

Prifysgol Bangor

Bangor University

# **Neuroimaging of Chronic Pain**

Aygul A. Khusnullina

Thesis submitted to the School of Psychology, Bangor University, in fulfilment of the requirements for the degree of Doctor of Philosophy

March 2016

## **Declaration and Consent**

### **Details of the Work**

I hereby agree to deposit the following item in the digital repository maintained by Bangor University and/or in any other repository authorized for use by Bangor University.

**Author Name:** Aygul Khusnullina

**Title:** Neuroimaging of Chronic Pain

**Supervisor/Department:** Dr Paul Mullins / School of Psychology

**Funding body (if any):** 125<sup>th</sup> Anniversary Scholarship

**Qualification/Degree obtained:** PhD

This item is a product of my own research endeavours and is covered by the agreement below in which the item is referred to as “the Work”. It is identical in content to that deposited in the Library, subject to point 4 below.

### **Non-exclusive Rights**

Rights granted to the digital repository through this agreement are entirely non-exclusive. I am free to publish the Work in its present version or future versions elsewhere.

I agree that Bangor University may electronically store, copy or translate the Work to any approved medium or format for the purpose of future preservation and accessibility. Bangor University is not under any obligation to reproduce or display the Work in the same formats or resolutions in which it was originally deposited.

## **Bangor University Digital Repository**

I understand that work deposited in the digital repository will be accessible to a wide variety of people and institutions, including automated agents and search engines via the World Wide Web.

I understand that once the Work is deposited, the item and its metadata may be incorporated into public access catalogues or services, national databases of electronic theses and dissertations such as the British Library's EThOS or any service provided by the National Library of Wales.

I understand that the Work may be made available via the National Library of Wales Online Electronic Theses Service under the declared terms and conditions of use (<http://www.llgc.org.uk/index.php?id=4676>). I agree that as part of this service the National Library of Wales may electronically store, copy or convert the Work to any approved medium or format for the purpose of future preservation and accessibility. The National Library of Wales is not under any obligation to reproduce or display the Work in the same formats or resolutions in which it was originally deposited.

### **Statement 1:**

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless as agreed by the University for approved dual awards.

Signed ..... (candidate)

Date .....

**Statement 2:**

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

All other sources are acknowledged by footnotes and/or a bibliography.

Signed ..... (candidate)

Date .....

**Statement 3:**

I hereby give consent for my thesis, if accepted, to be available for photocopying, for inter-library loan and for electronic repositories, and for the title and summary to be made available to outside organisations.

Signed ..... (candidate)

Date .....

**Statement 4:**

Choose **one** of the following options

a) I agree to deposit an electronic copy of my thesis (the Work) in the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University and where necessary have gained the required permissions for the use of third party material.	
b) I agree to deposit an electronic copy of my thesis (the Work) in the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University when the approved <b>bar on access</b> has been lifted.	
c) I agree to submit my thesis (the Work) electronically via Bangor University's e-submission system, however I <b>opt-out</b> of the electronic deposit to the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University, due to lack of permissions for use of third party material.	

Options B should only be used if a bar on access has been approved by the University.

In addition to the above I also agree to the following:

1. That I am the author or have the authority of the author(s) to make this agreement and do hereby give Bangor University the right to make available the Work in the way described above.
2. That the electronic copy of the Work deposited in the digital repository and covered by this agreement, is identical in content to the paper copy of the Work deposited in the Bangor University Library, subject to point 4 below.
3. That I have exercised reasonable care to ensure that the Work is original and, to the best of my knowledge, does not breach any laws – including those relating to defamation, libel and copyright.

4. That I have, in instances where the intellectual property of other authors or copyright holders is included in the Work, and where appropriate, gained explicit permission for the inclusion of that material in the Work, and in the electronic form of the Work as accessed through the open access digital repository, *or* that I have identified and removed that material for which adequate and appropriate permission has not been obtained and which will be inaccessible via the digital repository.
5. That Bangor University does not hold any obligation to take legal action on behalf of the Depositor, or other rights holders, in the event of a breach of intellectual property rights, or any other right, in the material deposited.
6. That I will indemnify and keep indemnified Bangor University and the National Library of Wales from and against any loss, liability, claim or damage, including without limitation any related legal fees and court costs (on a full indemnity bases), related to any breach by myself of any term of this agreement.

Signature: ..... Date: .....

## **Acknowledgments**

I would like to say sincere thanks to my first supervisor Dr Paul Mullins for giving me the opportunity to study a PhD at Bangor University. Also many thanks for the opportunity to attend many international conferences, which have broadened my horizons both academically and personally. Thank you very much for your time, support, academic guidance, and most importantly, for your patience and for believing in me.

My huge thanks to my second supervisor Dr Jeremy Jones for his clinical and academic guidance, time and support. I am very grateful for the opportunity to attend Dr Jones's fibromyalgia clinics, which gave me insight into fibromyalgia and the NHS. Thank you very much for the constructive feedbacks on my drafts and the sense of humour that helped me to survive this PhD.

I am also grateful to the other members of my PhD committee: Prof Charles Leek, Prof Bob Rafal and Prof Rob Ward for their input and help with my PhD. Many thanks to PhD administrator Everil McQuarrie for consistent availability and friendliness.

My special thanks to Dr Simon Tomlinson for tirelessly editing my endless drafts and teaching me sophisticated English (still learning though). I would also like to thank Dr Nia Goulden for her assistance and teaching of data analysis tools.

Huge thanks to all my participants and to Becca Henderson, Jacqui Delabertouche, and Gwyneth Rowlands for invaluable help with the recruitment of the participants.

Hearty thanks to my family (my parents and my brother), my big family-in-law and my friends both in Russia and the UK for giving me love and support during and beyond PhD. Especially my mum, who took care of my little son during the writing period.

And finally, I don't have enough words to thank my husband for being such a caring and loving man and our little boys for making me happy.

## **My role in the work described**

Studies conception and design (MRS, VBM, rs-FC): Aygul Khusnullina, Paul Mullins, and Jeremy Jones

Participants' recruitment:

Healthy participants – Aygul Khusnullina

Fibromyalgia and osteoarthritis participants – Aygul Khusnullina and Jeremy Jones

Questionnaires (administration and analysis) – Aygul Khusnullina

Acquisition of data – Aygul Khusnullina and Paul Mullins

Analysis and interpretation of data:

MRS – Aygul Khusnullina and Paul Mullins

VBM and rs-FC – Aygul Khusnullina and Nia Goulden

Statistical analysis – Aygul Khusnullina

## Table of contents

Declaration and Consent .....	2
Acknowledgments.....	7
Table of contents.....	9
List of tables.....	11
List of figures.....	12
List of abbreviations .....	13
Summary .....	15
CHAPTER 1 - General Introduction.....	16
Introduction.....	17
Pain and its purpose .....	18
The causes and mechanisms of pain .....	20
Chronic pain.....	22
Fibromyalgia .....	24
Osteoarthritis.....	26
Neuroimaging of Pain .....	28
Investigating the neural effects of chronic pain.....	30
Neuroimaging studies of fibromyalgia .....	32
Neuroimaging studies of osteoarthritis .....	38
Differences and Similarities of OA and FM .....	40
Implications and Hypotheses .....	41
CHAPTER 2 - Is chronic widespread pain (fibromyalgia) expressed differently from chronic local pain (osteoarthritis of the knee)? A Magnetic Resonance Spectroscopy study .....	44
Introduction.....	45
Methods and participants .....	47
Participants.....	47
Methods.....	51
MRS procedures.....	51
Questionnaires.....	54
Data Analysis .....	54
Statistics .....	56
Results.....	56

Discussion .....	65
Limitations .....	69
CHAPTER 3 - Functional connectivity in chronic pain .....	73
Introduction.....	74
Methods and participants .....	77
Participants.....	77
Methods.....	78
Functional Magnetic Resonance Imaging.....	79
Image Analysis.....	80
Analysis of resting state FC .....	80
Analysis of correlations of FC with pain measures .....	81
Results.....	82
Discussion .....	91
Summary of results .....	91
What my findings within and between brain networks of interest tell us?.....	91
Increased FC between areas of the DMN in pain patients.....	92
Decreased FC between areas of the EAN in pain patients.....	93
Increased between-network FC in pain patients .....	93
FC and clinical measures .....	94
What my findings within specific brain regions tell us?.....	95
CHAPTER 4 -VBM in chronic pain: differences and similarities between widespread and local pain.....	100
Introduction.....	101
Methods.....	103
Participants.....	103
Magnetic resonance imaging (MRI) and analysis .....	105
Results.....	107
Discussion .....	111
CHAPTER 5 - General Discussion.....	120
Summary of the investigations and key findings.....	121
Differences between CWP and CLP: summary and discussion of main research findings...	122
Similarities between CWP and CLP: summary and discussion of main research findings...	127
Limitations .....	129

General Limitations .....	129
Potential limitations of individual experiments .....	130
Clinical Implications and Applications.....	132
Future plans.....	133
Conclusion .....	134
References.....	136

## List of tables

<i>Table 1. Magnetic Resonance Spectroscopy studies of Fibromyalgia.</i> .....	37
<i>Table 2: Demographics of fibromyalgia subjects</i> .....	48
<i>Table 3: Demographics of osteoarthritis subjects</i> .....	49
<i>Table 4: Demographics of healthy controls</i> .....	50
<i>Table 5: Summary of demographic and clinical characteristics of participants (Mean / <math>\pm</math>SD). For full detail see tables 2, 3 and 4.)</i> .....	57
<i>Table 6. Signal to noise (S:N) ratio and tissue segmentation values</i> .....	58
<i>Table 7. Cramer-Rao Lower Bound (CRLB) percentages</i> .....	59
<i>Table 8: Neurometabolite concentrations (Mm, relative to water) for each region in participants with fibromyalgia (FM), osteoarthritis (OA) and healthy controls (HC)</i> .....	61
<i>Table 9: Significant results for correlations between levels of neurometabolites and pain rating scores (PRS), depression scores (DS), anxiety scores (AS), and pain duration (PD)</i> ..	64
<i>Table 10: MNI coordinates for seed regions</i> .....	79
<i>Table 11: Significant Results of Group Analysis with ROI1 – DMN</i> .....	86
<i>Table 12: Significant Results of Group Analysis with ROI2 – EANL</i> .....	87
<i>Table 13: Significant Results of Group Analysis with ROI3 – EANR</i> .....	88
<i>Table 14: The McGill Pain Questionnaire’s measures (mean scores)</i> .....	89
<i>Table 15: Significant correlations between McGill Scores and FC</i> .....	90
<i>Table 16: Areas of significant decrease in grey matter volume</i> .....	108

## List of figures

<i>Figure 1: Sagittal view of the left medial surface of human brain. The dorsal-caudal anterior cingulate cortex is indicated by the red boundary lines. The ventral-rostral anterior cingulate cortex is indicated by the blue boundary lines.....</i>	<i>30</i>
<i>Figure 2: Voxel locations for magnetic resonance spectroscopy (MRS) of the anterior cingulate cortex (ACC), Anterior Insula, Posterior Insula, and the Thalamus.....</i>	<i>52</i>
<i>Figure 3: Representative MRS spectra from each of the four regions from one participant in the study, demonstrating typical signal quality.....</i>	<i>53</i>
<i>Figure 4: Areas of significantly higher resting-state functional connectivity in fibromyalgia compared to knee osteoarthritis within the default mode network.....</i>	<i>83</i>
<i>Figure 5: Areas of significantly lower resting-state functional connectivity in fibromyalgia compared to healthy controls within the executive attention network left.....</i>	<i>84</i>
<i>Figure 6: Areas of significantly lower resting-state functional connectivity in knee osteoarthritis compared to healthy controls within the executive attention network left.....</i>	<i>85</i>
<i>Figure 7: Differences between FM and OA patients in drawing their pain.....</i>	<i>97</i>
<i>Figure 8: Areas of significant decrease in grey matter volume in fibromyalgia compared to knee osteoarthritis (FM&lt;OA).....</i>	<i>109</i>
<i>Figure 9: Areas of significant decrease in grey matter volume in knee osteoarthritis compared to fibromyalgia (OA&lt;FM).....</i>	<i>110</i>

## List of abbreviations

ACC	Anterior Cingulate Cortex
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AntIns	Anterior Insula
AS	Anxiety Score
BA	Brodmann Area
Cho	Choline
CLP	Chronic Localised Pain
CLBP	Chronic Lower Back Pain
Cr	Creatine
CRLB	Cramer-Rao Lower Bound
CRPS	Complex Regional Pain Syndrome
CSF	Cerebrospinal Fluid
CWP	Chronic Widespread Pain
dACC	Dorsal-caudal Anterior Cingulate Cortex
DH	Dorsal Horn
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
DS	Depression Score
EAN	Executive Attention Network
FC	Functional Connectivity
FM	Fibromyalgia
fMRI	Functional Magnetic Resonance Spectroscopy
FSS	Functional Somatic Syndrome
GABA	Gamma-Aminobutyric Acid
Gln	Glutamine
Glu	Glutamate
Glx	Glutamine and Glutamate
GM	Grey Matter
GMV	Grey Matter Volume
HADS	Hospital Anxiety and Depression Scale

HPA	Hypothalamic–pituitary–adrenal axis
m-Ins	Myo-Inositol
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MTG	Mid Temporal Gyrus
NAA	N-Acetylaspartic acid
NWC	Number of Words Chosen
OA	(knee) Osteoarthritis
PAG	Periaqueductal Grey
PD	Pain Duration
PFC	Prefrontal Cortex
PosIns	Posterior Insula
PPI	Present Pain Intensity
PRI	Pain Rating Index
PRS	Pain Rating Scale
ROI	Region of Interest
rs-FC	Resting-state Functional Connectivity
S1	Primary Somatosensory Cortex
S2	Secondary Somatosensory Cortex
SPL	Superior Parietal Lobule
SPM	Statistical Parametric Mapping
SMT	Spinomesencephalic tract
SRT	Spinoreticular tract
STT	Spinothalamic tract
Thal	Thalamus
TMD	Temporomandibular disorder
TNP	Trigeminal Neuropathic Pain
vACC	ventral-rostral Anterior Cingulate Cortex
VAS	Visual Analogue Scale
VBM	Voxel-based Morphometry
WM	White Matter

## Summary

Chronic pain is a debilitating symptom of a wide range of conditions. These conditions are both highly prevalent and create adverse consequences for individuals and society. Whilst understanding of chronic pain conditions has improved, in a number of cases the mechanisms of chronic pain are not fully understood and no cure is available. It is appreciated that chronic pain is not only unpleasant in itself, but can also lead to a reorganisation of the nervous system resulting in further suffering. These factors present a justification for further investigation into the mechanisms and effects of chronic pain to enable progress towards more effective treatments.

Neuroimaging techniques have helped our understanding the mechanisms and effects of chronic pain. Techniques have been developed to examine the structure, chemistry and activity of the brain. This thesis describes investigations that used neuroimaging to examine the effects of chronic pain on the human brain.

A distinction has been drawn between chronic widespread pain (CWP) and chronic localised pain (CLP). Historically, the latter was seen as a condition of the peripheral components of the pain system. More recently, however, an understanding has been gained that central mechanisms may also be a factor in these conditions. The purpose of my investigations was to examine differences and similarities in the effects of two CWP and CLP conditions on the human brain. Fibromyalgia (FM) and Knee Osteoarthritis (OA) were chosen as representatives of these classes of condition. The effects on neurochemistry, brain structure and coordinated brain activity in these conditions were compared using magnetic resonance spectroscopy (MRS), voxel-based morphometry (VBM) and resting state functional connectivity (rs-FC).

Using MRS I observed a reduction in N-Acetylaspartic acid (NAA) in the thalamus of OA patients when compared to FM. Using VBM I observed that grey matter volume (GMV) was reduced in the left brainstem and posterior cingulate cortex in FM patients when compared to OA. GMV was reduced in the left precentral, middle frontal and supramarginal gyri in OA when compared to FM. Using rs-FC I observed an increase in functional connectivity in the default mode network of FM patients when compared to OA. I observed increased functional connectivity within the default mode network (DMN) in both pain conditions compared to healthy controls. I also observed increased functional connectivity between the precuneus and regions in both the DMN and executive attention networks.

Consideration is given to these findings in the context of previous relevant research. The implications of the results are related the patients' own experience of their condition and links to clinical measures are also discussed. The findings provide further evidence for the neural basis of elements of patients' experience of their condition and further understanding of the differences between the wider presentations of these conditions. The findings are drawn together to demonstrate where the effects of CWP and CLP overlap and where their effects contrast. Consideration is given to the mechanisms at work in these conditions that suggest differing effects on the components of the pain system and also to demonstrate where prolonged abnormal peripheral input may be a factor driving adaptation in CLP.

## **CHAPTER 1 - General Introduction**

## **Introduction**

Pain and its avoidance has been a theme of a wide range of human activities since the evolution of our species. With the rare exception of those experiencing congenital analgesia, every human will experience pain during their lives. Whilst we are fortunate to live in an age where there is widespread access to mechanisms and treatments to manage and control pain, there are clinical conditions that demonstrate that our knowledge and mastery of pain is incomplete. The existence of chronic pain conditions that show limited response to medical intervention not only demonstrates the limits of our knowledge of pain, but also provides a pressing motivation for pain research given the adverse effects that these conditions have on the lives of their sufferers.

The purpose of this thesis is to describe and discuss a series of studies that examine the effects that two contrasting chronic pain conditions have on the human brain. The studies were conducted using three neuroimaging techniques to observe the effects of pain on the brains of chronic pain patients.

In this chapter I will consider the purpose of pain, the development of our understanding of the mechanisms of pain and examine research into the consequences of prolonged pain. I will contrast chronic localised pain and chronic widespread pain and consider why these conditions may differ in their effects on the human nervous system. I will then consider the use of neuroimaging for the study of pain in living humans. I will then examine how different neuroimaging techniques may be used to generate converging evidence to the nature of the effects of pain on the nervous system.

In the following chapters I describe three studies that were undertaken to use neuroimaging techniques to examine chronic localised and widespread pain. In each case comparisons are made both between groups of participants experiencing chronic localised or widespread pain conditions and between chronic pain patients and healthy control participants. Admission to the patient groups was based on prior diagnoses. To provide further insight into the nature of the patients' experience of their condition each patient was required to complete a series of questionnaires. The questionnaires were intended to capture the impact of the patients' condition on their mental health, to provide better understanding of patients' experience of

their pain and to provide further evidence of the distinction between the conditions. The use of the questionnaires is described in detail in the following chapters.

In Chapter 2 I describe the use of magnetic resonance spectroscopy to examine differences in levels of neurochemicals in areas of the brain associated with the processing and representation of pain.

Chapter 3 describes a study made to determine whether differences could be observed in the grey matter volumes of regions within the brains of chronic pain patients and those of healthy controls. The study used voxel-based morphometry to warp participants' brain images to a standard template to enable comparisons to be made between the levels of grey matter present across the areas of the brain.

Chapter 4 describes a study using functional connectivity to examine the correlation between levels of activity in areas of interest across the brain in both chronic pain conditions and healthy controls. This study examined the effects of chronic pain on activity within previously identified anticorrelated brain networks: the executive attention network associated with externally directed cognition and the default mode network associated with self-reflection and rest.

Chapter 5 summarises the results of the studies and discusses their implications for our knowledge of the nature of chronic pain. I consider the differences and similarities observed in the effects of chronic localised and widespread pain on the human brain. I also consider the implications of the observed differences in regard to the recommendation of further research and for the development of targeted therapies for the alleviation, or at least management, of these conditions.

## **Pain and its purpose**

In common with many species, humans have evolved a complex nervous system that continuously samples elements of the state of both the external world and the internal environment. From the principles of evolutionary theory, the primary force driving adaption is that which increases the chances of an individual surviving long enough to reproduce. It is clear that a signalling system capable of accurate representation of the threats and

opportunities in the external world is highly advantageous in ensuring continued survival. For this system to be effective, however, the avoidance of threats and use of opportunities needs to be considered in the context of the overall state of the organism to enable an effective survival strategy. The elements of the nervous system that have developed to register and represent the internal states of the organism are essential for the formation of a coherent representation of the organism's overall status and current needs. From this representation, a plan may be formed for the implementation of behaviours that will service the organism's current needs. These elements enable the "homeostatic" management of the organism's state (e.g. sweating in response to heat, shivering in response to cold), motivate behaviours to restore an optimal state and, within humans, develop the representations that underpin a sense of self (Damasio, 2003). In addition to the detection of an excess or a scarcity of survival-relevant substances within the internal environment, the internally-oriented nervous system has also evolved the capability to monitor the integrity of the components of the organism and signal when their integrity is compromised (Craig, 2003). The ability to monitor the integrity of an organism's own components and motivate a behavioural response to damage is of obvious survival value. Pain is the description given to the subjective experience that accompanies the activation of the elements of the nervous system that respond to damage. The most widely accepted definition of pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". (IASP Task Force on Taxonomy, 1994). The evolutionary purpose of pain is plausibly explained in terms of motivating behaviours to avoid bodily damage, to rest and protect damaged components and thus prevent further damage (Millan, 1999) and, within our own species, to motivate help-seeking behaviours (Thorstensson, Gooberman-Hill, Adamson, Williams, & Dieppe, 2009). Within this view the purpose of pain can be seen as an extension of the more basic homeostatic mechanisms of the nervous system in that it serves to motivate behaviours that seek to restore an individual to an optimal state. If an element of the evolved purpose of pain is to facilitate appropriate behaviours then it is understandable why pain is an unpleasant experience that, under normal circumstances, is difficult to ignore (Ecclestone & Crombez, 1999). It has been proposed that evolutionary purpose of pain is best served by the promotion of behaviours that minimise the risk of damage and enable bodily repair once damage has occurred (Wall, McMahon, & Koltzenburg, 2006). Within this thesis I will consider conditions where pain persists beyond the stage where these behaviours have been established and where the persistence of pain may have a transformative effect of the nervous

system of the sufferer. To enable this consideration I will first look at research into the causes and underlying mechanisms of the pain system.

## **The causes and mechanisms of pain**

Early theories about the causes of pain ranged from the imbalance of the vital humours, the actions of gods or demons, or the effects of negative emotions (as reviewed in Doleys, 2014). An idea of a system for conveying information about bodily damage from the periphery to the brain and for instigating compensatory behaviours was suggested by both Galen and Avicenna. Whilst elements within these theories find counterparts in the modern descriptions of the mechanisms of pain, a richer understanding of these mechanisms could not be developed before several discoveries had been made. The modern understanding of the mechanisms of pain grew from the 19<sup>th</sup> century studies of the nervous system, such as those performed by Schiff and von Frey (Perl, 2007); the discovery of the neuron and synaptic inter-cellular communication (Ramón y Cajal, 1911); and the description of nociceptors by Sherrington (1906). These elements combined to provide an understanding of a system comprising multiple specialised elements that could communicate with each other via electrochemical means to convey information about noxious stimuli from the periphery to the brain. Whilst the presence or absence of receptors with the specific purpose of detecting and signalling pain was the subject of intense debate throughout the 20<sup>th</sup> century (see Moayedi & Davis, 2012), the role of specific classes of pain receptors and those played by classes of afferent fibres came to be better understood. This led to an understanding of the key elements of the pain system outside the brain in that the importance of nociceptors, A $\delta$  and C afferent fibres, the neurons of the dorsal horn laminae and the ascending spinal tracts came to be appreciated (Wall et al., 2006). I will briefly summarise the contemporary view of the anatomy and neurobiology of pain using peripheral nociceptive pain for illustration: the reader is referred to reviews by Besson, Brooks and Tracey, and Millan for further detail (Besson, 1999; Brooks & Tracey, 2005; Millan, 1999). Pain most commonly entails the transmission of information from nociceptors located in the periphery to the CNS to enable an appropriate response to be made to actual or potential tissue damage. Nociception takes place at receptors on primary afferent fibres (PAFs), located within skin, muscle and joints. Different classes of PAF exist, which are either specialised to be responsive to a particular type of noxious stimulus (e.g. mechanical or thermal) or polymodal. The most important types of PAF for the transmission of pain-related information are the thin, unmyelinated A $\delta$

fibres and the thicker myelinated C fibres. These fibres use a range of neurotransmitters, excitatory amino acids, neuropeptides and other substances to transmit information onwards. The PAFs project to neurons within the laminae of the dorsal horn (DH) of the spine. Within the DH are located interneurons, which integrate sensory information and play a role in modulating nociceptive signals, including inhibiting the release of neurotransmitters from A $\delta$  and C fibres. Also within the DH are projection neurons, which carry pain information to supraspinal targets via mono- or polysynaptic pathways. Three major pathways from the DH to supraspinal targets are the spinothalamic (STT), spinomesencephalic (SMT) and spinoreticular tracts (SRT), which project to primarily contralateral targets (Almeida, Roizenblatt, & Tufik, 2004). The SMT projects to the pons and superior colliculus, which in turn pass pain-related signals to the basal ganglia. The SRT projects to the reticular formation and thence to the thalamus. The STT projects to a number of nuclei within the ventral, ventromedial and medial thalamus. The thalamic nuclei targeted by the STT have onward projections to cortical areas associated with sensory processing (postcentral gyrus), pain specific processing (insula), cognition (pre-frontal cortex) and affect (cingulate gyrus). These areas have been proposed as being components of the 'pain neuromatrix' i.e. brain areas whose combined action generate the overall representation of a patient's pain (Melzack, 2001).

With increased knowledge of the anatomy and neurobiology of the pain system, the limitations of available descriptions of the system's operation gained greater prominence.

An appreciation of the existence of distinct classes of pain and the diversity of responses to similar noxious stimuli (e.g. Beecher, 1946) led researchers to seek an improved model for interpreting the pain system. It was clear to Melzack and co-workers that the emotional state and the sufferer's attitude towards their pain could have a direct influence on the level of pain subjectively experienced. They proposed the Gate Control Theory (Melzack & Wall, 1965) which suggested that the level and type of pain experienced was influenced not only by input from the periphery, but could be modulated by control mechanisms descending from the brain. This led to a view of the pain system not merely as a passive reflector of reality, but as a complex system that could be influenced by an array of additional factors (e.g. a person's emotional state or psychological perception of pain). Other observations such as from cases where pain persisted after its original cause had been addressed or 'phantom pain' perceived to be present in a limb after amputation (e.g. Flor et al., 1995), led researchers to an

understanding that the response of the pain system was not necessarily optimal in all cases. Additionally, it was observed that exposure to pain could lead to alterations in the pain system's operation: the development of tolerance or hypersensitivity in response to pain could be considered adaptive (or maladaptive) responses to persisting pain conditions (e.g. Costigan, Scholz, & Woolf, 2010; Dar, Ariely, & Frenk, 1995). The initial view of adaptation within the pain system centred on alterations to peripheral components (Wall et al., 2006). Further research, however, uncovered distinctive forms of maladaptive response in persistent pain entailing a more fundamental reorganisation of the system's core components (Nielsen & Henriksson, 2007), with a suggestion of the 'central sensitisation' of the pain system. Central sensitisation occurs within the CNS at both DH neurons and supraspinal sites and consists of an increase in both membrane excitability and level of synaptic activity in response to noxious stimuli (Latremoliere & Woolf, 2009; Staud, 2002; Suzuki, Rygh, & Dickenson, 2004). Central sensitisation causes the recruitment of additional inputs to nociceptive pathways to lower the threshold at which stimuli are perceived as noxious or to amplify the effect of noxious stimuli, and persists after the original noxious stimulus has been withdrawn (Woolf, 2012). The growing appreciation of plasticity within the human pain system taken with the increasing understanding of the human nervous system has led to several theories concerning the effects of prolonged pain on this system. The investigation of central reorganisation of the human pain system in response to persistent pain is the primary focus of this thesis. I will now examine what is meant by chronic pain and contrast chronic widespread and chronic localised pain conditions.

## **Chronic pain**

Chronic pain has been defined by the International Association for the Study of Pain as pain that persists past the healing phase after injury, and is clinically determined as pain that has lasted longer than 12 weeks (Merskey & Bogduk, 1994). In practice, however, the use of this definition varies according to the condition creating the pain. For example, back pain often takes 6 months to be classed as chronic; while in post-herpetic neuralgia 3 months of continuous pain is the time at which the condition is typically deemed as chronic. Apkarian, Baliki and Geha (2009) proposed that the use of one definition of chronic pain does not give a full, correct explanation, and thus, for each specific type of chronic pain a specifically related definition must be given based on the peripheral and central mechanisms that underlie the development of each condition. Within the clinical and scientific literature two main

dimensions of pain emerge: one defined by site of injury (e.g. back, head, viscera) and the other by the cause of the discomfort experienced (e.g. neuropathic, arthritic, cancer, myofascial, diabetic). Clinically, symptoms can result from the presence of a number of pain-creating conditions that can make it difficult to assess the relative contribution of each condition to the overall pain experienced. An example of this situation is given by chronic low back pain where levels of joint degeneration, muscle, and nerve injuries differ from subject to subject. In some cases of lower back pain, clinical investigation reveals no joint abnormalities or injury to muscle or nerves. The wide variety in the results of clinical investigation in lower back pain greatly adds to the challenge of understanding the contribution of each component and the interaction between them (Apkarian et al., 2009).

The effect of chronic pain on the lives of sufferers can be profound and these conditions are highly prevalent. As an example, lower back pain is a very common condition that can arise due to a wide range of mechanical or non-mechanical factors. Mechanical causes are present in the overwhelming majority of lower back pain cases (Deyo & Weinstein, 2001).

Disruption of the components of the spinal system gives the possibility that lower back pain may be accompanied by numbness, loss of reflex and weakness (Chien & Bajwa, 2008). It has been estimated that lower back pain is one of the most common health problems in medical practice and is experienced by 70-85% of mature people (Frymoyer, 1988). In the UK chronic low back pain is the most common factor for worker absence and accounts for 12.5% of all days lost (Maniadakis & Gray, 2000).

Other examples of chronic pain conditions include temporomandibular joint pain syndrome (Moss & Garrett, 1984), chronic neuropathic pain (Woolf & Mannion, 1999), irritable bowel syndrome (Whitehead, Engel, & Schuster, 1980) and complex regional pain syndrome (Birklein, 2005).

As illustrated by these examples, chronic pain can result from a wide range of conditions. It is evident that there are different causes and expressions of chronic pain. One of the primary distinctions drawn between classes of chronic pain is derived from the extent, across the body, of the experienced pain. This distinction classifies chronic pain as localised, if limited to a single region of the body or widespread if the pain is more extensive. It is the purpose of this thesis to consider the similarities and differences in the effects on the human brain of chronic widespread and localised pain. For this purpose I have selected fibromyalgia (FM) as an example of chronic widespread pain and osteoarthritis of the knee (OA) as an example of

chronic localised pain. These conditions have been selected due to their high prevalence, the high level of disruption entailed for patients, and level of demand that these conditions create on the resources of global health care systems. I will now look in detail at these conditions.

## **Fibromyalgia**

FM is a chronic, painful, noninflammatory syndrome that has an adverse effect on the musculoskeletal system (Wolfe et al., 1990). This condition affects 2.7% of the world's population (Queiroz, 2013) with women being 11 times more likely to suffer FM than men (Clauw & Crofford, 2003). FM results in a greatly impaired quality of life for the patient and also entails economic consequences for society (Annemans et al., 2008). Despite its prevalence and adverse impact, the aetiology and pathophysiology of the disease is unclear.

The diagnosis of FM includes two specific criteria recommended by the American College of Rheumatology: 1) a record of widespread pain for at least three months; 2) pain in 11 of 18 specific anatomical sites or tender points on digital palpation with an estimated strength of 40 N or 4 kg (Wolfe et al., 1990). Other symptoms associated with FM include nonrestorative sleep, fatigue, anxiety, morning stiffness, cognitive disturbances, headache, paraesthesia, depression and symptoms associated with autonomic dysfunction (Crofford & Clauw, 2002; Solano et al., 2009; Yunus, Masi, & Aldag, 1989). As an example, nonrestorative sleep is common in FM: approximately about 75% of patients experience issues such as early middle or late insomnia, hypersomnia and frequent awakening. FM patients commonly experience more insomnia-related symptoms than either rheumatoid arthritis patients or healthy controls (Belt, Kronholm, & Kauppi, 2009).

It was noted (Wolfe, Ross, Anderson, Russell, & Hebert, 1995) that FM is often comorbid with affective disorders and functional somatic syndromes (FSSs) such as irritable bowel syndrome, irritable bladder and post-traumatic stress disorder. In addition to comorbidity, commonalities between FM and the other FSSs have been noted, though the overlap between the patients' experience of these conditions is far from complete (Kanaan, Lepine, & Wessely, 2007). A report made by Yunus (2007) proposed the unifying concept of 'central sensitivity syndrome' to account for the commonalities seen between FM and other FSS conditions. This proposal suggested an underlying mechanism of neuroendocrine deviations, which interact with psychological and other factors to generate the symptoms experienced, however, the

concept of a central sensitivity syndrome has not been universally accepted and this term has become uncommon in more recent literature

The similarities observed in FSSs may imply a common genetic mechanism. It has been identified in numerous FSSs that central sensitisation entails increased excitability of the central nervous system through diverse synaptic and neurotransmitter/neurochemical activations (Woolf, 2012). Recent research has proposed a role for polymorphisms of genes in the catecholaminergic systems in creating a predisposition to FM (Ablin, Cohen, & Buskila, 2006; Buskila, Sarzi-Puttini, & Ablin, 2007) and in shaping the patient's experience of the condition.

Significant progress has been made in understanding the mechanisms of underlying altered pain processing in FM (Ablin, Neumann, & Buskila, 2008). Buskila and Press (2001) suggested that this relates to atypical sensory processing in the CNS coupled with dysfunction of skeletal muscle nociception and the hypothalamic–pituitary–adrenal (HPA) axis. Adler and Geenen (2005) reported that FM patients have widespread anomaly in pain perception, particularly a reduction in pain threshold and tolerance to pressure, cold, and heat. Changes in the HPA axis have been verified in FM patients (Crofford et al., 1994). Additionally, alterations in the operation of the autonomic nervous system have often been described in FM. Various groups of researchers have suggested systematic hyperactivity in FM (Martinez-Lavin, 2007). Unusual sympathovagal balance in male FM patients with sympathetic hyperactivity and concurrently decreased parasympathetic activity has been reported recently (Cohen et al., 2001). Elevated sympathetic and lowered parasympathetic tones in women with FM were described as a basal autonomic state; and autonomic dysregulation may have insinuations related with the symptomatology, physical and psychological sides of health (Cohen et al., 2000). The observations of altered states associated with FM has led to a search for biomarkers for the diagnosis for the condition that would aid in understanding the disease's activity. It has been noted, however, as per Dadabhoy, Crofford, Spaeth, Russell and Clauw (2008) that many candidate biomarkers for FM have been rejected due to either a lack of specificity to FM or a reliance on subjective evaluation.

From the 1990s it has become evident that FM is not a homogeneous condition (Wolfe et al., 1996). Whilst there also are often chronic widespread pain and increased tenderness, other

symptoms are not present in all patients. It was demonstrated by Turk and colleagues that subgrouping by cluster analyses of the Multidimensional Pain Inventory in different chronic pain groups may be relevant to FM patients. They investigated three FM subgroups, which they described as dysfunctional, interpersonally distressed or adaptive copers. All subgroups undertook a standardised treatment program with the authors making the conclusion that applying treatment according to psychological needs increases treatment effectiveness (Turk, Okifuji, Sinclair, & Starz, 1998; Turk, Okifuji, Sinclair, & Starz, 1996). Subsequently, Thieme and colleagues (Thieme, Spies, Sinha, Turk, & Flor, 2005) subdivided FM patients into the three aforementioned categories according to their results on the Multidimensional Pain Inventory and applied hierarchical regression analyses to recognize predictors of pain behaviours for all subgroups. Their conclusions supported the heterogeneity of FM both in terms of the factors underlying a diagnosis of the condition and in the behaviours that result. Their research implies a challenge for gaining an understanding of the mechanisms that result in FM, but also supports the suggestion that FM treatment is most effective when it takes into account the patient's experience of their condition.

To conclude, while FM affects many people, its aetiology is currently unknown. The possibility remains that the term 'fibromyalgia' is currently used to refer to a number of different conditions, which contributes to the poor level of current understanding of FM (Apkarian et al., 2009).

## **Osteoarthritis**

Arthritis is the name given to a wide range of conditions that cause damage in one or more joints. Arthritis can result from a wide range of causes including autoimmune disorders, infection or physical damage to the affected site. Osteoarthritis (OA) is the most common form of arthritis. OA arises from either damage or accumulated wear to a joint, which results in the breakdown of cartilage and damage to bone. OA of the knee has been identified an important cause of pain and disability in elderly people (Jordan, 2003) and the fourth most common health problem in females (Murray & Lopez, 1997). The economic impact of OA is significant in terms of resultant disability and the cost of remedial surgery (Brooks, 2002). No cure is currently available for OA and therefore treatment objectives concentrate on maximising function and quality of life and minimising the pain experienced (Fries, Weinberger, Lorig, Jacobs, & Goldberg, 2000). Modern treatment regimens include

physiotherapy, exercise, diet management, alteration of mechanics with orthotic devices, analgesics, anti-inflammatories and management based on the pathophysiological mechanisms of pain (Woolf et al., 2004). However, despite the adoption of a wider basis for the treatment of OA, this is not sufficient to prevent a total replacement of the affected joint being required in some cases (Jordan, 2003).

Historically, OA patients were considered to be suffering from a peripheral disease with pain resulting solely from damage to joints. This is certainly the case for a subgroup of OA patients who experience a complete disappearance of their symptoms after replacement of the affected joints (March et al., 1999). However, from the 20<sup>th</sup> century an appreciation was gained that OA presented greater complexities. A disconnection between the radiographic severity of OA and pain experienced was noted in some cases (McAlindon, Cooper, Kirwan, & Dieppe, 1993). It was also noted that the high occurrence of referred pain, hyperalgesia and alterations in skin sensitivity away from the damaged joint could not be explained solely through a peripheral model of pain (Farrell, Gibson, McMeeken, & Helme, 2000; Hendiani et al., 2003; Imamura et al., 2008; Khan, McLoughlin, Giannakas, Hutchinson, & Andrew, 2004; Khan & Woolson, 1998; Ordeberg, 2004). An understanding has developed of the contributions of peripheral and central sensitisation to the pain experienced in OA (Jordan, 2003). It is also now known that regular and intensive nociceptive sensory information from painful structures generates considerable neurochemical and metabolic alterations (Siddall & Cousins, 2004). Neurologic reorganisation in OA was found within spinal cord segments (in animal models), which entailed an increased excitability of dorsal horn neurons that generates pain hypersensitivity in a segmental distribution (Bird et al., 2006; Schadrack et al., 1999). This demonstrated central sensitisation as a factor within OA and may offer some explanation for the range of sensations experienced by some OA patients.

There have been a number of studies examining the involvement of central pain modulation in OA (Farrell et al., 2000; Jordan, 2003; Kosek & Ordeberg, 2000). Bajaj, Bajaj, Graven-Nielsen, & Arendt-Nielsen (2001) illustrated deep hyperalgesia in the tibialis anterior muscle of subjects with knee OA. Creamer and colleagues displayed pain relief in the contralateral non-injected knee resulted from the injection of anaesthetic into another knee (Creamer, Hunt, & Dieppe, 1996). These studies both proposed a role for the central nervous system in the expression of chronic pain in patients with knee OA. More recently Imamura et al. (2008) demonstrated a generalised state of hyperalgesia in patients with knee OA in superficial and

deep structures. They similarly proposed that maintenance of the chronic pain depends on the involvement of the peripheral and central nervous system. This sensitisation phenomenon is not specific to knee OA, as Farrell and colleagues showed similar cutaneous and deep hyperalgesia in the forearm of patients with thumb-base OA (Farrell et al., 2000). Taken together, these studies suggest that as pain develops from acute to chronic a concomitant increase in central sensitisation is also present in some cases of OA.

Thus, despite the differences in patients' experience of chronic widespread pain (FM) and chronic localised pain (OA) there may be some underlying common factors since the effects of central sensitisation has been observed in both conditions. I will now consider neuroimaging of pain and the modern view of neuroimaging progress in the understanding of fibromyalgia and osteoarthritis.

## **Neuroimaging of Pain**

From the last quarter of the 20<sup>th</sup> century research into human pain has been greatly assisted by the availability of neuroimaging techniques that allow the living brains of pain patients to be noninvasively studied. A substantial contribution to our knowledge of pain has been made through the use of neuroimaging.

Neuroimaging is a set of techniques that enables the type, structure and extent of living brain tissue to be analysed based on its response to varying magnetic fields. Over several decades research into neuroimaging methods have enabled the generation of images of the brain's structure, the level of neurochemicals present within brain regions, and the level of coordinated activity between brain regions. The availability of neuroimaging techniques has been valuable in enabling the investigation of the areas of the brain that are involved in the processing of pain signals, in the representation of pain, and that play a role in the descending pain modulation pathways.

