Neuroimaging studies to understand neural mechanisms of pain in osteoarthritis

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Abstract

Osteoarthritis pain is a major cause of long-term disability and chronic pain in the adult population, which can lead to reduced quality of life and serious comorbidities such as depression. Together with the substantial burden chronic osteoarthritis pain (COAP) causes at the individual level, associated economical costs are alarming and set to rise given the ageing population. Yet there are very few effective treatments available, which include both pharmacological and non-pharmacological options. Around one in five COAP patients do not receive satisfactory pain relief, even after undergoing joint replacement surgery. This strongly supports the notion that chronic osteoarthritis pain has a central component, and reflects the need for improved understanding of neural mechanisms of chronic pain and chronic pain relief.

Substantial advances have been achieved over the past two decades towards unravelling the role of the central nervous system in predisposition, development and maintenance of chronic pain. Despite these efforts, there is still a major gap in integrating these advances and translating them into more meaningful treatments.

The studies presented here endeavour to shed light on these aspects by capturing multidimensional contributions from brain mechanisms, negative mood and pain characteristics to COAP and to the effect of treatment, with the overall aim of improving current understanding of central components of this condition and mechanisms of centrally acting analgesic treatment.

More specifically, the principal objectives consist of: **1.** Identifying markers indexing neuroplasticity changes linked with COAP, and molecular mechanisms in COAP; **2.** Studying neural correlates of the analgesic effects of opioids, the strongest pain killers

available, in individuals with COAP and explore whether pain phenotyping may predict the extent of treatment response; **3.** Assessing the neural effects of the antidepressant duloxetine and investigating potential predictors of treatment outcome.

In order to achieve these objectives four studies are undertaken. **First**, the microstructure of the brain in chronic osteoarthritis pain is investigated in a cross-sectional manner in 46 individuals (23 COAP), in an attempt to identify potential signs of structural neuroplasticity. Analysis of cerebral microstructure with diffusion imaging techniques is sensitive to minor changes in myelination and axonal loss, thus being a plausible marker of neuroplasticity. **Second**, in a similar study design, the brain of 39 participants (20 COAP) is examined on the molecular level, focusing on neurotransmitter levels within one key region involved in pain, namely the anterior cingulate cortex (ACC). Using the setting of randomised controlled trials (RCT), the **third** and **fourth** projects are planned and developed, with novel investigational protocols being established before recruitment takes place. Previously established multi-modal structural, blood oxygen level dependent (BOLD), and blood flow imaging of the brain are included, in addition to novel task-related functional paradigm and quantitative sensory testing. **Study three** consists of a mechanistic, saline controlled, acute intervention study of remifentanil involving 28 individuals with COAP, while a sub-chronic placebo controlled investigation of duloxetine (77 COAP) constitutes **study four**. Remifentanil is a $\mu-$opioid agonist, a strong analgesic, while duloxetine is a serotonin-norepinephrine reuptake inhibitor (SNRI), a novel class of antidepressants. All four studies are performed at 3T.

No abnormalities in brain structure were found in association with COAP, but an inverse correlation between ACC γ-aminobutyric acid levels and pain severity was seen, which was not dependent on negative affect or age, explaining nearly 60% of the variance.

Pinprick-related BOLD activity in COAP was characterised and partially modulated by the opioid remifentanil, preferentially in patients with more impaired conditioned pain modulation (CPM). 77 participants consented into study four. Fifty-six participants completed the study, from which 34 received duloxetine treatment for 6 weeks, while 22 received placebo equivalent. Around half (53%) of duloxetine intakers reported clinically meaningful pain relief, against 36% of placebo intakers. Interim analysis of the effects of duloxetine on pinprick-related activity did not detect activity changes, albeit lower baseline anterior insula activity showed association with better treatment outcomes after duloxetine, but not placebo. Additionally, responders to either duloxetine or placebo had a tendency for lower ACC activity prior to treatment.

These results suggest that, differently from other chronic pain conditions, such as back pain and fibromyalgia, COAP is not accompanied by major apparent structural reorganisation nor abnormal ACC neurotransmitter levels. However they also indicate some shared mechanisms between COAP and fibromyalgia, given cingulate GABA encoding of perceived pain severity, which supports use of GABA levels as a marker for mechanistic evaluation of GABAergic modulation for pain relief in COAP. The observed absence of structural abnormalities in COAP, suggests that this condition may be simpler to treat than other types of chronic pain. The GABA association seen in COAP, and previously shown in fibromyalgia, indicates that certain mechanism-based drugs may be useful for both COAP and other conditions.

Results from the opioid study, in conjunction with the published literature suggest shared neural mechanisms of opioidergic analgesia between chronic pain states and the healthy state. Additionally, they point out that CPM might allow prediction of the need for centrally acting drugs. In the duloxetine study however, CPM was not related

with differential treatment outcomes. Instead the anterior insula seem to play a role in determining the likelihood of clinically meaningful treatment response to duloxetine. However, results from both these analgesic studies must be interpreted with caution as they were based on a sub-sample of the full dataset acquired, as recruitment was still ongoing. Both RCTs have now been completed, and analyses of the full dataset is ongoing.

Collectively, findings in this dissertation provide new evidence to the field of chronic pain research, suggesting that the structural and neurochemical brain reorganisation seen in other chronic pain conditions are likely pain-type specific, besides some partially overlapping mechanisms which are characterised here. The analyses of the RCTs of opioid and SNRI treatments, albeit preliminary, elucidate some putative mechanisms and predictors of centrally-mediated analgesia in COAP. Nevertheless, the full dataset, based on the rich methodology and protocols developed as part of the PhD, which is now available and undergoing investigation, should allow firmer conclusions regarding these two major classes of drugs and placebo in chronic pain.

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Publications

Peer Reviewed Journals

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- Reckziegel D, Bailey HS, Cottam WJ, Tench CR, Mahajan RP, Walsh DA, Knaggs R, Auer DP (2017). Imaging Pain Relief in Osteoarthritis (IPRO): protocol of a double blind randomised controlled mechanistic study assessing pain relief and prediction of treatment outcome, *BMJopen*. Accepted.

- Cottam WJ, Condon L, Alshuft H, Reckziegel D, Auer DP (2016). Associations of limbic-affective brain activity and severity of ongoing chronic arthritis pain are explained by trait anxiety *NeuroImage: Clinical*, 12, S2213158216301206.

Conference Proceedings

- Reckziegel D, Bailey H, Chana A, Cottam WJ, Tench CR, Mahajan RP, Walsh DA, Knaggs R, Auer DP (2016). Opioid reduces nociceptive activation of the anterior

cingulum preferentially in patients with impaired conditioned pain modulation: a BOLD fMRI study in chronic osteoarthritis pain. *Proceedings of the World Congress on Pain*, Yokohama, Japan.

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- Reckziegel D, Dixon J, Auer DP (2014). Microstructural abnormalities related to the chronification of osteoarthritic pain: a DTI study. *Proceedings of the ISMRM Annual Meeting*, p.1901, Milan, Italy.

Other Conference Proceedings

- Kurien T, Reckziegel D, Cottam WJ, Petersen KK, Cooper A, Pearson RG, Arendt-Nielsen L, Graven-Nielsen T, Auer DP, Scammell BE (2016). Neural correlates of temporal summation in knee osteoarthritis pain - a preliminary study at 3T. *Osteoarthritis and Cartilage*, 24:S455, Amsterdam, Netherlands.

- Cottam WJ, Drabek M, Reckziegel D, Auer DP (2016). Subregion-specific resting-state amygdala connectivity in chronic knee osteoarthritis pain: towards a brain network signature of OA pain. *Proceedings of the ISMRM Annual Meeting*, p.1710 Singapore.

Organisation of the thesis

This thesis is organised into eleven chapters which have been split into five parts. Part I consists of a general introduction. Part II contains two chapters which introduce the principles of the neuroimaging techniques utilised in this dissertation's original research. Part III consists of the "Imaging Pain in Osteoarthritis" investigation, containing two cross-sectional studies, looking at both microstructure and neurochemical information in chronic knee osteoarthritis pain. Part IV consists of the main focus of this PhD and is comprised of four chapters based on the longitudinal mechanistic study "Imaging Pain Relief in Osteoarthritis", which has an emphasis on brain function. Part V gives a summary of the findings and discusses research impact and future directions.

In more detail, **Chapter** 1 introduces key concepts on pain perception and the problem of chronic pain. It also identifies some of the major gaps in the field of chronic pain research. This chapter should provide the reader with a general understanding and the importance of the research questions being discussed throughout the dissertation.

Chapter 2 describes the general aims and hypotheses for the entire thesis.

Chapter 3 gives an outline of the foundations of the NMR theory, together with an overview of the basic magnetic resonance imaging experiments required to understand the original work in the thesis.

Chapter 4 introduces some of the methodologies used to measure different types of information about the brain. A general overview of magnetic resonance techniques of diffusion, spectroscopy and functional imaging are presented, highlighting the points that are most relevant to the original contributions described in the later chapters.

Chapter 5 focuses on exploring whether chronic osteoarthritis pain is associated with neuroplasticity changes in cerebral microstructure, as a consequence or predisposition to chronic pain. Diffusion imaging is employed and white matter tracts are reconstructed using a tensor model for whole brain analysis. Additional region of interest analyses are performed focusing on key regions, which have been found abnormal in other chronic pain conditions.

Chapter 6 describes an investigation on the role of γ-aminobutyric acid levels in chronic osteoarthritis pain. Quantification of GABA is performed within the anterior cingulate cortex in a population of patients with knee osteoarthritis pain and healthy controls.

Chapter 7 provides a description of the developmental work towards the design and methodology of the longitudinal study "Imaging Pain Relief in Osteoarthritis" (IPRO). The study consists of two sub-studies, which follow the setting of randomised controlled trials, to investigate mechanisms of two classes of centrally active analgesic drugs, namely remifentanil and duloxetine, in individuals with chronic knee osteoarthritis pain.

Chapter 8 summarises general participation characteristics from IPRO, including participant demographics.

Chapter 9: Following IPRO developmental work and general results described in chapters 7 and 8, an interim analysis of the opioid component of IPRO study is presented here. More specifically, the analysis involves the pinprick fMRI paradigm,

quantitative sensory testing and psychometric data collected until January 2016, involving 28 participants with chronic knee osteoarthritis pain.

Chapter 10 presents an interim analysis of the main component of the IPRO study, which is a double blind, placebo controlled, mechanistic study assessing pain relief and prediction of treatment outcomes, following a sub-chronic treatment with the antidepressant duloxetine. Building on the framework presented in the previous chapter, the pinprick fMRI paradigm data collected until January 2016 is analysed (29 participants), together with quantitative sensory testing and psychometric data of the full cohort who completed the study (n=56).

Chapter 11 summarises and discusses the original research presented in **Chapters 5-10** and discusses future perspectives.

Additionally, some of the research chapters are supported by appendices. These are included at the end of the dissertation, and are briefly described below.

Appendix A contains copies of key questionnaires used in ImPOA.

Appendix B contains copies of questionnaires used in IPRO exclusively.

Appendix C displays conversation with the MHRA regarding the classification of IPRO.

Appendix D contains the most up to date version of the patient information sheet that was given to IPRO participants.

Appendix E includes the quantitative sensory testing protocols used to measure both pressure pain thresholds and conditioned pain modulation on IPRO participants.

Appendix F contains the numerical rating scale used for rating the perceived intensity of chronic osteoarthritis pain by IPRO participants.

Appendix G includes the quantitative sensory testing protocol used to measure

temporal summation of pain on IPRO participants.

Appendix H describes the developed procedure for measuring the neural correlates of conditioning pain modulation with fMRI.

Appendix I features the poster used for advertising IPRO recruitment.

Lastly, **Appendix** J lists medications being taken by IPRO participants who completed the duloxetine study.

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Part I.

Introduction

1

Introduction

Chronic pain is a major health care issue in the developed world, affecting around forty percent of the population in the United Kingdom (Fayaz et al., 2016). The socio-economic burden and levels of disability are alarming. In the United States, medical treatments and lost productivity due to chronic pain costs the nation up to $635 billion each year (Simon, 2012). Yet there are very few effective treatments available, largely due to the limited understanding of such conditions. In this dissertation the focus will be on chronic osteoarthritis pain which is one of the most common types of clinical pain (Breivik et al., 2006). However, before diving into such a complex condition it is important to characterize a few simpler concepts.

1.1. What is pain?

Pain is a highly subjective conscious experience and an important function of the nervous system, providing information about the presence or threat of an injury. The International Association for the Study of Pain (IASP) defines pain as *"an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."* (Merskey and Bogduk, 1994). The definition highlights its subjectivity, emphasizing that there are both physiological and psychological components to it, avoiding linearly coupling pain to the intensity of stimulus or amount of tissue damage, and naturally implicating an active role of the brain in pain perception.

Already in the seventeenth century, the French philosopher René Descartes in his *Traite de l'homme*, conceptualized a model of pain proposing that the sensation of pain is not perceived in the injured tissue, but is directionally transmitted from the periphery to the brain via the spinal cord (Fig 1.1). Albeit inaccurate, as the model failed to adequately address the role of the cortex in pain perception by considering that the brain simply passively and proportionally reflected the peripheral and spinal mechanisms, Descartes' proposal helped shape early research on the subject and yielded important advances in pain physiology (Melzack and Katz, 2004).

Until around twenty years ago and until the advent of non-invasive human neuroimaging methodologies, understanding of supra-spinal mechanisms of pain was limited and primarily based on anatomical and electrophysiological studies in animals (Apkarian et al., 2005). The specific role of the cortex was unclear and its involvement in human pain perception was often questioned (Penfield and Boldrey, 1937, Head and Holmes, 1911). Along with the rapid development of new technologies, there has been a major revolution in these concepts and a large body of evidence indicates a key active role of

the brain in modulating pain perception (Apkarian, 2015).



Figure 1.1.: **Descartes' concept of the pain pathway.** Schematic illustration of pain transmission from the periphery to the cerebral cortex as portrayed by René Descartes on *Traite de l'homme* 1648. Adapted from (Melzack and Katz, 2004).

1.2. Nociception

In order to understand how pain is processed, it is necessary to define nociception. Nociception is the neural process of encoding noxious stimuli (Merskey and Bogduk, 1994). The term was first introduced by Sherrington more than a century ago (Sherrington, 1900), who observed that there were specialized sensory receptors in the skin which

were activated by potentially damaging stimuli. These free nerve endings (nociceptors) detect and transduce the stimuli into long-ranging electrical signals that are relayed to higher brain centres (Dubin and Patapoutian, 2010). Depending on the rate of conduction and the type of stimuli they respond to, nociceptors can be divided into categories. $A\delta$ fibres respond primarily to extreme pressures and temperatures. They are thinly myelinated and smaller in diameter, thus sending impulses faster $(2 - 30m/s)$ than unmyelinated, C fibres $(1m/s)$. The difference in conduction speed determines the perception sensation. While $A\delta$ fibres give rise to sharp, immediate sensation (refereed to as first pain), sensation from C fibres comes later and is perceived as deep and prolonged. These fibres are classed as afferent, conveying input signals from the periphery to the dorsal horn of the spinal cord (Basbaum et al., 2009).

