conducted for the inclusion of multiple ROIs/conditions. This was done using the Bonferroni correction — dividing the p-value threshold for significance by the number of tests conducted.

2.5.3.2 Analysis 2: ROI Network analysis

The remaining analyses involved defining a network of ROIs and measuring connectivity changes within this network. In these ROI network analyses, all masks were bilateral to avoid issues with collinearity (highly correlated activity) (Bijsterbosch, Smith, and Bishop 2015). This is important because common activity is removed before performing the partial regression.

ROI Mask Creation

Masks were created for the following regions: hippocampus, putamen, thalamus (whole and sensory-only), anterior insula, posterior insula, SII, RVM, PAG, and amygdalae.

Automated Mask Creation in High-Resolution Structural Space

The thalamic, hippocampal, putamen, and amygdalae masks were generated using FIRST (Patenaude et al. 2011). These were transformed into functional space, restricted to a threshold of 25%, and binarised.

Masks Generated in Standard Space

Secondary Somatosensory Cortex

The SII mask was generated using the Jülich histological atlas (Eickhoff et al. 2005; Eickhoff et al. 2006; Eickhoff, Paus, et al. 2007) and comprises parietal operculum 1, 3, and 4 (Eickhoff, Grefkes, et al. 2007). The medial prefrontal, posterior, and anterior insular cortices masks were resourced from the PAIN Group seed library as standard space masks

(same insular masks as previously described). They were transformed into functional space and restricted to a 50% threshold.

Periaqueductal Gray

The mask of the PAG was hand drawn in standard space with reference to Ezra and colleagues (2015) and was restricted to a threshold of 50%.

Rostral Ventromedial Medulla

The spherical (3 mm in diameter) RVM mask was generated using the coordinates cited by Vincent and colleagues (2011). Due to its small size, thresholding would have erased the mask. As such, it was only binarised.

Thalamus

For the gabapentin trial, only the sensory thalamus was seeded. It was generated from the connectivity atlas published by Behrens and colleagues (2003).

Timeseries Extraction

Fslmeants was used to extract the principal component of the eigenvalue time series for each mask for each subject. In these analyses, eigenvalue timeseries was used since many of the masks were generated in standard space and then transformed back into each subject's functional space. In the context of functional connectivity analyses, there is debate as to whether use the principal component of the eigenvalue time series is better than using the mean time series. Discussions with FMRIB analysis experts led to the use of the principal component eigenvalue time series. The principal component is thought to be better at excluding noise, and might be particularly advantageous if the mask is imperfect and includes part of another region.

FSLNets: Network Correlations

The resultant time series were organised in matrices and fed into FSLNets (www.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets). FSLNets is a tool that analyses network dynamics of fMRI-derived activity. Each time series was normalised within each mask of each subject before running a ridge regression (partial correlation) (rho = 0.5). Partial correlation was chosen as it is thought to be the best measure of direct connectivity between regions (Marrelec et al. 2006). The output was subsequently run in RANDOMISE to compare the functional connectivity between groups or with respect to covariates of interest. The threshold for significance was $p < 0.05$ and all reported p-values are corrected for multiple comparisons (family-wise-error (FWE) correction based on number of tested connections between ROIs).

2.6 Analysis of Non-Imaging Datasets

All non-imaging data (questionnaire data and pain scores) and post-analysis imaging measures were analysed using Microsoft Office Excel, SPSS (Version 23), and R statistical software (Version 2.7.2). Before analysis, data was examined to ensure statistical assumptions were not violated. Outliers were identified by being two standard deviations away from the mean. Unless otherwise stated, the threshold for significance was $p < 0.05$.

Chapter 3

Modelling Chronic Pain: Capsaicin-Induced Ongoing Pain and Central Sensitisation in Healthy Individuals

Section 1

3.1 Evaluation of tSNR at 3T and 7T

3.1.1 Introduction

In Section 1, I examine data quality using a measure known as the tSNR. This was my first attempt analysing 7T data and the drop-out and distortion was slightly greater than what I had seen at 3T. As such, I wanted to ensure the data was of adequate quality by comparing the tSNR in this 7T dataset to previous reports. Since an identical 3T acquisition was acquired in the same subjects, I also compare the tSNR of the data at 3T and 7T.

In MRI, the signal-to-noise ratio (SNR) is defined as the ratio of signal from an anatomical region to the background noise associated with that region (Edelstein et al. 1986). tSNR is a measure of SNR over an fMRI acquisition and is considered one measure of data quality (Welvaert and Rosseel 2013). tSNR is an important consideration in experimental design since it impacts the measurement of fMRI signal (Murphy, Bodurka, and Bandettini 2007). Both thermal and physiological noise can impede the measurement of relevant neurophysiological signal (Krüger and Glover 2001). Thermal noise can result from multiple sources (MRI electronics and the person in the scanner) (Brooks et al. 2013) whilst physiological noise is a product of cardiac and respiratory rhythms (Krüger and Glover 2001).

Relevant signal in the amygdalae is vulnerable to dropout (loss of signal) due to their proximity to the sphenoid sinus (Robinson et al. 2004). Dropout increases with static field strength, which makes the amygdalae a challenging region to image at 7T (van der Kolk et al. 2013). Nonetheless, the amygdalae have been successfully examined at 7T. A 7T study that was specifically interested in the amygdalae reported a mean tSNR value of 116 in the region, which they deemed sufficient to measure BOLD-related activity (tSNR from this study was calculated from data with: 1.5 x 1.5 x 2.5mm voxel size, 6.0 mm spatial smoothing, and motion correction). However, there was notable variability in tSNR within the amygdalae (van der Zwaag et al. 2012).

The aim of this section was to 1) compare the global grey matter tSNR of the 3T and 7T data to determine if one dataset had more advantages than the other and 2) ensure the 7T tSNR was adequate to pursue a seed-based analysis in the amygdalae.

3.1.2 Methods

3.1.2.1 Subjects

Data from ten healthy subjects were included in these analyses with one exception. For the 7T and 3T global tSNR calculations, only nine subjects were used.

3.1.2.2 tSNR Calculation

The images had a spatial resolution of 2 x 2 x 2 mm, were motion corrected using MCFLIRT (Jenkinson et al. 2002), unwarped using fieldmaps, high-pass filtered (100 seconds), and smoothed using a Gaussian kernel of FWHM 5mm. tSNR was calculated in FSL by dividing the temporal mean signal by the temporal standard deviation of signal using pre-processed fMRI data (Welvaert and Rosseel 2013).

3.1.3 Results

3.1.3.1 Global Grey Matter tSNR Comparison of 3T and 7T

The mean global grey matter tSNR at 3T and 7T were 227.1 (SD ±36.1, N = 9) and 160.9 (SD ±44.2, N = 9), respectively. A paired t-test revealed that the tSNR at 3T was significantly higher (p = 0.0031) suggesting that with the sequence parameters used, the 3T is potentially better at detecting relevant fMRI signal across the brain (see Figure 3.01). There was also a greater range of tSNR at 7T, highlighting some of the susceptibility issues that might be magnified in some subjects but not others (for example, by different positioning of head in scanner).

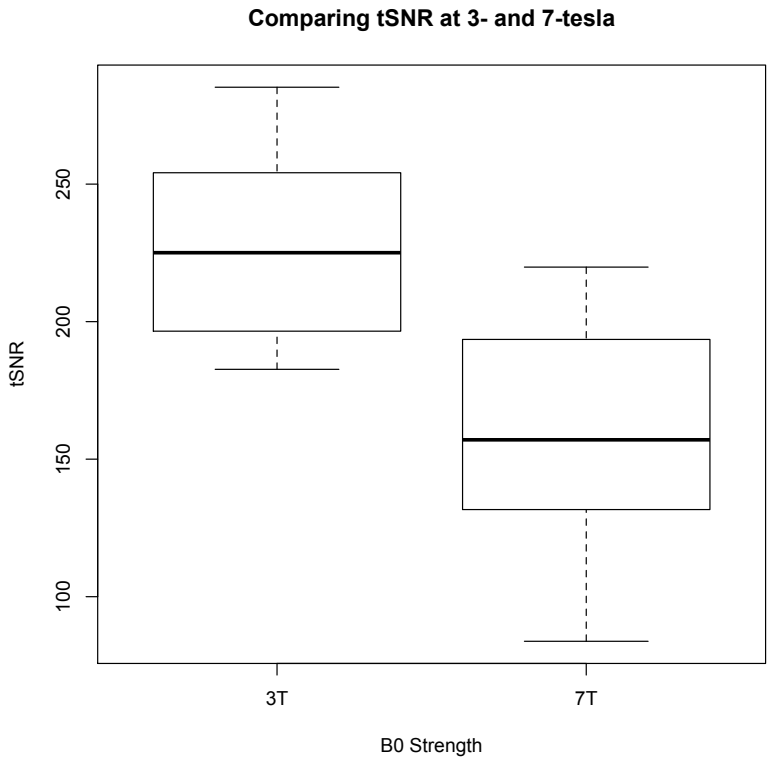


Figure 3.01 Boxplots Comparing Grey Matter tSNR at 3T and 7T

Data from the 3T had a significantly higher mean tSNR in the grey matter (Paired t-test: p = 0.0031). The whiskers represent the range, the box includes values within the 25 to 75th percentiles and the dark line is the median. N = 9.

3.1.3.2 tSNR in the Amygdalae at 3T and 7T

At 3T, the mean tSNR in the right and left amygdalae were 205.1 (SD ±23.4, N = 10) and 215.8 (SD ±26.7, N = 10), respectively. At 7T, the mean tSNR values in the right and left

amygdalae were 107.29 and 87.77, respectively (see Figures 3.02 & 3.03). Paired t-tests revealed that there were no significant differences between the tSNR of the left and right masks at 7T (p = 0.0664, N = 10) or 3T (p = 0.0756, N = 10). Overall, the seeds at 3T yielded significantly higher tSNR values than those at 7T (Paired t-test: *Right*: $p = 1.69 \times 10^{-5}$; *Left*: $p = 1.32 \times 10^{-6}$).

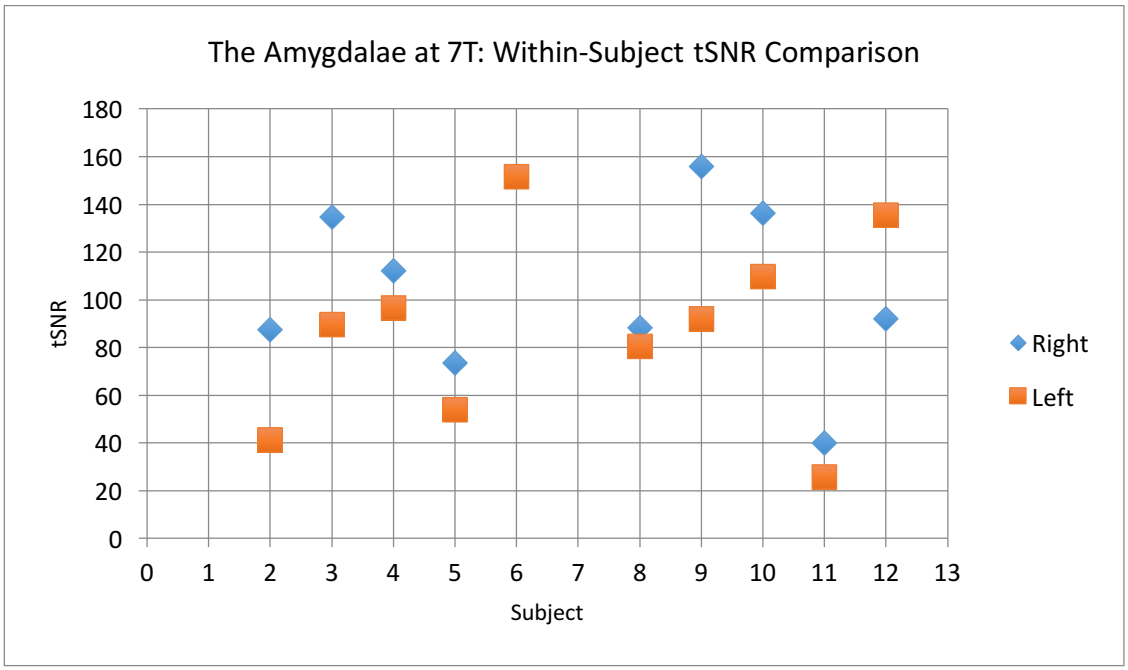


Figure 3.02 7T Amygdala Seed tSNR Comparison (Within-Subject)

A paired t-test determined that there was no statistically significant difference between the tSNR of the left and right amygdalae (p = 0.0664). Subjects 1 and 7 were excluded from this analysis due to corrupted physiological traces and different scan parameters, respectively. Additionally, subject 6's data points are overlapping. N = 10.

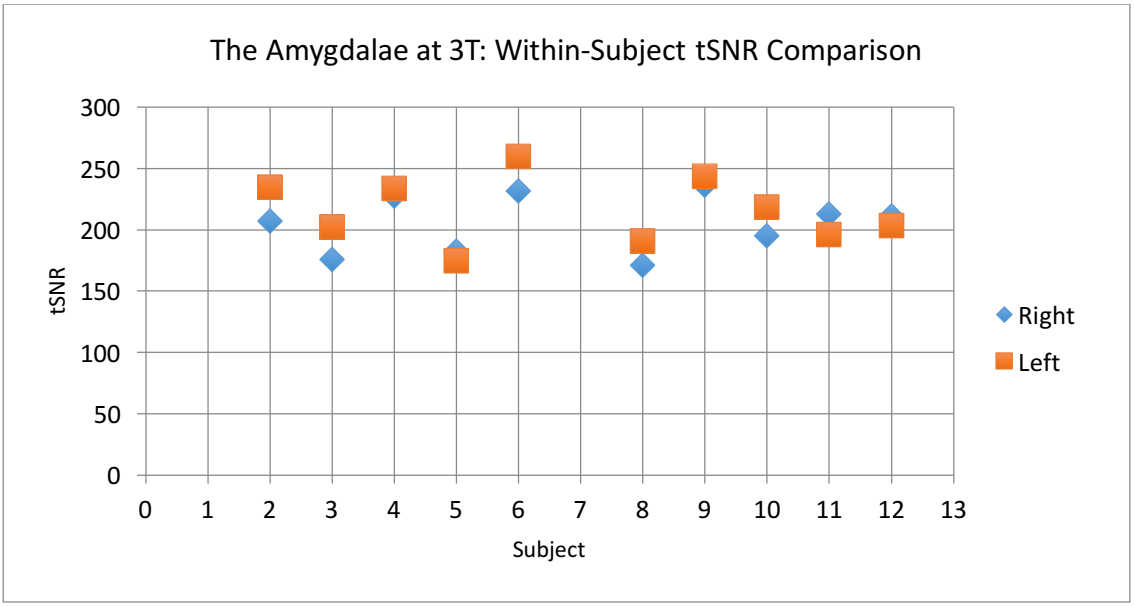


Figure 3.03 3T Amygdala Seed tSNR Comparison (Within-Subject)

A paired t-test determined that there was no statistically significant difference between the tSNR of the left and right amygdalae (p = 0.0756). Subjects 1 and 7 were excluded from this analysis due to a corrupted physiological trace and differing scan parameters, respectively. N = 10.

3.1.4 Discussion

When compared to the 7T, the 3T data had significantly higher mean tSNR values within the grey matter and amygdalae masks. At both field strengths, there were no significant differences in tSNR in the right and left amygdalae masks.

These results suggest that relevant neural-signal might be better detected at 3T. Unfortunately, the 3T dataset only had one condition (capsaicin-pain) whereas the 7T data had two conditions (both capsaicin-pain and sham). The lack of sham 3T dataset prevents a condition comparison, which will be described further in the following section.

When looking at the feasibility of using the 7T data, the tSNR 7T values in the amygdalae are slightly smaller than those previously reported by van der Zwaag and colleagues (2012) at 7T (Mean: 116). The higher tSNR values could potentially be attributed to

different pre-processing steps. As such, I believed it was worth pursuing this analysis, whilst keeping in mind the low tSNR could be a hindrance.

The low tSNR at 7T is likely due to the fact that this was some of the earliest data to be collected from the 7T. Similarly, for the sake of direct comparison, the acquisitions at 3T and 7T were made to be nearly identical, which did not allow for potential 7T benefits (including increased spatial resolution) to be taken advantage of.

3.1.5 Summary

The tSNR values at 3T data were significantly higher within the grey matter and amygdalae masks. The tSNR of the 7T data, whilst lower, was thought to be adequate to pursue a seed-based analysis.

Section 2

3.2 The Amygdalae and Nociception

3.2.1 Introduction

In the previous section I examined the tSNR in the grey matter and amygdalae to determine the feasibility of a functional connectivity analysis. Whilst the tSNR was slightly lower than previously reported values in that region on the 7T (and statistically lower that the 3T data), it seemed reasonable to proceed, keeping in mind that this could be a limitation. In this section I perform a functional connectivity analysis using the amygdalae as seeds. This first approach was exploratory due to the nature of the data quality and the desire to examine whole-brain connectivity changes. More ambitiously, it was an attempt to replicate findings from animal models in human imaging. It is important to highlight that this data was some of the first to be acquired on the 7T at FMRIB and thus it was used to form the basis for my understanding of functional connectivity analysis. Forming and following an analysis pipeline allowed me to learn the various methodologies used to conduct global and seed-based functional connectivity analyses. I began to understand the various caveats associated with resting analyses and which steps need to be taken to ensure those vulnerabilities are reduced. I also discovered the importance of optimizing MR sequence parameters for the question at hand as well as dealing with technical issues with 7T data. Given that this is early 7T data and the tSNR limitations described in the previous section, the following sections comprise exploratory analyses for future work. Importantly, this data is being re-acquired with improvements to the MRI sequences and increased subject numbers.

3.2.1.1 The Amygdalae and Pain Processing

It has been well-established that the amygdalae are not only critical in nociception but also descending pain modulation. As previously described, the amygdalae comprise multiple nuclei that play important roles in both the nociceptive and affective components of pain (Neugebauer et al. 2004). The CeA receives ascending nociceptive input directly from the spinal cord (Burstein and Potrebic 1993). It also receives input from the LA and BLA nuclei, which have connections to cortical structures (mPFC, insula) (Rizvi et al. 1991; Neugebauer et al. 2004; Likhtik et al. 2005; Baur et al. 2013). These connections then descend to the PAG and RVM as part of the descending modulatory system (Rizvi et al. 1991; Fields 2000). Despite both the right and left amygdalae containing these essential nuclei, animal research suggests that they do not play equal roles in nociceptive processing (Carrasquillo and Gereau IV 2008; Ji and Neugebauer 2009).

3.2.1.2 Potential Lateralisation in Pain Processing

In recent decades there has been increased interest in the lateralisation in pain processing, with particular interest in right hemisphere dominance (Brooks et al. 2002; Bingel et al. 2003; Symonds et al. 2006). Craig (2009) has argued that laterally opposing activity helps maintain homeostasis and that, from an evolutionary standpoint, lateralisation is beneficial in terms of energetics and efficiency. With the brain being the most energetically costly and sophisticated organ in the body, laterally coordinating tasks within the same context offers a better use of resources and allows for increased complexity in functioning (Craig 2009).

The amygdalae have been frequently evaluated for laterality in functioning; however, much of that research falls outside of the pain domain (Baas, Aleman, and Kahn 2004). Of

particular interest is the CeA because it has demonstrated a right-sided dominance in nociceptive processing (Carrasquillo and Gereau IV 2008; Ji and Neugebauer 2009).

Research in rodent models has produced biochemical and electrophysiological data that suggest the CeA of the right amygdala plays a dominant role in nociceptive processing. During inflammation, it has been shown that extracellular signal-regulated kinase (ERK) regulates pain sensitivity (Carrasquillo and Gereau 2007). Using peripheral inflammatory pain, the researchers found that by disrupting the ERK pathway in the CeA of the right amygdala, they could reduce the behavioural pain responses exhibited by the animals. Interestingly, disrupting this cascade in the left amygdala did not lead to any behavioural changes (Carrasquillo and Gereau IV 2008). Building on this research, an electrophysiological study found that neuronal activity in the right CeA increased more than in the left in the experimental chronic pain state. This right-sided dominance persisted during stimulation of both ipsi- or contra-lateral sites (Ji and Neugebauer 2009). These results led the authors to conclude that the right amygdala plays a unique role in nociception and pain perception. There are currently no published studies that have explicitly examined nociceptive laterality in the amygdalae in humans.

3.2.1.3 Central Sensitisation: Topical Capsaicin Induces Prolonged Pain States

In human research, there are far fewer ethical options for inducing prolonged pain states. Both topical and intradermal capsaicin have been used to induce long-lasting pain in humans. Capsaicin's mechanism of action is through activation of TRPV1; topical application can result in a constant burning sensation (Petersen and Rowbotham 1999). For those who respond to capsaicin, mechanical stimulation to the surrounding region will exhibit heightened sensitivity to noxious input, termed secondary hyperalgesia, which is

a consequence of central sensitisation (LaMotte, Lundberg, and Torebjörk 1992; Petersen and Rowbotham 1999). Understanding central sensitisation is important because it is thought to be an underlying mechanism of the chronic pain experience (Woolf 2011). Researchers have demonstrated the utility of topical capsaicin as an effective model for prolonged pain states and that its impact on the CNS can be captured using imaging (Segerdahl et al. 2015).

The aim of this section was to determine whether coupling of the right amygdala with other regions during the capsaicin-induced pain state could be detected using seed-based "resting" functional connectivity analysis. Given the animal literature, I hypothesised that there would be an increase in coupling between the right amygdala and key nociceptive regions. However, as previously stated, this was an exploratory analysis conducted on early 7T data.

3.2.2 Methods

3.2.2.1 Subjects

Ten healthy subjects (six men and four women) were included in both the 3T and 7T analyses. All subjects were right-handed and healthy (no chronic pain conditions) and completed the STAI.

One subject was missing State Anxiety scores for the 7T capsaicin visit. Another subject's 3T imaging data was incomplete due to an issue with the field map. This was discovered further down the analysis pipeline. As such, they were excluded from the 3T imaging analysis, reducing the sample size to 9.

As described in Chapter 2, this data was previously collected by Dr. Melvin Mezue as part of a study that was primarily interested in responses to evoked-pain. This dataset originally included twelve subjects; however, only ten subjects had resting scans collected.

3.2.2.2 Study Visits and Pain Paradigm

As mentioned in the introduction, for the sake of simplicity, the fMRI data analysed in this chapter will be occasionally referred to as "resting" scans. Where appropriate, there will be clear delineation between the resting sham and resting capsaicin conditions.

This study involved three MRI visits. There were 1 - 2 weeks between each scan and the order was determined by pseudo-randomisation. There were two 7T fMRI scans involving two conditions (sham or capsaicin) and one 3T fMRI involving capsaicin. The sham cream was not a true "sham" — subjects were not blinded to which cream they were receiving during the visit. Active 1% capsaicin or sham cream was administered bilaterally to the antero-medial part of the lower legs (14 cm above the medial malleolus) on a space of 4x4 cm^2 and left on for 1-hour preceding the scan. The capsaicin was not removed until the scan was complete. Four punctate scans took place before the resting state scan, so there were periods of painful stimulation preceding the resting-fMRI acquisition; this meant that capsaicin was on the leg for over 1.5 hours before the resting scan commenced. For the duration of the scan, subjects were asked to rate the pain they felt specifically from the capsaicin. Immediately before and after the resting-fMRI, subjects were asked to rate their pain from the capsaicin or sham cream using a VAS.

3.2.2.3 Functional Connectivity Analysis

In the first analysis, exploratory whole-brain connectivity of the left and right amygdalae was first examined for each condition (sham and capsaicin). The threshold for significance is Bonferroni corrected (for left and right amygdalae and each condition). The second analysis utilised a paired t-test to examine functional connectivity differences between capsaicin and sham conditions. In this analysis, the reported p-values were Bonferroni corrected. The aforementioned Bonferroni corrections were conducted in addition to the corrections calculated by RANDOMISE. Further details of the analysis pipeline have been previously outlined and can be found in Chapter 2 (Methods).

3.2.3 Results

The mean age of the participants was 26.3 years (N = 10).

3.2.3.1 State-Trait Anxiety Inventory Scores

The mean Trait Anxiety score was 29 (SD ±3.09, N =10) (scored from 20 - 80). The mean State Anxiety scores at each visit were 30.1 (SD ±5.80, N =10) at 3T, 33.44 (SD ±6.39, N = 9) at 7T-capsaicin, and 29.3 (SD ±6.13, N =10) at 7T-sham. The difference between the sham and capsaicin 7T scores was not statistically significant ($p = 0.0905$). Similarly, a paired t-test revealed that there was no significant difference between State Anxiety scores from the 3T and 7T capsaicin scans ($p = 0.1926$).

3.2.3.2 Capsaicin Pain Ratings

Pain intensity ratings during the scans preceding and during the resting-fMRI are plotted in Figures 3.04 & 3.05. There is both variation within and between subjects' pain intensity ratings. Over the entire 3T scan, the mean pain intensity score was 28.4 (scale from 0 - 100). For just the resting scan, the mean rating was 26.6. The mean pain scores during the 7T visit were 32.4 (over the entire scan) and 34.2 (during the resting scan).

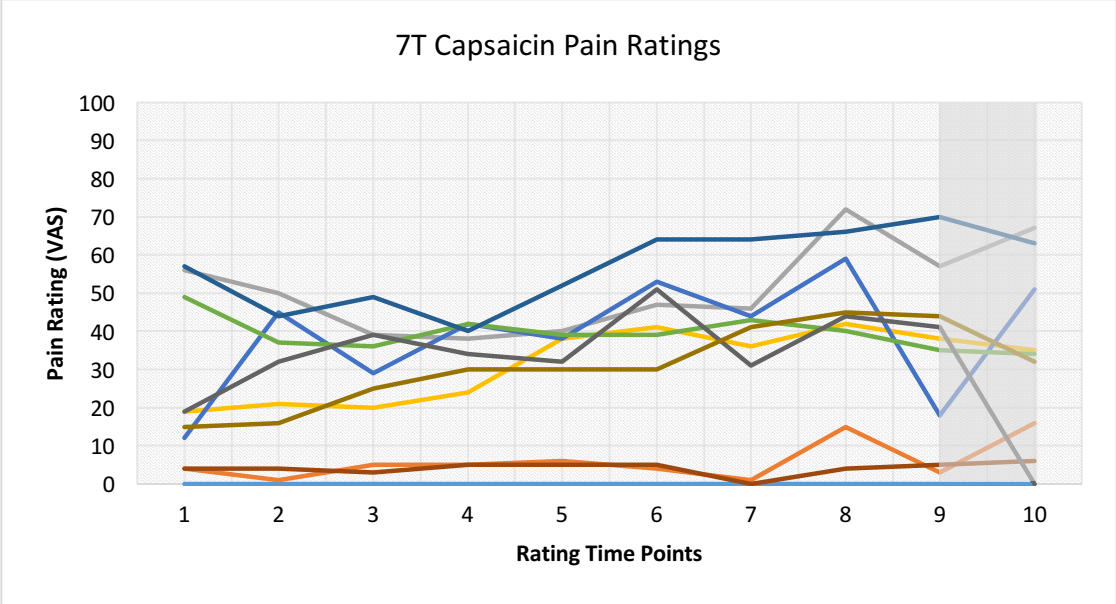


Figure 3.04 7T Scan: Capsaicin Pain Ratings

The dark grey box represents the time when the resting capsaicin scan was taking place. N = 10.

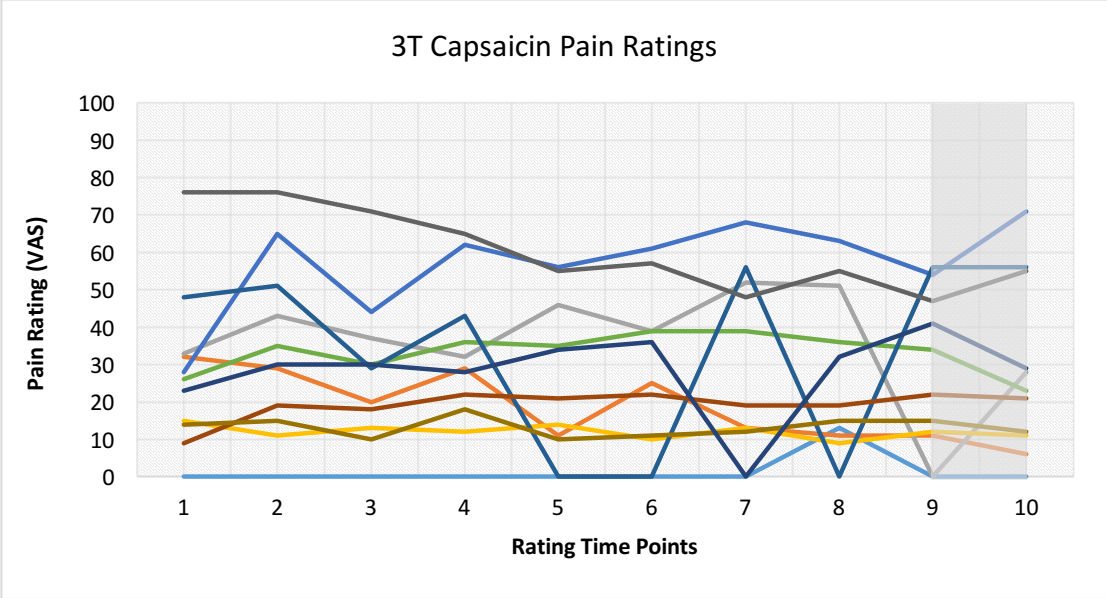


Figure 3.05 3T Scan: Capsaicin Pain Ratings

The dark grey box represents the time when the resting capsaicin scan was taking place. N = 10.

3.2.3.3 Pain Ratings and Anxiety Measures

For the 7T visit, State and Trait Anxiety scores and mean capsaicin pain ratings during all the scan sequences (not just the resting capsaicin scan) were not correlated (Pearson's Correlation: *State Anxiety*: r = 0.0230, p = 0.9532, N = 9; *Trait Anxiety*: r = -0.3837, p = 0.2737, N = 10). Similarly, State and Trait Anxiety scores were not significantly correlated

with the capsaicin pain ratings reported immediately before and after the resting scan (Pearson's Correlation: *State Anxiety*: r = -0.1221, p = 0.7543, N = 9; *Trait Anxiety*: r = 0.0167, p = 0.9635, N = 10).

3.2.3.4 Seed Volumes of the Amygdalae

7T Study

The volume of each amygdala seed mask was checked to ensure they fell within the range of previously reported data. Similarly, amygdala volumes were compared within each subject for each condition to ensure the segmentation was carried out correctly. Since there is hemispheric variability in amygdala size within individuals, a within subject paired t-test was run. There were no statistically significant differences between the left and right amygdalae between subjects (see Table 3.01).

Subjects	Sham Scan Amygdala Volumes (mm^3) Mean (± SD)		Capsaicin Scan Amygdala Volumes (mm^3) Mean (± SD)	
	Right Seed	Left Seed	Right Seed	Left Seed
All (10)	1031.20 (±422.59)	1180.80 (±383.34)	1024.79 (±423.19)	1175.99 (±408.69)

Table 3.01 7T Amygdala Volumes

A paired t-test determined that there were no significant differences between the left and right seeds for all subjects in either condition (Sham(all) p = 0.2079; Capsaicin(all) p = 0.2617). Furthermore, left and right amygdala volumes did not differ between sessions (p = 0.7959 and p = 0.4433, respectively). N = 10.

3T Study

At 3T, a paired t-test revealed that there were no significant bilateral differences in volumes of the seed masks (p = 0.0961) (see Table 3.02).

Subjects	Capsaicin Amygdala Volumes (mm^3) Mean (± SD)	
	Right Seed	Left Seed
All (10)	1367.99 (±378.14)	1542.39 (±395.85)

Table 3.02 3T Amygdala Volumes

A paired t-test determined that there were no statistical differences between the left and right amygdalae of all subjects (p = 0.0961). N = 10.

3.2.3.5 Seed-Based Analysis at 7T: Capsaicin and Sham Condition Comparison

In the first analysis, functional connectivity was examined separately for the left or right amygdalae and for each condition (corrected for multiple comparisons: p(corr) < 0.0125; N = 10). The only statistically significant result was left amygdala connectivity with the pre- and post-central gyri during the capsaicin state (see Figure 3.06). For the right side and both sides during the sham condition, there was non-significant activity that was below the corrected p-value threshold (p(corr) < 0.0125).

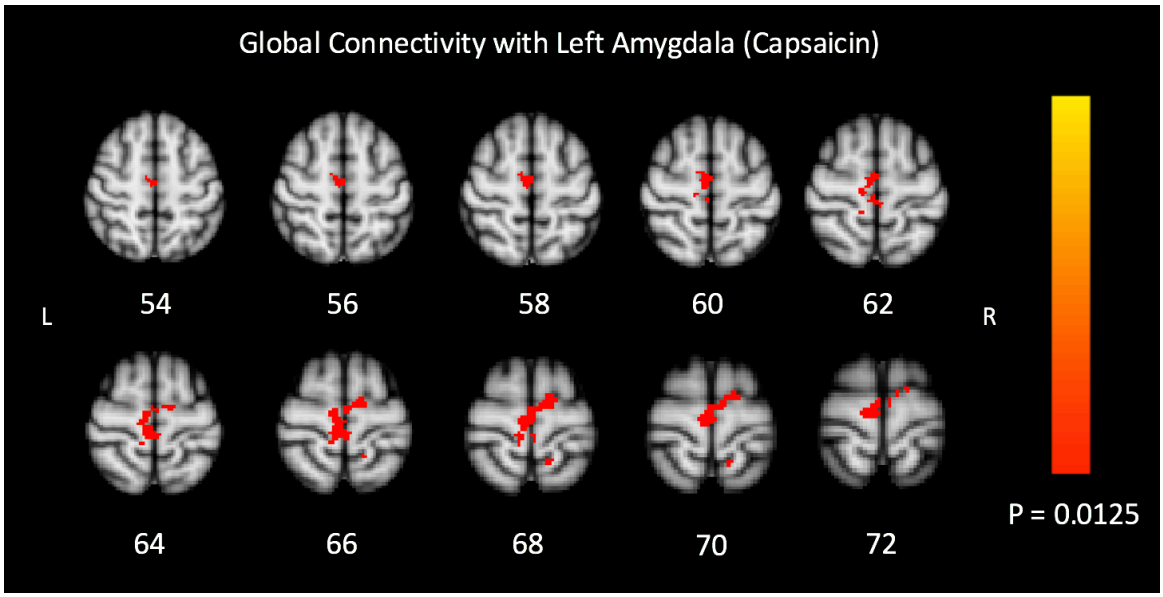


Figure 3.06 Global Connectivity with Left Amygdala During the Capsaicin Condition

There was significant connectivity between the left amygdala and pre- and post-central gyri during the capsaicin condition (p(corr) < 0.0125. N = 10)

Condition-Dependent Functional Connectivity of the Right and Left Amygdalae

Both the right and left amygdalae were evaluated for differences in functional connectivity between the capsaicin and sham conditions. When examining the right and left amygdalae separately (and not excluding shared activity between the two regions), none of the results reached significance (p-value threshold < 0.05). The closest result to the statistical threshold was an increase in connectivity between the right amygdala and the MCC (General Linear Model Paired t-test: p(corr) = 0.1113; N = 10) during the painful capsaicin condition when compared to sham. However, upon visual inspection of the uncorrected connectivity maps, this non-significant result appears to be driven by increased activation during the sham condition, rather than the capsaicin condition.

Condition-Dependent Functional Connectivity of the Bilateral Amygdalae

Since the laterality results were not statistically significant, the masks were combined to seed the bilateral amygdalae together to determine whether there were any condition specific differences that were not hemisphere-dependent. Again, none of the results reached statistical significance (p-value threshold < 0.05).

3.2.3.6 Examining Pain Intensity at 3T

Given the tSNR advantage at 3T, the dataset was also analysed. Since only capsaicin condition data were available, connectivity of the right and left amygdalae was examined with respect to their pain intensity ratings during the resting capsaicin scan. There was no significant correlation between pain scores and functional connectivity (p-value threshold < 0.05). There was a non-significant increase in activity between the left amygdala and precuneus with respect to pain ratings (General Linear Model with Covariate of Interest: p(corr) = 0.3928, N = 9).

3.2.4 Discussion

Only the left amygdala, during the capsaicin condition, demonstrated significant functional connectivity with other brain regions (pre- and post-central gyri). There were no statistically significant changes in right or left amygdala connectivity when comparing the capsaicin to the sham condition.

3.2.4.1 Limitations

Sample Size

As previously described, no power calculation was conducted prior to running this analysis. However, given 1) the study was not designed to conduct this analysis and 2) the resting scan sample size was lower than for other scans in the complete study (ten instead of twelve), it is conceivable that this study was underpowered.

Pain Model

When comparing this experiment to rodent research, the capsaicin model may not have elicited sufficiently strong pain intensity (average pain 28.4 and 32.3 during the painful resting scan at 3T and 7T, respectively). Whilst direct comparison of painful experiences between human subjects and rodents is impossible, it is conceivable that the more invasive arthritic and peripheral inflammatory pain paradigms in animal models were significantly more painful than those produced by topical capsaicin in this study. Some options for increasing the pain intensity include: only enrolling strong responders to capsaicin, increasing the capsaicin-pain with a hot water bottle (Segerdahl et al. 2015), or using a completely different type of stimulation altogether (i.e. continuous hypertonic saline injection as used in (Owen et al. 2010)).

Temporal and Spatial Resolution of fMRI

One important limitation of fMRI is temporal resolution. Given the rodent research involved electrophysiological measurements of actual nerve fibres, which measured rapid activity, it is possible that the fMRI sequence did not have the capacity to capture these changes. In this experiment, the repetition time (TR) was 4 seconds; TR is the frequency with which the brain is measured. Further, 4 seconds is much longer than typically used in fMRI research (the following experiments have TRs at or below 2.5 seconds); however, when this data was acquired, that was the shortest interval in which the entire brain could be functionally measured at the 7T.

The second fMRI limitation, which is by no means unique to this study, is the inability to accurately segment amygdala beyond its macrostructure. This study was particularly interested in CeA given its nociceptive role; however, as mentioned earlier, even segmenting the entire amygdala is challenging within the neuroimaging domain (Robinson et al. 2004). Thus, the signal from these seed masks includes more than pure CeA activity changes.