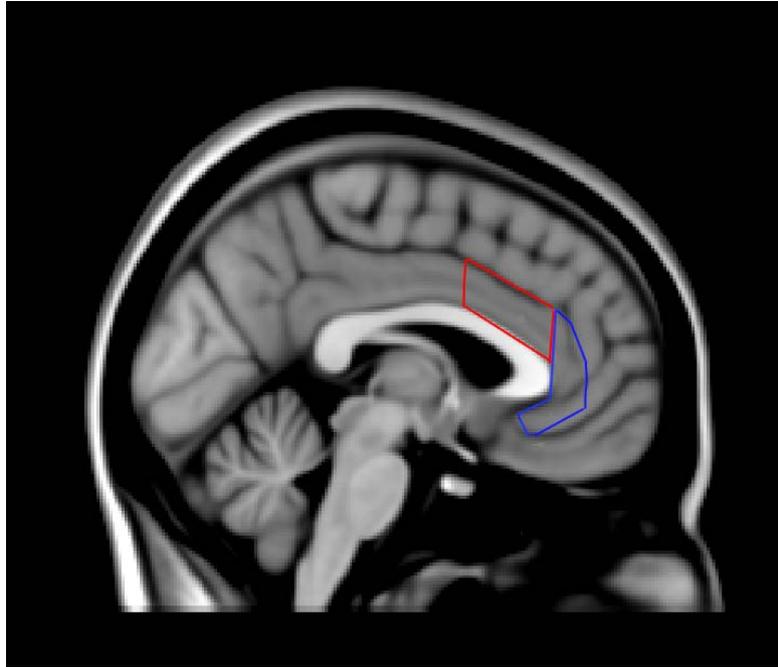
In the past two decades, numerous functional neuroimaging studies have identified a set of brain regions in which increased activity is associated with the presence of clinical or experimental pain. This group of regions has been referred to as the pain neuromatrix (Melzack, 1990). The inclusion of regions within the neuromatrix has been the subject of debate, but the current consensus identifies the following regions as playing a role in this

system: primary (S1) and secondary (S2) somatosensory cortex, anterior cingulate cortex (ACC), anterior and posterior insula, prefrontal cortex (PFC), thalamus and periaqueductal grey (PAG) (Apkarian et al., 2009; Derbyshire, 2000). The exact function performed by each of these areas in the pain neuromatrix is not yet fully understood, but a division into lateral (comprising S1, S2, thalamus and posterior insula) and medial (comprising ACC, PFC, anterior insula and PAG) systems has been proposed (Tracey, 2008). The lateral pain system is believed to be instrumental in the representation of the physical aspects of pain i.e. the location and characteristics of the noxious stimulus and the effects on the body. The medial system is proposed to be involved in the evaluative and affective responses to pain and the direction of attention to painful stimuli and, in the case of the PAG, modulating the effects of pain via the descending pathways (Xie, Huo, & Tang, 2009).

Research using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) has suggested that the anterior cingulate cortex (ACC) is particularly important in the processing of pain. It has been shown that the ACC is the brain area most regularly activated after the application of a painful stimulus (LaGraize & Fuchs, 2007).

As illustrated in Figure 1., the ACC comprises regions that differ in cytoarchitecture, connectivity and function (as reviewed in Bush, Luu, & Posner, 2000 and Vogt, 2005). A key distinction has been drawn between dorsal-caudal (dACC) and ventral-rostral (vACC) areas of the ACC. Functional and structural connectivity has been noted between dACC and lateral prefrontal cortex, parietal cortex, premotor cortex, motor and supplementary motor areas. In contrast, connections have been mapped between vACC and amygdala, PAG, nucleus accumbens, AntIns, hippocampus and hypothalamus. Imaging studies have shown that dACC plays a role in the modulation of cognition and attention, response selection and error detection. The vACC shows greater involvement in assessing the salience of emotional stimuli and the regulation of emotional responses (Mohanty et al., 2007; Whalen, Bush, McNally, & Wilhelm, 1998). The relevance of the vACC in emotional processing of pain has been demonstrated by a number of studies that demonstrated increased activity in this area particularly with increased attention to noxious stimuli or increased perceived unpleasantness (Kulkarni et al., 2005; Rainville, Duncan, Price, Carrier, & Bushnell, 1997; Vogt, Derbyshire, & Jones, 1996). Lesioning this area has been shown, in humans and animals, to eliminate the emotional element of pain sensation (that is, the subjective distress associated with the

presence of pain), yet leaving the sensory-discriminative component intact (Ballantine, Cassidy, Flanagan, & Marino, 1967; Fuchs, Balinsky, & Melzack, 1996; Johansen, Fields, & Manning, 2001; LaGraize, Borzan, Peng, & Fuchs, 2006).



*Figure 1. Sagittal view of the left medial surface of human brain. The dorsal-caudal anterior cingulate cortex is indicated by the red boundary lines. The ventral-rostral anterior cingulate cortex is indicated by the blue boundary lines.*

Research in the field of fMRI in humans illustrated that many analgesic procedures, including opioid analgesia, placebo and hypnotic analgesia, thalamic and motor cortex stimulation-induced analgesia, and cognitive modulation of pain are related with enhanced activation in the vACC (Casey, 1999; Davis, Taylor, Crawley, Wood, & Mikulis, 1997; deCharms et al., 2005; Faymonville et al., 2003; Petrovic & Ingvar, 2002). These findings support the idea that vACC holds an important position in the modulation of pain.

### **Investigating the neural effects of chronic pain**

Whilst we now have a better understanding of which brain regions are involved in the processing of pain, we have limited knowledge of the effects of prolonged pain on the structure and function of these regions. It is well understood that the brain is able to learn and adapt to changing circumstances through the reorganisation of synaptic connections between neurons (Pascual-Leone, Amedi, Fregni, & Merabet, 2005). This adaption can be achieved

through the establishment of new synapses, the removal of existing synapses or adjustments to the strength of the link between neurons. Neuroimaging studies have provided evidence of these mechanisms in action in response to either training or repeated presentation of stimuli (Recanzone, Merzenich, Jenkins, Grajski, & Dinse, 1992) or as a consequence of a change in sensory input. An example of the latter case is the reorganisation of the somatosensory map in response to limb amputation observed by Flor et al. (1995). The possible consequences of the prolonged application of a stimulus are the development of tolerance (Hammer, Egilmez, & Emmett-Oglesby, 1997), sensitivity (Karni & Sagi, 1991) and neuronal damage through excitotoxicity (Mayer, Mao, Holt, & Price, 1999). Given that chronic pain conditions can entail the prolonged presentation of sensory input, adaptation via one or more of these mechanisms is possible. The effects of chronic pain on the human nervous systems have been studied using a range of neuroimaging techniques. I will examine previous research using three of these techniques in detail in the coming chapters, however, I will provide examples of the findings of neuroimaging studies of pain here (several comprehensive reviews of neuroimaging studies of pain are available e.g. Apkarian, Bushnell, Treede, & Zubieta, 2005; Tracey, 2008). fMRI studies have shown that cortical reorganisation can be observed in chronic back pain and phantom pain patients (Flor, Braun, Elbert, & Birbaumer, 1997; MacIver, Lloyd, Kelly, Roberts, & Nurmikko, 2008; Tsao, Galea, & Hodges, 2008). Brain atrophy (i.e. a decrease in grey matter volume) has been observed in the of patients with a range of chronic pain conditions (Apkarian et al., 2004; Schmidt-Wilcke et al., 2005). Additionally, neurochemical changes in the brains of chronic pain patients have been observed (Grachev, Fredrickson, & Apkarian, 2000).

In this thesis I will describe studies that were conducted using three neuroimaging techniques: magnetic resonance spectroscopy (MRS), voxel-based morphometry (VBM) and resting state functional connectivity (FC). These techniques and their contributions to our knowledge of the mechanisms of chronic pain will be presented in detail in the relevant chapters. Each of these techniques examines a different aspect of brain composition that may be useful in generating converging evidence about chronic pain. MRS provides information about the levels of neurochemicals present in different regions of the brain. Of relevance to the examination of the effects of chronic pain on the brain, n-acetylaspartic acid (NAA) has been identified as a neurometabolite that is indicative of neuronal integrity, density and metabolism (Rae, 2014). Additionally, levels of the excitatory neurotransmitter Glutamate

have been found to increase in response to painful stimuli (Gussew et al., 2009; Mullins, Rowland, Jung, & Sibbitt, 2005) and may therefore provide information about differing mechanisms of action between pain conditions. VBM provides a method of mapping the brains of multiple participants to a standard template to enable an examination to be performed to the relative sizes of regions in the brain. FC examines the correlation in the activity of groups of neurons across regions of the brain. A strong correlation between the activity of neurons in regions distributed across the brain is taken to indicate their participation in functional networks i.e. collections of brain regions that contribute to a common task or purpose. These techniques were selected to give an opportunity to examine multiple aspects of the effects of chronic widespread and localised pain on the human nervous system and thus generate further understanding of these conditions.

I will now look at previous neuroimaging research that has been conducted into two examples of chronic pain conditions (FM and OA) and consider what this research can tell us about the mechanisms at work.

### **Neuroimaging studies of fibromyalgia**

The effects of FM on the human brain have been studied using neuroimaging techniques for a number of years (Gracely & Ambrose, 2011). Early investigations used SPECT and PET to examine alterations in activity levels in the areas of the brain relevant to pain processing.

Studies using SPECT to assess regional cerebral blood flow (rCBF) revealed alterations in the function of both cortical and subcortical pain processing areas in FM. Using a chemical tracer to highlight rCBF, reduced activity levels were observed in the thalamus and caudate nucleus in FM patients (Bradley et al., 1999; Mountz et al., 1995).

PET has been used to investigate neurochemical alterations to the brains of FM patients through the application of chemical tracers that selectively bind to receptors of interest. Through observing the interaction of the action of the chemical tracer with areas of interest in participants' brains an assessment can be made of the patients' binding potential for relevant substances. Wood et al. (Wood et al., 2007) used [<sup>11</sup>C]-raclopride to examine binding at D2/D3 dopamine receptors during the application of a painful stimulus. In normal

participants the release of endogenous dopamine (in response to the painful stimulus) reduced binding of [11C]-raclopride at the D2/D3 receptors. FM patients demonstrated disruption to the dopamine system in the basal ganglia this response was not observed. Harris et al. (Harris et al., 2007) conducted a similar study using the application of [11C]-carfentanil during PET to examine binding at the  $\mu$ -opioid receptor.  $\mu$ -opioid receptor binding potentials were shown to be reduced in FM patients compared to controls in several brain areas considered to be significant in pain processing and regulation, namely, the nucleus accumbens, dorsal cingulate, and amygdala).  $\mu$ -opioid receptors have been identified as the primary sites of action of several endogenous opioid peptides (Zadina, Hackler, Ge, & Kastin, 1997) and are the target of several analgesic opioid drugs (Trescot, Andrea M., Datta, Sukdeb, Lee, Marion, & Hansen, Hans, 2008). The action of endogenous opioids on these receptors has been associated with a reduction in the reported sensory and affective aspects of experienced pain (Zubieta et al., 2001). Given the role of  $\mu$ -opioid receptors in regulating the noxious experience of pain it is reasonable to suggest that a decrease in the presence of these receptors in FM may be accompanied with a decrease in the efficacy of endogenous opioids and may account for the reduced effectiveness of exogenous opiates in the treatment of FM (Harris et al., 2007).

Later studies observed the effects of FM on the intrinsic connectivity of the brain at rest. Using resting-state functional connectivity techniques (FC), researchers have shown an increased correlation of activity between areas of the insular cortex and areas of the cingulate cortex in FM patients (Ichesco et al., 2014).

In addition to alterations in brain activity, alterations to the structure of the brain have been noted in FM patients in studies using VBM. It was observed that reductions in grey matter volume were present in pain-relevant cortical and subcortical structures including the amygdala, cingulate cortex, hippocampus and the parahippocampal gyrus (Luerding, Weigand, Bogdahn, & Schmidt-Wilcke, 2008). It was further observed that FM patients suffered increased levels of age-related grey matter reduction in comparison to healthy controls (Kuchinad et al., 2007).

Diffusion tensor imaging has been used to observe the effects of FM on white matter connectivity throughout the brain and has demonstrated a reduction in the level of

connectivity (as reflected by fractional anisotropy) between the thalamus and other areas (Sundgren et al., 2007).

As illustrated in Table 1, several studies have used MRS to investigate changes to neurochemistry associated with FM. The majority of these studies have examined either the effects of FM on NAA or Glu/Glx, with some also including m-Ins, Cho and Cr. The studies have unsurprisingly focussed on areas of the brain known to be involved in the processing of either the sensory or emotional aspects of pain such as the insula, amygdala, basal ganglia, postcentral gyri and cingulate cortex. Also included in the studies are the hippocampus, thalamus and prefrontal cortex. Emerging from these studies is a view that an increase of Glu or Glx in pain relevant areas is related to the presence of FM and is proportional to clinical measures of the pain relating to the condition (for instance, as recorded in the Visual Analog Scale) (Feraco et al., 2011). As an example, Harris et al. (2008) observed that alterations in the ratio of Glutamate to Creatine in the posterior insula correlated with FM patients experience of clinical pain (after both sham and *verum* applications of a non-pharmacologic intervention). They further observed a negative correlation between the alteration of the Glu/Cr ratio in this patient group and their threshold to experimentally applied pain. As noted previously, an increase in the level of the excitatory transmitter Glu has been observed after the application of acute painful stimuli: these findings suggest a similar response to chronic clinical pain.

Several studies observed a decrease in the measured level of NAA in the hippocampi of FM patients, and a negative correlation between decreased hippocampal NAA and clinical pain. These findings can be viewed in the context of converging evidence of hippocampal vulnerability to the effects of a range of chronic conditions including pain and depression and evidenced in abnormalities in metabolite levels or connectivity between the hippocampus and cortical areas (Fasick, Spengler, Samankan, Nader, & Ignatowski, 2015; Mutso et al., 2014). Given the association between NAA and neuronal viability it is not surprising to note that a reduction in NAA is associated with adverse consequences of FM since it can be taken to indicate dysfunction within vulnerable structures.

The effects of FM on levels of other brain metabolites are less clear. A small number of studies have examined the effects of FM on m-Ins, Cho and Cr, but is difficult to build a

consensus view. FM has been associated with both an increase and decrease of hippocampal Cho (Emad et al., 2008; Fayed et al., 2010). An increase in m-Ins in amygdala and thalamus has been reported in FM (Valdés et al., 2010), which contrasts with a decrease observed in m-Ins in FM in hippocampus and sensorimotor cortex (Fayed et al., 2010). Further studies are required to determine if m-Ins can provide significant insights into the effects of FM on neurochemistry.

The differences observed in levels of NAA and Glu/Glx suggest that MRS may be able to provide a biomarker for FM to assist in the diagnosis and treatment of the condition.

Study	Metabolites	Brain Areas	Experimental Techniques	Groups	Participants	Measures	Results
Petrou et al (2008)	NAA, Cho, Cr, NAA/Cr, Cho/Cr, NAA/Cho	DLPFC, Basal ganglia, Supraventricular white matter, Insula	H-MRS, Pre- and post-contrast enhanced MR imaging, Pressure pain threshold	FM HC	21 27	Visual analog scale (VAS), Ctr. for Epidemiological Studies Depression Scale (CES-D), Spielberger Trait Personality Inventory Anxiety Qs, Catastrophizing Component of the Coping Strategies Qs	Cho/Cr variability in the right DLPFC was significantly different in the 2 groups A significant correlation between Cho/Cr in the DLPFC and clinical pain in FM Evoked pain threshold significantly correlated with NAA/Cho in the left insula and left basal ganglia in FM
Emad et al (2008)	NAA, Cho, Cr, NAA/Cho, NAA/Cr, Cho/Cr	Hippocampi	H-MRS	FM HC	15 10	FM Impact Qs (FIQ), Visual analogue scale (VAS), The Mini Mental State Examination (MMSE), The Hamilton Depression Scale, The structured sleep interview	NAA decrease of in hippocampi in FM Cho increase in R hippocampus in FM NAA/Cho and NAA/Cr decrease in hippocampi in FM Correlations between language score and R Cho and R Cr levels.
Harris et al (2008)	Glu	AntIns, PosIns	H-MRS, FMRI, Painful pressure	FM	10	Experimental pressure-evoked pain thresholds, Short form McGill Pain Questionnaire (SF-MPQ)	Changes in Glu/Cr in PosIns negatively correlated to experimental pain threshold Elevated Glu/Cr in FM pre-treatment correlates with clinical pain
Harris et al (2009)	Glu, Glx	Right AntIns, Right PosIns	H-MRS, Pressure pain test	FM HC	19 14	Gracely Box Scale (GBS) Qs	FM patients: elevated Glu, Glx in PosIns Glu and Glx levels negatively correlated with pressure pain thresholds
Wood et al (2009)	NAA, NAA-Cr	Hippocampi	H-MRS	FM HC	16 8	FM Impact Qs (FIQ)	NAA/Cr decrease in R hippocampus in FM Negative correlation between scores on the FIQ and NAA/Cr in R hippocampus
Valdes et al (2010)	Glx, NAA, Cr, Cho, M-Ins	Amygdala, Thalami, PFC	H-MRS	FM HC	28 24	Visual analog scale (VAS), Health Assessment Qs (HAQ), FM Impact Qs (FIQ), Hospital Anxiety and Depression Scale (HADS)	Glx increase and higher Glx/Cr ratio in the R amygdala in FM M-Ins increase in R amygdala and R thalamus in FM patients associated with higher levels of pain, fatigue and depression

Fayed et al (2010)	Glx, NAA, Cr, Cho, M-Ins	Somatosensory cortices, Thalamus, Hippocampi, Posterior cingulate gyrus	H-MRS, MRI, Diffusion tensor imaging (DTI), Diffusion-weighted images (DWI)	FM HC	10 10	Pain Catastrophizing Scale (PCS) Hospital Anxiety and Depression Scale (HADS) Pain Visual Analogue Scale (PVAS) Pain threshold Mini-Mental State Exam (MMSE) FM Impact Qs (FIQ)	Glx increase and higher Glx/Cr ratio in the posterior cingulate gurus in FM M-Ins decrease in in hippocampi in FM M-Ins/Cr decrease in the L sensory-motor area and the L hippocampus in FM Cho and NAA/NAAG decrease in L hippocampus in FM
Feraco et al (2011)	Glu, Glx, Cr, M-Ins	VLPFC, Thalamus	H-MRS	FM HC	12 12	Pain Visual Analogue Scale (PVAS) FM Impact Questionnaire (FIQ)	Glx/Cr and Glu/Cr increase in VLPFC in FM Correlation Glu/Cr in L thalamus and PVAS

*Table 1. Magnetic Resonance Spectroscopy studies of Fibromyalgia.*

A significant amount of research has used neuroimaging techniques to investigate central sensitisation in FM (as reviewed in Cagnie et al., 2014). A key theme to emerge from this research is increased activity in the brains of FM patients, as observed using fMRI, in response to experimentally applied pain. As demonstrated in Gracely, Petzke, Wolf and Clauw (2002) and by Cook et al. (2004), it has been noted that whilst the application of experimental pain to FM patients evoke activity in the same brain regions as in healthy controls, the levels of activation were higher in FM patients.

Functional neuroimaging techniques have also been used to investigate the cognitive and affective components of FM. It was demonstrated that pain catastrophising was correlated with enhanced activity, as assessed by fMRI, in some brain areas related to anticipation of pain (medial frontal cortex, cerebellum), attention to pain (dorsal anterior cingulate cortex, dorsolateral prefrontal cortex) and emotional aspects of pain (Gracely et al., 2004).

Additionally, FM patients showed increased activity in the PFC, posterior parietal cortex and PAG (Burgmer et al., 2011) during pain anticipation and catastrophisation. This observation reflects the cognitive and evaluative aspects of FM and also the effects of continuing pain on the descending pain modulation pathways. FMRI has been used to demonstrate alterations in brain perfusion in FM, as well as abnormalities in pain-stimulated perfusion (Foerster et al., 2011; Staud, 2002).

These findings suggest that FM is associated with a diverse range of alterations to both structure, function and chemistry within the brain, though further research is required to untangle causal relationships between the condition and these observations.

### **Neuroimaging studies of osteoarthritis**

As with FM, the effects of OA on the brain have been investigated using several neuroimaging techniques (as reviewed in Harvey, Taylor and Wise, 2012), though fewer studies have been made of OA using these methods. The lower number of neuroimaging studies of OA may reflect the consensus that peripheral factors are of greater importance in this condition.

Studies of the effects of OA on brain structure have used VBM to reveal grey matter reduction in the thalamus, anterior insula, hippocampus and somatosensory cortex (Baliki, Schnitzer, Bauer, & Apkarian, 2011), though Gwilym et al. (Gwilym, Filippini, Douaud, Carr, & Tracey, 2010) and Rodriguez-Raecke et al. (Rodriguez-Raecke, Niemeier, Ihle, Ruether, & May, 2009), also using VBM, noted that this reduction was eliminated in the months following replacement of the affected joint.

Neurochemical alterations of the thalamus have also been observed in OA. Shigemura et al. (2012) used MRS to observe that a decreased ratio of NAA to Creatine was present in the thalamus contralateral to joint damage in OA. Given that NAA is taken to be a marker of overall neuronal viability (Grachev, Thomas, & Ramachandran, 2002) it is perhaps not surprising that reversible thalamic atrophy may be accompanied by decreased relative levels of NAA.

Most studies that have used fMRI to investigate OA have examined the effects of applied experimental pain on OA patients. From these studies the application of acute pain to OA patients activates similar brain areas to those activated in healthy controls. Similarly to FM patients, levels of activation in these areas are somewhat increased in OA patients compared to HC. (Baliki, Geha, Jabakhanji, et al., 2008) and, in the case of the ACC, show differing extents of activation (Buffington, Hanlon, & McKeown, 2005).

The pain produced spontaneously in OA has been shown to activate areas associated with cognition, evaluation and affect. This has been achieved both by observing the resting brain via fMRI (Howard et al., 2012) and by using fMRI and PET to contrast activations produced spontaneously by OA with those produced by experimental procedures (Kulkarni et al., 2007; Parks et al., 2011).

Finally, abnormal activity in the brainstem especially the PAG has been noted in OA (Gwilym et al., 2009). It has been suggested that this represents an impairment of the descending pain modulation pathways in OA, which adds to the evidence for central mechanisms playing a role in the ongoing experience of the condition.

## **Differences and Similarities of OA and FM**

As previously stated, the purpose of this thesis is to present three neuroimaging studies that compare and contrast the effects of OA and FM on the human brain. I shall take the research described above as a starting point from which to develop the hypotheses to be investigated using MRS, VBM and FC.

The most obvious difference between FM and OA is the extent of painful areas across the bodies of patients. Whilst noting cases of referred pain and hyperalgesia, the areas of pain associated with OA are generally localised to the damaged joints. In contrast, FM pain is widely spread across patients' bodies, thus justifying the selection of these conditions as examples of CLP and CWP.

OA is associated, in most cases, with objectively observable joint damage and has a well-understood aetiology, whereas FM presents with no obvious signs of injury and its causes and mechanisms of progression are not yet known. OA is more common in women than men, but the ratio is much smaller than that for FM, which is much more common in women. The symptoms of OA are commonly restricted to physical impairments, whereas FM has been associated with impairments of mood, cognition and attention.

Taken together, these factors suggest sharply contrasting conditions whose similarities may be limited to the prolonged experience of pain and the effects that this continued experience may have on the brain. The factors described above suggest a contrast between a widespread disorder of the central nervous system and a condition that is maintained by peripheral nociception resulting from physical damage. The observation of elements of central sensitisation and resultant hyperalgesia in OA, however, support the suggestion that OA cannot be explained purely with reference to peripheral mechanisms. Examining the results of neuroimaging investigations also suggests a more complex relationship between the conditions.

Both OA and FM have been associated with grey matter volume reductions. The reductions in OA are in areas more associated with the lateral or somatosensory pain system (thalamus, insula, hippocampus, somatosensory cortex); the reductions in FM are associated with structures more associated with the affective and evaluative aspects of pain (amygdala,

cingulate cortex), though FM also showed GMV reduction in the hippocampus. Whilst it has been observed that thalamic GMV reduction in OA is reversible with treatment, no such observation has been made about FM. It is unclear if this is due to the peculiarities of the thalamus or whether different GMV reducing mechanisms are present in the conditions. Brainstem shape and volume alterations (across the pons and PAG) have been noted in FM to a greater degree than OA.

Both conditions show increased activity in pain processing brain areas (in patients relative to healthy controls) and both conditions show increased activity in areas associated with emotional processing of pain, though this has been much more pronounced in FM.

FM shows alterations in levels of glutamate in some pain processing regions, whilst OA shows reduced levels of NAA in the thalamus. Neurochemical disruption was observed to be more widespread in FM than OA with alterations to the neurochemistry of the nucleus accumbens, dorsal cingulate and amygdala being observed. Both conditions showed alteration to the functioning of the PAG, but a raised level of activity in FM contrasted with disrupted functioning in OA.

Considering the findings of this research suggests that FM would be expected to present more widespread differences than OA when compared with healthy controls in our studies. As such, one would expect to see FM presenting more marked differences in brain areas associated with cognition, emotion and attention than OA. The differences in the effects on the PAG of the conditions suggest that differences may be detected between the conditions in the areas influencing the behaviour of the descending pain modulation pathways. If the increased activity of the PAG in FM acts to limit ascending pain signals then it is possible that the maladaptive effects of continued pain stimulation would be lessened in the somatosensory pain system in FM when compared to OA.

## **Implications and Hypotheses**

As noted, three neuroimaging techniques will be used to generate converging evidence for the similarities and differences between the effects of OA and FM on the human brain. The techniques are MRS, VBM and FC, which examine neurochemistry, neuroanatomy and

neuronal activity respectively. I will describe the techniques and generate specific hypotheses in detail in the following chapters, but will now suggest broad hypotheses based on current knowledge of OA and FM and the opportunities afforded by MRS, VBM and FC. The research described above has suggested some differences (and commonalities) between the conditions of interest that may be uncovered through use of my selected techniques. My primary hypothesis is that CLP and CWP have different effects on the human brain and that these differences will be observable by MRS, VBM and FC.

Previous research has proposed two component systems within the overall pain representation and processing system. Given the more pronounced cognitive and emotional components of the experience of FM and I would hypothesise that greater differences would be observed between FM and HC in the medial cognitive-emotional pain system. Given the disruption of movement and proprioception associated with OA I hypothesise that greater differences would be observed between OA and HC in the lateral somatosensory pain system.

One of the themes to emerge from prior research into FM and OA is that whilst OA is influenced by central mechanisms their influence is more restricted than in FM. An example of this being the detection of neurochemical changes in a wider range of areas in FM when compared with OA: neurochemical changes were detected only in the thalamus in OA, but in a range of areas in FM. I hypothesise that my MRS investigation will reveal greater neurochemical differences in terms of brain regions and neurochemical levels in FM compared to OA and compared to HC.

Previous research has revealed greater cognitive impairment in FM than in OA. Using FC we are able to observe the level of cohesion of network activity in the resting brain of each participant. Since FM patients experience greater cognitive and attentional issues I hypothesise that greater dissimilarity will be observed between FM and HC than between OA and HC in the brain networks examined.

Prior research has noted GMV reductions in patients with chronic pain conditions. I hypothesise that the differences in the effects of OA on the lateral somatosensory pain system and FM on the medial cognitive-emotional pain system will result in observable differences in GMV reductions in the brain regions comprising these systems in the relevant patient groups.

Through the use of the selected techniques I will generate evidence that will provide greater understanding of the effects of CLP and CWP. By bringing together results from three neuroimaging techniques it is intended that this research will facilitate the development of better-targeted treatments for these conditions.

**CHAPTER 2 - Is chronic widespread pain (fibromyalgia) expressed differently from chronic local pain (osteoarthritis of the knee)? A Magnetic Resonance Spectroscopy study**

## Introduction

Pain has been defined as “an unpleasant sensory or emotional experience associated with actual or potential tissue damage” (Merskey & Bogduk, 1994). The experience of pain is subjective and is familiar to most people, but it can be debilitating when it becomes a chronic condition. For clinical purposes chronic pain is characterised as pain which persists longer than 12 weeks, usually existing after the healing phase of the injury that first caused the pain, but it may also develop without any obvious injury process (Merskey & Bogduk, 1994). Chronic pain can have different localisation in the body depending on the aetiology, being either localised or widespread.

Fibromyalgia (FM) and osteoarthritis (OA) are two common causes of chronic musculoskeletal pain with differing bodily representation. FM is a condition characterised by widespread pain, poor sleep and fatigue with no obvious peripheral pathological cause. In contrast, OA is a degenerative joint disease with obvious cartilage breakdown and bony deformity leading to dysfunction and pain in the affected area. OA is a common condition in the elderly, whereas FM can affect younger adults (Neumann & Buskila, 2003; Y. Zhang & Jordan, 2008). These diseases have a financial impact on society through public spending on treatment and time lost from work.

Interest has grown in the use of Magnetic Resonance Spectroscopy (MRS) to study the role of neurochemistry in chronic pain. A combination of improved technology and an increased appreciation of the value of neurochemical data in understanding physiological processes have made MRS an important technique in the investigation of the living brain. It shows great potential for the investigation of the biochemical processes associated with the sensation of pain (Grachev, Ramachandran, Thomas, Szeverenyi, & Fredrickson, 2003; Gussew, Rzanny, Güllmar, Scholle, & Reichenbach, 2011).

While MRS has been used widely to investigate FM (Emad et al., 2008; Fayed et al., 2010; Harris et al., 2009; Valdés et al., 2010; Wood, Ledbetter, Glabus, Broadwell, & Patterson, 2009), I am only aware of one *in vivo* MRS study of OA (Shigemura et al., 2012). Recently MRS has been used to examine possible neurochemical differences in two chronic pain conditions with different aetiologies (neuropathic versus non-

neuropathic) (Gustin et al., 2011). Gustin et al. used MRS to compare the effects of trigeminal neuropathic pain (TNP) with temporomandibular disorder (TMD) on patients' brains. Given the suggestion that TNP is maintained by mechanisms that alter brain anatomy, whereas TMD is driven by peripheral mechanisms, Gustin et al. hypothesised that TNP would also lead to observable changes in neurochemistry. A reduced ratio of NAA to Cr was observed in the Thalamus of TNP patients compared to healthy controls and to TMD patients. Furthermore, a negative correlation was observed between this ratio and TNP patients' recorded pain scores. These findings lend support to the suggestion that MRS is a viable method for observing differences in neurochemistry associated with the central mechanisms of chronic pain conditions and provide motivation for the use of MRS to investigate differences between FM and OA.

Using FM as a model of chronic widespread pain (CWP) and knee OA as a model of chronic localised pain (CLP), this study set out to compare the neurochemical profiles of these two conditions, using pain-free participants as controls. Since these pain conditions have different bodily representations, I hypothesized that there would be differences in central expression between CWP and CLP. In particular, I would expect to see significant differences in N-acetyl aspartate (NAA) and Glutamate (Glu) levels between the groups. NAA is widely believed to be a marker of neuronal density and viability (Govindaraju, Young, & Maudsley, 2000; Simmons, Frondoza, & Coyle, 1991), therefore I expected to observe a decrease in NAA level in CLP. Glu is a major excitatory neurotransmitter that has previously been found to increase in response to an acute painful stimulus (Gussew et al., 2009; Mullins et al., 2005). Increased levels of Glu may therefore be suggestive of an increase in excitatory activity consequently I predicted a Glu elevation in CWP.

The first aim of the study was to investigate whether statistical differences could be observed in the level of neurometabolites in four brain regions between CWP group (FM) and CLP group (OA) compared to healthy controls group (HC). The second aim was to examine neurometabolite differences between FM and OA groups whilst controlling for the effects of age. As noted, the typical age of onset differs between the two diseases therefore I anticipated a difference between the mean age of my patient groups: controlling for age differences would therefore protect the internal validity of my study. The third aim was to explore the relationships between the levels of neurometabolites and clinical measures, such as pain

rating scores (PRS), depression scores (DS), anxiety scores (AS), and pain duration (PD) between the groups.

## **Methods and participants**

### **Participants**

Consideration was given to the participant group sizes that would give a sufficiently high probability of detecting differences in metabolite levels between the groups. A review of 8 previous MRS studies of chronic pain (7 FM, 1 OA) revealed that a mean group size of 16 participants. These studies had made a comparison between metabolite levels in a single patient group and healthy controls and therefore had a mean total participant group size of 32. I examined the sample size that would be needed in such experiments to achieve an 80% power level, using the tool G\*Power 3 (Faul, Erdfelder, Lang, & Buchner, 2007). This analysis showed that to achieve 80% power in detecting a large effect size (Cohen's  $d = 0.8$ ) a total group of 42 participants would be required, hence the experiments, whilst successful, could be considered somewhat underpowered. It was my intention to make a 3-way comparison between two groups of chronic pain patients and a group of healthy controls, therefore I used G\*Power to determine what group sizes would be required to achieve a power level similar to previous experiments using an ANOVA. G\*Power revealed that to achieve 80% power in detecting a large effect size (Cohen's  $f = 0.4$ ) three groups of 22 participants would be required and therefore I aimed to recruit a total of 66 participants. For the purposes of this study it was possible to recruit 50 participants, therefore achieving a power of approximately 70%.

All participants were females aged 31-65. There were 17 participants with FM (mean +/- SD age 47.88 +/- 8.15 years), 17 participants with OA of the knee (mean +/- SD age 61.65 +/- 3.06 years), and 16 HC (mean +/- SD age 44.25 +/- 11.32 years) for this study. Demographic information for the FM, OA and HC participants is given in Tables 2, 3 and 4, respectively.

<b>Subject</b>	<b>Age</b>	<b>Pain duration (years)</b>	<b>Pain Rating Score</b>	<b>HADS Anxiety Score</b>	<b>HADS Depression Score</b>
2	43	14	5	17	13
3	51	30	3.5	1	6
6	36	3	6	8	9
8	42	18	6.5	0	2
10	50	7	5	7	9
13	41	2	2	20	9
17	65	14	8	0	7
18	46	10	8	8	10
22	51	20	6	15	12
25	55	2	7	7	6
27	34	5	6	9	16
28	55	12	7.5	14	17
29	61	24	3	12	15
30	46	13	8	16	18
31	49	4	2	12	16
38	45	2	4	18	13
53	44	22	7	15	17
Mean	47.88	11.88	5.56	10.53	11.47
±SD	8.15	8.66	2.04	6.26	4.69

*Table 2: Demographics of fibromyalgia subjects*

<b>Subject</b>	<b>Age</b>	<b>Pain duration (years)</b>	<b>Site</b>	<b>Pain Rating Score</b>	<b>HADS Anxiety Score</b>	<b>HADS Depression Score</b>
19	65	2	Left	2	1	6
20	63	40	Both	6	14	14
23	58	5	Left	6	7	8
24	64	3	Left	5	8	6
26	58	8	Both	7	13	10
32	62	3	Right	7	5	7
33	65	5	Left	5	9	17
34	62	4	Both	7	13	13
37	58	6	Right	9	13	11
45	64	3	Right	8	6	14
46	55	11	Both	6	14	15
47	65	5	Both	5	5	7
48	65	4	Right	7	2	8
49	62	5	Both	1	12	13
50	61	5	Right	4	8	6
51	62	44	Left	4	7	8
52	59	5	Both	3	3	9
Mean	61.65	9.29	N/A	5.41	8.24	10.12
±SD	3.06	12.50	N/A	2.12	4.31	3.57

*Table 3: Demographics of osteoarthritis subjects*

<b>Subject</b>	<b>Age</b>	<b>HADS Anxiety Score</b>	<b>HADS Depression Score</b>
1	31	0	2
4	46	5	5
5	62	1	3
7	46	0	1
9	47	0	0
11	32	6	2
12	36	4	10
14	56	3	0
15	35	0	0
16	65	3	1
35	34	1	0
36	34	2	0
39	60	7	6
40	47	3	4
41	36	1	0
42	41	2	5
Mean	44.25	2.38	2.44
±SD	±11.32	±2.22	±2.90

*Table 4: Demographics of healthy controls*

FM participants were classified using the American College of Rheumatology criteria (Wolfe et al., 1990) for the classification of FM, such that each subject fulfilled the following criteria:

- 1) widespread pain in all four quadrants of the body for at least 3 months;
- 2) tenderness or pain to touch in at least 11 out of 18 specific anatomical sites described as tender points.

FM participants were recruited from a district general hospital fibromyalgia clinic in North Wales. FM participants were excluded if they had knee OA. Participants with knee OA were

recruited from the pre-operative arthroplasty clinic at Ysbyty Gwynedd, Bangor. Participants with rheumatoid arthritis and OA of other joints were excluded, as was anyone who also had a diagnosis of co-morbid FM. HC participants without chronic pain were recruited using the Bangor University's School of Psychology's community participant panel and local advertisements. All participants were asked to refrain from using analgesic medication on the day of the scan until after scanning was completed. Normal contraindications for MRI were also used as exclusion criteria (e.g. pacemakers, metal implants, claustrophobia).

This study was approved by the Ethics Committee from the School of Psychology, Bangor University and North West Wales Research Ethics Committee (reference 10/WNo01/16). Research activities took place in Bangor Imaging Centre, Bangor University. All participants provided informed consent.

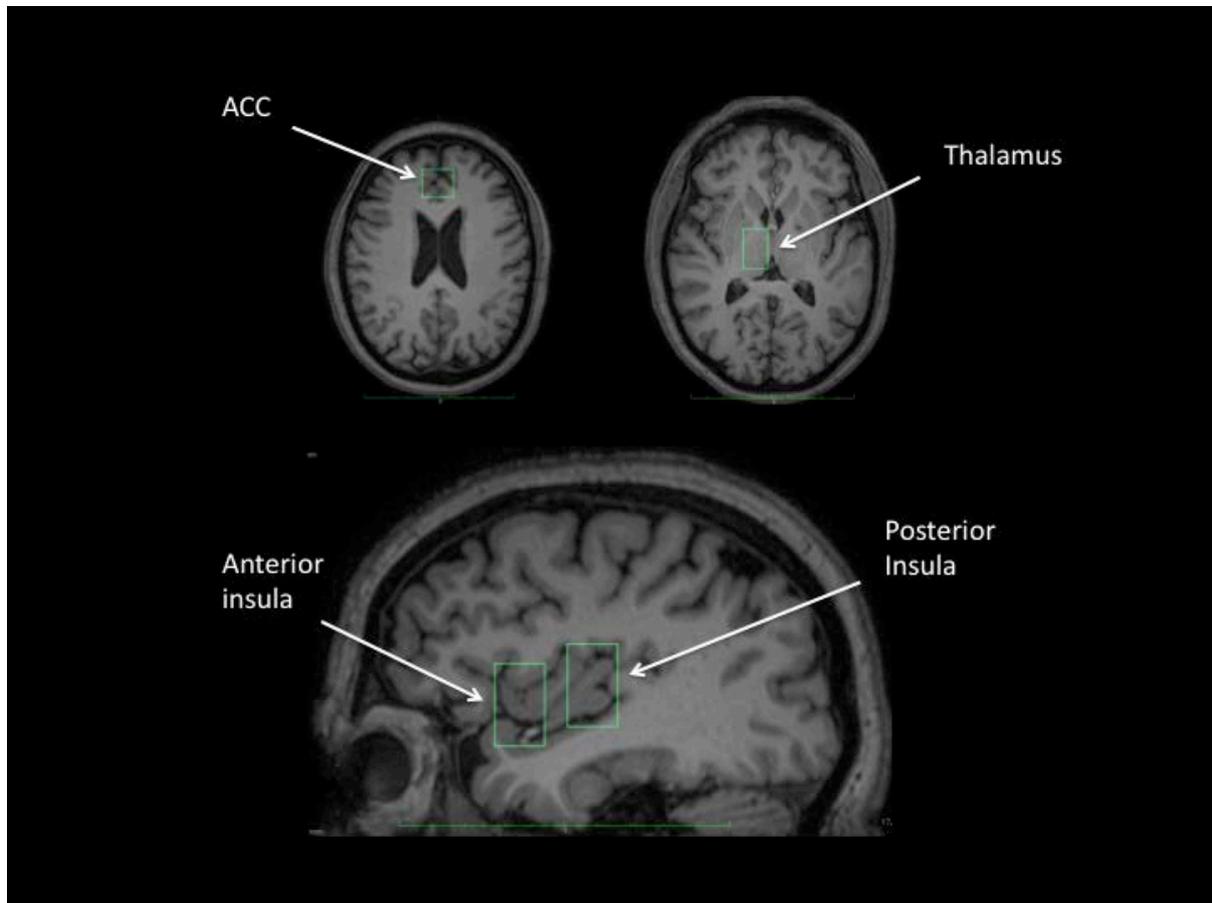
## **Methods**

### *MRS procedures*

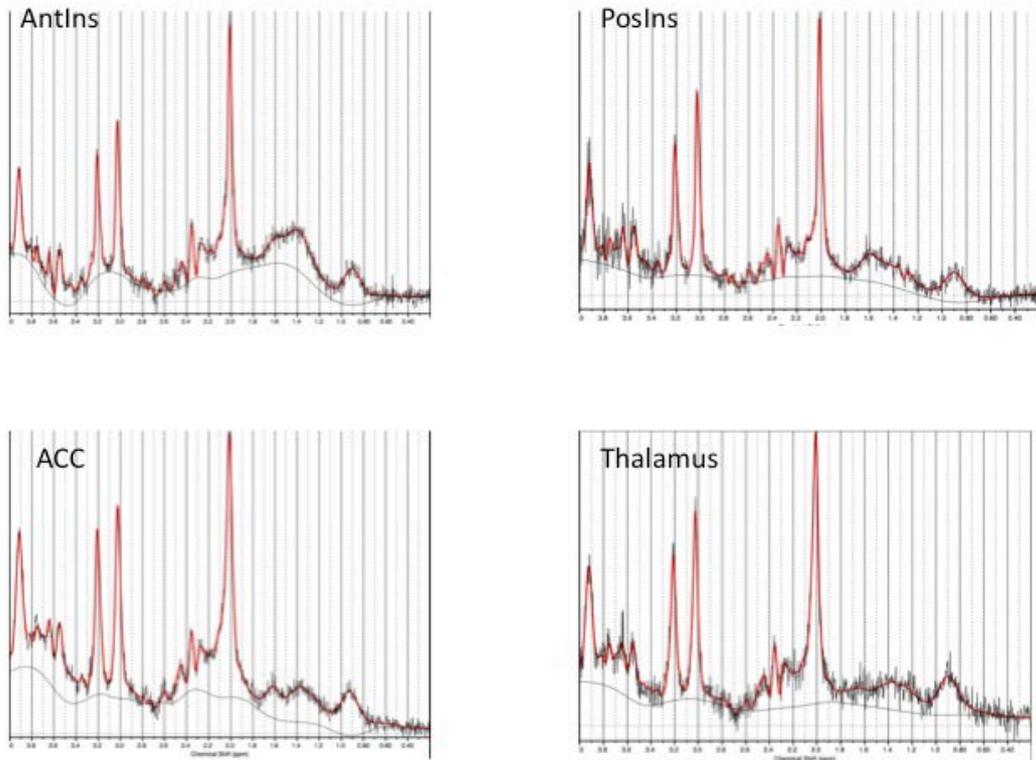
All participants underwent the same MRS protocol. MRS scanning was conducted using a 3 Tesla Phillips Achieva MRI system (Philips Health Care, Eindhoven, Netherlands). MRS was used to measure the levels of six neurometabolites in four different brain regions previously found to be implicated in chronic pain (Emad et al., 2008; Fayed et al., 2010; Grachev et al., 2000; Grachev et al., 2002; Gussew et al., 2011; Harris et al., 2009; Valdés et al., 2010; Wood, Ledbetter, et al., 2009). The neurochemicals investigated were N-acetyl aspartate (NAA), Choline (Cho), Creatine (Cr), Glutamate (Glu), Glutamine (Gln), Glu + Gln (Glx) and Myo-Inositol (m-Ins) within the anterior cingulate cortex (ACC), anterior insula (AntIns), posterior insula (PosIns), and the thalamus (Thal).