It is important to note that while nociception and pain are closely linked, and activation of nociceptors undoubtedly can give rise to pain, nociceptors can be active in the absence of perception of pain (Baliki and Apkarian, 2015).

1.3. Pain pathways

Once the afferent fibres enter the spinal cord, peripheral nociceptors can form three types of connections. Some create synapses with projection neurons which ascend directly to higher centres of the central nervous system. Others form local connections with either excitatory or inhibitory interneurons which regulate nociceptive information transmission and input to projection neurons.

Beyond the dorsal horn, nociceptive signals travel to the thalamus, the main relay site for nociceptive inputs to cortical and subcortical regions, via the contralateral spinothalamic tract (STT), and to the medulla and brainstem via a subreticular and

spinomesencephalic tracts. Each of these tracts serve distinct functions, depending on their lamina origin in the dorsal horn and final destination (Dostrovsky and Craig, 2006). Projections to the brainstem are especially important, as they both integrate nociceptive activity with homeostatic, arousal and autonomic processes, as well as indirectly conveying nociceptive signals to forebrain regions after brainstem processing, which indicates a key role of these specific pathways in pain perception (Tracey and Mantyh, 2007).

However, as the current definition of pain indicates, the brain is not simply a passive receiver of the sensory messages from the ascending nociceptive pathways, but rather a centre that interprets these signals and makes constant adjustments to them via descending pain control pathways. The mechanisms for the descending control of pain were first described in relation to the spinal cord by Melzack and Wall's gate theory, where they suggest that a mechanism in the dorsal horns act as a gate that inhibits or facilitates transmission of pain signals from periphery to the brain based on the dynamic action of brain processes (Melzack and Wall, 1967). Even though early research on the topic focused on supra-spinal inhibitory modulation, it is now well established that this modulation can be both inhibitory and excitatory. The dynamic equilibrium between these two systems is altered in certain psychological and pathological conditions, and may be key in the development of chronic pain states (Apkarian, 2015, Heinricher et al., 2009).

A large body of evidence suggests that the midbrain periaqueductal gray (PAG) has profound inhibitory role on the sensation of pain and forms a major centre in descending (top-down) regulation, as shown, for instance, by stimulation of the region producing selective loss of nocifensive behaviour without any changes in responses to innocuous

stimuli in animals (Mayer and Price, 1976). The PAG forms a hub of convergence between projections from the cortex, thalamus, hypothalamus, amygdala and spinal cord, which combined form the so-called descending pain modulatory system (DPMS) (Figure 1.2). Notably, some of these mechanisms are mediated by the various endogenous systems, including serotonin (also known as 5-hydroxytryptamine or 5-HT), noradrenaline (also called norepinephrine), γ-aminobutyric acid and opioid-related pathways (Ossipov et al., 2010). The main projection of the PAG is to the rostral ventromedial medulla (RVM), to which it is reciprocally connected. Chemical or electrical stimulation of either the PAG or RVM causes the release of serotonin in the spinal cord and increased levels of norepinephrine in the cerebral spinal fluid (CSF), both eliciting antinociception, as shown by numerous animal studies (Ossipov et al., 2014). Importantly, the PAG, together with the anterior cingulate cortex, amygdala and RVM, contains high concentrations of opioid receptors, and its involvement in endogenous pain modulation is directly linked to the opioid responsiveness of its cells, as evidenced by brain imaging studies (Petrovic et al., 2002, Apkarian, 2015). The stimulation of PAG, as well as analgesic doses of the opioid agonist morphine, have been shown to cause a subtype of RVM neurons called "off cells" to fire continuously. These cells block the sensation of pain and inhibit spinal withdrawal reflexes away from noxious peripheral input (Fields and Heinricher, 1985).

The DPMS is considered crucial for placebo response mechanisms in pain, with pain modulation occurring via changes in emotional and cognitive states from the limbic brain (Levine et al., 1978a, Apkarian, 2015). Opioid involvement in particular is thought to play a role, as placebo analgesia has been shown to be blocked by the opioid antagonist naloxone (Levine et al., 1978b).

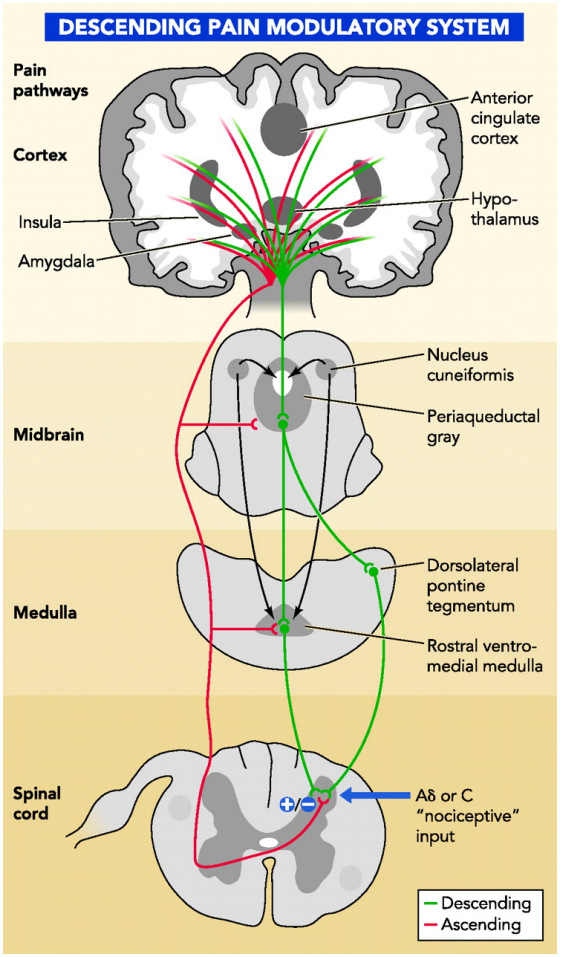


Figure 1.2.: **Schematic of the descending pain modulatory system.** Adapted from (Bingel and Tracey, 2008).

1.4. Cortical and subcortical processing of pain

Here it is important to emphasize that pain is a conscious, subjective experience and nociceptive activity is not necessarily accompanied by pain perception. For instance, a study on soldiers fighting during World War II found that many of those with severe injuries reported little pain or suffering and refused analgesic treatment (Yarnitsky and Dahan, 2015). Although pain is normally driven by nociceptive inputs, nociceptors can be active in the absence of pain perception (Baliki and Apkarian, 2015).

The term *"neuromatrix"* was first proposed by Melzack as a set of brain regions

which integrates diverse inputs to produce the output pattern that evokes pain (Melzack, 1999). With the advances in neuroimaging techniques, numerous researchers attempted to characterise such system, often referred to as "pain matrix" or "pain signature", in an endeavour to unravel how the perception of pain emerges from nociception within the human brain. Most of these studies examined cortical and subcortical responses evoked by transient nociceptive stimulation, which usually appears to be associated with activity in regions including the primary and secondary somatosensory cortices (S1 and S2), thalamus, the anterior cingulate cortex (ACC) and the insula (Apkarian, 2015, Iannetti and Mouraux, 2010). Other regions which, depending on the experiment, appear as responsive include the amygdala, hippocampus, prefrontal cortex, brainstem and basal ganglia (Tanasescu et al., 2016). Together, these regions make up a dynamic network modulated by emotion, attention, and arousal, among other processes, that help shaping the perception of pain (Figure 1.3).

Initial work on the topic made cautious conclusions on interpreting these findings, by taking into account that pain sensation was only one of the possibilities eliciting responses within that set of regions, as arousal, alertness to nociception, for instance, could be responsible for the activations instead (Apkarian, 2015). However, as such studies became more common, scientists started to interpret these findings in a more pain-specific manner, to the point of equivocally claiming that certain parts of the "neuromatrix" were specific to pain. One of the most recent cases include a *Nature Neuroscience* paper, claiming that the dorsal posterior insula (dpIns) is a pain-specific region based on the observation that the magnitude of regional cerebral blood flow fluctuations in the area correlated with the intensity of evoked pain (Segerdahl et al., 2015). Another example comes from a paper in *Proceedings of the National Academy*

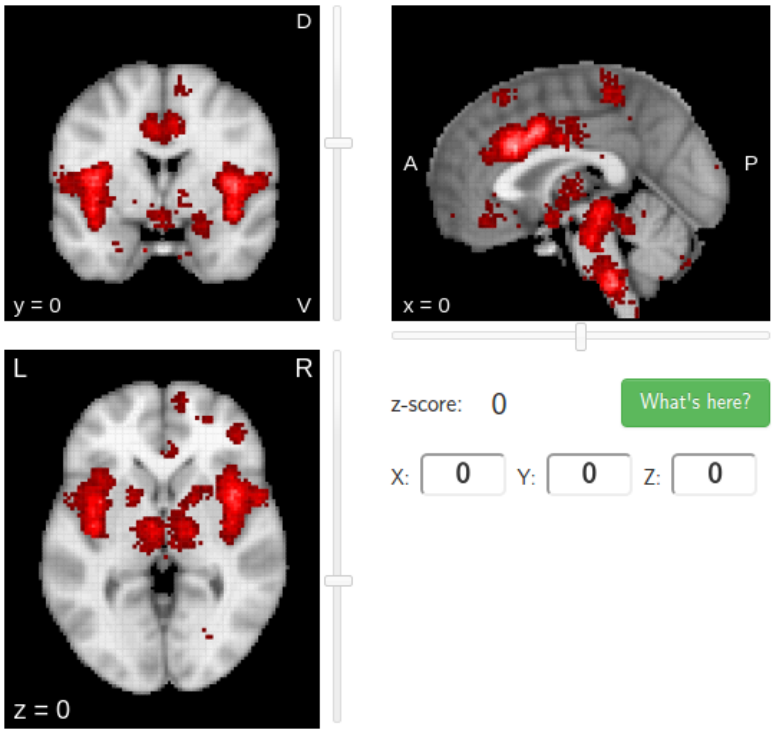


Figure 1.3.: **Meta-analytically derived map for acute pain.** In red, brain regions identified through reverse inference for the term "pain" on Neurosynth meta-analysis of 420 studies (www.neurosynth.org) (Yarkoni et al., 2011). Neurosynth uses text-mining and machine-learning techniques to generate mappings between search terms and brain activity. Strongest activations for "pain" appear on S2, ACC, bilateral anterior and posterior insula, thalamus and PAG.

of Sciences claiming that the "dorsal anterior cingulate cortex (dACC) is selective for pain" (Lieberman and Eisenberger, 2015), when only a small minority of dACC neurons are pain-related (Wager et al., 2016). Among other issues, the main problem with these examples has to do with the limitations of *reverse inference*, by which the engagement of a particular neurologic process is inferred from the activation of a particular brain region (Poldrack, 2006).

Recently, new approaches to characterise pain in the brain have been employed. Instead of looking at regional activations of spatial regions, sophisticated machine learning techniques have been used to identify patterns of brain activity that closely

predict symptoms. One study in particular claims to have characterised the neurologic signature of physical pain (Wager et al., 2013a). Even though this work was able to identify a distributed signal in the brain that could differentiate pain from other states, it remains unclear whether the identified signals are specifically linked to the perception of pain itself or whether they reflect secondary responses due to the presence of pain (Baliki and Apkarian, 2015).

Further studies, on the other hand, have provided evidence to the non-specificity of the "pain signature" (Iannetti et al., 2013), with an interesting example where investigators administered noxious stimulations to individuals with congenital insensitivity to pain and demonstrated intact "pain matrix" responses in these individuals (Salomons et al., 2016). Taken together, these studies reinforce the need for caution in interpreting attempts to identify signatures of pain. The consequences of reverse inference and careless use of more sophisticated machine learning approaches could be problematic as the "pain signature" is now considered a candidate biomarker for pain in medico-legal settings and a tool for drug discovery (Salomons et al., 2016).

1.5. The challenge of chronic pain

The mechanisms described above concern nociception and acute pain, the latter being crucial for suvival due to it calling for immediate action in order to avoid harm. Acute pain is short in duration and dissipates as part of the healing process. When pain persists and becomes chronic it loses its protective role and can lead to dramatically reduced quality of life. The current IASP definition of chronic pain consists of *"pain that persists beyond normal tissue healing time, which is assumed to be three months"* (Kehlet et al., 2006). If left untreated, chronic pain can become permanent and cause insomnia, anxiety,

severe depression, lower immune function, changes in eating patterns, impaired cognition, maladaptive stress responses, and other long-term problems (Baliki and Apkarian, 2015).

In conjunction with significant suffering, chronic pain remains one the most challenging problems faced by clinicians and health care policy makers, with a steady rise in the number of cases which is currently at around 28 million adults in the UK and 120 million Americans affected, despite improvements in standard health care (Fayaz et al., 2016, Nahin, 2015, Freburger et al., 2009). There is no one-pill-fits-all strategy and currently available pharmacological treatments, including strong analgesics such as opioids, do not provide satisfactory pain relief in about 70% of patients, as indicated by pooled analysis of placebo-controlled trials (Gilron et al., 2005). In summary, there is an urgent need for improved prevention, diagnosis and treatment of chronic pain conditions.

1.6. Chronic osteoarthritis pain

One of the most common types of clinical pain is chronic osteoarthritis pain. Osteoarthritis (OA) is a debilitating degenerative joint disease, characterised by degeneration of cartilage in the joint, commonly affecting the knee, hip, hand and spine. It also involves abnormal remodelling and attrition of subarticular bone, bone growth at the joint margins, ligamentous laxity and weakening of periarticular muscles, accompanied in some cases by synovial distension and inflammation (Arden and Nevitt, 2006). It is a leading cause of disability among older adults in the USA and is amongst the top ten causes of disability worldwide (Neogi, 2013). Approximately 8.5 million adults in the UK and 27 million Americans are estimated to suffer from osteoarthritis and these numbers will likely rise given the ageing population (Neogi, 2013). One third of the population at age 65 or older have OA (Neogi, 2013).

As with other chronic pain conditions, chronic OA pain (CPOA) can lead to severe damage to quality of life and is often accompanied by serious comorbidities such as depression. Together with the burden it causes at the individual level, the economic cost of OA is substantial, whether considering direct (those concerning treatment, hospital resources and management of complications arising from OA treatment) or indirect costs (such as loss of time from work, premature mortality and etc), these are very high, as estimated in 1997 to be between 1% and 2.5% of the gross domestic product in developed countries (Chen et al., 2012, March and Bachmeier, 1997).

Pain is the main complaint and the principal reason why individuals with OA seek medical care. The pain experience in knee OA in particular is well-recognized as typically transitioning from intermittent weight-bearing pain to a more persistent, chronic pain. The levels of pain and disability experienced by patients do not necessarily relate to the amount of joint damage, which suggests contribution from central nervous system mechanisms in the generation of COAP. In line with this idea, pain often persists after treatment, even after joint abnormalities have been reversed via joint replacement surgery.