Functional Connectivity and Volumes Collected

The number of volumes collected (91) and available for analysis (87 due to rating at the beginning of the scan) was comparatively low (see Chapter 2 for details of scan parameters). Whilst there are obvious reasons why so few volumes were collected (with the TR of 4 seconds the scan was over 6 minutes), it highlighted the statistical importance of collecting as many volumes as possible for functional connectivity analyses. These analyses rely on the fluctuation of the haemodynamic response and having more measurements allows for relevant correlations to be identified. Future studies on the 7T

will be able to take advantage of multi-band imaging, where multiple slices are collected at the same time, which substantially reduces how long it takes to acquire a whole-brain functional image.

3.2.5 Summary

No statistically significant changes were observed right amygdala connectivity during the capsaicin-condition. This data analysis used early 7T data, which is currently being re-acquired with improvements to scan sequences and sample size.

Section 3

3.3 Evaluating the Anterior and Posterior Divisions of the Insula During Prolonged Experimental Pain

3.3.1 Introduction

In the previous section, I performed an exploratory analysis examining nociceptive laterality in the amygdalae. None of the functional connectivity results were significant. I concluded that several components of this study needed to be improved to better address the question. With these limitations in mind, I decided to approach the dataset another way. In this section, I conduct a similar analysis but with the posterior and anterior divisions of the insula as our seeds of interest. Due to its location, the insular cortex is less prone to dropout than the amygdala and thus potentially a better region to evaluate condition-dependent connectivity changes at 7T.

3.3.1.1 Anterior and Posterior Divisions of the Insula

The insular cortex is one of the most frequently reported regions in pain imaging studies (Duerden and Albanese 2013). Both animal research and human imaging studies have demonstrated that the divisions of the insula play differing roles (Craig 2009).

The anterior division is thought to be very much a part of the cognitive-evaluative and affective-motivational component of pain processing. Research has demonstrated that the anterior insula is involved in integrating information about pain intensity (Kong et al. 2006) and harm (Wiech et al. 2010). Furthermore, its activation is influenced by attention and negative affect paradigms (Brooks et al. 2002; Ploner et al. 2011). Indeed, even at rest, the anterior insula is functionally connected to important limbic regions including the rostral anterior cingulate cortex (rACC) (Cauda et al. 2011); its functional connectivity

with the amygdala is positively correlated with anxiety (Baur et al. 2013). When compared to the posterior division, the anterior insula has comparatively increased connectivity with the prefrontal cortex (Wiech et al. 2014).

Unlike the anterior insula, the posterior division is thought to be more involved in the sensory-discriminatory component of pain processing. It is less influenced by attention-modulating paradigms (Brooks et al. 2002), hypnosis (Raij et al. 2005), and empathy (Singer et al. 2004) and there is significant evidence suggesting that the posterior division processes changes in stimulus intensity (Oertel et al. 2012). Specifically, activation in the posterior insula has been correlated with the temperature changes of heat stimulation (Kong et al. 2006). Furthermore, the dpINS has exhibited somatotopy (Brooks et al. 2005). Fitting with the posterior insula's role in sensory processing, it has been demonstrated that the division has increased connectivity with SI and SII when compared to the anterior division (Wiech et al. 2014).

More recently researchers have started to uncover the role of the insula during prolonged pain states. During continuous hypertonic saline injection, measures of cerebral blood flow (CBF) in affective-motivational pain regions (including the anterior insula) decreased relatively more than the CBF in the posterior insula (Owen et al. 2010). Similarly, by measuring CBF, researchers have demonstrated that the dpINS encodes changing pain intensity over prolonged pain experiences (Segerdahl et al. 2015).

The primary aim of this section was to determine if limitations of this data could be partially overcome by using seed regions less prone to dropout. If changes could be

captured, I hypothesised that during the capsaicin condition, the anterior division would exhibit more connections to limbic regions, prefrontal regions and the amygdalae, whilst the posterior division would be more heavily coupled with sensory-oriented regions, such as SI and SII.

3.3.2 Methods

3.3.2.1 Subjects

In this section, only 7T data was utilised. Ten healthy subjects were included in this analysis with data from both conditions (sham and capsaicin).

3.3.2.2 Functional Connectivity Analysis

Whole-brain connectivity of the anterior and posterior insula was first examined for each condition (sham and capsaicin). In this first analysis, the threshold for significance was Bonferroni corrected (for each division and condition). The second analysis utilised a paired t-test to examine functional connectivity differences between capsaicin and sham conditions. In this analysis, the reported p-values were Bonferroni corrected. The aforementioned Bonferroni corrections were conducted in addition to the voxel-wise multiple comparison corrections calculated by RANDOMISE. Further details of the analysis pipeline have been previously outlined and can be found in Chapter 2 (Methods).

3.3.3 Results

3.3.3.1 Examining Functional Connectivity in Each Division and Condition

In this first analysis, functional connectivity was examined separately for each condition and division (corrected for multiple comparisons: $p(corr) < 0.0125$). The mean pain intensity ratings during the capsaicin-condition were 32.4 (over the entire scan) and 34.2 (during the resting scan).

By visually inspecting the data, for both the posterior and anterior insula there is an increase in global connectivity during the capsaicin-induced pain state. When examining the posterior insula, there was an increase in coupling with the anterior insula, cerebellum, ACC, SI, brainstem, and thalamus during the capsaicin condition (see Figure 3.07). The anterior insula had an increase in connectivity with the caudate, thalamus, brainstem, cerebellum, and prefrontal regions (particularly the ACC) (see Figure 3.08).

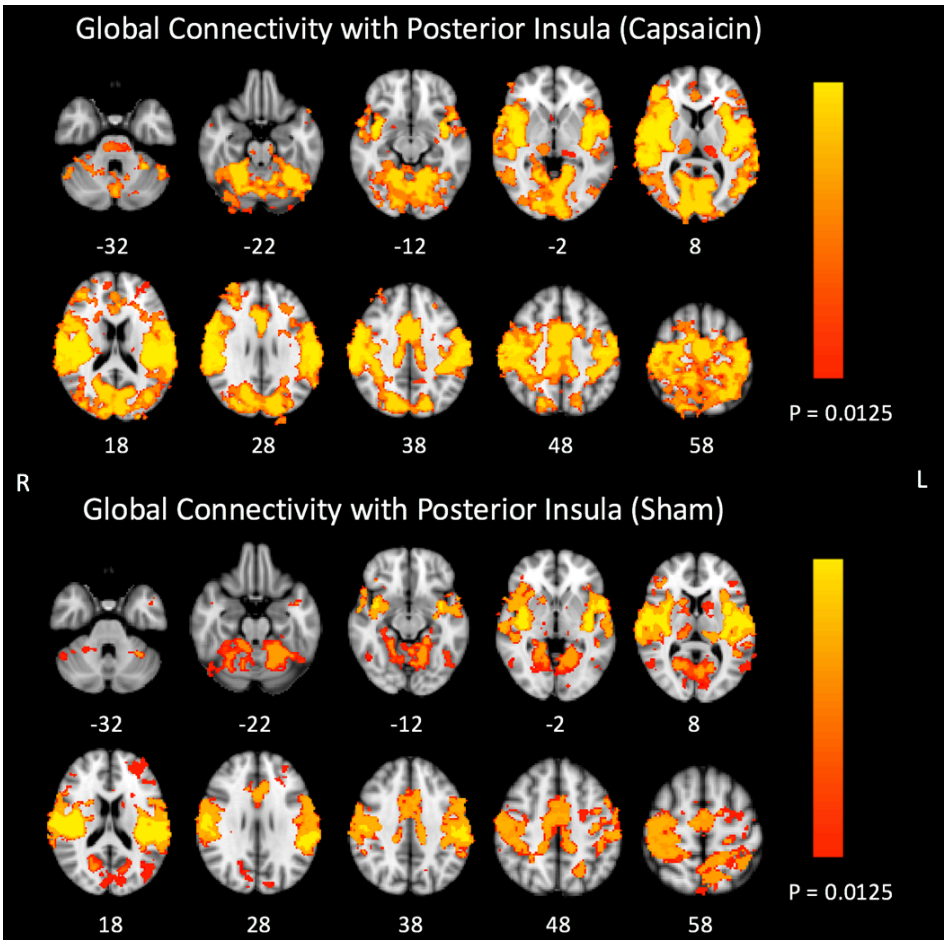


Figure 3.07 Global Functional Connectivity with the Posterior Insula During Capsaicin and Sham Conditions

Increased functional connectivity with the bilateral posterior insula during the capsaicin-pain and sham conditions (p(corr) < 0.0125). N = 10.

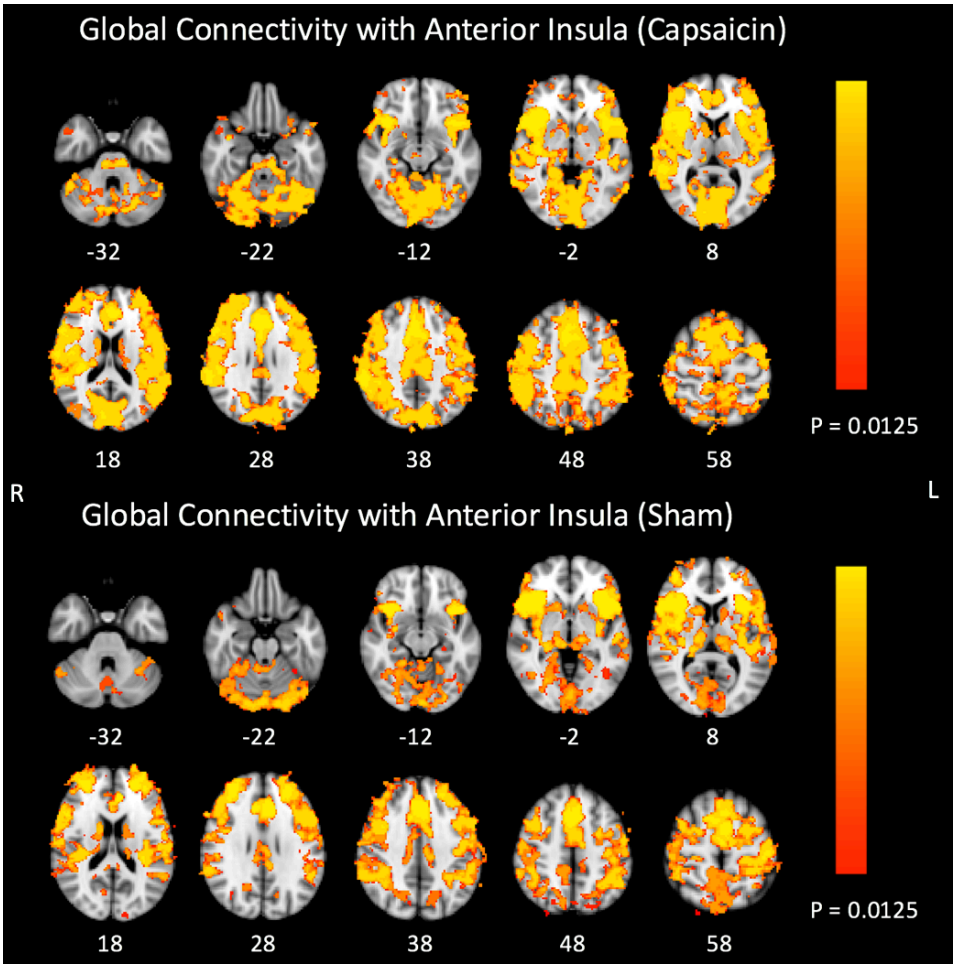


Figure 3.08 Global Functional Connectivity with the Anterior Insula During Capsaicin and Sham Conditions

Increased functional connectivity with the bilateral anterior insula during the capsaicin-pain and sham conditions (p(corr) < 0.0125). N = 10.

3.3.3.2 Condition-Specific Functional Connectivity Changes

Since this first analysis did not compare conditions statistically, a second analysis was run to determine if these observed changes were significant (paired t-test comparing conditions). None of the above changes were statistically significant in either the anterior or posterior insula. In the capsaicin condition, the anterior insula demonstrated a non-significant decrease in connectivity with the left middle temporal gyrus (General Linear Model Paired t-test: p(corr) = 0.1953). The posterior insula demonstrated a non-significant increase in connectivity with the left occipital pole during the capsaicin condition (General Linear Model Paired t-test: p(corr) = 0.3096).

3.3.4 Discussion

Upon visual inspection, unlike for the amygdalae, the condition-specific connectivity maps for the anterior and poster insula all demonstrated connectivity that survived the statistical threshold. However, when statistically comparing conditions, there were no significant global connectivity changes. Since the results did not reach significance, no further conclusions can be drawn.

3.3.4.1 Limitations

In Section 2, many of the limitations of this dataset were outlined. As previously described, the data from this chapter came from a study that was designed for pain-evoked work with a sample size of twelve. However, due to technical limitations, only resting data was collected for ten subjects.

There is a possibility that the imaging modality chosen (fMRI-BOLD) and the sequence parameters prevented the capture of pain-related changes. Recently, using arterial spin labelling (ASL), our lab demonstrated dpINS activity that was correlated with pain intensity during a similar capsaicin pain-paradigm (Segerdahl et al. 2015). Unlike fMRI-BOLD, which measures relative changes in BOLD signal, ASL can capture absolute CBF. As a result, it is thought to better measure changes associated with prolonged pain (Tracey and Johns 2010).

Furthermore, capsaicin-pain ratings in this and other experiments tend to fluctuate and will eventually decrease over time (demonstrated by Segerdahl and colleagues (2015)), which was not accounted for in this experiment. It is also known that even during consistent stimulation, functional coupling between regions is not always homogenous.

Owen and colleagues (2010) demonstrated this by plotting the CBF time courses during tonic pain. Whilst it primarily demonstrated gradual decreases in CBF over the pain paradigm, it also highlighted that between individual time points, CBF shifted, even in the posterior insula.

Again, as described in the previous section, increasing the number of acquired volumes during fMRI-BOLD could alleviate some problems. The 7T now has the capability of running multiband fMRI-BOLD acquisitions. Multiband involves simultaneously capturing activity in multiple regions of the brain. This has the advantage of increasing the number of measurements over a fixed scanning period.

This data is being re-acquired with a larger sample size, an increase in volumes, and improvements to scan parameters.

3.3.5 Summary

This section comprises exploratory functional connectivity analyses of the anterior and posterior insula. There were no statistically significant pain-related connectivity changes with either division. To better address this question, future studies are taking advantage of the sequence advancements at the 7T and larger sample sizes.

Section 4

3.4 Descending Pain Modulation and Central Sensitisation

3.4.1 Introduction

In the previous sections of this chapter, I was unable to identify statistically significant hemispheric differences in amygdala activity and condition-dependent global connectivity changes with the anterior and posterior insula. It is conceivable that these changes were not detected given the methods available at the time and the small sample size. Furthermore, these sections comprised my earliest functional connectivity analysis methods, which have since improved. Over the course of my D.Phil., I learned new approaches and techniques and wanted to apply them to this dataset. In this section, I use an ROI-network based approach to examine the descending modulatory system to see if changes in this network can be captured during the capsaicin condition.

3.4.1.1 Descending Modulatory Influences on Central Sensitisation

Maintenance of central sensitisation by the descending modulatory system (via the PAG) has been characterised in animal models (Pertovaara, Wei, and Hamalainen 1996). This brainstem circuitry can be engaged by higher cortical and sub-cortical structures, including the amygdala and insula. As previously described, the amygdala has a well-defined role in the descending opioidergic network which includes the PAG (Fields 2000). The anterior insula has also demonstrated engagement with brainstem regions. Ploner and colleagues (2010) observed that the categorisation of stimuli as "painful" could be predicted by anterior insula - PAG connectivity (increased coupling was associated with decreased pain perception).

Both the amygdalae and anterior insula are critical regions in the affective component of pain processing. Structural connectivity between these two regions have been positively correlated with anxiety and the PVAQ (Baur et al. 2013; Wiech et al. 2014). In fMRI research, negative emotion modulation has been shown to amplify pain-related activity in the anterior insula (Ploner et al. 2011). In the amygdalae, Berna and colleagues (2010) demonstrated that healthy individuals that responded most strongly to negative induction paradigms had higher pain-evoked activation. There is also evidence demonstrating that the amygdalae and anterior insula are involved in integrating information about pain intensity (Bornhovd et al. 2002; Kong et al. 2006).

Resting functional changes in the descending modulatory system are not well-characterised during states of central sensitisation. The primary aim of this section was to apply the skills gained in FSLNets on this dataset. Secondarily, I wanted to determine whether descending modulatory maintenance of central sensitisation could be observed in condition-dependent "resting" connectivity changes. However, given the limited nature of this dataset, this was an exploratory approach and analysis.

3.4.2 Methods

3.4.2.1 Subjects

The same ten subjects from the previous analyses were included in this section. Only 7T data was analysed in this chapter.

3.4.2.2 Functional Connectivity Analysis

This section examined connectivity changes between the anterior insula, amygdalae, and PAG by comparing the capsaicin and sham conditions. Given what is known about the multifactorial nature of pain processing and the roles of the amygdalae, anterior insula,

and PAG, I also wanted to explore this network connectivity beyond the strict pain vs. no-pain comparison. Consequently, functional connectivity measures were evaluated with respect to pain and anxiety measures. Reported p-values are corrected for multiple comparisons. The threshold for significance was $p < 0.05$.

3.4.3 Results

3.4.3.1 Comparing Conditions: Anterior Insula-Amygdala-PAG Functional Connectivity

When the capsaicin and sham conditions were compared, there were no significant differences in functional connectivity in the network. There were non-significant increases in anterior insula - amygdala connectivity during the capsaicin condition (N = 10; Paired t-test General Linear Model: p(corr) = 0.4204) and amygdala - PAG connectivity (Paired t-test General Linear Model: p(corr) = 0.4204) (see Figure 3.09).

Increases in Functional Connectivity During the Capsaicin Condition

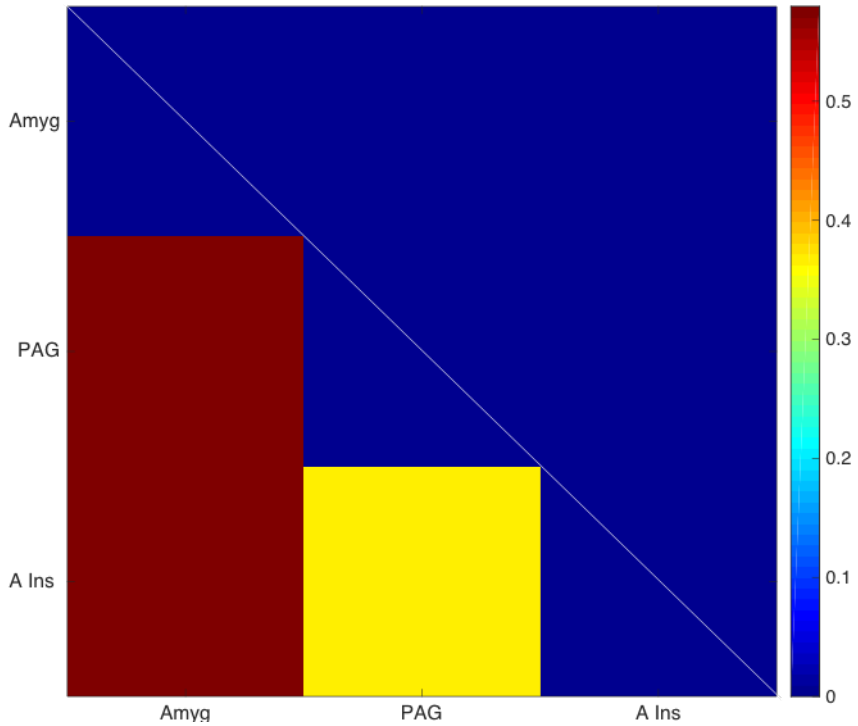


Figure 3.09 Increases in Functional Connectivity During the Capsaicin Condition

This matrix demonstrates the non-significant increases in functional connectivity in the capsaicin condition. There were non-significant increases in connectivity between the amygdala and anterior insula (General Linear Model Paired t-test: p(corr)=0.4204) and the amygdala and PAG (General Linear Model Paired t-test: p(corr)=0.4204). Amyg - amygdala; PAG - periaqueductal gray; A Ins - anterior insula. N = 10.

3.4.3.2 Functional Connectivity Relative to Pain and Pyschological Measures

As previously described, both the amygdala and anterior insula are important in the affective component of pain but also are involved in integrating information about pain intensity. Therefore, the nature of this non-significant connectivity was explored further. The z-transformed correlation coefficients of anterior insula - amygdala connectivity during the capsaicin condition were plotted against the pain ratings reported during the scan. When looking at the pain ratings from during the resting capsaicin scan, there was a significant positive correlation between the z-transformed correlation coefficients for the anterior insula and amygdala and the reported pain scores (Pearson's Correlation: r = 0.7382, p = 0.01476) (see Figure 3.10). State Anxiety scores were not significantly correlated with connectivity in the pain state (Pearson's Correlation: r = 0.30086, p = 0.3983); however, Trait Anxiety scores were significantly negatively correlated with the correlation coefficients (Pearson's Correlation: r = -0.64004, p = 0.04624) (see Figure 3.11).

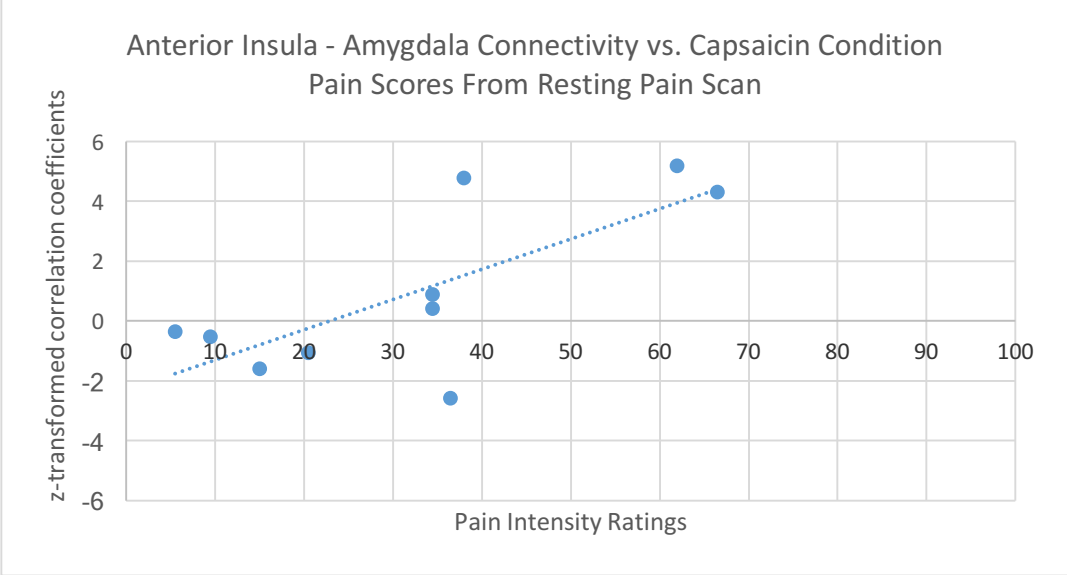


Figure 3.10 Anterior Insula - Amygdala Connectivity vs. Pain Scores During Resting Capsaicin Scan

The z-transformed correlation coefficients from the capsaicin condition plotted against the pain scores from the resting capsaicin scan-only. There was a significant positive correlation between the coefficients and the pain scores (Pearson's Correlation: r = 0.7382, p = 0.01476). N = 10.

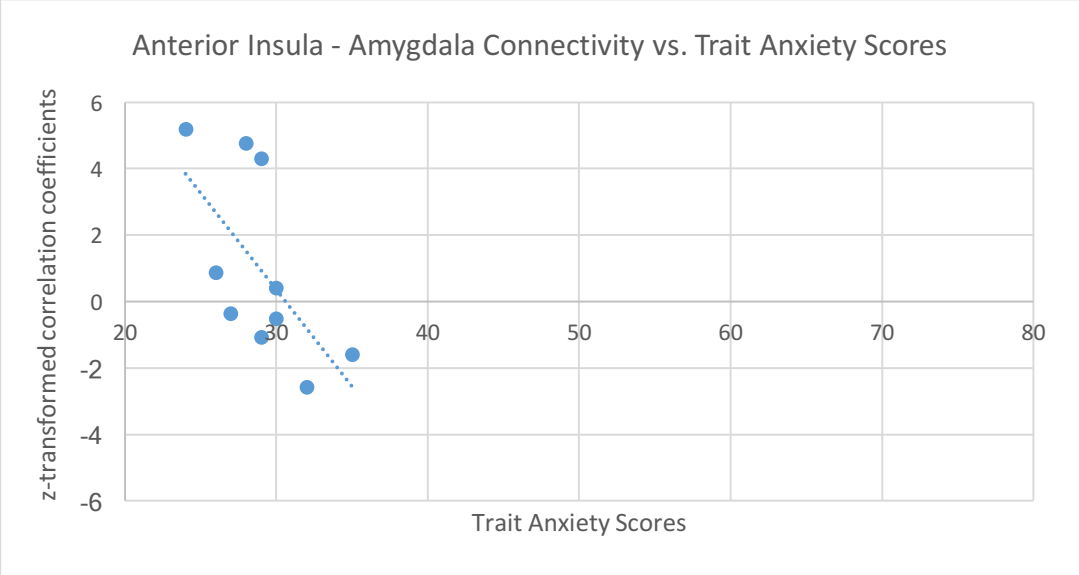


Figure 3.11 Anterior Insula - Amygdala Connectivity vs. Trait Anxiety Scores

The z-transformed correlation coefficients from the capsaicin condition scan plotted against Trait Anxiety scores. There was a negative correlation between the anterior insula-amygdala correlation coefficients and anxiety scores (Pearson's Correlation: r = -0.64004, p = 0.04624). N = 10.

The non-significant increase in connectivity between the amygdala and PAG (General Linear Model Paired t-test: p(corr) = 0.4204) was also evaluated with respect to the behavioural measures discussed above. Pain during the scan was not significantly correlated with the amygdala-PAG connectivity (Pearson's Correlation: r = 0.4254, p = 0.2203). Similarly, both Trait and State Anxiety scores were not significantly correlated with the connectivity coefficients (Pearson's Correlation: *Trait Anxiety*: r = 0.07781, p = 0.8308; *State Anxiety*: r = -0.4505, p = 0.1913).

3.4.4 Discussion

The network analyses in FSLNets did not reveal statistically significant condition-specific changes in the descending modulatory system. However, there was a significant positive correlation between anterior insula - amygdala connectivity and pain intensity ratings. Furthermore, Trait Anxiety scores were negatively correlated with the pain intensity scores. Before delving into a discussion of this one significant finding, it is again important

to highlight that the sample size used in these analyses were likely too small and the only significant result was the product of further exploration of the dataset.

3.4.4.1 Relevance of Anterior Insula and Amygdala Coupling

The anterior insula - amygdala connectivity finding fits with a recent neuroimaging study from our lab. In healthy individuals, Wiech and colleagues (2014) observed a positive correlation between anterior insula and amygdala structural connectivity and the scores from the PVAQ. As previously described, the PVAQ evaluates how attentive an individual is to their pain. Focussing on pain amplifies activation in the anterior insula and heightens intensity ratings (Brooks et al. 2002; Ploner et al. 2011). Given that attention paradigms have elicited changes in other structures in the descending modulatory system (Tracey et al. 2002), this coupling could potentially represent alterations at higher-levels of descending modulation. Unfortunately, PVAQ scores were not obtained in this experiment. As such, no conclusions can be made regarding anterior insula - amygdala connectivity and attention to pain.

One caveat with this result is the negative correlation between Trait Anxiety and amygdala - anterior insula connectivity. In this experiment, there was a non-significant positive correlation between State Anxiety and connectivity and a negative correlation between Trait Anxiety and connectivity. These regions are well-characterised affective regions, with previous reports of their functional connectivity being positively correlated with State Anxiety (Baur et al. 2013). Interestingly, the same study found no significant correlation between functional connectivity and Trait Anxiety.

3.4.4.2 Limitations

Despite the results fitting with previous reports of what is known about these regions, given the exploratory nature of the correlation analysis and small sample size, little can be concluded from these results. As previously described, this data comprises some of the earliest to be acquired on the FMRIB 7T and this study is being re-run with an increased sample size and improvements to scan parameters.

3.4.5 Summary

There were no statistically significant condition-dependent changes in the descending modulatory system; however, there was a significant positive correlation between anterior insula - amygdala connectivity and pain intensity scores. Previous work demonstrates structural connectivity between these regions is positively correlated with pain vigilance.

Chapter 4

Understanding the Pain Experience in Women with Chronic Pelvic Pain

4.1 The Heterogeneity of Chronic Pelvic Pain Patients

4.1.1 Introduction

In the previous chapter, I explored how prolonged pain experiences impact functional connectivity within well-defined pain networks. Most of the results did not reach statistical significance; however, during the pain condition, an exploratory analysis revealed a significant positive correlation between amygdala - anterior insula connectivity and pain intensity ratings. This connectivity was also negatively correlated with Trait Anxiety. The analysis techniques applied in the previous chapter have informed the approach for each of the following imaging analyses, with the remaining chapters focusing on women with CPP. In this chapter, I begin the examination of these patients by characterising their pain experience. Despite falling under the same diagnostic umbrella, women who suffer from CPP are a heterogeneous patient group. I first explore whether there is a relationship between psychological inventories and different measures of the pelvic pain experience (number of pain symptoms, pelvic pain duration, and overall pain intensity). Since each patient has a very different set of pain symptoms, I also examine how each pain experience relates to psychological measures. Understanding how different pain symptoms and measures of the pain experience relate to patients' psychological profiles potentially allows for the creation new multi-disciplinary treatment paradigms.

IASP defines chronic pelvic pain as "chronic or persistent pain perceived in structures related to the pelvis of either men or women. It is often associated with negative cognitive, behavioural, sexual and emotional consequences as well as with symptoms suggestive of lower urinary tract, sexual, bowel, pelvic floor or gynaecological dysfunction" (International Association for the Study of Pain 2012a). Recently, dysmenorrhoea has been included in the IASP definition of CPP; however, in the UK, dysmenorrhoea alone is not considered CPP (Moore and Kennedy 2012).

Given the breadth of the definition and the variety of viscera within the pelvis, many women diagnosed with CPP have heterogeneous pain experiences, with pain interfering with many aspects of their lives. These patients often report dysmenorrhoea, non-cyclical pain, dyspareunia, dysuria, and dyschezia as significant pain symptoms. However, not all women with CPP experience the same types of pain and, even if they do, there is a variation in pain intensity. For example, some women may experience only dyspareunia whilst others may experience non-cyclical pain and dysmenorrhoea. Whilst these patients may have a common diagnosis of CPP, the potential implications of pelvic pain on their psychological well-being are vastly different. There is a preponderance of literature demonstrating that anxiety and depression are co-morbid with chronic pain. Indeed, these psychological factors are thought to influence/be influenced by the pain experience. In patients with major depressive disorder, researchers demonstrated a positive correlation between degree of depression and the number of reported types of pain (Vaccarino et al. 2009); however, it appears this research was framed with pain as a side-effect of depression, when in fact, they potentially feed into each other cyclically (Gerrits et al. 2014). In chronic pain patients, there is evidence suggesting that depression and

clinical pain intensity are positively correlated (Arnstein et al. 1999). However, in the very unique population of women with CPP, it is not known if different types of pain or measures of the pain experience are related to psychological factors.

In 2008, a new phenotyping system, UPOINT, was published to assist in classifying chronic prostatitis/CPPS and IC patients (Shoskes, Nickel, Rackley, et al. 2009). There are six clinical symptom categories: "urinary, psychosocial, organ specific, infection, neurologic/systemic conditions, and tenderness of skeletal muscles" (Shoskes UPOINT System for the Clinical Phenotyping of Chronic Pelvic Pain). These categories are meant to facilitate earlier multi-disciplinary treatment since many factors often contribute to each patient's experience. In women with IC/PBS, duration of symptoms was positively correlated with the number of symptom categories fulfilled (Nickel, Shoskes, and Irvine-Bird 2009). A similar relationship between duration and symptom categories was noted in male patients with IC/CPPS, with more reported clinical categories in those with pain for longer than two years (Shoskes, Nickel, Dolinga, et al. 2009).

Women with CPP have long struggled to receive timely pain management (Grace 1995; Grace and Zondervan 2004; Moore and Kennedy 2012). One potential reason for this may be due to the over-characterisation of the condition as psychologically-driven (Castelnuovo-Tedesco and Krout 1970). As previously described, psychological state is certainly a well-known amplifier of both the acute and chronic pain experiences in both men and women; however, it is certainly not the only factor driving these experiences. Better characterisation of CPP and how it impacts psychological well-being has the

potential to improve future treatment paradigms by allowing for personalised clinical approaches based on each patient's needs.

The aim of this chapter was to characterise our cohort of women with CPP by determining 1) whether different measures of the pain experience (duration of pain, number of pain complaints, and pain intensity) were correlated with psychological measures (anxiety, depression, and catastrophising), and 2) whether different types of pain symptoms were correlated with anxiety, depression, or catastrophising. I hypothesised that 1) having more pain symptoms would be positively correlated with pain catastrophising, 2) functional symptoms (dyspareunia and dyschezia) would be associated with greater psychological distress, and 3) that pain intensity would be positively correlated with psychological measures.

4.1.2 Methods

Women who participated in the study were recruited from the endometriosis clinic at the John Radcliffe Hospital. They provided written consent and completed an extensive questionnaire within the 2-weeks prior to their diagnostic laparoscopy and/or endometriosis surgery. This questionnaire included the STAI, BDI, Pain Catastrophising Scale, and questions relating to their pain experience (types of pain and duration of symptoms). All pain ratings were recorded on an NRS.

4.1.2.1 Subjects

Twenty-six subjects were included in this analysis.

4.1.2.2 Analysis

Before interpreting the multiple linear regression models, several measures were checked to ensure statistical assumptions were not violated. The residuals of the response variable

were approximately normally distributed, collinearity between independent variables was not observed, homoscedasticity was confirmed graphically, and there was a linear relationship between the dependent and independent variables. All reported R^2 values are adjusted. The p-value threshold was defined at $p < 0.05$.

4.1.3 Results

Of the twenty-six women, twenty-two (85%) reported menstrual pain, twenty reported non-cyclical pain (77%), twenty-five reported dyspareunia (96%), nineteen reported dyschezia (73%), and twelve reported dysuria (46%) (see Figure 4.01). Fifteen (58%) reported pain related to their period as the worst pain. Other types of pain indicated to be the worst included dyschezia (seven, 27%), dyspareunia (five, 19%), dysuria (one, 4%), and general pain in the lower half of the body (one, 4%) (several patients reported more than one of these types of pain as their worst symptoms). Even in the women with confirmed endometriosis, there is a clear heterogeneity in the pain experience (see Figure 4.02).

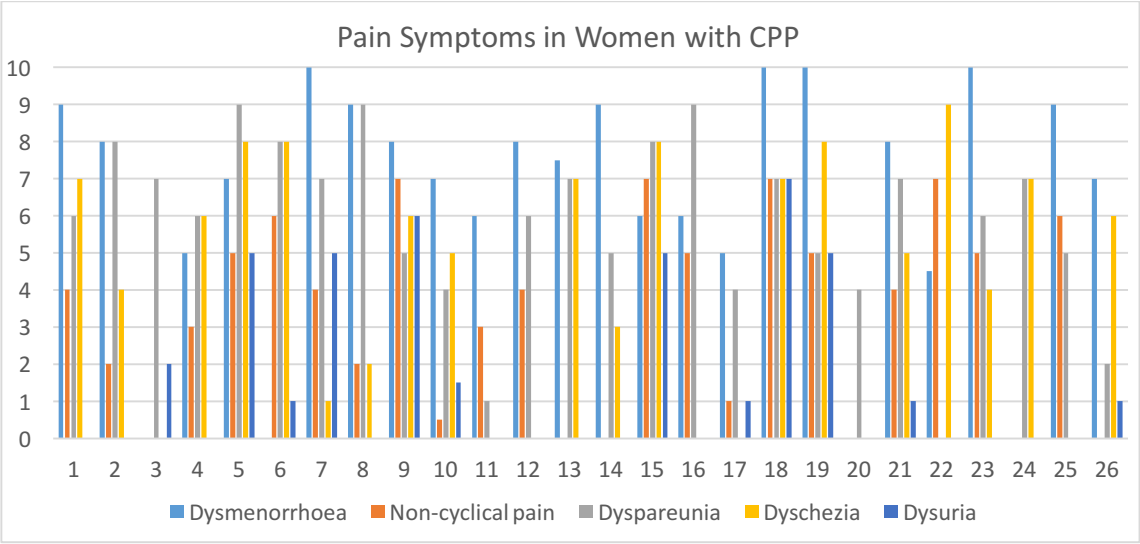


Figure 4.01 Intensity Ratings of Different Pain Symptoms in Women with CPP

Women with CPP suffer from different types of pain profiles and experience different degrees of pain intensity. The y-axis represents the pain intensity NRS. Some women will have one pain symptom whilst others will suffer from multiple. N = 26.

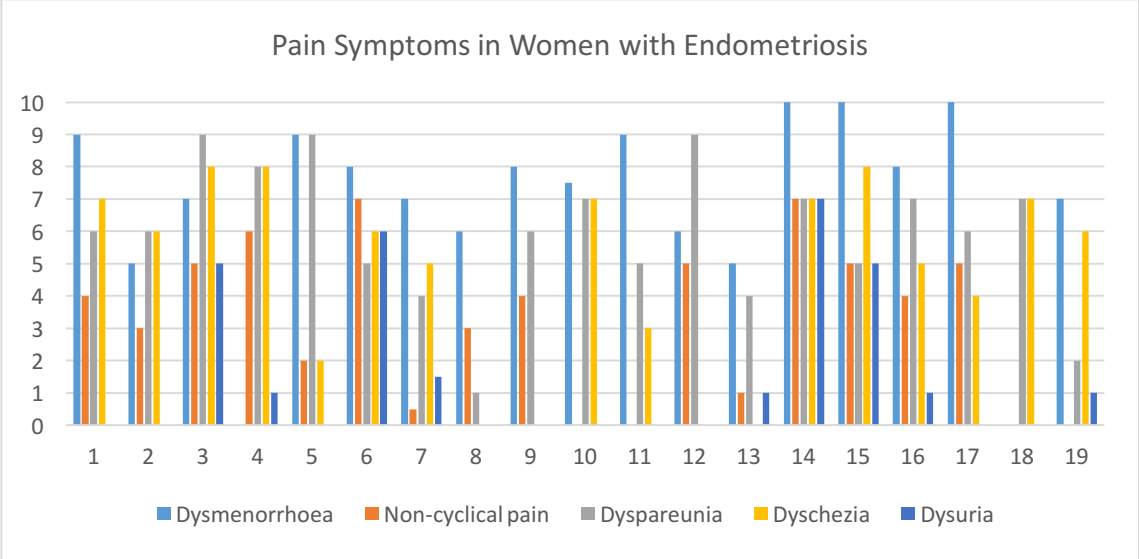


Figure 4.02 Intensity Ratings of Different Pain Symptoms in Women with Endometriosis-Associated CPP

Women with endometriosis-associated CPP suffer from different types of pain profiles and experience different degrees of pain intensity. The y-axis represents the pain intensity NRS. Some women will have one pain complaint whilst others will suffer from multiple. N = 19.