A high-resolution T<sub>1</sub>-weighted scan was used for localisation for the MRS scans. The T<sub>1</sub> weighted image was acquired as 5 echo MP-RAGE sequence (TE = 3.5, 5.1, 6.8, 8.5, 10.2 ms, TR = 12 ms, TI = 1150 ms, 3D acquisition, FOV = 240 mm X 220 mm X 130 mm, voxel dimensions = 0.7 X 0.7 X 0.7 mm<sup>3</sup>). Single voxel proton MRS (PRESS-TE=40 ms, TR=2000 ms, 196 averages) was localised to the ACC (2 x 2 x 2 cm<sup>3</sup>), AntIns (1.5 x 1.5 x 2.5 cm<sup>3</sup>), PosIns (1.5 x 1.5 x 2.5 cm<sup>3</sup>), and the Thal (1.5 x 1.5 x 2.5 cm<sup>3</sup>) based on anatomical

landmarks identified on the T<sub>1</sub> weighted image. Each examination lasted approximately one hour. Figures 2 and 3 show the selected voxel locations and MRS spectra respectively.



*Figure 2: Voxel locations for magnetic resonance spectroscopy (MRS) of the anterior cingulate cortex (ACC), Anterior Insula, Posterior Insula, and the Thalamus*



ACC - anterior cingulate cortex

AntIns - anterior insula

PosIns - posterior insula

*Figure 3: Representative MRS spectra from each of the four regions from one participant in the study, demonstrating typical signal quality*

### *Questionnaires*

Participants completed the Pain Rating Scale (PRS) and the Hospital Anxiety and Depression Scale (HADS). The questionnaires were completed by the participants immediately prior to scanning.

The Pain Rating Scale enables the level of pain on the day of scanning to be measured by assessing the intensity of the pain, how distressing it is, and how much it interferes with normal everyday activities (Jensen & Karoly, 2001). For this paper I focused solely on the intensity of pain on the day of scanning (PRS of 0-10).

The HADS is an uncomplicated and reliable instrument to assess both depression and anxiety in hospital practice and community settings, and provides a score of 0-21 for each measure. Participants complete a questionnaire by responding to statements applicable to generalised anxiety or depression (Snaith, 2003). Patients' responses are recorded as Anxiety Score (AS) and Depression Score (DS).

### *Pain Duration*

Pain duration (PD) was collected from medical notes and participant interviews. It was considered from the first report by patient.

### *Data Analysis*

The MRS data was transferred from the scanner in the form of Philips SDAT and SPAR files for analysis using LC-Model software (Provencher, 1993). LC-Model has a high level of acceptance within the MRS field as an appropriate fitting technique and normalizes the area under each fitted metabolite peak against the area of the unsuppressed water peak for the region, allowing metabolite concentrations to be calculated for NAA, Glu, Gln, Cho, and Cr and up to 14 other metabolites (Provencher, 1993). In addition, the use of LC-Model for spectral quantification provides a high level of reproducibility in clinical populations (Mullins et al., 2003). The default LC-Model setup contains an estimated correction for relaxation and assumed water concentration effects. However, these may not truly reflect the water content or relaxation properties of the tissues present in the voxels. To address these concerns the high-resolution T1 weighted images for each subject were segmented into grey

matter (GM), white matter (WM), and cerebrospinal fluid (CSF) using Statistical Parametric Mapping toolbox (SPM), so tissue content within the MRS voxels could be assessed (Gasparovic et al., 2006). Voxel registration was then performed using custom-made scripts developed in MATLAB, which can be accessed at <http://biu.bangor.ac.uk/projects.php.en>. The scripts generated a mask for each voxel location by combining location information from the Philips SPAR file with orientation and location information contained within the T1 image. The application of this mask to the GM, WM and CSF images enabled the calculation of partial volume within the region of interest by establishing the percentage of each tissue type within the relevant voxels. These percentages were used to correct metabolite concentrations referenced to water, for partial volume and relaxation effects as described by Gasparovic et al, (Gasparovic et al., 2006) using equation [1]:

$$[M] = \frac{S_{M\_obs} \times (f_{GM} \times R_{H2O_{GM}} + f_{WM} \times R_{H2O_{WM}} + f_{CSF} \times R_{H2O_{CSF}})}{S_{H2O\_obs} (1 - f_{CSF}) \times R_M} \times \frac{2}{\#H_M} \times [H_2O]$$

[1]

Where [M] is the concentration of the metabolite;  $S_{M\_obs}$  and  $S_{H2O\_obs}$  are the signals from the metabolite of interest and the unsuppressed water;  $f_{GM}$ ,  $f_{WM}$ , and  $f_{CSF}$  are the fractional volume of GM, WM and CSF in the voxel respectively.  $R_{H2O\_GM}$ ,  $R_{H2O\_WM}$ ,  $R_{H2O\_CSF}$ , and  $R_M$  refer to correction factors for  $T_1$  and  $T_2$  relaxation effects on GM, WM, CSF, metabolite signal and assumed water content for each tissue type (43300 mM for GM, 35880 mM for WM, 53883 mM for CSF, and assuming 55550 mM water in pure water). Relaxation effects are calculated as  $R_M = \exp[-TE/T2_M](1 - \exp[-TR/T1_M])$  where  $T_1$  for GM, WM and CSF was estimated from the literature at 1500 ms, 1000 ms, and 4000 ms respectively, while  $T_2$  was at 63 ms, 50 ms, and 200 ms respectively (Dieringer et al., 2014; Ganji et al., 2012; Prescott, Shi, Choi, & Renshaw, 2014; Träber, Block, Lamerichs, Gieseke, & Schild, 2004).  $T_1$  and  $T_2$  values for metabolites were likewise an average of estimates from the literature assuming mostly GM content of the voxels: NAA ( $T_1=1403$ ,  $T_2=272$ ); Cho ( $T_1=1182$ ,  $T_2=217$ ); Cr ( $T_1=1320$ ,  $T_2=217$ ); m-Ins ( $T_1=1100$ ,  $T_2 = 200$ ); with Glu and Gln signals assumed to have the same relaxation times ( $T_1=1220$ ,  $T_2=185$ ) (Choi & Frahm, 1999; Choi et al., 2006;

Träber et al., 2004; Tsai et al., 2007; Walaszek, Schubert, Mекle, & Ittermann, 2012; Zaaraoui et al., 2007).

For further description of the method for correction the reader is again directed to Gasparovic et al. (Gasparovic et al., 2006). This equation therefore gives a calculated concentration in units of mM/kg water, and all results are reported as such. However, due to the assumptions regarding water content, these may be thought of as institutional units and therefore would be different if other assumptions regarding water content and relaxation were used.

### *Statistics*

Each region of interest was analysed individually using SPSS 20 software (IBM, Armonk, NY, 2012). A one-way between-groups analysis of variance (ANOVA) was conducted to explore if there were any statistical differences in the levels of neurometabolites in four brain regions between three groups. Post-hoc comparisons using the Tukey HSD test were then performed to determine the level of significance and direction of potential differences in OA and FM groups compared to HC group. Likewise, ANOVAs with the Tukey HSD test were conducted to explore if there were any statistical differences in age between groups.

Furthermore, due to reports in the literature about changes in neurometabolite levels being associated with aging (Chang, Ernst, Poland, & Jenden, 1996; Christiansen, Toft, Larsson, Stubgaard, & Henriksen, 1993; Haga, Khor, Farrall, & Wardlaw, 2009; Saunders, Howe, van den Boogaart, Griffiths, & Brown, 1999), a one-way between-groups analysis of covariance (ANCOVA) was conducted to control for age in the levels of neurometabolites between the groups. The independent variables were groups, and the dependent variables were the levels of neurometabolites. Age was used as the covariate in this analysis.

In addition, the relationships between the levels of neurometabolites and clinical measures (PRS, DS, AS, and PD) were investigated using Pearson product-moment correlation coefficient.

## **Results**

A summary of the participants' characteristics are presented in Table 5 and include subjects' age, PD in years, PRS, AS, and DS. Mean PD, PRS, AS, and DS did not show any significant differences between the patient groups (an independent sample t-test). There was a

statistically significant difference in age for the three groups:  $F(2, 47) = 21.1, p = .00, \eta^2 = .47$  as revealed using a one-way between-groups ANOVA. The Tukey HSD test indicated that the mean age for OA group ( $M = 61.65, SD = 3.06$ ) was significantly different at the  $p < .01$  levels from FM group ( $M = 47.88, SD = 8.15$ ) and HC group ( $M = 44.25, SD = 11.32$ ). There was no significant difference in the mean age between FM and HC groups.

<b>Subject Group</b>	<b>Age</b>	<b>Pain duration (years)</b>	<b>Pain Rating Score</b>	<b>HADS Anxiety Score</b>	<b>HADS Depression Score</b>
FM	47.88 ±8.15	11.88 ±8.66	5.56 ±2.04	10.53 ±6.26	11.47 ±4.69
OA	61.65 ±3.06	9.29 ±12.50	5.41 ±2.12	8.24 ±4.31	10.12 ±3.57
HC	44.25 ±11.32	N/A	N/A	2.38 ±2.22	2.44 ±2.90

FM – fibromyalgia subjects

OA – knee osteoarthritis subjects

HC – healthy controls

*Table 5: Summary of demographic and clinical characteristics of participants (Mean / ±SD). For full detail see tables 2, 3 and 4.)*

As shown in Table 6, the acquired MRS data was of a satisfactory quality for further processing as expressed in the signal to noise ratios achieved.

Brain regions	Groups	S:N ratio Mean (SD)	Tissue segmentation		
			GM Mean (SD)	WM Mean (SD)	CSF Mean (SD)
ACC	FM	24.12 (3.66)	0.68 (0.09)	0.16 (0.08)	0.17 (0.11)
	OA	22.06 (3.38)	0.73 (0.03)	0.13 (0.03)	0.14 (0.04)
	HC	22.19 (4.76)	0.70 (0.08)	0.12 (0.03)	0.18 (0.09)
AntIns	FM	17.19 (3.64)	0.67 (0.06)	0.16 (0.08)	0.17 (0.08)
	OA	17.53 (2.43)	0.66 (0.05)	0.16 (0.07)	0.18 (0.06)
	HC	19.00 (2.63)	0.66 (0.06)	0.20 (0.08)	0.13 (0.04)
PosIns	FM	17.31 (2.65)	0.60 (0.07)	0.30 (0.07)	0.10 (0.04)
	OA	16.59 (1.70)	0.58 (0.05)	0.29 (0.06)	0.13 (0.04)
	HC	19.69 (3.38)	0.61 (0.06)	0.27 (0.07)	0.12 (0.06)
Thal	FM	16.18 (2.74)	0.48 (0.03)	0.48 (0.03)	0.04 (0.06)
	OA	15.24 (2.08)	0.49 (0.01)	0.49 (0.01)	0.03 (0.03)
	HC	16.93 (2.19)	0.49 (0.01)	0.49 (0.01)	0.03 (0.02)

ACC – anterior cingulate cortex, AntIns – anterior insula, PosIns – posterior insula, Thal – thalamus; FM – fibromyalgia subjects, OA – knee osteoarthritis subjects, HC – healthy controls; GM – grey matter, WM – white matter, CSF – cerebrospinal fluid

Table 6. Signal to noise (S:N) ratio and tissue segmentation values

I was able to derive good fits of the raw data as assessed by the Cramer-Rao Lower Bound (CRLB - an estimate of goodness of fit) from LC-Model. As shown in Table 7, CRLB for most metabolites was on average less than 10% (e.g. average CRLB for Glu was 7.7% in the ACC, 9% in the AntIns and PosIns, and 9.9% in the Thal, while Cr was < 3% in all regions), well below the cut-off of 20% as generally used in the literature (Choi & Frahm, 1999; Choi et al., 2006; Träber et al., 2004; Tsai et al., 2007; Walaszek et al., 2012; Zaaraoui et al., 2007). Gln, a small peak that is difficult to fit, was the only exception, with an average CRLB of 22%. Rather than to exclude data, I decided to extend my cut-off for Gln to 30%, and maintain awareness that there may be more variability in these measures than in other metabolites. I will discuss the implications of my CRLB analysis and use of cut-offs below.

Brain regions	Groups	Gln %SD	Glu %SD	Ins %SD	NAA %SD	Cho %SD	Cr %SD	Glu+Gln
ACC	FM	21.94 (6.25)	7.47 (1.18)	6.12 (0.86)	3.35 (0.70)	3.00 (0.00)	2.06 (0.24)	7.18 (1.02)
	OA	21.41 (4.56)	8.06 (1.52)	6.18 (0.95)	3.41 (0.80)	2.88 (0.33)	2.06 (0.24)	7.53 (1.42)
	HC	23.38 (8.19)	7.88 (1.54)	6.44 (0.96)	3.81 (0.98)	3.25 (0.58)	2.12 (0.34)	7.63 (1.41)
AntIns	FM	29.25 (10.50)	9.13 (1.15)	8.31 (1.78)	3.25 (0.68)	3.88 (0.62)	2.94 (0.44)	8.75 (1.57)
	OA	28.94 (10.90)	9.29 (0.77)	7.35 (0.79)	3.29 (0.47)	3.47 (0.52)	2.53 (0.52)	8.82 (0.88)
	HC	25.94 (9.57)	8.69 (1.08)	7.44 (1.26)	3.25 (0.58)	3.69 (0.60)	2.56 (0.51)	8.31 (1.02)
PosIns	FM	25.25 (4.20)	9.25 (1.18)	8.38 (1.67)	3.19 (0.40)	4.13 (0.34)	2.81 (0.40)	8.75 (1.00)
	OA	28.53 (8.60)	9.53 (1.01)	7.94 (1.30)	3.06 (0.24)	4.00 (0.50)	2.47 (0.52)	9.06 (1.09)
	HC	27.00 (14.82)	8.19 (1.38)	7.38 (1.15)	2.56 (0.51)	3.63 (0.50)	2.31 (0.48)	7.88 (1.26)
Thal	FM	33.12 (13.79)	9.35 (1.66)	9.94 (2.38)	3.88 (0.99)	3.35 (0.49)	2.71 (0.47)	8.88 (1.22)
	OA	35.77 (15.77)	10.71 (1.57)	10.18 (1.94)	4.06 (1.14)	3.53 (0.62)	2.71 (0.59)	10.35 (1.73)
	HC	28.07 (8.00)	9.80 (0.94)	9.73 (1.62)	3.47 (0.74)	3.33 (0.49)	2.47 (0.52)	9.07 (0.96)

ACC – anterior cingulate cortex, AntIns – anterior insula, PosIns – posterior insula, Thal – thalamus; FM – fibromyalgia subjects, OA – knee osteoarthritis subjects, HC – healthy controls; Gln – Glutamine, Glu – Glutamate, Ins – Inositol, NAA – N-acetyl aspartate, Cho – Choline, Cr – Creatine

Table 7. Cramer-Rao Lower Bound (CRLB) percentages

Figure 2 shows typical MRS spectra from all regions demonstrating the signal to noise and line shape acquired.

Neurometabolite levels across all brain regions are shown in Table 8, with significant results in bold, as discovered using one-way between-groups ANOVA. There were statistically significant differences at the  $p < .05$  level in the AntIns for the three groups: in Cr level  $F(2, 46) = 6.6, p < .01, \eta^2 = .22$  and in m-Ins level  $F(2, 46) = 4.34, p = .02, \eta^2 = .16$ . Post-hoc comparisons using the Tukey HSD test indicated that the mean Cr level for OA group ( $M = 10.18, SD = .94$ ) was significantly higher than HC group ( $M = 8.92, SD = 1.03$ ) with  $p < .01$ . Similarly, the mean m-Ins level was higher in OA group ( $M = 8.59, SD = 1.25$ ) compared to HC group ( $M = 7.42, SD = 1.00$ ) with  $p = .02$ . Furthermore, there was a trend towards significance ( $p = .07$ ) at the level of Glu and Glx in the AntIns (FM > HC).

There was a statistically significant difference in Thal in NAA level for the three groups:  $F(2, 46) = 3.89, p = .03, \eta^2 = .15$ . Post-hoc comparisons indicated that the mean NAA level for OA group ( $M = 10.37, SD = .60$ ) was significantly lower than FM group ( $M = 10.94, SD = .75$ ) with  $p = .03$ . No significant differences in any neurometabolite levels between the groups were found in ACC and PosIns.

	ACC			AntIns			PosIns			Thal		
	FM	OA	HC	FM	OA	HC	FM	OA	HC	FM	OA	HC
NAA	10.11	9.29	9.65	11.00	11.00	10.68	10.41	9.93	10.44	10.94	<b>10.37</b>	10.84
±SD	±2.93	±1.25	±1.84	±1.34	±1.06	±0.89	±0.49	±0.65	±0.91	±0.75	±0.60	±0.53
Cho	2.39	2.37	2.43	2.15	2.23	1.98	1.74	1.84	1.79	2.15	2.13	2.19
±SD	±0.60	±0.32	±0.38	±0.33	±0.35	±0.30	±0.17	±0.26	±0.25	±0.16	±0.16	±0.23
Cr	10.33	10.60	10.36	9.36	<b>10.18</b>	8.92	9.00	9.48	9.25	8.76	9.01	8.85
±SD	±2.10	±1.07	±1.23	±1.08	±0.94	±1.03	±0.63	±0.57	±0.66	±0.60	±0.67	±0.74
m-Ins	9.40	8.88	8.57	7.82	<b>8.59</b>	7.42	6.81	7.27	6.78	5.72	5.86	5.85
±SD	±2.09	±1.29	±2.93	±1.20	±1.25	±1.00	±0.78	±0.86	±0.97	±1.46	±1.05	±0.90
Glu	14.22	13.23	13.93	13.74	12.45	11.61	11.46	10.99	11.14	12.11	10.94	10.95
±SD	±4.22	±3.04	±3.28	±3.14	±2.35	±2.00	±2.44	±1.84	±1.38	±2.69	±1.46	±1.71
Gln	2.86	3.11	3.12	3.10	2.79	2.84	3.01	2.57	2.47	3.09	3.07	3.01
±SD	±1.08	±1.51	±2.15	±1.15	±0.79	±1.46	±1.37	±0.73	±0.80	±1.37	±1.56	±1.10
Glx	17.08	16.34	17.05	16.84	15.24	14.45	14.47	13.56	13.61	15.30	14.10	14.05
±SD	±4.89	±4.27	±4.79	±4.03	±2.61	±3.25	±3.58	±2.11	±1.81	±2.99	±2.61	±2.23

Significant differences are shown in bold

ACC – anterior cingulate cortex, AntIns – anterior insula, PosIns – posterior insula, Thal – thalamus, NAA – N-acetyl aspartate, Cho – Choline, Cr – Creatine, Glu – Glutamate, Gln – Glutamine, m-Ins – Myo-Inositol

*Table 8: Neurometabolite concentrations (Mm, relative to water) for each region in participants with fibromyalgia (FM), osteoarthritis (OA) and healthy controls (HC)*

After adjusting for age with a one-way between-groups ANCOVA, there was no significant difference in the levels of Cr in AntIns between the groups,  $F(2, 45) = 1.77, p = .18, \eta^2 = .07$ . Similarly, there was no significant difference in the levels of m-Ins in AntIns between the groups,  $F(2, 45) = 1.95, p = .15, \eta^2 = .08$ , after controlling for age. There were no significant relationships between age and levels of Cr and levels of m-Ins in AntIns, while controlling for groups ( $\eta^2 = .03$  for Cr and  $.00$  for m-Ins).

However, there was still a significant difference in the levels of NAA in the Thal between the groups,  $F(2, 45) = 4.02, p = .03, \eta^2 = .15$ , after controlling for age. There was no significant relationship between age and levels of NAA in the Thal, while controlling for groups ( $\eta^2 = .03$ ).

Also I ran an exploratory analysis (one-way between-groups ANOVA) of the tissue content (GM, WM, and CSF) in each region of interest and did not find a significant difference between the groups.

Given the previously discussed consideration of NAA as a measure of neuronal viability, I analysed the observed levels of NAA data uncorrected for partial volumes together with the results from the tissue segmentation. This procedure was intended to determine whether lower levels of NAA correlated with a lower proportion of grey matter within the ROIs. No significant correlations were observed in ACC, AntIns or PosIns. A significant positive correlation was observed between the proportion of Thalamic grey matter and uncorrected NAA levels, Spearman's  $\rho = .19, p < .01$ .

Finally, the relationships between the levels of neurometabolites and clinical measures were examined. Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity and homoscedasticity. Significant results for correlations between the levels of neurometabolites and PRS, DS, AS, and PD ( $r$  ranging from  $|0.49|$  to  $|0.75|$ ) are presented in Table 9. FM showed negative correlations of Cr and m-Ins with DS and AS, NAA and Cho with PRS, and Glu and Glx with PD, while OA revealed positive correlations of Glu with PRS, and Gln and Glx with DS. It must be noted that comparisons between patients' metabolite levels and 4 clinical measures were made. Applying a Bonferroni correction to these comparisons gives a p-value of  $.0125$  for a significant difference. For the

FM patients the correlations between Cr levels in AntIns and both DS and AS achieved this level of significance. For FM, correlations between Cr in PosIns and DS, m-Ins in AntIns and DS, m-Ins in Ant Ins and AS, Cho in Thal and PRS and Glu in PosIns and PD approached the required level of significance. For OA patients the correlation between Gln in Thal and DS achieved this level of significance. Also for OA, Gln in PosIns and DS approached significance.

<b>Correlations</b>	<b>Correlation Coefficient</b>	<b>Significance Level</b>	<b>Number of Cases</b>	<b>Brain Region</b>	<b>Pain Group</b>
Cr - DS	- 0.62	p = .01	17	AntIns	FM
	- 0.59	p = .017	17	PosIns	FM
Cr - AS	- 0.75	p = .001	17	AntIns	FM
m-Ins - DS	- 0.6	p = .014	17	AntIns	FM
m-Ins - AS	- 0.61	p = .013	17	AntIns	FM
NAA - PRS	- 0.53	p = .034	17	PosIns	FM
Cho - PRS	- 0.59	p = .013	17	Thal	FM
Glu - PD	- 0.6	p = .013	17	PosIns	FM
Glu – PRS	0.59	p = .013	17	PosIns	OA
Gln - DS	0.62	p = .008	17	ACC	OA
	0.57	p = .017	17	PosIns	OA

*Table 9: Significant results for correlations between levels of neurometabolites and pain rating scores (PRS), depression scores (DS), anxiety scores (AS), and pain duration (PD)*

My results demonstrated changes in OA patients (decreased NAA in Thal and increased Cr and m-Ins in AntIns), which were not observed in FM patients. Glu was found to correlate at a trend level with clinical measures associated with pain, but in differing ways between the two pain cohorts (with PD in FM, and with PRS in OA). After correcting for multiple

comparisons, depression and anxiety scores were found to be negatively correlated with measures of Cr in FM.

## **Discussion**

To my knowledge, this is the first MRS study of the effects of CLP (OA) on the brain in comparison with CWP (FM). My hypothesis of different central expression of chronic pain in CWP and CLP has been supported by my results, which indicate that these conditions have different neurochemical profiles in particular with regard to the relationship between clinical questionnaire measures and neurochemical levels. This is similar to a previous report, which studied trigeminal neuropathic pain (TNP) and temporomandibular disorder (TMD) (Gustin et al., 2011). Differences in some neurometabolite levels were seen in the raw data; however, only the NAA decrease within the Thal in OA group was found to be statistically significant after correcting for age. This finding is similar to what was reported in hip OA (Shigemura et al., 2012), and supports my hypothesis of reduced NAA in CLP although only in a single region.

I did find the hypothesised elevation in Glu in CWP, however this was only at the trend level and must therefore discussion of this finding must be treated as speculative, pending further research. Further to this, an examination of the relationships between neurochemical profiles and clinical measures showed marked differences between the two patient groups. In particular, Glu levels were found to correlate at marginal significance with pain intensity in the PosIns in OA participants, but with duration of pain in FM. In addition, Cr showed a negative relationship with depression in FM (in both AntIns and PosIns), while in OA depression was found to correlate positively with Gln measures (in ACC and at a trend level in PosIns). These differing neurochemical profiles and relationships suggest that the neural mechanisms in FM are different from those found in OA. I will examine each of these findings individually.

The first substantive finding that I report is the reduction of NAA in the Thal in OA. NAA is a marker of neuronal density and viability (Simmons et al., 1991), however, dynamic changes of NAA concentrations suggest that NAA levels may reflect neuronal dysfunction rather than loss (Govindaraju et al., 2000). Recovery of NAA levels has been detected in brain injury

(De Stefano, Matthews, & Arnold, 1995), reversible ischemia (Brulatout et al., 1996), multiple sclerosis (MS), AIDS, and temporal lobe epilepsy (Barker & Lin, 2006). Reduced NAA levels have also been observed with many neurodegenerative diseases such as leukodystrophies, hypoxic encephalopathy (Rosen & Lenkinski, 2007), and multiple sclerosis (Rosen & Lenkinski, 2007; Tsai & Coyle, 1995). My finding of NAA reduction in OA compared to HC suggests that long-term chronic pain from OA may lead to degeneration or dysfunction of central nervous system tissue. It would be informative to examine if NAA levels recover after knee surgery in OA patients.

There are some similarities between this finding and previous MRS research in pain. A reduction of NAA has been found in patients with chronic back pain (CBP), although in different brain regions than in OA, specifically the dorsolateral prefrontal cortex (DLPFC) (Grachev et al., 2000), the AntIns, and the ACC (Gussew et al., 2011). Chronic regional pain syndrome (CRPS) has also shown reduced levels of NAA in bilateral DLPFC when compared with HC (Grachev et al., 2002). Taken together with my results in OA participants, it seems that NAA reduction is a common finding in CLP, although the location of these changes may differ, providing further support for the heterogeneity of neural involvement in different chronic pain conditions.

Interestingly, there was also a positive correlation between uncorrected NAA levels and GM content in the Thalamus, but no other regions. Given that NAA is often reported to be at a higher level of concentration in GM compared to WM (Wang & Li, 1998), this is perhaps not entirely surprising. However, given that GM levels did not vary between the three groups in the Thal, it is unlikely this relationship impacted on our findings of reduced NAA in the Thal in OA.

In contrast to my finding of reduced NAA in the Thal in OA, Cr and m-Ins were found to be elevated in the AntIns in this patient group, although this finding did not survive correction for age. This is not surprising given previous reports of increases in Cr and m-Ins with age (Chang et al., 1996; Christiansen et al., 1993; Haga et al., 2009; Saunders et al., 1999) and the fact that my OA group was older than two other groups. This suggests that the increase I observed in these two metabolites in this cohort may be related to the effects of aging rather

than disease. The effects of the difference in mean age of the participant groups will be considered in the limitations below.

Providing a degree of support for my initial hypothesis, I did find a trend towards significance in Glu levels between the groups (FM > HC) using a one-way between-groups ANOVA. In post hoc analysis I found a Glu elevation in FM ( $M = 13.74$ ,  $SD = 3.14$ ) compared with HC ( $M = 11.61$ ,  $SD = 2.00$ ) using an independent-samples t-test:  $t(25.42) = 2.29$ ,  $p = .03$  (two-tailed). This result is in line with previous research in FM (Fayed et al., 2010; Harris et al., 2009; Valdés et al., 2010). In addition, I did find the differing relationships between Glu levels and clinical measures within PosIns in my two chronic pain cohorts. In contrast to AntIns, which is considered to play a role in the managing saliency of external stimuli and the direction of attention (Menon & Uddin, 2010), PosIns is considered to play a role in somatosensory representation and modulating response to painful stimuli (Sawamoto et al., 2000; Segerdahl, Mezue, Okell, Farrar, & Tracey, 2015). An alteration in levels of the excitatory neurotransmitter Glu may indicate increased activity within PosIns in response to pain. In my results Glu within PosIns was found to negatively correlate with duration of pain in FM, whereas in OA it was pain intensity that showed a strong positive relationship with Glu levels. This suggests that Glu levels in PosIns are higher at the onset of FM, and decrease in later stages of the condition; while in OA higher Glu levels in PosIns are linked to higher levels of reported pain on the day.

Early reports of correlations between measures of Glu metabolites in cerebral spinal fluid and FM patients' pain ratings (Larson, Giovengo, Russell, & Michalek, 2000; Sarchielli, Di Filippo, Nardi, & Calabresi, 2007) support this suggestion. More recent evidence comes from reports of correlations between Glu in PosIns and evoked pain ratings in FM patients (Harris et al., 2009), and increased Glu in PosIns in FM patients compared to HC (Fayed et al., 2010). It would be beneficial for future studies to examine the correlation between neurometabolites and pain intensity in longitudinal studies to determine if levels of reported pain fluctuate with levels of neurometabolites.

Within my study, differing relationships were observed between neurometabolite levels and clinical measures in FM and OA. As seen in Table 9 these differences are varied across metabolites and regions. The different relationships that exist between neurometabolite levels

and clinical measures within OA and within FM are an indication that different mechanisms are at work in these two conditions.

It is informative to consider my results regarding relationships between neurochemicals and clinical measures in the light of previous, similar studies. A negative correlation between NAA and pain intensity was demonstrated recently, but in a different region – the thalamus – in patients with chronic neuropathic pain compared to patients without pain after spinal cord injury (Pattany et al., 2002). In contrast, another study showed a positive correlation between Cho/Cr and clinical pain in DLPFC in FM (Petrou et al., 2008), whereas my finding was a negative correlation between Cho and PRS in the Thal of FM participants. However, given a relationship between Cr and anxiety and depression scores, use of ratios to Cr as a concentration reference for neurometabolites is potentially problematic, as it can be difficult to determine whether the numerator or the denominator is changing. This issue was addressed in my study through the use of water as a reference in the calculation of neurometabolite concentrations.

Similarly to my findings, other researchers have found that Cr was negatively correlated with depression, but in a different region (left thalamus in FM) (Valdés et al., 2010). However, they also report a positive relationship between Cho and duration of disease in right amygdala and right thalamus in FM patients, while I observed a negative correlation of duration of pain with another neurometabolite - Glu in a different region - PosIns. Unfortunately, differences in MRS acquisition strategies and field strength make it difficult to directly compare results, though further studies may be able to explain the physiological significance of these effects.

Previous research (Harris et al., 2009) has demonstrated that FM patients have a negative correlation between elevated Glu and Glx levels and lower pain threshold within the PosIns, while in my research Glu was negatively correlated with duration of pain. It would therefore be interesting to examine how pain thresholds change over time in FM patients, as based on these two findings: one might expect lower pain thresholds at first presentation, with a gradual increase in these thresholds over time. Longitudinal studies investigating all factors, including pain thresholds, duration of pain, pain intensity, and Glu levels would be instructive in elucidating the nature of this relationship.

Similarly, while others showed positive correlations between Glu/Cr in the left thalamus and pain intensity (VAS) and m-Ins/Cr in the right VLPFC and pain intensity in FM patients (Feraco et al., 2011), I showed that elevated Glu was related with higher pain level in PosIns in OA. This may reflect a basic role for Glu in pain response, a suggestion that is in agreement with acute pain studies that have shown an increase in Glu with painful stimulus administration (Gussew et al., 2009; Mullins et al., 2005).

## **Limitations**

I am aware of some limitations within this study: participants were asked to refrain from using analgesic medication on the day of scanning whereas the washout period for some drugs is much longer. However, I felt that it would not be ethical to ask participants with chronic pain to refrain from taking analgesic drugs for a longer period.

Age matching for the three groups presented a difficulty due to patients with OA typically being older. In order to obtain sufficiently sized participant groups it proved necessary to tolerate a mean age in the OA group that was significantly higher to the FM and HC groups. There was no significant difference in mean age between the FM and HC groups. Future studies should attempt to recruit older FM participants and ensure a closer match between the ages of patient groups and controls. To consider the potential effects of age differences within this study the literature was consulted to examine previous observations. The literature does not provide a definitive statement of the effects of aging on levels of brain metabolites. A systematic review (Haga et al., 2009) was consulted with several of the more relevant studies being examined in detail (Chang et al., 1996; Maudsley et al., 2009; Saunders et al., 1999). Haga et al. reported that most studies had observed no significant change in levels of NAA, Cr, Cho and m-Ins with aging. A meta-analysis, conducted by Haga et al., of a subset (4) of the studies considered found a NAA decrease with age of borderline significance and significant increases of Cr and Cho with age. Haga et al. noted the difficulty of achieving a synthesis of the results of previous studies given the diversity of MRS and data analysis methods employed. It is instructive to note that the difference in mean ages between the (aggregated) groups for comparison in Haga et al.'s study was far larger than in my study. The overall groups used in Haga et al. had a mean age difference of 56.1 years, compared to a

maximum difference of 17.1 years in my study. Looking in detail at several studies showed that where changes in metabolites had been detected the changes were modest in size and not uniform across the brain. The overall consensus is that there *may* be a small decrease in levels of NAA and an increase in Cho, Cr, but this cannot be taken as a robust observation. In regard to my study this suggests that the ANOVA results are most likely unaffected by the difference in age between participant groups. However, a degree of caution is warranted given the literature's suggestion of a possible increase in Cr with age and that the significance of the finding of increased Cr (between OA and HC) did not survive age-controlled analysis. Given the literature's equivocal findings about the effects of age on NAA and that the significance of my observation of increased Thalamic NAA (between FM and OA), this observation can be considered robust. Further studies of the effects of aging on levels of brain metabolites are required, ideally with similar methods, to give a clear statement of the requirement to control for age differences between groups. Wherever possible, however, age matching of groups should be ensured to remove uncertainty in interpreting a study's findings.

As noted, I was unable to recruit sufficient numbers of participants to achieve the desired level of power within the study. An impairment to a study's power level raises the possibility of type 2 errors occurring. This may have been a salient factor in this study as several correlation analyses were observed to be at the trend level of significance when corrected for multiple comparisons. Further studies that achieve a greater level of power may be able to offer confirmation of the validity of the correlations between metabolite levels and clinical measures presented above.

I was also rather limited in exclusive female participation in this study, which precludes the investigation of possible gender effects. However, as FM patients are primarily female (Neumann & Buskila, 2003; Wolfe et al., 1995; Yunus, Masi, Calabro, Miller, & Feigenbaum, 1981), it was decided to only recruit female participants for the other two groups, avoiding any potential gender bias effects. Future studies investigating both male and female participants may therefore be of use.

Building on my results, I intend to investigate if a decrease of NAA in the Thal in OA is correlated with a decrease of grey matter volume in the same region and the same group since previous VBM studies have been equivocal on that matter (Baliki, Schnitzer, et al., 2011; Gwilym et al., 2010). Further to this, I intend to examine if decreased NAA levels in Thal of

OA patients recover after knee surgery as may be suggested from the post-operative recovery of grey matter volume as observed using VBM (Gwilym et al., 2010; Rodriguez-Raecke et al., 2009). If this the case, it will suggest that low NAA level reflects dysfunction of the nervous system rather than degeneration, which might explain why drugs like NSAIDs and simple analgesics, and joint surgery work in OA. Additional investigation of the differences in the neural expression of chronic pain may also be of interest, in particular, studies comparing CWP and CLP to investigate differences in functional connectivity using fMRI.

A potential methodological limitation was my use of the Cramer-Rao Lower Bound (CRLB) as a technique for setting a cut-off above which spectra would be excluded from analysis. As noted, CRLB is a technique, which gives an indication of the goodness of fit between a spectrum and a subsequently fitted model. The CRLB is the minimum possible level of error resulting *from the fitting process*, but should not be taken to indicate the level of error within the measured data itself (Kreis, 2016). CRLBs expressed as a percentage of the estimated value of error are generated by LC-Model and have been used in a large number of studies to establish a threshold above which spectra would be excluded from analysis (Choi & Frahm, 1999; Choi et al., 2006; Träber et al., 2004; Tsai et al., 2007; Walaszek et al., 2012; Zaaraoui et al., 2007). As noted by Kreis, using CRLBs in this manner can distort MRS data since exclusion may apply unequally to groups of participants therefore creating a biased set of data for subsequent analysis. Caution must therefore be applied with the application of thresholds to ensure that the exclusion of data does not lead to erroneous conclusions. The CRLBs of all metabolites within my study, with the exception of Glutamine, were less than 10%. Analysis of the standard deviations revealed that mean percentages were at least 5 standard deviations from the cut-off of 20%. An inspection of these data revealed that no spectra were excluded from the analysis as a result of the 20% cut-off. The CRLB associated with Glutamine was 22% and therefore I decided to extend the cutoff to 30% rather than exclude more data than was necessary. An inspection of the data revealed that approximately one quarter of the spectra were excluded from analysis and therefore any findings related to Gln in this study must be treated with caution.

In conclusion, I have demonstrated differences in the neurochemistry and relationships with clinical measures between two different types of chronic pain conditions, CWP and CLP. OA participants, my model for CLP, exhibited changes usually associated with degeneration

(decreased NAA in the Thal) and ageing (increased Cr and m-Ins in the AntIns), while FM, my CWP model, did not. In addition, Glu was found to correlate with clinical measures associated with pain, but in differing ways between the two pain cohorts, supporting again the existence of differing mechanisms between CLP and CWP. Furthermore, depression and anxiety were found to be negatively associated with measures of Cr and m-Ins in FM, suggesting that the use of Cr as a reference in studies of chronic pain may be problematic. Future MRS investigations of other chronic pain conditions would therefore benefit from using absolute measures of neurometabolites.

Overall, the main difference between CLP and CWP was that in OA there were changes in neurometabolite levels, associated with anatomy and brain function, whereas in FM there were mainly changes in the relationships between neurometabolite levels and clinical measures (depression, anxiety, pain intensity), associated with mood and behavioural functional abilities. This might explain why OA patients give better response to physically targeted therapies (pain killers, anti-inflammatories, surgery, physiotherapy), whereas FM patients have a better response to behavioural therapies (CBT, meditation, hypnosis, yoga) and drugs working centrally such as amitriptyline, pregabalin, and duloxetine. Future MRS investigations of other chronic pain conditions would enable further elucidation of the true relationship between neurometabolites and clinical measures of pain, thus allowing better understanding of pain mechanisms in CLP and CWP. Gaining an understanding of the nature of chronic pain at all its levels is essential in developing novel therapies and the achievement of efficient pain management.

## **CHAPTER 3 - Functional connectivity in chronic pain**

## Introduction

As previously mentioned, pain is “an unpleasant sensory or emotional experience associated with actual or potential tissue damage” and is classified as acute or chronic based on its duration (Merskey & Bogduk, 1994). Malinen et al. noted that acute pain has an important role in protecting the body, which is lost when pain becomes chronic. Further to this, chronic pain decreases quality of life and interferes with cognitive, affective, and physical function (Malinen et al., 2010). Chronic pain is classified as localised or widespread according to the extent of affected areas (Kamaleri, Natvig, Ihlebaek, & Bruusgaard, 2008). Fibromyalgia (FM) is an example of chronic widespread pain (CWP) and knee osteoarthritis (OA) is an example of chronic localised pain (CLP) (Chapter 2). OA is a common condition in the elderly, whereas FM can affect younger adults (Neumann & Buskila, 2003; Zhang & Jordan, 2008). Both diseases have a financial impact on society through public spending on treatment and/or time lost from work (Breivik, Collett, Ventafridda, Cohen, & Gallacher, 2006). FM and OA are two common causes of chronic musculoskeletal pain with contrasting bodily representations. FM is characterised by widespread pain, poor sleep and fatigue with no obvious peripheral pathological cause. OA is a degenerative joint disease with obvious cartilage breakdown and bony deformity leading to dysfunction and pain in the affected area (Altman et al., 1986).