Treatment options include a number of pharmacological and non-pharmacological interventions (McAlindon et al., 2014). Non-pharmacological possibilities range from moderate exercises, physiotherapy and cognitive behavioural therapy to joint replacement surgery (the final option). Pharmacological routes include non-steroidal anti-inflammatory drugs (NSAID), opioid analgesics, antidepressants or intra-articular injection of corticosteroids with local anaesthetics (McAlindon et al., 2014). All these therapies aim to reduce joint pain and stiffness. However, besides pain persisting in about 20% of patients (Beswick et al., 2012), many of the commonly prescribed medications have associated issues such as strong side effects, including tolerance, addiction and gastrointestinal complications

related to long-term use (Chou et al., 2009). As evidenced by a meta-analysis of randomised controlled trials, placebo is effective in the treatment of OA (Zhang et al., 2008) and may in fact be more effective than most drugs.

Such poor outcomes in randomised trials of analgesic drugs may be a result of the heterogeneity of mechanisms between different patients, which have been all grouped together. However, by incorporating to these trials the concept of *precision medicine*, which consist of treatment strategies that take individual variability into account, one could expect increased efficiency if each study participant was to receive the intervention most likely to work for them.

Attempts have started to be made towards characterizing OA populations according to phenotyping. Variables considered for sub-grouping of OA patients include sex, age, questionnaires and quantitative sensory testing (QST) (Moreton et al., 2015, Egsgaard et al., 2015). The latter consists of a technique to define individual somatosensory characteristics, allowing assessment of both large and small sensory nerve fibre function by use of temperature, vibration and pressure. Facilitated or impaired levels of certain QST measures were found to be associated with treatment response following joint arthoplasty (Petersen et al., 2015, Petersen et al., 2016). However, such variables could only explain a small percentage of the variance in treatment outcomes (less than 10%).

Despite these efforts the field of stratified medicine in COAP remains in its infancy. There are no established markers available for prediction of differential treatment response. Instead, patients follow a trial and error approach, trying different types of drugs, until one that gives them satisfactory pain relief is found. This is a reflection of the complexity of COAP and how little is known about the mechanisms by which drugs work to provide pain relief on an individual level.

1.7. Neural circuitry of chronic pain

Chronic pain is associated with numerous molecular changes in the spinal cord which result in heightened sensitivity of afferent fibres which is generally referred to as "central sensitisation" (Basbaum et al., 2009). Also at the brain level, changes in neural circuitry have been shown to take place in association with chronic pain conditions. Significant progress has been made towards unravelling the neural mechanisms of such conditions over the past two decades. A brief outline of the underlying neurobiology is presented below, highlighting some important recent developments in the field.

Most initial neuroimaging pain research focused on investigating how the healthy brain responded to nociceptive stimuli, under the idea that nociceptive stimulation would mimic pain and potentially the brain in chronic pain if nociceptive stimuli were applied for a prolonged time. However, accumulating evidence shows that the human brain undergoes extensive neurochemical, morphometric and functional reorganisation in certain chronic pain conditions (Apkarian et al., 2011a, Baliki and Apkarian, 2015), and in fact, recent pioneering longitudinal studies show that chronic back pain (CBP) is characterised by predisposing neurostructural and neurofunctional properties (Vachon-Presseau et al., 2016, Mansour et al., 2013, Baliki et al., 2012). These advances evidence the need to investigate clinical pain populations and the field of human neuroimaging research of pain is now slowly converging from experiments in healthy volunteers to chronic pain cohorts (Baliki and Apkarian, 2015).

Such studies have shown that brain reorganisation is associated with chronic pain. In terms of morphology, mostly decreases (but also increases) in grey matter density have been shown in multiple brain regions in chronic back pain, with some partial overlap maps with other conditions such as COAP and chronic regional pain syndrome (CRPS) (Baliki

et al., 2011). As identified by meta-analysis, under the viewpoint of resting-state networks, salience and attention networks seem to be the main circuits commonly affected in terms of grey matter density changes across chronic pain conditions, while sensory regions seem to show more condition-specific grey matter density alterations (Cauda et al., 2014). A longitudinal study indicates that thalamic atrophy found in COAP could be renormalised following successful treatment (Gwilym et al., 2010). Also in COAP, an association between cortical thinning and pain duration was found in fronto-temporal areas but no abnormalities in cortical thickness were detected (Alshuft et al., 2016).

When examining white matter microstructure, abnormalities have been found in CRPS, irritable bowel syndrome and chronic back pain (Chen et al., 2011, Geha et al., 2008, Mansour et al., 2013). In CRPS these alterations were linked to gray matter abnormalities in the same cohort of patients (Geha et al., 2008), suggesting shared mechanisms between the two processes. It is unclear whether COAP is accompanied by similar alterations as there has been no published studies investigating the underlying brain microstructure.

In an attempt to identify metabolite information in chronic pain conditions, researchers have employed magnetic resonance spectroscopy techniques and, despite inconsistent results across early studies, abnormal metabolite levels in multiple brain regions have been found. With more recent advances in data acquisition, studies in fibromyalgia and neuropathic pain indicate alterations in levels of GABA and glutamate or the ratio between the two (Foerster et al., 2012, Foerster et al., 2015, Petrou et al., 2012).

At the functional level, studies have shown that brain activity during acute pain perception is distinct from that in chronic pain, the latter activating more emotional and limbic brain regions, with distinct patterns of limbic system activation being seen

across clinical pain conditions (Apkarian et al., 2011b). Growing evidence also shows that the brain activity at rest appears disrupted in multiple ways across conditions, with some consistency regarding the dissociation of the medial prefrontal cortex from the default-mode network (Baliki et al., 2008, Bolwerk et al., 2013, Loggia et al., 2013). In COAP, investigators found no functional abnormalities when examining cerebral blood flow (CBF) levels (Cottam et al., 2016). What they did find was an association between CBF and reported pain intensity within nociception-processing regions and emotional and limbic regions, which was in fact, apart from the middle anterior cingulate cortex, shown to be driven by trait anxiety scores (Cottam et al., 2016).

Additionally, neuroimaging researchers have recently started to investigate predictors of development of chronic pain. In an extensive design, individuals with sub-acute episodes of back pain were imaged and tracked over a period of one to three years (Vachon-Presseau et al., 2016, Mansour et al., 2013, Baliki et al., 2012). It was found that brain white matter properties of the corticolimbic system impart risk for chronic pain (Mansour et al., 2013). Another potential identified predictor was based on corticostriatal functional connectivity: increased functional connectivity between the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) was present at baseline in patients who developed persistent pain in comparison to those who recovered (accuracy of 80%) (Baliki et al., 2012). With a larger dataset from the same study, researchers found that stronger functional and structural corticolimbic connectivity between mPFC, amygdala and NAc at baseline predicted persistence of back pain, with only the white-matter connectivity remaining constant after three years (Vachon-Presseau et al., 2016). Amygdala and hippocampal volumes also distinguished between persistent and recovery groups while gene polymorphisms and negative mood indirectly contributed to the risk for chronic

pain (Vachon-Presseau et al., 2016). These findings indicate that structural properties of the corticolimbic brain, not the initial acute back injury, determine the risk for developing chronic back pain. It is unclear, however, the extent to which this can be generalised to other chronic pain conditions.

Based on these advances, models of chronification of pain have been proposed. As illustrated in Figure 1.4, chronification of pain seems to be mediated by dysregulation of homeostatic reward processes, yielding impairments in sensory, psychological, behavioural and social functions which in turn contribute to chronification (Elman and Borsook, 2016).

Collectively, these observations show that chronic pain is not an unitary system mediated by an isolated mechanism or a neurotransmitter. Instead, these advances point to chronic pain being an integration of emotional and motivational functions, and derived cognitive, behavioural and social manifestations (Elman and Borsook, 2016). It seems to be a maladaptive response, contingent on corticolimbic systems that represent the brain addicted to pain (Vachon-Presseau et al., 2016, Elman and Borsook, 2016).

1.8. Outstanding work

Finally, even though a major contribution to understanding of chronic pain mechanisms have been achieved over the past decade by investigating the CNS role in clinical pain, there is still a major gap in comprehending and integrating clinical symptomatology and effective analgesic strategies, along with basic mechanisms. It is still unclear why certain treatments work for some patients and not for others, and there are currently no established markers that determine whether a patient is likely to respond to a given treatment. Additionally, it is unknown whether several of the underlying neuro

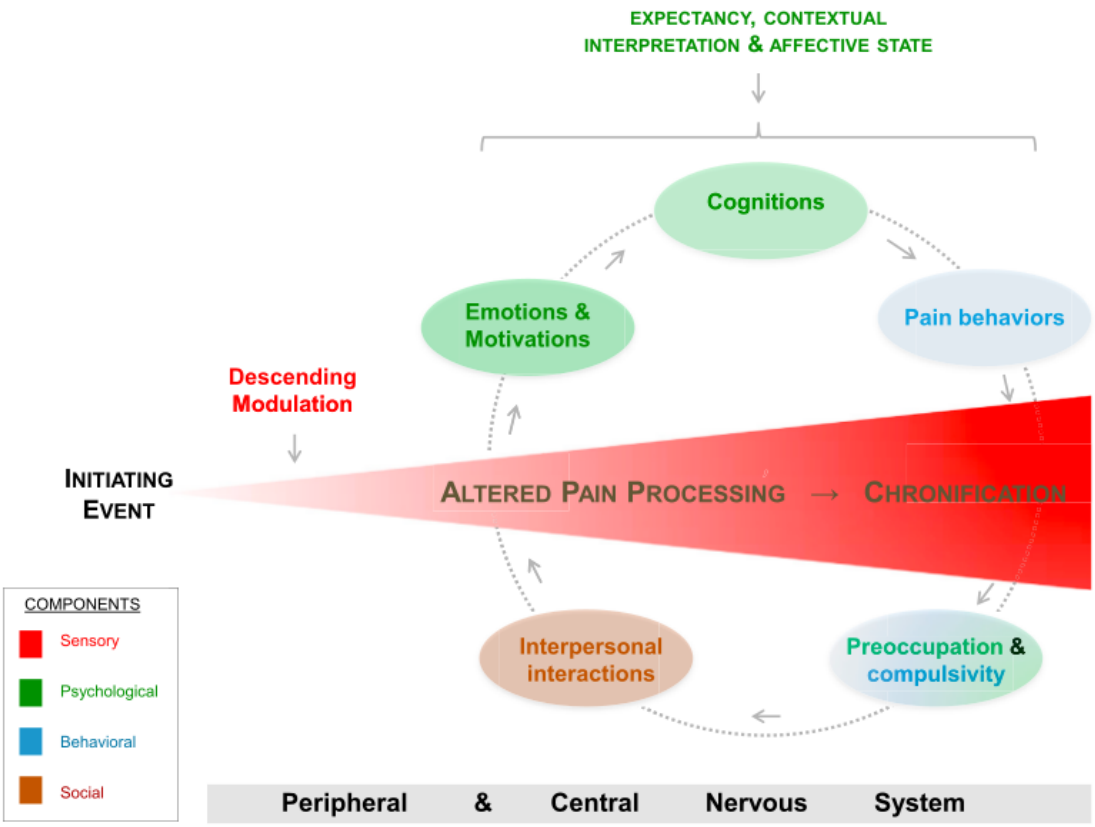


Figure 1.4.: **Chronification of pain.** The figure illustrates a proposed model for the transition from acute tissue injury, detected by nociceptors, to the development of chronic pain via abnormal reward processes resulting in impaired sensory, psychological, behavioural and social functions. *Reproduced from (Elman and Borsook, 2016).*

mechanisms observed in other pain conditions can be also seen in COAP.

Given the generally poor outcomes of currently available treatments, together with the substantial burden both at economic and individual levels, and the large and increasing predominance of COAP, further and urgent work is required in order to fully comprehend and integrate the complex, multidimensional contributions from both psychological and physiological factors, to the development, maintenance and relief of COAP. Going towards this goal, it is necessary to elucidate COAP-specific underlying central components by integrating their neural, psychometric and sensory contributions, and comparing these

to those seen in other chronic pain conditions. Based on such processes and mechanisms of pain relief, strategies for stratification of patients according to likelihood of treatment response may ultimately be developed.

2

General aims and hypotheses

The overall aim of this thesis is to improve understanding of central components of chronic osteoarthritis pain and mechanisms of centrally active analgesic treatment. In an effort to elucidate such processes, a multidimensional approach is followed, where multiple layers of characterization of cerebral components are integrated with psychological, sensory and demographic factors. These are further compared with the published literature in other chronic pain conditions and, when relevant, also with the literature in psychiatric conditions or the healthy state. Four studies were undertaken.

The **first study** aims to identify and characterize potential alterations in brain microstructure, underlying adaptive or predisposing abnormalities in association with chronic osteoarthritis pain (COAP). An additional aim is to contrast these potential changes to findings in other chronic pain conditions. It is hypothesized that COAP is associated with changes in myelination or axonal quantities in white matter tracts

as indexed by mean diffusivity and fractional anisotropy, and that these changes are related to pain duration and the development of neuropathic-like symptoms. It is further hypothesised that findings from other musculoskeletal types of chronic pain would be replicated in COAP.

Study two focuses on the molecular level with the aim of characterizing the role of γ-aminobutyric acid levels in COAP. The hypothesis is that GABAergic disinhibition (low GABA) in the mid anterior cingulate cortex (mACC) is a molecular marker of ongoing pain severity in COAP. More specifically, it is hypothesized that mACC GABA is decreased in COAP and correlates with the level of perceived ongoing pain intensity.

Study three has two major aims. One is to study neural correlates of the analgesic effects of the short-acting μ-opioidergic agonist, remifentanil, in individuals with COAP. The second aim is to explore whether pain phenotyping, based on questionnaires and psychophysiological testing, allows prediction of the extent of opioid response. Here it is hypothesised that nociceptive activity in pain-related brain regions with high opioid receptor densities (Baumgärtner et al., 2006), and shown to be down-regulated by opioids in healthy subjects, is modulated by remifentanil in COAP. In addition, it is hypothesised that opioid action is related to levels of impairment in pain modulatory systems.

The **fourth study** aims to investigate the mechanisms of pain relief in COAP following treatment with the serotonin-norepinephrine reuptake inhibitor duloxetine, and to establish predictors of treatment response. It is hypothesised that duloxetine will induce reduction in nociceptive brain response and that responding and non-responding subgroups of COAP patients differ in their limbic brain signatures and may show differences in negative affect and/or sensory pain phenotypes. Specifically, for responders to either treatment (duloxetine or placebo) it is hypothesised that pain intensity encoding regions

such as the anterior insula and the mid anterior cingulate cortex are down-regulated after treatment. Duloxetine responders in particular, are expected to additionally present treatment-induced activity modulation within limbic regions including the amygdala and putamen, as opposed to placebo responders. Finally, activity modulation or enhancement after duloxetine treatment is expected as direct drug effect.

Part II.

Neuroimaging Background

3

Nuclear magnetic resonance

Overview

Before tackling some of the knowledge gaps in chronic pain research stated in Part I, it is necessary to describe some of the theoretical foundations of relevant techniques, utilized to extract information about the brain. Nuclear Magnetic Resonance (NMR) is the basis of such tools, and refers to the behaviour of a nucleus in the presence of a magnetic field (Levitt, 2008, De Graaf, 2013). This chapter provides an outline of the foundations of the NMR theory, together with an overview of the magnetic resonance techniques required to understand the original work presented in this thesis.