The mean duration of pain for all twenty-six patients with CPP was 73.67 months or 6.14 years. Patients reported a mean of 3.77 pain symptoms (out of 5). There was a mean of 3.12 pain symptoms that were of moderate to high intensity ($\geq$3 out of 10). Mean pain intensities for the different symptoms ranged from 1.56 to 6.5 out of 10. When examining psychological measures, State and Trait Anxiety Inventory scores fell within the moderate anxiety range, pain catastrophising scores were below the clinical threshold (30), and Beck Depression Inventory scores fell into the mild category (see Table 4.01).

Demographic and Clinical Measures	**Mean (±SD)**
Age (Years)	31.85 (±6.47)
Duration of Pain	73.67 Months (±69.66) 6.14 Years (±5.81)
Pain Catastrophising Scale (0 – 52)	24.54 (±13.27)
Beck Depression Inventory (0 – 63)	16.12 (±10.52)
State Anxiety Inventory (20 – 80)	43.42 (±12.63)
Trait Anxiety Inventory (20 – 80)	48.37 (±13.39)
Pain Measures (0 - 10)	**Mean (±SD)**
Overall Pain Intensity (past 4-weeks)	5.29 (±2.09)
Dysmenorrhoea	6.50 (±3.24)
Non-Cyclical Pain	3.50 (±2.52)
Dyspareunia	5.85 (±2.34)
Dyschezia	4.27 (±3.24)
Dysuria	1.56 (±2.29)

Table 4.01 Demographic, Clinical, and Pain Measures

Summary of demographics, pain measures, and psychological inventories for the patients included in this chapter. N = 26.

4.1.3.1 Characterising the Pain Experience

First, I examined whether measures of the pain experience (duration of pelvic pain, pain intensity, and number of pain symptoms) were correlated with State-Trait Anxiety Inventory, Beck Depression Inventory, and Pain Catastrophising Scale scores. Each psychological inventory was examined separately with respect to the three pain measures. I found that pain intensity scores were positively correlated with State-Trait Anxiety Inventory and Beck Depression Inventory scores (Multiple Linear Regression: *State Anxiety*: p = 0.0199, *Trait Anxiety*: p = 0.00506, *Beck Depression*: p = 0.0490) (see Table 4.02 and Figures 4.03-4.05). Additionally, longer duration of pelvic pain was positively correlated with Trait Anxiety scores (Multiple Linear Regression: p = 0.0472) (see Figure 4.06).

N = 26	**Number of Pain Symptoms** (0 - 5)	**Overall Pain Intensity** (4-weeks preceding visit)	**Duration of Pain** (Months)
Pain Catastrophising Scale (0 - 52)	p = 0.2460	p = 0.3310	p = 0.1240
Trait Anxiety Inventory (20 - 80)	p = 0.2704	p = 0.0132	p = 0.0472
State Anxiety Inventory (20 - 80)	p = 0.1061	p = 0.0199	p = 0.5690
Beck Depression Inventory (0 - 63)	p = 0.1720	p = 0.0490	p = 0.3600

Table 4.02 Multiple Linear Regression for Different Measures of the Pain Experience

A multiple linear regression was run for each psychological measure. In the model, the different measures of the pain experience were included. Trait and State Anxiety and Beck Depression scores were significantly positively correlated with the mean reported pain intensity (average of the four weeks preceding Visit 1). N = 26.

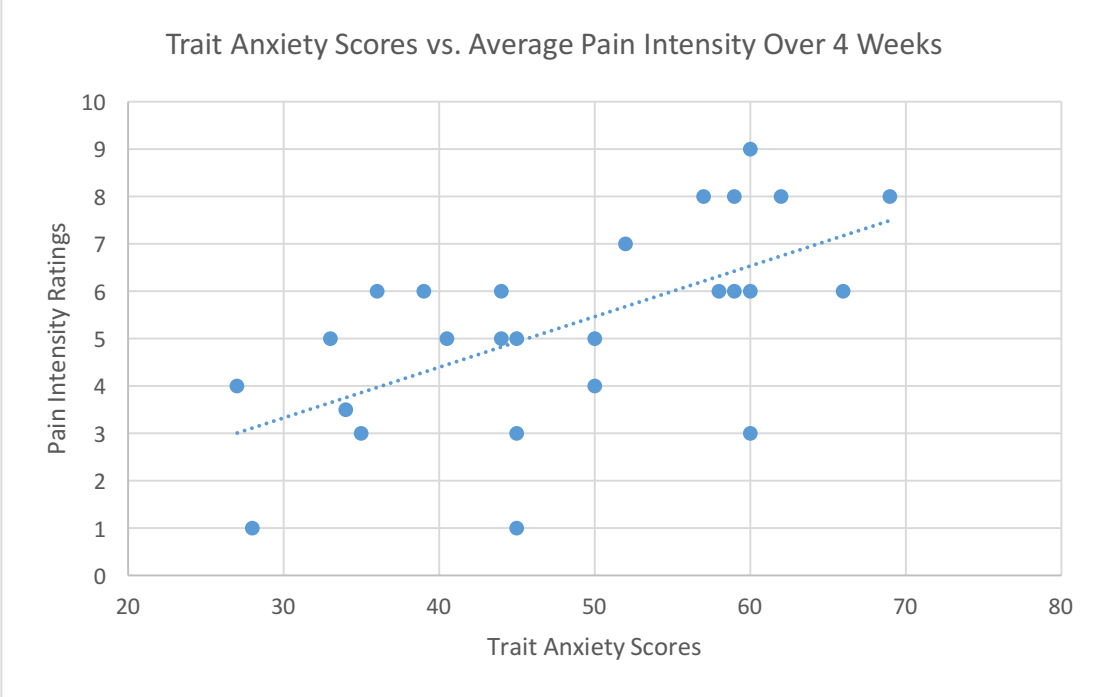


Figure 4.03 Pain Intensity Ratings vs. Trait Anxiety Scores

There was a significant positive correlation between Trait Anxiety scores and overall pain intensity (the pain intensity from the four weeks prior to the research visit). Statistics from multiple linear regression: R^2 = 0.3862, p = 0.0031 (full model); p = 0.0132 (Trait Anxiety-only). N = 26.

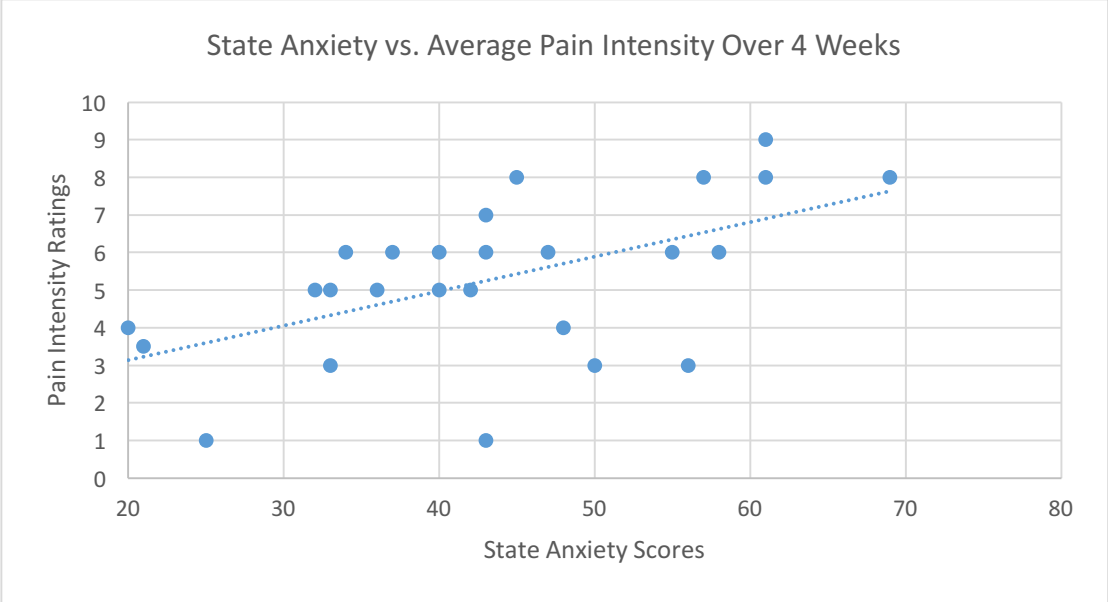


Figure 4.04 Pain Intensity Ratings vs. State Anxiety Scores

There was a significant positive correlation between State Anxiety scores and overall pain intensity (the pain intensity from the four weeks prior to the research visit). Statistics from multiple linear regression: R^2 = 0.3279 p = 0.0081 (full model); p = 0.0199 (State Anxiety-only). N = 26.

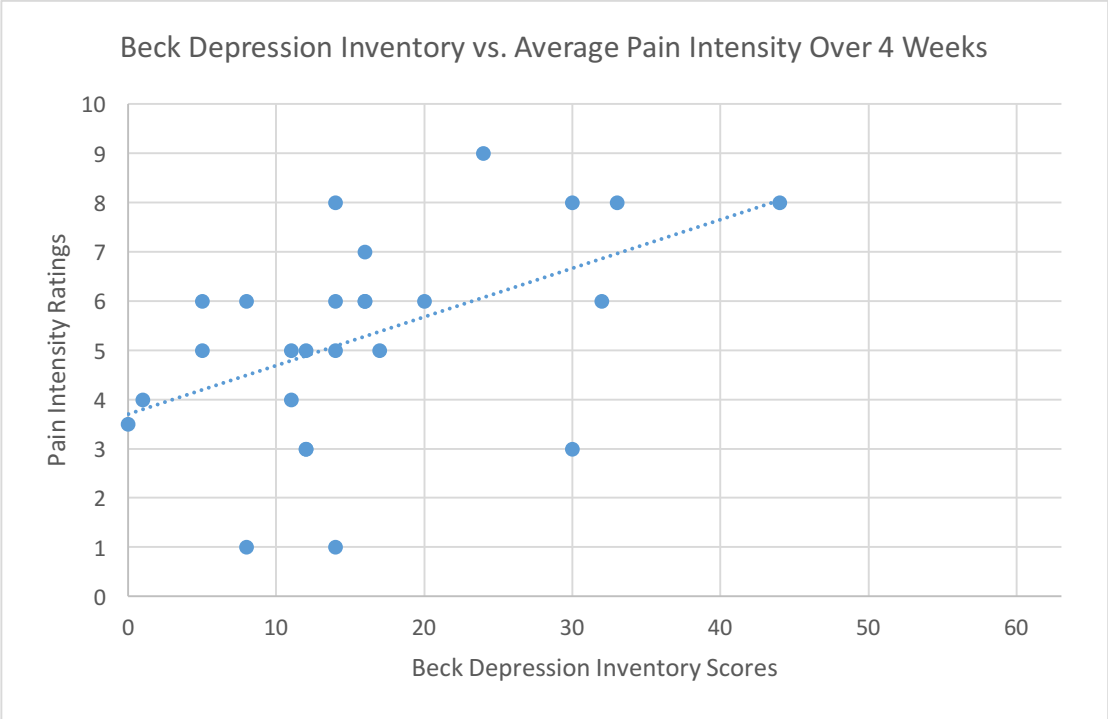


Figure 4.05 Pain Intensity Ratings vs. Beck Depression Inventory

There was a significant positive correlation between Beck Depression Inventory scores and overall pain intensity (the pain intensity from the four weeks prior to the research visit). Statistics from multiple linear regression: R^2 =0.2667, p = 0.0199 (full model); p = 0.0490 (Beck Depression-only). N = 26.

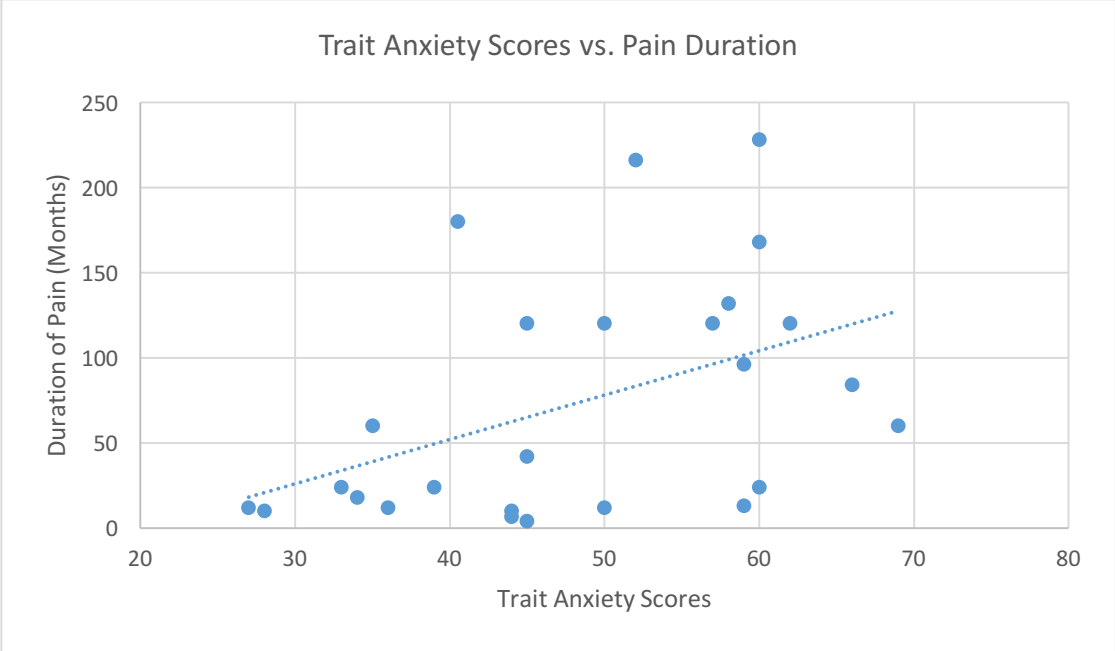


Figure 4.06 Pain Duration vs. Trait Anxiety Scores

There was a significant positive correlation between Trait Anxiety scores and duration of pelvic pain symptoms (in months). Statistics from multiple linear regression: R^2 = 0.3862, p = 0.0031 (full model); p = 0.0472 (Trait Anxiety-only). N = 26.

4.1.3.2 The Impact of Heterogeneous Pain Symptoms in Women with Chronic Pelvic Pain

Next, I examined how different types of pain (dysmenorrhoea, non-cyclical pain, dyspareunia, dysuria, and dyschezia) related to patients' anxiety, depression, and catastrophising scores. The only pain score that was correlated with any psychological measure was dyschezia with State Anxiety (Positive correlation; Multiple Linear Regression: p = 0.0162) (see Table 4.03 and Figure 4.07).

	Dysmenorrhoea	Dyspareunia	Non-Cyclical Pain	Dyschezia	Dysuria
Pain Catastrophising Scale (0 - 52)	p = 0.0811	p = 0.3740	p = 0.1895	p = 0.1813	p = 0.7497
Trait Anxiety Inventory (20 - 80)	p = 0.2773	p = 0.5787	p = 0.9607	p = 0.0512	p = 0.3618
State Anxiety Inventory (20 - 80)	p = 0.8801	p = 0.5843	p = 0.6203	p = 0.0162	p = 0.2412
Beck Depression Inventory (0 - 63)	p = 0.4765	p = 0.6813	p = 0.7494	p = 0.6307	p = 0.0899

Table 4.03 Multiple Linear Regression for Different Pelvic Pain Symptoms

A multiple linear regression was run for each psychological measure. In the model, the pain intensity scores for each pain symptom were included. The only significant correlation was dyschezia with State Anxiety (p = 0.0162). N = 26.

State Anxiety scores were plotted against the dyschezia pain ratings to further examine the relationship (see Figure 4.07).

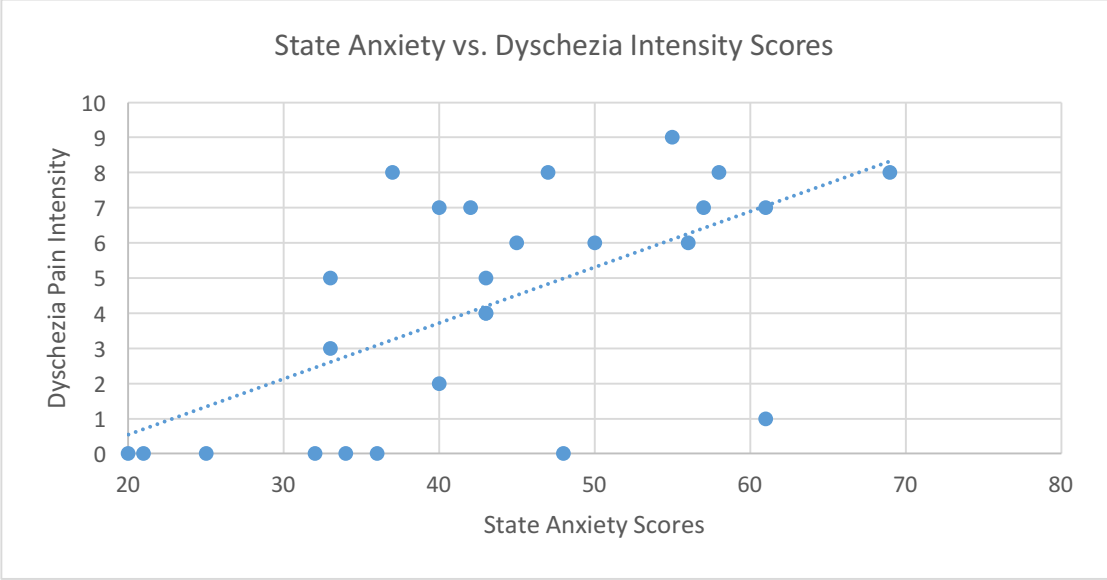


Figure 4.07 Dyschezia Pain Intensity vs. State Anxiety Scores

Dyschezia pain ratings plotted against State Anxiety scores. There was a significant positive correlation. Statistics from multiple linear regression: R^2 = 0.3213, p = 0.0268 (full model); p = 0.0162 (dyschezia). N = 26.

4.1.4 Discussion

Overall pain intensity scores were significantly positively correlated with State and Trait Anxiety scores as well as Beck Depression Inventory scores. Additionally, having

experienced pelvic pain for longer was significantly correlated with higher Trait Anxiety scores. When examining the relationship between each pain symptom and psychological profile, the only significant correlation was a positive one between State Anxiety scores and dyschezia ratings.

4.1.4.1 Psychological Measures and the Pain Experience

As previously described, State Anxiety is a measure of anxiety at the time of questionnaire completion whereas Trait Anxiety is a static measure of inherent anxiety (Spielberger 1983). The Beck Depression Inventory measures depression within the "past few days" that the questionnaire is completed (Beck et al. 1961; Beck, Steer, and Brown 1996). It has been well-documented that experimentally induced anxiety and depression, even in healthy individuals, can amplify the pain experience (Ploghaus et al. 2001; Berna et al. 2010). My results suggest that depression and anxiety potentially play a part in intensifying the chronic pain experience; however, the impact of chronic pain on the worsening of psychological symptoms cannot be ignored (Tracey and Bushnell 2009; Gerrits et al. 2014). Indeed, the static measure of Trait Anxiety was positively correlated with pain duration in these women, so perhaps inherent predisposition to higher anxiety levels facilitates prolonged chronic pain experiences.

No pain measure, including number of pain symptoms, was significantly correlated with pain catastrophising (rumination, magnification, helplessness) which is noteworthy as it is a different, pain-specific measure of anxiety. Since the average duration of pelvic pain symptoms exceeds 6 years, the patients could be beyond the threshold where this would be a distinguishing measure (they have become accustomed to their pain). Since the study is ongoing, specific components of the Pain Catastrophising Scale can be evaluated with a

larger sample size. Duration of pain could be correlated with helplessness, given that there is a significant diagnosis and treatment delay (Grace 1995; Grace and Zondervan 2004).

When examining pain symptoms, only dyschezia was correlated with State Anxiety. In the age of the internet, it is highly likely these women have researched their symptoms online and discovered that dyschezia can be a sign of advanced disease (i.e. deep infiltrating endometriosis) (Seracchioli et al. 2008). The first research visit for this study was within the 2-weeks prior to, for many women, their first diagnostic laparoscopy. It is likely that this correlation is driven by fear of having extensive endometriosis and thus having more of tissue removed during the surgical procedure. Additionally, their anxiety could also be a result of the high frequency with which this pain occurs and its unavoidable nature.

4.1.4.2 Limitations

However, there are several important limitations that must be addressed. First of all, the patients included were referred from a tertiary endometriosis care clinic at the John Radcliffe Hospital. These women were all suspected of having endometriosis (a few had confirmed diagnoses) and were on a waiting list for a diagnostic laparoscopy and/or surgical intervention for the pathology. As such, they do not necessarily represent the broader CPP patient population. For example, in our cohort from the John Radcliffe Hospital, 85% experienced dysmenorrhoea and 96% experienced dyspareunia. In a survey of the community (not associated with any clinic), Zondervan and colleagues (2001a) found that in women with CPP, 81% experienced dysmenorrhoea and 41% experienced dyspareunia. Given our patients were recruited from a specialised clinic, the findings from

this chapter cannot necessarily be extended to the greater population of women with CPP.

The second critical limitation is that these types of analyses are better performed on much larger datasets. This study was not designed to examine these relationships. Again, the epidemiological study performed by Zondervan and colleagues (2001a) included 483 women with CPP whereas this chapter only included 26 patients.

4.1.5 Summary

This this chapter, I demonstrated that pain intensity and pain duration are positively correlated with psychological distress in our cohort of women with CPP. Dyschezia scores were positively correlated with anxiety as well. Given the aforementioned limitations, these results are likely specific to our group of patients and cannot necessarily be extrapolated to the broader population of women with CPP.

Chapter 5

Central Representation of Neuropathic Pain in Women with Chronic Pelvic Pain

5.1 Using PainDETECT to Identify Central Modulators of Neuropathic Pain

5.1.1 Introduction

In the previous chapter, I demonstrated that there were correlations between psychological and pain measures in our cohort of CPP patients. The chapter also highlights that there is a diversity of pain symptoms and severity in this patient population. In this section, I explored whether the distinction between nociceptive and neuropathic pain using a standardised questionnaire (PainDETECT) (Whitaker et al. 2016) is reflected in central networks in women with CPP. Specifically, I examined if there were differences in underlying neuropathic mechanisms of central sensitisation in the descending modulatory system (Woolf 2011). A measurable divergence in underlying modulatory mechanisms in these patients would suggest that different analgesic approaches might be needed for effective treatment of pelvic pain (Gwilym et al. 2009).

5.1.1.1 Descending Modulatory Mechanisms and Neuropathic Pain

In animal models of neuropathic pain, researchers have demonstrated that central sensitisation is maintained through supraspinal modulation by the PAG and RVM. Chemical interference with the PAG and RVM, by injection of a local anaesthetic, has been shown to reduce allodynia in rodents with experimentally induced neuropathic pain (Pertovaara, Wei, and Hamalainen 1996).

Supraspinal modulators of neuropathic pain have been observed in chronic pain patients. In the imaging domain, PainDETECT is a commonly used tool to classify patients with neuropathic components to their pain. Researchers observed that in the PAG, PainDETECT scores predicted pain-related signal, which corresponded to the level of hyperalgesia experienced by the patients (Gwilym et al. 2009).

5.1.1.2 Neuropathic Pain in Women with Chronic Pelvic Pain

Recent evidence suggests that neuropathic mechanisms could be responsible for the pain experienced by women with CPP. In women with painful bladder syndrome (PBS), neuropathic PainDETECT scores were positively correlated with worse urinary and psychological symptoms (Cory et al. 2012). Similar mechanisms could exist in women with CPPS and endometriosis-associated CPP. Recently, Whitaker and colleagues (2016) found that of the 72 women with CPP included in their study, 19 (26%) had neuropathic components to their pain (based on PainDETECT). Interestingly, they found that PainDETECT scores did not significantly differ between women who had endometriosis-associated pain and those with pelvic pain but no pathology (Whitaker et al. 2016).

The aim of this section was to determine whether possible neuropathic mechanisms driving CPP are differentially represented in resting activity of the amygdala, PAG, and RVM, key regions in the descending modulatory system. I hypothesised that there would be PainDETECT-associated differences in functional connectivity, which would suggest that there are differences in underlying pain modulatory mechanisms.

5.1.2 Methods

The same twenty-six subjects from the previous analysis were included in this section. Resting fMRI-BOLD data and PainDETECT scores were collected. PainDETECT has three score categories: nociceptive (0-12), unclear (13-18), and neuropathic (19-38).

In the first analysis, scores were included in the model as a continuous covariate (N = 26). In the second analysis, the goal was to run a group comparison based on the reported PainDETECT scores. However, there was an imbalance of patients in the "nociceptive" category (N = 17) when compared to the "unclear" (N = 5) and "neuropathic" (N = 4) categories. Therefore, those with potential neuropathic components (unclear) and those with a high likelihood (>90%) of neuropathic pain (neuropathic) where combined to form one group. Thus, the group comparison is between patients who potentially have a neuropathic component and patient with low likelihood of neuropathic components of pain (<15%). Since there were still many more nociceptive scorers, only those with the lowest scoring nociceptive PainDETECT scores were included. This resulted in a group comparison of unclear/neuropathic (N = 9) and nociceptive (N = 11). Eleven were included in the nociceptive score group because three shared the same score.

5.1.3 Results

Since the patient population includes both women with endometriosis-associated pain and women with CPPS, PainDETECT scores were grouped based on diagnostic category to evaluate whether there was a difference due to pathology. Despite PainDETECT scores being, on average, higher in the endometriosis cohort (12.58 versus 9.86), a Mann Whitney U test revealed that the difference was not statistically significant ($p = 0.2790$) (see Figure 5.01).

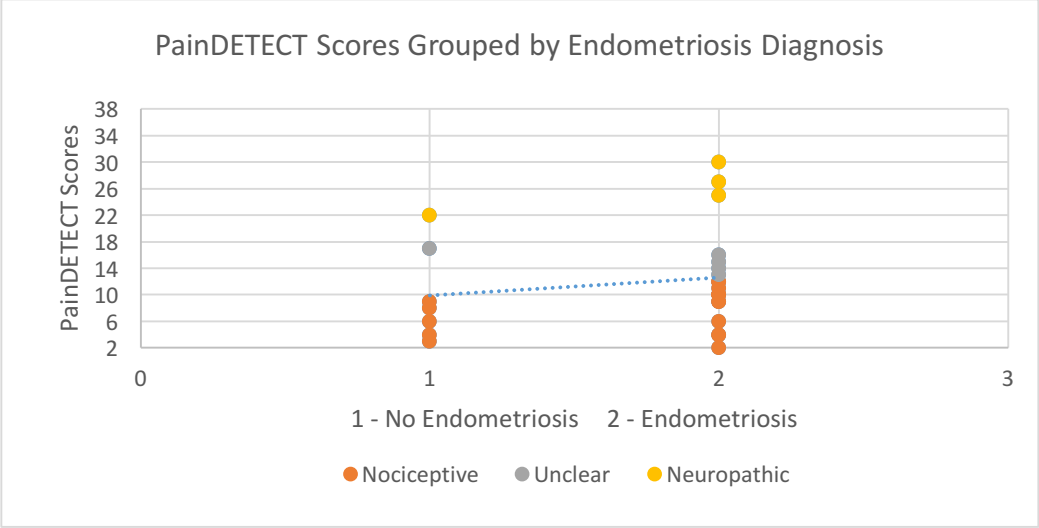


Figure 5.01 PainDETECT Scores Grouped by Endometriosis Diagnosis

Women with endometriosis had higher average PainDETECT scores (Mean = 12.58, N = 19) than women without pathology (Mean = 9.86, N = 7); however, the difference was not statistically significant (Mann Whitney U: p = 0.2790).

5.1.3.1 PainDETECT and the Descending Modulatory System

When PainDETECT scores were included as a continuous covariate of interest in FSLNets, there was a non-significant increase in functional connectivity between the amygdala and PAG (General Linear Model: p(corr) = 0.2948) and decrease in functional connectivity between the amygdala and RVM (General Linear Model: p(corr) = 0.322800) (see Figure 5.02).

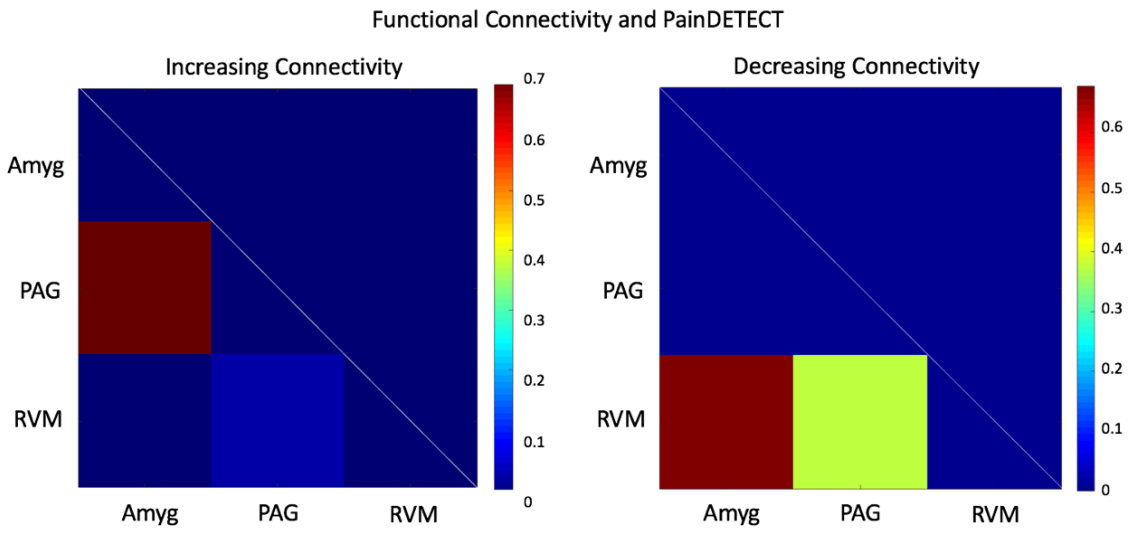


Figure 5.02 Functional Connectivity Changes with Respect to Increasing PainDETECT Scores

Above are the connectivity matrices demonstrating non-significant increases (General Linear Model: p(corr) = 0.2948) and decreases (General Linear Model: p(corr) = 0.3228) in connectivity with respect to higher neuropathic pain scores. The values above the diagonal represent those that survived the threshold, while the ones below are not significant at the threshold ($p < 0.05$). The colour bar represents 1-p-values. N = 26. Amyg - amygdala; PAG - periaqueductal gray; RVM - rostral ventromedial medulla.

The z-transformed correlation coefficients for amygdala - PAG connectivity were plotted against PainDETECT scores (see Figure 5.03). There is no clear trend between connectivity and scores, likely due to the nature of the questionnaire, which does not have a linear scoring system.

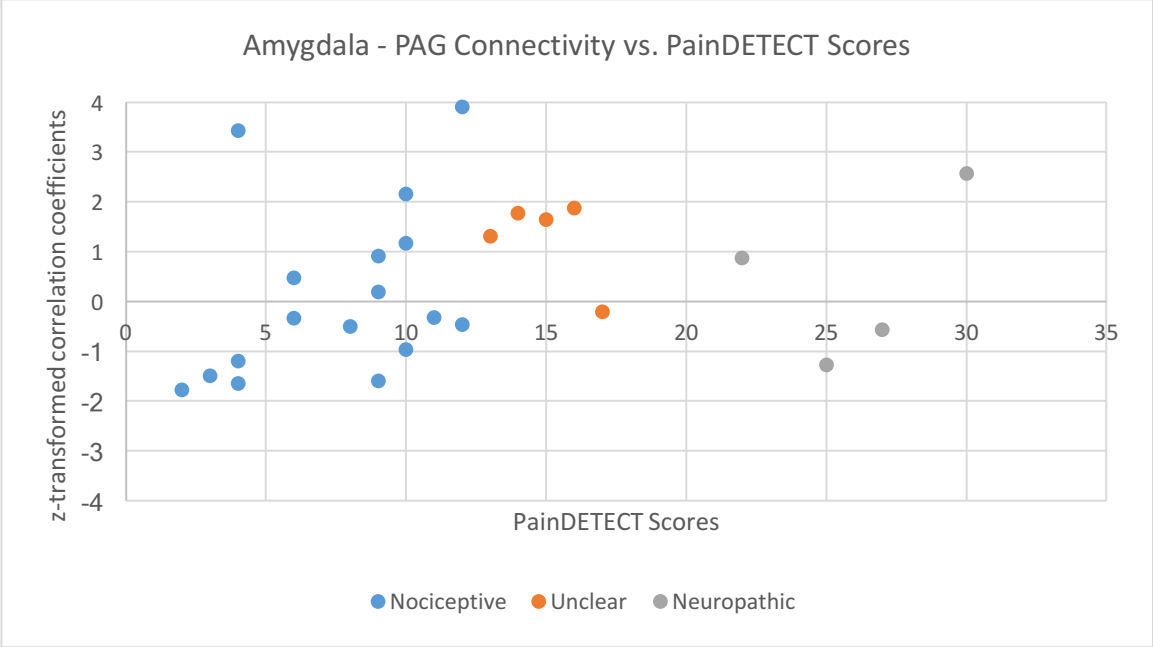


Figure 5.03 Amygdala - PAG Connectivity vs. PainDETECT Scores

The z-transformed correlation coefficients for amygdala - PAG connectivity are plotted against the PainDETECT scores (blue - nociceptive, orange - unclear, grey - neuropathic). There is a non-significant positive trend between connectivity and PainDETECT scores. N = 26.

The patients were then separated into an unclear/neuropathic group (unclear or neuropathic PainDETECT scores, N = 9) and the lowest scorers on the questionnaire (all nociceptive, N = 11) (see Table 5.01).

Demographic, Pain, and Clinical Measures	**Nociceptive** (N = 11) Mean (±SD)	**Unclear / Neuropathic** (N = 9) Mean (±SD)	**p-values**
Age (Years)	33.45 (±7.84)	29.89 (±4.76)	p = 0.2483
Duration of Pain (Months)	46.41 (±58.14)	77.44 (±70.14)	p = 0.0950
Overall Pain Intensity (0 - 10)	4.50 (±2.22)	6.22 (±1.86)	p = 0.0805
Beck Depression Inventory (0 - 63)	13.36 (±8.29)	23.00 (±12.23)	p = 0.0504
*State Anxiety Inventory (20 - 80)	38.82 (±11.37)	51.33 (±11.86)	p = 0.0273
Trait Anxiety Inventory (20 - 80)	43.36 (±13.44)	55.33 (±12.23)	p = 0.0539
*Pain Catastrophising Scale (0 - 52)	18.00 (±10.05)	34.00 (±13.41)	p = 0.0069

Table 5.01 Demographic, Clinical, and Pain Measures of the Women with CPP

Unpaired t-tests revealed that women with neuropathic and unclear PainDETECT scores had statistically significant higher State Anxiety (p = 0.0273) and Pain Catastrophising (p = 0.0069) scores than women with nociceptive scores. Group differences in duration of pain were evaluated using Mann Whitney U. N = 20.

FSLNets revealed that there was a non-significant increase in connectivity between the PAG and amygdala in the neuropathic group (General Linear Model Group Comparison: p(corr) = 0.1072) (see Figure 5.04).

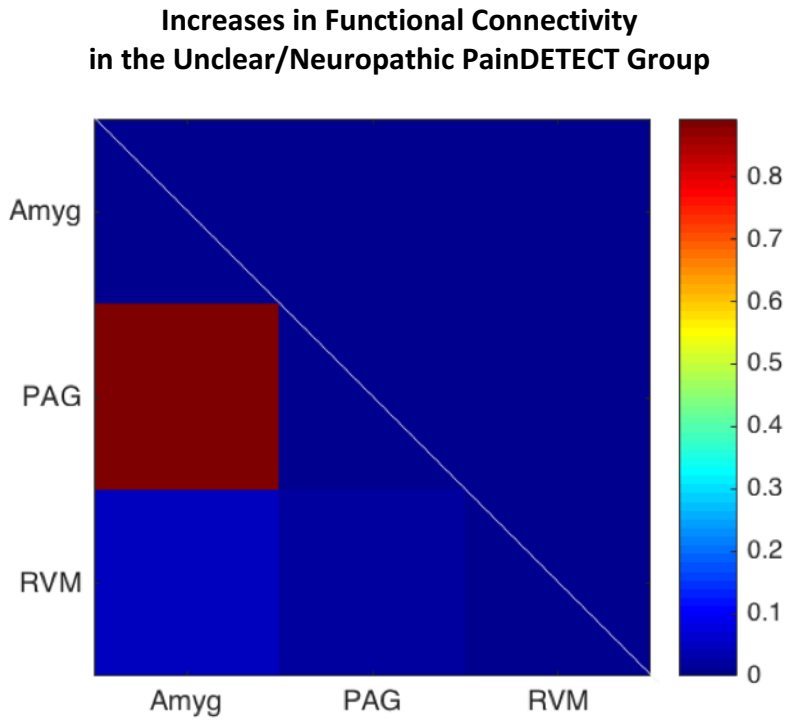


Figure 5.04 Increases in Functional Connectivity in the Unclear/Neuropathic PainDETECT Group

Above are the connectivity matrices demonstrating non-significant increases in connectivity in the neuropathic group (General Linear Model Group Comparison: p(corr) = 0.1072). The values above the diagonal represent those that survived the threshold, while the ones below are not significant at the threshold ($p < 0.05$). The colour bar represents 1-p-values. N = 20. Amyg- amygdala; PAG - periaqueductal gray; RVM - rostral ventromedial medulla.

The increase in amygdala and PAG activity was further examined by plotting the z-transformed correlation coefficients against the PainDETECT groups (see Figure 5.05).