Chronic pain has previously been associated with changes in brain activity: FM, chronic low back pain (CLBP), neuropathic pain, temporomandibular disorder (TMD), OA and other chronic pain conditions have all been investigated using fMRI (task-based and resting-state) (Baliki, Baria, & Apkarian, 2011; Baliki et al., 2012; Baliki, Geha, Apkarian, & Chialvo, 2008; Cauda et al., 2010; Franco Cauda, Sacco, D’Agata, et al., 2009; Franco Cauda, Sacco, Duca, et al., 2009; Cifre et al., 2012; Geha et al., 2007; Gracely, Petzke, Wolf, & Clauw, 2002; Ichesco et al., 2012; Jensen et al., 2013; Malinen et al., 2010; Napadow et al., 2010; Napadow, Kim, Clauw, & Harris, 2012; Parks et al., 2011). Whilst Giesecke et al. compared CLBP and FM brain responses using painful stimuli (Giesecke et al., 2004), there has not yet been a study that contrasts the effects of chronic localised and widespread pain using resting-state fMRI. Indeed, López-Solà et al., who used fMRI to assess brain response to auditory, visual and tactile-motor stimulation in FM patients, acknowledged the absence of a direct

comparison between the neural effects of two different chronic pain conditions as being a limitation of their study (López-Solà et al., 2014).

One aspect of the effects of chronic pain on the central nervous system is altered functional connectivity (FC) within and between regions comprising identified brain networks (Baliki, Mansour, Baria, & Apkarian, 2014; Cauda et al., 2010; Cifre et al., 2012; IchESCO et al., 2012; Napadow et al., 2012). FC is a measure of the level of correlation of activity within two or more brain regions: strong positive correlations in fluctuations in the neural activity of brain regions are taken to represent a high level of FC (Fox et al., 2005). FC is assessed using resting-state fMRI, which measures the fluctuations in BOLD signal, taken to reflect changes in neural and metabolic activity, in a resting participant over a short period of time (Napadow et al., 2010). Studies of the effects of chronic pain on FC between brain regions have shown altered connectivity between elements of the default mode network (DMN) and areas associated with the processing of painful stimuli, such as the insula (Cifre et al., 2012; IchESCO et al., 2012; Napadow et al., 2012). Other studies have demonstrated altered FC within the DMN and between the DMN and attentional networks (Baliki, Mansour, Baria, & Apkarian, 2014; Cauda et al., 2010). I shall examine the nature of the DMN and attentional networks below, but given the attentional and cognitive disruption reported by chronic pain patients (Cifre et al., 2012) and the complementary nature of attentional and default mode networks it is reasonable to examine FC within and between these networks in the context of chronic pain conditions with differing aetiologies to gain a better understanding of their effects.

The DMN (Greicius, Krasnow, Reiss, & Menon, 2003) comprises brain regions believed to be involved in self-referential thinking and includes brain areas such as the inferior parietal lobule, the posterior cingulate cortex (PCC) and precuneus (PC), the medial frontal gyri, the hippocampal formation, and lateral temporal cortex (Greicius, Krasnow, Reiss, & Menon, 2003; Napadow et al., 2010). The PCC and PC are considered to constitute the “core hub” of the DMN and exercise influence on activity in the other regions of the network (Fransson & Marrelec, 2008; Utevsky, Smith, & Huettel, 2014). The DMN is more active at rest and “deactivated” during externally directed tasks (Napadow et al., 2010; Uddin, Kelly, Biswal, Castellanos, & Milham, 2009). This reduction in DMN activity during task-based fMRI is more pronounced in participants with chronic pain when compared to healthy controls (Baliki,

Geha, Apkarian, et al., 2008). Previous examinations of the effects of chronic pain on resting state FC in the DMN have found increased FC within the DMN (Baliki et al., 2014); increased FC between the DMN and areas associated with pain (Tagliazucchi, Balenzuela, Fraiman, & Chialvo, 2010); and it has been suggested (Loggia et al., 2013) that increased FC within the DMN encodes the intensity of pain experienced by chronic pain patients. Given these observations it is plausible to expect that a higher level of DMN FC would be expected in chronic pain patients when compared to healthy controls.

The EAN (Posner & Dehaene, 1994), in contrast, consists of brain regions involved in cognitive processing of working memory and attention and includes the frontal and parietal lobe regions, specifically the dorsolateral prefrontal cortex (DLPC) and posterior parietal regions overlapping the superior parietal lobule (SPL) and intraparietal sulcus (Fan, McCandliss, Fossella, Flombaum, & Posner, 2005; Napadow et al., 2010). Of these areas, it has been suggested that the SPL plays a critical role in the transfer of attention between sources, the recruitment of other network regions and the establishment of attention (Behrmann, Geng, & Shomstein, 2004; Wolpert, Goodbody, & Husain, 1998). Behrmann et al. made the observation that whilst the SPL is pivotal in the establishment or alteration of attention it is not the primary EAN component required for maintenance of task-specific attention. Given that this study examined activity in the resting brain and considered ‘spontaneous’ activity in brain networks, the SPL is an appropriate candidate for investigation since it is reasonable to expect activation in this area during a spontaneous shift of activity from the DMN to the EAN. The SPL’s role in establishing attention suggests that a strong anticorrelation of activity between the core hub of the DMN and the SPL would be expected in normal brain operation.

The objective of this study was to investigate the degree of connectivity within and between three brain networks – the DMN, the left EAN (EANL) and the right EAN (EANR) – using resting-state fMRI in patients with chronic pain, comparing between FM, OA and healthy controls (HC). In addition, given previously noted differences in the experience of pain suffered by FM and OA patients (for example, Marques, Rhoden, de Oliveira Siqueira, & Joao, 2001) I compared how activity across these networks correlates with clinical measures taken from the McGill questionnaire (Melzack, 1975).

My hypotheses for this study were as follows:

1. Since differences between CWP and CLP have been found in clinical measures and neurochemical profiles (Chapter 2), I hypothesized that there may be differences in the connectivity of the components of the DMN and EAN between the two chronic pain conditions. Given that the normal DMN response is usually disrupted during a state of chronic pain and cognitive deficits specific to working memory and attention are common in patients with chronic pain (Glass, 2009), I hypothesized that intrinsic connectivity between critical regions of DMN (PCC) and EAN (SPL) and the other regions within the networks would be altered in patients with CWP and CLP compared to HC.
2. Given the previously observed effects on chronic pain conditions on the direction of attention and that this process entails interaction between core regions of the DMN and EANs, I was motivated to investigate FC between the EAN and DMN seed regions and components of the other network. I hypothesized that increased FC between the SPL and components of the DMN would be observed in CWP and CLP compared to HC. I similarly hypothesized that increased FC would be observed between the PC and regions of the EAN in CWP and CLP compared to HC.
3. I hypothesized that these alterations in the FC observed in pain patients would be associated with altered clinical measures. Specifically, I hypothesised that there would be a correlation between clinical measures of patients' pain and between-network FC, and that observed correlations would differ between pain conditions.

## **Methods and participants**

### **Participants**

All participants were females aged 31-65. FC data was collected from 9 participants with FM (mean +/- SD age 47 +/- 7.87 years), 14 participants with OA of the knee (mean +/- SD age 62.07 +/- 3.12 years), and 12 HC (mean +/- SD age 44.5 +/- 11.04 years) for this study.

FM participants were classified using the American College of Rheumatology criteria (Wolfe et al., 1990) for the classification of FM, such that each subject fulfilled the following criteria: 1) widespread pain in all four quadrants of the body for at least 3 months; 2) tenderness or pain to touch in at least 11 out of 18 specific anatomical sites described as tender points. FM participants were recruited from a district general hospital fibromyalgia clinic in North Wales. FM participants were excluded if they had knee OA. Participants with knee OA were recruited from the pre-operative arthroplasty clinic at Ysbyty Gwynedd, Bangor. Participants with rheumatoid arthritis and OA of other joints were excluded, as was anyone who also had a diagnosis of co-morbid FM. HC participants without chronic pain were recruited using the Bangor University's School of Psychology's community participant panel and local advertisements. All participants were asked to refrain from using analgesic medication on the day of the scan until after scanning was completed. Normal contraindications for MRI were also used as exclusion criteria (e.g. pacemakers, metal implants, claustrophobia).

This study was approved by the Ethics Committee from the School of Psychology, Bangor University and North West Wales Research Ethics Committee (reference 10/WNo01/16). Research activities took place in Bangor Imaging Centre, Bangor University. All participants provided informed consent.

## **Methods**

Functional connectivity (FC) was examined using the seed-based correlation method (Fox et al., 2005) within and between three brain networks: the default mode network (DMN), the executive attention network left (EANL), and the executive attention network right (EANR). Seeds (12 mm diameter spheres, as per Fox et al. within core regions of the networks of interest were selected in the posterior cingulate cortex for the DMN (ROI1), the left superior parietal lobule for EANL (ROI2), and the right superior parietal lobule for EANR (ROI3) (see Table 10).

Network (ROI)	Seed region	X	Y	Z
DMN (ROI1)	Posterior cingulate cortex	0	-48	30
EANL (ROI2)	Superior parietal lobule left	-36	-62	50
EANR (ROI3)	Superior parietal lobule right	36	-62	50

ROI – Region of Interest

DMN – Default Mode Network

EANL – Executive Attention Network Left

EANR – Executive Attention Network Right

*Table 10: MNI coordinates for seed regions*

To enable analysis of the FC between the selected seed regions and the networks of interest, masks of the DMN, EANL and EANR were created. This was accomplished using WFU Pickatlas to combine templates of brain regions within the networks (Maldjian, Laurienti, Kraft, & Burdette, 2003).

### *Functional Magnetic Resonance Imaging*

Resting state fMRI images were acquired using a single-shot echo-planar pulse sequence on a 3 Tesla Phillips Achieva MRI system (Philips Health Care, Eindhoven, Netherlands). Each three-dimensional volume comprised 35 contiguous axial slices in an ascending order (TR/TE = 2000/30ms, flip angle 65 degrees, voxel size 3x3x3mm). During the 6-minute (~360 second) resting state fMRI acquisition period (175 scans), the subjects were asked to remain still with their eyes open. An anatomical, T1-weighted gradient echo data set (TR 17ms, TE 6.5ms and 12.2ms, voxel size 0.7x0.7x0.7mm) was also acquired for each subject to assist with registration to standard stereotactic space. During the resting state data collection, 175 volumes were acquired. No volumes were discarded before analysis, as the Philips scanner automatically excluded T1 magnetisation equilibrium volumes.

## *Image Analysis*

Pre-processing of the data was carried out using Data Processing Assistant for Resting-State fMRI (DPARSF) (Yan and Zang, 2010) which is based on Statistical Parametric Mapping (SPM8) (<http://www.fil.ion.ucl.ac.uk/spm>) and Resting-State fMRI Data Analysis Toolkit (REST, (Song et al., 2011)). Data were slice timing corrected and realigned to the first volume. The anatomical image was coregistered to the mean functional image and then segmented using the 'New Segment' method into grey matter, white matter and cerebrospinal fluid (CSF). Functional data were then normalised to standard space and smoothed with a 9 mm Gaussian kernel using DARTEL (Ashburner, 2007). A group-specific template for the entire data set was created for this purpose.

## *Analysis of resting state FC*

For each of the defined seed regions the procedure described below was performed. The resting state time series of the seed region was correlated with resting state activity in each of the networks (DMN, EANL, EANR), for each group of participants (FM, OA, HC). Three one-way ANOVAs were performed to examine relative differences in FC between the seed region and each network between groups of participants. Thus, as an example, the first ANOVA examined differences in FC between the DMN seed region (the PCC) and the rest of the DMN across participant groups (i.e. an examination of FC differences **within** the DMN **between** participant groups); the second ANOVA examined differences in FC between the PCC and the EANL across participant groups (i.e. an examination of FC differences **between** the DMN seed and the EANL **between** participant groups); and the third ANOVA examined differences in FC between the PCC and the EANR across the participant groups (**between** the DMN seed and EANR **between** participant groups). The following planned comparisons were used within the ANOVAs: FM>HC, FM>OA, OA>HC, OA>FM, HC>FM, and HC>OA. Thresholds for significance were set at  $p < .05$  and included an extent threshold of 100 voxels, which means that all clusters are significant at the family-wise corrected level. Regions showing significant differences in FC with the seed region between participant groups were labelled using the Automatic Anatomic Labelling toolbox (AAL) (Tzourio-Mazoyer et al., 2002).

FC between the seed regions of all pairs of networks (DMN-EANL, DMN-EANR, EANL-EANR) was assessed using DPARSF to examine correlation between the activity of each

network's seed region. The seed regions were taken to be representative of overall activity within the networks of interest, as discussed earlier. These levels of between-network FC were generated to enable correlations to be made between the patient groups' clinical measures and the level of FC between the networks of interest.

#### *Analysis of correlations of FC with pain measures*

The McGill Pain Questionnaire was designed to provide quantitative measures of clinical pain. It consists of three classes of word descriptors, such as sensory, affective and evaluative, which are used by subjects to describe subjective pain experience. There are three major measures: the pain-rating index (PRI) – sum and rank, the number of words chosen (NWC), and the present pain intensity (PPI) (Melzack, 1975). In the present study PRI and NWC measures were collected. The measures were then compared between the patients groups (FM and OA) using an independent-samples t-test.

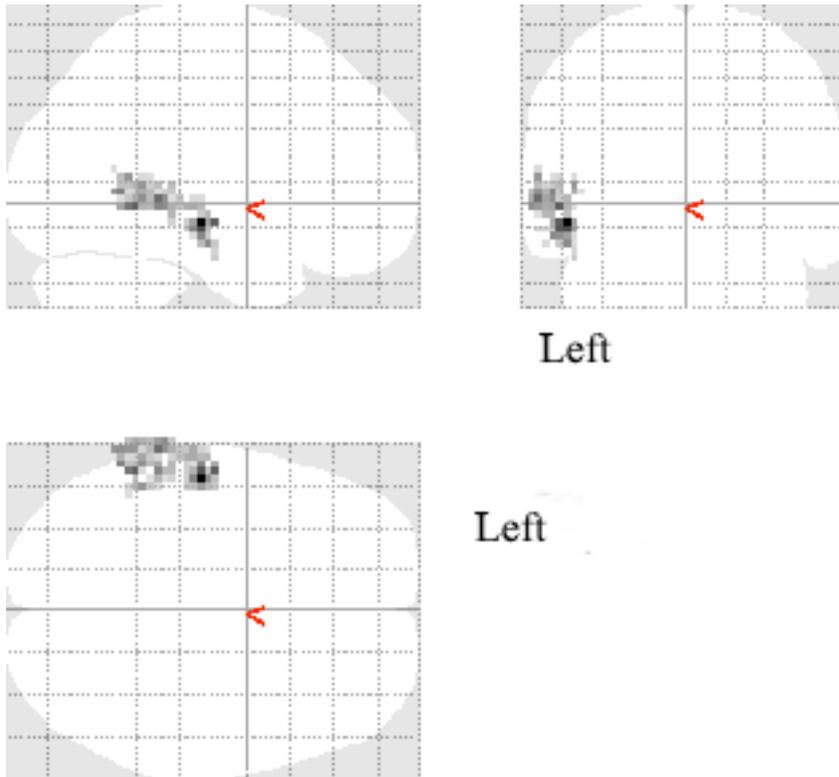
The relationships between the McGill Pain Questionnaire's measures and resting state FC between networks, as represented by the level of FC observed between each network's seed voxel was performed. The relationships between these variables were investigated using Spearman's Rank Order Correlation ( $\rho$ ) coefficient as the variables were related in non-linear fashion.

## Results

I observed that functional connectivity (FC) between the DMN seed region (in the PCC) and the rest of the DMN during resting state was higher within areas of the DMN in both chronic pain conditions compared to HC (see Table 11). FC between the PCC and the left middle temporal gyrus was higher in FM when compared to OA (see Figure 4), and higher between the PCC and both middle temporal gyri in FM when compared to HC. FC between the PCC and the precuneus (left and right) was higher in OA when compared to HC. No areas of significantly different FC between the PCC and areas of the EANs were observed between participant groups.

FC was lower between the EANL seed region (in the left SPL) and areas of the EANs in both pain conditions compared to HC (see Table 12). FC between the left SPL and the inferior and middle frontal gyri was lower in both pain conditions in comparison with HC (see Figures 5 and 6). FC was lower in OA compared to HC between the EANR seed region (in the right SPL) and the left inferior and middle frontal gyri (see Table 13).

FC between the EANL seed region (in the left SPL) and areas of the DMN was higher in both pain conditions compared to HC (see Table 12). FC between the left SPL and the precuneus (left and right) was higher in FM compared to HC. FC between the left SPL and right middle temporal gyrus was higher in OA compared to HC. I observed areas of higher FC between the EANR seed region (in the right SPL) and the precuneus (left and right) in the DMN in FM compared to HC (see Table 13).



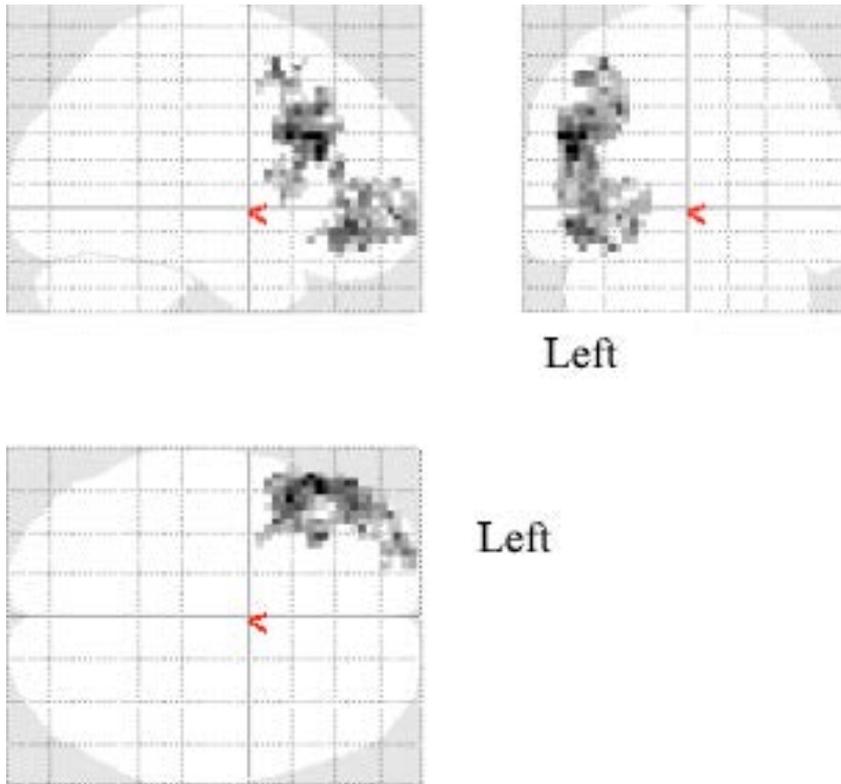
rs-FC – resting-state functional connectivity

FM – fibromyalgia

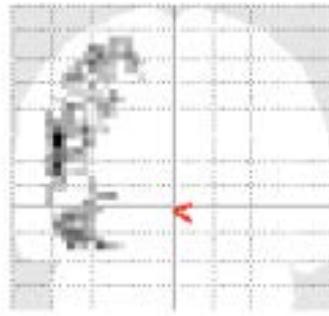
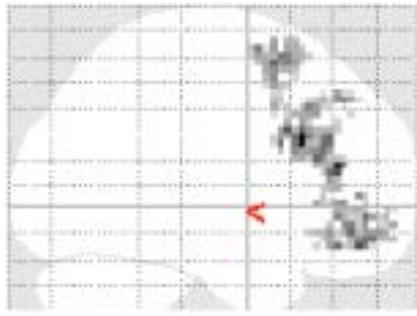
OA – knee osteoarthritis

DMN – default mode network

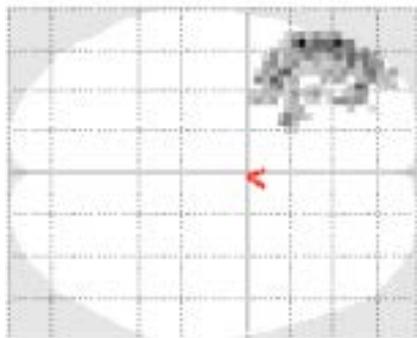
*Figure 4: Areas of significantly higher resting-state functional connectivity in fibromyalgia compared to knee osteoarthritis within the default mode network*



*Figure 5: Areas of significantly lower resting-state functional connectivity in fibromyalgia compared to healthy controls within the executive attention network left*



Left



Left

*Figure 6: Areas of significantly lower resting-state functional connectivity in knee osteoarthritis compared to healthy controls within the executive attention network left*

Name of Brain Region	PFWE - corrected (Cluster Level)	% Cluster	Cluster Size (N of voxels)	Z Score (Peak Value)	MNI Coordinates X, Y, Z mm
Contrast FM>OA, Masked by DMN					
MTGL	0.018	97.47	158	4.65	-51 -21 -9
Contrast FM>HC, Masked by DMN					
MTGR	0.011	94.71	170	3.85	57 -9 -12
MTGL	0.010	95.95	173	3.72	-51 -21 -9
Contrast OA>HC, Masked by DMN					
PR	0.012	59.52	168	3.35	0 -54 54
PL		37.50		3.21	9 -39 51

MTGL - Middle Temporal Gyrus Left

MTGR - Middle Temporal Gyrus Right

PR - Precuneus Right

PL - Precuneus Left

*Table 11: Significant Results of Group Analysis with ROI1 – DMN*

Name of Brain Region	PWE - corrected (Cluster Level)	% Cluster	Cluster Size (N of voxels)	Z Score (Peak Value)	MNI Coordinates X, Y, Z mm
Contrast FM>HC, Masked by DMN					
PL	0.013	55.56	153	3.55	-6 -51 51
PR		39.87		3.29	9 -39 51
Contrast OA>HC, Masked by DMN					
MTGR	0.003	96.20	184	4.01	51 -42 9
Contrast FM<HC, Masked by EANL					
IFGL, PT	0.000	30.96	533	4.23	-51 27 27
MFGL		28.89		4.16	-45 15 27
IFGL, PO		12.38		3.70	-51 39 -9
Contrast OA<HC, Masked by EANL					
IFGL, PT	0.001	63.94	208	4.63	-51 21 27
MFGL		26.44		4.16	-51 36 15
Contrast FM<HC, Masked by EANR					
MFGR	0.016	54.36	149	3.83	51 36 18
IFGR, PT		39.60		3.23	51 33 30

PL - Precuneus Left

PR - Precuneus Right

MTGR - Middle Temporal Gyrus Right

IFGL, PT - Inferior Frontal Gyrus Left, Pars Triangularis

MFGL - Middle Frontal Gyrus Left

IFGL, PO - Inferior Frontal Gyrus Left, Pars Orbitalis

MFGR - Middle Frontal Gyrus Right

IFGR, PT - Inferior Frontal Gyrus Right, Pars Triangularis

*Table 12: Significant Results of Group Analysis with ROI2 – EANL*

Name of Brain Region	PFWE - corrected (Cluster Level)	% Cluster	Cluster Size (N of voxels)	Z Score (Peak Value)	MNI Coordinates X, Y, Z mm
Contrast FM>HC, Masked by DMN					
PL	0.006	60.77	181	3.33	3 -57 45
PR		33.15		3.30	-15 -66 60
Contrast OA<HC, Masked by EANL					
IFGL, PT	0.027	71.62	148	3.59	-45 30 18
MFGL		12.84		3.13	-51 24 30

PL - Precuneus Left

PR - Precuneus Right

IFGL, PT - Inferior Frontal Gyrus Left, Pars Triangularis

MFGL - Middle Frontal Gyrus Left

*Table 13: Significant Results of Group Analysis with ROI3 – EANR*

An independent-samples t-test was conducted to compare the McGill pain questionnaire's measures for FM and OA. There was a significant difference in mean scores for evaluative rank of pain rating index (PRI) between FM (M=4.11, SD=1.45) and OA (M=2.93, SD=1.27);  $t(21) = 2.06, p < .05$  (two-tailed). The magnitude of the differences in the means was large (eta squared = .18). The McGill Pain Questionnaire's measures are presented in Table 14 (significant differences in bold).

Patient Group	S	A	Mean E	(SD) M	T	NWC
FM	24.67 (7.11)	6.11 (3.48)	<b>4.11</b> (1.45)	9.00 (4.09)	43.89 (13.68)	15.89 (3.48)
OA	25.29 (7.57)	5.14 (2.85)	<b>2.93</b> (1.27)	8.79 (3.77)	42.14 (14.22)	15.86 (3.72)

FM – fibromyalgia                      OA – knee osteoarthritis

S – sensory rank of pain rating index (PRI)

A – affective rank of PRI

E – evaluative rank of PRI

M – miscellaneous rank of PRI

T – total rank of PRI

NWC – number of words chosen

*Table 14: The McGill Pain Questionnaire’s measures (mean scores)*

I examined the relationships between the McGill Questionnaire’s measures and resting state FC. Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity and homoscedasticity. Significant results for correlations between the McGill Questionnaire’s measures and resting state FC ( $r$  ranging from  $|0.54|$  to  $|0.84|$ ) are presented in Table 15. FM showed positive correlations of affective rank of pain rating index (PRI) with FC between all three ROI (DMN-EANL, DMN-EANR, EANL-EANR) and PRI’s evaluative rank with FC between two ROI (DMN-EANL and EANL-EANR), whereas OA revealed a negative correlation of affective rank of PRI with FC between DMN and EANR. It must be noted that comparisons between patients’ FC and 7 clinical measures were made. Applying a Bonferroni correction to these comparisons gives a p-value of .007 for a significant difference. It can be seen that only the correlation between FM patients’ evaluative measure of their pain and FC between DMN and EANL achieved this level of significance: all other observations must be treated speculatively.

Correlations	Correlation Coefficient	Significance Level	Number of Cases
FM			
A – DMN/EANL	0.66	p = .051	9
A – DMN/EANR	0.72	p = .028	9
A – EANL/EANR	0.77	p = .015	9
E – DMN/EANL	0.84	<b>p = .005</b>	9
E – EANL/EANR	0.78	p = .014	9
OA			
A – DMN/EANR	- 0.54	p = .048	14

DMN – Default Mode Network

EANL – Executive Attention Network Left

EANR – Executive Attention Network Right

A – PRI (AR) – Pain Rating Index (Affective Rank)

E – PRI (ER) – Pain Rating Index (Evaluative Rank)

*Table 15: Significant correlations between McGill Scores and FC*

## **Discussion**

### **Summary of results**

My hypothesis of altered FC between the core hub of the DMN (the PCC) and other areas of the network in chronic pain patients when compared to healthy controls was supported by the results. My hypothesis of altered FC in chronic pain patients between the SPL and other areas of the EANs was also supported. My hypothesis of rs-FC differences between CWP and CLP was supported by the observation of areas of higher connectivity between the PCC and the middle temporal gyrus left (MTGL) in CWP when compared to CLP. Additionally, the alterations observed in FC correlated with differing clinical measures in FM and OA.

I observed greater FC in FM compared to OA in the MTGL within the DMN. Furthermore, higher FC in FM compared to HC was found in the MTGL and MTGR. In contrast, lower FC in FM compared to HC was found in the inferior frontal gyrus left (IFGL), pars triangularis (PT) and orbitalis (PO) and the middle frontal gyrus left (MFGL) within the EANL. Greater between-network FC was observed in FM compared to HC between the SPL in the EAN and the precuneus (left and right) in the DMN.

OA participants showed higher FC compared to HC in the left and right precuneus (PL and PR) within the DMN. In contrast, lower FC in OA compared to HC was found in the IFGL, PT and the MFGL within the EANL. Greater between-network FC was observed in OA compared to HC between the SPL in the EANL and the MTGR in the DMN.

Finally, a correlation between the evaluative element of the McGill Questionnaire and resting state FC were found in FM. Other correlations were observed, but must be treated with caution given their failure to survive Bonferroni correction.

### **What my findings within and between brain networks of interest tell us?**

My findings demonstrated increased FC between areas of the DMN and decreased FC between areas of the EANs in both chronic pain conditions when compared to healthy controls. There were also altered FC between areas of the DMN and EAN observed in the pain patients. These suggestions have parallels with observations in previous studies of the

effects of chronic pain on FC across brain networks. I will consider each of these findings in turn.

### *Increased FC between areas of the DMN in pain patients*

Resting-state FC in areas of the DMN was increased in both chronic pain conditions in comparison with HC. It is instructive to consider what increased connectivity between the core of the DMN and other DMN regions may imply. Recent studies have shown that enhanced DMN FC has been associated with a range of adverse conditions. Gardini et al. demonstrated increased DMN FC in participants with mild cognitive impairment (Gardini et al., 2015); Cowdrey et al. observed an increased DMN FC in participants who had experienced anorexia nervosa (Cowdrey, Filippini, Park, Smith, & McCabe, 2014); Luo et al. demonstrated a link between higher levels of DMN FC and lower levels of happiness (Luo, Kong, Qi, You, & Huang, 2015); and McCormick et al. observed a link between impaired memory performance and increased DMN FC (McCormick et al., 2014). McCormick et al. specifically highlighted that abnormally high connectivity between the MTGs and the posterior DMN, including PCC, was associated with impaired episodic memory performance. I will consider these findings, as related to individual DMN regions, in detail in the following section, but it is noteworthy that increased FC within the DMN has been observed after a range of chronic conditions and has been associated with adverse outcomes. Adding to these findings, my observations suggest adaptation of brain connectivity in response to prolonged atypical experience: with a higher level of alteration in CWP than CLP as PCC-MTG FC connectivity was increased to a greater degree in FM than OA.

This observation is in accord with previous studies, which found that supratentorial processing of neuropathic stimuli in chronic pain may alter FC at resting state. For example, it was reported that rs-FC was decreased between the thalamus and the cortex in diabetic neuropathic pain patients compared to HC (Cauda, Sacco, D'Agata, et al., 2009). Cifre et al. demonstrated a considerable imbalance of the FC within the pain network during rest in patients with FM, 'suggesting that chronic pain may also lead to changes in brain activity during internally generated thought processes such as occur at rest' (Cifre et al., 2012). Cifre's observation is in agreement with the conclusion drawn by Luo that increased FC within areas of the DMN was associated with lower levels of happiness as the increased FC was strongly associated with internally generated 'ruminative' negative thought.

### *Decreased FC between areas of the EAN in pain patients*

I observed that FC in both chronic pain conditions was lower in areas of EANs compared to HC. Had the participants in this study been performing an externally directed task it would be reasonable to take this result as evidence of the disruption of the direction of attention associated with chronic pain (Dick & Rashiq, 2007). However given the nature of rs-FC, my participants were necessarily observed at rest and therefore it is challenging to interpret decreased FC in the EAN. No other studies have observed decreased FC in the EAN in either OA or FM in comparison to HC. A study by Napadow et al (2010) observed increased FC in areas of the EANR in FM patients at rest when compared to HC. Napadow et al interpreted this observation as suggesting that potentially indicative of pain causing the consumption of attentional resources that would be available for executive functioning during task performance i.e. the aberrant activity observed at rest would also be present during task related EAN activity and would therefore impair intended function. I did not observe any areas of increased FC in the EAN when comparing pain conditions to HC, however, it is possible that the areas of abnormally low FC I observed in the EAN of pain patients at rest may also have implications for the activity of this network during execution of a task. Disruption of directed attention in chronic pain is especially pronounced in FM patients as manifest in deficits of episodic memory and cognition (Glass, 2009). Given Luo's (Luo et al., 2015) observation about elevated levels of rumination in FM an investigation of the correlation between rumination and altered levels of FC in FM patients is warranted.

### *Increased between-network FC in pain patients*

I observed greater FC between- areas of the DMN and EAN in both pain conditions when compared to HC. These observations are in broad agreement with those of previous researchers who have recorded increased FC between areas of both the DMN and EAN and other areas of other brain networks. Ichesco et al. revealed an increased FC between the left anterior insular cortex (IC) and pregenual anterior cingulate cortex (ACC) in TMD patients, during both resting state and the application of a painful stimulus to the face (Ichesco et al., 2012). Napadow et al. showed that FM patients had greater connectivity within the DMN and greater connectivity between the DMN and the insular cortex, which is a brain region known to process evoked pain (Napadow et al., 2010). This observation is in accord with the findings of Martucci et al. (2015) observed increased functional connectivity within the DMN

and between the DMN and insula in chronic pelvic pain syndrome patients. Likewise, Malinen et al. showed altered spatial connectivity between insula and ACC in chronic pain patients using seed point-based correlation analysis (Malinen et al., 2010). It implies ‘both temporally and spatially aberrant activity of the affective pain processing areas in patients suffering from chronic pain’ (Malinen et al., 2010). These findings give support to the suggestion that chronic pain conditions disrupt the activity of the resting brain and, as suggested by Napadow et al., that abnormal inter-network activity ‘may also have broader implications for how subjective experiences such as pain arise from a complex interplay among multiple brain networks’ (Napadow et al., 2010).

Taken together with my observations, these findings demonstrate alterations in FC within brain networks resulting from chronic pain, highlight that aberrant network activity alters a participant’s experience of acute stimuli and also affects ongoing interaction between brain networks.

#### *FC and clinical measures*

The only correlation between FC and patients’ clinical measures found to be significant after Bonferroni correction was the evaluative element of the McGill Questionnaire and FC between the DMN and EANL in FM. I noted previously that increased levels of between-network FC may relate to cross-network activity entailed in spontaneous attention shifts towards pain, but it is challenging to suggest why this increased activity would correlate specifically with FM patients’ evaluation of their pain. It is possible that this is indicative of a more general set of relationships between clinical measures and between-network FC that my study was not sufficiently powerful to capture. I present below the other observed correlations, which did not survive Bonferroni correction as speculative observations, which may inform future studies with greater power.

A positive correlation was observed between affective rank of pain rating index (PRI) and with between-network FC using all seed regions for the FM group. Higher affective ranks of PRI in FM were associated with higher levels of FC between seed regions (i.e. between the DMN and EANs). Given the typically anticorrelated activity of the DMN and EAN, the increased FC between network regions in FM may indicate dyscoordination of network based brain activity. That is, the transitions between DMN and EAN activity represented by

anticorrelation between these networks are impaired in FM and thus increased cross-network activity between DMN and EANs can be observed.

It has been observed that the affective component of experienced pain increases in FM (Gracely et al., 2004). This observation, coupled with the increased FC in the DMN may suggest the presence of a feedback loop where patients concentrate on their pain, increasing their awareness of this sensation, which in turn increases the focus of their concentration on their condition.

In contrast, the OA patients showed a negative correlation of affective rank of PRI with FC between DMN and EANR. This might suggest that the affective component of pain is less pronounced in OA compared to FM. However, a comparison of affective ranks of PRI between FM and OA did not show a significant difference. Further research in revealing different components of pain between the two chronic pain conditions is needed.

### **What my findings within specific brain regions tell us?**

It is also informative to consider brain regions with altered FC within and between DMN and EAN in the context of their function. Within the DMN, FC in FM was higher compared to OA and HC between PCC and precuneus left and right, PCC and medial temporal gyrus left and right, PCC and medial frontal gyrus left and right.

The precuneus, a part of the medial posterior parietal cortex, is engaged in reflective, self-related processing (Kjaer, Nowak, & Lou, 2002; Lou et al., 2004). It is plausible to suggest that, given its role, the precuneus may be active with rumination in FM, and therefore may be increasing the level of patients' experienced pain. The precuneus is also involved in awareness and conscious information processing (Kjaer, Nowak, Kjaer, Lou, & Lou, 2001; Vogt & Laureys, 2005), episodic memory (Dörfel, Werner, Schaefer, Von Kummer, & Karl, 2009; Lundstrom et al., 2003; Lundstrom, Ingvar, & Petersson, 2005), and visuospatial processing (Kawashima, Roland, & O'Sullivan, 1995; Wenderoth, Debaere, Sunaert, & Swinnen, 2005), as well as showing greater activity during resting as compared to responding to an external task (Cavanna & Trimble, 2006; Cavanna, 2007; Fransson & Marrelec, 2008;

Zhang & Li, 2012). Future fMRI research looking at the involvement of the precuneus in ruminative processes in FM would therefore be of interest.

The middle (medial) temporal gyrus is involved in a number of cognitive processes, including semantic memory processing, language processes, and integrating information from different senses (Calvert, Campbell, & Brammer, 2000; Squire & Zola-Morgan, 1991). Together with the inferior temporal gyrus, it has been implicated in recognising and interpreting information about faces. Both structures are a part of the ventral visual pathway, which identifies “what” things are. The inferior temporal gyrus also participates in visual perception and some forms of mental imagery (Eskandar, Richmond, & Optican, 1992; Andrea Mechelli, Price, Friston, & Ishai, 2004). The medial temporal gyrus has been connected with processes as different as contemplating distance, recognition of known faces, and accessing word meaning while reading (Acheson & Hagoort, 2013). Kirkham (2010) observed marked differences between the responses of FM and OA patients when asked to draw their pain (see Figure 7). Despite the differences in the distribution of physical sources of the patients’ pain, it is worth considering the role played by the MTGL and the involvement of mental imagery in the patients’ experience of their condition. Differences within FC in the MTGL between pain conditions may suggest a differing use of mental imagery by patients in relation to their condition. Further research is warranted to uncover the links between activity within the MTGL and patients’ use of self-referential or external imagery in relation to their experience of pain.

Draw on this diagram whatever the shape or form your distress takes (Black pen)

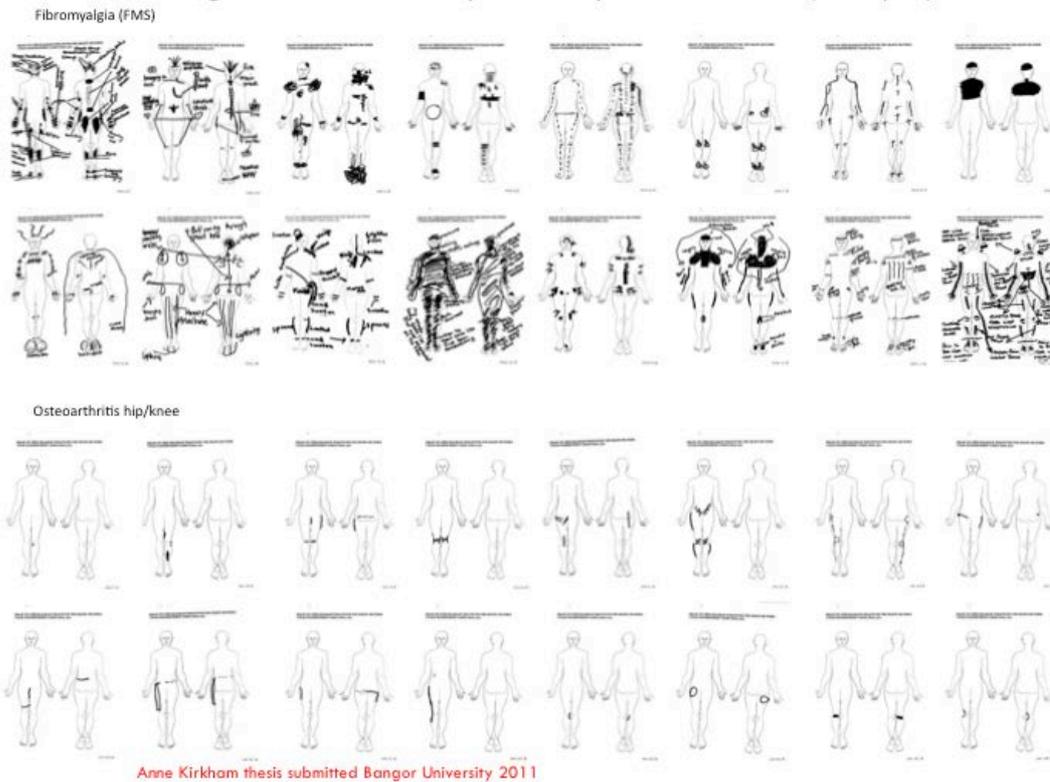


Figure 7: Differences between FM and OA patients in drawing their pain

The left inferior frontal gyrus left (IFGL) has consistently been associated with both phonologic and semantic operations in functional neuroimaging studies (Costafreda et al., 2006; Katzev, Tuscher, Hennig, Weiller, & Kaller, 2013). There is growing interest regarding the role of the inferior frontal gyrus right (IFGR) during a particular form of executive control referred to as response inhibition (Hampshire, Chamberlain, Monti, Duncan, & Owen, 2010).

Inferior frontal gyrus, pars triangularis, is a part of Broca's area, which is involved in semantic tasks. Inferior frontal gyrus, pars orbitalis, is associated with controlled semantic retrieval (Sabb, Bilder, Chou, & Bookheimer, 2007) and processing the semantic aspects of a sentence (Dapretto & Bookheimer, 1999).