3.1. NMR principles

An elementary particle is conventionally described in terms of the physical properties of electric charge, mass, magnetism and spin. An atom is constituted by a nucleus surrounded by electrons, which are negatively charged. The nucleus contains protons, positively charged, and neutrons, which have no charge. It is the strong electrostatic interactions between positively charged nuclei and negatively charged electrons that bound atoms and molecules together. Nuclei with spin angular momentum can interact with a magnetic field and the resonant angular frequency, or *Larmor frequency* ω_L, is given by the following equation:

$$\omega_L = -\gamma B_0 \tag{3.1}$$

where γ is the gyromagnetic ratio, which is characteristic to each nucleus. Table 3.1 gives the gyromagnetic ratio and spin I of selected nuclei.

Nucleus	Nuclear spin	*gamma* (MHz/T)
^{1}H	1/2	42.58
^{13}C	1/2	10.71
^{19}F	1/2	40.08
^{17}O	5/2	5.77

Table 3.1.: Properties of commonly used nuclei in magnetic resonance (Levitt, 2008)

A given nucleus is said to be MR active if either the mass number or atomic number is odd. In this case the nucleus may align to an external magnetic field due to its magnetic momentum, the latter being proportional to the spin by a constant which is the gyromagnetic ratio

$$\hat{\mu} = \gamma \hat{S} \tag{3.2}$$

where $\hat{\mu}$ and $\hat{S}$ are quantum mechanical operators of magnetic momentum and spin respectively.

Therefore the interaction of a particle with an external magnetic field is specific to each nucleus, which results in specific NMR sensitivities. The magnetic energy E_{mag} of the interaction with an external magnetic field is the dot product of the nucleus' magnetic momentum $\vec{\mu}$ and the field $\vec{B}$

$$E_{mag} = -\vec{\mu} \cdot \vec{B} \tag{3.3}$$

which indicates that the magnetic energy depends on the relative direction of the $\vec{B}$ and $\vec{\mu}$, and the lowest energy is achieved when the magnetic momentum is parallel to the external magnetic field.

The work contained in this thesis is concerned with the hydrogen atom, which is the most abundant in biological tissues and most commonly used for in-vivo NMR. The remaining of the dissertation will therefore focus on this isotope.

3.1.1. Nuclear spin and Zeeman splitting

In quantum mechanics, the angular momentum is quantised. For a nucleus with unpaired number of nucleons (neutrons and protons) the net angular momentum $\mathbf{p}$ is given by

$$\mathbf{p} = \hbar \sqrt{I(I+1)} \tag{3.4}$$

where $\hbar = 1.054 \times 10^{34} Js$ is the Planck constant divided by 2π. That is the case for the hydrogen atom, which contains a single proton and one electron.

The z component of the angular momentum $\mathbf{p}$ is a measurable quantity and is related to the spin quantum number m_I

$$p_z = \hbar m_I \tag{3.5}$$

where m_I can take discrete values $-I, -I+1, -I+2, ..., I-1, I$ (Dirac, 1928). A nucleus with spin I has $2I+1$ degenerate energy states m_I. Since hydrogen has $I = \frac{1}{2}$ (Table 3.1), m_I can be $\frac{1}{2}$ and $-\frac{1}{2}$. This splitting into discrete energy states $E = \gamma \hbar m_I B_0$ in the presence of an external magnetic field B_0 is known as Zeeman splitting. The separation between the two energy states is given by

$$\Delta E = \hbar \gamma B_0 = h\nu \tag{3.6}$$

where ν is the frequency of electromagnetic radiation required to move the proton between spin states (Figure 3.1).

NMR is the spectroscopy of the Zeeman sublevels, where an electromagnetic field is applied in order to cause transitions between the nuclear spin states.

3.1.2. Bulk magnetisation

So far, the behaviour of a single proton was described. However, real systems consist of numerous individual spins and the observed macroscopic quantities are a reflection of the average response from the sample. In order to describe this, it is appropriate to refer to the classical description of bulk magnetisation $\mathbf{M}$, which is the superposition of

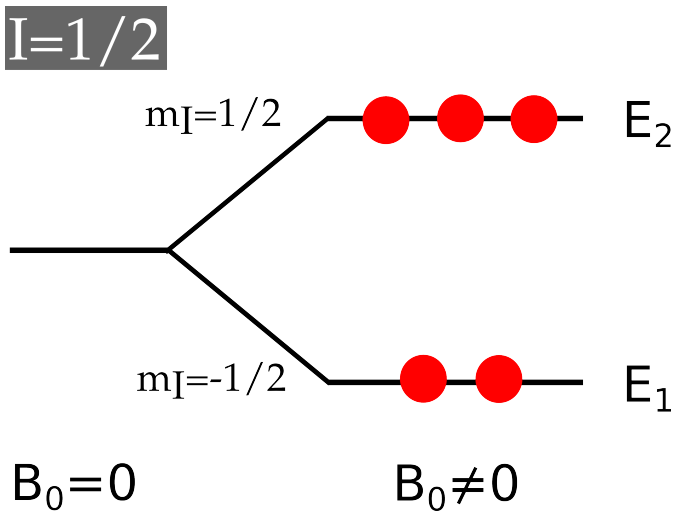


Figure 3.1.: Illustration of Zeeman splitting under the influence of an external magnetic field $\vec{B}_0$. Spin states are indistinguishable in the absence of an external magnetic field, $B_0 = 0$. Under the application of an external magnetic field, the spin population is split into two different levels of energy E_1 and E_2, where $E_2 - E_1 = \hbar\gamma B_0$.

the multiple magnetic moments of a sample when an external magnetic field is applied. Boltzmann statistics can be employed to predict the behaviour of the population of states for an ensemble of non-interacting spins. For a temperature T above absolute zero and a magnetic field B_0, the distribution of protons in the two energy states described above is given by

$$\frac{N_\beta}{N_\alpha} = e^{\frac{\Delta E}{k_B T}} = e^{-\frac{\hbar\gamma B_0}{k_B T}} \tag{3.7}$$

where $k_B = 1.38 \times 10^{-23} JK^{-1}$ is the Boltzmann constant. For normal room temperatures, $k_B \gg \hbar\gamma B_0$, expression 3.7 can be expanded using a power series and the difference in population between the two states can be approximated by

$$N_\alpha - N_\beta \approx \frac{N\hbar\gamma B_0}{2k_B T} \tag{3.8}$$

where N is the total number of protons in the system. Therefore, the magnetisation depends on the strength of the external magnetic field B_0 and the temperature. Since it

is not possible to decrease the temperature of the sample when analysing *in-vivo* human tissues, a higher field strength is required for increasing the NMR signal.

3.1.3. Bloch equations and relaxation

Based on the fact that the bulk magnetisation $\mathbf{M}$ is the sum over all magnetic moments, the rate of change of bulk magnetisation can be described as

$$\frac{d\mathbf{M}(t)}{dt} = \mathbf{M}(t) \times \gamma \mathbf{B}(t) \tag{3.9}$$

At thermal equilibrium, no NMR signal can be detected since there are no net x and y components of $\mathbf{M}$. From equation 3.9 it follows that $\mathbf{M_z}$ may be perturbed by a secondary magnetic field, with lower amplitude than B_0, perpendicular to $\mathbf{M_z}$. As this field is rotating at the Larmor frequency in the radiofrequency (RF) range of the electromagnetic spectrum, it is often referred to as the RF magnetic field. The magnetic component of this field $\mathbf{B_1}$, that is linearly polarized along the x axis, can be described as

$$\mathbf{B_1}(t) = 2B_1^{max}(t) \cos \omega t \vec{x} \tag{3.10}$$

where B_1^{max} is the maximum amplitude of the applied field, ω is the carrier frequency of the RF field and $\vec{x}$ is a unit vector along the x axis. In the presence of both $\mathbf{B_0}$ and $\mathbf{B_1}$, equation 3.9 can be expanded to yield the Bloch equations in the laboratory frame of reference:

$$\frac{dM_x(t)}{dt} = \gamma[M_y(t)B_0 - M_z(t)B_{1y}] \tag{3.11}$$

$$\frac{dM_y(t)}{dt} = \gamma[M_z(t)B_{1x} - M_x(t)B_0] \tag{3.12}$$

$$\frac{dM_z(t)}{dt} = \gamma[M_x(t)B_{1y} - M_y(t)B_{1x}] \tag{3.13}$$

From these equations one can see that the application of an electromagnetic pulse at the Larmor frequency will cause magnetisation to be tipped into the transverse plane.

Upon magnetisation being tipped into the transverse plane, a key phenomenon, called relaxation, occurs. Relaxation is the process of returning to thermal equilibrium following a perturbation. Each component of the magnetisation **M** return to equilibrium in an exponential manner and the longitudinal (M_z) and transverse (M_x and M_y) components to $\mathbf{B_0}$ relax with distinct time constants (Huettel et al., 2004).

Longitudinal T1 relaxation

Longitudinal relaxation refers to the decay of the z component of the bulk magnetisation and may be written as follows

$$\frac{dM_z}{dt} = \frac{(M_0 - M_z)}{T_1} \tag{3.14}$$

where T_1 is a time constant called *longitudinal relaxation time* or *spin-lattice relaxation time* and M_0 is the longitudinal magnetisation at thermal equilibrium. The decay of the longitudinal magnetisation is due to energy from the spins being transferred to the surrounding lattice. T_1 is specific for different kinds of matter and varies according to magnetic field strength, being longer at stronger fields.

Taking a 180^o inversion pulse and a 90^o saturation pulse, it is possible to solve

equation 3.14 as follows. For the first pulse, the magnetisation is fully inverted, meaning that $M_z(t) = -M_0$. Therefore, in this case, equation 3.14 becomes

$$M_z(t) = M_0(1 - 2e^{-t/T1}) \tag{3.15}$$

While, for the 90^o, $M_z(t) = 0$ and therefore

$$M_z(t) = M_0(1 - M_0 e^{-t/T1}). \tag{3.16}$$

An illustration of the longitudinal decay following an inversion pulse is given in Figure 3.2.

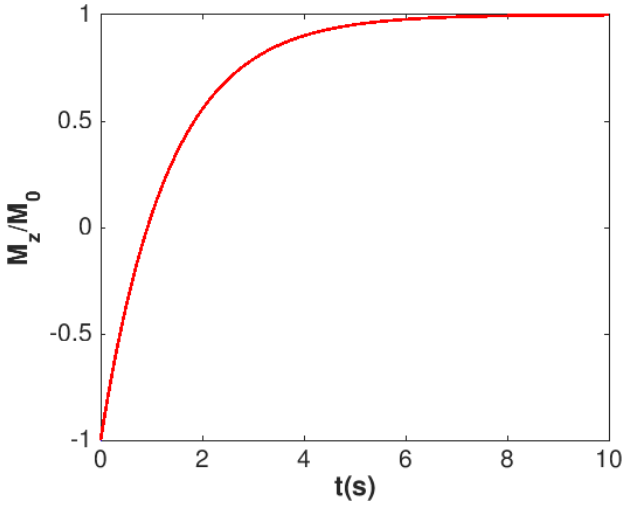


Figure 3.2.: Longitudinal decay at 3T in the putamen of the human brain ($T_1 = 1.33$s) (Wright et al., 2008) following an inversion pulse, described by Equation 3.15.

Transverse T2 relaxation

The other component of relaxation, the so called transverse or T_2 relaxation, occurs due to spin-spin interactions and describes the disappearance of the transverse magnetisation. This can be seen as an entropy process since spins exchange energy between themselves,

causing a decrease in phase coherence. Similarly to T_1, T_2 is dependent on the magnetic field strength and varies according to the type of matter. The transverse relaxation process can be written as

$$\frac{dM_{x,y}}{dt} = -\frac{M_{x,y}}{T_2} \tag{3.17}$$

T_2 is always shorter than T_1, and it is normally longer where the spins are freer. For instance, in water molecules, they are more mobile and therefore have longer T_2.

After the application of 90^o pulse, the decay of transverse magnetisation is described as follows

$$M_{x,y} = M_0 e^{-t/T_2} \tag{3.18}$$

which can be visualised in Figure 3.3.

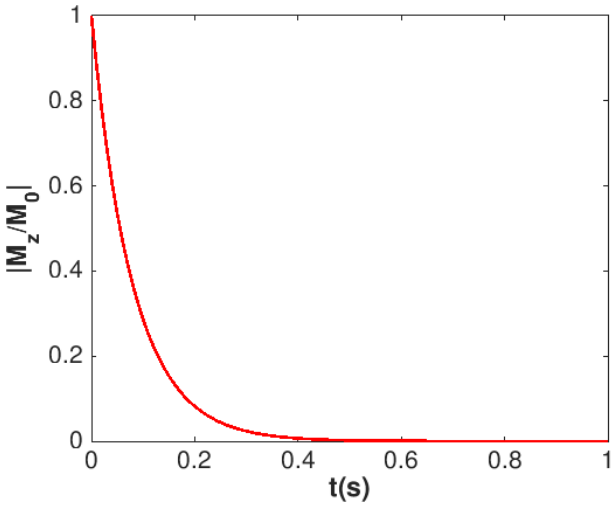


Figure 3.3.: Relaxation of the transverse magnetisation component, given by Equation 3.18, for grey matter at 3T ($T_2 = 80$ms) (Wansapura et al., 1999)

This signal is called a free induction decay (FID) and it is important when considering signal "echoes".

However, in real systems, spins behaviour is affected by inhomogeneities in the external magnetic field, which causes further dephasing of the magnetic moments in the transverse plane. Therefore, in real systems, the rate of decay is faster than described above. The so-called effective T_2, T_2^*, is a time constant that accounts for the effects of B_0 inhomogeneities. Factors that can impact T_2* include flow and diffusion, for example. Its relationship with T_2 is described in the equation below

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'} \tag{3.19}$$

where T_2' is the correction needed to be made to T_2 due to B_0 inhomogeneities.

3.1.4. Spin echo

Magnetic dipoles naturally decay in random phase. In NMR, for a coherent decay, it is necessary to re-focus the magnetic dipoles, by applying an additional radiofrequency pulse or gradient upon excitation. This re-focusing process is referred to as an *echo*. All spin echo sequences contain a 90^o excitation pulse followed by one or more 180^o pulse (Bernstein et al., 2004). After the excitation pulse is applied, the magnetisation decays freely during a time t, until the refocusing pulse is applied. Upon this, the magnetisation decay will result in accumulation of phase coherence and, after a time t, the bulk magnetisation will return to a coherent state, forming a spin echo. This is when the signal acquisition takes place (Figure 3.4).