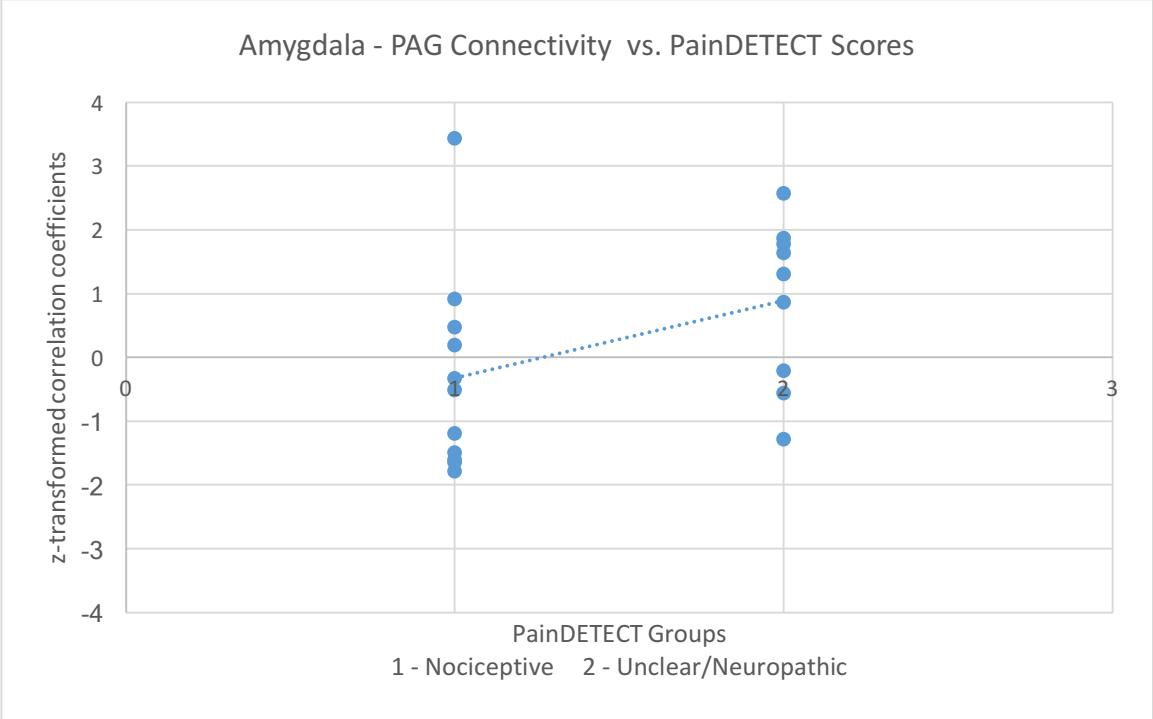


Figure 5.05 Amygdala - PAG Connectivity vs. PainDETECT Score Groups

The z-transformed correlation coefficients for amygdala - PAG connectivity are separated into the two PainDETECT groups. There is a non-significant positive trend between connectivity and PainDETECT scores (N = 20). There was one outlier in the nociceptive group.

There was a clear outlier in the nociceptive group. Upon re-examining the study's medical questionnaire for this participant, it became apparent that there was a cardiological condition that could potentially influence the BOLD-fMRI response and thus was excluded. With the outlier removed, there is a trend suggesting that connectivity is higher in the unclear/neuropathic pain group (see Figure 5.06).

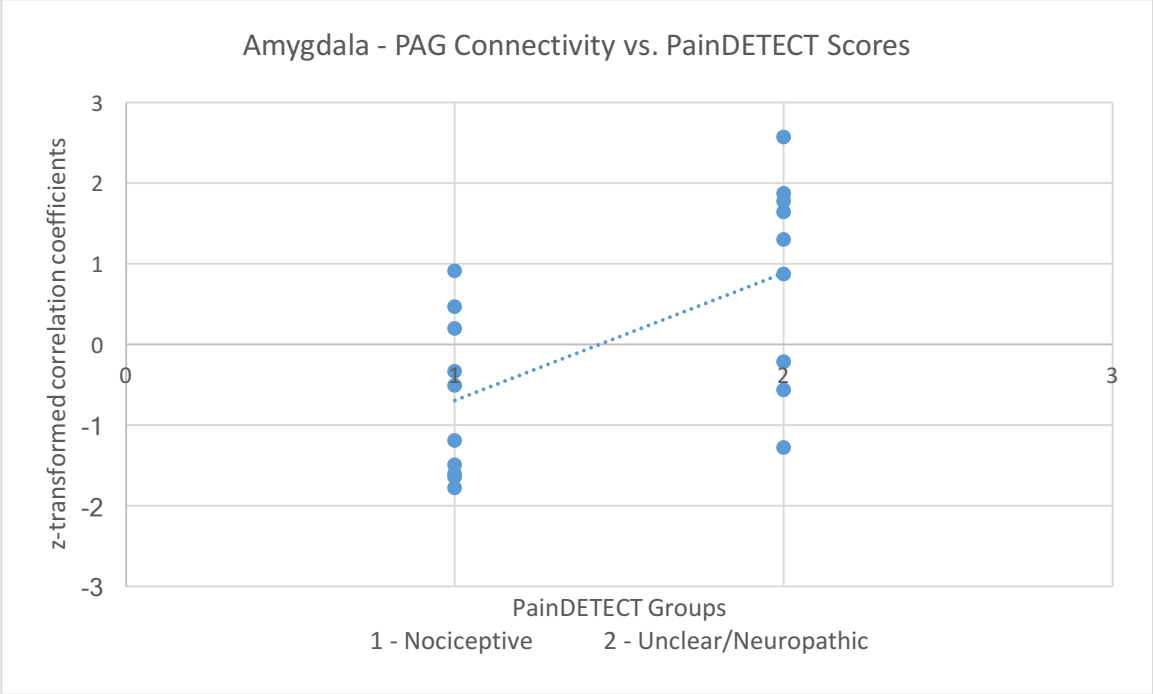


Figure 5.06 Amygdala - PAG Connectivity vs. PainDETECT Score Groups (Without Outlier)

The z-transformed correlation coefficients for amygdala - PAG connectivity separated into the two PainDETECT groups with the outlier removed. N = 19.

The FSLNets network analysis was run again excluding the outlier in the nociceptive group. Again, State Anxiety ($p = 0.0299$) and Pain Catastrophising ($p = 0.0166$) scores were higher in the unclear/neuropathic group (see Table 5.02).

Demographic, Pain, and Clinical Measures	**Nociceptive** Mean (±SD) N = 10	**Unclear / Neuropathic** Mean (±SD) N = 9	**p-values**
Age (Years)	33.70 (±8.22)	29.89 (±4.76)	p = 0.2400
Duration of Pain (Months)	46.85 (±61.27)	77.44 (±70.14)	p = 0.0790
Pain Intensity (0 - 10)	4.85 (±2.00)	6.22 (±1.86)	p = 0.1409
Beck Depression Inventory (0 - 63)	13.90 (±8.53)	23.00 (±12.23)	p = 0.0748
*State Anxiety Inventory (20 - 80)	38.40 (±11.89)	51.33 (±11.86)	p = 0.0299
Trait Anxiety Inventory (20 - 80)	43.20 (±14.16)	55.33 (±12.23)	p = 0.0632
*Pain Catastrophising Scale (0 - 52)	18.50 (±10.45)	34.00 (±13.41)	p = 0.0116

Table 5.02 Demographic, Clinical, and Pain Measures of the Women with CPP

Unpaired t-tests revealed that women with neuropathic and unclear PainDETECT scores had statistically significant higher State Anxiety (p = 0.0299) and Pain Catastrophising (p = 0.0116) scores than women with nociceptive scores (outlier removed). Group differences in duration of pain were evaluated using Mann Whitney U. N = 19.

The network analysis revealed a significant increase in connectivity between amygdala and the PAG in the unclear/neuropathic group when compared to the nociceptive group (General Linear Model Group Comparison: p(corr) = 0.0112) (see Figure 5.07).

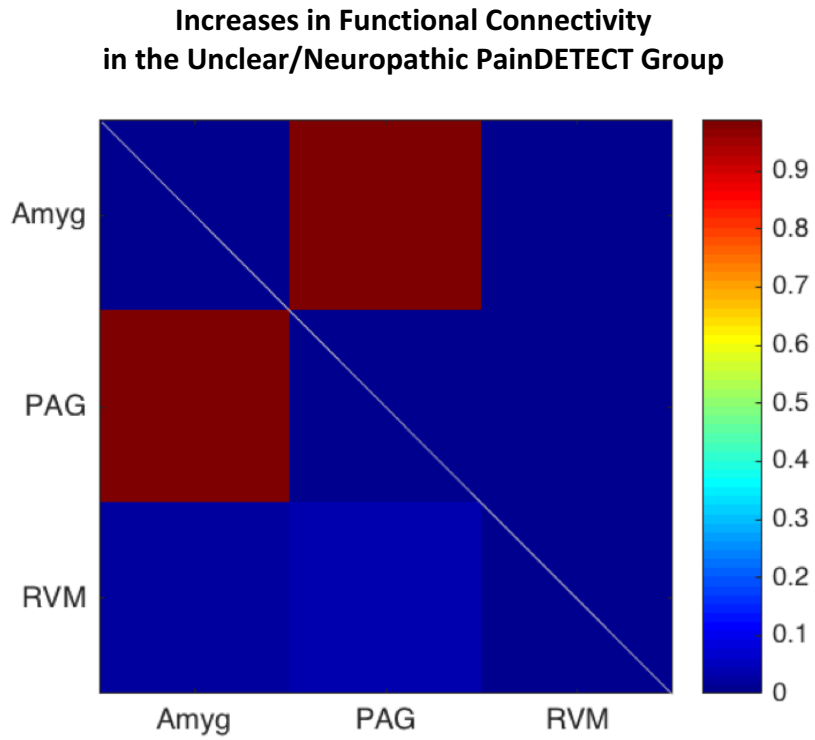


Figure 5.07 Increases in Functional Connectivity in the Unclear/Neuropathic PainDETECT Group (Without Outlier)

Above are the connectivity matrices demonstrating the increase in amygdala-PAG connectivity in the neuropathic group when compared to the nociceptive group (General Linear Model Group Comparison: p(corr) = 0.0112). The values above the diagonal represent those that survived the threshold, while the ones below are not significant at the threshold ($p < 0.05$). The colour bar represents 1-p-values. N = 19. Amyg - amygdala; PAG - periaqueductal gray; RVM - rostral ventromedial medulla.

Since there were significant differences in State Anxiety and Pain Catastrophising scores between the groups, the amygdala and PAG connectivity correlation coefficients were evaluated with respect to these scores. Neither State Anxiety (Pearson's Correlation: r = 0.3628, p = 0.1268) nor Pain Catastrophising was close to significance (Pearson's Correlation: r = 0.2257, p = 0.3527).

5.1.4 Discussion

5.1.4.1 Functional Connectivity

PainDETECT scores were not significantly different between women with CPP-only and those with endometriosis-associated CPP. In the imaging analysis, I demonstrated that women with unclear to neuropathic PainDETECT scores have increased connectivity

between the amygdala and PAG. Patients in the unclear/neuropathic category also had higher State Anxiety and Pain Catastrophising scores; however, these differences were not significantly correlated with connectivity.

Rodent models of neuropathic pain have demonstrated that central sensitisation is potentially modulated and maintained by supraspinal structures including the RVM and PAG (Pertovaara, Wei, and Hamalainen 1996; Urban and Gebhart 1999). Altered descending activity has been observed in patients with neuropathic components to their pain. In osteoarthritis patients, Gwilym and colleagues (2009) observed that PainDETECT scores were positively correlated with hyperalgesia-associated increases in PAG activation. As previously described, the animal literature has demonstrated that the amygdalae are directly connected to the PAG via ascending and descending connections making the amygdala a key component of pain modulatory circuitry (Rizvi et al. 1991; Neugebauer et al. 2004).

In our patients with CPP, the increased coupling between the amygdala and PAG in unclear/neuropathic patients could reflect increased descending facilitatory processes sustaining central sensitisation (Pertovaara, Wei, and Hamalainen 1996; Urban and Gebhart 1999; Gwilym et al. 2009). This lends support to Gwilym and colleagues' (2009) suggestion that tailored pharmacological interventions that address the underlying mechanisms would be highly advantageous for neuropathic pain patients.

5.1.4.2 Neuropathic Pain in CPP

This is the second report of neuropathic pain in women with CPPS and endometriosis-associated CPP. In a recent study, Whitaker and colleagues (2016) reported that 26% of

the women with CPP had neuropathic pain. In the patient cohort included in this chapter, only 15% of the patients reported neuropathic scores on PainDETECT. This distinction could be explained by the differing surgical histories. Our group of patients had never previously undergone surgical treatment for endometriosis whereas their cohort included patients who had been operated on. With this in mind, it is possible that the scores reported in this experiment are more representative of the underlying neuropathic component of CPP itself rather than follow-up surgical procedures that could potentially induce nerve damage.

5.1.4.3 Limitations

The distribution of PainDETECT scores in our cohort was skewed towards nociceptive pain, which limited the options for a group-comparison. Only four of the twenty-six women (15%) fell in the neuropathic category. Five were classified as unclear (19%), leaving the majority of patients in the nociceptive category (66%). To balance the group comparison, only the lowest scorers in the nociceptive category (N = 11) were compared to the combined unclear and neuropathic patients (N = 9). Despite the significant results, little can be concluded because 1) nociceptive and neuropathic PainDETECT categories were not directly compared and 2) five of the patients in the nociceptive category were excluded. As such, these findings may not be reproducible. This study is ongoing so this data will be re-analysed with a more complete dataset and, hopefully, with a more even distribution of PainDETECT scores.

5.1.5 Summary

In this section, I demonstrated that women with unclear/neuropathic pain scores on PainDETECT have increased functional coupling between the PAG and amygdala. These results suggest that there could be neuropathic-specific alterations in descending

modulatory mechanisms. However, given the aforementioned limitations, further work is needed to verify the validity of these results.

Chapter 6

Central Changes and Duration of Pelvic Pain

6.1 The Impact of Delayed Treatment on Pain Networks in Women with Chronic Pelvic Pain

6.1.1 Introduction

In Chapter 5, I demonstrated that examining functional connectivity is a potentially useful method for understanding prolonged pain states and characterising underlying mechanisms of pelvic pain. In this chapter, I evaluate functional connectivity in women with CPP with particular interest in how duration of pain is related to central networks. As previously discussed, women with CPP experience both extended diagnosis and treatment delays, so it is critical to understand whether this has maladaptive consequences on the central nervous system as seen in many other pain conditions.

6.1.1.1 Treatment and Diagnostic Delays

Women with CPP experience a tremendous diagnostic delay for potential underlying pathologies (endometriosis). In the US, on average, the delay is twelve years, whilst in the UK and Norway the delay is eight and seven years, respectively (Hadfield et al. 1996; Husby, Haugen, and Moen 2003). Furthermore, there is evidence that women with fertility concerns received diagnostic laparoscopies, and thus appropriate treatment, several years faster than women with pain symptoms (Dmowski et al. 1997; Arruda et al. 2003). Whilst not all women should undergo a diagnostic laparoscopy, in the interim there should be appropriate medical management of pain symptoms. However, this too is significantly delayed, leaving women without potentially effective pain treatment (Grace and Zondervan 2004).

6.1.1.2 Diagnostic Threshold: Transition from Acute to Chronic Pain

As previously described, in pain management, there are specific diagnostic cut-offs used to clinically separate those with acute and chronic pain; however, they have little biological basis in terms of associated-central changes (Apkarian, Baliki, and Geha 2009). Pain is classified as chronic if the pain continues beyond the threshold of typical tissue healing (Treede et al. 2015). For many conditions, like headache and back pain, this threshold is three months; however, pelvic pain is only defined as chronic after six months (Treede et al. 2015). Studies have employed the three-month cut-off as inclusion criteria and observed central changes in patients with chronic back pain (Buckalew et al. 2008), complex regional pain syndrome (Geha et al. 2008), temporomandibular joint disorder (Gerstner et al. 2011), fibromyalgia (Kuchinad et al. 2007; Schmidt-Wilcke et al. 2007), chronic facial pain (Schmidt‐Wilcke, Hierlmeier, and Leinisch 2010) when compared to healthy individuals (full meta-analysis by Smallwood and colleagues (2013)). Similarly, the six-month cut-off was used by As-Sanie and colleagues (2012; 2016) and, as previously discussed, the researchers found structural and functional changes.

6.1.1.3 Duration of Pain and Central Changes

One important caveat is that these findings do not truly address whether significant changes are taking place at the three or six-month threshold since the average duration of pain of the included research participants is frequently much higher (for example, median duration of pain was over five years in a study by As-Sanie and colleagues (2012)). As such, the true threshold for when these central changes start/become measurable remains somewhat elusive. However, it has been well documented that central changes correspond to important aspects of the clinical pain experience, like dysmenorrhoea intensity, but also anxiety and depression (Vincent et al. 2011; As-Sanie et al. 2016).

Fortunately, there is evidence that suggests that these pain-related changes can be reversed, even well beyond the diagnostic thresholds. In osteoarthritis patients, Gwilym and colleagues (2010) observed a reversal in pain-associated thalamic atrophy after surgical treatment (hip arthroplasty). Seminowicz and colleagues (2011) demonstrated that in individuals that have suffered from chronic back pain for more than one year (mean = 5 years), treatment reversed pain-associated structural and functional changes after six months.

To date, no published studies have explicitly reported how duration of pain impacts functional connectivity in the pelvic pain population. Given the many delays encountered by these women, it is important to determine if there are maladaptive consequences on the central nervous system.

6.1.1.4 Central Changes in Women with Chronic Pelvic Pain

It is well-established that women with pelvic pain have altered central pain mechanisms. Imaging studies have demonstrated altered structural and chemical changes in key pain processing regions in these patients (thalamus, putamen, anterior insula, hippocampus, PAG, amygdala) (Seminowicz et al. 2010; Tu et al. 2010; As-Sanie et al. 2012; As-Sanie et al. 2016). In response to noxious stimulation, functional changes have also been observed in important pain regions (mPFC, thalamus, hippocampus, entorhinal cortex, amygdala, anterior insula) (Wilder-Smith et al. 2004; Elsenbruch et al. 2010; Vincent et al. 2011; Hampson et al. 2013; As-Sanie et al. 2016).

To date, only two studies have looked at resting functional connectivity changes in women with CPP. One study evaluated women with interstitial cystitis/painful bladder syndrome

(IC/PBS) whilst the other examined women with endometriosis-associated CPP (Kilpatrick et al. 2014; As-Sanie et al. 2016). In the first published functional connectivity study, Kilpatrick and colleagues (2014) observed that, compared to healthy women, the women with IC/PBS had decreased resting connectivity between the posterior and mid insula. In the patient cohort, both the cerebellum and midbrain had resting connectivity increases with the supplementary motor area and the anterior paracentral lobule. More recently, As-Sanie and colleagues (2016) demonstrated that women with endometriosis-associated CPP had increased resting functional connectivity from the anterior insular to the mPFC and occipital cortex. These functional findings were supplemented with chemical data — they observed increases in excitatory neurochemicals, glutamate and glutamine (Glx), in the anterior insula. Functional connectivity between the mPFC and anterior insula was positively correlated with these Glx concentrations, depression, anxiety, and pain intensity measures.

Given the treatment and diagnostic delay experienced by women with CPP, the aim of this chapter was determine whether there was a relationship between pain duration and functional connectivity in a network of regions that have previously exhibited changes in this patient population. Additionally, data from this chapter was compared with the previous findings reported by As-Sanie and colleagues (2016) to examine reproducibility.

6.1.2 Methods

Twenty-six subjects were included in this analysis. The MRI included one resting fMRI-BOLD. Each woman completed a questionnaire including the State-Trait Anxiety Inventory, Beck Depression Inventory, and Pain Catastrophising Scale. They also

completed questionnaires relating to their pain experience (types of pain, duration of symptoms, pain intensity). All pain ratings were recorded on an NRS.

6.1.2.1 fMRI Network Analysis: Justification for Regions of Interest

Seven ROIs were included in the functional connectivity analysis (fMRI-BOLD): anterior insula, hippocampus, amygdala, putamen, PAG, thalamus, and mPFC (see Figure 6.01).

In women with pelvic pain, functional changes have been observed in the anterior insula (Pukall et al. 2005; Elsenbruch et al. 2010; As-Sanie et al. 2016), thalamus (Hampson et al. 2013), amygdala (Wilder-Smith et al. 2004), mPFC (Elsenbruch et al. 2010; As-Sanie et al. 2016), and hippocampal formation (Wilder-Smith et al. 2004; Vincent et al. 2011), and midbrain (Kilpatrick et al. 2014).

Grey matter atrophy has been observed in the anterior insula (Blankstein et al. 2010), thalamus (Seminowicz et al. 2010; As-Sanie et al. 2012), and putamen (As-Sanie et al. 2012). Increases in grey matter have been observed in the amygdala (As-Sanie et al. 2012) and the hippocampal gyrus (Tu et al. 2010). As-Sanie and colleagues (2012) found increased grey matter in the PAG in women with endometriosis but without pain suggesting an inherent or adaptive protection in these pain-free women.

During dysmenorrhoea, increased metabolic activity in thalamus has been observed (measured with PET) (Tu et al. 2009). More recently, increased Glx concentrations were observed in the the anterior insula (As-Sanie et al. 2016).

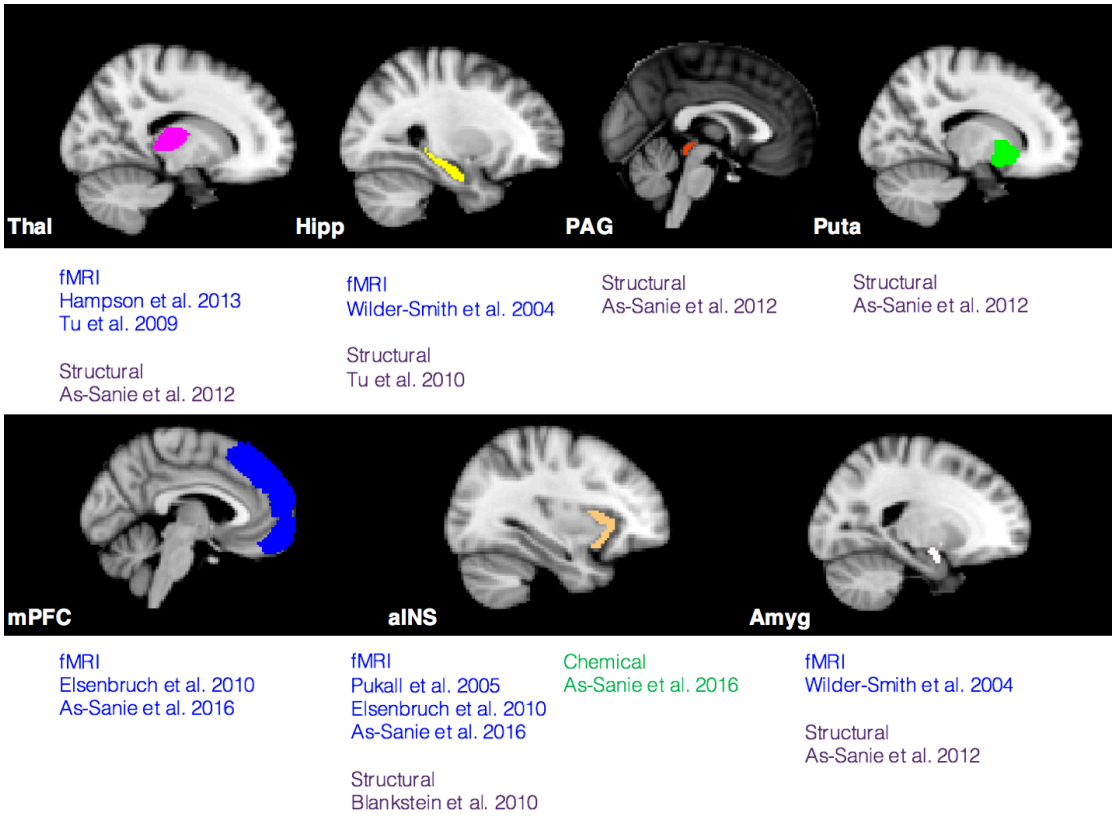


Figure 6.01 Regions of Interest for the Functional Connectivity Analysis

Studies have found structural, functional, and chemical changes in important pain processing regions when comparing women with CPP to healthy controls. These regions include the: thalamus (Thal), hippocampus (Hipp), periaqueductal grey (PAG), putamen (Puta), medial prefrontal cortex (mPFC), anterior insula (aINS), and amygdala (Amyg).

6.1.2.2 Replicating findings of As-Sanie and colleagues (2016)

Finally, in order to connect this chapter to the broader literature, I investigate some of the functional connectivity trends noted by As-Sanie and colleagues (2016). In the first approach, the same mPFC and anterior insula masks from my functional connectivity were used. In the second analysis, I more closely mimic the analysis by As-Sanie and colleagues by using their peak PFC coordinate for the mPFC mask.

6.1.3 Results

6.1.3.1 Part 1: Functional Connectivity and Duration of Pain

The mean age of the women included in this analysis was 31.85 years. The mean reported overall pain intensity was 5.29 out of 10 (rating from four weeks prior to the study visit). Mean Pain Catastrophising scores were below the clinical threshold (30), whilst mean

State-Trait Anxiety scores fell within the moderate anxiety range. Mean depression scores fell into the mild category (see Table 6.01).

Demographic and Clinical Measures	**Mean (±SD)**
Age (Years)	31.85 years (±6.47)
Duration of Pain	73.67 months (±69.66) 6.14 years (±5.81)
Pain Catastrophising Scale (0 – 52)	24.54 (±13.27)
Beck Depression Inventory (0 – 63)	16.12 (±10.52)
State Anxiety Inventory (20 – 80)	43.42 (±12.63)
Trait Anxiety Inventory (20 – 80)	48.37 (±13.39)
Pain Measures (0 - 10)	**Mean (±SD)**
Overall Pain Intensity (past 4-weeks)	5.29 (±2.09)
Dysmenorrhoea	6.50 (±3.24)
Non-Cyclical Pain	3.50 (±2.52)
Dyspareunia	5.85 (±2.34)
Dyschezia	4.27 (±3.24)
Dysuria	1.56 (±2.29)

Table 6.01 Demographic and Clinical Measures from the Women with CPP

Summary of demographic, pain, and psychological data for the patients included in this analysis. N = 26.

Functional Connectivity Analysis 1: Duration of Pain as a Continuous Covariate

With pain duration as a continuous covariate, no changes in functional connectivity between regions were statistically significant. However, connectivity between the hippocampus and the PAG demonstrated a trending positive correlation with duration of pain (General Linear Model: p(corr) = 0.1394) (see Figure 6.02).

Increases in Functional Connectivity with Increasing Pain Duration

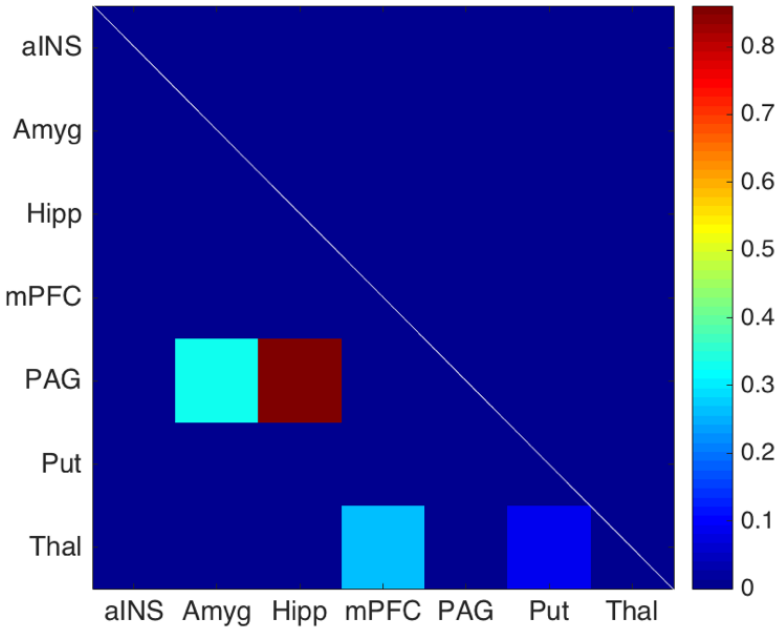


Figure 6.02 Increases in Functional Connectivity with Increasing Pain Duration

There were no statistically significant changes in connectivity correlated with pain duration; however, the hippocampus and PAG demonstrated a non-significant increase in connectivity as pain duration increased (General Linear Model: p(corr)=0.1394). The values above the diagonal represent those that survived the threshold, while the ones below are not significant at the threshold ($p < 0.05$). The colour bar represents 1-p-values. N = 26. Thal - thalamus; Hipp - hippocampus; PAG - periaqueductal grey; Puta - putamen; mPFC - medial prefrontal cortex; aINS - anterior insula; Amyg - amygdala.

Since the correlation was trending towards significance, I decided to examine the data further. The z-transformed correlation coefficients for hippocampus - PAG connectivity were plotted against pain duration (see Figure 6.03).

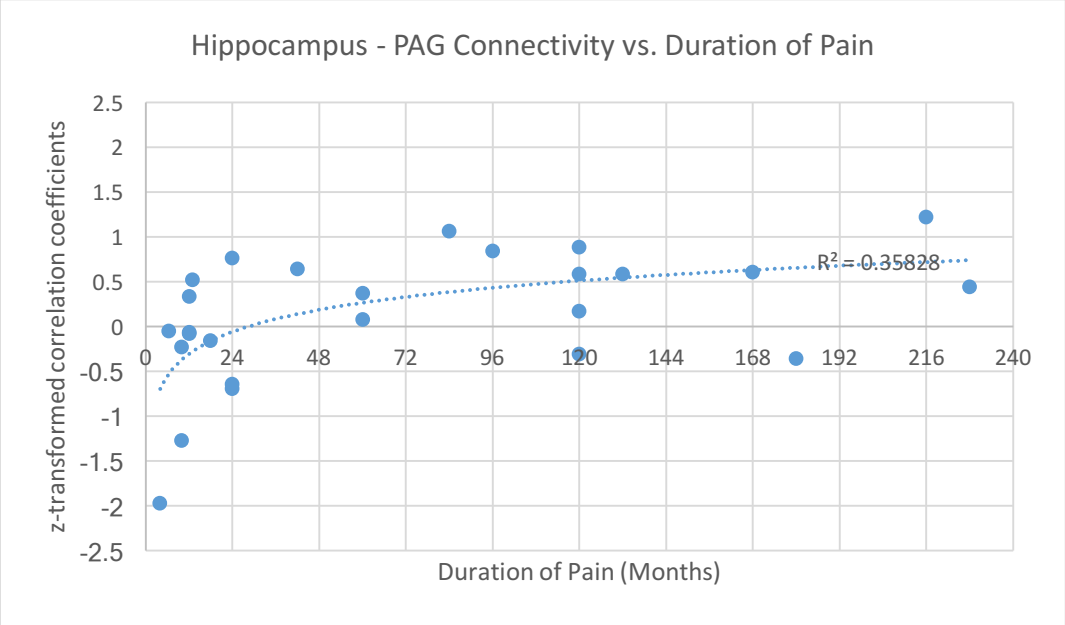


Figure 6.03 Hippocampus - PAG Connectivity vs. Duration of Pain

The z-transformed correlation coefficients plotted against pain duration (months) with the trend line demonstrating the trending, positive correlation (F = 13.40, p = 0.0010, R^2 = 0.3583). N = 26.

The curve was examined for linear and non-linear trends, with the best fit being a logarithmic function (F = 13.40, p = 0.0010, R^2 = 0.3583). The x-intercept is 24-months, suggesting an important shift at this time point. Therefore, the patients were separated at the 24-month mark and correlation coefficients were plotted again (see Figure 6.04).

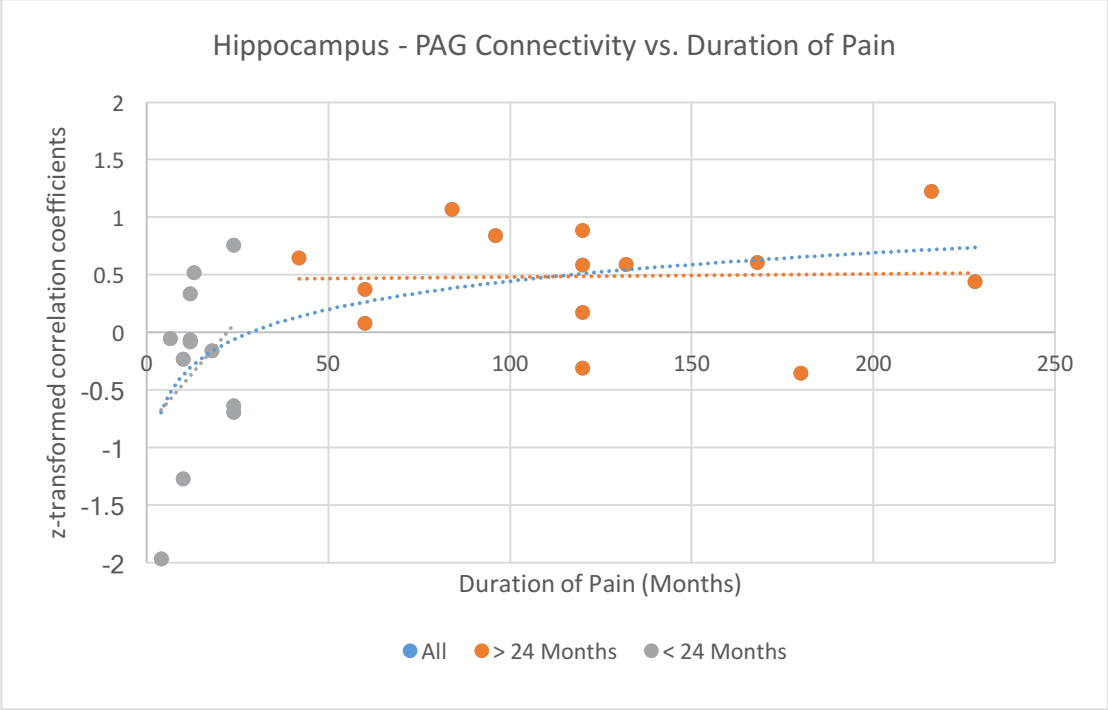


Figure 6.04 Hippocampus - PAG Connectivity vs. Duration of Pain

The z-transformed correlation coefficients plotted against pain duration (months) for patients with pain for more than 24 months (orange, N = 14), and patients with pain for 24 months or less (grey, N = 12). The blue dotted line represents the trend line for all the data points (N = 26).

The plot demonstrates that there is a greater positive trend in the group of patients who have had pain for 24-months or less. Correlations for each group were then calculated and compared to determine if the trends in each pain duration group were significantly different. For those with pain for 24-months or less, the positive trend was not significant (Spearman's Correlation: rho = 0.266, p = 0.402) and for those with pain for longer than 24-months, the slightly negative trend was not significant (Spearman's Correlation: rho = -0.027, p = 0.828). The correlation did not significantly differ (z = 0.67, p = 0.5029).

Functional Connectivity Analysis 2: 24-month split

In this second analysis, a group comparison was run. The patients were split at the 24-month mark: Group 1 - pain for longer than 24 months (n = 14) and Group 2 - pain for 24 months or less (n = 12). The mean pain duration for the > 24-month group was 124 months, whilst the ≤ 24-month group had a mean pain duration of 14 months. Age and

pain intensity were not statistically different between the groups; however, depression, anxiety, and catastrophising scores were significantly higher in those with longer pain duration (see Table 6.02).

Demographic, Pain, and Clinical Measures	**≤ 24 Months of Pain** Mean (±SD) N = 12	**> 24 Months of Pain** Mean (±SD) N = 14	**p-values**
Age (Years)	32.75 (±7.64)	31.07 (±5.44)	p = 0.5205
Overall Pain Intensity (past 4-weeks) (0 - 10)	4.88 (±2.00)	5.64 (±2.17)	p = 0.3606
*Beck Depression Inventory (0 - 63)	10.83 (±6.66)	20.64 (11.29)	p = 0.0144
*State Anxiety Inventory (20 - 80)	37.17 (±13.06)	48.79 (±9.78)	p = 0.0160
*Trait Anxiety Inventory (20 - 80)	39.42 (±10.88)	54.18 (±9.92)	p = 0.0051
*Pain Catastrophising Scale (0 - 52)	18.33 (±10.60)	29.86 (±13.32)	p = 0.0239

Table 6.02 Demographic, Clinical, and Pain Measures in Women with CPP

Demographic, pain, and clinical measures of the patient included in this analysis. Women with pain for longer than 24 months had statistically higher depression, anxiety, and catastrophising scores. N = 26.

When the FSLNets seed-based functional connectivity analysis was run, I observed that women with pain for longer than 24 months had a statistically significant increase in hippocampus - PAG connectivity when compared to the group that had pain for 24 months or less (General Linear Model Group Comparison: p(corr) = 0.0346). No other regions displayed near-threshold changes in functional connectivity (see Figure 6.05).

Increases in Functional Connectivity in Women with CPP for > 24 Months

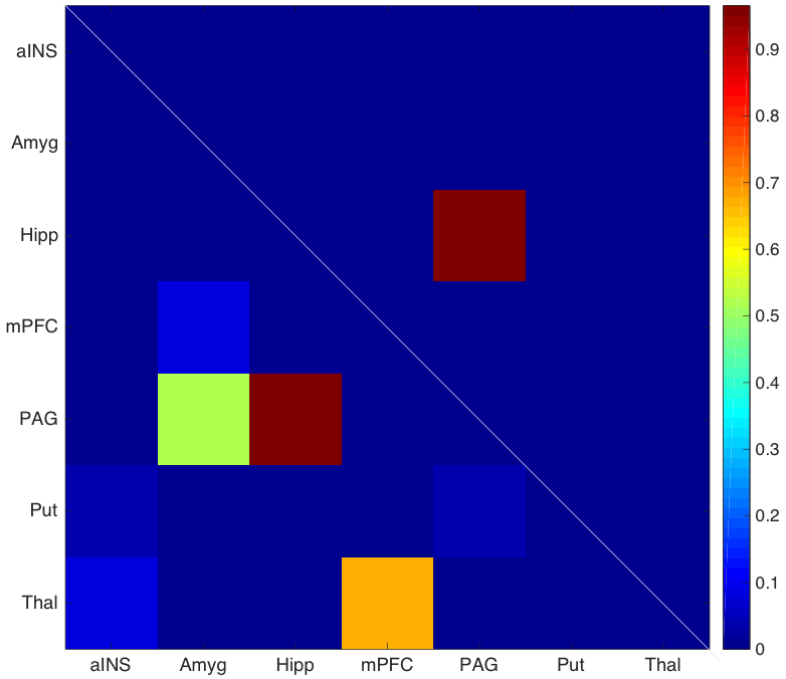


Figure 6.05 Increases in Functional Connectivity in Women with CPP for > 24 Months

There was an increase in functional connectivity between the hippocampus and PAG in women with pain for longer than 24 months (General Linear Model Group Comparison: p(corr)=0.0346). The values above the diagonal represent those that survived the threshold, while the ones below are not significant at the threshold (p < 0.05). The colour bar represents 1-p-values. Pain > 24 months N = 14, Pain ≤ 24 months N = 12. Thal - thalamus; Hipp - hippocampus; PAG - periaqueductal grey; Puta - putamen; mPFC - medial prefrontal cortex; aINS - anterior insula; Amyg - amygdala.