Overall, from analysing the functions of brain areas involved in DMN and EAN, it may be suggested that those brain networks intersect morphologically and functionally. Both brain networks are altered in chronic pain. For example, the increased FC in the FM cohort may

indicate increased emotional processing (ACC), increased monitoring of sensory input and an attempt to make sense of it (MTG), as well as increased rumination and reflective self-referential processing of that input (precuneus).

There are some limitations to my study, the biggest being the age difference and small populations. A plausible suggestion of the age difference may be that greater FC in FM compared to OA could be a result of OA group being older than FM. However, FC in OA is greater than in HC (being older as well), where aging usually results in a decrease, not an increase of rs-FC (Damoiseaux et al., 2008). Larger participant groups may have led to more powerful results, but numbers of participants between 16-32 have also been suggested to be useful in imaging studies (Friston, 2012). As a preliminary exploration of the effects of chronic pain on brain networks connectivity at rest my results have shown interesting alterations in FC that may suggest more directed research questions for future studies.

The broader implications of my study suggest that differences between resting-state FC measures in chronic pain conditions support the use of differing pain therapies in different conditions, especially when considering behavioural based therapies addressing the affective component of pain. Alterations in FC in chronic pain may also serve as biomarkers for chronic pain, but further research of FC in various and larger clinical pain populations is needed. However, differences in FC in various chronic pain conditions may provide another index for the development of individualized pain management.

In conclusion, I have presented the first study to compare resting-state FC in two different chronic pain conditions. Differences in FC between CWP and CLP were found, such as an increased FC in FM participants compared to OA within DMN (PCC – MTGL). Furthermore, I observed a suggestion of differing correlations between FC and clinical measures in FM and OA, though only a single correlation achieved robust significance. The other correlations observed may be suggestive of both affective and evaluative clinical measures showing a greater role in predicting FC in FM, compared to the affective measure in OA, though a further study of greater power would be required to confirm this suggestion. This suggestion fits with an increased attention to pain in FM, consistent with increased rumination, suggesting FM patients concentrate on their pain, increasing their awareness of it, which further increases their attention to it in a feedback loop. FC in DMN in chronic pain was

increased compared to HC, with a greater effect in FM. In contrast, FC in EAN in chronic pain was decreased in comparison with HC. Both these results are in line with my hypotheses. These results showing changes within the brain in chronic pain also provide some justification for the use of central painkillers in chronic pain as well as suggesting that some cognitive therapies, which have also been shown to alter FC, may be useful as treatments for chronic pain, especially in FM.

## **CHAPTER 4 -VBM in chronic pain: differences and similarities between widespread and local pain**

## Introduction

Chronic pain is defined as pain that lasts longer than 3 months (Merskey & Bogduk, 1994). This is a highly prevalent condition that has adverse effects across many areas of patient health including sleep, cognition, mental health and general quality of life (Fine, 2011). The effects of chronic pain can be exacerbated by the development of increasingly complex pathophysiology that renders effective treatment challenging (Fine, 2011). The far reaching effects of chronic pain mean that many patients suffer serious economic consequences (Fine, 2011).

Osteoarthritis (OA) and fibromyalgia (FM) are chronic pain conditions with different bodily representation: osteoarthritis is a local pain condition whereas fibromyalgia is a widespread pain condition. Fibromyalgia and knee osteoarthritis are described in detail in Chapter 2.

The neurobiology and pathophysiology of chronic pain have been studied using several neuroimaging techniques: voxel-based morphometry (VBM) - which examines brain morphology, fMRI and resting-state functional connectivity (FC) - which examines brain function, and magnetic resonance spectroscopy (MRS) - which reveals differences in brain neurochemistry (Bandettini, 2009). In earlier chapters, I looked at the differences between chronic local and widespread pain in neurochemistry (Chapter 2) and brain function (Chapter 3). I found differences between the two chronic pain conditions using MRS: NAA level in the thalamus of OA participants was significantly lower than in FM. Furthermore, there were differences in the relationship of neurometabolites and clinical measures between OA and FM (Chapter 2). I additionally found differences in resting-state FC between the two chronic pain conditions. The relationships between FC and clinical measures (McGill questionnaire) were also different between local and widespread chronic pain (Chapter 3). These differences suggest that these two chronic pain conditions may benefit from different therapeutic approaches (Chapters 2 and 3).

In this study I further examine the differences in the brain representation of chronic pain, specifically the differences in brain morphology between chronic widespread pain (FM) and chronic local pain (knee OA), relative to healthy controls using VBM. VBM is a neuroimaging technique, which involves a voxel-wise comparison of the relative amounts of

each tissue type (grey matter – GM and white matter - WM) in a region of interest between two or more groups of subjects (Mechelli, Price, Friston, & Ashburner, 2005). VBM provides high sensitivity to regional differences in grey or white matter using the estimation of smooth, low frequency deformation fields (Mechelli et al., 2005). VBM is a simple and pragmatic approach for localising small scale changes, which is feasible for most research groups (Ashburner & Friston, 2000).

There are relatively few VBM studies in chronic pain. In the studies published reductions in grey matter volume (GMV) in the thalamus have been found: in chronic back pain (Apkarian et al., 2004); in FM (Schmidt-Wilcke et al., 2007); in hip OA (Gwilym et al., 2010); and in trigeminal neuropathy (Gustin et al., 2011). Apkarian et al. suggested there are at least two types of decrease in the thalamic GMV. One possible explanation is tissue shrinkage due to changes in microvascular volume and extracellular space without impacting neurons substantially (Apkarian et al., 2004). This type of decrease in most cases is reversible after appropriate treatment (Apkarian et al., 2004). In contrast, the other type of decrease – atrophy - is irreversible. Thalamic atrophy in chronic pain was suggested to be due to neurodegeneration caused by changes in glial numbers and microglial activity and apoptosis of thalamic interneurons (Apkarian et al., 2004). A decrease in the brain GMV in chronic pain may be result of a change in cell size, shrinkage or atrophy of neurons or glia, and synaptic loss (May, 2008; May & Gaser, 2006).

One research group found a thalamic GMV decrease and a reduction of thalamic NAA level in trigeminal neuropathy patients compared to trigeminal neuralgia patients (Gustin et al., 2011). As was shown in Chapter 2, there is a decrease in the level of NAA in the left thalamus of OA patients compared to FM. Since NAA is a marker of neuronal density and viability (Govindaraju et al., 2000; Simmons et al., 1991) it would be expected that GMV of the thalamus would be decreased in OA compared to FM. Therefore I propose that OA patients would have a reduction of thalamic GMV positively correlated to the decreased NAA level in the thalamus.

Another research group compared chronic back pain, complex regional pain syndrome (CRPS) and knee OA, relative to healthy controls (Baliki, Schnitzer, et al., 2011). They found that different chronic pain conditions reveal distinctive anatomical ‘brain signatures’ (Baliki,

Schnitzer, et al., 2011). I likewise propose that the brain morphology will be different between the two chronic pain conditions. As these two chronic pain conditions have different bodily representation (Chapter 2) I propose that structural reorganization of the brain would be different as well between FM and OA participants. I would expect a decrease of GMV in FM participants in the anterior cingulate cortex – ACC (Burgmer et al., 2009; Jensen et al., 2013; Kuchinad et al., 2007; Robinson, Craggs, Price, Perlstein, & Staud, 2011; Wood, Glabus, Simpson, & Patterson, 2009), posterior cingulate cortex – PCC (Kuchinad et al., 2007; Robinson et al., 2011; Wood, Glabus, et al., 2009), the prefrontal cortex - PFC (Burgmer et al., 2009; Kuchinad et al., 2007), insula (Robinson et al., 2011), thalamus (Schmidt-Wilcke et al., 2007), left precuneus (Fallon et al., 2013), the pons (brainstem) (Fallon et al., 2013), and parahippocampal gyri (Kuchinad et al., 2007; Wood, Glabus, et al., 2009). In OA participants I would expect a decrease of GMV in primary and secondary somatosensory cortex, insula, ACC, DLPFC, brainstem and motor cortex (Davis & Moayed, 2012; May, 2011; Rodriguez-Raecke et al., 2009), and the thalamus (Gwilym et al., 2010).

In this study I compared grey matter volume (GMV) of FM and OA participants, relative to healthy controls. I hypothesised that reductions in GMV would be seen when the patient groups were compared to healthy controls. Furthermore, I hypothesised that different brain areas would be affected (a decrease in GMV) when FM and OA participants compared to each other and healthy controls. Moreover, since NAA levels were reduced in the thalamus of OA patients (Chapter 2), I additionally hypothesised that there would be a positive relationship between the thalamus GMV and the thalamus NAA in OA participants compared to FM and HC.

## **Methods**

### **Participants**

A review of 8 previous VBM studies of chronic pain (7 FM, 1 OA) revealed a mean group size of 20.5 participants. These studies had, except in one case, made a comparison between grey matter volumes in a single patient group and healthy controls and therefore had a mean total participant group size of 41. As per the previous analysis (Chapter 2) a total participant group size of 42 would be required to achieve an 80% power level in detecting a large effect size (Cohen's  $d = 0.8$ ) therefore these studies were adequately powered. Since it was my

intention to make a 3-way comparison between two groups of chronic pain patients and a group of healthy controls I was able to reuse the previous analysis to determine the group sizes that would be required to achieve a power level similar to previous experiments using an ANOVA. To achieve 80% power in detecting a large effect size (Cohen's  $f = 0.4$ ) three groups of 22 participants would be required and therefore I aimed to recruit a total of 66 participants. It was possible to recruit 50 participants for this study, thus achieving a power of approximately 70%. The achieved power level was lower than ideal and therefore raised the possibility of type 2 errors.

All participants were females aged 31-65. There were 17 participants with FM (mean  $\pm$  SD age 47.88  $\pm$  8.15 years), 17 participants with OA of the knee (mean  $\pm$  SD age 61.65  $\pm$  3.06 years), and 16 HC (mean  $\pm$  SD age 44.25  $\pm$  11.32 years) for this study.

FM participants were classified using the American College of Rheumatology criteria (Wolfe et al., 1990) for the classification of FM, such that each subject fulfilled the following criteria: widespread pain in all four quadrants of the body for at least 3 months and tenderness or pain to touch in at least 11 out of 18 specific anatomical sites described as tender points. FM participants were recruited from a district general hospital fibromyalgia clinic in North Wales. FM participants were excluded if they had knee OA. Participants with knee OA were recruited from the pre-operative arthroplasty clinic at Ysbyty Gwynedd, Bangor. Participants with rheumatoid arthritis and OA of other joints were excluded, as was anyone who also had a diagnosis of co-morbid FM. HC participants without chronic pain were recruited using Bangor University School of Psychology's community participant panel and local advertisements. All participants were asked to refrain from using analgesic medication on the day of the scan until after scanning was completed. Normal contraindications for MRI were also used as exclusion criteria (e.g. pacemakers, metal implants, claustrophobia).

This study was approved by the Ethics Committee from the School of Psychology, Bangor University and North West Wales Research Ethics Committee (reference 10/WNo01/16). Research activities took place in Bangor Imaging Centre, Bangor University. All participants provided informed consent.

## **Magnetic resonance imaging (MRI) and analysis**

MRI was performed on a Phillips Achieva MRI system (Philips Health Care, Eindhoven, Netherlands), operating at 3.0 Tesla. For each subject a T1 weighted gradient echo MP-RAGE sequence (TE = 3.5, 5.1, 6.8, 8.5, 10.2 ms, TR = 12 ms, TI = 1150 ms, 3D acquisition, FOV = 240 mm X 220 mm X 130 mm, voxel dimensions = 0.7 X 0.7 X 0.7 mm<sup>3</sup>) was acquired. The T1 MR-images showed no morphological abnormalities or artefacts across any of the groups.

The aim of VBM is to identify differences in the local composition of brain tissue, while discounting large-scale differences in gross anatomy and position (Mechelli et al., 2005). This is achieved by spatially normalising all the structural images to the same stereotactic space, segmenting the normalised images into grey and white matter, smoothing the grey and white matter images and finally performing a statistical analysis to localise significant differences between the groups (Mechelli et al., 2005).

The analysis in VBM included the following steps:

- 1) High-resolution MR images from all subjects were spatially normalised to the same stereotaxic space, which was achieved by registering each image to the same template.
- 2) The spatially normalised images were then segmented into grey and white matter using the segmentation technique 'New segment' (Ashburner & Friston, 2005; Ashburner, 2007).
- 3) Next grey matter images were smoothed by convolving with an isotropic Gaussian kernel. The use of Gaussian smoothing makes the subsequent voxel by voxel analysis comparable to a region of interest approach, because each voxel in the smoothed images contains the average amount of grey matter from around the voxel. The region around the voxel is defined by the form of the smoothing kernel (Ashburner & Friston, 2000, 2001).
- 4) Following pre-processing, the next step was to perform a voxel-wise parametric statistical analysis (a one-way between-groups ANOVA), which compare the smoothed GM images from the groups (FM, OA, and HC). Corrections for multiple

comparisons were made using the theory of Gaussian random fields (Ashburner & Friston, 2000, 2001). The output from the method is a statistical parametric map (SPM) showing regions where grey matter concentration differs significantly between the groups. A whole-brain search for significant clusters (extent threshold=1000 voxels) was performed using a voxel-wise threshold of  $p < .05$  corrected for multiple comparison using family-wise error (Hsu et al., 2009; Kuchinad et al., 2007; Schmidt-Wilcke et al., 2006, 2007). Then a whole-brain search for significant clusters was performed using independent-samples t-tests with contrasts of interest as relevant to the hypotheses (HC>FM+OA, HC<FM+OA, FM>OA, and FM<OA). A voxel-wise threshold of  $p < .05$  corrected for multiple comparison using family-wise error was applied. In addition, a ROI-based search using a mask, which was specially created, using WFU Pickatlas by combining templates of brain regions within the left thalamus was conducted (Maldjian et al., 2003). For the ROI-based search, all clusters with an uncorrected voxel-level  $p < .001$  and those with a voxel-level  $p < .05$  (corrected for small volume and family-wise error) were considered significant (Brett, Anton, Valabregue, & Poline, 2002; Hsu et al., 2009). The contrasts of interest were again HC>FM+OA, HC<FM+OA, FM>OA, and FM<OA.

- 5) Finally, mean voxel values for any significant clusters inside the left thalamus mask were extracted from each subject for a correlation analysis with NAA levels. The correlation analysis was planned to be performed using SPSS, version 14.0 (SPSS Inc., Chicago, IL). Between-group differences were planned to test using a one-way ANOVA, with a significance threshold of  $p < .05$ . For any significant clusters, the standardized residuals from a linear regression between mean voxel value - dependent and total intracranial volume (TIV) - independent were calculated (Hsu et al., 2009). The relationships between the left thalamus GMV and the left thalamus NAA in all three groups were planned to investigate using Spearman's Rank Order Correlation (rho) coefficient.

## Results

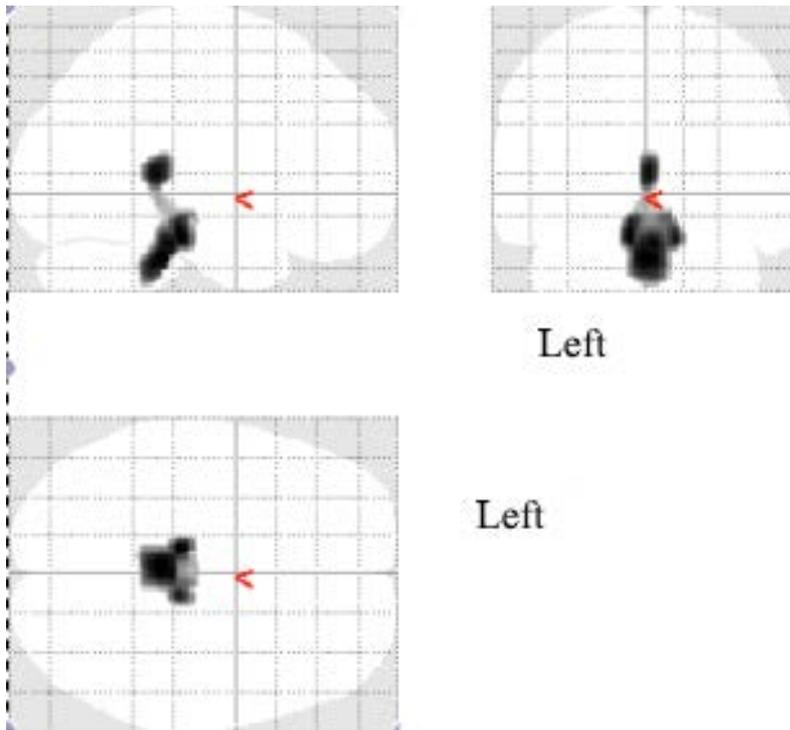
The initial whole-brain analysis of revealed no clusters (above the threshold of 1000 voxels) of significantly different GMV between the three groups of participants when the results had been corrected for multiple comparisons. The results of the whole-brain analysis between the 3 groups are therefore not presented in detail.

Using the whole-brain search approach with the planned contrasts of interest (t-tests), the main effect of group revealed the following clusters of significantly different GMV between patient groups. The FM<OA contrast revealed clusters of difference within the left brainstem – midbrain, the left brainstem – pons, and the posterior cingulate ( $p < .05$ , corrected). The FM>OA contrast ( $p < .05$ , corrected) showed differences in the left precentral gyrus (Brodmann areas 4 and 6) and the left postcentral gyrus (BA 40). There were no significant alterations in any other brain areas. There were no significant GMV changes in patient groups compared to HC. These results are shown in detail (i.e. peak coordinates, T-values, Z-values, and cluster sizes) in Table 16. Figures 8 and 9 demonstrate areas of significant decrease in grey matter volume in FM and OA patient groups compared to each other.

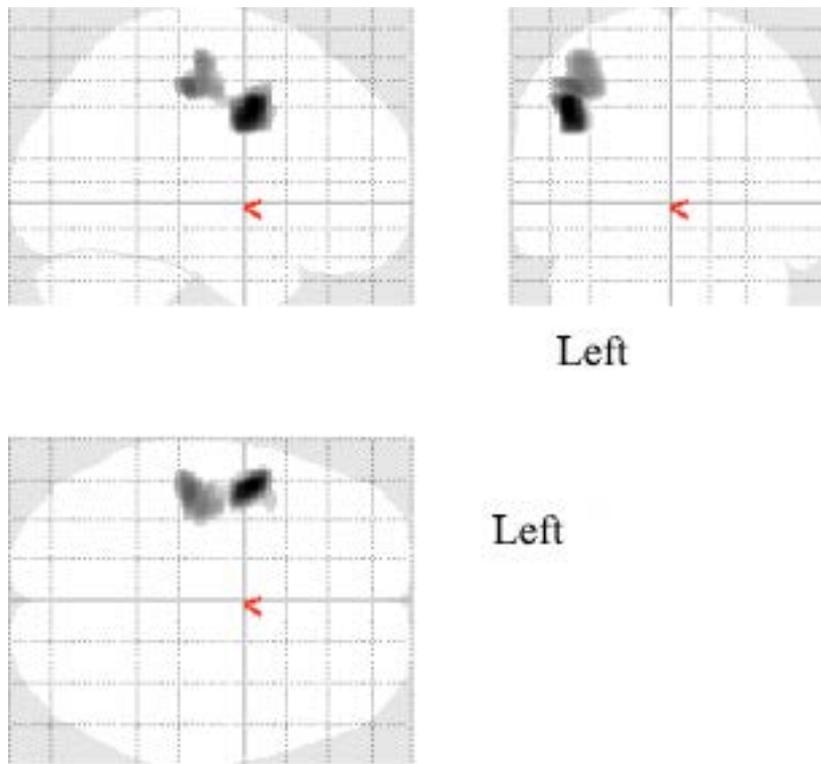
Cluster Location	MNI coordinates of highest peaks within cluster X, Y, Z mm	Peak t value	Uncorrected <i>p</i> value	FWE corrected <i>p</i> value	Cluster Size (N of Voxels)
<b><i>Contrast FM &lt; OA</i></b>					
Left Brainstem (Midbrain)	-9 -27 -20	5.02			
Left Brainstem (Pons)	-2 -36 -30	5.01	< .0001	.003	2088
Posterior Cingulate	0 -37 7	4.89			
<b><i>Contrast OA &lt; FM</i></b>					
Left Middle Frontal Gyrus (BA 6)	-45 2 37	4.88			
Left Suprmarginal Gyrus (BA 40)	-42 -25 46	4.21	.002	.012	1482
Left Precentral Gyrus (BA 4)	-39 -19 57	3.97			

FM – fibromyalgia; OA – osteoarthritis; BA – Brodmann area  
P < 0.001, uncorrected; Extent threshold k = 1000 voxels

*Table 16: Areas of significant decrease in grey matter volume*



*Figure 8: Areas of significant decrease in grey matter volume in fibromyalgia compared to knee osteoarthritis (FM<OA)*



*Figure 9: Areas of significant decrease in grey matter volume in knee osteoarthritis compared to fibromyalgia (OA<FM)*

Using the ROI-based search approach, applying the left thalamus mask, the main effect of group revealed no clusters of significantly different GMV between the three groups (a one-way between-groups ANOVA), uncorrected and small-volume corrected. In addition, using 6 contrasts of interest and the left thalamus mask in the ROI-based search approach did not reveal any clusters of significantly different GMV between the groups (independent-samples t-tests), uncorrected and small-volume corrected. Despite the absence of the expected decrease in GMV in the left thalamus of OA patients, a correlation analysis was performed between left thalamus GMV and thalamic NAA. No significant correlation was found between thalamic GMV and corrected NAA levels in any of the participant groups. However, as mentioned in chapter 2, a positive correlation was found between the uncorrected NAA and grey matter levels. This contrasting result indicates that our partial volume correction

procedures were effective and suggests that the NAA differences seen are not a result of tissue content changes, but may instead reflect alterations in neuronal viability in OA.

## **Discussion**

My most notable finding was a decrease of GMV in FM patients compared to OA in the left brainstem (midbrain and pons) and the posterior cingulate. In contrast, in OA patients GMV was decreased compared to FM in the left precentral (BA 4), middle frontal (BA 6) and the postcentral gyri (BA 40). No significant GMV changes in either patient group compared to HC were found. No decrease of thalamic GMV in OA patients was found. No correlation between thalamic GMV and NAA levels were found in OA patients. I will analyse each of these findings individually.

### 1) FM < OA: left brainstem – midbrain and pons

The midbrain, particularly periaqueductal grey (PAG) and reticular formation, is involved in the pain desensitization pathway (Zambreau, Wise, Brooks, Iannetti, & Tracey, 2005).

Decrease of GMV in the midbrain of FM patients may inhibit pain desensitization function in these chronic pain patients, which may explain hypersensitivity in FM. This dysfunction (or incoordination) of the pain desensitization pathway may result in central sensitization being more prominent in FM patients than in OA patients.

The pons, on the other hand, carries sensory signals to the thalamus. So, decrease of GMV in the pons of FM patients may interrupt the spinothalamic tract's function, which in turn may cause a disruption in the pain sensitivity control of those patients. This may result in FM patients having some sensory incoordination, which may explain the existence of paraesthesias in FM patients.

The spinothalamic tract for pain sensation passes through the brainstem. Brainstem has integrative functions within cardiovascular system control, respiratory system control, pain sensitivity control, consciousness and the sleep cycle (Nicholls & Paton, 2009). Given the brainstem's involvement in this range of functions, a decrease of GMV in this region in FM may provide an explanation for the diversity of the symptoms of FM: widespread pain, fatigue, nonrestorative sleep, morning stiffness, headache, cognitive disturbance, anxiety, and paraesthesias, in addition to chronic pain.

My finding is in agreement with previous research by Fallon et al. (Fallon et al., 2013) who also reported a decrease in GMV within the brainstem (pons) in FM patients. Fallon et al. suggested that brainstem GMV loss may contribute to sensitivity to pressure pain in FM patients, as they noted the role of brainstem areas within descending nociceptive control.

Another research group suggested that a decrease of GMV in the brainstem may promote the chronification of back pain (Schmidt-Wilcke et al., 2006). A correlation between the grey matter decrease in the brainstem and pain intensity and unpleasantness at the time of scanning was demonstrated, which was suggested to account for the level of impaired antinociception at that time (Schmidt-Wilcke et al., 2006). My finding of both a reduction in brainstem GMV and increased pain intensity in FM patients adds support to the conclusions of Schmidt-Wilcke et al.

## 2) FM < OA: posterior cingulate

Posterior cingulate cortex (PCC) has a well-documented role in cognitive function (working memory and episodic memory retrieval) (Shimizu et al., 2007). Working memory is a key component of effective cognition and has a major bearing on problem solving (Baddeley & Hitch, 1974). Fibromyalgia affects patients' cognition, especially memory and ability to focus and express their thoughts clearly, which is often referred to as "fibro fog" (Arnold et al., 2008). Decrease of GMV in the PCC of FM patients compared to OA may suggest that cognitive function is more affected in FM compared to OA.

Hypofunction of the PCC has been found to be associated with cognitive decline (Shimizu et al., 2007). It was suggested that a decreased NAA/Cr in the PCC has a contribution to the pathophysiology of chronic schizophrenia with cognitive deficit (Shimizu et al., 2007). In addition, a relationship between the NAA/Cr in the PCC and verbal memory was found and it was suggested that the NAA/Cr in the PCC might be associated with episodic memory function (Shimizu et al., 2007). Previous PET studies also have shown the usage of PCC in episodic memory (Andreasen et al., 1995; Desgranges, Baron, & Eustache, 1998; Nyberg et al., 1996; Shallice et al., 1994). Furthermore, a role of the PCC in cognitive impairment (learning and memory) was demonstrated in Alzheimer's disease (Minoshima et al., 1997; Minoshima, Foster, & Kuhl, 1994).

In addition, involvement of the PCC in working memory has been shown in fMRI studies (Hampson, Driesen, Skudlarski, Gore, & Constable, 2006). In particular, the PCC was activated during standardized and autobiographical memory retrieval, allowing concluding about an important role of the PCC in successful memory retrieval (Maddock, Garrett, & Buonocore, 2001). Furthermore, it was proved that PCC plays a key role in the default mode network (DMN), which was investigated to link intrinsic connectivity to cognition (Fransson & Marrelec, 2008). Also, abnormal connectivity in the PCC and hippocampus was demonstrated in early Alzheimer disease and mild cognitive impairment (Zhou et al., 2008).

Thus, my finding of decreased GMV in the PCC of FM patients compared to OA and cognitive impairments in those patients (Glass, 2009; Reyes Del Paso, Pulgar, Duschek, & Garrido, 2012; Suhr, 2003) give additional evidence for the role of PCC in cognitive function.

In addition to the involvement of the PCC in cognition, it was demonstrated the PCC has a role in pain (Bromm, 2001; Hsieh, Belfrage, Stone-Elander, Hansson, & Ingvar, 1995; Lekander, Fredrikson, & Wik, 2000; Nielsen, Balslev, & Hansen, 2005; Vogt et al., 1996; Willoch et al., 2000). A PET study showed the activation of the PCC in neuropathic pain patients (Hsieh et al., 1995). Another PET study correlated regional cerebral blood flow to immune function in fibromyalgia: activity in the PCC was negatively correlated with natural killer cell activity (Lekander et al., 2000). Furthermore, activations in the PCC were positively correlated to phantom limb pain sensations (Willoch et al., 2000). Thus, my finding of decreased GMV in the PCC in FM gives additional support to the role of this region in pain.

Bromm noted that, given the position of the PCC within a network involving amygdala and widespread areas of temporal and parietal cortex, there is support for pain-related activation in the PCC representing the “emotional-aversive component of pain” (Bromm, 2001). I found a GMV reduction in the PCC in FM patients and higher evaluative rank of pain rating index (PRI) in FM compared to OA (see McGill Questionnaire in Chapter 3). My findings are in accord with Bromm’s suggestion that the PCC plays a key role in providing the “emotional-aversive component of pain” (Bromm, 2001).

### 3) OA < FM: BA 4 and BA 6 – primary motor cortex and premotor cortex

Brodmann areas 4 (left precentral gyrus) and 6 (middle frontal gyrus) are primary motor cortex (motor execution) and premotor cortex areas (motor planning and execution) respectively (Hanakawa, Dimyan, & Hallett, 2008; Rizzolatti & Luppino, 2001). I found a decrease in GMV in those areas in OA patients compared to FM. This suggests the possibility that changes to brain structure in OA may contribute to, or arise from, the motor dysfunction and consequent disability observed in this condition.

My finding is in agreement with previous research. Motor cortex stimulation was used for central and neuropathic facial pain. 88% of pain relief was demonstrated postoperatively and 75% after 10 months. It was suggested that motor cortex stimulation alters cortical plasticity and inhibits thalamic hyperactivity (Brown & Pilitsis, 2005). Pain relief and improvement of daily functioning was observed after applying transcranial direct current stimulation (tDCS) over primary motor cortex in FM patients (Fagerlund, Hansen, & Aslaksen, 2015). Many studies have reported significant pain relief by repetitive transcranial magnetic stimulation over primary motor cortex in central and peripheral neuropathic pain, fibromyalgia, CRPS, low back pain, irritable bowel syndrome, and postoperative pain syndrome (Galhardoni et al., 2015). My finding of decreased GMV in motor cortex in OA invites the question if stimulation over the primary motor cortex would have the same analgesic effect in patients with OA of the knee.

### 4) OA < FM: BA 40 – supramarginal gyrus

Supramarginal gyrus (BA 40) is a component within the somatosensory system, which plays an important role in proprioception (De Ridder, Van Laere, Dupont, Menovsky, & Van de Heyning, 2007; Filimon, Nelson, Huang, & Sereno, 2009; Goble et al., 2011; Shimada, Hiraki, & Oda, 2005). Proprioception is awareness of the body's disposition and the position of its components relative to each other, including joint static position and movement (Sharma, 1999). Proprioception is mediated by the central nervous system, and depends upon input from the somatosensory system (proprioceptive input), as well from the visual and the vestibular systems (Lephart, Pincivero, & Rozzi, 1998). It has previously been demonstrated that proprioception is impaired in OA of the knee (Sharma, 1999; Sharma, Pai, Holtkamp, & Rymer, 1997). I found a decrease in GMV in the supramarginal gyrus in OA patients

compared to FM. My suggestion is that a decrease in GMV of the supramarginal gyrus is related to the proprioceptive impairment in knee OA. Whether this is cause or consequence is difficult to say at this stage. Further research on OA of the knee and other joints is needed. Furthermore, it would be of interest to investigate whether a decrease in GMV in the supramarginal gyrus reverses after a total knee replacement as it was shown that proprioception was more accurate in replaced knees than in osteoarthritic knees (Barrett, Cobb, & Bentley, 1991; Gauchard, Vançon, Meyer, Mainard, & Perrin, 2010; Isaac et al., 2007; Reider et al., 2003; Swanik, Lephart, & Rubash, 2004).

#### 5) No significant differences in GMV between patients groups and HC

I did not find significant GMV changes in patients groups when compared to HC. Most previous VBM studies of the effects of chronic pain have made a comparison between a single group of patients and HC rather than between pain groups (see Smallwood et al. (2013) for review). The failure to detect changes between patients and healthy controls may be due in part to this study's relatively small sample size (as can be seen in comparison to the studies listed by Smallwood et al.) and the difference in mean ages between the groups (as discussed as limitations below). Those differences were detected between the patient groups rather than with HC suggest that the pain conditions can create opposing alterations in GMV in affected areas rather than being solely responsible for reduction in GMV. This suggestion is supported to an extent by the findings of Smallwood et al. who observe that several studies recorded GMV increases in chronic pain patients when compared to HC. The suggestion of opposing effects on brain areas by CLP and CWP is worthy of further study as greater understanding of the mechanisms underlying these changes may lead to better-targeted treatments.

#### 6) No decrease of GMV in the thalamus of OA patients

In my previous MRS study (Chapter 2) I found a decrease of the thalamus NAA levels in OA patients. NAA is a marker of neuronal density and viability (Govindaraju et al., 2000; Simmons et al., 1991). Reduced NAA levels have been observed with many neurodegenerative diseases such as leukodystrophies, hypoxic encephalopathy (Rosen & Lenkinski, 2007), and multiple sclerosis (Rosen & Lenkinski, 2007; Tsai & Coyle, 1995). Because of my previous finding of thalamic NAA reduction in OA I suggested that long-term chronic pain from OA might lead to degeneration or dysfunction of central nervous system

tissue (Chapter 2). In this study I hypothesised a reduction of GMV in the thalamus of OA patients. However, I did not find the expected decrease in GMV in this region. This may suggest that the neurochemical changes seen are related to functional rather than structural (morphological) effects.

Furthermore, dynamic changes of NAA concentrations suggest that NAA levels may reflect neuronal dysfunction rather than loss (Govindaraju et al., 2000). Recovery of NAA levels has been detected in brain injury (De Stefano et al., 1995), reversible ischemia (Brulatout et al., 1996), multiple sclerosis, AIDS, and temporal lobe epilepsy (Barker & Lin, 2006). My findings of a reduction in NAA level in the thalamus, but not a reduction of GMV supports possible functional, but not structural changes in the thalamus of OA patients.

#### 7) No correlation between thalamic GMV and NAA levels in OA patients

Despite the absence of the hypothesised reduction in thalamic GMV in OA patients, the data were analysed to determine if a relationship could be observed between NAA levels and thalamic GMV. No significant correlations between these values were observed for any of the participant groups. This finding, taken with the lack of thalamic atrophy in OA adds further support to the suggestion that reduced NAA levels in this region are indicative of functional rather than structural alterations in response to chronic pain. A degree of caution must be exercised in the synthesis of data from the MRS study described in Chapter 2 and the current VBM study. The data used to form the correlations with GMV had been corrected for partial volumes and relaxation effects therefore the relationship between GMV and metabolite levels may be obscured.

There are some limitations of this study. The OA group is older than FM and HC groups, the numbers of participants in each group were smaller than would have been ideal, and only female participants were recruited. I will consider each of these limitations in turn.

It is possible that the age difference between participant groups may have introduced confounding factors given the effects of age on brain morphology. I examined the literature to consider the effects that an older participant group may have had on the results derived by VBM. A review conducted by Matsuda (2013) of 12 previous VBM studies of the healthy, aging brain revealed a broad consensus of cortical GMV reduction in older subjects. The

observed reduction was most marked in frontal and insular areas of the cortex. In contrast, non-cortical structures, including the thalamus, were largely unaffected. Examination of studies outside the scope of Matsuda's review (Callaert, Ribbens, Maes, Swinnen, & Wenderoth, 2014; Crivello, Tzourio-Mazoyer, Tzourio, & Mazoyer, 2014; Riello et al., 2005; Van Laere & Dierckx, 2001) were broadly in agreement with Matsuda's observations, with Crivello et al. demonstrating GMV loss in the frontal and parietal cortices, middle occipital gyri, temporal cortex and hippocampus in healthy older adults (65-82 years). Given these findings it is possible that OA participants had reduced GMV compared to other groups as a result of age, which may have obscured findings in the FM < OA contrast. The one significant finding from this contrast was a reduction of brainstem GMV in FM, which occurred in an area not noted by previous studies as affected by age-related GMV reduction. The observation of GMV reductions in the younger FM cohort is interesting, as it would appear to be in the opposite direction to healthy aging related changes. It is possible that GMV decrease in FM patients relates to premature aging in FM (Hassett, Clauw, & Williams, 2015; Kuchinad et al., 2007). I found one significant result in the OA < FM contrast, namely a cluster of GMV reduction across the middle frontal, supramarginal, and precentral gyri. These are cortical areas in which age-related GMV reductions have been observed, therefore it is possible that age may be a contributing factor to these results. A further study with more closely matched participant groups may determine if this is the case.

As noted previously, I was unable to recruit sufficient numbers of participants to achieve the desired level of experimental power. It is possible that existing differences in GMV between the chronic pain conditions were not detected by this experiment. The original intention for recruitment to this study was to form participant groups of sufficient size to achieve 80% power. Logistical constraints enable the recruitment of only 50 participants, which reduced the sensitivity of my experiment.

Additionally, as I only recruited female participants, possible gender effects may limit the generalizability of my findings (for detailed consideration of the limitations of my studies the reader is referred to Chapter 5). Certain methodological limitations of VBM have been noted, for instance in normalisation and segmentation confounds, accuracy of localisation and the interpretation of results (Mechelli et al., 2005). However, as Whitwell reported, there is a

growing body of literature that provides validation of VBM as a technique for assessing structural changes in the brain (Whitwell, 2009).

My results suggest future research to perform a longitudinal study with OA patients being scanned before and after a total knee replacement. There is some evidence that brain morphology is reversible in chronic pain (Gwilym et al., 2010; Rodriguez-Raecke et al., 2009; Seminowicz et al., 2011). For example, Rodriguez-Raecke et al. have shown reversible alterations of GMV in the ACC, DLPFC, amygdala, brainstem and right insula in patients with hip OA before and after endoprosthetic joint replacement (Rodriguez-Raecke et al., 2009). Gwilym et al., likewise, have demonstrated reversible GMV changes in the left thalamus of hip OA patients before and after arthroplasty (Gwilym et al., 2010). Reversibility of brain changes has also been shown in chronic low back pain with increased cortical thickness in the left DLPFC, primary motor cortex, and right anterior insula in CLBP patients after spine surgery or facet joint injections (Seminowicz et al., 2011). Furthermore, another research group has concluded that CLBP due to lumbar disk herniation can induce structural brain alterations, which are potentially reversible after microsurgical lumbar discectomy (Luchtman et al., 2015). Thus I expect that GMV of the left precentral gyrus (BA 4 and 6) and the left postcentral gyrus (BA 40) would increase (normalise) after a total knee replacement in OA patients.

Subsequently, it would be interesting to evaluate whether non-surgical treatment of FM patients (CBT, acupuncture) would have the same effect i.e. normalising brain morphology. Possible future research is to investigate the influence of cognitive behavioural therapy (CBT) on FM patients. I speculate that GMV of the posterior cingulate in FM would recover to normal levels after the treatment. There is evidence that CBT is an effective method in FM (Ang et al., 2010; Bennett & Nelson, 2006; Glombiewski et al., 2010; Goldenberg et al., 1994), which improves cognitive function, especially attention, and reduces nociceptive responding in FM (Ang et al., 2010).

In conclusion, different brain areas were affected in two chronic pain conditions: in FM there were brain areas more associated with cognition, whereas in OA the areas were implicated in motor function. These findings suggest that the two chronic pain conditions would benefit from different therapeutic strategies: for FM patients an accentuation on CBT, psychotherapy

and relaxation techniques and for OA patients emphasis on movement therapies and physiotherapy. Further VBM studies of a wider range of chronic pain conditions would allow better comprehension of pain mechanisms in CLP and CWP. Gaining an understanding of the nature of different chronic pain conditions is essential in the development of targeted therapies and the achievement of efficient pain management.

## **CHAPTER 5 - General Discussion**

This programme of research sought to determine whether differences can be identified in the effects of chronic widespread and chronic localised pain on the human brain. I selected osteoarthritis (OA) as an example of chronic localised pain (CLP) and fibromyalgia (FM) as representative of chronic widespread pain (CWP) to make a comparison with each other and with healthy controls (HC). My investigations used a range of neuroimaging techniques to examine the brains of participants at rest. It is my intention in this chapter to bring together the results of these investigations and consider what these results can tell us about the effects of chronic pain on the human brain.

## **Summary of the investigations and key findings**

My research used Magnetic Resonance Spectroscopy (MRS), Voxel-based Morphometry (VBM) and Functional Connectivity (FC) to examine tissue composition (neurochemistry), relative size of brain regions (structure), or correlations between activity (function) in the brains of chronic pain patients and healthy controls. In this section I will summarise the investigations that were performed and their main findings.

I used MRS to determine the levels of seven neurochemicals in four brain regions that have been found to be relevant to chronic pain conditions. My most notable finding was a reduction of N-acetyl aspartate (NAA) in the thalamus of OA patients compared to FM patients.

I used VBM to compare the relative amounts of tissue types present across the brain. Further motivation for this investigation was provided by the results of the preceding study: since NAA levels are taken to be indicative of neuronal viability, I considered it plausible that the observed reduction may suggest loss of neurons in the thalamus of OA patients. Contrary to expectations, I did not find a reduction of grey matter volume (GMV) in the thalamus of OA patients. I did observe a reduction in GMV in OA in the left middle frontal and supramarginal gyri. I also observed a reduction of GMV in FM patients in the brainstem (primarily midbrain and pons) and in the posterior cingulate cortex (PCC).

I used FC to examine the effects of chronic pain conditions on the level of correlated activity in networks of regions in the participants' brains. Given that an altered level of NAA in the thalamus without corresponding neuronal loss was observed, it was considered plausible that

the differing effects of CLP and CWP on patients' brains may be functional rather than structural. I noted a higher level of FC between areas of the Default Mode Network (DMN) in FM when compared to OA. I also noted that FC in both chronic pain conditions was greater compared to HC between selected regions within the networks and the precuneus in the DMN. In contrast, I noted that FC in both chronic pain conditions was lower than in HC between SPLs and the inferior frontal gyri (IFGs) and the middle frontal gyri (MFGs) within the Executive Attention Network (EAN). I will consider now what these results tell us about the differences and similarities of the effects of CWP and CLP on the human brain.

## **Differences between CWP and CLP: summary and discussion of main research findings**

My main hypothesis of the existence of observable differences in the effects of patients' brains of CWP and CLP was supported by the following findings across the clinical measures (questionnaires) and neuroimaging techniques:

- 1) A significant decrease in the level of NAA in the thalamus of CLP (OA) patients compared to CWP (FM) group (MRS).