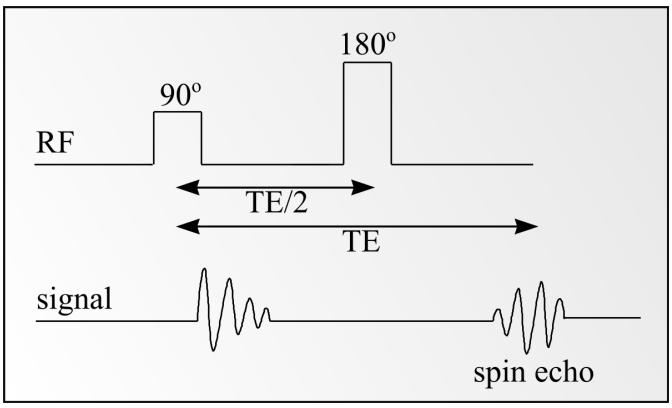


Figure 3.4.: Illustration of a basic spin echo sequence. (Taken from http://www.voxelcube.com/articles/1/the-principles-of-magnetic-resonance-imaging in September 2016).

3.2. Magnetic resonance imaging

This section outlines how pulse sequences can be used, in a magnetic resonance imaging scanner, to create images with different types of contrasts.

In order to acquire spatially specific signals from a given volume, a combination of linear magnetic fields can be employed using additional electromagnetic induction coils, called gradient coils. Spatial location methods can be divided into slice selection, frequency encoding and phase encoding. Further details are given below.

3.2.1. Slice selection

Slice selection consists of isolating a single plane in the volume being imaged, by only exciting the spins in that plane. This is done by applying a gradient simultaneously to the application of a radiofrequency (RF) pulse. The pulse will then only excite those spins whose Larmor frequency corresponds to the applied RF pulse (Figure 3.5).

The thickness of the selected slice is inversely proportional to the applied gradient strength (Garroway et al., 1974). By altering the central frequency and the bandwidth $\Delta\omega$ of the RF pulse, one can manipulate the location z of the slice

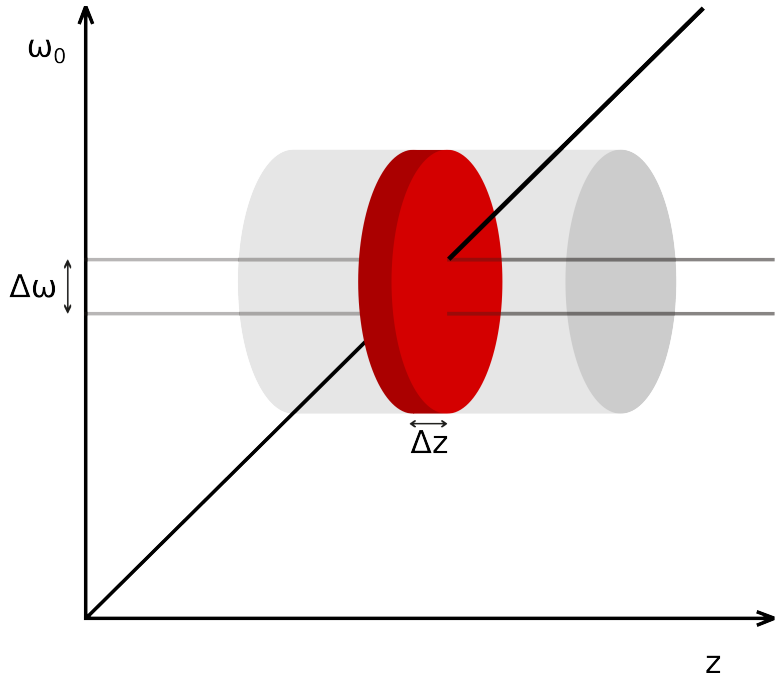


Figure 3.5.: **Slice selection is achieved by varying the magnetic field strength.** A slice selective gradient applied over an object will linearly vary the frequency of resonance of the spins with spatial distance in the direction of the applied gradient. The intersection points between the transversal line (black) and the cylindrical object indicates the limits of the slice selection, and consequently, the frequencies being excited. The stronger the gradient field (*i.e* steeper black line), the smaller the slice thickness will be, due to the increased resonance frequency.

$$\Delta z = \frac{\Delta\omega}{\gamma G_z} \tag{3.20}$$

3.2.2. Phase encoding

Once the slice has been selected, one can spatially vary the phase of the magnetisation by introducing an additional gradient in order to locate signal from within the selected slice. This phase encoding gradient is typically applied after the excitation pulse and prior to the readout gradient. Applying a phase encoding gradient $\vec{G_y}$ for a duration t, will introduce a phase shift ϕ given by

$$\phi = \gamma G_y y t \tag{3.21}$$

3.2.3. Frequency encoding

Based on the concept of frequency space, also known as k-space (where k is the wave number $k = \frac{2\pi}{\lambda}$), frequency encoding is another spatial localisation method. It consists of applying a frequency encoding gradient following the phase encoding gradient, which causes the excited spins to precess at a range of different frequencies. These will determine how different areas of the sample will absorb, and thus radiate, different frequencies. The received signal will be a function sum of the individual frequencies. When taking the inverse Fourier transform of the signal, it is possible to identify the individual frequencies and therefore, the spatial position of the signal source (Kumar et al., 1975).

Moving the centre of the voxel by a a distance Δx makes the magnetic field experienced by the centre of the voxel to change according to

$$\Delta B_z = G \cdot \Delta x \tag{3.22}$$

which also results in a change in the angular frequency

$$\Delta \omega = \gamma G \cdot \Delta x \tag{3.23}$$

If the signal $S(t)$ received at time t, following the refocusing pulse and during the application of a gradient, is in form of a free induction decay, it can be treated as if it is a Fourier transform of the proton density function $\rho(\vec{r})$ at a vector $\vec{r}$, that is, $S(t) = \rho(k(t))$

$$S(t) = \int \rho(r) e^{i\gamma \int_0^t r \cdot G(t')dt'} dr \tag{3.24}$$

If $S(t)$ is a function of time, then $k(t')$ must too be a function of time, as it is a vector in k-space and is dependent on the applied B_z and the gradient $G(t)$, where t' represents

time in the Fourier transformed domain

$$k(t) = \gamma \int_0^t G(t')dt' \tag{3.25}$$

Since equation 3.24 can be described as an inverse Fourier transform, then applying a Fourier transform gives the proton density function in terms of k

$$S(k) = transform(\rho(k)) = \int \rho(r) e^{i\vec{r}\cdot\vec{r}} dr \tag{3.26}$$

and therefore

$$\rho(r) = \int S(\vec{k}) e^{i\vec{r}\cdot\vec{r}} d\vec{r} \tag{3.27}$$

Thus, by acquiring sufficient signal $S(t)$ at different frequencies, k-space can be sufficiently populated.

3.2.4. Echo planar imaging

Mansfield and colleagues showed, in 1977, that it is possible to fill the whole k-space after a single excitation pulse (Mansfield, 1977). Previous to this, available methods would only allow for the equivalent of one line of k-space to be filled after each excitation pulse. The sequence proposed by Mansfield et al, generally called *echo planar imaging*, has high signal to noise ratio, sensitivity to transverse relaxation time and temporal resolution, being therefore useful for functional imaging, which will be discussed in the next chapter.

One example of echo planar imaging sequence is illustrated in Figure 3.6. Following a radiofrequency pulse and a slice selection gradient, two further short gradients align the frequency and phase to the extreme edge of the k-space, prior to the start of

measurements. Gradient echoes (GREs) are then collected with each oscillation of the readout (frequency) gradient. The first gradient echo represents the sampling of k-space from $-k_x$ to 0. After a short phase encoding gradient G_y, the sampling progresses from 0 to $+k_x$ under another frequency encoding gradient G_x, of opposite polarity. The next sampling goes incrementally up to the next k-space line, until the whole k-space is sampled.

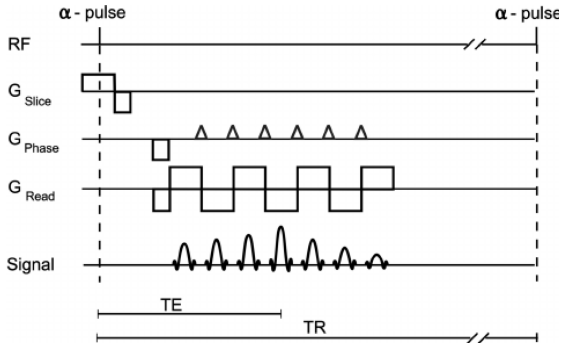


Figure 3.6.: Pulse diagram of a gradient echo, echo planar imaging acquisition. Taken from (Gebker et al., 2007)

3.2.5. Parallel imaging

In order to reduce scan time, receiver coils with multiple elements may be employed. One of the common designs was first proposed in 1997 and is known as sensitivity encoding (SENSE) (Sodickson and Manning, 1997). In such coils, where the field of view of each coil element is overlapping, knowledge of the sensitivity map of each coil element is used to partially fill k-space, so that some trajectories do not need to be sampled. The signal from regions detectable by two or more elements is under-sampled, without compromise to the spatial resolution. Alternatively, the field of view may be expanded without augmenting the scan acquisition time. When the data is reconstructed to form an image, the sensitivity maps are used to calculate weighting values that are used to fill the k-space without aliasing. Scan time is reduced by an acceleration factor, R. Higher values of R

at the same spatial resolution, however, result in higher noise in the image.

4

Investigating the human brain

Overview

Based on the theoretical foundations introduced in Chapter 3, key general concepts about the human brain, and methodologies used to measure different aspects of the organ are described in this chapter. This is by no means a comprehensive review; instead, a general overview is presented, highlighting the points that are most relevant for the original research contributions presented in the proceeding chapters.

4.1. Brain cells and signaling

The central nervous system consists of the *brain* and the *spinal cord*. These are composed by two categories of cells, named *neurons* and *glia*, and are protected by three layers of connective tissue that form the *meninges*. Within each of those categories there are many

types of cells that differ in structure, chemistry and function. Neurons sense changes in the environment, communicate these changes to other neural cells and send commands to make the body react to these sensations. The glial cells, on the other hand, provide support, insulation and nourishment to neighbouring neurons.

A neural cell may be divided into three main parts: *axon*, *dendrites* and *soma* (cell body) [see Figure 4.5]. While dendrites cluster together forming the brain grey-matter, axons cluster together forming axonal tracts or bundles, constituting the brain white-matter (Kandel et al., 2000).

The transfer of information between neurons is known as *neuronal signalling*. There are two means by which this communication may happen. One is via chemical changes, which is done via signalling molecules called *neurotransmitters*, which are released by a neuron, binding and activating receptors of another neural cell. There are more than 100 distinct types of neurotransmitters available. The main excitatory neurotransmitter is the amino acid glutamate, while the principal inhibitory type of such molecules is gamma aminobutyric acid. These are covered in more detail on section 4.2.1. The second type of neural signalling is electrical. In general terms, ion imbalances across the neuron membrane lead to a negative electrical potential inside the cell. The action potential transiently extinguishes the negative resting potential and makes the trans-membrane potential positive. Action potentials propagate along the length of axons, and are the fundamental signals that convey information through the nervous system.

4.2. Neuroimaging methods

This section provides an overview of the available methods to investigate the in-vivo brain non-invasively. Further details are provided for the methods used in the original

research of this dissertation.

Neuroimaging is a rapidly evolving field and offers a wide variety of methods for measuring brain structure, chemistry and function. Magnetic resonance imaging (MRI) is a non-invasive imaging modality capable of providing excellent structural quality and contrast between different brain tissues. Newer anatomical modalities include diffusion magnetic resonance imaging which, more specifically, enables visualisation and quantification of, for example, characteristics of myelinated tracts.

In order to gather metabolic details about concentration of various biochemical compounds in the brain, magnetic resonance spectroscopy (MRS) is a unique technique for non-invasive, in-vivo analysis measurements of such information. Methods for imaging brain function may be divided into two major categories. The first, consists of approaches using direct measurement of neuronal electromagnetic activity such as electroencephalography (EEG) and magnetoencephalography (MEG). The second is formed by techniques of indirect brain activity measures, which operate under the assumption that neural activity is supported by elevated local blood flow and metabolic activity. Examples on this category include positron emission tomography (PET), and functional magnetic resonance imaging (fMRI) methods such as blood-oxygen level dependent and arterial spin labelling (ASL). Direct measurements of neural activity have the advantage of high temporal resolution, being sensitive to changes on the order of a millisecond. However, they suffer from poor spatial resolution, which makes it difficult to identify the specific origin of the signal. Indirect techniques, on the other hand, offer improved spatial resolution, albeit poorer temporal resolution.

In this thesis we are more concerned with improved sensitivity on the spatial rather than temporal dimension and therefore, the functional imaging methods chosen to

investigate chronic osteoarthritis pain are part of the indirect measurements category. The relevant functional, structural and chemical methods for the original research in this dissertation are briefly reviewed below.

4.2.1. Measuring brain metabolites with proton MRS

In vivo ^{1}H-MRS has been applied in numerous neurological studies and is sometimes used in the clinical evaluation of certain tumours.It allows non-invasive evaluation of brain metabolite and neurochemical concentrations and can be performed by most modern MR systems with field strengths equal or higher than 1.5T, provided that these are able to optimize field homogeneity. Different neurochemicals may be detected by manipulating variables such as resolution, TR, TE and the type of acquisition sequence. Spectra can be obtained either from one selected region, as single-voxel spectroscopy, or from multiple brain regions in MR spectroscopic imaging.

Single volume localization

In-vivo MRS generally requires restriction of signal detection to a well-defined region of interest (ROI). Spatial location is also used for removing signals from outside the ROI, such as extra-cranial lipids. Localisation methods rely on the selection of a slice by the application of a frequency-selective RF pulse in the presence of a magnetic field gradient.

Point Resolved Spectroscopy (PRESS) PRESS is a multi-echo single shot technique consisting of a 90^o excitation pulse and two 180^o refocusing pulses. The 90^o RF pulse rotates the spins in the yx-plane, followed by the first 180^o pulse applied at $\frac{T_{E1}}{2}$. An echo is then generated at time T_{E1}, in the region intersected by the 90^o and first 180^o pulses. The second 180^o pulse refocuses this spin echo during a second delay T_{E2}, such that the final echo is formed at $TE = T_{E1} + T_{E2}$ from those spins that were excited

by all three RF pulses. The three RF pulses are frequency-selective and are applied in combination with magnetic field gradients, hence allowing three dimensional spatial localisation. Each pulse is applied perpendicularly to each other such that only spins within the ROI are excited to produce a signal, whereas spins outside the ROI are either not excited or not refocused, leading to rapid de-phasing of signal by the TE magnetic field gradients.

In order to accommodate the three RF pulses, TE is relatively large in the PRESS sequence, which makes PRESS less suitable for measuring metabolites with short T_2.