Given that the two pain duration groups had multiple factors that were statistically different (State-Trait Anxiety, Beck Depression, and Pain Catastrophising scores), the correlation between the hippocampus and PAG was examined in relation to these measures. Separately, depression, anxiety, and catastrophising scores were examined for correlations with the hippocampus - PAG z-transformed correlation coefficients. State Anxiety and Pain Catastrophising scores were significantly positively correlated with functional connectivity between the two regions (Pearson's Correlation: *State Anxiety*: r = 0.4159 p = 0.0346; *Pain Catastrophising*: r = 0.4551 p = 0.0195) (see Figures 6.06 & 6.07). Conversely, Trait Anxiety and Beck Depression were not significantly correlated (Pearson's Correlation: *Trait Anxiety*: r = 0.3464, p = 0.0829; *Beck Depression*: r = 0.2941, p = 0.1447).

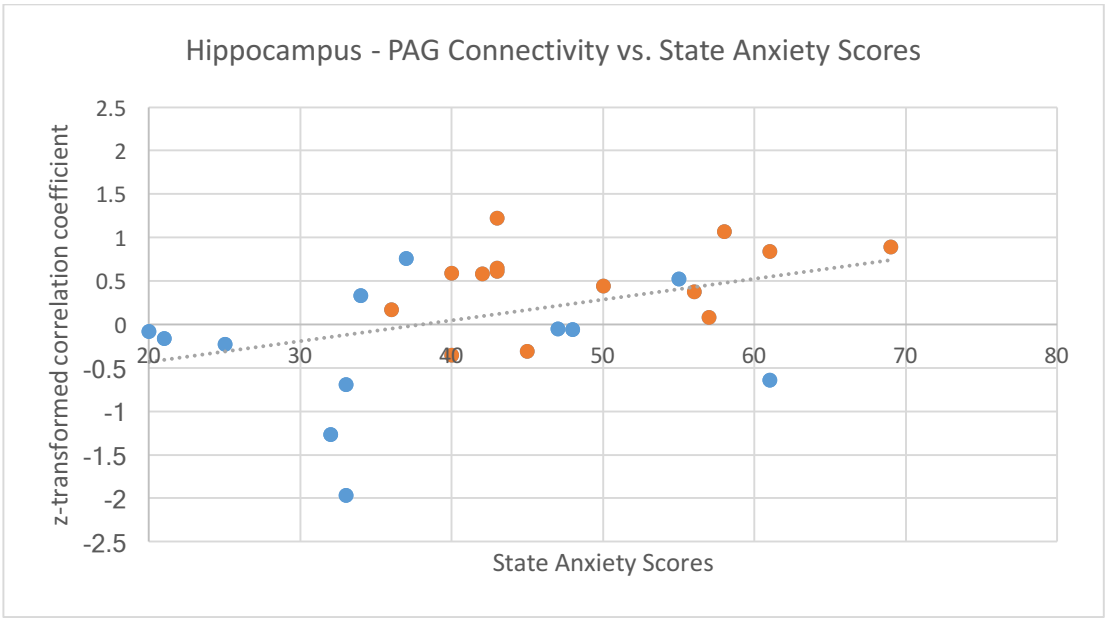


Figure 6.06 Hippocampus - PAG Connectivity vs. State Anxiety Scores

The z-transformed correlation coefficients are plotted against State Anxiety scores with the positive trend line demonstrating the non-significant correlation (Orange - pain for longer than 24 months; Blue - pain for 24 months or less; Pearson's Correlation: r = .4159, p = 0.03459). N = 26.

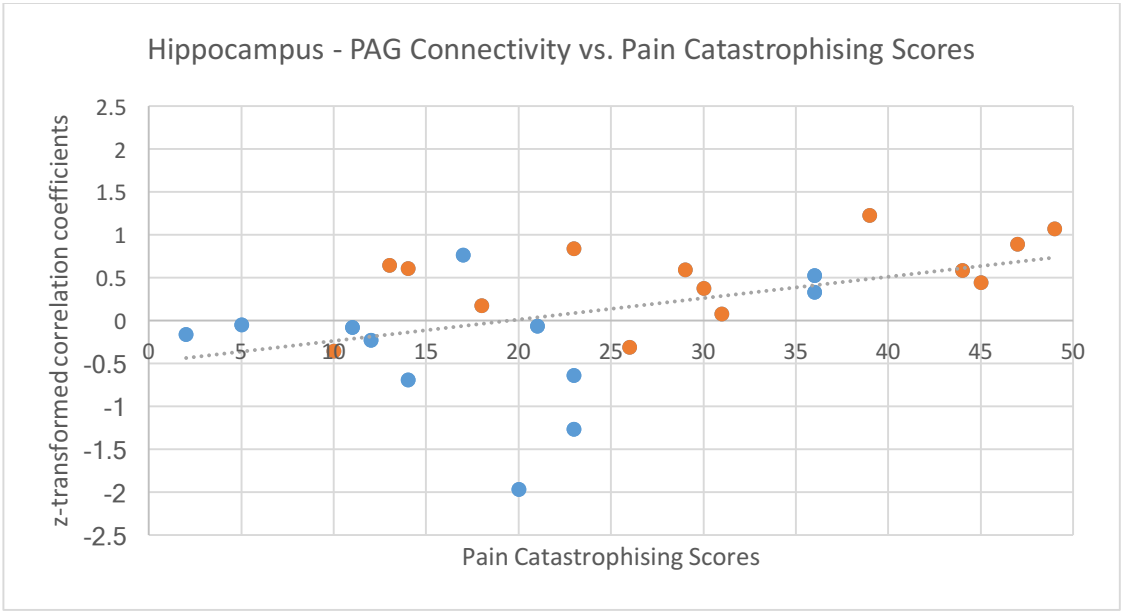


Figure 6.07 Hippocampus - PAG Connectivity vs. Pain Catastrophising Scores

The z-transformed correlation coefficients are plotted against Pain Catastrophising scores with the positive trend line demonstrating the significant correlation (Orange - pain for longer than 24 months; Blue - pain for 24 months or less; Pearson's Correlation: r = 0.4551, p = 0.0195). N = 26.

Both State Anxiety and Pain Catastrophising scores are highly correlated (r = 0.5821, p = 0.0018) so it was not possible to examine their individual relationships with the observed connectivity changes.

Functional Connectivity Analysis 3: Median Split of Pain Duration

Median splits have also been employed in pain imaging literature (Leknes et al. 2011), so it seemed reasonable to adopt that approach to see if the results changed. The median reported duration of pelvic pain was 51 months (N = 13 in each group). Again, Beck Depression Inventory, State-Trait Anxiety Inventory, and Pain Catastrophising scores were higher in the pain for longer than 51-months group (see Table 6.03).

Demographic, Pain, and Clinical Measures	**< 51 months** Mean (±SD) N = 13	**> 51 months** Mean (±SD) N = 13	**p-values**
Age (Years)	32.62 (±7.33)	31.08 (±5.66)	0.5549
Overall Pain Intensity (past 4-weeks) (0 - 10)	4.58 (±2.19)	6 (±1.78)	0.0821
*Beck Depression Inventory (0 - 63)	10.62 (±6.42)	21.62 (±11.12)	0.0050
*State Anxiety Inventory (20 - 80)	37.62 (±12.61)	49.23 (±10.03)	0.0157
*Trait Anxiety Inventory (20 - 80)	41.85 (±10.46)	54.88 (±9.95)	0.0034
*Pain Catastrophising Scale (0 - 50)	17.92 (±10.26)	31.15 (±12.92)	0.0080

Table 6.03 Demographic, Clinical, and Pain Measures in Women with CPP

Women with pain for longer than the median (51 months) had statistically higher depression (p = 0.0050), anxiety (State p = 0.0157; Trait p = 0.0022), and catastrophising (p = 0.0080) scores. N = 26.

There was no significant change in connectivity; however, the closest correlation was, again, the increase in connectivity between the hippocampus and PAG in women who have had pain for longer than 51 months (General Linear Model Group Comparison: p(corr) = 0.1004) (see Figure 6.08).

Increases in Functional Connectivity in Women with CPP for > 51 Months

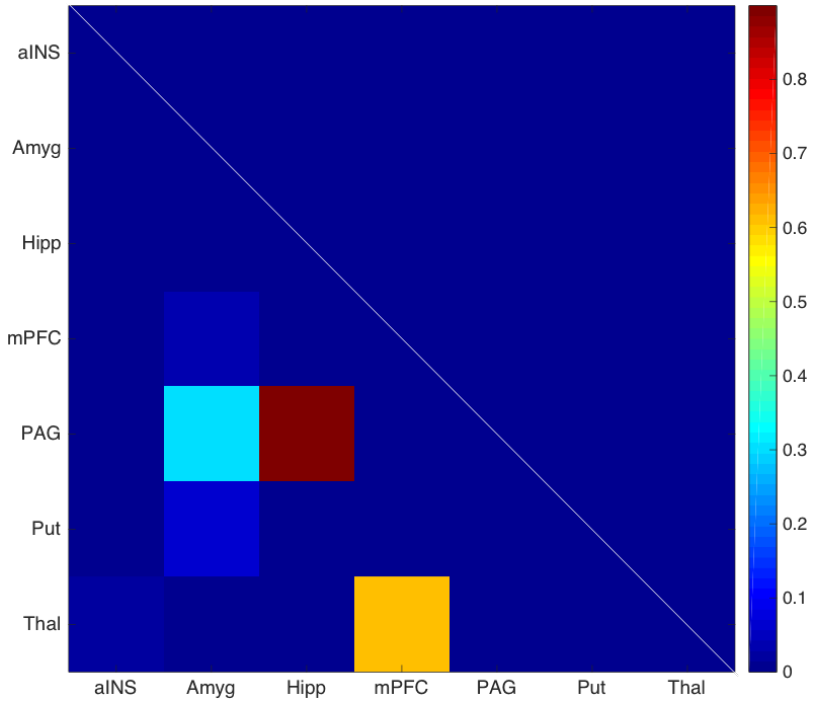


Figure 6.08 Increases in Functional Connectivity in Women with CPP for > 51 Months

There was a non-significant increase in functional connectivity between the hippocampus and PAG in women with pain for longer than the median for the cohort (General Linear Model Group Comparison: p(corr)=0.1004). The values above the diagonal represent those that survived the threshold, while the ones below are not significant at the threshold (p < 0.05). The colour bar represents 1-p-values. N = 13 in each group. Thal - thalamus; Hipp - hippocampus; PAG - periaqueductal grey; Puta - putamen; mPFC - medial prefrontal cortex; aINS - anterior insula; Amyg - amygdala.

6.1.3.2 Part 2: Reproducibility of findings from As-Sanie and Colleagues 2016

Using the correlation coefficients generated above for the connectivity of the mPFC and anterior insula, I calculated each correlation with respect to anxiety, depression, and pain intensity separately. I observed significant negative correlations between functional connectivity and measures of depression and State Anxiety (Pearson's Correlation: *Beck Depression*: r = -0.5013, p = 0.0091; *State Anxiety*: r = -0.4576, p = 0.0187). Trait Anxiety was close to significance (Pearson's Correlation: r = -0.3731, p = 0.0604) (see Figures 6.09 - 6.11). The correlation between pain intensity and functional connectivity was not significant (Pearson's Correlation: r = 0.0475, p = 0.8179). As-Sanie and colleagues (2016) reported significant positive correlations for all the measures.

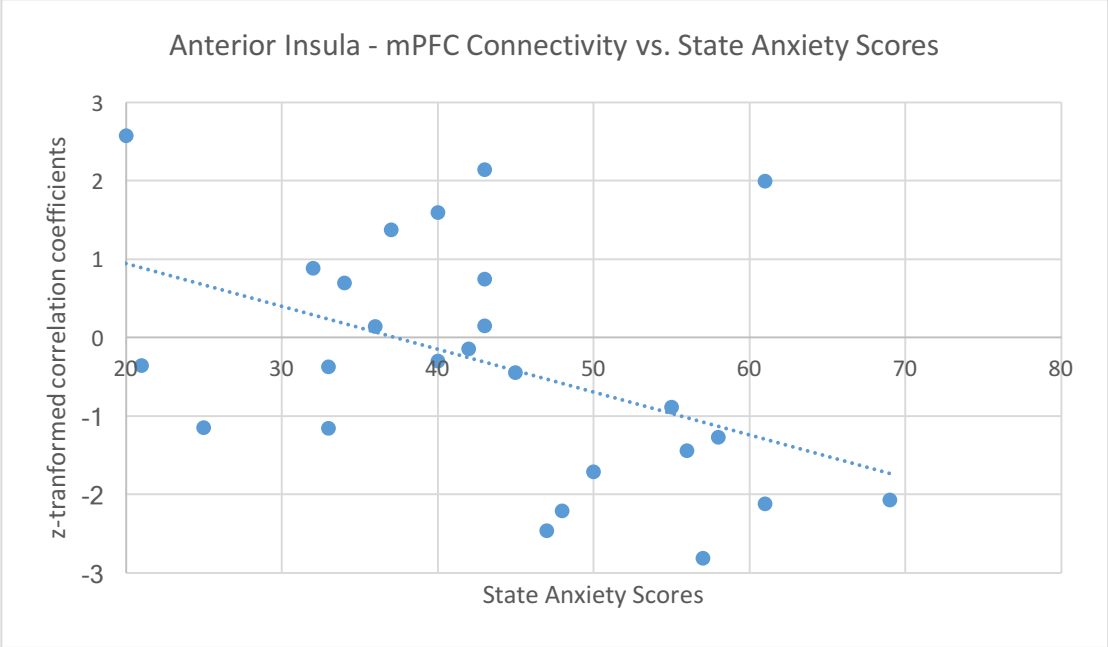


Figure 6.09 Anterior Insula - mPFC Connectivity vs. State Anxiety Scores

The z-transformed correlation coefficients are plotted against State Anxiety scores with the negative line demonstrating the correlation (Pearson's Correlation: r = -0.4576, p = 0.0187). N = 26.

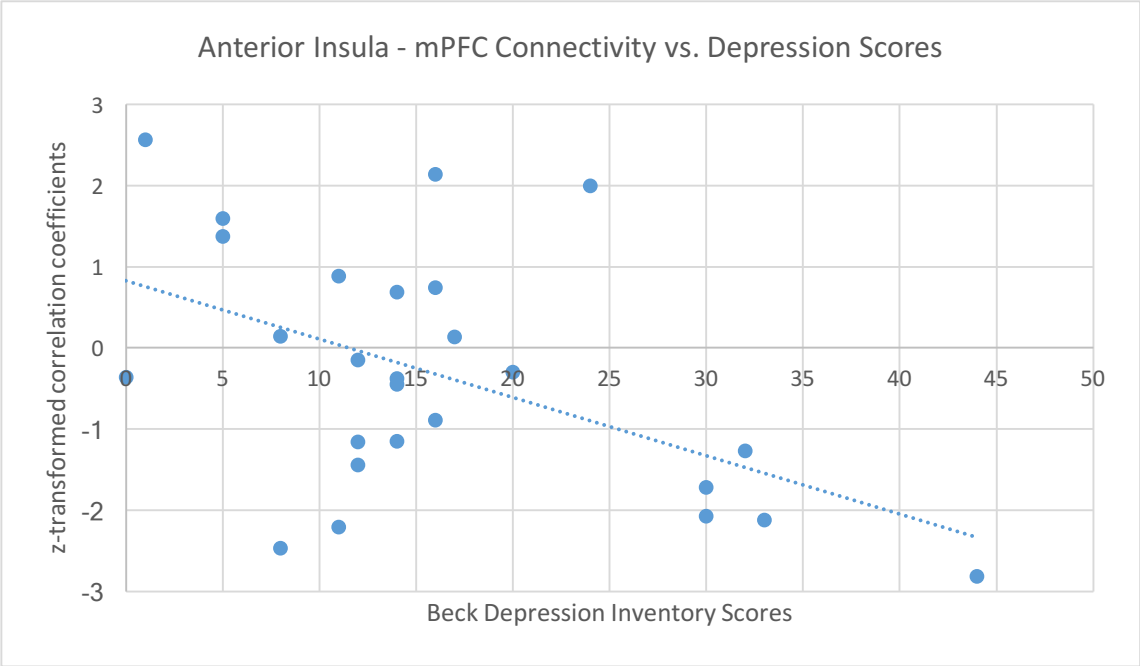


Figure 6.10 Anterior Insula - mPFC Connectivity vs. Depression Scores

The z-transformed correlation coefficients are plotted against Beck Depression scores with the negative trend line demonstrating the correlation (Pearson's Correlation: r = -0.5013, p = 0.0091). N = 26.

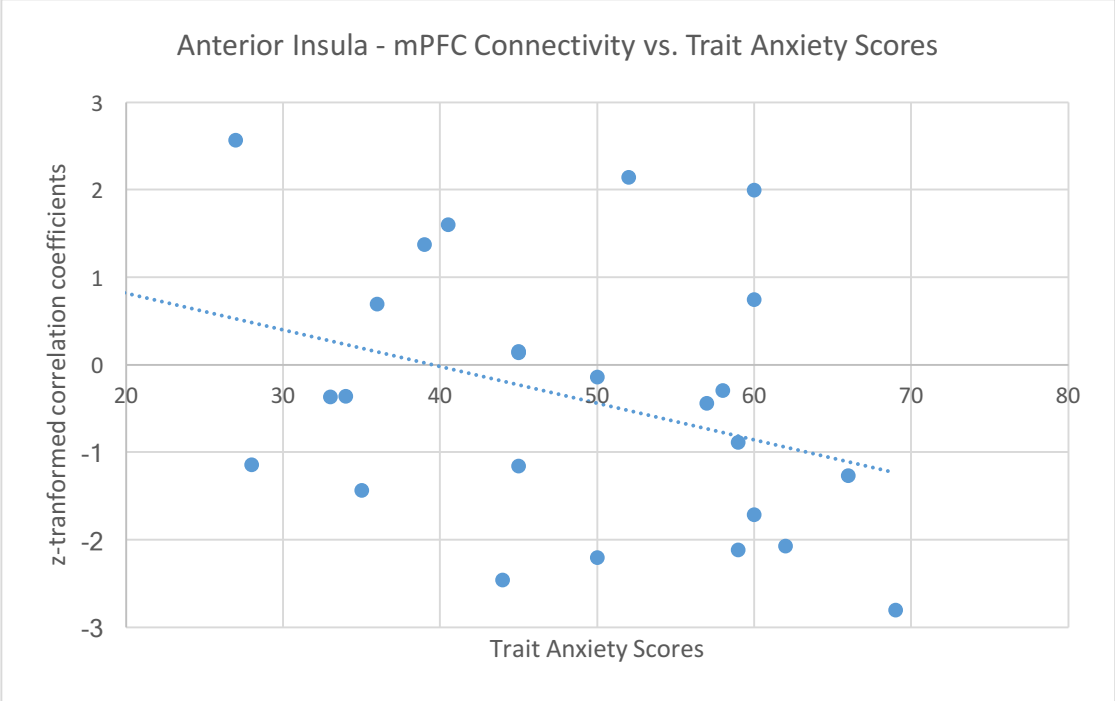


Figure 6.11 Anterior Insula - mPFC Connectivity vs. Trait Anxiety Scores

The z-transformed correlation coefficients are plotted against Trait Anxiety scores with the negative line demonstrating the correlation (Pearson's Correlation: r = -0.3731, p = 0.0605). N = 26.

Since these initial findings did not align with (As-Sanie et al. 2016), I re-analysed the data using the exact region of the mPFC that they reported in their study. There was a trending negative relationship between connectivity and depression scores (Pearson's Correlation: r = -0.3493, p = 0.0803). However, for pain intensity (Pearson's Correlation: r = 0.1119, p = 0.5863) and both anxiety measures, there were no significant correlations with functional connectivity changes (Pearson's Correlation: *State Anxiety*: r = -0.3069, p = 0.1272; *Trait Anxiety*: r = -0.2064, p = 0.3116).

6.1.4 Discussion

I demonstrated that women with pelvic pain for longer than 24 months have increased connectivity between the hippocampus and PAG. This connectivity was positively correlated with State Anxiety and Catastrophising, suggesting that this shift could be partly attributed to psychological mechanisms. The mPFC - anterior insula connectivity results did not align with As-Sanie and colleagues (2016) as the significant correlations

were opposite to what they reported (positive correlations). In this study, there were negative correlations between mPFC - anterior insula connectivity and State Anxiety and Beck Depression scores. In the second iteration of the analysis, their mPFC mask was used and none of the correlations were significant.

6.1.4.1 Relating Pain Duration and Central Changes

The majority of published research examining the relationship between central changes and pain duration centres on volumetric grey matter changes (May 2008, 2011). However, recent work is beginning to shed light on how the prolonged pain experience might be reflected in functional connectivity. In back pain, two studies have demonstrated that there may be relevant central changes taking place within the first 12-months of the onset of pain. In a longitudinal study, Baliki and colleagues (2012) observed changes in functional connectivity when comparing back pain patients with and without symptom improvement. After 12-months, in those without pain improvements, there was increased functional coupling between the mPFC and nucleus accumbens and decreased coupling between the insula, posterior cingulate, and dorsolateral PFC. As the mPFC - nucleus accumbens coupling was present at the first MRI, the authors postulated that this connectivity could be potentially predictive of the development of chronic pain.

Subsequently, the same research group demonstrated that over 12-months, those with back pain had a functional decoupling between the mPFC and hippocampus when compared to patients whose pain improved. Again, the authors concluded that this change in coupling could relate to the shift towards chronic pain and the associated affective components (Mutso et al. 2014).

Both studies demonstrated functional changes within 12-months in back pain populations while this chapter reported functional changes around 24-months. When compared to the results reported in this chapter, these studies have several advantages including the use of multiple visits over 12-months. Their patients were enrolled in the study within 4 months of the onset of pain, which allowed the researchers to examine potential central changes at the very beginning (Baliki et al. 2012; Mutso et al. 2014). This chapter included cross-sectional data with many of the patients having experienced years of pain (median: 51 months; mean: 64 months) so it is unlikely that the shift to chronic pain was captured, but instead the implications of long-term, largely unmanaged chronic pain.

6.1.4.2 Hippocampus, PAG, and Pain

It has been argued that chronic pain is a physiological stressor (Borsook et al. 2012). The hippocampus is critical in the stress response, particularly through its relationship with the hypothalamic-pituitary-adrenal (HPA) axis (McEwen 2001). Increased glucocorticoids damage the hippocampus; however, it has been observed that damage is still done even if circulating cortisol levels are not high. In rodents, consistent stressors lead to a plateau of the HPA response (McEwen 2001; McEwen and Kalia 2010). Indeed, researchers have demonstrated that women with dysmenorrhoea (Vincent et al. 2011) and endometriosis-associated CPP (Petrelluzzi et al. 2008; Quiñones et al. 2015) have significantly lower cortisol levels than healthy individuals. This holds true even in patients with high stress (Ehrström et al. 2009). Furthermore, these low cortisol levels have been demonstrated over repeated measures (Petrelluzzi et al. 2008). Interestingly, Vincent and colleagues (2011) observed that cortisol levels were lower in women who had experienced dysmenorrhoea for longer. Pain and cortisol levels have been correlated: women with endometriosis-associated CPP who reported incapacitating pain were more likely to have

lower cortisol levels (Quiñones et al. 2015). Even when cortisol levels are low, the hippocampus is a site where stress-related damage can occur (McEwen and Kalia 2010). This can have demonstrable consequences on cognitive ability and possibly pain processing (McEwen 2001; Frodl and O'Keane 2013).

As discussed in the introductory chapter of this dissertation, more recent evidence has demonstrated that the hippocampus is also critical in pain processing. Negative expectation, depression, and anxiety have been shown to amplify pain-induced activation in the hippocampal formation (Ploghaus et al. 2001; Kong et al. 2008; Berna et al. 2010). In women with dysmenorrhoea, entorhinal cortex activity was amplified in response to noxious experimental stimulation when compared to healthy individuals (Vincent et al. 2011).

As previously described, the PAG has a better characterised role in pain with changes in activity relating to pain perception (Tracey et al. 2002; Ploner et al. 2010). In healthy women, Rosenberger and colleagues (2009) linked chronic stress and heightened PAG activation in response to visceral pain.

Several studies have reported resting connectivity changes between the PAG and hippocampus. Coupling between these regions has been observed in healthy individuals and in osteoarthritis patients with increased clinical pain intensity (Kong et al. 2010; Egorova, Gollub, and Kong 2015). In terms of anatomy, there is some evidence in the rodent literature supporting connectivity between the PAG and hippocampus (Castle, Comoli, and Loewy 2005) and hippocampal formation (Marchand and Hagino 1983;

Cavdar et al. 2008). However, there are conflicting reports across animal species (rodents and cats) (Nauta 1958). Given that there does not appear to be conclusive evidence in primates (Mantyh 1982; Linnman et al. 2012), it is more likely that indirect connections underlie these functional changes but the potential for direct connectivity cannot be excluded entirely.

Given the well-established treatment delays experienced by women with CPP (Hadfield et al. 1996; Husby, Haugen, and Moen 2003; Grace and Zondervan 2004), it is conceivable that this increased hippocampus - PAG coupling represents the physiological consequences of the prolonged, and generally clinically unmanaged, chronic pain state. Since both the PAG and hippocampus have demonstrated altered pain processing with respect to anxiety and stress, the increased hippocampus - PAG connectivity could reflect, or be associated with, changes to relevant circuitry to affective-motivational processes. This is supported by the fact that women who had pain for longer than 24 months had significantly higher anxiety and depression measures. Further, there were significant positive correlations between hippocampus - PAG connectivity and anxiety and catastrophising measures (anxiety and catastrophising). It is critical to highlight that this result does not suggest that women suffer from CPP because of their psychology (a damaging misconception that was discussed in the introduction), but instead that the physiological burden of long-term CPP is potentially associated with maladaptive strengthening of pain-related circuitry.

6.1.4.3 Comparing Results to As-Sanie and colleagues (2016)

There are multiple reasons why the results presented in this chapter did not align with previously published findings by As-Sanie and colleagues (2016). One important

distinguishing factor is the surgical history of our respective patient cohorts. Our study had surgically naive women whilst all their patients had undergone at least one surgery. Approximately 31% of those patients had undergone two or more surgical procedures for their endometriosis. Previous surgical procedures have been correlated with musculoskeletal pain in these women (Tu, As-Sanie, and Steege 2006), which could result in altered central responses.

Finally, the analysis methodology used here was different from As-Sanie and colleagues (2016) (different fMRI analysis software, time series extraction, anterior insula mask). It would be valuable to copy their methods more precisely using our patient cohort to see if their results are reproducible. If not, perhaps the differences are reflective of differing surgical histories, which would be a worthwhile issue to explore given endometriosis is treated surgically.

6.1.4.4 Future Directions

This study is ongoing. As such, this connectivity will be re-examined once all scans are complete. Importantly, salivary cortisol measures have been collected at every visit of this study, which, as previously mentioned, are known to be lower in women with CPP (Petrelluzzi et al. 2008; Vincent et al. 2011; Quiñones et al. 2015). Once data collection is complete, they will be analysed. This hormone data will help clarify whether there is HPA axis dysfunction in these women and if it does indeed relate to the observed hippocampus and PAG connectivity changes.

6.1.5 Summary

I demonstrated altered connectivity between the hippocampus and PAG in women with pain durations longer than 24 months. This connectivity was positively correlated with

State Anxiety and Catastrophising, suggesting that this shift could be partly attributed to psychological stress. Future work will include cortisol levels into the analysis to determine if the HPA axis is playing a role in this connectivity shift.

Chapter 7

Evaluating Clinical Measures After Diagnostic Laparoscopy and Surgical Treatment of Endometriosis

Section 1

7.1 The Impact of Laparoscopy on Women with Chronic Pelvic Pain

7.1.1 Introduction

I previously demonstrated that women with pelvic pain for longer than 24-months have increased hippocampus and PAG connectivity. Characterising these changes is important due to the diagnosis and treatment delay experienced by this patient population. However, it is also critical to understand which patients benefit from the current surgical and pharmaceutical interventions. This chapter comprises two sections. The first section evaluates the impact of laparoscopy on pain and psychological measures in women with CPP. The second section examines the efficacy of surgery on pain scores and attempts to disentangle predictive measures for success of the surgery.

7.1.1.1 Laparoscopy to Diagnose Endometriosis

Endometriosis is diagnosed by laparoscopy; two or three small incisions are made in the abdomen (one to visually inspect for pathology and the other two to move organs/perform surgery if necessary). The abdomen is filled with CO_2 to allow the surgeon better visual range and to increase distance between the abdominal wall and the viscera during insertion of trocars. If endometriosis is found, lesions are excised or ablated (with either diathermy or laser) at the discretion of the surgeon. Randomised placebo-controlled clinical trials have revealed that surgical treatment reduces pelvic pain in over

half of women enrolled. However, it is not clear how long these pain changes last outside the trials (1 year) and why some women do not respond to surgery (Abbott et al. 2004).

7.1.1.2 Potential Impact of Laparoscopy on All Women with CPP

For all women who undergo laparoscopy (even those who are not treated for endometriosis), there are some potential benefits due to the nature of the surgical procedure. Firstly, the tenting of the abdomen with CO_2 could potentially alleviate some of the musculoskeletal-components of pain associated with CPP (Tu, As-Sanie, and Steege 2006). Indeed, the treatment of trigger points in men with CPPS contributes to alleviating overall pain (Anderson et al. 2005). Secondly, the influence of general anaesthetic on the central nervous system could also alter pain processing post-operatively. However, the mechanisms driving this are poorly understood particularly with respect to clinical pain (Wilder-Smith et al. 1998).

Despite these potential benefits, surgical diagnoses have one obvious downfall for the patients: all women suspected of having endometriosis are exposed to a surgical procedure. While this is certainly less of an issue for those that have the pathology and diagnosis turns into treatment, it does expose women with a negative diagnosis to the risks of surgery.

The aim of this section was to determine how different pain and psychological measures changed over recovery from the diagnostic laparoscopy/endometriosis treatment. An fMRI pilot dataset was interrogated to determine whether changes in pain intensity are reflected centrally in descending modulatory circuitry.

7.1.2 Methods

As this study is ongoing, this section comprises an early examination of a growing dataset. Due to drop-out being a common problem in longitudinal research, the sample sizes utilised here are small. It is therefore important to highlight that the analyses presented below are exploratory in nature.

7.1.2.1 Subjects

Twenty-four women were included in this analysis. Two women were excluded from the original twenty-six due to pregnancies after the first visit. There was drop-out due to pregnancy and missed questions in the questionnaires that led to varying subject numbers at Visit 2 (6-weeks), Visit 3 (3-months), and Visit 4 (6-months).

7.1.2.2 Questionnaires

As previously described, the women completed an extensive questionnaire at each of the study visits. The women recorded their pain scores on a numeric rating scale (NRS). They were asked to rate their average overall pain intensity (average pain during the month before the research visit), dysmenorrhoea, non-cyclical pain, dyspareunia, dyschezia, and dysuria. Psychological measurements included the Beck Depression Inventory, State-Trait Anxiety Inventory, Pain Catastrophising Scale, and PainDETECT.

7.1.2.3 Pre- and Post-Surgical MRIs

The first of the two MRIs (Visit 1) was within two weeks of each patient's diagnostic laparoscopy and/or surgical treatment. The second MRI (Visit 4) took place six months after the surgery. Only five women completed both the pre- and post-surgical MRI.

7.1.2.4 Analysis

Due to dropout, changes in pain and psychological scores were evaluated using two methods: 1) analysis of all individuals who completed the questionnaires at each follow-up and 2) analysis of only those who completed all four study visits.

Prior to performing the paired t-tests, normality and homoscedasticity assumptions were assessed. For data that violated these assumptions, the Sign Rank Test was used. The p-value threshold for statistical significance was adjusted for multiple comparisons (Bonferroni correction) for each analysis. The p-value threshold (0.05) was divided by the number of tests run (each follow-up comparison multiplied by each pain or psychological measure evaluated). The adjusted statistical threshold is clearly stated with each analysis.

7.1.3 Results

7.1.3.1 Pain Intensity Ratings Pre- and Post-Surgery

Each pain rating was plotted over time, starting with the pre-surgical visit (baseline), followed by the three post-surgical follow-up visits (6-weeks, 3-months, 6-months) (see Figure 7.01).

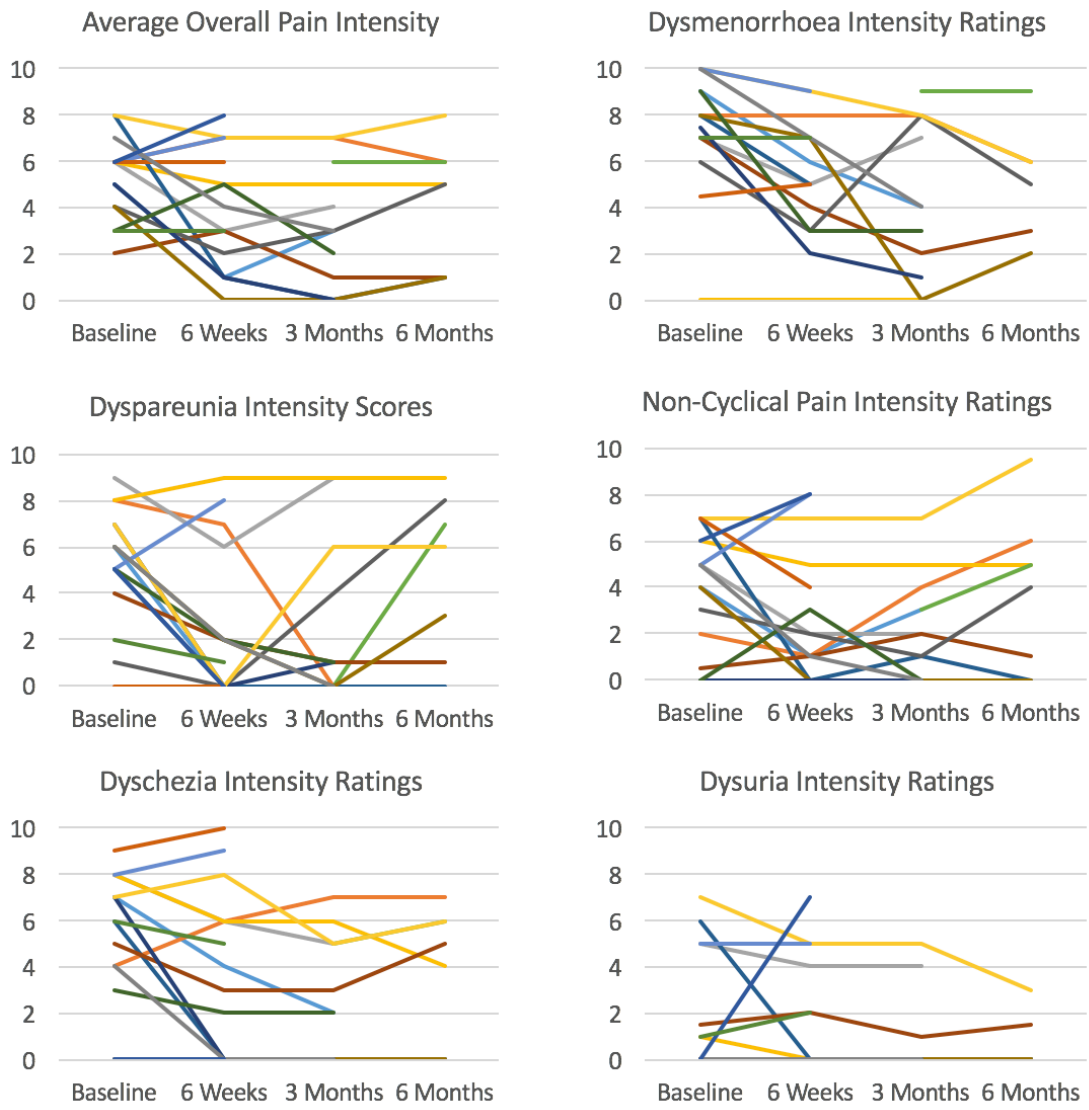


Figure 7.01 Pain Intensity Ratings Plotted Over Study Visits

The pain intensity measures varied over the course of the study. Average overall pain intensity is a measure of pain 4-weeks prior to the research visit. Baseline was acquired in the 2-weeks prior to surgery whilst ratings from the follow-up visits were acquired post-surgically at 6-weeks, 3-months, and 6-months. The y-axis represents the pain ratings on an NRS whilst the x-axis indicates the visit time point.

Paired t-tests were run comparing each post-surgical pain intensity at each follow-up visit (6-weeks, 3-months, and 6-months) to the pre-surgical baseline ratings (Threshold for significance: $p(corr) < 0.0028$) (Sign Rank Test was used for dysuria, see Table 7.01). Since there was drop-out (or missing responses to certain questions), the sample size for the paired comparison varied depending on which post-surgical visit was of interest. There were significant decreases in pain ratings at the 6-week (only dyspareunia and dysmenorrhoea) and 3-month follow-ups (only dyspareunia).