Reduced levels of thalamic NAA have been observed in a range of conditions. Gustin et al. (2014, 2011) found that this reduction could be used to draw a distinction between groups of trigeminal neuropathy and spinal injury patients who experienced neuropathic pain and those who did not. Shigemura et al. (2012) observed a similar reduction of thalamic NAA in hip OA patients. Other instances of reduced thalamic NAA have been observed in schizophrenia, multiple sclerosis, epilepsy, essential tremor, ecstasy use, cocaine tolerance and alcoholism (Bernasconi et al., 2003; De Win et al., 2008; Jagannathan, Desai, & Raghunathan, 1996; Li, Wang, Pankiewicz, & Stein, 1999; Wylezinska et al., 2003). The thalamus acts as a hub for information directed to the cortex and plays a role in managing the direction of consciousness and arousal through oscillatory, inhibitory circuits (Steriade & Llinás, 1988). Since reduced levels of NAA levels are taken to indicate neuronal dysfunction (Tsai & Coyle, 1995) it is clear that the thalamus is vulnerable to damage by a range of factors. It is interesting to note that abnormal and, in some cases, prolonged input to the brain is present in several of these cases. In CLP this may suggest that chronic nociceptive input causes damage to the thalamus,

which disrupts inhibitory thalamocortical circuits. Thalamocortical dysrhythmia has been implicated as a factor in which central mechanisms maintain the experience of pain (Llinás, Ribary, Jeanmonod, Kronberg, & Mitra, 1999). The absence of a similar effect in CWP may indicate that abnormal input from the periphery is not the primary factor in the generation of pain i.e. thalamic damage does not occur in FM since central rather than peripheral factors are primarily responsible for pathogenesis.

- 2) A significant decrease in grey matter volume (GMV) in the left brainstem and PCC of the CWP group compared to CLP (VBM).

The brainstem plays a key role in facilitating descending pain modulation and desensitisation, signal integration and in a range of the brain's fundamental functions (Nicholls & Paton, 2009). The GMV loss observed in FM patients in my study may indicate damage to the structures fulfilling these functions. This finding may suggest a neurological basis for FM patients' paraesthesias (due to disruption of sensory integration), hypersensitivity (due to impairment of the descending pain desensitization pathway) and the spectrum of additional symptoms experienced by FM patients (due to dyscoordination of the integrative control functions of the brainstem). My observation of reduced brainstem GMV is agreement with Fallon et al. (2013) who observed a reduction accompanied by brainstem shape alteration. Further analysis of my data may add to our understanding if volume change or structural distortion is the main component of functional loss.

It is interesting to note that these findings are relatively novel: prior studies that have noted GMV reduction in pain patients have compared the brains of CLP patients with healthy controls (Rodriguez-Raecke et al., 2009; Schmidt-Wilcke et al., 2005). Rodriguez-Raecke et al. examined the effects on GMV associated with hip OA and found GMV reduction in the patient group, which was reversible after treatment of the affected joint. Rather than suggesting a different mechanism of action between hip and knee OA it is more plausible to consider the difference in CLP findings between my studies to be due to the larger sample size (roughly twice as many in each group) and the closer match that Rodriguez-Raecke were able to achieve in the ages of their participant groups.

My finding of reduced GMV in the PCC in CWP is in agreement with the observation made by Kuchinad et al. (2007) and suggests a neurological basis for the cognitive symptoms experienced by FM. As noted, the PCC plays a role in working memory and cognitive control therefore a loss of GMV in this region may impair these functions in FM patients. The PCC has also been suggested as a key component of the pain representation system, particularly in the “emotional-aversive component of pain” (Bromm, 2001). This finding adds support to the division of the pain system into two component systems: a lateral somatosensory system and a medial emotional-aversive system, of which the PCC forms a part. No studies have been published which indicate GMV loss in the PCC associated with OA. Neuronal loss or damage to the PCC may therefore contribute to the higher level of emotional symptoms experienced by FM patients when compared to OA. It must be noted, however, that a reduction in GMV in PCC (and ACC) was noted by Gerstner et al. (2011) in patients with myofascial-type temporomandibular disorders. This latter observation suggests that whilst a division may exist in the impact of OA and FM on the medial and lateral pain systems, caution should be used when looking to separate the effects of all CWP and CLP conditions.

- 3) A significant decrease in GMV in the left precentral (BA 4), middle frontal (BA 6) and supramarginal (BA 40) gyri of the CLP patients compared to CWP (VBM).

The decrease of GMV in the motor cortex suggests that changes to brain structure are associated with the motor dysfunction and consequent disability. However, a clear statement cannot yet be made of the direction of any causal relationship. Reorganisation of the motor cortex has been observed in OA (Shanahan, Hodges, Wrigley, Bennell, & Farrell, 2015) therefore it is not surprising to observe structural changes to these areas. Increases in the GMV of these areas has been observed in OA patients who have received treatment (Tetreault et al., 2015), which suggests that as per the findings of Rodriguez-Raecke et al. GMV alterations associated with OA are reversible in some cases, suggesting neuronal damage rather than loss.

Whilst alterations to the motor cortex in OA may primarily result from an adjustment to a patient’s use of the affected limb, there is evidence to suggest that some reorganisation may be driven by alterations to the mechanisms that support motor imagery and anticipation (Stanton et al., 2012). An effect on motor imagery has been discovered in complex regional

pain syndrome: another CLP (Moseley et al., 2008). It has also been observed that stimulation of the motor cortex has an analgesic effect on some chronic pain conditions (Brown & Pilitsis, 2005; Fagerlund, Hansen, & Aslaksen, 2015). Taken together, this suggests a more complex role for the motor cortex in CLP than was previously appreciated.

A reduction in GMV was found in the supramarginal gyrus (BA 40) within the somatosensory association cortex in OA patients. This finding is novel as I am not aware of any other studies that have observed GMV alterations in this area in OA (or FM). Gerstner et al. (Gerstner et al., 2011) observed an increase in white matter volume in the supramarginal gyrus, but no significant difference in GMV in patients with temporomandibular pain (a CLP condition). Impaired proprioception has been observed in OA patients (Sharma, 1999; Sharma et al., 1997). The impairment observed being generalised rather than relating to the joint or limb affected by OA. This finding taken with my observation of GMV reduction in OA may suggest a neurological basis for the impaired general proprioception in OA.

- 4) A significant increase in FC within DMN between PCC and MTGL in the CWP group compared to CLP. Increased DMN FC in CWP.

It is challenging to interpret this result in the context of the hypothesis that increased FC would be observed in the DMN in pain conditions that in healthy controls. No difference was found between the pain conditions and healthy controls, but a higher level of FC was observed between the seed region of the DMN and a component of the DMN in FM when compared to OA. The MTG is believed to play a role in language and memory (Binder et al., 1997; Squire & Zola-Morgan, 1991). The different effects may reflect the more cognitive elements of FM, but this cannot be stated confidently. Further research is required to investigate whether opposing effects on the DMN FC of the MTGL can show significantly differences between the pain conditions and healthy controls. It would be instructive to rerun this study with larger and more closely matched participant groups.

It is interesting to consider the observation of increased DMN FC in FM with the previous finding of decreased GMV within the PCC in FM compared to OA. Taken together, this suggests a somewhat counterintuitive situation entailing increased network cohesion being generated by a region experiencing neuronal damage or loss. Further research is required to

determine the underlying nature of the reduction of GMV and whether increased FC can cause neuronal loss or FC increases as a result of neuronal damage.

- 5) Significant differences between CWP and CLP in relationships between the clinical measures (questionnaires) and the measures from neuroimaging techniques.

FM patients showed positive correlations of affective rank of pain rating index (PRI) with FC between DMN and the EANs. OA, on the other hand, revealed a negative correlation of affective rank of PRI with FC between DMN and the right EAN. Activity in the DMN and EANs is anticorrelated under normal conditions. The observation of opposing shifts in correlation between the patients' experience of CWP or CLP and FC between the networks is illustrative of the greater emotional component of FM. This observation aligns with the suggestion made by Gracely et al. (2004) of a ruminative feedback loop within FM, where patients' reflection on their pain enhances their awareness and this effects their emotional state in relation to their condition. Increased FC across DMN and EANs accompanying FM was observed by Napadow et al. (2010). Increased FC between the networks may indicate that the intrusion of pain into a patient's resting state causes attention shifts entailing switching between DMN and EAN. Within FM these shifts may contribute to the direction of attention to pain, rumination and the enhancement of the affective component of the patient's condition. A role for the insula in the shifting of activity between DMN and EAN has been demonstrated (Menon & Uddin, 2010). The insula is also a component of the pain neuromatrix (Melzack, 1990). It would be instructive to review the activity of the insula in conjunction with the DMN and EAN in FM patients to determine if further understanding can be gained of the interaction of the insula's role in both pain processing and the co-ordination of shifting activity between networks.

- 6) A significant difference in mean scores for evaluative rank of pain rating index (PRI) was observed between CWP and CLP (McGill Questionnaire).

The observation of significantly increased evaluative PRI in FM was anticipated given prior research that has emphasised the cognitive and affective component of the condition. This finding emphasised that patients' experience of CWP and CLP can vary not only in the location of pain, but also disruption to other psychological and physical systems. This finding

is closely related to the catastrophisation of their condition expressed by some FM patients (Gracely et al., 2004), which marks a significant difference in the subjective experience of this group with than of OA patients. Whilst this finding is not novel it is informative to consider in the context of the other differences observed between CLP and CWP particularly with a view towards determining new methods for managing the conditions. The correlation found between the increased between network FC and PRI in FM, coupled with the higher evaluative component of FM suggests that working with FM patients to reappraise their experience and attempt to manage their reaction to the intrusion of pain may be appropriate in this condition.

I will consider now the findings from my studies that indicated similarities between CWP and CLP. Additionally, I will discuss the findings where the pain conditions shared a difference between themselves and HC.

## **Similarities between CWP and CLP: summary and discussion of main research findings**

- 1) Increased FC in the DMN in both chronic pain conditions compared to HC. Increased FC between the seed regions (SPLL, SPLR and PCC) and the precuneus.

Both chronic pain conditions were associated with increased levels of FC within the DMN when compared to healthy controls. Previous research has indicated that enhanced DMN FC is associated with a range of adverse effects including mild cognitive impairment, lower levels of happiness and impaired episodic memory performance (Gardini et al., 2015; Luo, Kong, Qi, You, & Huang, 2015; McCormick et al., 2014). My findings are in accord with the work of the other research groups, but they leave questions unanswered to whether increased FC is caused by the pain conditions and why enhanced DMN FC should be associated with adverse effects. I recommend a further study to compare areas of enhanced DMN FC with the behavioural and emotional effects presented in OA and FM: whilst the cognitive effects of FM are well-documented (“fibro fog” etc.) less is known about the effects of OA on cognition. It would be informative to know whether a link can be drawn between enhanced DMN FC in OA and impaired cognition.

Increased FC between the precuneus and areas between and within my networks of interest was noted in both pain conditions when compared to healthy controls. In Chapter 3 I considered the role of the precuneus and noted its extensive connectivity with areas of the brain believed to support a wide range of behaviours. Activity in the precuneus has been associated with awareness, self-reflection, episodic memory and rumination (Kjaer, Nowak, & Lou, 2002; Lou et al., 2004; Kjaer, Nowak, Kjaer, Lou, & Lou, 2001; Vogt & Laureys, 2005; Dörfel, Werner, Schaefer, Von Kummer, & Karl, 2009; Lundstrom et al., 2003; Lundstrom, Ingvar, & Petersson, 2005). The pronounced atypicality of FC between the precuneus and other brain areas and, as noted, the proposed role of the precuneus as a core hub of the DMN, gives plausibility that the abnormal FC observed in chronic pain patients is a key factor in the effects of their condition: both sensory and beyond.

It was noted that increased FC between the seed regions of both the DMN and the EANs and the precuneus was observed. An increase in between network FC (i.e. between the precuneus of the DMN and the EAN seed regions) may be indicative of pain-driven activity shifts between the networks (as discussed above). Given the previously identified role of the insula in between network activity shifts, it would be informative to examine FC between the precuneus and insula to determine if FC is increased between these areas in pain conditions. It is recommended that further studies of the disruptive effects of chronic pain examine the role of the precuneus and insula.

- 2) A significant decrease in FC in both chronic pain conditions compared to HC between SPLs and IFGs and MFGs within and between EANs.

I observed that network coherence within (and between) the EANs was decreased in both pain conditions when compared to healthy controls. As noted, EAN activity corresponds with externally directed, goal oriented cognition (Seeley et al., 2007). This network incoherence adds further support for the suggestion that the effects of chronic pain are not limited to sensory discomfort, but also result from the disruption of coordinated activity that supports other functions (e.g. Dick & Rashedi, 2007; Luo et al., 2015). My observations revealed shared differences between the pain conditions and healthy controls. These observations suggest a level of overlap in the effects of these conditions on the brain, but also invite

further questions to why similar neurological effects can be associated with differing levels of behavioural dysfunction.

## **Limitations**

I am aware of several limitations of my investigation, both as a whole and within the individual studies. I shall consider these in turn.

### **General Limitations**

I chose two conditions to represent CLP and CWP and to generalise from them, rather than select participants from a range of CLP and CWP conditions. My patient population was small, but well-defined, and represents two common pain conditions. I selected knee OA as an example of CLP because of its prevalence in adult population. However, it would be difficult to make a generalisation about other CLP conditions, as the changes in the brain might be different from those I found. An investigation of other CLP conditions is required in future studies.

The selection of FM as a ‘typical’ CWP condition is worthy of further consideration. Our understanding of the underlying organic condition that drives FM is still somewhat limited (Chinn, Caldwell, & Gritsenko, 2016), indeed it is possible that this investigation’s FM group may comprise a set of individuals with different underlying problems that share only a common set of symptoms. This may have had the effect of diluting the ‘purity’ of my CWP sample and may account for the absence of some of the expected findings. Future studies may be guided by increased knowledge of the underlying causes of FM or may select other conditions as exemplars of CWP.

For logistical reasons I formed participants groups whose mean age differed according to condition. It is the case that OA is more common in older people and that the mean age of onset of FM is much younger and therefore recruiting age-matched participants is challenging. It is important to try to match age, because of the effects of age on the brain chemicals, brain volume and functional connectivity. Future researchers may benefit from either selecting a CLP condition with an earlier mean onset age, or/and a CWP condition with a later mean onset age. It is possible that researchers with access to a larger group of patients may be able to select FM patients of a more comparable age to those with OA, but this may

come at the price of introducing another possible confound to the study in an increased number of years that one of the conditions has been experienced.

I included only female participation in my studies, which precluded the investigation of possible gender effects. However, as FM patients are primarily female (Neumann & Buskila, 2003; Wolfe et al., 1995; Yunus et al., 1981), it was decided to only recruit female participants for all groups to avoid any potential gender bias effects. Studying females is important as females generally report a greater level of chronic pain (Andersson, Ejlertsson, Leden, & Rosenberg, 1993). However, future studies investigating both male and female participants may provide further knowledge.

### **Potential limitations of individual experiments**

MRS is a relatively less well-known technique in the neuroimaging of pain, which has evolved rapidly over recent years. As such, there is not yet full agreement regarding elements of the methodology of MRS and the conclusions that can be drawn using this technique. As an example, there is still discussion about appropriate peak referencing and calibration (Jansen, Backes, Nicolay, & Kooi, 2006), although in recent years a consensus have developed around the use of water as a concentration reference.

The conclusions drawn from MRS are reliant on an accurate assessment of neurochemicals present within the subjects' brains on the day of scanning. Neurochemical levels can, however, be affected by a variety of factors in addition to a pain condition including age, fitness, vascular health, diet and drug usage (e.g. Yeo et al., 2012). The participants were asked to refrain from using analgesic medication on the day of scanning whereas the washout period for some drugs is much longer. It was felt, however, that it would not be ethical to ask participants with chronic pain to refrain from taking analgesic drugs for a longer period.

Potential methodological vulnerabilities of VBM have been noted, for instance in normalisation and segmentation confounds, inaccuracy of localisation and potential misinterpretation of results (Mechelli et al., 2005). It has been suggested that VBM may produce type 1 errors if the possibility of systematic misregistration of participants' brain images is not controlled (Ashburner & Friston, 2001). Systematic differences between

scanning parameters, or the movement or positioning of a subject group within the scanner may produce artefacts that manifest as observed differences between-group grey matter volumes that do not reflect the morphometry of the participants' brains. Within my study I controlled for this possibility by ensuring that images were generated using identical scanning parameters within the same scanning unit. I further ensured that all participants were positioned identically in the scanner and all image sequences were analysed for motion artefacts.

Other potential limitations of VBM have related to the introduction of artefacts due to the pre-processing of images of atypical brains (Mechelli et al., 2005). Mechelli et al. found that apparent differences in grey matter volume may be produced by the presence of pathological features within participants' brains (e.g. tumours or abnormal artero-venous structures) that are not accounted for in the template employed for normalisation and segmentation. To control for this possibility, high-resolution anatomical scans of each participant were obtained and the absence of significant atypicality was confirmed. I am confident that my use of VBM was in accordance with appropriate methodological recommendations and I note, as per Whitwell (2009), the presence of a growing body of literature that provides validation of VBM as a technique for assessing structural changes in the brain.

FC requires a relatively lengthy scanning sequence during which the participant is instructed to rest. These requirements lead to the potential impairment of this technique through the introduction of head motion and to differences in participant response to the "Rest" instruction (Buckner, Krienen, & Yeo, 2013). The interpretation of FC results has been found to be vulnerable to observation of spurious correlations resulting from head motion (van Dijk, Sabuncu, & Buckner, 2012). I believe that I accounted for the possibility of head motion artefacts through regression and realigning. I acknowledge, however, that the possibility of performance differences in response to the "Rest" instruction is more difficult to control. Whilst each participant was briefed as to the response required during the FC scanning sequence and emphasis was placed on the need for an absence of directed thought, I cannot be fully confident that the participants' responses to the instruction were uniform. I believe that there is no reason to suspect a systematic difference in the responses between groups within my experiment, but I cannot exclude this possibility.

A further methodological consideration is specific to the form of FC that was used within my study. Seed-based component analysis examines the FC between a selected group of voxels and the rest of the brain. Whilst there are several advantages associated with this method (Cole, Smith, & Beckmann, 2010), the drawing of correct inferences from results derived from this method is entirely dependent on the selection of a well-defined seed that is appropriate for the research question being answered. As noted earlier, I believe I selected appropriate seed voxels based on prior knowledge of the networks of interest and a review of the literature.

Another possible limitation of the FC study is that I only examined resting state when considering attention – it is possible that differences in EAN need the participants to be engaged in a suitable task to reveal themselves. I am also mindful that the power of my FC experiment may have been limited due to the size of the available participant groups. Friston (2012) recommended groups of between 16 and 32 participants to reduce the possibility of type 2 errors. The groups in my study were, however, limited by access to suitable patients. I would suggest that the results of this experiment be considered a preliminary exploration of the effects of chronic pain on brain networks' connectivity at rest. I also suggest that my results have shown interesting alterations in FC that may help directed research questions for future studies with larger participant groups. I will consider now clinical implications and applications of the results.

## **Clinical Implications and Applications**

The differences discovered in the neurological presentation of chronic pain between CWP and CLP give us grounds to consider differential therapy and management of the two conditions. For example, the MRS study identified differences between the two chronic pain conditions: OA gave changes in neurochemical levels, associated with anatomy and brain function, whereas FM presented with changes in the relationships between neurochemical levels and clinical measures (depression, anxiety, pain intensity), associated with mood and behavioural functional abilities. This gives us a basis for using physically targeted therapies (pain killers, anti-inflammatories, surgery, physiotherapy) in OA patients, whereas FM patients may derive more benefit from behavioural therapies (CBT, meditation, hypnosis, yoga) and drugs working centrally such as amitriptyline, pregabalin, and duloxetine. Findings

from the VBM study also suggest that the two chronic pain conditions would benefit from different therapeutic strategies: for FM patients an emphasis on CBT, psychotherapy and relaxation techniques (changes in the brain area involved in cognition) and for OA patients a focus on movement therapies and physiotherapy (changes in the brain areas involved in movement). Differences found between CWP and CLP within the FC study also suggested that cognitive and behavioural therapies would be more effective in FM than in OA. I found an increase in FC between PCC and MTGL (within DMN) in FM compared to OA. The PCC, especially within the precuneus, has been found to be active in rumination in FM, and therefore may play a role in increasing the level of patients' experienced pain. Thus, FM patients would benefit from hypnosis, rational emotive behaviour therapy and group psychotherapy. MTGL is involved in a number of cognitive processes and increased FC between PCC and MTGL of FM patients suggests that CBT would be more effective in FM compared to OA. The observation of the differing emotional relationship to patients' pain conditions suggests that FM sufferers may derive more benefit from therapies that examine their subjective experience of pain and aim to control rumination. However, further research is required to understand the atypical FC that is observed within this condition to determine whether this has an effect in reinforcing rumination and would therefore work against re-evaluative therapies.

## **Future plans**

My future plans include scanning OA patients after knee surgery to see whether the brain changes that I observed are reversible. Also I am planning to scan FM patients after a full course of CBT to investigate if CBT can improve cognitive performance of FM patients and play a role in altering the effects of this condition on the brain.

In future studies I am planning to make sure that participants are age-matched and also to include male participants as well. My future research would include larger population groups to have more powerful results. Additionally, I intend to investigate differences in brain presentation of chronic pain between other chronic pain conditions: chronic low back pain, hip osteoarthritis, CRPS, chronic pelvic pain syndrome to determine whether my results can be generalised.

## Conclusion

These investigations have provided additional evidence to enhance our understanding of the effects of CLP and CWP on the human brain. Differences were observed in the effects of FM on the medial cognitive-emotional elements of the pain system and of OA on the lateral somatosensory pain systems. The differences observed via neuroimaging are in good alignment with previous research and with patients' own reports of their experience of these conditions. The correlation between the FM patients' evaluation of their condition and the observation of alterations to components of the cognitive-emotional pain system suggest a neural basis for this part of their experience. I believe that the evidence provided by my investigations on the separation of affected brain areas may assist in the search for brain biomarkers, which would be particularly useful in the case of FM.

Of further value in my investigations has been the addition of evidence that may assist in understanding the mechanisms through which reorganisation of the brain becomes a factor in CLP. My observation of functional alteration in OA (and not in FM) is in accord with previous research that highlighted the vulnerability of the thalamus to prolonged abnormal input from the periphery. The alignment of my findings with this previous work and the known role of the thalamus in thalamocortical oscillatory, inhibitory circuits (which are in turn known to play a role in managing the descending pain modulation pathways), suggests that thalamic disruption may lead to dysfunction of pain modulation and to the prolongation of CLP through central mechanisms.

My investigations have also provided evidence for the role of the precuneus in the activity of brain networks in patients experiencing chronic pain. The increased FC observed in pain conditions between the precuneus and areas within the DMN and EANs suggests a role for the precuneus in between-network co-ordination in these conditions. As noted, a detailed investigation of FC between the precuneus and insula of CLP and CWP patients would be of great value.

In conclusion, my studies have demonstrated differences in the effects of CLP and CWP on the pain system and provided additional understanding of the neural substrates of these

conditions. I have also demonstrated where the conditions overlap in their effects on the human brain and given an indication of shared mechanisms. I believe the results of my investigations have added to our knowledge of these conditions and may be useful in the development of further treatment.

## References

- Ablin, J. N., Cohen, H., & Buskila, D. (2006). Mechanisms of disease: genetics of fibromyalgia. *Nature Clinical Practice Rheumatology*, 2(12), 671–678.
- Ablin, J., Neumann, L., & Buskila, D. (2008). Pathogenesis of fibromyalgia - A review. *Joint Bone Spine*, 75(3), 273–279. <https://doi.org/10.1016/j.jbspin.2007.09.010>
- Acheson, D. J., & Hagoort, P. (2013). Stimulating the Brain's Language Network: Syntactic Ambiguity Resolution after TMS to the Inferior Frontal Gyrus and Middle Temporal Gyrus. *Journal of Cognitive Neuroscience*, 25(10), 1664–1677.
- Adler, G. K., & Geenen, R. (2005). Hypothalamic–pituitary–adrenal and autonomic nervous system functioning in fibromyalgia. *Rheumatic Disease Clinics of North America*, 31(1), 187–202.
- Almeida, T. F., Roizenblatt, S., & Tufik, S. (2004). Afferent pain pathways: A neuroanatomical review. *Brain Research*, 1000(1–2), 40–56. <https://doi.org/10.1016/j.brainres.2003.10.073>
- Altman, R., Asch, E., Bloch, D., Bole, G., Borenstein, D., Brandt, K., ... Hochberg, M. (1986). Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis and Rheumatism*, 29(8), 1039–1049. <https://doi.org/10.1002/art.1780290816>
- Andersson, H. I., Ejlertsson, G., Leden, I., & Rosenberg, C. (1993). Chronic pain in a geographically defined general population: studies of differences in age, gender, social class, and pain localization. *The Clinical Journal of Pain*, 3, 174–182.
- Andreasen, N. C., O'Leary, D. S., Cizadlo, T., Arndt, S., Rezai, K., Watkins, G. L., ... Hichwa, R. D. (1995). Remembering the past: two facets of episodic memory explored with positron emission tomography. *Am J Psychiatry*, 152(11), 1576–1585.
- Ang, D. C., Chakr, R., Mazzuca, S., France, C. R., Steiner, J., & Stump, T. (2010). Cognitive-behavioral therapy attenuates nociceptive responding in patients with fibromyalgia: A pilot study. *Arthritis Care and Research*, 62(5), 618–623. <https://doi.org/10.1002/acr.20119>
- Annemans, L., Wessely, S., Spaepen, E., Caekelbergh, K., Caubère, J. P., Le Lay, K., & Taïeb, C. (2008). Health economic consequences related to the diagnosis of fibromyalgia syndrome. *Arthritis and Rheumatism*, 58(3), 895–902.

<https://doi.org/10.1002/art.23265>

- Apkarian, A. V., Bushnell, M. C., Treede, R. D., & Zubieta, J. K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain*, 9(4), 463–484. <https://doi.org/10.1016/j.ejpain.2004.11.001>
- Apkarian, A. V., Baliki, M. N., & Geha, P. Y. (2009). Towards a theory of chronic pain. *Progress in Neurobiology*, 87(2), 81–97. <https://doi.org/10.1016/j.pneurobio.2008.09.018>
- Apkarian, A. V., Sosa, Y., Sonty, S., Levy, R. M., Harden, R. N., Parrish, T. B., & Gitelman, D. R. (2004). Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 24(46), 10410–10415. <https://doi.org/10.1523/JNEUROSCI.2541-04.2004>
- Arnold, L. M., Crofford, L. J., Mease, P. J., Burgess, S. M., Palmer, S. C., Abetz, L., & Martin, S. a. (2008). Patient perspectives on the impact of fibromyalgia. *Patient Education and Counseling*, 73(1), 114–120. <https://doi.org/10.1016/j.pec.2008.06.005>
- Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *NeuroImage*, 38(1), 95–113. <https://doi.org/10.1016/j.neuroimage.2007.07.007>
- Ashburner, J., & Friston, K. J. (2000). Voxel-based morphometry--the methods. *NeuroImage*, 11(6 Pt 1), 805–821. <https://doi.org/10.1006/nimg.2000.0582>
- Ashburner, J., & Friston, K. J. (2001). Why voxel-based morphometry should be used. *NeuroImage*, 14(6), 1238–1243. <https://doi.org/10.1006/nimg.2001.0961>
- Ashburner, J., & Friston, K. J. (2005). Unified segmentation. *NeuroImage*, 26(3), 839–51. <https://doi.org/10.1016/j.neuroimage.2005.02.018>
- Baddeley, A. D., & Hitch, G. J. (1974). Working memory. *The Psychology of Learning and Motivation*, 8, 47–89.
- Bajaj, P., Bajaj, P., Graven-Nielsen, T., & Arendt-Nielsen, L. (2001). Osteoarthritis and its association with muscle hyperalgesia: An experimental controlled study. *Pain*, 93(2), 107–114. [https://doi.org/10.1016/S0304-3959\(01\)00300-1](https://doi.org/10.1016/S0304-3959(01)00300-1)
- Baliki, M. N., Baria, A. T., & Apkarian, a V. (2011). The cortical rhythms of chronic back pain. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 31(39), 13981–90. <https://doi.org/10.1523/JNEUROSCI.1984-11.2011>
- Baliki, M. N., Geha, P. Y., Apkarian, a V., & Chialvo, D. R. (2008). Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. *The Journal of*

- Neuroscience : The Official Journal of the Society for Neuroscience*, 28(6), 1398–1403.  
<https://doi.org/10.1523/JNEUROSCI.4123-07.2008>
- Baliki, M. N., Geha, P. Y., Jabakhanji, R., Harden, N., Schnitzer, T. J., & Apkarian, a V. (2008). A preliminary fMRI study of analgesic treatment in chronic back pain and knee osteoarthritis. *Molecular Pain*, 4, 47. <https://doi.org/10.1186/1744-8069-4-47>
- Baliki, M. N., Mansour, A. R., Baria, A. T., & Apkarian, a V. (2014). Functional reorganization of the default mode network across chronic pain conditions. *PloS One*, 9(9), e106133. <https://doi.org/10.1371/journal.pone.0106133>
- Baliki, M. N., Petre, B., Torbey, S., Herrmann, K. M., Huang, L., Schnitzer, T. J., ... Apkarian, a V. (2012). Corticostriatal functional connectivity predicts transition to chronic back pain. *Nature Neuroscience*, 15(8), 1117–1119.  
<https://doi.org/10.1038/nn.3153>
- Baliki, M. N., Schnitzer, T. J., Bauer, W. R., & Apkarian, a V. (2011). Brain morphological signatures for chronic pain. *PloS One*, 6(10), e26010.  
<https://doi.org/10.1371/journal.pone.0026010>
- Ballantine, H. T., Cassidy, W. L., Flanagan, N. B., & Marino, R. (1967). Stereotaxic Anterior Cingulotomy for Neuropsychiatric Illness and Intractable Pain. *Journal of Neurosurgery*, 26, 488–495.
- Bandettini, P. A. (2009). What's New in Neuroimaging Methods? *Annals of the New York Academy of Sciences*, 1156, 260–293. <https://doi.org/10.1111/j.1749-6632.2009.04420.x>
- Barker, P. B., & Lin, D. D. M. (2006). In vivo proton MR spectroscopy of the human brain. *Progress in Nuclear Magnetic Resonance Spectroscopy*, 49(2), 99–128.  
<https://doi.org/10.1016/j.pnmrs.2006.06.002>
- Barrett, D. S., Cobb, A. G., & Bentley, G. (1991). Joint proprioception in normal, osteoarthritic and replaced knees. *J Bone Joint Surg Br.*, 73(1), 53–56.
- Beecher, H. K. (1946). Pain in Men Wounded in Battle. *Annals of Surgery*, 123(1), 96–105.
- Behrmann, M., Geng, J. J., & Shomstein, S. (2004). Parietal cortex and attention. *Current Opinion in Neurobiology*, 14(2), 212–217. <https://doi.org/10.1016/j.conb.2004.03.012>
- Belt, N. K., Kronholm, E., & Kauppi, M. J. (2009). Sleep problems in fibromyalgia and rheumatoid arthritis compared with the general population. *Clin Exp Rheumatol*, 27(1), 35–41.
- Bennett, R., & Nelson, D. (2006). Cognitive behavioral therapy for fibromyalgia. *Nature Clinical Practice. Rheumatology*, 2(8), 416–424. <https://doi.org/10.1038/ncprheum0245>

- Bernasconi, A., Bernasconi, N., Natsume, J., Antel, S. B., Andermann, F., & Arnold, D. L. (2003). Magnetic resonance spectroscopy and imaging of the thalamus in idiopathic generalized epilepsy. *Brain*, *126*(Pt 11), 2447–2454.  
<https://doi.org/10.1093/brain/awg249>
- Besson, J. M. (1999). The neurobiology of pain. *Lancet*, *353*(9164), 1610–1615.  
[https://doi.org/10.1016/S0140-6736\(99\)01313-6](https://doi.org/10.1016/S0140-6736(99)01313-6)
- Binder, J. R., Frost, J. A., Hammeke, T. A., Cox, R. W., Rao, S. M., & Prieto, T. (1997). Human brain language areas identified by functional magnetic resonance imaging. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *17*(1), 353–362.
- Bird, G. C., Han, J. S., Fu, Y., Adwanikar, H., Willis, W. D., & Neugebauer, V. (2006). Pain-related synaptic plasticity in spinal dorsal horn neurons: role of CGRP. *Molecular Pain*, *2*(1), 31. <https://doi.org/10.1186/1744-8069-2-31>
- Birklein, F. (2005). Complex regional pain syndrome. *Journal of Neurology*, *252*(2), 131–138. <https://doi.org/10.1007/s00415-005-0737-8>
- Biswal, B., Yetkin, F., Haughton, V., & Hyde, J. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med.*, *34*(4), 537–541.
- Bradley, L. A., Sotolongo, A., Alberts, K. R., Alarcon, G. S., Mountz, J. M., Liu, H. G., ... Blalock, J. E. (1999). Abnormal regional cerebral blood flow in the caudate nucleus among fibromyalgia patients and non-patients is associated with insidious symptom onset. *Journal of Musculoskeletal Pain*, *7*(1–2), 285–292.  
[https://doi.org/10.1300/J094v07n01\\_29](https://doi.org/10.1300/J094v07n01_29)
- Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2006). Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *European Journal of Pain*, *10*(4), 287–333. <https://doi.org/10.1016/j.ejpain.2005.06.009>
- Brett, M., Anton, J.-L., Valabregue, R., & Poline, J.-B. (2002). Region of interest analysis using an SPM toolbox [abstract]. Available on CD-ROM in NeuroImage, Vol 16, No. 2. In *8th International Conference on Functional Mapping of the Human Brain*.
- Bromm, B. (2001). Brain images of pain. *News in Physiological Sciences : An International Journal of Physiology Produced Jointly by the International Union of Physiological Sciences and the American Physiological Society*, *16*, 244–249.
- Brooks, J., & Tracey, I. (2005). From nociception to pain perception: Imaging the spinal and

- supraspinal pathways. *Journal of Anatomy*, 207(1), 19–33.  
<https://doi.org/10.1111/j.1469-7580.2005.00428.x>
- Brooks, P. M. (2002). Impact of osteoarthritis on individuals and society: how much disability? Social consequences and health economic implications. *Current Opinion in Rheumatology*, 14(5), 573–577.
- Brown, J. A., & Pilitsis, J. G. (2005). Motor Cortex Stimulation for Central and Neuropathic Facial Pain: A Prospective Study of 10 Patients and Observations of Enhanced Sensory and Motor Function during Stimulation. *Neurosurgery*, 56(2), 290–297.  
<https://doi.org/10.1227/01.NEU.0000148905.75845.98>
- Brulatout, S., Méric, P., Loubinoux, I., Borredon, J., Corrèze, J. L., Roucher, P., ... Seylaz, J. (1996). A one-dimensional (proton and phosphorus) and two-dimensional (proton) in vivo NMR spectroscopic study of reversible global cerebral ischemia. *Journal of Neurochemistry*, 66(6), 2491–2499. <https://doi.org/10.1046/j.1471-4159.1996.66062491.x>
- Buckner, R. L., Krienen, F. M., & Yeo, B. T. T. (2013). Opportunities and limitations of intrinsic functional connectivity MRI. *Nature Publishing Group*, 16(7), 832–837.  
<https://doi.org/10.1038/nn.3423>
- Buffington, A. L. H., Hanlon, C. A., & McKeown, M. J. (2005). Acute and persistent pain modulation of attention-related anterior cingulate fMRI activations. *Pain*, 113(1–2), 172–184. <https://doi.org/10.1016/j.pain.2004.10.006>
- Burgmer, M., Gaubitz, M., Konrad, C., Wrenger, M., Hilgart, S., Heuft, G., & Pfliegerer, B. (2009). Decreased gray matter volumes in the cingulo-frontal cortex and the amygdala in patients with fibromyalgia. *Psychosomatic Medicine*, 71(5), 566–573.  
<https://doi.org/10.1097/PSY.0b013e3181a32da0>
- Burgmer, M., Petzke, F., Giesecke, T., Gaubitz, M., Heuft, G., & Pfliegerer, B. (2011). Cerebral activation and catastrophizing during pain anticipation in patients with fibromyalgia. *Psychosomatic Medicine*, 73(9), 751–759.
- Bush, G., Luu, P., & Posner, M. I. (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends in Cognitive Sciences*, 4(6), 215–222.  
[https://doi.org/http://dx.doi.org/10.1016/S1364-6613\(00\)01483-2](https://doi.org/http://dx.doi.org/10.1016/S1364-6613(00)01483-2)
- Buskila, D., & Press, J. (2001). Neuroendocrine mechanisms in fibromyalgia-chronic fatigue. *Best Practice and Research: Clinical Rheumatology*, 15(5), 747–758.  
<https://doi.org/10.1053/berh.2001.0191>

- Buskila, D., Sarzi-Puttini, P., & Ablin, J. N. (2007). The genetics of fibromyalgia syndrome.
- Cagnie, B., Coppieters, I., Denecker, S., Six, J., Danneels, L., & Meeus, M. (2014). Central sensitization in fibromyalgia? A systematic review on structural and functional brain MRI. *Seminars in Arthritis and Rheumatism*, *44*(1), 68–75.  
<https://doi.org/10.1016/j.semarthrit.2014.01.001>
- Callaert, D. V., Ribbens, A., Maes, F., Swinnen, S. P., & Wenderoth, N. (2014). Assessing age-related gray matter decline with voxel-based morphometry depends significantly on segmentation and normalization procedures. *Frontiers in Aging Neuroscience*, *6*(JUN), 1–14. <https://doi.org/10.3389/fnagi.2014.00124>
- Calvert, G., Campbell, R., & Brammer, M. (2000). Evidence from functional magnetic resonance imaging of crossmodal binding in the human heteromodal cortex. *Current Biology*, *10*(11), 649–657. Retrieved from <http://discovery.ucl.ac.uk/146998/>
- Casey, K. L. (1999). Forebrain mechanisms of nociception and pain: analysis through imaging. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(14), 7668–7674. <https://doi.org/10.1073/pnas.96.14.7668>
- Cauda, F., D'Agata, F., Sacco, K., Duca, S., Cocito, D., Paolasso, I., ... Geminiani, G. (2010). Altered resting state attentional networks in diabetic neuropathic pain. *Journal of Neurology, Neurosurgery, and Psychiatry*, *81*(7), 806–811.  
<https://doi.org/10.1136/jnnp.2009.188631>
- Cauda, F., Sacco, K., D'Agata, F., Duca, S., Cocito, D., Geminiani, G., ... Isoardo, G. (2009). Low-frequency BOLD fluctuations demonstrate altered thalamocortical connectivity in diabetic neuropathic pain. *BMC Neuroscience*, *10*(138). <https://doi.org/10.1186/1471-2202-10-138>
- Cauda, F., Sacco, K., Duca, S., Cocito, D., D'Agata, F., Geminiani, G. C., & Canavero, S. (2009). Altered resting state in diabetic neuropathic pain. *PLoS ONE*, *4*(2).  
<https://doi.org/10.1371/journal.pone.0004542>
- Cavanna, A. E. (2007). The precuneus and consciousness. *CNS Spectrums*, *12*(7), 545–552.
- Cavanna, A. E., & Trimble, M. R. (2006). The precuneus: A review of its functional anatomy and behavioural correlates. *Brain*, *129*(3), 564–583.  
<https://doi.org/10.1093/brain/awl004>
- Chang, L., Ernst, T., Poland, R. E., & Jenden, D. J. (1996). In vivo proton magnetic resonance spectroscopy of the normal aging human brain. *Life Sciences*, *58*(22), 2049–2056. [https://doi.org/10.1016/0024-3205\(96\)00197-X](https://doi.org/10.1016/0024-3205(96)00197-X)