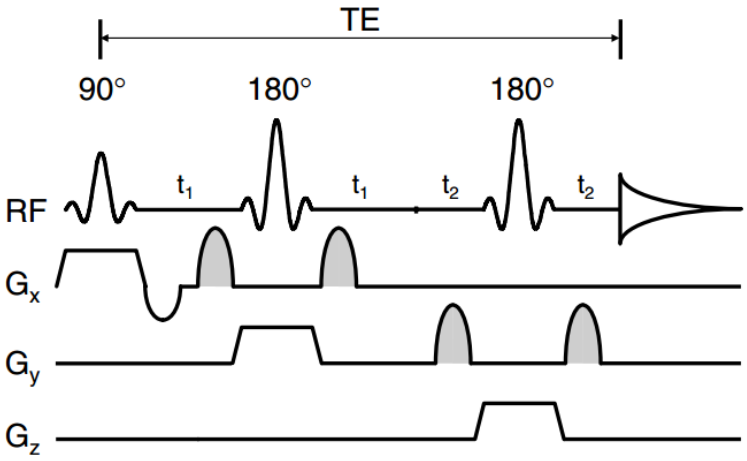


Figure 4.1.: Illustration of the PRESS sequence for a 3D spatial localisation. Adapted from (De Graaf, 2013)

Stimulated Echo Acquisition Mode (STEAM) STEAM is used for detecting compounds with short $T2$. Like PRESS, the spatial selection is performed by three RF pulses, however using only 90^o pulses. The first 90^o pulse, in combination with its respective gradients, rotates all the spins into the transverse plane. During a time $\frac{T_E}{2}$ after the first pulse, the magnetisation dephases for the effect of TE crusher gradient and B_0 inhomogeneities. Following that a second 90^o pulse is applied perpendicularly to the first, which causes rotation of the magnetisation into the xz plane. During time TM, the

mixing time or the delay during which the magnetisation is in the longitudinal rather than the transverse plane, between the second and third pulses half of this magnetisation is dephased by the TM crusher gradient and only a net of coherent longitudinal component remains. The third 90^o pulse rotates the remaining magnetisation back into the xy plane where a second, matched TE crasher gradient refocuses at time $TE + TM$ to give the stimulated echo. In order to eliminate spurious signals in the stimulated echo, a crusher gradient is applied in the TM period, leaving only the stimulated echo and free induction decay arising from the last RF pulse. The FID component can be easily removed by including identical crusher gradients in both $\frac{T_E}{2}$ periods.

A disadvantage of the STEAM sequence is that only half of the magnetization is refocussed and therefore, SNR is lower compared to the PRESS sequence. However, STEAM can achieve very short echo-times and therefore, differently from PRESS, can be used to measure compounds with short T_2. Since STEAM only uses 90^o pulses, which give better slice profile than 180^o pulses, it provides improved localisation accuracy in comparison to PRESS.

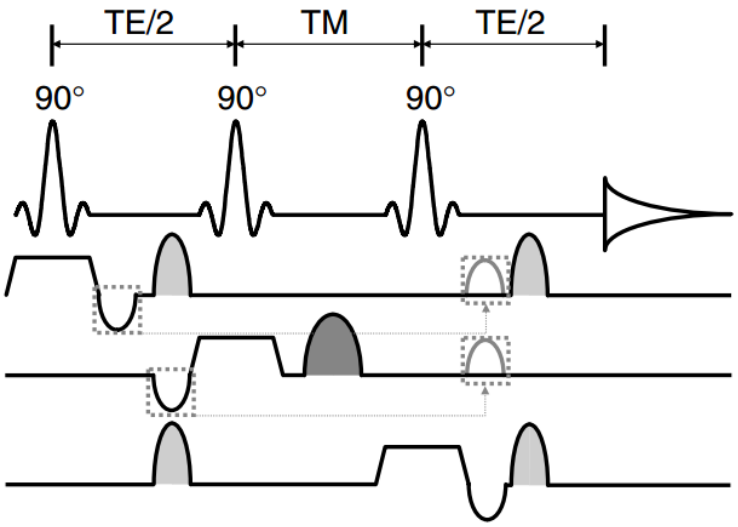


Figure 4.2.: Illustration of the STEAM sequence for a 3D spatial localisation. Adapted from (De Graaf, 2013)

Spectra quantification

Popular fitting algorithms include the jMRUI package (Naressi et al., 2001) and LCModel (Provencher, 1993, Provencher, 2011). The LCModel software uses a linear combination algorithm where the total measured NMR signal is considered as linear combination of the NMR signals of each isolated compounds. For this, a basis set is necessary, containing the spectra of all metabolites to be modelled. It is important that all metabolites present in the measured NMR spectrum are also included in the basis set as the omission of a metabolite would potentially lead to a systematic bias in the estimated parameters.

Water suppression techniques

As the water signal is $10,000$ times stronger than the neurochemical signals, it is necessary to suppress the water signal in order to measure metabolite concentrations. There are a number of methods available for water suppression and they rely on differences between magnetic properties of water and metabolites.

^{1}H-MRS Detectable Neurochemicals

^{1}H-MRS focuses on carbon-bound protons in the 1–5 ppm range of the chemical shift scale [Fig 4.3]. The metabolites that can be identified with this technique are those present at sufficiently high concentrations, including the neuronal metabolite N-acetylaspartate, the glial metabolite myo-inositol, choline-containing compounds, neurotransmitters glutamate and γ-aminobutyric acid, creatine, glutamine and lactate, amongst other lipids and proteins (Soares and Law, 2009, Kwock, 1998).

^{1}H spectra show a dominant resonance of water (at 4.7 ppm) which is nearly ten thousand times higher than the resonance signals metabolites. And in order to enable evaluation of metabolites of interest, the water resonance has to be suppressed.

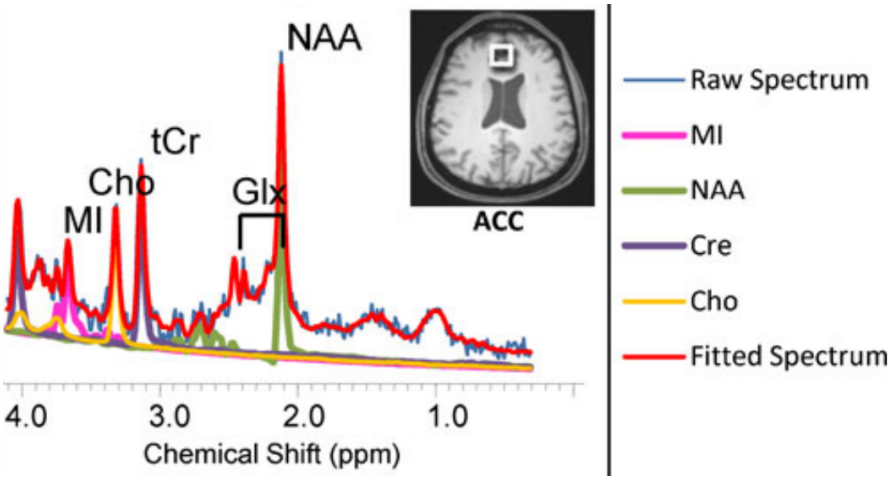


Figure 4.3.: Representation of typical MR spectra of the frontal gray matter from a healthy volunteer (Chang et al., 2013)

N-acetylaspartate (NAA) and NAA-glutamate (NAAG)

NAA is an amino acid present in a relatively large quantity in the healthy human brain and therefore represents the highest peak after water on the proton spectra, resonating at 2.01 ppm [Fig 4.3]. It has been frequently measured in neurological, psychiatric and chronic pain conditions (Birken and Oldendorf, 1989), with greater precision than other compounds of interest. Considered to be a marker of the density and viability of neurons, NAA appears in slightly different concentrations in white (6-9mM) and grey matter (8-11mM) (Meyerhoff et al., 1993). The metabolic function of this amino acid is not entirely known but most observations suggest hypotheses of NAA involvement in the formation and maintenance of myelin and fatty acids (Miller, 1991, Castillo et al., 1998, Baslow, 2000), and as a breakdown product of the neurotransmitter NAAG (Blakely and Coyle, 1988). Numerous studies have identified decreased NAA concentrations in multiple sclerosis, stroke and tumours (Rosen and Lenkinski, 2007, Ross and Bluml, 2001). The only disease known to exhibit increased NAA levels is Canavan's disease (Matalon et al., 1988).

Glutamate (Glu) and Glutamine (Gln)

Glu is the main excitatory neurotransmitter and also the most abundant amino acid in the healthy brain. It is present in all cells, more predominantly in glutamatergic neurons and less in GABAergic neurons and astroglia. Its concentration varies between 6 and 12.5 mM with significant differences between white and gray matter.

Gln is an amino acid synthesised from Glu and is mainly located in astroglia. It also has an important role in ammonia detoxification (De Graaf, 2013).

Due to the similar chemical structures, Glu and Gln peaks overlap (between 2.05 and 2.5 ppm) in the proton spectra [Fig 4.3], resulting in difficult distinct quantification in medium magnetic field strengths, when a measure of combined concentrations of Gln and Glu is given as Glx (De Graaf, 1998, Glitz et al., 2002). For improved accuracy in individual quantification, high and ultra-high magnetic fields are needed (Mason et al., 1994, Srinivasan et al., 2006, Ugurbil et al., 2003).

$\gamma-$Aminobutyric acid (GABA)

GABA, in contrast, is the major inhibitory neurotransmitter in the central nervous system, being responsible for managing the brain excitability (Yildiz-Yesiloglu and Ankerst, 2006, Hertz, 2006). Its concentration in the brain is around 1 mM, though several factors including menstrual cycle and substance abuse are known to cause variations in those concentration levels. As for Glu and Gln, at low magnetic fields, significant coupling effects are observed. Given the low concentration of GABA in the brain and considerable spectral overlap with highly concentrated metabolites, *in-vivo* detection by proton MR spectroscopy is challenging. Sophisticated acquisition approaches are necessary for GABA quantification, such as spectral editing techniques, J-coupling or optimised sequences (Mullins et al., 2014, Napolitano et al., 2013, Duncan et al., 2014a).

Choline (Cho) compounds Choline signal, resonating at 3.2 ppm [Fig 4.3], arises

from free choline, glycerophosphorylcholine (GCP) and phosphorylcholine (PC). Hence this peak is often referred to as "total choline" (tCho) and its concentration is around 1-2 mM in the healthy brain. Levels of Cho are higher (30%) in white matter than grey matter (Kusumakar et al., 2001). Considered a potential metabolic marker of membrane density and integrity, variations in tCho concentration have been found in several pathological states such as multiple sclerosis, stroke and Alzheimer's and liver diseases. Malignant tumours, on the other hand, show an increase in Cho peak because of increased cellularity (Danielsen and Ross, 1999).

Myo-inositol (mI) mI is a cyclic sugar involved in the regulation of neuronal osmolarity and resonates at 3.5 ppm [Fig 4.3], with concentrations of 4 to 8 mM in the healthy brain. Besides its important role in osmotic regulation in the kidney, the exact function of mI is unknown. It is considered to be a glial marker and hence increased levels of mI are believed to represent glial proliferation or an increase in glial cell size, both of which may occur in inflammation (Rosen and Lenkinski, 2007, Govindaraju et al., 2000). Increases in mI concentrations have been found in Alzheimer's disease, frontotemporal dementia and HIV infection (Valk et al., 2002).

Creatine (Cre) and Phosphocreatine (PCr) Cr and PCr can be found in both neuronal and glial cells and, together with ATP, play an essential role in the energy metabolism of tissues (Castillo et al., 1996,Maheshwari et al., 2000) . The peak of Cr+PCr (often referred to as "total creatine" (tCr)) is displayed at 3.02ppm [Fig 4.3] and is very high and stable, hence often used as an internal reference for comparison. Its concentration is significantly higher on grey matter than on white matter, by approximately 20% (Ross and Bluml, 2001). Besides being used as a reference, changes in tCr levels have been observed in pathologies such as tumours and stroke (Soares and Law, 2009, Lowry et al.,

1977).

Lactate Lc peak is displayed as a doublet at 1.33ppm in the proton spectra [Fig 4.3] and is normally present at a low concentration of circa 0.5 mM in the healthy brain. Augmented Lc levels have been observed in conditions which involve failure in the aerobic oxidation mechanisms and anaerobic glycolysis takes over such as ischemia, hypoxia and seizures (López-Villegas et al., 1995).

In addition to these, several other metabolites may be studied with ^{1}H MRS, including Acetate (Ace), Aspartate (Asp) and Scyllo-inositol. However those are not covered here as they are less relevant for this thesis. In the following section, fundamentals of diffusion imaging are covered.

4.2.2. Measuring brain microstructure with diffusion MRI

Diffusion magnetic resonance imaging (dMRI) enables reconstruction of the white-matter tracts of the brain and study of brain microstructure, based on the assumption that the measured restraint of water diffusion reflects the underlying tissue structure (Mori et al., 2005). To date, this is the only technique allowing non-invasive reconstruction of white-matter structure *in-vivo*. Here only a few key basic concepts on dMRI are reviewed and only briefly, as an in-depth comprehensive description has been written as part of the MSc dissertation.

Molecular diffusion

Diffusion is a process that results from the random translational motion behaviour presented by molecules in fluids at non-zero absolute temperatures, also known as Brownian motion. The mean square of the diffusion displacement $\langle \mathbf{r}^2 \rangle$ is directly proportional to the observation time τ and the diffusion coefficient D, with D being

dependent on both the diffusion molecules fluid viscosity and temperature. In a n-dimensional space, with a homogeneous and barrier-free medium with sufficiently large number of molecules, $\langle \mathbf{r}^2 \rangle$ can be described by

$$\langle \mathbf{r}^2 \rangle = 2nD\tau, \qquad n = 1, 2, 3. \tag{4.1}$$

For the case of a homogeneous and barrier-free medium, diffusion displacement of a given molecule is equal in all directions and is classified as *isotropic* diffusion [see Figure 4.4]. In this case, one parameter is sufficient to fully describe diffusion, that being the diffusion constant D. On the other hand, in a medium where barriers are highly oriented, diffusion is highly dependent on the direction and is termed *anisotropic* [see Figure 4.4].

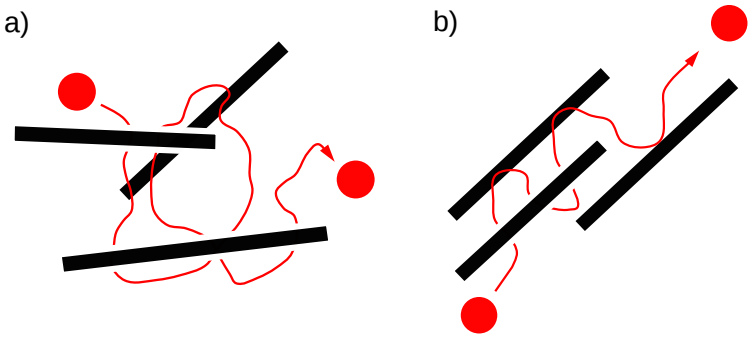


Figure 4.4.: **Example of isotropic (a) and anisotropic (b) diffusion behaviour.** *Adapted from MSc dissertation.*

In living tissues, cell membranes and macromolecules, for instance, serve as barriers to the diffusion of water molecules, which results in water molecules displace along certain preferential directions. As pictured in Figure 4.5, around neural cells, water molecules tend to displace along axons bundles, while perpendicular displacement is restricted. Conversely, although water displacement is also restricted in gray-matter tissues, there is no particular preferred axis for displacement (Beaulieu, 2002).