Type of Pain (0 - 10)	**Pre-Surgery**	**Post-Surgery**		
	Baseline Median (±SD)	**6-weeks** Median (±SD)	**3-months** Median (±SD)	**6-months** Median (±SD)
Average Overall Pain Intensity	6 (±1.81) N=24	3.5 (±2.57) N=16 p = 0.0519	3 (±2.54) N=13 p = 0.0061	5 (±2.75) N=5 p = 0.1725
Dysmenorrhoea	7.75 (±3.06) N=24	5 (±2.61) N=16 p = 0.0012	4 (±3.37) N=12 p = 0.0097	6 (±2.37) N=7 p = 0.0194
Non-Cyclical Pain	4 (±2.52) N=23	2 (±2.96) N=16 p = 0.2199	2 (±2.19) N=13 p = 0.1559	4.5 (±3.32) N=8 p = 0.9332
Dyspareunia	6 (±2.43) N=24	1.5 (±3.18) N=17 p = 0.0014	1 (±3.45) N=13 p = 0.0024	4.5 (±3.31) N=7 p = 0.5853
Dyschezia	5 (±3.24) N=24	0 (±3.23) N=16 p = 0.0399	2 (±2.63) N=13 p = 0.0374	4.5 (±3.02) N=8 p = 0.6807
Dysuria	0 (±2.37) N=24	1 (±2.09) N=16 p = 1.0000	0 (±1.16) N=13 p = 0.0625	0 (±1.12) N=8 p = 0.2500

Table 7.01 Comparing Pain Intensity Ratings Over Study Visits

Paired t-tests were run for every post-surgical visit (6-weeks, 3-months, and 6-months) with respect to the pre-surgical baseline (Sign Rank Test was run for dysuria). Baseline pain ratings were obtained within 2-weeks of surgery. Overall pain intensity is a measure of the average pain each patient experienced over the preceding 4-weeks for each study visit. The N represents the number of patients who reported the pain scores. For the follow-up visits, the N represents the number of patients that could be included in the paired comparison. Each p-value is the outcome of a paired comparison of reported pain scores at that time point with respect to baseline. Threshold for significance: p(corr) < 0.0028.

Pain scores were evaluated again, but this time only including those who attended every study visit (Threshold for significance: p(corr) < 0.0028). None of the results reached significance (see Table 7.02).

Type of Pain (0 - 10)	**Pre-Surgery**	**Post-Surgery**		
	Baseline Median (±SD)	**6-weeks** Median (±SD)	**3-months** Median (±SD)	**6-months** Median (±SD)
Average Overall Pain Intensity N = 6	5 (±2.09)	2.5 (±2.61) p = 0.1747	2 (±2.88) p = 0.1345	3 (±2.40) p = 0.1769
Dysmenorrhoea N = 5	8 (±1.48)	7 (±2.59) p = 0.0560	8 (±3.89) p = 0.2174	5 (±1.82) p = 0.0175
Non-Cyclical Pain N = 7	4 (±2.55)	1 (±2.69) p = 0.1027	2 (±2.54) p = 0.2615	4 (±3.54) p = 0.7054
Dyspareunia N = 6	5.5 (±2.48)	1 (±3.49) p = 0.0522	2.5 (±3.67) p = 0.2543	4.5 (±3.73) p = 0.7181
Dyschezia N = 7	5 (±3.19)	3 (±3.40) p = 0.3559	3 (±3.06) p = 0.2626	4 (±3.08) p = 0.3476
Dysuria N = 7	1 (±2.99)	0 (±1.91) p = 0.6250	0 (±1.86) p = 0.1250	0 (±1.18) p = 0.2500

Table 7.02 Comparing Pain Intensity Ratings Over Study Visits

Above are the pain intensity scores for the patients who completed all four study visits. Paired t-tests were run for every post-surgical visit (6-weeks, 3-months, and 6-months) with respect to the pre-surgical baseline (Sign Rank Test was run for dysuria). Baseline pain ratings were obtained within 2-weeks of surgery. Overall pain intensity is a measure of the average pain each patient experienced over the preceding 4-weeks for each study visit. Each p-value is the outcome of a paired comparison of reported pain scores at that time point with respect to baseline. Threshold for significance: p(corr) < 0.0028.

7.1.3.2 PainDETECT Scores Pre- and Post-surgery

PainDETECT scores were plotted over the four study visits (see Figure 7.02).

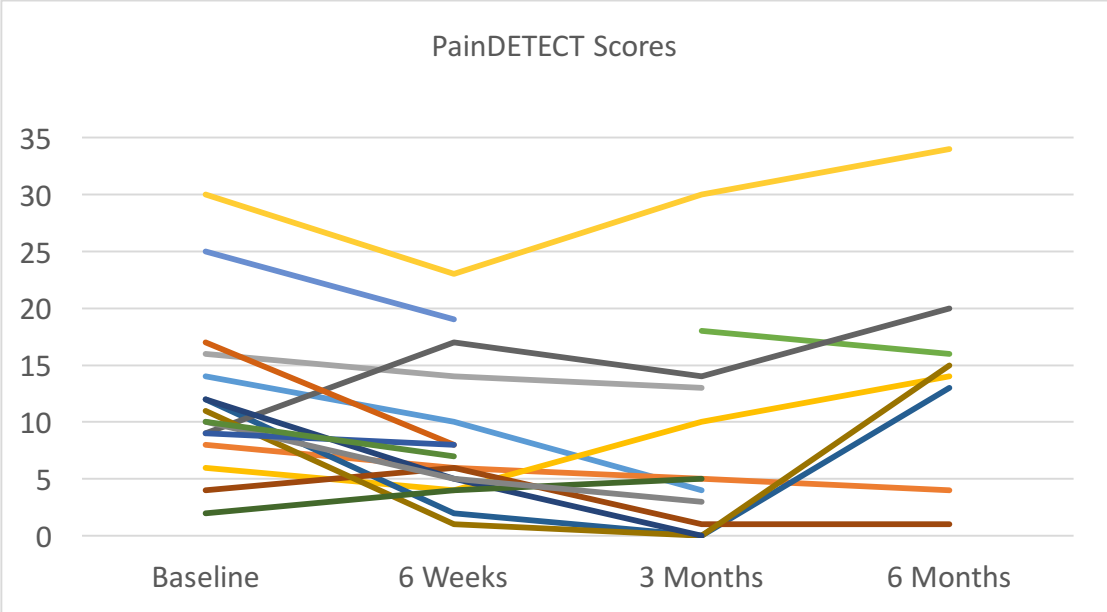


Figure 7.02 PainDETECT Scores Plotted Over Study Visits

PainDETECT scores varied over the course of the study. Baseline was acquired in the 2-weeks prior to surgery whilst ratings from the follow-up visits were acquired post-surgically at 6-weeks, 3-months, and 6-months. The y-axis represents the pain ratings on an NRS whilst the x-axis indicates the visit time point.

A paired t-test revealed that PainDETECT scores decreased significantly at six weeks when compared to baseline; however, the scores were not significantly lower at three months (Threshold for significance: $p(corr) < 0.0167$). Interestingly, at six months, PainDETECT scores were higher than at baseline but the difference was not statistically different (see Table 7.03).

Neuropathic Measure	**Pre-Surgery**	**Post-Surgery**		
	Baseline Median (±SD)	**6-weeks** Median (±SD)	**3-months** Median (±SD)	**6-months** Median (±SD)
PainDETECT Scores	10 (±7.49) N = 24	6.5 (±6.34) N = 16 p = 0.0114	7.92 (±8.89) N = 13 p = 0.1405	14.5 (±10.06) N = 8 p = 0.1021

Table 7.03 Comparing PainDETECT Scores Over Study Visits

Paired t-tests were run for every post-surgical visit (6-weeks, 3-months, and 6-months) with respect to the pre-surgical baseline. For the follow-up visits, the N represents the number of patients that could be included in the paired t-test. Each p-value is the outcome of a paired t-test of reported pain scores at that time point with respect to baseline. Threshold for significance: p(corr) < 0.0167.

For the subjects that completed all four visits, each of the follow-up scores were compared to baseline using a paired t-test (Threshold for significance: p(corr) < 0.0167) (see Table 7.04).

Neuropathic Measure	**Pre-Surgery**	**Post-Surgery**		
	Baseline Median (±SD)	**6-weeks** Median (±SD)	**3-months** Median (±SD)	**6-months** Median (±SD)
PainDETECT Scores N = 7	9 (±8.64)	6 (±8.30) p = 0.2749	5 (±10.86) p = 0.3003	14 (±10.85) p = 0.1974

Table 7.04 Comparing PainDETECT Scores Over Study Visits

PainDETECT scores for the patients who completed all four study visits. Paired t-tests were run for every post-surgical visit (6-weeks, 3-months, and 6-months) with respect to the pre-surgical baseline (N = 7). Threshold for significance: p(corr) < 0.0167.

7.1.3.3 Psychological Measures Pre- and Post-Surgery

State Anxiety, Beck Depression Inventory, and Pain Catastrophising scores were plotted over the four research visits (see Figure 7.03).

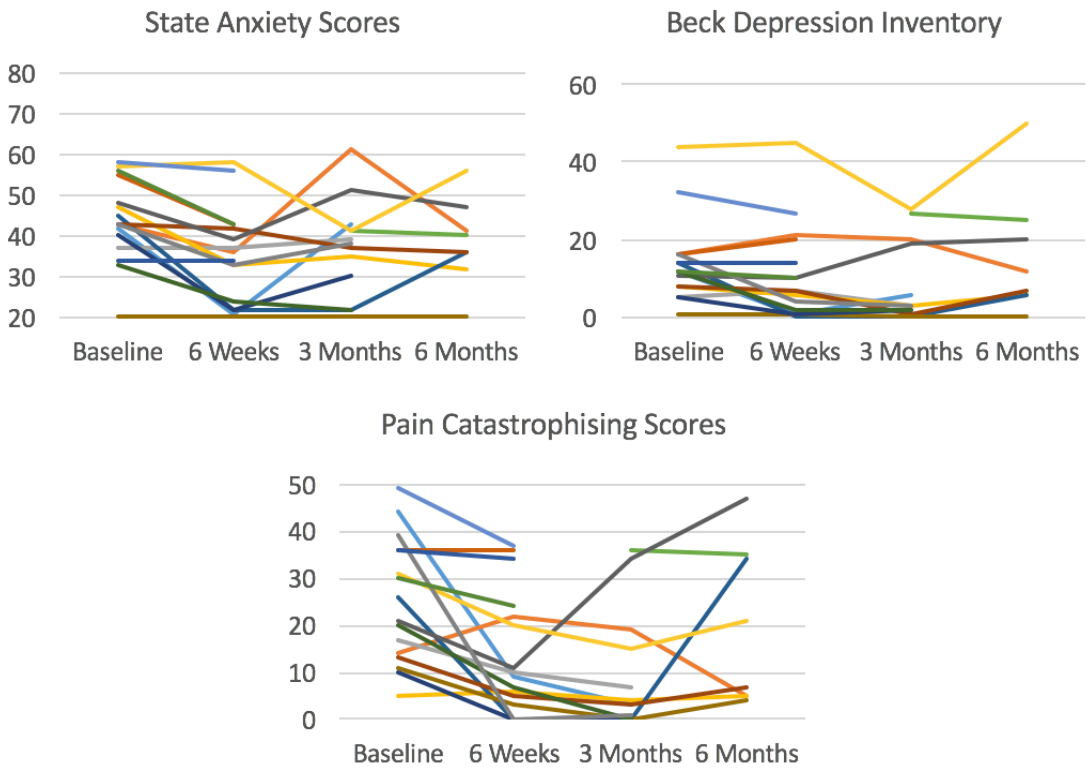


Figure 7.03 Psychological Measures Plotted Over Study Visits

State Anxiety, Beck Depression Inventory, and Pain Catastrophising scores plotted over the study visits. Baseline was acquired in the 2-weeks prior to surgery whilst ratings from the follow-up visits were acquired post-surgically at 6-weeks, 3-months, and 6-months. The y-axis represents the pain ratings on an NRS whilst the x-axis indicates the visit time point.

State Anxiety and Pain Catastrophising were statistically lower at the 6-week follow-up when compared to baseline (Threshold for significance: $p(corr) < 0.0056$) (see Table 7.05).

Psychological Measures	**Pre-Surgery**	**Post-Surgery**		
	Baseline Median (±SD)	**6-weeks** Median (±SD)	**3-months** Median (±SD)	**6-months** Median (±SD)
State Anxiety Inventory (20 - 80)	43 (±12.51) N = 24	35 (±11.71) N = 16 p = 0.0005	38 (±11.68) N = 13 p = 0.1469	38 (±10.58) N = 8 p = 0.0574
Beck Depression Inventory (0 - 63)	14 (±10.56) N = 24	7 (±12.14) N = 16 p = 0.0473	3 (±10.59) N = 13 p = 0.0592	9.5 (±16.06) N = 8 p = 0.8106
Pain Catastrophising Scale (0 - 52)	23 (±12.89) N = 24	9.5 (±13.08) N = 16 p = 0.0029	3 (±12.84) N = 13 p = 0.0199	14 (±17.01) N = 8 p = 0.8226

Table 7.05 Comparing Psychological Scores Over Study Visits

Paired t-tests were run for every post-surgical visit (6-weeks, 3-months, and 6-months) with respect to the pre-surgical baseline. The N represents the number of patients with reported scores for the psychological measures. For the follow-up visits, the N represents the number of patients that could be included in the paired t-test. Each p-value is the outcome of a paired t-test of reported pain scores at that time point with respect to baseline. Threshold for significance: p(corr) < 0.0056.

For the subjects that completed all four visits, each of the follow-up scores were compared to baseline; none of the scores were significantly reduced at any of the follow-up visits (Threshold for significance: p(corr) < 0.0056) (see Table 7.06).

Psychological Measures	**Pre-Surgery**	**Post-Surgery**		
	Baseline Median (±SD)	**6-weeks** Median (±SD)	**3-months** Median (±SD)	**6-months** Median (±SD)
State Anxiety Inventory (20 - 80) N = 7	45 (±11.32)	36 (±12.84) p = 0.0612	37 (±14.70) p = 0.3563	36 (±11.41) p = 0.0551
Beck Depression Inventory (0 - 63) N = 7	11 (±13.85)	7 (±15.78) p = 0.4697	3 (±11.79) p = 0.2336	7 (±16.87) p = 0.9503
Pain Catastrophising Scale (0 - 52) N = 7	14 (±9.11)	6 (±8.50) p = 0.1023	4 (±12.67) p = 0.2359	7 (±17.13) p = 0.9553

Table 7.06 Comparing Psychological Scores Over Study Visits

Psychological measures for the patients who completed all four study visits. Paired t-tests were run for every post-surgical visit (6-weeks, 3-months, and 6-months) with respect to the pre-surgical baseline. Threshold for significance: p(corr) < 0.0056.

7.1.3.4 Neural-Correlates and Behavioural Measures

As demonstrated above, pain intensity scores did not consistently decrease from baseline to 6-months post-surgery. In fact, five women had an increase in their reported pain intensity and three reported no change. Next, I examine whether these patterns could be predicted in pre-surgical connectivity between descending modulatory regions. Using the correlation coefficients generated in Chapter 5 for the amygdala, PAG, and RVM, the women were separated into groups based on their pain changes from baseline to each follow-up visit (decrease, no change, increase) and were plotted (see Figure 7.04). A multiple linear regression revealed non-significant decreases in amygdala - PAG connectivity (Threshold for significance: p(corr) < 0.0167).

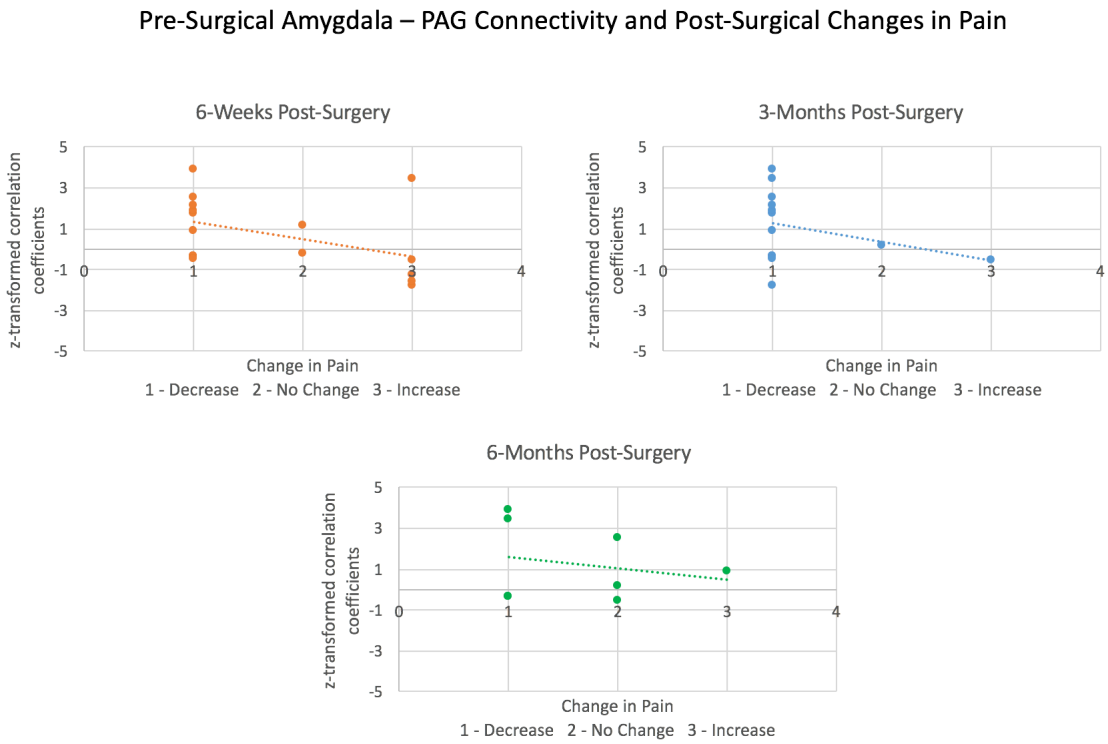


Figure 7.04 Pre-Surgical Amygdala - PAG Connectivity vs. Post-Surgical Changes in Pain

The z-transformed correlation coefficients for pre-surgical amygdala - PAG connectivity are plotted against changes in pain (relative to baseline) at each of the follow-up visits: six-weeks (Multiple Linear Regression: p = 0.100, N = 16), 3-months (Multiple Linear Regression: p = 0.372, N = 13), and 6-months (Multiple Linear Regression: p = 0.756, N = 8). Threshold for significance: p(corr) < 0.0167.

7.1.3.5 Pre- and Post-Surgery Pilot fMRI Dataset

As previously described, five patients completed pre- and post-surgical fMRI visits. Connectivity between the amygdalae, PAG, and RVM was examined in this pilot dataset to determine if there were any surgically-related changes. Given the small sample size of this pilot dataset, this was an exploratory analysis.

The clinical measures for the baseline visit (pre-surgery) and 6-month follow-up (post-surgery) MRI visits can be found in Table 7.07. Paired t-tests did not reveal any significant differences in these measures between sessions.

Clinical Measures	**Pre-Surgery** Mean (±SD)	**Post-Surgery** Mean (±SD)	**p-value**
Overall Pain Intensity (0 - 10)	4.2 (±2.05)	3.8 (±2.59)	p = 0.5870
Pain Catastrophising Scale (0 - 52)	17.6 (±7.40)	19.6 (±20.02)	p = 0.7753
Beck Depression Index (0 - 63)	11.2 (±7.33)	12.8 (±9.98)	p = 0.5347
State Anxiety Inventory (20 - 80)	38.8 (±10.89)	36.8 (±10.18)	p = 0.1998
PainDETECT (0 - 38)	8.2 (±2.59)	11.2 (±8.23)	p = 0.3565

Table 7.07 Comparing Pain Intensity Ratings Over Study Visits

Pain and psychology measures for the five patients that underwent a pre-surgical (baseline) scan and a post-surgical scan (6-months). Paired t-tests did not reveal statistically significant differences in the measures between sessions. N = 5.

Correlation coefficients for PAG - amygdala connectivity pre- and post-surgery were plotted for each subject (see Figure 7.05). At 6-months, with respect to baseline, three subjects had a non-statistically significant decrease in connectivity whilst two had an increase.

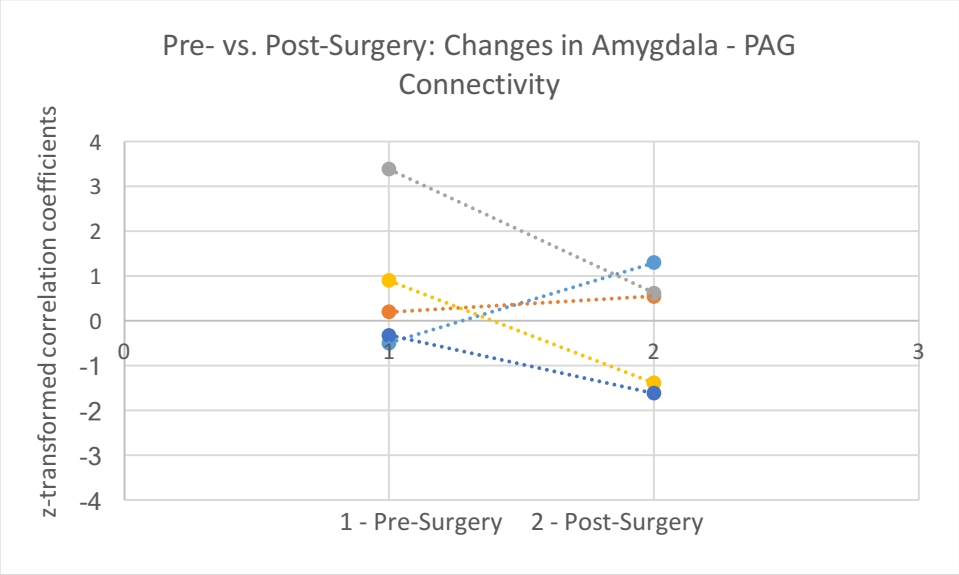


Figure 7.05 Pre- vs. Post-Surgical Changes in Amygdala - PAG Connectivity

The z-transformed correlation coefficients are separated by pre- and post-surgery visits. Pre-surgical visits were within 2-weeks of the procedure whilst post-surgical visits were 6-months after surgery. Each colour represents the pre- and post- correlation coefficients for each patient, with the dotted line illustrating the change in connectivity. N = 5.

When the subjects are separated into those who had a decrease (grey), no change (blue), or increase (orange) in their pain scores, all groups demonstrate decrease in connectivity post-surgery (see Figure 7.06).

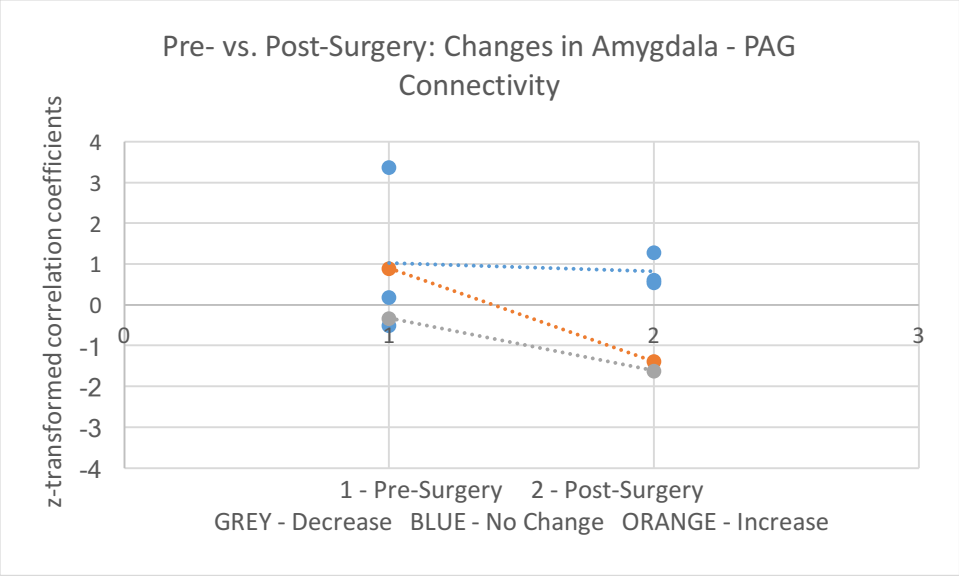


Figure 7.06 Pre- vs. Post-Surgical Changes in Amygdala - PAG Connectivity

The z-transformed correlation coefficients separated by pre- and post-surgery visits. Pre-surgical visits were within 2-weeks of the procedure whilst post-surgical visits were 6-months after surgery. Each colour represents patients with a decrease (grey), no change (blue), or increase (orange) in post-surgical pain scores, with the dotted line illustrating the change in connectivity. N = 5.

7.1.4 Discussion

When comparing pain measures at each follow-up to baseline scores, paired t-tests revealed that only dysmenorrhoea and dyspareunia were significantly lower at the 6-week follow-up. At 3-months, only dyspareunia scores remained significantly (when compared to baseline). When this analysis was re-run only including patients that completed all four study visits, there were no statistically significant changes. Compared to baseline, PainDETECT scores significantly decreased at the 6-week follow-up. When only including patients that completed all the visits, there were no statistically significant changes. State Anxiety and Pain Catastrophising scores were significantly lower at 6-weeks. When only examining those who completed all visits, the baseline to follow-up t-tests yielded no significant results. None of the functional connectivity results were significant, therefore any further discussion would be highly speculative. As this study is ongoing, more subjects will be included in future analyses. These preliminary approaches will help inform the future use of the completed dataset.

7.1.4.1 Limitations

For all the above analyses, larger sample sizes are needed for these questions to be appropriately addressed. Since this study was longitudinal and followed women for over half a year, dropout was a significant limiting factor. As previously described, there were different patterns in post-surgical pain and psychological measures depending on whether all patients were included or just those who completed all the follow-ups. Consequently, it is conceivable that there is a bias in our sample size towards those who did not respond well to the surgery.

7.1.4.2 Future Directions

Despite the limitations of this data set in its current form, it is important to discuss future directions given this study is ongoing. By just examining the raw data, in many instances, there are sharp decreases in pain scores and psychological measures immediately after surgery that are not maintained (albeit not statistically significant). As such, future work should examine the placebo effect. The placebo-effect has been witnessed in other surgical procedures (Turner et al. 1994). In some cases, sham operations (performed by merely making an incision) have been shown to be just as effective as full-fledged procedures (Beecher 1961). Diagnostic laparoscopy alone has been shown to be effective in reducing pain symptoms in 22.6% of women even at the 6-month mark (Sutton et al. 1994).

7.1.5 Summary

Paired t-tests revealed that pain (dysmenorrhoea, dyspareunia) and psychological (catastrophising and State Anxiety) measures decreased 6-weeks post-surgery. Dyspareunia ratings were also significantly lower at the 3-month follow-up. However, these results were not replicated when analyses included only those who completed all the follow-up visits. The functional connectivity result was not close to significance. Larger datasets are needed for future analyses.

Section 2

7.2 Surgical Treatment of Endometriosis: Efficacy of Treatment and Predicting Post-Surgical Pain Improvement

7.2.1 Introduction

In the previous section, I observed a decrease in some of the pain and psychological measures at 6-weeks post-diagnostic laparoscopy/endometriosis surgery; however, no conclusions can be drawn since these changes were not statistically significant when only patients who completed all four study visits were included. The following section focuses on women who had a positive endometriosis diagnosis and underwent surgical treatment. However, given that this study is ongoing and still has a small sample size, this section is exploratory. Since there are only three publications (of two trials) that examine the benefits of this surgery, I wanted to explore the efficacy of surgical treatment on pain scores. Secondly, since it is already known that surgery does not help alleviate pain in all patients, I wanted to examine if simple, bed-side tools, such as the Pain Catastrophising Scale and PainDETECT, could predict the post-surgical pain outcomes for these patients. Finally, I explored the connectivity trends between the amygdala and PAG in this cohort of women.

7.2.1.1 Surgical Intervention in Women with Endometriosis

Surgery is an invasive, expensive, and potentially risky treatment option for patients. There is risk of tissue and/or nerve damage, which can lead to the development or worsening of pain symptoms, and is especially significant for patients who undergo surgery to alleviate said symptoms. Indeed, there is a relationship between past pelvic pain surgery and musculoskeletal pain in women with CPP (Tu, As-Sanie, and Steege

2006). Given the risks of surgery and requisite recovery time, ideally patients would be characterised as good or bad candidates before the procedure.

7.2.1.2 Efficacy of Laser Ablation and Surgical Excision of Endometrial Tissue

Whilst endometriosis is often treated surgically, to date, only two studies have evaluated the efficacy of surgical intervention in endometriosis (laser and excision of endometrial lesions). In the first blinded randomised controlled trial, one group of patients received laser treatment while the other only received a diagnostic laparoscopy (N = 63). Six-months after treatment, of the patients who received the surgical intervention, 62.5% had declining pain scores. For those who had the diagnostic laparoscopy-only, 22.6% of the patients had a decrease in pain (Sutton et al. 1994). In a one-year follow-up, 90% of the improvement group for the surgical intervention still had less pain as a result of the treatment (Sutton et al. 1997). A more recent study has evaluated the efficacy of endometrial tissue excision (N = 39) (Abbott et al. 2004). In the surgical group (N = 20), 80% of the patients' pain decreased (average reduction was 30 on a 0 - 100 scale) whilst 32% in the placebo arm (N = 19) reported lower pain scores.

Both these trials demonstrate that surgery is an effective treatment for many women with endometriosis; however, it is not known why some women do not see an improvement. In the second trial, 20% of the patients did not see a decrease in their pain scores or their pain symptoms became worse (Abbott et al. 2004). Understanding the characteristics that separate the 80% and 20% is key to effectively and efficiently treating all patients and reducing exposure to potentially unnecessary procedures.

7.2.1.3 Predicting Post-Surgical Pain Improvement

Several studies have evaluated predictive factors in post-surgical pain

improvement/response to surgery; however, to date, none have evaluated surgical predictors for women with endometriosis. Surgical studies have observed that male patients tend to have more positive outcomes (Theodosopoulos et al. 2002; Gepstein et al. 2006). Other factors such as high education (lumbar spinal stenosis procedure; (Gepstein et al. 2006)), optimistic expectations (lumbar spinal stenosis procedure; (Gepstein et al. 2006)), trigger points (trigeminal neuralgia; (Tyler-Kabara et al. 2002)), and longer pain duration (trigeminal neuralgia; (Theodosopoulos et al. 2002); radicular pain; (Derby et al. 1992)) have been associated with reductions in pain.

Whilst it was not a surgical study, Martin and colleagues (2011) followed the treatment of 115 women with endometriosis for one year. They found that endometriosis patients with lower catastrophising were more likely to have their pain decrease after one year of treatment in the clinic.

7.2.1.4 Defining Response to Surgery

There are a variety of methods for determining response to treatment (both surgical and non-surgical). In Moore and colleagues' (2013) review, they highlight that most trials use the "change in pain" between two time points as the metric of improvement. Both Martin and colleagues (2011) and Abbott and colleagues (2004) employ the difference in pre- and post-surgical pain ratings when defining response to treatment. Looking at raw changes in pain pre- and post-surgically takes into account magnitude differences, which is a significant characteristic, but puts somewhat dissimilar patients into the same category (Patient 1: reduction from 9/10 to 7/10 versus Patient 2: reduction from 2/10 to 0/10). Responders can also be defined as those who experience a 50% decrease in pain intensity. However, as outlined by Moore and colleagues (2013), one caveat with that method is

that it places Patient 1 (10/10 to 5/10) in the same category as Patient 2 (4/10 to 2/10). It has been argued that from a patient standpoint, reduction to mild pain (≤3 out of 10) is perhaps the most important indicator of improvement (Moore, Straube, and Aldington 2013). This categorisation takes into account the variation of pain intensities post-surgically, placing Patient 1 (10/10 to 5/10) and Patient 2 (4/10 to 2/10) into non-responder and responder categories, respectively (Moore, Straube, and Aldington 2013).

The aims of this section were twofold: 1) to determine whether the surgical intervention was effective in this patient cohort and, if it was, which pain symptoms improved the most and 2) to determine whether bed-side tools (Pain Catastrophising Scale and PainDETECT) can predict reduction in pain post-surgery in women with endometriosis-associated CPP.

7.2.2 Methods

As described in the previous section, this study is ongoing. The analyses in this section comprise an early examination of a growing dataset. Due to drop-out being a common problem in longitudinal research, the sample sizes utilised here are small. It is therefore important to highlight that the analyses presented below are exploratory in nature.

7.2.2.1 Subjects

Of the twenty-six women included in the previous section, nineteen had a positive endometriosis diagnosis and received surgical treatment. At the six-week follow-up, thirteen of the patients with a positive endometriosis diagnosis completed the second questionnaire. At three and six months, eleven and seven women, respectively, completed the follow-up questionnaires (see Figure 7.07).

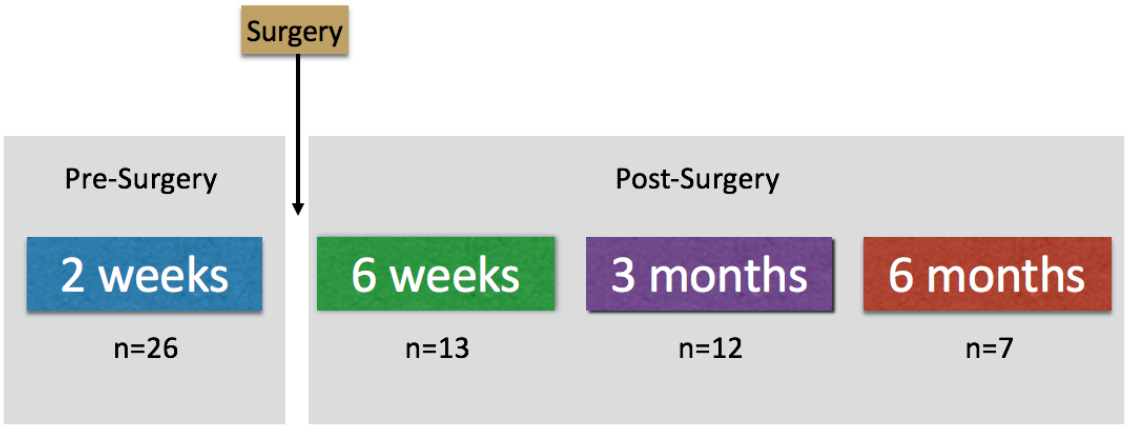


Figure 7.07 Study Visits and Longitudinal Enrolment

A total of 26 women with CPP underwent a diagnostic laparoscopy/surgery. Nineteen had endometriosis, thirteen women completed the 6-week follow-up, twelve completed the 3-month follow-up, and seven completed the 6-month follow-up. The study is still running so we are anticipating having fourteen at 6 weeks, fourteen at 3 months, and ten at 6 months.

7.2.2.2 Pain Measures

The women recorded their pain ratings on an NRS in addition to completing PainDETECT and the Pain Catastrophising Scale. They were asked to rate their dysmenorrhoea, non-cyclical pain, dyspareunia, dyschezia, and dysuria. In addition, they recorded their average overall pain intensity from the 4-weeks prior to the research visit.

7.2.2.3 Analysis

Changes in Pain Measures

As described in the previous section: due to dropout, changes in pain scores were evaluated in two ways: 1) analysis of all individuals who completed the questionnaires at each follow-up and 2) analysis of only those who completed all four study visits.

Prior to performing the paired t-tests, normality and homoscedasticity assumptions were assessed. For data that violated these assumptions, the Sign Rank Test was used. The p-value threshold for statistical significance was adjusted for multiple comparisons (Bonferroni correction) for each analysis. The p-value threshold (0.05) was divided by the

number of tests run (each follow-up comparison multiplied by each pain or psychological measure evaluated). The adjusted statistical threshold is clearly stated with each analysis.

Response to Surgery

Response to surgery was defined using the three methods outlined above. The first used the *raw change in pain scores* from baseline to post-surgery to evaluate response (i.e. 6/10 to 4/10 is a -2 in pain intensity). The second method split women into responders and non-responders based on a *50% reduction in pain* (i.e. 8 out of 10 to 4 out of 10). The third method categorized those with a *reduction to mild pain* ($\leq$ 3 out of 10) as responders, leaving all others as non-responders.

When response to surgery was evaluated using the *raw change in pain scores*, patients were separated into groups based on their expectations, PainDETECT, and Pain Catastrophising scores. Responses to the surgical expectation questionnaire were separated into three groups: 1) no/minimal improvement, 2) marked improvement and 3) very marked improvement. As previously described, PainDETECT has three score categories: nociceptive (0-12), unclear (13-18), and neuropathic (19-38). Similarly, the Pain Catastrophising Scale can be divided into clinically relevant catastrophising ($\geq$ 30) and those with sub-clinical catastrophising (Sullivan, Bishop, and Pivik 1995).

When response to surgery was defined by *50% reduction* or a *reduction to mild pain*, PainDETECT and Pain Catastrophising were evaluated as predictors by using their scores on a continuous scale.

7.2.3 Results

7.2.3.1 Pain Intensity Ratings Pre- and Post-surgery

Similar to the previous section, patients' pain ratings at baseline and each follow-up visit were plotted (see Figure 7.08).

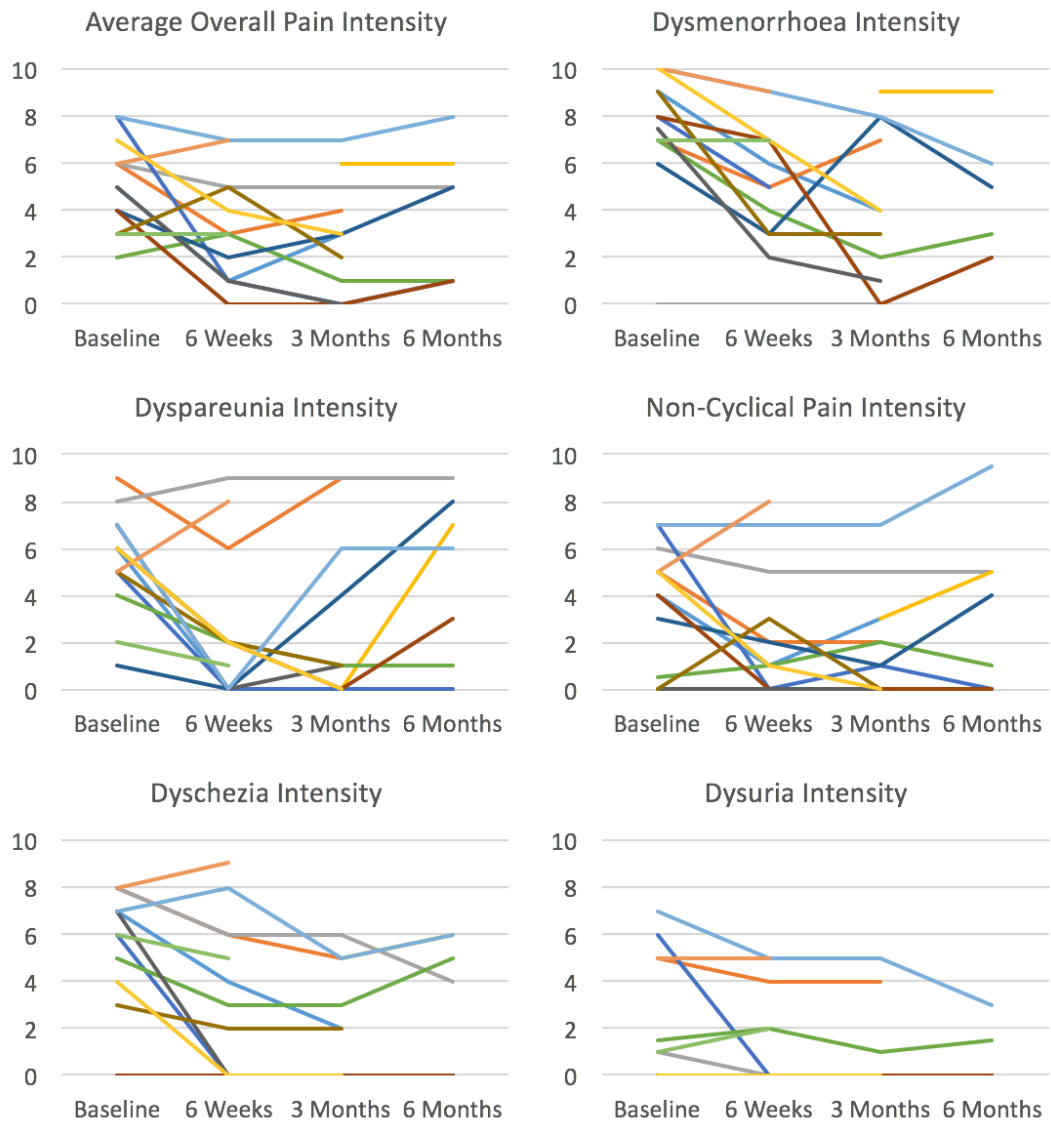


Figure 7.08 Pain Intensity Ratings Plotted Over Study Visits

Pain intensity ratings plotted over the study visits. Average overall pain intensity is a measure of pain 4-weeks prior to the research visit. Baseline was acquired in the 2-weeks prior to surgery whilst ratings from the follow-up visits were acquired post-surgically at 6-weeks, 3-months, and 6-months. The y-axis represents the pain ratings on an NRS whilst the x-axis indicates the visit.