- Chien, J. J., & Bajwa, Z. H. (2008). What is mechanical back pain and how best to treat it? *Current Pain and Headache Reports*, *12*, 406–411. <https://doi.org/10.1007/s11916-008-0069-3>
- Chinn, S., Caldwell, W., & Gritsenko, K. (2016). Fibromyalgia Pathogenesis and Treatment Options Update. *Current Pain and Headache Reports*, *20*(4), 25. <https://doi.org/10.1007/s11916-016-0556-x>
- Choi, C., Coupland, N. J., Bhardwaj, P. P., Kalra, S., Casault, C. A., Reid, K., & Allen, P. S. (2006). T2 measurement and quantification of glutamate in human brain in vivo. *Magnetic Resonance in Medicine*, *56*(5), 971–977.
- Choi, C. G., & Frahm, J. (1999). Localized proton MRS of the human hippocampus: Metabolite concentrations and relaxation times. *Magnetic Resonance in Medicine*, *41*(1), 204–207.
- Christiansen, P., Toft, P., Larsson, H. B. W., Stubgaard, M., & Henriksen, O. (1993). The concentration of N-acetyl aspartate, creatine + phosphocreatine, and choline in different parts of the brain in adulthood and senium. *Magnetic Resonance Imaging*, *11*(6), 799–806.
- Cifre, I., Sitges, C., Fraiman, D., Munoz, M. A., Balenzuela, P., Gonzalez-Roldan, A., ... Montoya, P. (2012). Disrupted Functional Connectivity of the Pain Network in Fibromyalgia. *Psychosomatic Medicine*, *74*(1), 55–62. <https://doi.org/10.1097/PSY.0b013e3182408f04>
- Clauw, D. J., & Crofford, L. J. (2003). Chronic widespread pain and fibromyalgia: what we know, and what we need to know. *Best Practice & Research Clinical Rheumatology*, *17*(4), 685–701. [https://doi.org/10.1016/S1521-6942\(03\)00035-4](https://doi.org/10.1016/S1521-6942(03)00035-4)
- Cohen, H., Neumann, L., Alhoshhle, A., Kotler, M., Abu-Shakra, M., & Buskila, D. (2001). Abnormal sympathovagal balance in men with fibromyalgia. *Journal of Rheumatology*, *28*(3), 581–589.
- Cohen, H., Neumann, L., Shore, M., Amir, M., Cassuto, Y., & Buskila, D. (2000). Autonomic dysfunction in patients with fibromyalgia: Application of power spectral analysis of heart rate variability. *Seminars in Arthritis and Rheumatism*, *29*(4), 217–227. [https://doi.org/10.1016/S0049-0172\(00\)80010-4](https://doi.org/10.1016/S0049-0172(00)80010-4)
- Cole, D. M., Smith, S. M., & Beckmann, C. F. (2010). Advances and pitfalls in the analysis and interpretation of resting-state fMRI data. *Frontiers in Systems Neuroscience*, *4*(April), 8. <https://doi.org/10.3389/fnsys.2010.00008>

- Cook, D. B., Lange, G., Ciccone, D. S., Liu, W. C., Steffener, J., & Natelson, B. H. (2004). Functional imaging of pain in patients with primary fibromyalgia. *J Rheumatol*, *31*(2), 364–378.
- Costafreda, S. G., Fu, C. H. Y., Lee, L., Everitt, B., Brammer, M. J., & David, A. S. (2006). A systematic review and quantitative appraisal of fMRI studies of verbal fluency: Role of the left inferior frontal gyrus. *Human Brain Mapping*, *27*(10), 799–810. <https://doi.org/10.1002/hbm.20221>
- Costigan, M., Scholz, J., & Woolf, C. J. (2010). Neuropathic Pain: A Maladaptive Response of the Nervous System to Damage. *Annu Rev of Neurosci.*, *32*, 1–32. <https://doi.org/10.1146/annurev.neuro.051508.135531.Neuropathic>
- Cowdrey, F. a., Filippini, N., Park, R. J., Smith, S. M., & McCabe, C. (2014). Increased resting state functional connectivity in the default mode network in recovered anorexia nervosa. *Human Brain Mapping*, *35*(2), 483–491. <https://doi.org/10.1002/hbm.22202>
- Craig, A. D. (2003). Interoception: The sense of the physiological condition of the body. *Current Opinion in Neurobiology*, *13*(4), 500–505. [https://doi.org/10.1016/S0959-4388\(03\)00090-4](https://doi.org/10.1016/S0959-4388(03)00090-4)
- Creamer, P., Hunt, M., & Dieppe, P. (1996). Pain mechanisms in osteoarthritis of the knee: effect of intraarticular anesthetic. *The Journal of Rheumatology*, *23*(6), 1031–1036.
- Crivello, F., Tzourio-Mazoyer, N., Tzourio, C., & Mazoyer, B. (2014). Longitudinal Assessment of Global and Regional Rate of Grey Matter Atrophy in 1,172 Healthy Older Adults: Modulation by Sex and Age. *PLoS ONE*, *9*(12), e114478. <https://doi.org/10.1371/journal.pone.0114478>
- Crofford, L. J., & Clauw, D. J. (2002). Editorial: Fibromyalgia: Where are we a decade after the American College of Rheumatology classification criteria were developed? *Arthritis and Rheumatism*, *46*(5), 1136–1138. <https://doi.org/10.1002/art.10217>
- Crofford, L. J., Pillemer, S. R., Crofford, L. J., Pillemer, S. R., Kalogeras, K. T., Cash, J. M., ... Wilder, R. L. (1994). Hypothalamic–pituitary–adrenal axis perturbations in patients with fibromyalgia. *Arthritis & Rheumatism*, *37*(11), 1583–1592.
- Dadabhoy, D., Crofford, L. J., Spaeth, M., Russell, I. J., & Clauw, D. J. (2008). Biology and therapy of fibromyalgia. Evidence-based biomarkers for fibromyalgia syndrome. *Arthritis Research & Therapy*, *10*(4), 211. <https://doi.org/10.1186/ar2443>
- Damasio, A. (2003). Feelings of Emotion and the Self. *Annals of the New York Academy of Sciences*, *1001*, 253–261. <https://doi.org/10.1017/CBO9781107415324.004>

- Damoiseaux, J. S., Beckmann, C. F., Arigita, E. J. S., Barkhof, F., Scheltens, P., Stam, C. J., ... Rombouts, S. a R. B. (2008). Reduced resting-state brain activity in the “default network” in normal aging. *Cerebral Cortex*, *18*(8), 1856–1864.  
<https://doi.org/10.1093/cercor/bhm207>
- Dapretto, M., & Bookheimer, S. Y. (1999). Form and Content: Dissociating Syntax and Semantics in Sentence Comprehension. *Neuron*, *24*(2), 427–432.  
[https://doi.org/10.1016/S0896-6273\(00\)80855-7](https://doi.org/10.1016/S0896-6273(00)80855-7)
- Dar, R., Ariely, D., & Frenk, H. (1995). The effect of past injury on pain threshold and tolerance. *Pain*, *60*, 189–193.
- Davis, K. D., & Moayedi, M. (2012). Central mechanisms of pain revealed through functional and structural MRI. *Journal of Neuroimmune Pharmacology*, *8*(3), 518–534.  
<https://doi.org/10.1007/s11481-012-9386-8>
- Davis, K. D., Taylor, S. J., Crawley, a P., Wood, M. L., & Mikulis, D. J. (1997). Functional MRI of pain- and attention-related activations in the human cingulate cortex. *Journal of Neurophysiology*, *77*(6), 3370–80. Retrieved from  
<http://www.ncbi.nlm.nih.gov/pubmed/9212281>
- De Ridder, D., Van Laere, K., Dupont, P., Menovsky, T., & Van de Heyning, P. (2007). Visualizing out-of-body experience in the brain. *The New England Journal of Medicine*, *357*(18), 1829–1833. <https://doi.org/10.1056/NEJMoa070010>
- De Stefano, N., Matthews, P. M., & Arnold, D. L. (1995). Reversible decreases in N-acetylaspartate after acute brain injury. *Magnetic Resonance in Medicine*, *34*(5), 721–727. <https://doi.org/10.1002/mrm.1910340511>
- De Win, M. M. L., Jager, G., Booij, J., Reneman, L., Schilt, T., Lavini, C., ... Van Den Brink, W. (2008). Neurotoxic effects of ecstasy on the thalamus. *British Journal of Psychiatry*, *193*(4), 289–296. <https://doi.org/10.1192/bjp.bp.106.035089>
- deCharms, R. C., Maeda, F., Glover, G. H., Ludlow, D., Pauly, J. M., Soneji, D., ... Mackey, S. C. (2005). Control over brain activation and pain learned by using real-time functional MRI. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(51), 18626–31. <https://doi.org/10.1073/pnas.0505210102>
- Derbyshire, S. W. (2000). Exploring the pain “neuromatrix”. *Current Review of Pain*, *4*(6), 467–477. <https://doi.org/10.1007/s11916-000-0071-x>
- Desgranges, B., Baron, J. C., & Eustache, F. (1998). The functional neuroanatomy of episodic memory: the role of the frontal lobes, the hippocampal formation, and other

- areas. *NeuroImage*, 8(2), 198–213. <https://doi.org/10.1006/nimg.1998.0359>
- Deyo, R. A., & Weinstein, J. N. (2001). Low Back Pain. *N Engl J Med*, 344(5), 363–370. <https://doi.org/10.1056/NEJM200102013440508>
- Dick, B. D., & Rashiq, S. (2007). Disruption of attention and working memory traces in individuals with chronic pain. *Anesthesia and Analgesia*, 104(5), 1223–1229. <https://doi.org/10.1213/01.ane.0000263280.49786.f5>
- Dieringer, M. A., Deimling, M., Santoro, D., Wuerfel, J., Madai, V. I., Sobesky, J., ... Niendorf, T. (2014). Rapid parametric mapping of the longitudinal relaxation time T1 using two-dimensional variable flip angle magnetic resonance imaging at 1.5 Tesla, 3 Tesla, and 7 Tesla. *PLoS ONE*, 9(3), 3–10. <https://doi.org/10.1371/journal.pone.0091318>
- Doleys, D. M. (2014). *Pain: dynamics and complexities*. New York: Oxford University Press.
- Dörfel, D., Werner, A., Schaefer, M., Von Kummer, R., & Karl, A. (2009). Distinct brain networks in recognition memory share a defined region in the precuneus. *European Journal of Neuroscience*, 30(10), 1947–1959. <https://doi.org/10.1111/j.1460-9568.2009.06973.x>
- Ecclestone, & Crombez. (1999). Pain Demands Attention: A Cognitive-Affective Model of the Interruptive Function of Pain, 125(3), 1–11. Retrieved from <papers3://publication/uuid/01C07091-6AF1-4814-8BB2-D6403C7D954B>
- Emad, Y., Ragab, Y., Zeinhom, F., El-Khouly, G., Abou-Zeid, A., & Rasker, J. J. (2008). Hippocampus dysfunction may explain symptoms of fibromyalgia syndrome. A study with single-voxel magnetic resonance spectroscopy. *The Journal of Rheumatology*, 35(7), 1371–7. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/18484688>
- Eskandar, E. N., Richmond, B. J., & Optican, L. M. (1992). Role of inferior temporal neurons in visual memory. I. Temporal encoding of information about visual images, recalled images, and behavioral context. *Journal of Neurophysiology*, 68(4), 1277–1295. Retrieved from <http://jn.physiology.org/content/68/4/1277.abstract>
- Fagerlund, A. J., Hansen, O. A., & Aslaksen, P. M. (2015). Transcranial direct current stimulation as a treatment for patients with fibromyalgia: a randomized controlled trial. *Pain*, 156(1), 62–71.
- Fallon, N., Alghamdi, J., Chiu, Y., Sluming, V., Nurmikko, T., & Stancak, A. (2013). Structural alterations in brainstem of fibromyalgia syndrome patients correlate with sensitivity to mechanical pressure. *NeuroImage: Clinical*, 3, 163–170. <https://doi.org/10.1016/j.nicl.2013.07.011>

- Fan, J., McCandliss, B. D., Fossella, J., Flombaum, J. I., & Posner, M. I. (2005). The activation of attentional networks. *NeuroImage*, *26*(2), 471–479.  
<https://doi.org/10.1016/j.neuroimage.2005.02.004>
- Farrell, M., Gibson, S., McMeeken, J., & Helme, R. (2000). Pain and hyperalgesia in osteoarthritis of the hands. *The Journal of Rheumatology*, *27*(2), 441–447.
- Fasick, V., Spengler, R. N., Samankan, S., Nader, N. D., & Ignatowski, T. A. (2015). The hippocampus and TNF: Common links between chronic pain and depression. *Neuroscience and Biobehavioral Reviews*, *53*, 139–159.  
<https://doi.org/10.1016/j.neubiorev.2015.03.014>
- Faul, F., Erdfelder, E., Lang, A.-G. G., & Buchner, A. (2007). G \* Power 3 : A flexible statistical power analysis program for the social , behavioral , and biomedical sciences. *Behavior Research Methods*, *39*(2), 175–191. <https://doi.org/10.3758/bf03193146>
- Fayed, N., Garcia-Campayo, J., Magallón, R., Andrés-Bergareche, H., Luciano, J. V., Andres, E., & Beltrán, J. (2010). Localized 1H-NMR spectroscopy in patients with fibromyalgia: a controlled study of changes in cerebral glutamate/glutamine, inositol, choline, and N-acetylaspartate. *Arthritis Research & Therapy*, *12*(4), R134.  
<https://doi.org/10.1186/ar3072>
- Faymonville, M. E., Roediger, L., Del Fiore, G., Delguedre, C., Phillips, C., Lamy, M., ... Laureys, S. (2003). Increased cerebral functional connectivity underlying the antinociceptive effects of hypnosis. *Cognitive Brain Research*, *17*(2), 255–262.  
[https://doi.org/10.1016/S0926-6410\(03\)00113-7](https://doi.org/10.1016/S0926-6410(03)00113-7)
- Feraco, P., Bacci, A., Pedrabissi, F., Passamonti, L., Zampogna, G., Pedrabissi, F., ... Leonardi, M. (2011). Metabolic abnormalities in pain-processing regions of patients with fibromyalgia: a 3T MR spectroscopy study. *AJNR. American Journal of Neuroradiology*, *32*(9), 1585–90. <https://doi.org/10.3174/ajnr.A2550>
- Filimon, F., Nelson, J. D., Huang, R.-S., & Sereno, M. I. (2009). Multiple parietal reach regions in humans: cortical representations for visual and proprioceptive feedback during on-line reaching. *The Journal of Neuroscience*, *29*(9), 2961–2971.  
<https://doi.org/10.1523/JNEUROSCI.3211-08.2009>
- Fine, P. G. (2011). Review and President’s Message: Long-Term Consequences of Chronic Pain : Mounting Evidence for Pain as a Neurological. *Pain*, 996–1004.
- Flor, H., Braun, C., Elbert, T., & Birbaumer, N. (1997). Extensive reorganization of primary somatosensory cortex in chronic back pain patients. *Neuroscience Letters*, *224*(1), 5–8.

- Flor, H., Elbert, T., Knecht, S., Wienbruch, C., Pantev, C., Birbaumer, N., ... Taub, E. (1995). Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. *Nature*, *375*(6531), 482–484. <https://doi.org/10.1038/375482a0>
- Foerster, B. R., Petrou, M., Harris, R. E., Barker, P. B., Hoeffner, E. G., Clauw, D. J., & Sundgren, P. C. (2011). Cerebral blood flow alterations in pain-processing regions of patients with fibromyalgia using perfusion MR imaging. *American Journal of Neuroradiology*, *32*(10), 1873–1878. <https://doi.org/10.3174/ajnr.A2614>
- Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(27), 9673–8. <https://doi.org/10.1073/pnas.0504136102>
- Fransson, P., & Marrelec, G. (2008). The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: Evidence from a partial correlation network analysis. *NeuroImage*, *42*(3), 1178–84. <https://doi.org/10.1016/j.neuroimage.2008.05.059>
- Fries, J. F., Weinberger, M., Lorig, K. R., Jacobs, J. J., & Goldberg, V. (2000). Osteoarthritis: New Insights. Part 2: Treatment Approaches. *Annals of Internal Medicine*, *133*(9), 726–729. Retrieved from [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\\_uids=11074906](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11074906)
- Friston, K. (2012). Ten ironic rules for non-statistical reviewers. *NeuroImage*, *61*(4), 1300–10. <https://doi.org/10.1016/j.neuroimage.2012.04.018>
- Frymoyer, J. W. (1988). Back pain and sciatica. *New England Journal of Medicine*, *318*(5), 291–300.
- Fuchs, P. N., Balinsky, M., & Melzack, R. (1996). Electrical stimulation of the cingulum bundle and surrounding cortical tissue reduces formalin-test pain in the rat. *Brain Research*, *743*(1–2), 116–23. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9017238>
- Galhardoni, R., Correia, G. S., Araujo, H., Yeng, L. T., Fernandes, D. T., Kaziyama, H. H., ... de Andrade, D. C. (2015). Repetitive Transcranial Magnetic Stimulation in Chronic Pain: A Review of the Literature. *Archives of Physical Medicine and Rehabilitation*, *96*(4), S156–S172. <https://doi.org/10.1016/j.apmr.2014.11.010>
- Ganji, S. K., Banerjee, A., Patel, A. M., Zhao, Y. D., Dimitrov, I. E., Browning, J. D., ... Choi, C. (2012). T 2 measurement of J-coupled metabolites in the human brain at 3T.

*NMR in Biomedicine*, 25(4), 523–529. <https://doi.org/10.1002/nbm.1767>

- Gardini, S., Venneri, A., Sambataro, F., Cuetos, F., Fasano, F., Marchi, M., ... Caffarra, P. (2015). Increased functional connectivity in the default mode network in mild cognitive impairment: a maladaptive compensatory mechanism associated with poor semantic memory performance. *J Alzheimers Dis.*, 45(2), 457–470. <https://doi.org/10.3233/JAD-142547>
- Gasparovic, C., Song, T., Devier, D., Bockholt, H. J., Caprihan, A., Mullins, P. G., ... Morrison, L. a. (2006). Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magnetic Resonance in Medicine : Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 55(6), 1219–26. <https://doi.org/10.1002/mrm.20901>
- Gauchard, G. C., Vançon, G., Meyer, P., Mainard, D., & Perrin, P. P. (2010). On the role of knee joint in balance control and postural strategies: effects of total knee replacement in elderly subjects with knee osteoarthritis. *Gait & Posture*, 32(2), 155–60. <https://doi.org/10.1016/j.gaitpost.2010.04.002>
- Geha, P. Y., Baliki, M. N., Chialvo, D. R., Harden, R. N., Paice, J. a, & Apkarian, a V. (2007). Brain activity for spontaneous pain of postherpetic neuralgia and its modulation by lidocaine patch therapy. *Pain*, 128(1–2), 88–100. <https://doi.org/10.1016/j.pain.2006.09.014>
- Gerstner, G., Arbor, A., Ichesco, E., Pain, C., Arbor, A., Quintero, A., ... Arbor, A. (2011). Changes in Regional Gray and White Matter Volume in Patients with Myofascial-type Temporomandibular Disorders: A Voxel-based Morphometry Study. *Journal of Orofacial Pain*, i, 99–106.
- Giesecke, T., Gracely, R. H., Grant, M. a B., Nachevson, A., Petzke, F., Williams, D. a., & Clauw, D. J. (2004). Evidence of Augmented Central Pain Processing in Idiopathic Chronic Low Back Pain. *Arthritis and Rheumatism*, 50(2), 613–623. <https://doi.org/10.1002/art.20063>
- Glass, J. M. (2009). Review of cognitive dysfunction in fibromyalgia: a convergence on working memory and attentional control impairments. *Rheumatic Diseases Clinics of North America*, 35(2), 299–311. <https://doi.org/10.1016/j.rdc.2009.06.002>
- Glombiewski, J. a., Sawyer, A. T., Gutermann, J., Koenig, K., Rief, W., & Hofmann, S. G. (2010). Psychological treatments for fibromyalgia: A meta-analysis. *Pain*, 151(2), 280–295. <https://doi.org/10.1016/j.pain.2010.06.011>

- Goble, D. J., Coxon, J. P., van Impe, A., Geurts, M., Doumas, M., Wenderoth, N., & Swinnen, S. P. (2011). Brain Activity during Ankle Proprioceptive Stimulation Predicts Balance Performance in Young and Older Adults. *Journal of Neuroscience*, *31*(45), 16344–16352. <https://doi.org/10.1523/JNEUROSCI.4159-11.2011>
- Goldenberg, D. L., Kaplan, K. H., Nadeau, M. G., Brodeur, C., Smith, S., & Schmid, C. H. (1994). A Controlled Study of a Stress-Reduction, Cognitive-Behavioral Treatment Program in Fibromyalgia. *Journal of Musculoskeletal Pain*, *2*(2), 53–66.
- Govindaraju, V., Young, K., & Maudsley, A. A. (2000). Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR in Biomedicine*, *13*(3), 129–53. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10861994>
- Gracely, R. H., & Ambrose, K. R. (2011). Neuroimaging of fibromyalgia. *Best Practice and Research: Clinical Rheumatology*, *25*(2), 271–284. <https://doi.org/10.1016/j.berh.2011.02.003>
- Gracely, R. H., Geisser, M. E., Giesecke, T., Grant, M. A. B., Petzke, F., Williams, D. A., & Clauw, D. J. (2004). Pain catastrophizing and neural responses to pain among persons with fibromyalgia. *Brain*, *127*(4), 835–843. <https://doi.org/10.1093/brain/awh098>
- Gracely, R. H., Petzke, F., Wolf, J. M., & Clauw, D. J. (2002). Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis and Rheumatism*, *46*(5), 1333–43. <https://doi.org/10.1002/art.10225>
- Grachev, I. D., Fredrickson, B. E., & Apkarian, A. V. (2000). Abnormal brain chemistry in chronic back pain: an in vivo proton magnetic resonance spectroscopy study. *Pain*, *89*(1), 7–18. [https://doi.org/S0304-3959\(00\)00340-7](https://doi.org/S0304-3959(00)00340-7) [pii]
- Grachev, I. D., Ramachandran, T. S., Thomas, P. S., Szeverenyi, N. M., & Fredrickson, B. E. (2003). Association between dorsolateral prefrontal N-acetyl aspartate and depression in chronic back pain: an in vivo proton magnetic resonance spectroscopy study. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *110*(3), 287–312. <https://doi.org/10.1007/s00702-002-0781-9>
- Grachev, I. D., Thomas, P. S., & Ramachandran, T. S. (2002). Decreased levels of N-acetylaspartate in dorsolateral prefrontal cortex in a case of intractable severe sympathetically mediated chronic pain (complex regional pain syndrome, type I). *Brain and Cognition*, *49*(1), 102–13. <https://doi.org/10.1006/brcg.2001.1489>
- Greicius, M. D., Krasnow, B., Reiss, A. L., & Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the*

- National Academy of Sciences of the United States of America*, 100(1), 253–258.  
<https://doi.org/10.1073/pnas.0135058100>
- Gussew, A., Rzanny, R., Erdtel, M., Scholle, H. C., Kaiser, W. A., Mentzel, H. J., & Reichenbach, J. R. (2009). Time-resolved functional 1H MR spectroscopic detection of glutamate concentration changes in the brain during acute heat pain stimulation. *NeuroImage*, 49(2), 1895–902. <https://doi.org/10.1016/j.neuroimage.2009.09.007>
- Gussew, A., Rzanny, R., Güllmar, D., Scholle, H.-C., & Reichenbach, J. R. (2011). 1H-MR spectroscopic detection of metabolic changes in pain processing brain regions in the presence of non-specific chronic low back pain. *NeuroImage*, 54(2), 1315–23.  
<https://doi.org/10.1016/j.neuroimage.2010.09.039>
- Gustin, S. M., Peck, C. C., Wilcox, S. L., Nash, P. G., Murray, G. M., & Henderson, L. A. (2011). Different pain, different brain: thalamic anatomy in neuropathic and non-neuropathic chronic pain syndromes. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 31(16), 5956–5964.  
<https://doi.org/10.1523/JNEUROSCI.5980-10.2011>
- Gustin, S. M., Wrigley, P. J., Youssef, A. M., McIndoe, L., Wilcox, S. L., Rae, C. D., ... Henderson, L. A. (2014). Thalamic activity and biochemical changes in individuals with neuropathic pain after spinal cord injury. *Pain*, 155(5), 1027–1036.  
<https://doi.org/10.1016/j.pain.2014.02.008>
- Gwilym, S. E., Filippini, N., Douaud, G., Carr, A. J., & Tracey, I. (2010). Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study. *Arthritis and Rheumatism*, 62(10), 2930–40. <https://doi.org/10.1002/art.27585>
- Gwilym, S. E., Keltner, J. R., Warnaby, C. E., Carr, A. J., Chizh, B., Chessell, I., & Tracey, I. (2009). Psychophysical and functional imaging evidence supporting the presence of central sensitization in a cohort of osteoarthritis patients. *Arthritis Care and Research*, 61(9), 1226–1234. <https://doi.org/10.1002/art.24837>
- Haga, K. K., Khor, Y. P., Farrall, A., & Wardlaw, J. M. (2009). A systematic review of brain metabolite changes, measured with 1H magnetic resonance spectroscopy, in healthy aging. *Neurobiology of Aging*, 30(3), 353–63.  
<https://doi.org/10.1016/j.neurobiolaging.2007.07.005>
- Hammer, R. P., Egilmez, Y., & Emmett-Oglesby, M. W. (1997). Review article Neural mechanisms of tolerance to the effects of cocaine. *Behavioural Brain Research*, 84,

225–239.

- Hampshire, A., Chamberlain, S. R., Monti, M. M., Duncan, J., & Owen, A. M. (2010). The role of the right inferior frontal gyrus: inhibition and attentional control. *NeuroImage*, *50*(3), 1313–1319. <https://doi.org/10.1016/j.neuroimage.2009.12.109>
- Hampson, M., Driesen, N. R., Skudlarski, P., Gore, J. C., & Constable, R. T. (2006). Brain connectivity related to working memory performance. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(51), 13338–13343. <https://doi.org/10.1523/JNEUROSCI.3408-06.2006>
- Hanakawa, T., Dimyan, M. A., & Hallett, M. (2008). Motor planning, imagery, and execution in the distributed motor network: A time-course study with functional MRI. *Cerebral Cortex*, *18*(12), 2775–2788. <https://doi.org/10.1093/cercor/bhn036>
- Harris, R. E., Clauw, D. J., Scott, D. J., McLean, S. A., Gracely, R. H., & Zubieta, J.-K. (2007). Decreased Central -Opioid Receptor Availability in Fibromyalgia. *Journal of Neuroscience*, *27*(37), 10000–10006. <https://doi.org/10.1523/JNEUROSCI.2849-07.2007>
- Harris, R. E., Sundgren, P. C., Craig, A. D., Kirshenbaum, E., Sen, A., Napadow, V., & Clauw, D. J. (2009). Elevated insular glutamate in fibromyalgia is associated with experimental pain. *Arthritis and Rheumatism*, *60*(10), 3146–52. <https://doi.org/10.1002/art.24849>
- Harris, R. E., Sundgren, P. C., Pang, Y., Hsu, M., Petrou, M., Kim, S.-H., ... Clauw, D. J. (2008). Dynamic levels of glutamate within the insula are associated with improvements in multiple pain domains in fibromyalgia. *Arthritis and Rheumatism*, *58*(3), 903–7. <https://doi.org/10.1002/art.23223>
- Harvey, A. K., Taylor, A. M., & Wise, R. G. (2012). Imaging pain in arthritis: Advances in structural and functional neuroimaging. *Current Pain and Headache Reports*, *16*(6), 492–501. <https://doi.org/10.1007/s11916-012-0297-4>
- Hassett, A. L., Clauw, D. J., & Williams, D. . (2015). Premature aging in fibromyalgia. *Current Aging Science*, *8*(2), 178–185.
- Hendiani, J. A., Westlund, K. N., Lawand, N., Goel, N., Lisse, J., & McNearney, T. (2003). Mechanical sensation and pain thresholds in patients with chronic arthropathies. *Journal of Pain*, *4*(4), 203–211. [https://doi.org/10.1016/S1526-5900\(03\)00557-1](https://doi.org/10.1016/S1526-5900(03)00557-1)
- Howard, M. A., Sanders, D., Krause, K., O’Muircheartaigh, J., Fotopoulou, A., Zelaya, F., ... Williams, S. C. R. (2012). Alterations in resting-state regional cerebral blood flow

- demonstrate ongoing pain in osteoarthritis: An arterial spin-labeled magnetic resonance imaging study. *Arthritis and Rheumatism*, 64(12), 3936–3946.  
<https://doi.org/10.1002/art.37685>
- Hsieh, J.-C., Belfrage, M., Stone-Elander, S., Hansson, P., & Ingvar, M. (1995). Central representation of chronic ongoing neuropathic pain studied by positron emission tomography. *Pain*, 63(2), 225–236. [https://doi.org/10.1016/0304-3959\(95\)00048-W](https://doi.org/10.1016/0304-3959(95)00048-W)
- Hsu, M. C., Harris, R. E., Sundgren, P. C., Welsh, R. C., Fernandes, C. R., Clauw, D. J., & Williams, D. a. (2009). No consistent difference in gray matter volume between individuals with fibromyalgia and age-matched healthy subjects when controlling for affective disorder. *Pain*, 143(3), 262–267. <https://doi.org/10.1016/j.pain.2009.03.017>
- Iadarola, M. J., Max, M. B., Berman, K. F., Byas-Smith, M. G., Coghill, R. C., Gracely, R. H., & Bennett, G. J. (1995). Unilateral decrease in thalamic activity observed with positron emission tomography in patients with chronic neuropathic pain. *Pain*, 63(1), 55–64. [https://doi.org/0304-3959\(95\)00015-K](https://doi.org/0304-3959(95)00015-K) [pii]
- IASP Task Force on Taxonomy. (1994). *A Current List with Definitions and Notes on Usage. Part III: Pain Terms*. (H. Merskey & N. Bogduk, Eds.) (Second). Seattle, Washington: IASP Press.
- Ichesco, E., Quintero, A., Clauw, D. J., Peltier, S., Sundgren, P. M., Gerstner, G. E., & Schmidt-Wilcke, T. (2012). Altered functional connectivity between the insula and the cingulate cortex in patients with temporomandibular disorder: A pilot study. *Headache*, 52(3), 441–454. <https://doi.org/10.1111/j.1526-4610.2011.01998.x>
- Ichesco, E., Schmidt-Wilcke, T., Bhavsar, R., Clauw, D. J., Peltier, S. J., Kim, J., ... Harris, R. E. (2014). Altered resting state connectivity of the insular cortex in individuals with fibromyalgia. *Journal of Pain*, 15(8), 815–826.e1.  
<https://doi.org/10.1016/j.jpain.2014.04.007>
- Imamura, M., Imamura, S. T., Kaziyama, H. H. S., Targino, R. A., Wu, T. H., De Souza, L. P. M., ... Camanho, G. L. (2008). Impact of nervous system hyperalgesia on pain, disability, and quality of life in patients with knee osteoarthritis: A controlled analysis. *Arthritis Care and Research*, 59(10), 1424–1431. <https://doi.org/10.1002/art.24120>
- Isaac, S. M., Barker, K. L., Danial, I. N., Beard, D. J., Dodd, C. A., & Murray, D. W. (2007). Does arthroplasty type influence knee joint proprioception? A longitudinal prospective study comparing total and unicompartmental arthroplasty. *The Knee*, 14(3), 212–7.  
<https://doi.org/10.1016/j.knee.2007.01.001>

- Jagannathan, N. R., Desai, N. G., & Raghunathan, P. (1996). Brain metabolite changes in alcoholism: An in vivo proton magnetic resonance spectroscopy (MRS) study. *Magnetic Resonance Imaging*, *14*(5), 553–557. [https://doi.org/10.1016/0730-725X\(96\)00048-3](https://doi.org/10.1016/0730-725X(96)00048-3)
- Jansen, J. F. a, Backes, W. H., Nicolay, K., & Kooi, M. E. (2006). <sup>1</sup>H MR spectroscopy of the brain: absolute quantification of metabolites. *Radiology*, *240*(2), 318–332. <https://doi.org/10.1148/radiol.2402050314>
- Jensen, K. B., Srinivasan, P., Spaeth, R., Tan, Y., Kosek, E., Petzke, F., ... Kong, J. (2013). Overlapping structural and functional brain changes in patients with long-term exposure to fibromyalgia pain. *Arthritis and Rheumatism*, *65*(12), 3293–3303. <https://doi.org/10.1002/art.38170>
- Jensen, M. P., & Karoly, P. (2001). Self-report scales and procedures for assessing pain in adults. In D. C. Turk & R. Melzack (Eds.), *Handbook of Pain Assessment* (2nd ed., pp. 15–34). New York: Guilford Press.
- Johansen, J. P., Fields, H. L., & Manning, B. H. (2001). The affective component of pain in rodents: direct evidence for a contribution of the anterior cingulate cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(14), 8077–82. <https://doi.org/10.1073/pnas.141218998>
- Jordan, K. M. (2003). EULAR Recommendations 2003: an evidence based approach to the management of knee osteoarthritis: Report of a Task Force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). *Annals of the Rheumatic Diseases*, *62*(12), 1145–1155. Retrieved from <http://ard.bmj.com/content/62/12/1145.full>
- Kamaleri, Y., Natvig, B., Ihlebaek, C., & Bruusgaard, D. (2008). Localized or widespread musculoskeletal pain: does it matter? *Pain*, *138*(1), 41–46.
- Kanaan, R. A. A., Lepine, J. P., & Wessely, S. C. (2007). The Association or Otherwise of the Functional Somatic Syndromes. *Psychosomatic Medicine*, *69*(9), 855–859. <https://doi.org/10.1097/PSY.0b013e31815b001a>.
- Karni, A., & Sagi, D. (1991). Where practice makes perfect in texture discrimination : Evidence for primary visual cortex plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(June), 4966–4970.
- Katzev, M., Tuscher, O., Hennig, J., Weiller, C., & Kaller, C. P. (2013). Revisiting the Functional Specialization of Left Inferior Frontal Gyrus in Phonological and Semantic Fluency: The Crucial Role of Task Demands and Individual Ability. *Journal of*

- Neuroscience*, 33(18), 7837–7845. <https://doi.org/10.1523/JNEUROSCI.3147-12.2013>
- Kawashima, R., Roland, P., & O’Sullivan, B. (1995). Functional anatomy of reaching and visuomotor learning: a positron emission tomography study. *Cereb Cortex*, 5(2), 111–122.
- Khan, A. M., McLoughlin, E., Giannakas, K., Hutchinson, C., & Andrew, J. G. (2004). Hip osteoarthritis: where is the pain? *Annals of the Royal College of Surgeons of England*, 86(2), 119–121. <https://doi.org/10.1308/003588404322827518>
- Khan, N., & Woolson, S. (1998). Referral patterns of hip pain in patients undergoing total hip replacement. *Orthopedics*, 21(2), 123–126.
- Kirkham, M. A. (2010). *Accessing and assessing fibromyalgia through pain drawings*. Bangor University.
- Kjaer, T. W., Nowak, M., Kjaer, K. W., Lou, A. R., & Lou, H. C. (2001). Precuneus-prefrontal activity during awareness of visual verbal stimuli. *Consciousness and Cognition*, 10(3), 356–65. <https://doi.org/10.1006/ccog.2001.0509>
- Kjaer, T. W., Nowak, M., & Lou, H. C. (2002). Reflective Self-Awareness and Conscious States: PET Evidence for a Common Midline Parietofrontal Core. *NeuroImage*, 17(2), 1080–1086. <https://doi.org/10.1006/nimg.2002.1230>
- Kosek, E., & Ordeberg, G. (2000). Abnormalities of somatosensory perception in patients with painful osteoarthritis normalize following successful treatment. *Eur J Pain*, 4(3), 229–238. Retrieved from doi: 10.1053/eujp.2000.0175
- Kreis, R. (2016). The trouble with quality filtering based on relative Cramer-Rao lower bounds. *Magnetic Resonance in Medicine*, 75(1), 15–18. <https://doi.org/10.1002/mrm.25568>
- Kuchinad, A., Schweinhardt, P., Seminowicz, D. A., Wood, P. B., Chizh, B. A., & Bushnell, M. C. (2007). Accelerated brain gray matter loss in fibromyalgia patients: premature aging of the brain? *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 27(15), 4004–4007. <https://doi.org/10.1523/JNEUROSCI.0098-07.2007>
- Kulkarni, B., Bentley, D. E., Elliott, R., Julyan, P. J., Boger, E., Watson, A., ... Jones, A. K. P. (2007). Arthritic pain is processed in brain areas concerned with emotions and fear. *Arthritis and Rheumatism*, 56(4), 1345–1354. <https://doi.org/10.1002/art.22460>
- Kulkarni, B., Bentley, D. E., Elliott, R., Youell, P., Watson, A., Derbyshire, S. W. G., ... Jones, A. K. P. (2005). Attention to pain localization and unpleasantness discriminates the functions of the medial and lateral pain systems. *The European Journal of*

- Neuroscience*, 21(11), 3133–42. <https://doi.org/10.1111/j.1460-9568.2005.04098.x>
- LaGraize, S. C., Borzan, J., Peng, Y. B., & Fuchs, P. N. (2006). Selective regulation of pain affect following activation of the opioid anterior cingulate cortex system. *Experimental Neurology*, 197(1), 22–30. <https://doi.org/10.1016/j.expneurol.2005.05.008>
- LaGraize, S. C., & Fuchs, P. N. (2007). GABAA but not GABAB receptors in the rostral anterior cingulate cortex selectively modulate pain-induced escape/avoidance behavior. *Experimental Neurology*, 204(1), 182–94. <https://doi.org/10.1016/j.expneurol.2006.10.007>
- Larson, A. A., Giovengo, S. L., Russell, I. J., & Michalek, J. E. (2000). Changes in the concentrations of amino acids in the cerebrospinal fluid that correlate with pain in patients with fibromyalgia: implications for nitric oxide pathways. *Pain*, 87(2), 201–211.
- Latremoliere, A., & Woolf, C. J. (2009). Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *Journal of Pain*, 10(9), 895–926. <https://doi.org/10.1016/j.jpain.2009.06.012>
- Lee, Y. C., Nassikas, N. J., & Clauw, D. J. (2011). The role of the central nervous system in the generation and maintenance of chronic pain in rheumatoid arthritis, osteoarthritis and fibromyalgia. *Arthritis Research & Therapy*, 13, 211. <https://doi.org/10.1186/ar3306>
- Lekander, M., Fredrikson, M., & Wik, G. (2000). Neuroimmune relations in patients with fibromyalgia: a positron emission tomography study. *Neuroscience Letters*, 282(3), 193–196. [https://doi.org/10.1016/S0304-3940\(00\)00901-0](https://doi.org/10.1016/S0304-3940(00)00901-0)
- Lephart, S. M., Pincivero, D. M., & Rozzi, S. L. (1998). Proprioception of the ankle and knee. *Sports Med*, 25, 149.
- Li, S. J., Wang, Y., Pankiewicz, J., & Stein, E. A. (1999). Neurochemical adaptation to cocaine abuse: Reduction of N-acetyl aspartate in thalamus of human cocaine abusers. *Biological Psychiatry*, 45(11), 1481–1487. [https://doi.org/10.1016/S0006-3223\(98\)00230-3](https://doi.org/10.1016/S0006-3223(98)00230-3)
- Llinás, R. R., Ribary, U., Jeanmonod, D., Kronberg, E., & Mitra, P. P. (1999). Thalamocortical dysrhythmia: A neurological and neuropsychiatric syndrome characterized by magnetoencephalography. *Proceedings of the National Academy of Sciences of the United States of America*, 96(26), 15222–7. <https://doi.org/10.1073/pnas.96.26.15222>
- Loggia, M. L., Kim, J., Gollub, R. L., Vangel, M. G., Kirsch, I., Kong, J., ... Napadow, V. (2013). Default mode network connectivity encodes clinical pain: An arterial spin

- labeling study. *Pain*, *154*(1), 24–33. <https://doi.org/10.1016/j.pain.2012.07.029>
- López-Solà, M., Pujol, J., Wager, T. D., Garcia-Fontanals, A., Blanco-Hinojo, L., Garcia-Blanco, S., ... Deus, J. (2014). Altered fMRI responses to non-painful sensory stimulation in fibromyalgia patients. *Arthritis & Rheumatism*. <https://doi.org/10.1002/art.38781>
- Lou, H. C., Luber, B., Crupain, M., Keenan, J. P., Nowak, M., Kjaer, T. W., ... Lisanby, S. H. (2004). Parietal cortex and representation of the mental Self. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(17), 6827–6832. <https://doi.org/10.1073/pnas.0400049101>
- Luchtman, M., Baecke, S., Steinecke, Y., Bernarding, J., Tempelmann, C., Ragert, P., & Firsching, R. (2015). Changes in gray matter volume after microsurgical lumbar discectomy: a longitudinal analysis. *Frontiers in Human Neuroscience*, *9*(February), 1–7. <https://doi.org/10.3389/fnhum.2015.00012>
- Luerding, R., Weigand, T., Bogdahn, U., & Schmidt-Wilcke, T. (2008). Working memory performance is correlated with local brain morphology in the medial frontal and anterior cingulate cortex in fibromyalgia patients: Structural correlates of pain-cognition interaction. *Brain*, *131*(12), 3222–3231. <https://doi.org/10.1093/brain/awn229>
- Lundstrom, B. N., Ingvar, M., & Petersson, K. M. (2005). The role of precuneus and left inferior frontal cortex during source memory episodic retrieval. *NeuroImage*, *27*(4), 824–834. <https://doi.org/10.1016/j.neuroimage.2005.05.008>
- Lundstrom, B. N., Petersson, K. M., Andersson, J., Johansson, M., Fransson, P., & Ingvar, M. (2003). Isolating the retrieval of imagined pictures during episodic memory: activation of the left precuneus and left prefrontal cortex. *NeuroImage*, *20*(4), 1934–1943. <https://doi.org/10.1016/j.neuroimage.2003.07.017>
- Luo, Y., Kong, F., Qi, S., You, X., & Huang, X. (2015). Resting-state functional connectivity of the default mode network associated with happiness. *Social Cognitive and Affective Neuroscience Advance Access*.
- MacIver, K., Lloyd, D. M., Kelly, S., Roberts, N., & Nurmikko, T. (2008). Phantom limb pain, cortical reorganization and the therapeutic effect of mental imagery. *Brain*, *131*(8), 2181–2191. <https://doi.org/10.1093/brain/awn124>
- Maddock, R. ., Garrett, A. ., & Buonocore, M. . (2001). Remembering familiar people: the posterior cingulate cortex and autobiographical memory retrieval. *Neuroscience*, *104*(3), 667–676. [https://doi.org/10.1016/S0306-4522\(01\)00108-7](https://doi.org/10.1016/S0306-4522(01)00108-7)

- Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage*, *19*(3), 1233–1239. [https://doi.org/10.1016/S1053-8119\(03\)00169-1](https://doi.org/10.1016/S1053-8119(03)00169-1)
- Malinen, S., Vartiainen, N., Hlushchuk, Y., Koskinen, M., Ramkumar, P., Forss, N., ... Hari, R. (2010). Aberrant temporal and spatial brain activity during rest in patients with chronic pain. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(14), 6493–6497. <https://doi.org/10.1073/pnas.1001504107>
- Maniadakis, N., & Gray, A. (2000). The economic burden of back pain in the UK. *Pain*, *84*(1), 95–103. [https://doi.org/10.1016/S0304-3959\(99\)00187-6](https://doi.org/10.1016/S0304-3959(99)00187-6)
- March, L. M., Cross, M. J., Lapsley, H., Brnabic, A. J., Tribe, K. L., Bachmeier, C. J., & Brooks, P. M. (1999). Outcomes after hip or knee replacement surgery for osteoarthritis. A prospective cohort study comparing patients' quality of life before and after surgery with age-related population norms. *The Medical Journal of Australia*, *171*(5), 235–238.
- Marques, A. P., Rhoden, L., de Oliveira Siqueira, J., & Joao, S. M. (2001). Pain evaluation of patients with fibromyalgia, osteoarthritis, and low back pain. *Revista Do Hospital Das Clinicas*, *56*(1), 5–10.
- Martinez-Lavin, M. (2007). Biology and therapy of fibromyalgia. Stress, the stress response system, and fibromyalgia. *Arthritis Research & Therapy*, *9*(4), 216. <https://doi.org/10.1186/ar2146>
- Martucci, K. T., Shirer, W. R., Bagarinao, E., Johnson, K. A., Farmer, M. A., Labus, J. S., ... Mackey, S. C. (2015). The posterior medial cortex in urologic chronic pelvic pain syndrome: detachment from default mode network—a resting-state study from the MAPP Research Network. *Pain*, *156*(9), 1755–64. <https://doi.org/10.1097/j.pain.0000000000000238>
- Matsuda, H. (2013). Voxel-based Morphometry of Brain MRI in Normal Aging and Alzheimer's Disease. *Aging and Disease*, *4*(1), 29–37. Retrieved from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3570139&tool=pmcentrez&rendertype=abstract>
- Maudsley, A. A., Domenig, C., Govind, V., Darkazanli, A., Studholme, C., Arheart, K., & Bloomer, C. (2009). Mapping of brain metabolite distributions by volumetric proton MR spectroscopic imaging (MRSI). *Magnetic Resonance in Medicine*, *61*(3), 548–559. <https://doi.org/10.1002/mrm.21875>
- Maudsley, A., Govind, V., & Arheart, K. L. (2012). Associations of age, gender and body

- mass with <sup>1</sup>H MR-observed brain metabolites and tissue distributions. *NMR in Biomedicine*, 25(4), 580–93. <https://doi.org/10.1002/nbm.1775>
- May, A. (2008). Chronic pain may change the structure of the brain. *Pain*, 137(1), 7–15. <https://doi.org/10.1016/j.pain.2008.02.034>
- May, A. (2011). Structural brain imaging: a window into chronic pain. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, 17(2), 209–220. <https://doi.org/10.1177/1073858410396220>
- May, A., & Gaser, C. (2006). Magnetic resonance-based morphometry: a window into structural plasticity of the brain. *Current Opinion in Neurology*, 19(4), 407–411. <https://doi.org/10.1097/01.wco.0000236622.91495.21>
- Mayer, D. J., Mao, J., Holt, J., & Price, D. D. (1999). Cellular mechanisms of neuropathic pain, morphine tolerance, and their interactions. *Proceedings of the National Academy of Sciences of the United States of America*, 96(July), 7731–7736. <https://doi.org/10.1073/pnas.96.14.7731>
- McAlindon, T. E., Cooper, C., Kirwan, J. R., & Dieppe, P. A. (1993). Determinants of Disability in Osteoarthritis of the Knee. *Annals of the Rheumatic Diseases*, 52(4), 258–262. <https://doi.org/10.1136/ard.52.4.258>
- McCormick, C., Protzner, A. B., Barnett, A. J., Cohn, M., Valiante, T. a., & McAndrews, M. P. (2014). Linking DMN connectivity to episodic memory capacity: What can we learn from patients with medial temporal lobe damage? *NeuroImage: Clinical*, 5, 188–196. <https://doi.org/10.1016/j.nicl.2014.05.008>
- Mechelli, A., Price, C. J., Friston, K. J., & Ashburner, J. (2005). Voxel-based morphometry of the human brain: Methods and applications. *Current Medical Imaging Reviews*, 1, 105–113. <https://doi.org/10.2174/1573405054038726>
- Mechelli, A., Price, C. J., Friston, K. J., & Ishai, A. (2004). Where Bottom-up Meets Top-down: Neuronal Interactions during Perception and Imagery. *Cerebral Cortex*, 14(11), 1256–1265. <https://doi.org/10.1093/cercor/bhh087>
- Melzack, R. (1975). The McGill Pain Questionnaire: Major Properties and Scoring Methods. *Pain*, 1, 277–299.
- Melzack, R. (1990). Phantom limbs and the concept of a neuromatrix. *Trends in Neurosciences*, 13(3), 88–92. [https://doi.org/10.1016/0166-2236\(90\)90179-E](https://doi.org/10.1016/0166-2236(90)90179-E)
- Melzack, R. (2001). Pain and the neuromatrix in the brain. *Journal of Dental Education*, 65(12), 1378–1382.