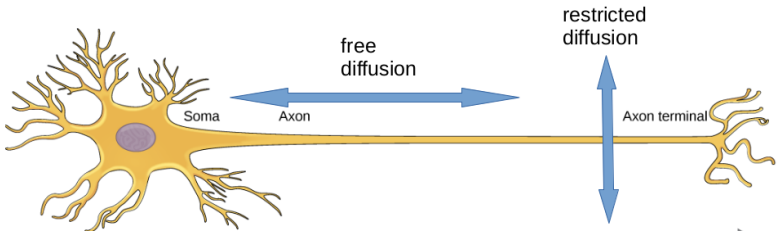


Figure 4.5.: Water diffusion behaviour around a neural cell. Adapted from *Khan Academy* (www.khanacademy.org/science/biology/human-biology/neuron-nervous-system/a/the-synapse).

Based on the diffusion behaviour of water around neural cells, it is possible to use MRI to characterise the underlying structure of such tissues.

MR Diffusion sensitization

A modified spin echo sequence is used in order to measure apparent diffusion in the human brain (Le Bihan et al., 1986). The sequence is called pulsed gradients spin echo (PGSE) (Stejskal, 1965), which uses two magnetic field gradients that are applied along a particular direction, but opposite magnitudes. At a time when the second gradient is applied causing re-phasing of spins, those protons that have significantly moved during the time interval between the two gradient pulses, will not have perfect refocus, resulting in attenuated signal to be captured by the MRI scanner.

With such measurements it is possible to characterise the diffusion coefficient D, which is also referred to as the apparent diffusion coefficient (ADC) as it reflects an observation, rather than a true measure of intrinsic diffusion. In gray-matter, where water diffusion is mostly isotropic, it is normally sufficient to describe diffusion with ADC. However, in white-matter, where diffusion is highly dependent on the tissue orientation, it is necessary to describe anisotropic diffusion. One option for doing so is to replace the scalar diffusion coefficient with a symmetric effective or apparent diffusion tensor of water, $\mathbf{D}$.

Diffusion Tensor Imaging

Under the assumption that the 3D diffusion profile in each voxel takes the shape of a symmetric ellipsoid (Basser and Jones, 2002), diffusion can be characterised using a tensor model, which replaces the scalar diffusion coefficient by the symmetric and three dimensional diffusion tensor **D**:

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{xz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} \tag{4.2}$$

In order to fully describe such tensor, at least six diffusion-weighted images along six non-collinear and non-coplanar directions are required. Higher number of directions will allow more accurate estimation of the diffusion ellipsoid.The diffusion tensor may be fully characterised by its eigenvectors $\vec{v_1}, \vec{v_2}, \vec{v_3}$ and corresponding eigenvalues $\lambda_1, \lambda_2, \lambda_3$, which give information about the main directions and intensity of diffusion within each voxel. Scalar indices have been defined for extracting meaningful characteristics such tensor, namely fractional anisotropy (FA) and mean diffusivity (MD), among others.

Fractional anisotropy represents the degree of directionality of diffusion within a voxel and it can assume values between 0 and 1. Values near 1 reflect strongly restricted diffusion, *i.e.*, highly anisotropic region; whereas FA values near to zero represent free diffusion, or nearly fully isotropic voxel.

$$FA = \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}}{\sqrt{2}\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \tag{4.3}$$

MD is the average of the three eigenvalues, describing the means diffusivity within a given region. It corresponds to ADC.

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \tag{4.4}$$

Higher axonal integrity and organisation is considered to be represented by higher FA values, due to elevated directional coherence of diffusion. Nevertheless, FA also depends on other factors, including the presence of crossing fibre tracts and axonal density. Even though FA is usually decreased in regions with crossing fibres [See Figure 4.6], in a particular fibre tract, decreased FA may be indicative of reduced restricted movement due to less axonal integrity or myelination. Thus, it may be useful to additionally explore MD, as once combined, FA and MD provide information on both the direction and magnitude of water diffusion, indicative of the organization and orientation of axonal bundles and myelination.

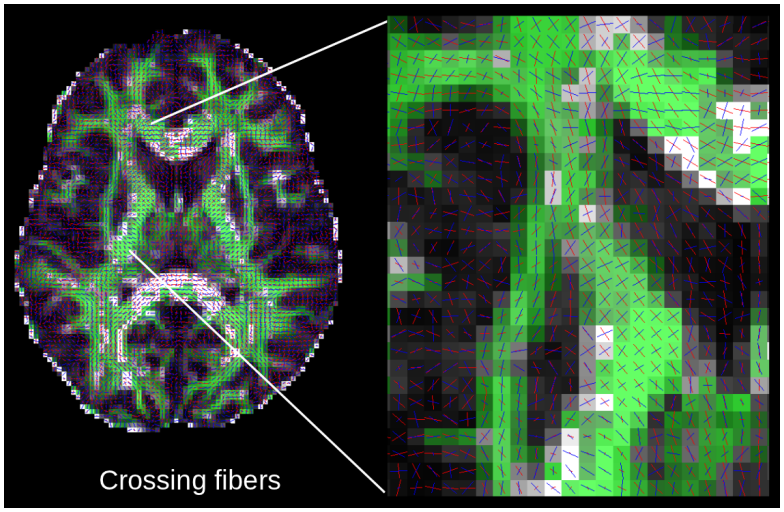


Figure 4.6.: **Example of dMRI-derived image from a healthy subject at 3T.** Two tensors have been fitted within each voxel in order to visualize the crossing fibres profile. The main eigenvectors of each tensor have been overlaid on the corresponding FA map. *Adapted from MSc dissertation.*

By estimating FA and MD it is possible to study both the orientation and integrity of white matter tracts. Typical analysis methods of such parameters may be divided between whole brain (WBA) and region-of-interest (ROI) analyses. The latter usually consists

of manually drawing masks of a fixed size and shape over a predefined region. This approach may avoid registration errors but is susceptible to user bias. The subjectivity of regions masking may also limit comparability across studies. Despite this, the ROI technique is a powerful way to investigate pre-defined cerebral areas, based on existing hypotheses.

Conversely, the WBA approach allows more exploratory analyses, and is useful when no localised hypotheses are available. It may characterise the full gray and white matter in the brain, albeit limited by quality of the challenging inter-subject diffusion image registration and the amount of spatial smoothing of data (Smith et al., 2006). An elegant way of dealing with crucial issues of cross-subject data alignment, is using tract-based spatial statistics (TBSS). TBSS is a pipeline within FSL (FMRIB's software library), which works by transforming the data from the centres of the main tracts, which are consistent across subjects, into a common space. More specifically, it projects each subject's FA maps onto an average FA skeleton before applying voxelwise statistics. TBSS is more sensitive and robust than a simple whole brain voxel-based analysis, despite its limitations regarding interpretation of differences in crossing-fibres regions (Smith et al., 2006).

4.2.3. Measuring brain function with BOLD fMRI

Measuring brain function involves capturing brain activity in consecutive temporal windows. In order to acquire information on the dynamics of activation, fast acquisition is required as well as a contrast that is sensitive to changes in brain activation.

In 1977 an imaging sequence that enabled rapid acquisitions was proposed by researchers at the University of Nottingham (Mansfield, 1977). The sequence, called

echo-planar imaging (EPI), currently allows full brain coverage with spatial resolution of a couple of millimetres in each dimension, acquired at sub-second rates.

Although active neurons generate electromagnetic signals, functional magnetic resonance imaging methods are not directly sensitive to those. Instead, they make inferences from physiological events that are associated with neuronal activity, such as blood flow or oxygen demand. The most widely used contrast for measuring brain activity is based on oxygen demand and is named blood-oxygen level dependent (BOLD) contrast.

The proof of concept of the BOLD contrast was provided by Ogawa and colleagues, after observing that blood vessel contrast in T_2* weighted images varied with changes in blood oxygen demand (Ogawa et al., 1990). Deoxygenated blood induces field inhomogeneities in surrounding tissues in the form of microscopic magnetic field gradients neighbouring the capillaries. These gradients cause acceleration in dephasing of the transverse magnetisation of the dipole moments, resulting in decreased T_2*. This occurs due to oxygenated and deoxygenated haemoglobin molecules having different magnetic properties. While oxygenated molecules are diamagnetic and therefore absent of magnetic moment and weakly repelled by magnetic fields, deoxygenated molecules are paramagnetic, meaning that its non-zero magnetic moment causes it to have a weak attraction to a magnetic field.

Whilst the precise mechanisms of the BOLD signal remain a subject of research, it can be generally summarised as follows: the augmented neuronal activity provokes an increase in blood flow and volume, accompanied by a less pronounced oxygen consumption rate. This leads to a decrease in tissue deoxygenated hemoglobin fraction and an increase in signal intensity on T_2* weighted images.

Haemodynamic response function

The shape of the BOLD response to brief neuronal activity is referred to as the haemodynamic response function (HRF). It is considered to have three main features, consisting of an initial dip, a positive overshoot and a post-stimulus undershoot Figure 4.7. The initial dip is believed to be due to oxygen extraction taking place ahead of the inflow of oxygenated blood (Malonek and Grinvald, 1996). And the post-stimulus undershoot is due to the fact that blood flow decreases more rapidly than blood volume, resulting in augmented concentrations of deoxygenated haemoglobin in regions of previous neural activity. The HRF peak lags considerably behind the peak neuronal activation, by about six seconds, decaying back to baseline after about $25s$.

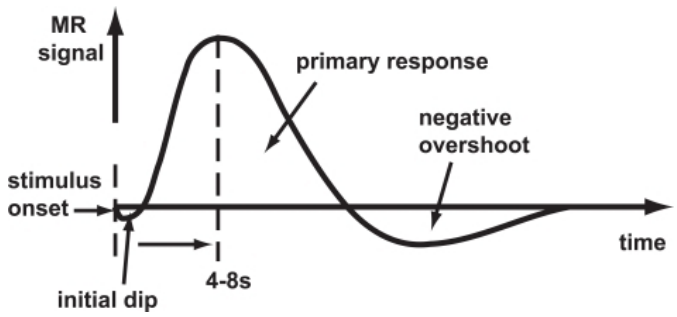


Figure 4.7.: **Illustration of the haemodynamic response function.** Reproduced from (Kornak et al., 2011).

Acquisition of BOLD FMRI

Acquisition of BOLD contrast images can be optimised by choosing the echo time that maximises BOLD contrast in the tissue of interest, which would be approximately equal to the T_2*. T_2* depends on the field strength, being shorter at stronger fields (Peters et al., 2007). At 3T, gray matter T_2* assumes values around 40 to $60ms$, depending on the cortical region (Peters et al., 2007).

One of the complications with BOLD fMRI is the potential problem of structured

noise, also known as imaging artifacts. Some of the most common types are:

- motion artifacts, connected to physical motion of the subject during acquisition;
- cardiac and respiratory activity related;
- scanner related, such as susceptibility, scanner drifts, signal inhomogeneity artifacts.

Some of these can be overcome during preprocessing, as described on the next section.

Basic preprocessing

Some of the basic preprocessing steps normally applied to BOLD fMRI data before statistical analysis are outlined below.

- **Slice-timing correction:** Given that the acquisition is done slice by slice, not all the slices within a 3D volume actually reflect the same moment in time. One simple way of compensating for these time of slice acquisition is to use linear interpolation, according to the slice acquisition order.
- **Head motion correction:** Even small, nearly imperceptible physical or apparent (scanner-drift related) movement of the head as well as pulsation effects may corrupt fMRI data by causing spatial misalignment of the 3D volumes. Correction for head movement is typically performed treating the brain as a rigid body, using simple linear transformations consisting of translations and rotations.
- **Spatial smoothing** Spatial smoothing is used for increasing the signal to noise ratio by averaging out the noise, assuming that the noise is spatially independent and the signal is smooth. In this step, the BOLD value in each voxel is replaced by a weighted average of the BOLD response in neighbouring voxels.

- **Temporal filtering** Like with spatial smoothing, temporal filtering is used for improving the signal to noise ratio. It smoothes the data at each TR across neighbouring voxels, the data being one-dimensional time series. It is used to eliminate low-frequency noise likely to be physiological artifacts or scanner drift.

- **Filtering out physiological signals** As a complement to temporal filtering, simultaneous physiological recordings can be done and filtered out from each voxel of the imaging data. One commonly used method is RETROICOR (Glover et al., 2000) and is currently part of the FSL library (FMRIB's software library).

Part III.

Brain imaging studies in painful osteoarthritis

Preamble to Part III - ImPOA: study design and protocol

Overview

Here a description of the general design and protocol of the cross-sectional study "Imaging Pain in Osteoarthritis" (ImPOA) is provided. Several researchers actively worked on running the investigation which was already ongoing when the present PhD programme started. Only a subset of data from ImPOA is included in this dissertation, namely the diffusion MRI and MR spectroscopy datasets and associated psychometric and psychophysical scores. These datasets are used in the two proceeding chapters (Chapter 5 and Chapter 6)

Introduction

ImPOA was designed to provide a multidimensional characterisation of the brain in chronic knee osteoarthritis pain. By undertaking a cross sectional approach, investigation

of neuroplasticity in chronic osteoarthritis pain was the primary general aim of the study, where brain function, neurochemistry and structure were set to be researched, in addition to contributions from behaviour, psychological and demographic factors.

Methods and Materials

All study components were conducted in accordance with the Declaration of Helsinki (Saif et al., 2000). Ethical approval was given by the Nottingham Research Ethics Committee 2 (10/H0408/115). The study was also approved by the local Research and Innovation department. Research governance documentation and approvals were dealt with by Laura Condon and Hamza Alshuft.

Participants

Individuals with chronic knee osteoarthritis pain were recruited from orthopaedic outpatient clinics at Nottingham University Hospital and from previous studies carried out within the Rheumatology department at the University of Nottingham. Additional participants were identified via general practices in Nottinghamshire and Derbyshire. Recruitment, screening and booking of participants was carried out by Maggie Wheeler and Hamza Alshuft.

Inclusion criteria for patients consisted of:

- Radiographically defined osteoarthritis changes in the knee;
- Chronic knee pain;
- Unmedicated (but over-the-counter painkillers, anti-hypertensive and diuretic drugs allowed);

- Able to give informed consent.

Pain was considered chronic if it was present during the past month, for most of the days and most part of the day, as described in (Peat et al., 2006). Exclusion criteria were as follows:

- Current pregnancy or breastfeeding;
- Metals in the body (such as pacemaker, shrapnel, iron filings, etc);
- Claustrophobia;
- Large tattoos in the head, neck or shoulder regions;
- Major medical, neurological or psychiatric conditions (such as diabetes, stroke, cancer, epilepsy, Alzheimer's disease, chronic hepatitis, chronic renal failure);
- Under 18 years of age.

Age-matched healthy individuals were included if they were free of pain, able to consent and if none of the exclusion criteria described above was met. They were recruited via posters which were advertised in public places such as libraries, community centres and hospitals waiting areas. Additionally, partners of other participants were invited to be screened into the study.