Paired t-tests compared pain scores at each follow-up visit to scores at baseline (Threshold for significance: p(corr) < 0.0028; Sign Rank Test was used for dysuria) (see

Table 7.08). Only dysmenorrhoea scores were significantly lower at the 6-week follow-up.

At 3-months, the only significantly lower score was dyschezia.

Type of Pain (0 - 10)	**Pre-Surgery**	**Post-Surgery**		
	Baseline Median (±SD)	**6-weeks** Median (±SD)	**3-months** Median (±SD)	**6-months** Median (±SD)
Overall Pain Intensity	5 (±1.85) N = 18	3 (±2.28) N = 13	3 (±2.37) N = 12	5 (±2.85) N = 7
Dysmenorrhoea	7.75 (±2.93) N = 18	5 (±2.70) N = 13 p = 0.0005	3.5 (±3.09) N = 10 p = 0.0077	5 (±2.07) N = 5 p = 0.0299
Non-Cyclical Pain	4 (±2.44) N = 17	1.5 (±2.75) N = 12 p = 0.1362	1 (±2.30) N = 11 p = 0.0260	2.5 (±3.71) N = 6 p = 0.3992
Dyspareunia	6 (±2.26) N = 18	2 (±3.15) N = 13 p = 0.0036	1 (±3.60) N = 11 p = 0.0121	4.5 (±3.73) N = 6 p = 0.7181
Dyschezia	5.5 (±3.03) N = 18	3 (±3.28) N = 13 p = 0.0132	2 (±2.24) N = 11 p = 0.0021	2 (±2.81) N = 6 p = 0.1401
Dysuria	0.5 (±2.38) N = 18	0 (±2.02) N = 13 p = 0.6875	0 (±1.81) N = 11 p = 0.0625	0 (±1.25) N = 6 p = 0.2500

Table 7.08 Comparing Pain Intensity Ratings Over Study Visits

Paired t-tests were run for every post-surgical visit (6-weeks, 3-months, and 6-months) with respect to the pre-surgical baseline (Sign Rank Test was run for dysuria). Baseline pain ratings were obtained within 2-weeks of surgery. Overall pain intensity is a measure of the average pain each patient experienced over the preceding 4-weeks for each study visit. The N represents the number of patients who reported the pain scores. For the follow-up visits, the N represents the number of patients that could be included in the paired comparison. Each p-value is the outcome of a paired comparison of reported pain scores at that time point with respect to baseline. Threshold for significance: p(corr) < 0.0028.

The paired t-tests were re-run including only those that completed all four visits (Threshold for significance: p(corr) < 0.0028) (see Table 7.09). No pain scores were significantly reduced at any of the follow-up visits.

Type of Pain (0 - 10)	**Pre-Surgery**	**Post-Surgery**		
	Baseline Median (±SD)	**6-weeks** Median (±SD)	**3-months** Median (±SD)	**6-months** Median (±SD)
Overall Pain Intensity N = 5	4 (±2.28)	2 (±1.92) p = 0.1293	1 (±2.17) p = 0.0951	1 (±2.19) p = 0.1802
Dysmenorrhoea N = 4	7.5 (±1.71)	5.5 (±2.75) p = 0.0405	5 (±4.12) p = 0.2255	4 (±1.83) p = 0.0358
Non-Cyclical Pain N = 6	5 (±2.58)	1.5 (±2.88) p = 0.1356	1.5 (±2.73) p = 0.1459	2.5 (±3.71) p = 0.3992
Dyspareunia N = 6	5.5 (±2.48)	1 (±3.49) p = 0.0522	2.5 (±3.67) p = 0.2543	4.5 (±3.73) p = 0.7181
Dyschezia N = 6	5.5 (±3.50)	1.5 (±3.49) p = 0.2031	1.5 (±2.73) p = 0.0756	2 (±2.81) p = 0.1401
Dysuria N = 6	1.25 (±3.11)	0 (±2.04) p = 0.6250	0 (±2.00) p = 0.1250	0 (±1.25) p = 0.2500

Table 7.09 Comparing Pain Intensity Ratings Over Study Visits

Above are the pain intensity scores for the patients who completed all four study visits. Paired t-tests were run for every post-surgical visit (6-weeks, 3-months, and 6-months) with respect to the pre-surgical baseline (Sign Rank Test was run for dysuria). Baseline pain ratings were obtained within 2-weeks of surgery. Overall pain intensity is a measure of the average pain each patient experienced over the preceding 4-weeks for each study visit. Each p-value is the outcome of a paired comparison of reported pain scores at that time point with respect to baseline. Threshold for significance: p(corr) < 0.0028.

7.2.3.2 PainDETECT Scores Pre- and Post-surgery

PainDETECT scores were plotted for all four research visits (see Figure 7.09).

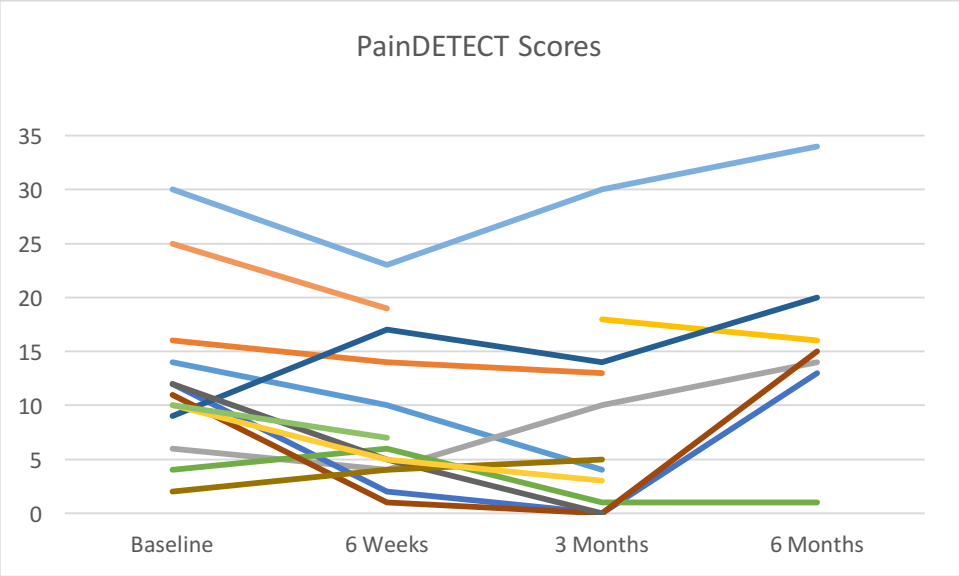


Figure 7.09 PainDETECT Scores Plotted Over Study Visits

PainDETECT ratings plotted over the study visits. Baseline was acquired in the 2-weeks prior to surgery whilst ratings from the follow-up visits were acquired post-surgically at 6-weeks, 3-months, and 6-months. The y-axis represents the PainDETECT scores whilst the x-axis indicates the visit.

Paired t-tests revealed that PainDETECT scores did not significantly decrease at any of the follow-up visits when compared to baseline (Threshold for significance: p(corr) < 0.0167) (see Table 7.10).

	Pre-Surgery	**Post-Surgery**		
	Baseline Median (±SD)	**6-weeks** Median (±SD)	**3-months** Median (±SD)	**6-months** Median (±SD)
PainDETECT Scores	10.5 (±7.81) N = 18	6 (±7.04) N = 13 p = 0.0347	4.5 (±9.24) N = 12 p = 0.1736	15 (±9.82) N = 7 p = 0.0406

Table 7.10 Comparing PainDETECT Scores Over Study Visits

Post-surgical PainDETECT scores compared to baseline. Baseline PainDETECT scores were obtained within 2-weeks of surgery. The N represents the number of patients who reported the pain scores. For the follow-up visits, the N represents the number of patients that could be included in the paired t-test. Each p-value is the outcome of a paired t-test of reported pain scores at that time point with respect to baseline. Threshold for significance: p(corr) < 0.0167.

PainDETECT scores were re-examined to understand the specific trend for the women who completed all four study visits (see Table 7.11). None of the results were statistically significant; however, there was a non-significant decrease in scores at 6-weeks and 3-months, and a non-significant increase in scores at 6-months.

	Pre-Surgery	**Post-Surgery**		
	Baseline Median (±SD)	**6-weeks** Median (±SD)	**3-months** Median (±SD)	**6-months** Median (±SD)
PainDETECT Scores N = 6	10 (±9.32)	5 (±9.02) p = 0.3319	5.5 (±11.25) p = 0.3857	14.5 (±10.76) p = 0.0945

Table 7.11 Comparing PainDETECT Scores Over Study Visits

Post-surgical PainDETECT scores compared to baseline. Baseline PainDETECT scores were obtained within 2-weeks of surgery. Only the scores from patients who completed all four study visits are included in this table. Each p-value is the outcome of a paired t-test of reported PainDETECT scores at that time point with respect to baseline. Threshold for significance: p(corr) < 0.0167.

Unfortunately, there was no placebo-control group to determine whether indeed these changes were specific to the removal of endometrial lesions. As an alternative, I examined the cohort that did not have a positive endometriosis diagnosis. Unfortunately, at the 6-week follow-up only three subjects provided overall pain intensity scores, with only one score available at 3-months and 6-months. These numbers obviously prevented any statistical analysis from taking place.

7.2.3.3 Expectation and Response to Surgery at 6-Week Follow-up

Next, the patients' responses to the questionnaire examining expectation for surgery were examined (Threshold for significance: p(corr) < 0.0167). Overall, the women had positive expectations with respect to surgical outcome for all their symptoms. Only one patient anticipated there would be no pain improvement. Improvement in pain symptoms was expected to last, on average, 1 - 2 years and the patients expressed anxiety about the surgery (6 out of 10) (see Tables 7.12 & 7.13).

N = 13	**Dysmenorrhoea** Mean (Range)	**Dyspareunia** Mean (Range)	**Non-Cyclical Pain** Mean (Range)	**Length of Pain Changes** Mean (Range)
Expected Improvement	**Minimal - Marked** (No Change - Very Marked)	**Marked** (No Change - Very Marked)	**Marked - Very Marked** (No Change - Very Marked)	**1 - 2 years** (Less than 3 months - longer than 5 years)

Table 7.12 Expectations of Surgery: Improvement to Pelvic Pain

The patients included had a positive endometriosis diagnosis and completed the 6-week follow-up. N = 13.

N = 13	**Fertility** Mean (Range)	**Overall Change to Health** Mean (Range)	**Anxiety About Surgery** Mean (Range)	**Overall Feelings About Surgery** Mean (Range)
Expected improvement	**Marked - Very Marked** (No Change - Very Marked)	**Marked - Very Marked** (No Change - Very Marked)	**6 / 10** (0 - 10)	**5.39 on scale from -10 to 10** (-5 to 10) positive number = positive expectation

Table 7.13 Expectations and Anticipation of Surgery

The patients included had a positive endometriosis diagnosis and completed the 6-week follow-up. N = 13.

Again, based on how the questionnaire was designed, expectations in pain reduction were set as categories and magnitude of pain changes (continuous variable) were set as the response to surgery. At 6-weeks post-surgery, the mean reduction in pain was -2.71 for dysmenorrhoea, -3.23 for dyspareunia, and -1.5 for non-cyclical pain.

When patients were separated into very marked (N = 5), marked (N = 5), and minimal/no improvement (N =2) expectation categories for their dysmenorrhoea, expectations did not predict the change in their dysmenorrhoea (Multiple Linear Regression: p = 0.1548; one patient was excluded for not having dysmenorrhoea). Expectation for dyspareunia improvement (very marked (N = 4), marked (N = 6), minimal/no (N = 3)) did not predict post-surgical pain reduction (Multiple Linear Regression: p = 0.5233). Similarly, expectations for improvement of non-cyclical pain (very marked (N = 5), marked (N = 6),

minimal/no (N = 2)) did not predict pain reduction (Multiple Linear Regression: p = 0.4810).

7.2.3.4 Pre-Surgical PainDETECT Scores and Response to Surgery at 6-Week Follow-up

Next, the predictive value of PainDETECT was examined (Threshold for significance: p(corr) < 0.0167). Of the women who completed the 6-week follow-up, eight women had pre-surgical PainDETECT scores that were categorised as nociceptive, three women had unclear scores, and two women had neuropathic scores (see Table 7.14).

PainDETECT Scores Mean: 12.62, Range: 2 - 30		
Nociceptive	Unclear	Neuropathic
N = 8	N = 3	N = 2

Table 7.14 PainDETECT Scores

Distribution of patients in each PainDETECT score group. Eight women had nociceptive pain (nociceptive), three women had pain of either nociceptive or neuropathic origins (unclear), and two women had neuropathic pain (neuropathic).

When response to surgery was categorised as either 1) reduction to mild pain or 2) 50% reduction in pain, against the continuous PainDETECT scores, the relationship was not significant (Multiple Linear Regression: p = 0.4902 and p = 0.9301, respectively). When PainDETECT scores were placed into the categories of nociceptive, unclear, and neuropathic, they did not significantly predict response to surgery (defined by change in pain from baseline to 6 weeks) (Multiple Linear Regression: p = 0.2343). Since there were far fewer patients in the unclear and neuropathic categories, they were grouped together for a second analysis (see Table 7.15) focusing on the most significant relationship: PainDETECT categories versus the magnitude of pre- to post-surgical pain changes (continuous variable).

PainDETECT Scores Mean: 12.62, Range: 2 - 30		
Group 1 (N = 8)	Group 2 (N = 5)	
Nociceptive	Unclear	Neuropathic
N = 8	N = 3	N = 2

Table 7.15 PainDETECT Scores

Distribution of patients in each PainDETECT score group. Eight women had nociceptive pain (nociceptive), three women had pain of either nociceptive or neuropathic origins (unclear), and two women had neuropathic pain (neuropathic). The unclear and neuropathic groups were combined (N = 5).

There was a shift closer towards significance in this analysis (Multiple Linear Regression: p = 0.1768) (see Figure 7.10).

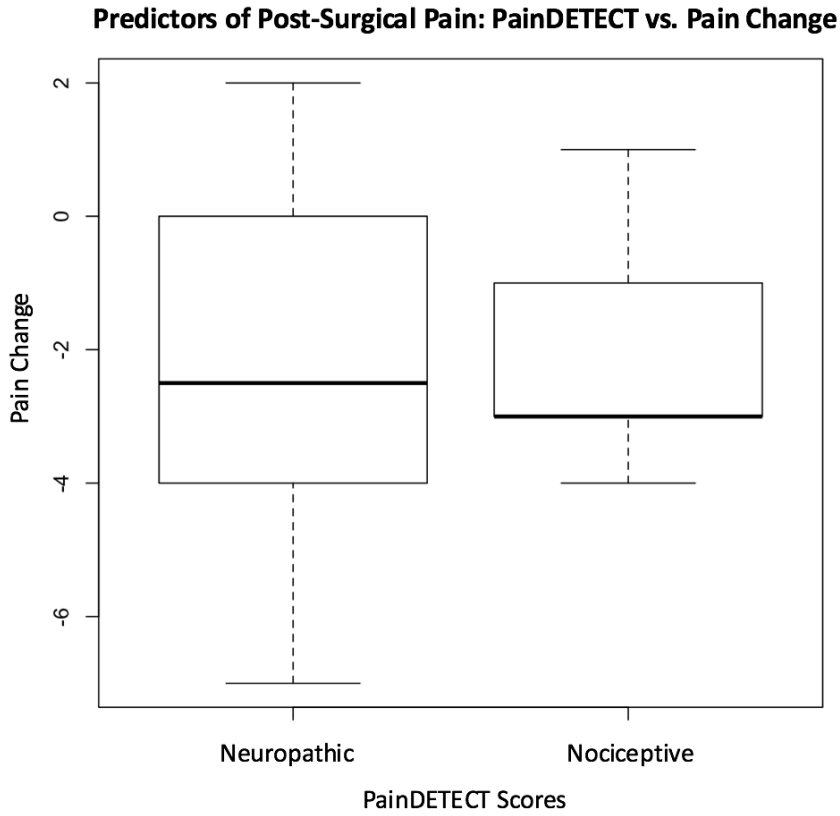


Figure 7.10 Predictors of Post-Surgical Pain

Boxplot of change in pain (difference between baseline and 6-weeks) and the two PainDETECT categories. The whiskers represent the range, the box includes values within the 25 to 75th percentiles and the dark line is the median.

7.2.3.5 Pain Catastrophising and Response to Surgery

The predictive value of Pain Catastrophising scores was also evaluated (Threshold for significance: p(corr) < 0.0167). Five women had catastrophising scores that were at or

above the clinical threshold (≥30), whilst eight had scores below said threshold (see Table 7.16).

Pain Catastrophising Scale Mean: 25.46, Range: 5 - 49	
Sub-clinical catastrophising	Clinically relevant catastrophising
N = 8	N = 5

Table 7.16 Pain Catastrophising Scale Scores

Distribution of patients in each pain catastrophising group. Eight women had sub-clinical catastrophising and five had clinical levels of catastrophising.

When response to surgery was categorised as either 1) a reduction to mild pain or 2) a 50% reduction in pain, catastrophising scores (on a continuous scale) did not predict response to surgery (Multiple Linear Regression: p = 0.544 and p = 0.882, respectively). Similarly, when Pain Catastrophising scores were placed into the categories of clinical and sub-clinical levels and modelled against continuous pain change (defined by change in pain from baseline to 6 weeks), the relationship was not significant (Multiple Linear Regression: p = 0.8701).

7.2.3.6 Central Markers of Response to Surgery

Since the women with endometriosis were surgically treated for the pathology, the same pre-surgical connectivity from the previous section (amygdala - PAG) was evaluated with respect to changes in pain scores at each follow-up visit (Threshold for significance: p(corr) < 0.0167). There was a non-significant relationship between higher connectivity and decreasing pain scores; however, all the results were highly non-significant (Multiple Linear Regression) (see Figure 7.11).

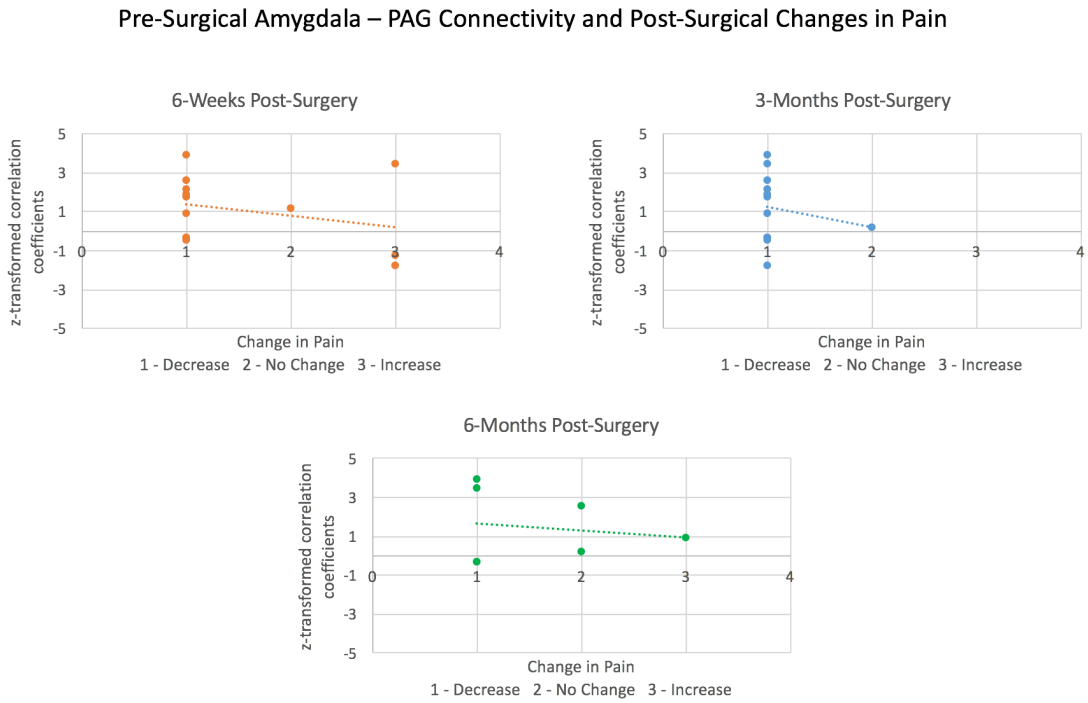


Figure 7.11 Pre-Surgical Amygdala - PAG Connectivity vs. Post-Surgical Changes in Pain

The z-transformed correlation coefficients for pre-surgical amygdala and PAG connectivity are plotted against changes in pain (relative to baseline) at each of the follow-up visits: six-weeks (Multiple Linear Regression: p = 0.351, N = 13), 3-months (Multiple Linear Regression: p = 0.588, N = 12), and 6-months (Multiple Linear Regression: p = 0.771, N = 7). Threshold for significance: p(corr) < 0.0167.

7.2.4 Discussion

When compared to baseline, only dysmenorrhoea and dyschezia pain scores were significantly lower at follow-up (6-weeks and 3-months, respectively). However, there were no statistically significant changes when only patients who completed all the study visits were included. There were non-significant increases in PainDETECT scores at follow-up.

When examining surgical outcomes, expectation did not significantly predict changes in dysmenorrhoea, non-cyclical pain, or dyspareunia. Similarly, PainDETECT and Pain Catastrophising scores did not predict the magnitude of change in pain, 50% reduction in pain, or reduction to mild pain at a statistically significant level. Finally, amygdala - PAG connectivity did not significantly predict post-surgical pain.

7.2.4.1 Limitations

As previously described, the sample size was a limiting factor in this chapter. As such, little can be concluded without the completion of the larger dataset.

7.2.4.2 Future Directions

Of the two clinical trials published, only one explicitly evaluated the impact of surgery on each individual pain symptom. In the first trial, the investigators collected pain ratings for dysmenorrhoea, dyspareunia, and non-cyclical pain, but they did not report response to surgery separately for each type of pain (Sutton et al. 1994; Sutton et al. 1997). In the second trial, the researchers found that there were significant decreases in pain ratings over 12-months for dysmenorrhoea, non-cyclical pain, dyspareunia, and dyschezia (Abbott et al. 2004). An important difference between our cohort and Abbott and colleagues' (2004) was that 17% of the women in their study were not surgically naive whereas our entire cohort was. This should be kept in mind when performing future analyses and interpreting results.

7.2.5 Summary

This section comprised exploratory analyses of an ongoing study. There were inconsistent reductions in pain scores at the post-surgical follow-up visits. Expectation, PainDETECT, Pain Catastrophising, and amygdala - PAG connectivity did not significantly predict post-surgical pain reductions.

Chapter 8

Non-Surgical Treatment: Gabapentin for Women with Chronic Pelvic Pain

8.1 The Impact of Gabapentin on Resting Functional Connectivity in Women with CPP (Pilot)

8.1.1 Introduction

When examining post-surgical pain scores, I demonstrated that endometriosis surgery does result in some decreases in pain, making it a potentially effective treatment option for some women. When comparing pain at follow-up to baseline, dysmenorrhoea, and dyschezia pain scores were significantly reduced at 6-weeks and 3-months, respectively. However, there were no statistically significant changes when only patients who completed all the study visits were included. Furthermore, I did not identify a significant predictor of surgical success. Expectation, PainDETECT and Pain Catastrophising scores did not significantly predict reductions in pain intensity at the 6-week follow-up. Larger datasets are needed to address these types of questions. However, previous clinical trials demonstrate that surgical treatment does not provide pain relief for all women with endometriosis-associated CPP (Abbott et al. 2004). Moreover, it is not an option for women with CPPS (i.e. no pathology). In this chapter, I explore pharmaceutical treatment for these women by evaluating the impact of gabapentin on neural networks that are known to be influenced by the drug (in both pain patients and healthy individuals). The results are a product of a preliminary analysis of a pilot dataset and therefore little can be concluded from this chapter. The acquisition of this early data assisted in better structuring the full-scale trail, which began in the fall of 2015.

8.1.1.1 Mechanisms of Action: Gabapentin and Pregabalin

Both gabapentin (brand name: Neurontin) and pregabalin (brand name: Lyrica) are structural analogues of gamma-aminobutyric acid (GABA), a naturally occurring inhibitory neurotransmitter (Taylor 2009). Of the two, gabapentin was the first to be developed. Gabapentin's first clinical application was for the treatment of epilepsy (UK Gabapentin Study Group 1990). However, further investigations into gabapentin's anti-epileptic properties led to the discovery of its potential as an analgesic agent (Rosner, Rubin, and Kestenbaum 1996). Over a decade after gabapentin's initial approval in the UK, pregabalin was approved for the treatment of seizures and chronic pain (Bockbrader et al. 2010).

Despite both medications being structurally analogous to GABA, their mechanism of action is thought to differ from the neurotransmitter. Currently, the complete picture of gabapentin and pregabalin's mechanism of action is not entirely understood (Taylor 2009). They are believed to work through a variety of cellular pathways including voltage-gated calcium channels. The most recent conclusion is that gabapentin and pregabalin binds specifically to the α2δ1 and α2δ2 receptors (Taylor 2009). Tanabe and colleagues (2008) demonstrated that gabapentin also has analgesic properties by engaging the descending pain inhibitory mechanisms.

8.1.1.2 Gabapentin and Pregabalin as a Treatment for Chronic Pain

Clinical trials have demonstrated that both gabapentin and pregabalin are potentially effective drugs in treating a variety of pain conditions including: diabetic neuropathy (Backonja et al. 1998; Rosenstock et al. 2004), post-herpetic neuralgia (Rowbotham et al. 1998; Sabatowski et al. 2004), and post-surgical pain (Fassoulaki et al. 2002; Clarke et al. 2012). Within the context of CPP, only gabapentin has been evaluated as an effective

treatment (Sator-Katzenschlager et al. 2005). The researchers observed that gabapentin treatment significantly reduced pain in these women at 1, 3, 6, 12, and 24 months. After one month of treatment, the women reported pain of 7.7 on a scale from 0 to 10. At three and six months, their scores were 3.7 and 2.1, respectively. And finally, at twelve and twenty-four months, the reported pain scores decreased to below 3 out of 10. The impact of amitriptyline on pain scores was also examined and, when compared to gabapentin, it reduced pain significantly less (Sator-Katzenschlager et al. 2005). Unfortunately, this study was not blinded and lacked a placebo arm, so it is difficult to make concrete the conclusions about the efficacy of either drug.

8.1.1.3 Central Pain Processing: Gabapentin and Pregabalin

Whilst gabapentin and pregabalin clearly have benefits in chronic and/or neuropathic pain, their specific influence on central pain processing is not well-characterised. Rodent models have demonstrated that gabapentin influences activity in the descending modulatory system (specifically the PAG) (Governo et al. 2008). In humans, several fMRI studies have found that gabapentin and pregabalin alter activity in key pain regions. With topical capsaicin-induced central sensitisation, Iannetti and colleagues (2005) observed that gabapentin decreased brainstem activity, which suggested a shift in modulatory mechanisms.

The PAG is not the only region to demonstrate changes. In fibromyalgia patients, Harris and colleagues (2013) observed decreased glutamate and glutamine levels, excitatory neurochemicals, in the posterior insula during pregabalin treatment.

In a more recent study, relative to to ibuprofen and placebo treatments, researchers demonstrated decreasing connectivity between SII and the left thalamus in healthy subjects during gabapentin treatment (Wanigasekera et al. 2016). This connectivity was further validated with plasma-derived gabapentin levels, with a negative correlation between the two measures.

These results suggest that gabapentin plays an important role in altering central pain networks. To date, there have been no published randomised placebo-controlled trials to determine whether gabapentin is an effective treatment for women with CPP.

The aim of this chapter was to perform a preliminary analysis using pilot data to determine whether gabapentin alters central networks in women with CPP. As such, the results presented in this chapter are exploratory. The full-scale trial is currently on-going.

8.1.2 Methods

8.1.2.1 Subjects

As part of a larger randomised placebo-controlled pilot trial (Horne et al. 2012), twelve women with CPP and negative endometriosis diagnosis were included in this analysis.

8.1.2.2 Study Visits

The trial comprised 4-visits: baseline and three follow-up visits at 6-weeks (MRI), 3- and 6-months (after the medication had been started). Treatment (gabapentin or placebo) began at the baseline visit. At each visit, excluding the MRI visit, patients reported their pain intensity on a VAS.

8.1.2.3 Medication Ramp-Up

Gabapentin treatment began at 300 mg and continued with 300 mg weekly increases until pain was reduced by 50%, the maximum dose was reached (2700 mg), or negative side effects were reported. The continuation of other treatments was permitted during this time (i.e. acupuncture and analgesics). CPP-related pain ratings were recorded at the beginning of the study (Horne et al. 2012).

8.1.2.4 MRI

The MRI took place 6-weeks after the baseline visit (halfway between the baseline and 3-month follow-up). Thus, each of the women had undergone six weeks of either gabapentin or placebo treatment before the MRI.

8.1.3 Results

8.1.3.1 Pain Ratings in the Gabapentin and Placebo Groups

There were no significant differences in age and duration of pelvic pain between the groups (see Table 8.01).

Patient Characteristics	**Gabapentin Group** Mean (±SD) N=6	**Placebo Group** Mean (±SD) N=6	**p-value**
Age (Years)	29.83 (±10.78)	28.50 (±7.18)	p = 0.8059
Duration of Pain	111 months (±8.34) 9.25 years	84 months (±2.64) 7 years	p = 0.6773

Table 8.01 Patient Characteristics Separated by Medication Group

Unpaired t-tests revealed that age and duration of pain were not statistically different between groups. Five subjects could not recall when they first developed CPP (3 in placebo-arm and 2 in gabapentin-arm). N = 12.

Pain scores were plotted for each group over the 3-visits (see Figures 8.01 & 8.02).

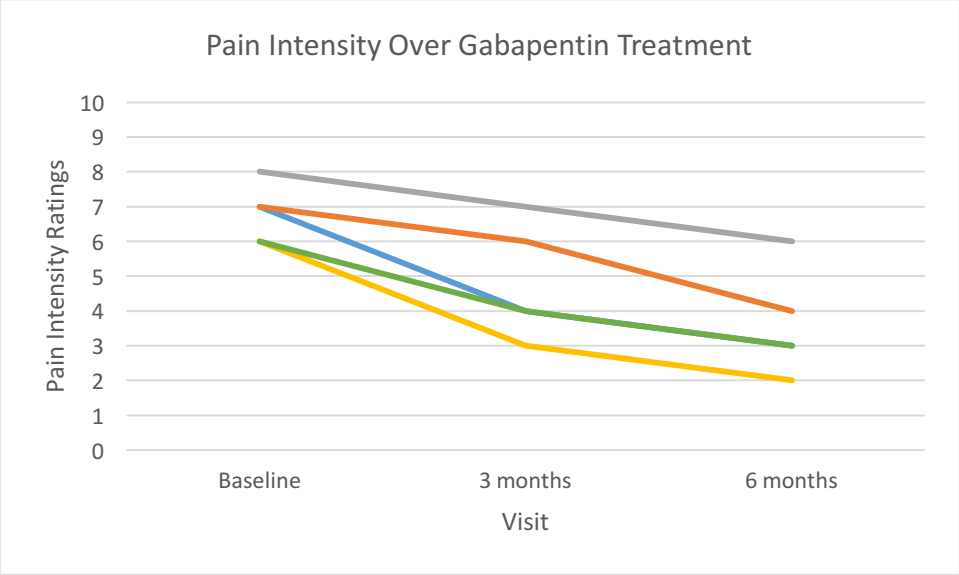


Figure 8.01 Pain Intensity Over Gabapentin Treatment

Pain intensity ratings over the trial in the gabapentin-arm. Baseline pain ratings represent pain intensity before gabapentin treatment began. Patients are still taking gabapentin at the 3- and 6-month follow-ups. N = 6.

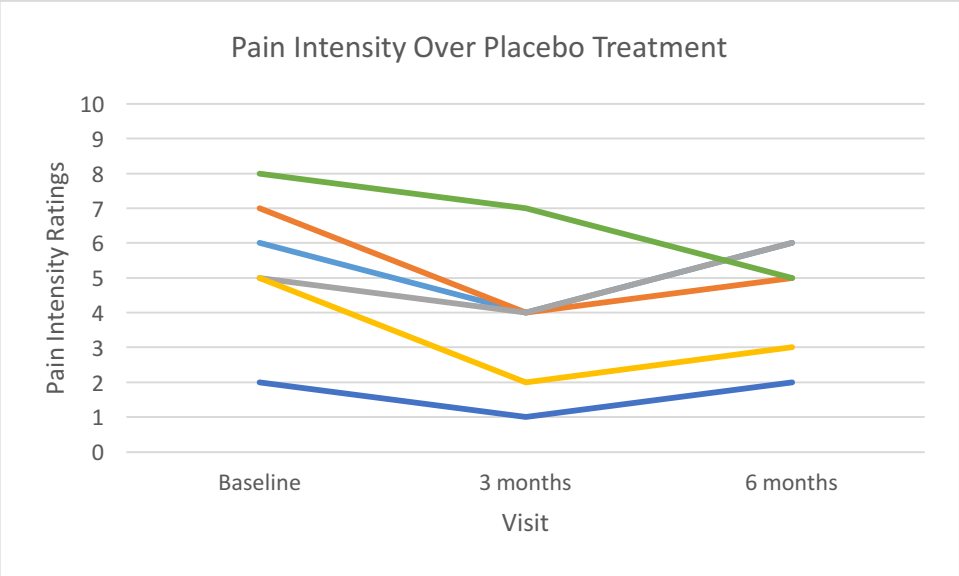


Figure 8.02 Pain Intensity Over Placebo Treatment

Pain intensity ratings over the trial in the placebo-arm. Baseline pain ratings represent pain intensity before placebo treatment began. Patients are still taking placebo at the 3- and 6-month follow-ups. N = 6.

Paired t-tests revealed that both the gabapentin and the placebo group had significantly decreased pain ratings at the 3-month follow-up; however, only the gabapentin group had significantly lower pain ratings at the 6-month mark (see Table 8.02).

Pain Ratings (0 - 10)	**Baseline** Median (±SD)	**3-Months** Median (±SD)	**6-Months** Median (±SD)
Gabapentin N = 6	6.5 (±0.81)	4 (±1.64) p = 0.0111	3.5 (±1.47) p = 0.0019
Placebo N = 6	5.5 (±2.07)	4 (±2.07) p = 0.0060	5 (±1.64) p = 0.1747

Table 8.02 Pain Ratings Over Trial

For both groups, paired t-tests revealed decreases in pain ratings at the 3-month follow-up (Gabapentin: p = 0.0111, Placebo: p = 0.0060). At 6-months, only the gabapentin group had significantly lower pain scores compared to baseline (p = 0.0019). Baseline pain scores represent the pre-medication visit whilst 3-months and 6-months are post-medication (either placebo or gabapentin). There is one missing pain score at the 3-month follow-up in the gabapentin group.

ANOVA revealed that both the gabapentin-arm (F = 41.263, p = 0.001, N = 5) and the placebo-arm (F = 5.482, p = 0.036, N = 6) had significant reductions in pain scores over the follow-ups. When comparing the gabapentin and placebo cohorts, unpaired t-tests demonstrated that the pain scores were not significantly different between groups at each follow-up (3-month: p = 0.3478, 6-month: p = 0.4762).

8.1.3.2 Gabapentin-Related Increases in Connectivity

Next, functional connectivity was examined in the four seed regions. There were no statistically significant network changes between the groups. There was a non-significant increase in connectivity between SII and the PAG in those taking gabapentin; however, it was highly non-significant (General Linear Model Group Comparison: p(corr) = 0.1613) (see Figure 8.03).

Increases in Functional Connectivity in the Gabapentin Group

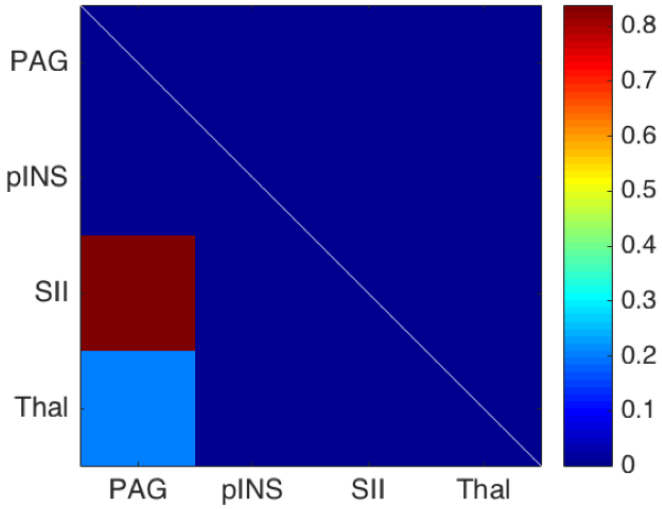


Figure 8.03 Comparing Increases in Functional Connectivity in Gabapentin and Placebo Groups

The connectivity matrix demonstrating non-significant increases in connectivity in the gabapentin group (General Linear Model Group Comparison: p(corr) = 0.1613). The values above the diagonal represent those that survived the threshold, while the ones below are not significant at the threshold ($p < 0.05$). The colour bar represents 1-p-values. N = 12. PAG - periaqueductal gray; pINS - posterior insula; SII - secondary somatosensory cortex; Thal - thalamus.