- Melzack, R., & Wall, P. (1965). Pain Mechanism: A new Theory. *Science*.  
<https://doi.org/10.1126/science.150.3699.971>
- Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Structure and Function*, 1–13.  
<https://doi.org/10.1007/s00429-010-0262-0>
- Merskey, H., & Bogduk, N. (1994). *Classification of chronic pain: Descriptions of chronic pain syndromes and definitions of pain terms* (2nd ed.). Seattle, Washington: IASP Press.
- Millan, M. J. (1999). The induction of pain an integrative review. *Progress in Neurobiology*, 57(98), 1–164.
- Minoshima, S., Foster, N. L., & Kuhl, D. E. (1994). Posterior cingulate cortex in Alzheimer's disease. *Lancet*, 344(8926), 895.
- Minoshima, S., Giordani, B., Berent, S., Frey, K. A., Foster, N. L., & Kuhl, D. E. (1997). Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Ann Neurol.*, 42(1), 85–94.
- Moayedi, M., & Davis, K. D. (2012). Theories of pain: from Specificity to Gate Control. *Journal of Neurophysiology*, (Rey 1995), 5–12. <https://doi.org/10.1152/jn.00457.2012>
- Mohanty, A., Engels, A. S., Herrington, J. D., Heller, W., Ringo Ho, M. H., Banich, M. T., ... Miller, G. A. (2007). Differential engagement of anterior cingulate cortex subdivisions for cognitive and emotional function. *Psychophysiology*, 44(3), 343–351.  
<https://doi.org/10.1111/j.1469-8986.2007.00515.x>
- Moseley, G. L., Zalucki, N., Birklein, F., Marinus, J., Van Hilten, J. J., & Luomajoki, H. (2008). Thinking about movement hurts: The effect of motor imagery on pain and swelling in people with chronic arm pain. *Arthritis Care and Research*, 59(5), 623–631.  
<https://doi.org/10.1002/art.23580>
- Moss, R. A., & Garrett, J. C. (1984). Temporomandibular joint dysfunction syndrome and myofascial pain dysfunction syndrome: a critical review. *Journal of Oral Rehabilitation*, 11(1), 3–28.
- Mountz, J. M., Bradley, L. A., Modell, J. G., Alexander, R. W., Triana-Alexander, M., Aaron, L. A., ... Mountz, J. D. (1995). Fibromyalgia in women. *Arthritis & Rheumatism*, 38(7), 926–938. <https://doi.org/10.1002/art.1780380708>
- Mullins, P. G., Rowland, L., Bustillo, J., Bedrick, E. J., Lauriello, J., & Brooks, W. M. (2003). Reproducibility of 1H-MRS measurements in schizophrenic patients. *Magnetic Resonance in Medicine : Official Journal of the Society of Magnetic Resonance in*

- Medicine / Society of Magnetic Resonance in Medicine*, 50(4), 704–7.  
<https://doi.org/10.1002/mrm.10598>
- Mullins, P. G., Rowland, L. M., Jung, R. E., & Sibbitt, W. L. (2005). A novel technique to study the brain's response to pain: proton magnetic resonance spectroscopy. *NeuroImage*, 26(2), 642–6. <https://doi.org/10.1016/j.neuroimage.2005.02.001>
- Murray, C. J. L., & Lopez, A. D. (1997). Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet*, 349(9064), 1498–1504. [https://doi.org/10.1016/S0140-6736\(96\)07492-2](https://doi.org/10.1016/S0140-6736(96)07492-2)
- Mutso, A. A., Petre, B., Huang, L., Baliki, M. N., Torbey, S., Herrmann, K. M., & ... Apkarian, A. V. (2014). Reorganization of hippocampal functional connectivity with transition to chronic back pain. *Journal Of Neurophysiology*, 111(5), 1065–1076. <https://doi.org/10.1152/jn.00611.2013>
- Napadow, V., Kim, J., Clauw, D. J., & Harris, R. E. (2012). Decreased intrinsic brain connectivity is associated with reduced clinical pain in fibromyalgia. *Arthritis and Rheumatism*, 64(7), 2398–2403. <https://doi.org/10.1002/art.34412>
- Napadow, V., LaCount, L., Park, K., As-Sanie, S., Clauw, D. J., & Harris, R. E. (2010). Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. *Arthritis and Rheumatism*, 62(8), 2545–2555. <https://doi.org/10.1002/art.27497>
- Neumann, L., & Buskila, D. (2003). Epidemiology of fibromyalgia. *Current Pain and Headache Reports*, 7(5), 362–8. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12946289>
- Nicholls, J. G., & Paton, J. F. R. (2009). Brainstem: neural networks vital for life. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 364(1529), 2447–2451. Retrieved from <http://rstb.royalsocietypublishing.org/content/364/1529/2447.abstract>
- Nielsen, F. Å., Balslev, D., & Hansen, L. K. (2005). Mining the posterior cingulate: Segregation between memory and pain components. *NeuroImage*, 27(3), 520–532. <https://doi.org/10.1016/j.neuroimage.2005.04.034>
- Nielsen, L. A., & Henriksson, K. G. (2007). Pathophysiological mechanisms in chronic musculoskeletal pain (fibromyalgia): the role of central and peripheral sensitization and pain disinhibition. *Best Practice and Research: Clinical Rheumatology*, 21(3), 465–480. <https://doi.org/10.1016/j.berh.2007.03.007>
- Nyberg, L., McIntosh, A. R., Cabeza, R., Nilsson, L. G., Houle, S., Habib, R., & Tulving, E.

- (1996). Network analysis of positron emission tomography regional cerebral blood flow data: ensemble inhibition during episodic memory retrieval. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 16(11), 3753–3759.
- Ordeberg, G. (2004). Characterization of joint pain in human OA. *Novartis Found Symp*, 260, 105–121.
- Parks, E. L., Geha, P. Y., Baliki, M. N., Katz, J., Schnitzer, T. J., & Apkarian, A. V. (2011). Brain activity for chronic knee osteoarthritis: Dissociating evoked pain from spontaneous pain. *European Journal of Pain London England*, 15(8), 843.e1-e843.e14. Retrieved from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3113642&tool=pmcentrez&rendertype=abstract>
- Parks, E. L., Geha, P. Y., Baliki, M. N., Katz, J., Schnitzer, T. J., & Apkarian, A. V. (2011). Brain activity for chronic knee osteoarthritis: Dissociating evoked pain from spontaneous pain. *Eur J Pain*, 15(8), 843.e1-843.e14. <https://doi.org/10.1016/j.ejpain.2010.12.007>
- Pascual-Leone, A., Amedi, A., Fregni, F., & Merabet, L. (2005). the Plastic Human Brain Cortex. *Annual Review of Neuroscience*, 28(1), 377–401. <https://doi.org/10.1146/annurev.neuro.27.070203.144216>
- Pattany, P. M., Yeziarski, R. P., Widerström-Noga, E. G., Bowen, B. C., Martinez-Arizala, A., Garcia, B. R., & Quencer, R. M. (2002). Proton magnetic resonance spectroscopy of the thalamus in patients with chronic neuropathic pain after spinal cord injury. *AJNR. American Journal of Neuroradiology*, 23(6), 901–5. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12063213>
- Perl, E. R. (2007). Ideas about pain, a historical view. *Nature Reviews. Neuroscience*, 8(1), 71–80. <https://doi.org/10.1038/nrn2042>
- Petrou, M., Harris, R. E., Foerster, B. R., McLean, S. A., Sen, A., Clauw, D. J., & Sundgren, P. C. (2008). Proton MR spectroscopy in the evaluation of cerebral metabolism in patients with fibromyalgia: comparison with healthy controls and correlation with symptom severity. *AJNR. American Journal of Neuroradiology*, 29(5), 913–8. <https://doi.org/10.3174/ajnr.A0959>
- Petrovic, P., & Ingvar, M. (2002). Imaging cognitive modulation of pain processing. *Pain*, 95(1–2), 1–5. [https://doi.org/10.1016/S0304-3959\(01\)00467-5](https://doi.org/10.1016/S0304-3959(01)00467-5)
- Prescot, A. P., Shi, X., Choi, C., & Renshaw, P. F. (2014). In vivo T2 relaxation time

- measurement with echo-time averaging. *NMR in Biomedicine*, 27(8), 863–869.  
<https://doi.org/10.1002/nbm.3115>
- Provencher, S. W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med*, 30(6), 672–679.  
<https://doi.org/10.1002/mrm.1910300604>
- Queiroz, L. P. (2013). Worldwide epidemiology of fibromyalgia topical collection on fibromyalgia. *Current Pain and Headache Reports*, 17(8), 356.  
<https://doi.org/10.1007/s11916-013-0356-5>
- Rae, C. D. (2014). A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochemical Research*, 39(1), 1–36.  
<https://doi.org/10.1007/s11064-013-1199-5>
- Rainville, P., Duncan, G. H., Price, D. D., Carrier, B., & Bushnell, M. C. (1997). Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science*, 277(5328), 968–971. [https://doi.org/DOI: 10.1126/science.277.5328.968](https://doi.org/DOI:10.1126/science.277.5328.968)
- Ramón y Cajal, S. (1911). *Histologie du Système Nerveux de l'Homme et des Vertébrés II*. Paris: Maloine.
- Recanzone, G. H., Merzenich, M. M., Jenkins, W. M., Grajski, K. A., & Dinse, H. R. (1992). Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task. *Journal of Neurophysiology*, 67(5), 1031–56.  
<https://doi.org/1597696>
- Reider, B., Arcand, M. A., Diehl, L. H., Mroczek, K., Abulencia, A., Stroud, C. C., ... Staszak, P. (2003). Proprioception of the knee before and after anterior cruciate ligament reconstruction. *Arthroscopy: The Journal of Arthroscopic & Related Surgery: Official Publication of the Arthroscopy Association of North America and the International Arthroscopy Association*, 19(1), 2–12. <https://doi.org/10.1053/jars.2003.50006>
- Reyes Del Paso, G. A., Pulgar, A., Duschek, S., & Garrido, S. (2012). Cognitive impairment in fibromyalgia syndrome: the impact of cardiovascular regulation, pain, emotional disorders and medication. *European Journal of Pain (London, England)*, 16(3), 421–9.  
<https://doi.org/10.1002/j.1532-2149.2011.00032.x>
- Riello, R., Sabbatoli, F., Beltramello, A., Bonetti, M., Bono, G., Falini, A., ... Frisoni, G. B. (2005). Brain volumes in healthy adults aged 40 years and over: a voxel-based morphometry study. *Aging Clinical and Experimental Research*, 17(4), 329–36.  
 Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/16285200>

- Rizzolatti, G., & Luppino, G. (2001). The Cortical Motor System. *Neuron*, *31*, 889–901.
- Robinson, M. E., Craggs, J. G., Price, D. D., Perlstein, W. M., & Staud, R. (2011). Gray Matter Volumes of Pain-Related Brain Areas Are Decreased in Fibromyalgia Syndrome. *The Journal of Pain*, *12*(4), 436–443. <https://doi.org/10.1016/j.jpain.2010.10.003>
- Rodriguez-Raecke, R., Niemeier, A., Ihle, K., Ruether, W., & May, A. (2009). Brain gray matter decrease in chronic pain is the consequence and not the cause of pain. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(44), 13746–50. <https://doi.org/10.1523/JNEUROSCI.3687-09.2009>
- Rosen, Y., & Lenkinski, R. E. (2007). Recent advances in magnetic resonance neurospectroscopy. *Neurotherapeutics : The Journal of the American Society for Experimental NeuroTherapeutics*, *4*(3), 330–45. <https://doi.org/10.1016/j.nurt.2007.04.009>
- Sabb, F. W., Bilder, R. M., Chou, M., & Bookheimer, S. Y. (2007). Working memory effects on semantic processing: priming differences in pars orbitalis. *NeuroImage*, *37*(1), 311–322. <https://doi.org/10.1016/j.neuroimage.2007.04.050>
- Sarchielli, P., Di Filippo, M., Nardi, K., & Calabresi, P. (2007). Sensitization, glutamate, and the link between migraine and fibromyalgia. *Current Pain and Headache Reports*, *11*(5), 343–51. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/17894924>
- Saunders, D. E., Howe, F. a, van den Boogaart, a, Griffiths, J. R., & Brown, M. M. (1999). Aging of the adult human brain: in vivo quantitation of metabolite content with proton magnetic resonance spectroscopy. *Journal of Magnetic Resonance Imaging : JMRI*, *9*(5), 711–6. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10331768>
- Sawamoto, N., Honda, M., Okada, T., Hanakawa, T., Kanda, M., Fukuyama, H., ... Shibasaki, H. (2000). Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/posterior insula: an event-related functional magnetic resonance imaging study. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(19), 7438–7445.
- Schadrack, J., Neto, F. L., Ableitner, A., Castro-Lopes, J. M., Willoch, F., Bartenstein, P., ... Tölle, T. R. (1999). Metabolic activity changes in the rat spinal cord during adjuvant monoarthritis. *Neuroscience*, *94*(2), 595–605. [https://doi.org/10.1016/S0306-4522\(99\)00186-4](https://doi.org/10.1016/S0306-4522(99)00186-4)
- Schmidt-Wilcke, T., Leinisch, E., Gänßbauer, S., Draganski, B., Bogdahn, U., Altmeyen, J.,

- & May, a. (2006). Affective components and intensity of pain correlate with structural differences in gray matter in chronic back pain patients. *Pain, 125*(1–2), 89–97.  
<https://doi.org/10.1016/j.pain.2006.05.004>
- Schmidt-Wilcke, T., Leinisch, E., Straube, A., Kampfe, N., Draganski, B., Diener, H. C., ... May, A. (2005). Gray matter decrease in patients with chronic tension Headache history. *Neurology, 65*, 1483–1486.
- Schmidt-Wilcke, T., Luerding, R., Weigand, T., Jürgens, T., Schuierer, G., Leinisch, E., & Bogdahn, U. (2007). Striatal grey matter increase in patients suffering from fibromyalgia - A voxel-based morphometry study. *Pain, 132*(SUPPL. 1).  
<https://doi.org/10.1016/j.pain.2007.05.010>
- Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., ... Greicius, M. D. (2007). Dissociable Intrinsic Connectivity Networks for Salience Processing and Executive Control. *Journal of Neuroscience, 27*(9), 2349–2356.  
<https://doi.org/10.1523/JNEUROSCI.5587-06.2007>
- Segerdahl, A. R., Mezue, M., Okell, T. W., Farrar, J. T., & Tracey, I. (2015). The dorsal posterior insula subserves a fundamental role in human pain. *Nature Neuroscience, 18*(4), 499–500. <https://doi.org/10.1038/nn.3969>
- Seminowicz, D. a, Wideman, T. H., Naso, L., Hatami-Khoroushahi, Z., Fallatah, S., Ware, M. a, ... Stone, L. S. (2011). Effective treatment of chronic low back pain in humans reverses abnormal brain anatomy and function. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 31*(20), 7540–7550.  
<https://doi.org/10.1523/JNEUROSCI.5280-10.2011>
- Shallice, T., Fletcher, P., Frith, C. D., Grasby, P., Frackowiak, R. S., & Dolan, R. J. (1994). Brain regions associated with acquisition and retrieval of verbal episodic memory. *Nature, 368*(6472), 633–635.
- Shanahan, C. J., Hodges, P. W., Wrigley, T. V, Bennell, K. L., & Farrell, M. J. (2015). Organisation of the motor cortex differs between people with and without knee osteoarthritis. *Arthritis Research & Therapy, 17*, 164. <https://doi.org/10.1186/s13075-015-0676-4>
- Sharma, L. (1999). Proprioceptive impairment in knee osteoarthritis. *Rheumatic Disease Clinics of North America, 25*(2), 299–314. [https://doi.org/10.1016/S0889-857X\(05\)70069-7](https://doi.org/10.1016/S0889-857X(05)70069-7)
- Sharma, L., Pai, Y. C., Holtkamp, K., & Rymer, W. Z. (1997). Is knee joint proprioception

- worse in the arthritic knee versus the unaffected knee in unilateral knee osteoarthritis? *Arthritis Rheum.*, 40(8), 1518–1525.
- Sherrington, C. (1906). *The Integrative Action of the Nervous System*. Oxford: Oxford University Press.
- Shigemura, T., Kishida, S., Eguchi, Y., Ohtori, S., Nakamura, J., Kojima, M., ... Takahashi, K. (2012). Proton magnetic resonance spectroscopy of the thalamus in patients with osteoarthritis of the hip. *Bone and Joint Research*, 1(1), 8–12.  
<https://doi.org/10.1302/2046-3758.1.1.2000018>
- Shimada, S., Hiraki, K., & Oda, I. (2005). The parietal role in the sense of self-ownership with temporal discrepancy between visual and proprioceptive feedbacks. *NeuroImage*, 24(4), 1225–32. <https://doi.org/10.1016/j.neuroimage.2004.10.039>
- Shimizu, E., Hashimoto, K., Ochi, S., Fukami, G., Fujisaki, M., Koike, K., ... Iyo, M. (2007). Posterior cingulate gyrus metabolic changes in chronic schizophrenia with generalized cognitive deficits. *Journal of Psychiatric Research*, 41(1–2), 49–56.  
<https://doi.org/10.1016/j.jpsychires.2005.04.015>
- Siddall, P. J., & Cousins, M. J. (2004). Persistent pain as a disease entity: implications for clinical management. *Anesthesia and Analgesia*, 99(2), 510–520, table of contents.  
<https://doi.org/10.1213/01.ANE.0000133383.17666.3A>
- Simmons, M. L., Frondoza, C. G., & Coyle, J. T. (1991). Immunocytochemical localization of N-acetyl-aspartate with monoclonal antibodies. *Neuroscience*, 45(1), 37–45.  
[https://doi.org/10.1016/0306-4522\(91\)90101-S](https://doi.org/10.1016/0306-4522(91)90101-S)
- Smallwood, R. F., Laird, A. R., Ramage, A. E., Parkinson, A. L., Lewis, J., Clauw, D. J., ... Robin, D. A. (2013). Structural brain anomalies and chronic pain: A quantitative meta-analysis of gray matter volume. *Journal of Pain*, 14(7), 663–675.  
<https://doi.org/10.1016/j.jpain.2013.03.001>
- Snaith, R. P. (2003). The Hospital Anxiety And Depression Scale. *Health Qual Life Outcomes*, 1, 29. <https://doi.org/10.1186/1477-7525-1-29>
- Solano, C., Martinez, A., Becerril, L., Vargas, A., Figueroa, J., Navarro, C., ... Martinez-Lavin, M. (2009). Autonomic dysfunction in fibromyalgia assessed by the Composite Autonomic Symptoms Scale (COMPASS). *Journal of Clinical Rheumatology*, 15(4), 172–176.
- Squire, L. R., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science (New York, N.Y.)*, 253(5026), 1380–1386. <https://doi.org/10.1126/science.1896849>

- Stanton, T. R., Lin, C. W. C., Smeets, R. J. E. M., Taylor, D., Law, R., & Lorimer Moseley, G. (2012). Spatially defined disruption of motor imagery performance in people with osteoarthritis. *Rheumatology (United Kingdom)*, *51*(8), 1455–1464.  
<https://doi.org/10.1093/rheumatology/kes048>
- Staud, R. (2002). Evidence of involvement of central neural mechanisms in generating fibromyalgia pain. *Current Rheumatology Reports*, *4*(4), 299–305.  
<https://doi.org/10.1007/s11926-002-0038-5>
- Steriade, M., & Llinás, R. R. (1988). The functional states of the thalamus and the associated neuronal interplay. *Physiological Reviews*, *68*(3), 649–742.
- Suhr, J. A. (2003). Neuropsychological impairment in fibromyalgia. *Journal of Psychosomatic Research*, *55*(4), 321–329. [https://doi.org/10.1016/S0022-3999\(02\)00628-1](https://doi.org/10.1016/S0022-3999(02)00628-1)
- Sundgren, P. C., Petrou, M., Harris, R. E., Fan, X., Foerster, B., Mehrotra, N., ... Welsh, R. C. (2007). Diffusion-weighted and diffusion tensor imaging in fibromyalgia patients: a prospective study of whole brain diffusivity, apparent diffusion coefficient, and fraction anisotropy in different regions of the brain and correlation with symptom severity. *Academic Radiology*, *14*(7), 839–846.
- Suzuki, R., Rygh, L. J., & Dickenson, A. H. (2004). Bad news from the brain: Descending 5-HT pathways that control spinal pain processing. *Trends in Pharmacological Sciences*, *25*(12), 613–617. <https://doi.org/10.1016/j.tips.2004.10.002>
- Swanik, C. B., Lephart, S. M., & Rubash, H. E. (2004). Proprioception, Kinesthesia, and Balance After Total Knee Arthroplasty with Cruciate-Retaining and Posterior Stabilized Prostheses. *The Journal of Bone & Joint Surgery*, *86*(2), 328–334. Retrieved from <http://jbjs.org/content/86/2/328.abstract>
- Tagliazucchi, E., Balenzuela, P., Fraiman, D., & Chialvo, D. (2010). Brain resting state is disrupted in chronic back pain patients. *Neurosci Lett.*, *485*(1), 26–31.  
<https://doi.org/doi:10.1016/j.neulet.2010.08.053>
- Tetreault, P., Baliki, M., Vachon-Presseau, E., Yeasted, R., Schnitzer, T. J., & Apkarian, A. V. (2015). Cortical singularity of knee osteoarthritis patients and their response to duloxetine or placebo treatment. *Osteoarthritis and Cartilage*, *23*(2015), A354.  
<https://doi.org/10.1016/j.joca.2015.02.653>
- Thieme, K., Spies, C., Sinha, P., Turk, D. C., & Flor, H. (2005). Predictors of pain behaviors in fibromyalgia syndrome. *Arthritis Care and Research*, *53*(3), 343–350.

<https://doi.org/10.1002/art.21158>

- Thorstensson, C. A., Gooberman-Hill, R., Adamson, J., Williams, S., & Dieppe, P. (2009). Help-seeking behaviour among people living with chronic hip or knee pain in the community. *BMC Musculoskeletal Disorders*, *10*, 153. <https://doi.org/10.1186/1471-2474-10-153>
- Träber, F., Block, W., Lamerichs, R., Gieseke, J., & Schild, H. H. (2004). 1H Metabolite Relaxation Times at 3.0 Tesla: Measurements of T1 and T2 Values in Normal Brain and Determination of Regional Differences in Transverse Relaxation. *Journal of Magnetic Resonance Imaging*, *19*(5), 537–545.
- Tracey, I. (2008). Imaging pain. *British Journal of Anaesthesia*, *101*(1), 32–39. <https://doi.org/10.1093/bja/aen102>
- Trescot, Andrea M., M., Datta, Sukdeb, M., Lee, Marion, M., & Hansen, Hans, M. (2008). Opioid Pharmacology. *Pain Physician*, *1975*(3), S133–S153.
- Tsai, G., & Coyle, J. T. (1995). N-acetylaspartate in neuropsychiatric disorders. *Prog Neurobiol*, *46*(5), 531–40. [https://doi.org/10.1016/0301-0082\(95\)00014-M](https://doi.org/10.1016/0301-0082(95)00014-M)
- Tsai, S. Y., Posse, S., Lin, Y. R., Ko, C. W., Otazo, R., Chung, H. W., & Lin, F. H. (2007). Fast mapping of the T2 relaxation time of cerebral metabolites using proton echo-planar spectroscopic imaging (PEPSI). *Magnetic Resonance in Medicine*, *57*(5), 859–865.
- Tsao, H., Galea, M. P., & Hodges, P. W. (2008). Reorganization of the motor cortex is associated with postural control deficits in recurrent low back pain. *Brain*, *131*(8), 2161–2171. <https://doi.org/10.1093/brain/awn154>
- Turk, D. C., Okifuji, A., Sinclair, J. D., & Starz, T. W. (1996). Pain, disability, and physical functioning in subgroups of patients with fibromyalgia. *The Journal of Rheumatology*, *23*(7), 1255–1262.
- Turk, D. C., Okifuji, a, Sinclair, J. D., & Starz, T. W. (1998). Differential responses by psychosocial subgroups of fibromyalgia syndrome patients to an interdisciplinary treatment. *Arthritis Care and Research : The Official Journal of the Arthritis Health Professions Association*, *11*(5), 397–404. <https://doi.org/10.1002/art.1790110511>
- Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., ... Joliot, M. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage*, *15*(1), 273–89. <https://doi.org/10.1006/nimg.2001.0978>
- Uddin, L. Q., Kelly, a. M. C., Biswal, B. B., Castellanos, F. X., & Milham, M. P. (2009).

- Functional Connectivity of Default Mode Network Components: Correlation, Anticorrelation, and Causality. *Human Brain Mapping*, 30(2), 625–637.  
<https://doi.org/10.1002/hbm.20531>
- Utevsky, A. V., Smith, D. V., & Huettel, S. A. (2014). Precuneus Is a Functional Core of the Default-Mode Network. *Journal of Neuroscience*, 34(3), 932–940.  
<https://doi.org/10.1523/JNEUROSCI.4227-13.2014>
- Valdés, M., Collado, A., Bargalló, N., Vázquez, M., Rami, L., Gómez, E., & Salamero, M. (2010). Increased glutamate/glutamine compounds in the brains of patients with fibromyalgia: a magnetic resonance spectroscopy study. *Arthritis and Rheumatism*, 62(6), 1829–36. <https://doi.org/10.1002/art.27430>
- van Dijk, K. R. A., Sabuncu, M. R., & Buckner, R. L. (2012). The influence of head motion on intrinsic functional connectivity MRI. *NeuroImage*, 59(1), 431–438.  
<https://doi.org/10.1016/j.neuroimage.2011.07.044>
- Van Laere, K. J., & Dierckx, R. A. (2001). Brain perfusion SPECT: age- and sex-related effects correlated with voxel-based morphometric findings in healthy adults. *Radiology*, 221(3), 810–7. <https://doi.org/10.1148/radiol.2213010295>
- Vogt, B. A. (2005). Pain and Emotion Interactions in Subregions of the Cingulate Gyrus. *Nature Reviews Neuroscience*, 6(7), 533–544. <https://doi.org/10.1038/nrn1704>.Pain
- Vogt, B. A., Derbyshire, S., & Jones, A. K. (1996). Pain processing in four regions of human cingulate cortex localized with co-registered PET and MR imaging. *The European Journal of Neuroscience*, 8(7), 1461–73. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8758953>
- Vogt, B. A., & Laureys, S. (2005). Posterior cingulate, precuneal and retrosplenial cortices: cytology and components of the neural network correlates of consciousness. *Progress in Brain Research*, 150, 205–17. [https://doi.org/10.1016/S0079-6123\(05\)50015-3](https://doi.org/10.1016/S0079-6123(05)50015-3)
- Walaszek, B., Schubert, F., Mекle, R., & Ittermann, B. (2012). Age dependence of NAA and tCr transverse relaxation times determined in hippocampus and frontal cortex at 3T. In *ISMRM 20th Annual Meeting & Exhibition*.
- Wall, P. D., McMahon, S. B., & Koltzenburg, M. (2006). *Wall and Melzack's textbook of pain*. Philadelphia: Elsevier/Churchill Livingstone.
- Wang, Y., & Li, S. J. (1998). Differentiation of Metabolic Concentrations Between Gray Matter and White Matter of Human Brain by *in vivo* <sup>1</sup>H Magnetic Resonance Spectroscopy. *Magnetic Resonance in Medicine : Official Journal of the Society of*

- Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 39(1), 28–33. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9438434>
- Wenderoth, N., Debaere, F., Sunaert, S., & Swinnen, S. P. (2005). The role of anterior cingulate cortex and precuneus in the coordination of motor behaviour. *European Journal of Neuroscience*, 22(1), 235–246. <https://doi.org/10.1111/j.1460-9568.2005.04176.x>
- Whalen, P. J., Bush, G., McNally, R. J., & Wilhelm, S. (1998). The emotional counting stroop paradigm: a functional magnetic resonance imaging probe of the .... *Biological Psychiatry*, 3223(98), 1219–1228. Retrieved from [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list\\_uids=17529439282385510445related:LeBdAicURfMJ](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=17529439282385510445related:LeBdAicURfMJ)
- Whitehead, W. E., Engel, B. T., & Schuster, M. M. (1980). Irritable bowel syndrome: Physiological and psychological differences between diarrhea-predominant and constipation-predominant patients. *Digestive Diseases and Sciences*, 25(6), 404–413.
- Whitwell, J. L. (2009). Voxel-based morphometry: an automated technique for assessing structural changes in the brain. *The Journal of Neuroscience*, 29(31), 9661–9664. <https://doi.org/10.1523/JNEUROSCI.2160-09.2009>
- Willoch, F., Rosen, G., Tölle, T. R., Øye, I., Wester, H. J., Berner, N., ... Bartenstein, P. (2000). Phantom limb pain in the human brain: Unraveling neural circuitries of phantom limb sensations using positron emission tomography. *Annals of Neurology*, 48(6), 842–849. [https://doi.org/10.1002/1531-8249\(200012\)48:6<842::AID-ANA4>3.0.CO;2-T](https://doi.org/10.1002/1531-8249(200012)48:6<842::AID-ANA4>3.0.CO;2-T)
- Wolfe, F., Allen, M., Bennett, R. M., Bombardier, C., Broadhurst, N., Cameron, R. S., & Yunus, M. B. (1996). The fibromyalgia syndrome: A consensus report on fibromyalgia and disability. *Journal of Rheumatology*, 23(3), 534–539.
- Wolfe, F., Ross, K., Anderson, J., Russell, I. J., & Hebert, L. (1995). The prevalence and characteristics of fibromyalgia in the general population. *Arthritis and Rheumatism*, 38(1), 19–28. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/7818567>
- Wolfe, F., Smythe, H. A., Yunus, M. B., Bennett, R. M., Bombardier, C., Goldenberg, D. L., ... Clark, P. (1990). The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum*, 33(2), 160–172.
- Wolpert, D. M., Goodbody, S. J., & Husain, M. (1998). Maintaining internal representations: the role of the human superior parietal lobe. *Nature Neuroscience*, 1(6), 529–533.

<https://doi.org/10.1038/2245>

- Wood, P. B., Glabus, M. F., Simpson, R., & Patterson, J. C. (2009). Changes in gray matter density in fibromyalgia: correlation with dopamine metabolism. *The Journal of Pain : Official Journal of the American Pain Society*, *10*(6), 609–18.  
<https://doi.org/10.1016/j.jpain.2008.12.008>
- Wood, P. B., Ledbetter, C. R., Glabus, M. F., Broadwell, L. K., & Patterson, J. C. (2009). Hippocampal metabolite abnormalities in fibromyalgia: correlation with clinical features. *The Journal of Pain : Official Journal of the American Pain Society*, *10*(1), 47–52.  
<https://doi.org/10.1016/j.jpain.2008.07.003>
- Wood, P. B., Schweinhardt, P., Jaeger, E., Dagher, A., Hakyemez, H., Rabiner, E. A., ... Chizh, B. A. (2007). Fibromyalgia patients show an abnormal dopamine response to pain. *European Journal of Neuroscience*, *25*(12), 3576–3582.  
<https://doi.org/10.1111/j.1460-9568.2007.05623.x>
- Woolf, A. D., Zeidler, H., Haglund, U., Carr, A. J., Chaussade, S., Cucinotta, D., ... Martin-Mola, E. (2004). Musculoskeletal pain in Europe: its impact and a comparison of population and medical perceptions of treatment in eight European countries. *Annals of the Rheumatic Diseases*, *63*(4), 342–7. <https://doi.org/10.1136/ard.2003.010223>
- Woolf, C. J. (2012). Central Sensitization: Implications for the diagnosis and treatment of pain. *Pain*, *152*(3 Supplemental), 1–31.  
<https://doi.org/10.1016/j.pain.2010.09.030>
- Woolf, C. J., & Mannion, R. J. (1999). Neuropathic pain: aetiology, symptoms, mechanisms, and management. *The Lancet*, *353*(9168), 1959–1964. [https://doi.org/10.1016/S0140-6736\(99\)01307-0](https://doi.org/10.1016/S0140-6736(99)01307-0)
- Wylezinska, M., Cifelli, A. I., Jezard, P., Palace, J., Alecci, M., & Matthews, P. (2003). Thalamic neurodegeneration in relapsing: Remitting multiple sclerosis. *Journal of Neurology Neurosurgery and Psychiatry*, *74*(8), 1177.
- Xie, Y., Huo, F., & Tang, J. (2009). Cerebral cortex modulation of pain. *Acta Pharmacologica Sinica*, *30*(1), 31–41. <https://doi.org/10.1038/aps.2008.14>
- Yeo, R. A., Thoma, R. J., Gasparovic, C., Monnig, M., Harlaar, N., Calhoun, V. D., ... Hutchison, K. E. (2012). Neurometabolite concentration and clinical features of chronic alcohol use: A proton magnetic resonance spectroscopy study. *Psychiatry Research*, *211*(2), 141–147. <https://doi.org/10.1016/j.psychresns.2012.05.005>
- Yunus, M. B. (2007). Fibromyalgia and Overlapping Disorders: The Unifying Concept of

- Central Sensitivity Syndromes. *Seminars in Arthritis and Rheumatism*, 36(6), 339–356.  
<https://doi.org/10.1016/j.semarthrit.2006.12.009>
- Yunus, M. B., Masi, A. T., & Aldag, J. C. (1989). A controlled study of primary fibromyalgia syndrome: clinical features and association with other functional syndromes. *The Journal of Rheumatology*, 19 (Supple), 62–71.
- Yunus, M., Masi, A. ., Calabro, J. J., Miller, K. A., & Feigenbaum, S. L. (1981). Primary fibromyalgia (fibrositis): Clinical study of 50 patients with matched normal controls. *Seminars in Arthritis and Rheumatism*, 11(1), 151–171.  
[https://doi.org/http://dx.doi.org/10.1016/0049-0172\(81\)90096-2](https://doi.org/http://dx.doi.org/10.1016/0049-0172(81)90096-2)
- Zaaraoui, W., Fleysheer, L., Fleysheer, R., Liu, S., Soher, B. J., & Gonen, O. (2007). Human brain-structure resolved T2 relaxation times of proton metabolites at 3 Tesla. *Magnetic Resonance in Medicine*, 57(6), 983–989.
- Zadina, J. E., Hackler, L., Ge, L. J., & Kastin, A. J. (1997). A potent and selective endogenous agonist for the mu-opiate receptor. *Nature*, 386(6624), 499–502.  
<https://doi.org/10.1038/386499a0>
- Zambreanu, L., Wise, R. G., Brooks, J. C. W., Iannetti, G. D., & Tracey, I. (2005). A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging. *Pain*, 114(3), 397–407. <https://doi.org/10.1016/j.pain.2005.01.005>
- Zhang, S., & Li, C.-S. R. (2012). Functional connectivity mapping of the human precuneus by resting state fMRI. *NeuroImage*, 59(4), 3548–3562.  
<https://doi.org/10.1016/j.neuroimage.2011.11.023>Functional
- Zhang, Y., & Jordan, J. M. (2008). Epidemiology of osteoarthritis. *Rheumatic Diseases Clinics of North America*, 34(3), 515–29. <https://doi.org/10.1016/j.rdc.2008.05.007>
- Zhou, Y., Dougherty, J. H., Hubner, K. F., Bai, B., Cannon, R. L., & Hutson, R. K. (2008). Abnormal connectivity in the posterior cingulate and hippocampus in early Alzheimer's disease and mild cognitive impairment. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association*, 4(4), 265–70. <https://doi.org/10.1016/j.jalz.2008.04.006>
- Zubieta, J., Smith, Y., Bueller, J., Xu, Y., Kilbourn, M., Jewett, D., ... Stohler, C. (2001). Regional mu opioid receptor regulation of sensory and affective dimensions of pain. *Science*, 293(5528), 311–5. <https://doi.org/10.1126/science.1060952>