The z-transformed correlation coefficients for SII - PAG connectivity were plotted for each group (placebo and gabapentin). The non-significant relationship is being driven, in part, by the outlier in the gabapentin cohort (see Figure 8.04).

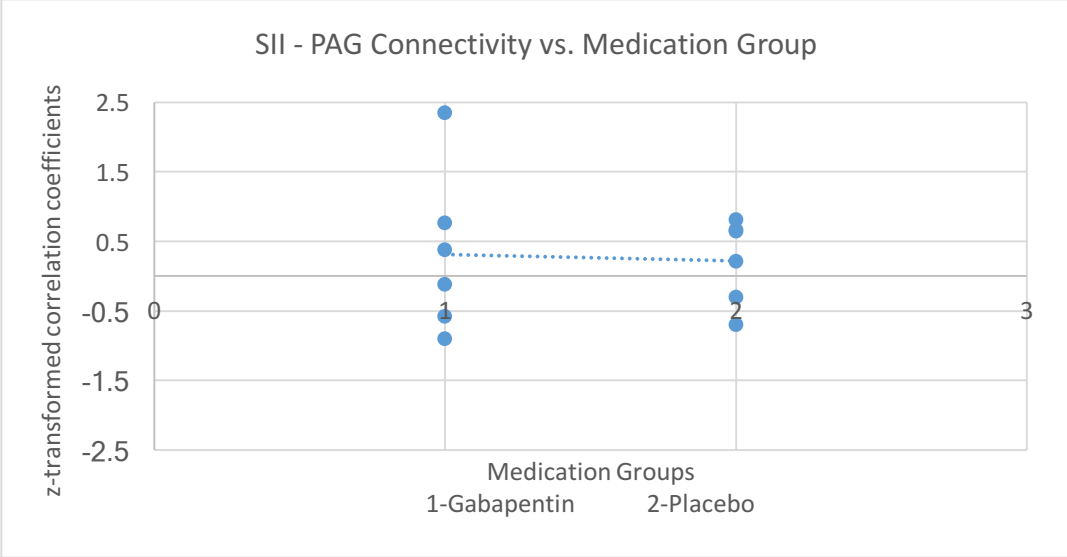


Figure 8.04 SII - PAG Connectivity in the Gabapentin and Placebo Groups

The z-transformed correlation coefficients are separated by medication group. The outlier in the gabapentin group is driving the correlation seen in Figure 8.03. N = 12.

8.1.3.3 Gabapentin-Related Decreases in Connectivity

When decreases in connectivity were evaluated, there were no significant differences between the gabapentin and placebo groups (see Figure 8.05).

Decreases in Functional Connectivity in the Gabapentin Group

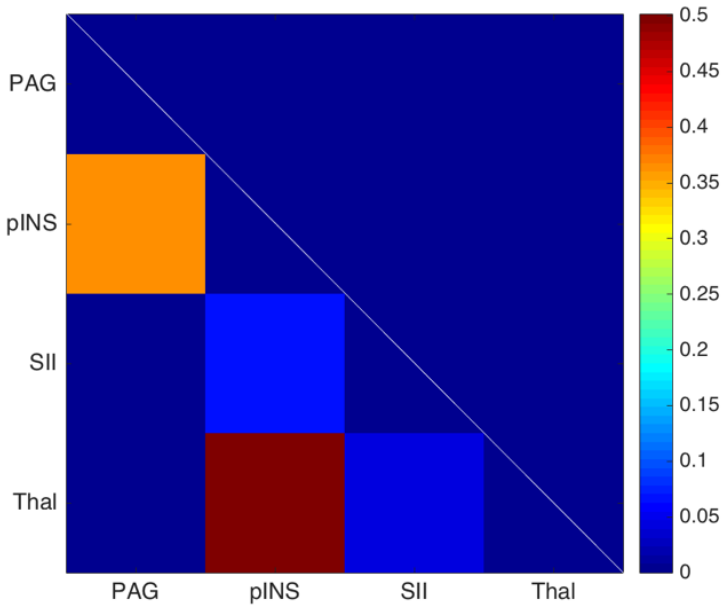


Figure 8.05 Comparing Decreases in Functional Connectivity in Gabapentin and Placebo Groups

The connectivity matrix demonstrating non-significant decreases in connectivity in the gabapentin group. The values above the diagonal represent those that survived the threshold, while the ones below are not significant at the threshold ($p < 0.05$). The colour bar represents 1-p-values. N = 12. PAG - periaqueductal gray; pINS - posterior insula; SII - secondary somatosensory cortex; Thal - thalamus.

8.1.4 Discussion

ANOVA demonstrated that both the gabapentin and placebo groups reported significant decreases in pain at the follow-up visits. In the placebo group, paired t-tests revealed a statistically significant decrease at the 3-month follow-up; however, it is not maintained at 6-months. On the other hand, the gabapentin group had significantly lower pain scores at 3- and 6-months compared to baseline. However, when comparing these reductions between the groups, there were no significant differences in pain scores at each of the follow-up visits.

All the functional connectivity results were highly non-significant. The finding closest to significance from the ROI-network analysis was increased functional coupling between

the PAG and SII in the gabapentin treatment group; however, this was likely driven by an outlier.

This pilot was the first fMRI study to evaluate the impact of pain medications on women with CPP. As previously outlined, women who suffer from CPP often do not receive timely treatment for pelvic pain so more studies are required to elucidate key central mechanisms that might assist in their pain management. Not only is it important in helping uncover the underlying analgesic mechanisms in drugs like gabapentin but it is vital to convince the clinicians who treat these women that they will benefit from pharmaceutical intervention. Hopefully the clinical trial that has just begun will provide data with answers to how gabapentin alters pain processing in these women and women in general.

8.1.4.1 Limitations

The functional connectivity analysis was largely hampered by the study design. It is important to note that study limitations outlined here have been rectified in the full-scale trial and that I assisted in setting up the full trial's MRI scan parameters.

The first important limitation was the lack of a pre-medication baseline MRI in the pilot design — there was only a post-medication scan after 6-weeks of treatment. Without pre-medication functional connectivity measures, it is impossible to know whether gabapentin's central influence relates to the changing pain experience in these women. In this chapter, I was able to conduct a group comparison evaluating potential medication differences; however, it would have been ideal to examine the impact of gabapentin within the patients (with respect to their changing pain scores). The aforementioned fMRI

studies discussed in the introduction of this chapter included a pre-medication baseline scan (Iannetti et al. 2005; Harris et al. 2013; Wanigasekera et al. 2016). The full-scale trial is conducting a pre-medication baseline MRI, which will allow such an analysis to take place.

The small sample size was another significant limitation in this pilot. With only six patients in each group, it is not surprising that none of the group comparisons reached statistical significance. Similar studies examining pregabalin and gabapentin have included much large sample sizes. Harris and colleagues (2013) included seventeen patients whilst Wanigasekera and colleagues (2016) included twenty-four healthy participants. The MRI-arm of the full trial at the University of Edinburgh has increased sample sizes (N = 25 per group).

The trial is also acquiring important clinical and behavioural measures on the day of each MRI. As previously discussed, pain ratings were obtained at baseline and the 3- and 6-month follow-ups but not on the day of the MRI. In this pilot analysis, that data would have allowed for a better characterisation of the chronic pain experience and how pain scores potentially relate to gabapentin-induced connectivity changes. Similarly, no psychological data was collected on these patients on the day of the MRI (data were collected at other time points), which, as demonstrated in Chapters 5 & 6, is important when understanding clinical populations and functional connectivity data. This obviously prevents the further characterisation of this diverse patient population, as previously seen in Chapter 4.

Whilst great care was taken in choosing the seed regions, it is possible that the chosen regions were not the best to evaluate this specific patient population. As previously mentioned, gabapentin's mechanism of action is not well characterised. There are only a handful of studies that have examined its impact on the central nervous system in general and it is possible that the reported results are condition- and sex-specific, and are not necessarily reflective of the overall story. As previously described, women with CPP are a unique patient population even within chronic pain domain, so perhaps there are other networks that gabapentin disrupts. Furthermore, no studies have examined the central impact of gabapentin in women specifically.

8.1.5 Summary

In this pilot dataset, there were no significant differences in the four seed regions when comparing the two medication groups. Given the preliminary nature of this dataset, little can be gleaned from these results. The full clinical trial (N = 25 per medication group in the MRI-arm) is now underway, which has the potential to contribute to the literature on gabapentin's influence on central pain processing and its efficacy in this patient population.

Chapter 9

Conclusion

9.1 Summary of Results

The aim of this dissertation was to demonstrate my evolving analysis techniques and characterise markers of the pain experience in healthy individuals and women with CPP. Using FSLNets functional connectivity analyses, I observed network changes that may represent the maintenance of the chronic pain state or are a consequence of unmanaged pain.

Chapter 3 was an exploratory chapter, limited by the small sample size (N = 10) and the use of early 7T data. I examined seed-based connectivity changes during capsaicin-induced central sensitisation. Condition-dependent global connectivity changes between the amygdalae, posterior, and anterior insula did not reach statistical significance. Nonetheless, the data were further examined to determine if connectivity changes could be seen in a defined network of regions involved in descending modulation (amygdala, anterior insula, and PAG). There were no significant differences between the capsaicin and sham conditions; however, in an exploratory analysis, the pain intensity reported during the capsaicin paradigm was significantly positively correlated with anterior insula - amygdala connectivity.

Chapter 4 introduced the patient population that would be examined for the remainder of the dissertation. Since women with CPP are heterogeneous in their clinical presentation, this chapter aimed to characterise this population to better understand

important components of their pain experience. Pain intensity and pain duration were associated with increased anxiety and depression in our CPP cohort. Additionally, dyschezia was associated with increased psychological distress (State Anxiety). Given the small sample size and that the patients included in this study were recruited from a highly specialised tertiary care centre, these findings cannot necessarily be extrapolated to the larger CPP population.

Chapter 5 introduced the first functional connectivity analysis in women with CPP. This analysis was limited by the uneven distribution of patients across PainDETECT categories. I examined whether activity in descending modulatory circuitry (amygdala, PAG, and RVM) differed between women with nociceptive scores on the PainDETECT questionnaire and those with unclear/neuropathic scores. Women with unclear/neuropathic scores had increased functional coupling between the PAG and amygdala, which suggests neuropathic-specific alterations in descending modulatory mechanisms. This experiment is only the second report of neuropathic measures in women with CPP (Whitaker et al. 2016). However, since our cohort had few patients with unclear and neuropathic scores, which required them to be grouped together as one category in the analysis, future work should examine the reproducibility of these results.

Chapter 6 explores the consequences of pain duration on resting functional connectivity in a network of important CPP brain regions. I demonstrated that women who have experienced pelvic pain for longer than 24 months have increased functional coupling between hippocampus and PAG. I postulated that perhaps this shift represents the physiological consequences of unmanaged chronic pain. There was a significant positive

correlation between hippocampus - PAG connectivity and State Anxiety and Pain Catastrophising scores, which could suggest these changes are associated with affective processes. The results from Chapter 6 underscore the need for more timely pain management in this patient population.

Using preliminary, growing datasets, Chapters 7 & 8 explored surgical and pharmaceutical treatment options for women with CPP. With all the findings in these chapters, larger sample sizes are needed and the analyses will be rerun once the study is complete. In Chapter 7, I examined both the impact of surgery and treatment of endometriosis on reported symptoms. Pain intensity scores were not consistently reduced after either of the interventions. Furthermore, I did not identify any significant predictors (expectation for surgery, PainDETECT, pain catastrophising) of reductions in pain in those who had their endometriosis surgically treated. When examining the impact of gabapentin treatment on resting functional connectivity, gabapentin treatment did not significantly alter network activity between the thalamus, SII, PAG, and posterior insula. This analysis will be rerun upon completion of the full trial.

In this dissertation, I explored analysis methodologies and attempted to utilise measures of functional connectivity to characterise both prolonged experimental pain and chronic pelvic pain. With increasing interest in functional connectivity, new tools have allowed for hypothesis-driven questions within resting state. Functional connectivity is an incredibly useful tool in characterising "baseline" functioning and its respective clinical and experimental correlates. There were many challenges involved in conducting these experiments, particularly using relatively new analysis tools (FSLNets) and working with

7T data (Chapter 3). Further, coordinating the MRIs for the women with CPP was difficult given their surgical schedules.

The results presented in this dissertation may serve as an indicator of inherent changes that relate to their pain experience — particularly the delays in pain management experienced by women with CPP.

9.2 Future Directions

Many of the analyses in this dissertation were hampered by small sample sizes. This was largely due to the challenge of recruiting appropriate patients into the pelvic pain study. With the exception of Chapter 3, all the studies are still running or have full-fledged trials in place (as is the case for Chapter 8). Once the studies are complete, these analyses will be repeated to determine whether the results presented hold in larger patient cohorts. Nonetheless, the findings presented in this dissertation provide a baseline for future experiments and analysis approaches and serve to propel research towards better characterising the pain experience.

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Appendix

Supplementary Tables

The following tables were published in Human Reproduction Update (Brawn et al. 2014).

Table 1

Summary of research studies investigating the response to experimental pain at the site chronic pain is experienced at or referred to. Healthy control women (HC), women with dysmenorrhoea (DYSMEN), endometriosis (ENDO), vulvodynia (VVD), provoked vestibulodynia (PVD), irritable bowel syndrome (IBS), and interstitial cystitis (IC)/Painful bladder syndrome (PBS).

Study	Number of subjects	Stimulation	Location	Results
Dysmenorrhoea (DYSMEN)				
(Giamberardino et al. 1997)	10 DYSMEN 10 HC	Electrical	Left and right abdomen i) skin ii) subcutaneous iii) muscle	• DYSMEN had ↓ pain thresholds in the left rectus abdominis muscle than HC (at each of the 4 points over the menstrual cycle; $p < 0.05$). • There were some subcutaneous threshold differences between groups (not consistent over 4 testing points). • Skin thresholds did not statistically differ between the groups.
(Bajaj et al. 2002)	20 DYSMEN (back and abdominal or abdominal-only pain referral) 15 HC	• Thermal heat • Mechanical Pressure	• Left and right abdomen • Lower back	• DYSMEN (with referral pain to abdomen-only) had ↓ pressure pain thresholds at the abdomen and back compared to HC. • Pressure pain thresholds did not statistically differ between DYSMEN (with back and abdominal pain referral) and HC. • Heat pain thresholds did not statistically differ between groups.
(Brinkert et al. 2007)	11 DYSMEN 9 HC	1-Electrical 2-Balloon distension	1-Abdomen (skin) 2-Sigmoid colon & rectum	• Electrical pain threshold and tolerance measures did not statistically differ between DYSMEN and HC (statistics for all somatic sites: $p > 0.2$). • DYSMEN required ↓ volumes for balloon distension in the sigmoid colon ($p < 0.001$) and rectum ($p = 0.03$) to reach pain threshold (statistics include other sensory outcome measures).
(Vincent et al. 2011)	12 DYSMEN 12 HC	Thermal heat	Abdomen	• DYSMEN required ↓ heat to reach a rating of 5 out of 10 than HC ($p = 0.02$).
Endometriosis (ENDO)				

(Bajaj et al. 2003)	10 ENDO 10 HC	1-Intramuscular saline injection 2-Mechanical pressure	1,2-Lower back muscle 2-Abdomen	• No statistical differences in pain intensity rating measures in response to intramuscular saline injection in the back between ENDO and HC (measured by area under the rating curve and highest rating). • ENDO had ↓ pressure pain thresholds in the lower back and abdomen both pre- ($p = 0.026$) and post-injection ($p = 0.01$) compared to HC.
(Laursen et al. 2005)	10 ENDO 41 HC	Mechanical pressure	Abdomen Lower back	• ENDO had ↓ pressure pain thresholds at all sites compared to HC ($p < 0.001$).
Vulvodynia (VVD) and provoked vestibulodynia (PVD)				
(Bohm-Starke et al. 2001)	19 PVD 20 HC	1-Mechanical punctate 2-Thermal heat and cold 3-Vibration 4-Balloon distension	1,2,3-Vulvar vestibule (2 sites) 4-Vaginal introitus	• PVD had ↓ mechanical punctate pain thresholds ($p < 0.001$; $p < 0.0001$) and heat pain thresholds ($p < 0.05$; $p < 0.0001$) at both vulvar vestibule sites. • Vaginal introitus distension pain thresholds were at ↓ pressures in PVD ($p < 0.001$) than in HC. • More PVD reported pain from cold stimulation than did HC ($p = 0.02$, one HC reported pain). • Vibration did not consistently elicit pain in either group.
(Pukall et al. 2002)	<u>Visit 1</u> 13 PVD 13 HC <u>Visit 2</u> 9 PVD 11 HC	Mechanical punctate	• Labium minus • Inner thigh • Vulvar vestibule (4 sites)	• PVD had ↓ punctate pain thresholds than HC at the vulvar vestibule (all sites visit 1: $p < 0.001$; two sites individually visit 2: $P < 0.01$) and labium minus ($p < 0.01$) but not at the inner thigh (both visits). • PVD and HC did not have significantly different supra-threshold pain ratings in response to punctate at the vulvar vestibule.
(Giesecke et al. 2004)	17 VVD 23 HC	Mechanical pressure	Vulva (23 different sites)	• VVD had ↓ pain thresholds than HC (range for all sites: $p < 0.001$ to $p = 0.03$).
(Lowenstein et al. 2004)	35 PVD 22 HC	1-Mechanical punctate 2-Mechanical pressure (2 devices) 3-Thermal heat	Posterior fourcette	• PVD had ↓ heat ($p < 0.0001$), punctate ($p < 0.0001$), and pressure pain thresholds ($p < 0.0001$; $p < 0.0001$). • PVD had ↑ supra-threshold pain intensity ratings for all aforementioned stimuli ($p < 0.0001$).
(Pukall et al. 2005)	14 PVD 14 HC	Mechanical pressure	Vulvar vestibule	• PVD had ↑ intensity ($p < 0.001$) and unpleasantness ($p < 0.001$) ratings than HC in response to the same pressure.
(Schweinhardt et al. 2008)	14 PVD 14 HC	Mechanical pressure	Vulva	• PVD had ↓ pain thresholds ($p < 0.01$) than HC.

(Sutton, Pukall, and Chamberlain 2009)	25 PVD 25 HC	1 – Mechanical pressure 2 – Thermal heat	1-Vulvar vestibule 2-Vulvar vestibule (3 sites)	• PVD had ↓ heat ($p < 0.001$) and pressure ($p < 0.001$) pain thresholds and lower heat tolerance ($p < 0.001$) than HC. • Pressure pain (from threshold testing paradigm) and heat pain (from tolerance and threshold rating paradigm) unpleasantness and intensity ratings were not significantly different between groups.
(Hampson et al. 2013)	19 VVD 8 HC	Mechanical pressure	Vulva	• VVD had ↓ faint ($p = 0.00001$), mild ($p = 0.00001$), and slightly intense ($p = 0.01$) pressure pain thresholds compared to HC.
Irritable bowel syndrome (IBS)				
(Wilder-Smith et al. 2004)	10 IBS (5 with constipation, 5 with diarrhoea) 10 HC	Balloon distension	Rectum	• IBS (with constipation) had ↑ distension threshold and tolerance volumes than HC and the other IBS cohort ($p < 0.05$). • There were no differences in pain ratings between groups during continuous distension (different volumes used).
(Song et al. 2006)	12 IBS 12 HC	Balloon distension	Rectum	• IBS had ↓ pain thresholds ($p < 0.05$) compared to HC. • Pain intensity ratings for supra-threshold distensions did not differ significantly between groups.
(Wilder-Smith and Robert-Yap 2007)	40 IBS (20 with constipation, 20 with diarrhoea) 20 HC	Balloon distension	Rectum	• IBS had ↓ distension pain thresholds than HC ($p = 0.05$). • During continuous, supra-threshold stimulation, both groups' intensity ratings did not differ significantly.
(Ringel et al. 2008)	10 IBS 10 HC	Balloon distension	Rectum	• There were no differences between groups for pain intensity ratings from distension (same pressures).
(Elsenbruch et al. 2010)	15 IBS 12 HC	Balloon distension	Rectum	• Pain thresholds did not differ significantly between the two groups. • IBS had ↑ discomfort ($p < 0.05$) and pain intensity ($p < 0.001$) ratings than HC.
(Piche et al. 2010)	27 IBS 19 HC	Balloon distension	Rectum	• IBS had ↓ pain thresholds than HC ($p < 0.01$). • IBS had ↑ pain intensity ($p < 0.001$) and unpleasantness ($p < 0.001$) ratings (from threshold testing paradigm) than HC.
Interstitial cystitis (IC) /Painful bladder syndrome (PBS)				
(Ness et al. 2005)	8 IC 13 HC	Balloon distension	Bladder	• IC had ↓ distension pressure and volume pain tolerance than HC ($p < 0.05$). • IC rated distension pain and unpleasantness ↑ than HC ($p < 0.05$).
(Powell-Boone et al. 2005)	6 IC 7 HC	Balloon distension	Bladder	• IC had ↓ distension pressure and volume pain tolerance than HC (p

				< 0.05) at both the luteal and follicular phase. • IC had ↑ distension volume pain tolerance during the luteal phase than the follicular phase ($p < 0.05$).

Table 2

Summary of research studies investigating the response to experimental pain at sites distal to where clinical pain was experienced. Healthy control women (HC), women with dysmenorrhoea (DYSMEN), endometriosis (ENDO), vulvodynia (VVD), provoked vestibulodynia (PVD), irritable bowel syndrome (IBS), and interstitial cystitis (IC)/Painful bladder syndrome (PBS).

Study	Number of subjects	Type of stimulation	Location	Results
Dysmenorrhoea				
(Amodei and Nelson-Gray 1989)	35 DYSMEN 12 HC	1 – Mechanical pressure 2 – Blood-pressure cuff (ischemic)	1 - Finger 2 - Arm	• No statistical differences in pain tolerance and threshold between DYSMEN and HC.
(Giamberardino et al. 1997)	10 DYSMEN 10 HC	Electrical	Quadriceps and deltoid muscles (skin, subcutaneous, muscle)	• DYSMEN had ↓ pain thresholds in the deltoid muscle than HC (at each of the 4 points over the menstrual cycle; $P < 0.05$). • There were some subcutaneous threshold differences between groups (not consistent over 4 testing points). • Skin thresholds did not statistically differ between the groups.
(Granot et al. 2001)	22 DYSMEN 31 HC	1 - Thermal heat 2 – Laser	Hand	• For thermal heat, DYSMEN and HC did not have different thresholds but DYSMEN did have ↑ pain ratings for supra-threshold pain ($p < 0.001$). • For laser stimulation, DYSMEN rated supra-threshold pain as more painful ($p = 0.047$) than HC.
(Bajaj et al. 2002)	20 DYSMEN (back and abdominal or abdominal-only pain referral) 15 HC	• Thermal heat • Mechanical Pressure	Upper arm Thigh	• DYSMEN (both groups) had ↓ pressure ($p = 0.05$; $p < 0.001$) and heat ($p = 0.05$; $p = 0.005$) pain thresholds at both sites than HC.
(Brinkert et al. 2007)	11 DYSMEN 9 HC	Electrical	Dermatomes: C5, L2, and dorsal T10	• Electrical pain threshold and tolerance measures did not statistically differ between DYSMEN and HC (statistics for all somatic sites: $p > 0.2$).
(Vincent et al. 2011)	12 DYSMEN 12 HC	Thermal heat	Arm	• To reach a pain intensity rating of 5 out of 10, DYSMEN required ↓ heat compared to HC ($p = 0.02$).
Endometriosis				
(Bajaj et al. 2003)	10 ENDO 10 HC	1 - Intramuscular saline injection 2 – Mechanical pressure	1,2 - Palm of hand 2 - Thigh 2 - Upper Arm	• ENDO had ↑ pain intensity ratings (highest rating only, not area under the rating curve) in response to intramuscular saline injection on hand higher compared to HC ($p < 0.01$). • ENDO had ↓ pressure pain thresholds on the hand, thigh, and upper arm pre- ($P=0.022$) and post-injection ($p = 0.003$) than HC.

(Laursen et al. 2005)	10 ENDO 41 HC	Mechanical pressure	• Arm • Finger (2 sites) • Leg • Upper back	• ENDO had ↓ pressure pain thresholds than HC at all sites ($p < 0.001$).
(He et al. 2010)	100 ENDO 70 HC	1 - Electrical 2 – Blood pressure cuff (ischemic)	1 - Finger 2 - Arm	• ENDO had ↓ electrical pain thresholds than HC ($p < 0.01$). • ENDO rated pain from the blood pressure cuff at ↑ intensity than HC ($p < 0.01$). • Post-surgery, ENDO rated ischemic pain ↓ and had ↑ electrical pain thresholds compared to baseline ($p < 0.01$).
(Neziri et al. 2010)	20 ENDO 25 HC	Electrical	Foot	• ENDO had ↓ electrical pain thresholds than HC ($p < 0.001$).
(As-Sanie et al. 2012)	ENDO 17 with CPP 15 no CPP No ENDO 6 with CPP 26 healthy controls	Mechanical pressure	Finger	• ENDO with CPP had ↓ pain thresholds (only for "slightly intense pain") than ENDO without CPP ($p < 0.05$) and HC ($p = 0.05$). • There were no statistical differences for any other pain intensity levels between the groups of women with CPP, CPP and ENDO or just ENDO.
Irritable bowel syndrome				
(Song et al. 2006)	12 IBS 12 HC	Cold pressor (cold water bath)	Foot	• There were no differences in pain intensity ratings between groups.
(Wilder-Smith and Robert-Yap 2007)	40 IBS (20 with constipation, 20 with diarrhoea) 20 HC	Cold pressor (cold water bath)	Foot	• IBS had ↑ pain intensity ratings than HC ($p = 0.02$).
(Piche et al. 2010)	27 IBS 24 HC	Thermal heat	• Calf (3 sites) • Forearm (3 sites)	• IBS had ↓ pain thresholds at the calf ($p < 0.05$) compared to HC but no statistical differences were observed in forearm pain thresholds between groups. • Pain tolerance at the calf ($p < 0.05$) and forearm ($p < 0.05$) were ↓ in IBS than HC. • At both sites, IBS reported ↑ pain and unpleasantness ratings in response to painful heat (from threshold and tolerance testing paradigm) ($p < 0.001$).
Vulvodynia and provoked vestibulodynia				
(Granot et al. 2002)	44 PVD 41 HC	Thermal heat	Forearm	• PVD had ↓ pain thresholds than HC ($p = 0.006$). • At 2 supra-threshold temperatures, PVD had ↑ pain intensity ($p = 0.0001$; $p < 0.0001$) and unpleasantness ($p < 0.01$; $p = 0.001$) ratings than HC.
(Pukall et al. 2002)	Visit 1 13 PVD 13 HC Visit 2	1-Mechanical punctate 2-Mechanical pressure	1-Forearm 1,2-Deltoid 1,2-Tibia	• PVD had ↓ punctate pain thresholds on the deltoid and forearm than HC (visit 2; $p < 0.05$).

	9 PVD 11 HC			• There was no difference in tibia punctate pain thresholds between groups (visit 2). • PVD had ↓ pressure pain tolerance at the deltoid (p = 0.01). There was no difference in tolerance at the tibia between groups (visit 1 with only 11 PVD; p > 0.05).
(Giesecke et al. 2004)	17 VVD 23 HC	Mechanical pressure	1-Deltoid 2-Shin 3-Thumb	• VVD had ↓ pressure pain thresholds on the deltoid (p = 0.04), shin (p = 0.05), and thumb (p = 0.04) compared to HC. • At the thumb, the amount of pressure resulting in faint (p = 0.02, p = 0.03), mild (p < 0.001, p = 0.002), and slightly intense (p = 0.001, p = 0.001) pain was ↓ in VVD.
(Foster, Dworkin, and Wood 2005)	10 PVD 10 HC	• Chemical (capsaicin injection) • Mechanical punctate	• Foot • Forearm	• PVD rated the capsaicin-induced spontaneous pain at ↑ intensity on both the foot (p = 0.003) and forearm (p < 0.0001) than HC. • PVD had larger areas of punctate hyperalgesia (p = 0.005; p = 0.005) and dynamic allodynia (p = 0.007; p 0.01) on both the forearm and the foot than HC.
(Granot and Lavee 2005)	28 PVD 50 HC	Thermal heat	Forearm	• PVD had ↓ pain thresholds (p < 0.01) and rated supra-threshold episodic pain at ↑ intensity (p < 0.05) than HC. • There were no group differences in pain intensity rating during the tonic supra-threshold pain.
(Granot 2005)	98 PVD 135 HC	Thermal heat	Forearm	• PVD had ↓ pain thresholds (p < 0.001) and rated supra-threshold pain at ↑ intensity (p < 0.001) than HC.
(Pukall et al. 2006)	16 PVD 16 HC	Pressure from hand	Bilateral: Occiput, trapezius, supraspinatus, low cervical, second rib, lateral epicondyle, gluteal, greater trochanter, knee	• PVD had ↑ number of painful points (p < 0.05) and rated pressure to them at ↑ intensity and unpleasantness (p < 0.001) than HC.
(Johannesso n et al. 2007)	20 PVD 20 HC 20 HC taking combined oral contraceptives	Mechanical pressure	• Tibial muscle • Deltoid	• Pain thresholds in PVD were significantly ↓ compared to HC on the tibial and deltoid muscles. • Both groups had no significant difference between pain intensity ratings (rating pain threshold).
(Sutton, Pukall, and Chamberlain 2009)	25 PVD 25 HC	1 – Mechanical pressure 2 – Thermal heat	1-Forearm 2-Forearm (3 sites)	• No differences in pressure pain or heat pain thresholds or heat tolerance between groups. • No differences in unpleasantness and intensity ratings from pressure (from

				threshold testing paradigm) and heat (from tolerance and threshold rating paradigm) between groups ($p > 0.05$).
(Sutton, Pukall, and Chamberlain 2012)	23 PVD 23 HC	1 – Thermal heat 2- Cold pressor (cold water bath)	1-Forearm 2-Foot	• PVD did not have statistically different heat pain thresholds or intensity ratings (from heat tolerance temperature) compared to HC. • PVD had ↓ heat pain tolerance than HC ($p < 0.05$).
(Hampson et al. 2013)	24 VVD 13 HC	Mechanical pressure	Thumb	• VVD had ↓ mild pressure pain thresholds ($p = 0.01$) compared to HC. • Statistically significant differences were not found between groups for faint ($p = 0.10$) or slightly intense ($p = 0.10$) pressure pain thresholds.
Interstitial cystitis/Painful bladder syndrome				
(Clauw et al. 1997)	25 IC 25 HC	Mechanical pressure	Bilateral: Occiput, trapezius, supraspinatus, low cervical, second rib, lateral epicondyle, gluteal, greater trochanter, knee, forehead, and anterior tibia	• IC had ↓ pain thresholds ($p = 0.002$; $p = 0.02$) and tolerance ($p = 0.01$; $p = 0.001$) than HC.
(Ness et al. 2005)	13 IC (12 for ischemic tolerance) 13 HC (11 in mechanical pressure paradigm)	1-Thermal heat 2-Mechanical pressure 3-Blood pressure cuff (ischemic)	1-Forearm 2-Trapizius, masseter, ulna 3-Arm	• No difference in heat threshold between groups ($p = 0.17$). • IC had ↓ tolerance for ischemic pain than HC ($p < 0.05$). • No statistical differences between groups for ischemic pain threshold, intensity or unpleasantness ratings.
(Powell-Boone et al. 2005)	6 IC 7 HC	1-Thermal heat 2-Blood pressure cuff (ischemic)	1-Forearm 2-Arm	• There were no statistical differences in pain threshold and tolerance between the luteal and follicular phase in either group. • Group comparisons for pain threshold and tolerance were not reported.

Table 3

Pain imaging studies conducted in women with chronic pelvic pain.

Study	Type of imaging	Subjects	Type/site of stimulation	Results
(Wilder-Smith et al. 2004)	fMRI	10 women with IBS (5 with constipation, 5 with diarrhoea) 10 healthy women	Balloon distension of rectum	• Women with IBS (both groups) had significant deactivation in the amygdala/hippocampus compared to healthy women. • Healthy women had significantly increased activation compared to both IBS groups in the anterior cingulate cortex (no activation of anterior cingulate cortices in either IBS group). • Women with IBS (with diarrhoea) had decreased activation in the superior and inferior prefrontal cortex compared to healthy women.
(Ringel et al. 2008)*	fMRI	10 women with IBS 10 healthy women	Balloon distension of rectum	• No significant differences between women with IBS and healthy women in pre-specified regions of interest: posterior cingulate cortex, midcingulate cortex, supragenual anterior cingulate cortex.
(Elsenbruch et al. 2010)	fMRI	15 women with IBS 12 healthy women	Balloon distension of rectum	• Increased activation in women with IBS compared to controls in the left prefrontal and anterior insular cortices. However, significance was lost when anxiety and depression measures were included.
(Pukall et al. 2005)	fMRI	14 women with provoked vestibulodynia 14 healthy women	Mechanical pressure to vulvar vestibule	• In women with provoked vestibulodynia compared to healthy women, there was increased activation in the left anterior insula, bilateral mid-posterior insula, left Brodmann Area 40 and bilateral Brodmann Area 6.
(Hampson et al. 2013)	fMRI	<u>Vulva</u> 19 women with vulvodynia 8 healthy women <u>Thumb</u> 24 women with vulvodynia 13 healthy women	Mechanical pressure to thumb and vulva	***Pressure to thumb*** • Increased activation in: insula, posterior cingulate, dorsal midcingulate, and thalamus (ventral posterolateral nuclei) of women with vulvodynia compared to healthy controls. ***Pressure to vulva*** • No global differences between women with vulvodynia and healthy controls. • Assessment of multiple regions of interest found significant differences in the inferior parietal lobule: women with

				vulvodynia had less decreases in activity than healthy women.
(Tu et al. 2009)	PET	17 women with dysmenorrhoea 16 healthy women	Painful menstruation compared to no menstruation. No experimental pain used.	***During painful menstruation*** • Compared to healthy controls, women with dysmenorrhoea had increased metabolic activity in: posterior cingulate cortex, superior frontal gyrus, left thalamus, and left parahippocampal gyrus. • Compared to healthy controls, women with dysmenorrhoea had decreased metabolic activity in: right superior frontal gyrus, left middle frontal gyrus, left precentral gyrus, left posterior insula, and left secondary somatosensory cortex.
(Vincent et al. 2011)	fMRI	12 women with dysmenorrhoea 12 healthy women	Heat to 1 - Abdomen 2 - Forearm	• Women with dysmenorrhoea had increased activation in the entorhinal cortex, with strength of activation positively correlated with reported pain at menstruation.

* Main purpose of the study was to evaluate the relationship between abuse and IBS

Table 4

Summary of research studies investigating morphological brain changes in women with chronic pelvic pain.

Study	Subjects	Volumetric Grey Matter Changes	
		Grey Matter Increases	**Grey Matter Decreases**
Endometriosis			
(As-Sanie et al. 2012)	Endometriosis 17 with CPP 15 no CPP No endometriosis 6 with CPP 26 healthy controls	Women ***with endometriosis without pain*** compared to healthy women. • Right periaqueductal grey • Right middle frontal gyrus • Right inferior frontal gyrus Women ***with endometriosis with pain*** compared to healthy women. • Left amygdala	Women ***with endometriosis without pain*** compared to healthy women. • Right inferior temporal gyrus Women ***with endometriosis with pain*** compared to healthy women. • Right putamen • Right insula • Left thalamus • Left middle frontal gyrus • Bilateral cingulate gyrus Women ***without endometriosis with pain*** compared to healthy women. • Left thalamus
Vulvodynia and provoked vestibulodynia			
(Schweinhardt et al. 2008)	14 women with vestibulodynia 14 healthy women	Women ***with vestibulodynia*** compared to healthy women. • Left caudate nucleus • Left substantia nigra • Bilateral parahippocampal gyrus • Left globus pallidus	No reported grey matter decreases in women ***with vestibulodynia*** compared to healthy women.
Dysmenorrhoea			
(Tu et al. 2010)	32 women with dysmenorrhoea 32 healthy women	Women ***with dysmenorrhoea*** compared to healthy women. • Right hippocampal gyrus • Right cerebellar tonsil • Left anterior cingulate cortex/dor posterior cingulate cortex • Left precuneus • Left hypothalamus • Left midbrain • Left superior temporal gyrus/mid temporal gyrus	Women ***with dysmenorrhoea*** compared to healthy women. • Right and left secondary somatosensory cortices/posterior insula • Right cerebellar tonsil • Right culmen • Right precuneus • Right medial frontal gyrus • Right superior temporal gyrus/mid insula • Right superior parietal lobule

(Tu et al. 2013)	32 women with dysmenorrhoea 32 healthy women	**<u>Grey Matter Increases</u>** during the menstrual phase compared to the periovulatory phase. Women ***with dysmenorrhoea*** compared to healthy women over the menstrual cycle. • Right postcentral gyrus • Right precuneus • Right hypothalamus • Left orbital gyrus • Left precentral gyrus	**<u>Grey Matter Decreases</u>** in the menstrual phase compared to the periovulatory phase. Women ***with dysmenorrhoea*** compared to healthy women over the menstrual cycle. • Left secondary somatosensory cortex • Left anterior cingulate cortex/dorsal posterior cingulate cortex
Irritable bowel syndrome			
(Blankstein et al. 2010)	11 women with IBS 16 healthy women	Women ***with IBS*** compared to healthy women. • Hypothalamus	Women ***with IBS*** compared to healthy women. • Cortical thinning: anterior insula (only cohort with recent development of IBS) and anterior midcingulate cortex
(Seminowicz et al. 2010)	55 women with IBS 48 healthy women	Women ***with IBS*** compared to healthy women. • Left orbitofrontal cortex • Midline pregenual anterior cingulate cortex	Women ***with IBS*** compared to healthy women. • Bilateral temporal lobe • Bilateral thalamus/midbrain • Bilateral ventral striatum • Bilateral ventrolateral prefrontal cortex • Medial prefrontal cortex • Left posterior parietal cortex • Left precuneus • Left middle frontal gyrus • Left premotor cortex • Occipital cortex