All participants underwent comprehensive verbal safety screening to ensure none of the exclusion criteria for magnetic resonance experimentation was met. Moreover, subjects taking any pain medication were asked to refrain from it during a 24-hour period prior to their research visit. All participants provided written informed consent during their visit at the Queen's Medical Centre, where they were attended to by at least one of the investigators (Maggie Wheeler, Laura Condon, Hamza Alshuft, Maryam Abaei, William Cottam and myself).

Clinical and psychometric assessment

Participants were asked to complete a series of pain and behavioural questionnaires, including an assessment of depression levels, through the Beck Depression Inventory II (BDI) (Beck et al., 1961); neuropathy, through the painDETECT questionnaire (Freynhagen et al., 2006a); anxiety, with the State-trait Anxiety Inventory (STAI) (Spielberger, 2010); pain catastrophisation, using the Pain Catastrophizing Scale (PCS) (Sullivan et al., 1995); handedness and pain severity and duration. Other questionnaires were additionally used, including assessments of cognition and suggestibility, however these will not be covered here. Questionnaires were usually sent to participants prior to their visit, by Maggie Wheeler. A brief description of each of the relevant questionnaires is given below and copies can be found in Appendix A.

The BDI (Beck et al., 1961) is a multiple-choice self-report inventory, used for measuring the severity of depression. It consists of twenty-one questions about how the individual felt over the past week. Each question has a set of at least four possible answer choices, ranging in intensity. Raw scores, out of a maximum of 63, are recorded.

The painDETECT questionnaire (Freynhagen et al., 2006a) consists of a series of questions about the characteristics of an individual's perception of pain. It has been developed and validated with the objective of identifying neuropathic elements of pain (Freynhagen et al., 2006a). The final score ranges from 0 to 39, where higher values indicate the presence of more neuropathic-like symptoms as opposed to lower scores, the latter suggesting predominance of nociceptive features.

The PCS (Sullivan et al., 1995) attempts to measure the extent to which individuals negatively misinterpret or exaggerate pain experiences. It invites participants to reflect on past painful events to indicate how they feel and what they think about when they

are in pain. It contains thirteen items, with 5-point scales each, where 0 corresponds to "not al all" and 4 corresponds to "all the time".

STAI (Spielberger, 2010) assesses levels of both state and trait anxiety. There are twenty items for each of these sub-scales. All items are rated on a 4-point scale, ranging from "Almost Never" to "Almost Always". Higher scores indicate greater anxiety.

Participants also underwent quantitative sensory testing (QST). Pressure pain thresholds (PPT) were obtained applying pressure (algometer by Somedic AB, Sweden) stimulations to the medial aspect of the patella and a site 3 cm inferior to the sternal notch. These were respectively taken as examples of PPT local and remote to the affected joint.

Finally, directly before the MRI scan session, participants rated the intensity of their spontaneous pain, using a Visual Analogue Scale (VAS) ranging from 0 to 100, where 0 was classed as "no pain" and 100 corresponded to "excruciating pain". This rating took place soon after participants walked into the clinical examination room, prior to walking to the MRI unit.

MRI acquisition

Participants underwent multi-modal MRI scan of the brain on a clinical MR scanner (General Electric, Milwaukee, WI - MR Discovery 750 Whole Body), equipped with a 32-channel head coil. Acquisition protocols included diffusion-weighted imaging, T1-weighted volumes, MR spectroscopy, arterial spin labelling and blood-oxygen level dependent fMRI at rest. Total scan duration was around one hour and most scans were performed by the radiographer Anita French.

Contributions

The ImPOA study was conducted with support from a large team of researchers who participated contributing to research governance, recruitment, data collection, or data scoring and digitalisation. I did not design the study, but helped with coordination and with several (around n=40 participants) of the later participant visits, by operating or supervising scanner procedures, as well as with QST, data digitalisation and scoring.

Analyses of datasets with available structural T1-weighted, arterial spin labelling (ASL) and resting state fMRI (rsfMRI) data have been published or is being prepared for publication in scientific journals and conferences (Alshuft et al., 2016, Cottam et al., 2016). Hamza Alshuft used the T1-weighted datasets in his PhD thesis, and William Cottam is currently writing his PhD dissertation based on the ASL and rsfMRI datasets. The only in-house analysis programs written that were available when I joined the study consisted of tools for conversion of DICOM data, developed by Paul Morgan.

Datasets with available dMRI data and MRS data were used to characterise both the cerebral microstructure, and neurotransmission profiles in chronic knee osteoarthritis pain. These will be discussed in the two chapters that follow (Chapters 5 and 6).

5

Is cerebral microstructure affected in COAP?

Overview

Here diffusion MRI tools are used to investigate whether chronic knee osteoarthritis pain is associated with neuroplastic changes, as a consequence or predisposition to chronic pain. This particular technique was chosen as it can be sensitive to neuroplasticity indicators such as changes in myelination and cell death or growth.

This work fits the overall aims of the thesis as it elucidates whether the microstructural brain reorganisation shown in other pain conditions can be also seen in chronic osteoarthritis pain. Data acquired as part of ImPOA (described in the previous chapter) is used and white matter tracts are reconstructed with a tensor model for whole brain

analysis. Additional region of interest analyses are performed focusing on key brain regions, which have been previously found abnormal in other chronic pain conditions.

It must be noted that this is an extension of the work performed during my MSc studies. A partially overlapping, but different dataset was used during the MSc programme. That dataset contained several inconsistencies on the data acquisition, which were only detected during the PhD and are addressed here.

Contributions of this chapter

- Diffusion-weighted brain volumes are acquired along thirty diffusion directions in order to characterise the brain microstructure in chronic osteoarthritis pain;
- Tract based spatial tractography is employed for whole brain white matter investigation, followed by *post-hoc* region of interest analysis of cortical and subcortical structures;
- No major structural abnormalities were detected in the chronic knee osteoarthritis pain cohort here investigated.

Publications

Contributions from this chapter have appeared in the following:

- Reckziegel, D., Dixon, J., Auer, D. P. (2014). Microstructural abnormalities related to the chronification of osteoarthritic pain: a DTI study. *Proceedings of the ISMRM Annual Meeting, p. 1901, Milan, Italy, April 2014.*

5.1. Introduction

The ability of a system to undergo structural or functional modifications, according to need and biological advantage, is refereed to as plasticity. It occurs, for instance, in neural tissues with the activity of learning (Zatorre et al., 2012) or following limb amputation (Preißler et al., 2012). Persistent pain input is also associated with sensitisation of neural networks related to pain processing due to repetitive activation of spinal nociceptors. In this case, the biological advantage is the increased sensitivity to peripheral inputs, which maximises the probability of tissue healing and minimises the risk of secondary injury (Moseley and Flor, 2012). However, over time sensitisation due to pain looses its protective role becoming chronic pain (Woolf and Mannion, 1999).

In addition to nociceptive mechanisms, central sensitisation may also contribute to chronic pain in some patient populations (Mease et al., 2011, Hochman et al., 2013). Cumulative data across different chronic pain conditions suggest changes in both grey and white matter integrity within brain regions, mainly the ones involved in perception and behavioural response to pain such as the thalamus, insula and anterior cingulate cortex (Geha et al., 2008, Ellingson et al., 2013). After reviewing the published literature using diffusion imaging to investigate chronic pain (performed during my MSc studies) (Table 5.1), it is noted that the thalamus, followed by the cingulate cortex, are the brain structures most commonly reported as abnormal across all reviewed studies. These have detected both altered regional fractional anisotropy (FA) [Fig 5.1] and mean diffusivity (MD) [Fig 5.2].

Based on these observations, the aim of this chapter was to identify potential key microstructural disruptions, underlying any adaptive or predisposing changes to the networks in the brain in response to ongoing knee osteoarthritis (OA) pain. In addition to

Table 5.1.: Population and acquisition characteristics of the reviewed studies. Only studies comparing clinical pain populations to healthy volunteers, using either TBSS or region of interest analyses, and published between July 2000 and July 2013 were included. P, patients; C, healthy controls; CP, chronic pain; TMD, temporomandibular disorder; IBS, irritable bowel syndrome; SCI, spinal cord injury; CRPS, chronic regional pain syndrome

Subject details					Acquisition			
Study	Journal	Number of subjects	Mean age±SD or (age range)	Diagnosis	Scanner	Acquisition voxel size (mm^3)	Number of directions	b value (s/mm^2)
Absinta et al., 2011	Cephalalgia	P=15 CP C=19	P=44(29-64) C=42(27-69)	Cluster headache	Philips 3T	2.5x2.5x2.3	35	900
Chen et al., 2011	Brain Research	P=10 CP C=16	P=32.8±10.4 C=29.1±7.9	IBS	GE 3T	1.875x1.875x3	23	1000
DaSilva et al., 2006	NeuroReport	P=24 CP C=12	P1=33.8±9.2 P2=36.0±7.7 C=31.0±8.0	Migraine	Siemens 3T	2x2x2	60	700
Fayed et al., 2010	Arthritis Research & Therapy	P=10 CP C=10	P=40.0±6.2 C=37.8±8.7	Fibromyalgia	GE 1.5T	2x2x3	25	1000
Frokjaer et al., 2011	Gut	P=23 CP=12 C=14	P=52(23-73) C=47(27-64)	Chronic Pancreatitis	GE 3T	2.4x2.4x2.4	32	1300
Geha et al., 2008	Neuron	P=21 CP C=21	P=39.4±2.4 C=39.2±2.4	CRPS	Siemens 3T	1.7x1.7x2 2x2x2	60	1000
Gustin et al., 2010	Cerebral Cortex	P=23 CP=12 C=45	P=43±3 C=33±2	Neuropathic SCI	Philips 3T	2x2x2.5	32	1000
Lutz et al., 2008	Arthritis & Rheumatism	P=30 CP C=30	P=54±8 C=49±7	Fibromyalgia	Siemens 1.5T	1.8x1.8x3.6	6	1000
Moayedi et al., 2012	PAIN	P=17 CP C=17	P=33.1±11.9 C=32.8±9.8	TMD	GE 3T	1.875x1.875x3	23	1000
Rocca et al., 2008	Cephalalgia	P=15 C=11	P=43.3(30-58) C=41.6(29-55)	Migraine	Philips 3T	0.94x0.94x2.5	32	1000
Sundgren et al., 2007	Academic Radiology	P=19 CP C=25	P=41.0(20-57) C=43.9(22-59)	Fibromyalgia	GE 1.5T	2x2.5x4	9	1000
Szabo et al., 2012	PAIN	P=17 CP C=17	P=34.65±10.86 C=33.27±11.32	Migraine	GE 1.5T	2.4x2.4x2.4	60	1000

explorative, whole brain analysis, regions of interest were defined based on the literature in order to compare OA pain with other types of pain. It was hypothesised that OA pain chronification is associated with microstructural abnormalities, and that these changes are related to pain duration and to the development of neuropathic-like symptoms. It was also hypothesised that findings from other musculoskeletal types of chronic pain would be replicated in OA pain.

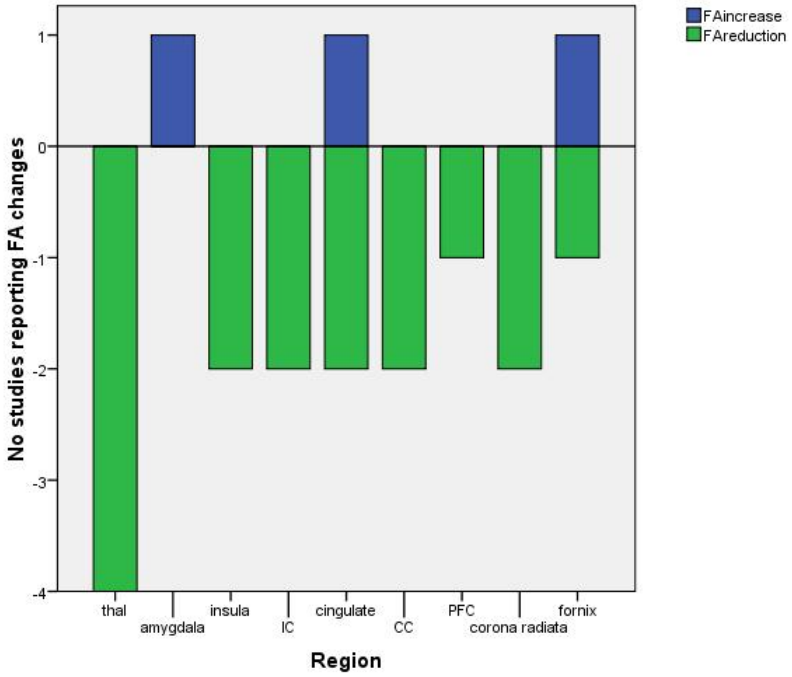


Figure 5.1.: Reported changes in regional FA values. Green represents the number of studies included in the literature review (Table 5.1) reported regional FA decreases in the brain of chronic pain patients in relation to healthy controls. Blue represents reported FA increases. IC, internal capsule; thal, thalamus; CC, corpus calossum; PFC, prefrontal cortex.

5.2. Materials and Methods

Data presented here has been collected as part of the ImPOA study which had the general design and methodology explained in the preamble of Part III. Here the focus

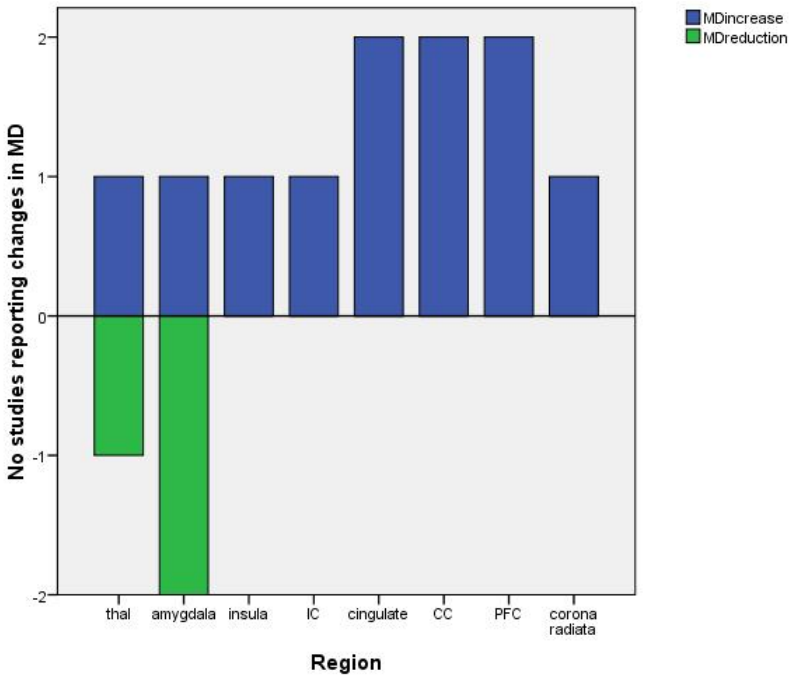


Figure 5.2.: Reported changes in regional MD values. Green represents the number of studies included in the literature review (Table 5.1) which reported regional MD decreases in the brain of chronic pain patients in relation to healthy controls. Blue represents reported MD increases.

will be on the additional specific methods and materials for this dMRI investigation. Corresponding MRS findings are presented on the next chapter [Chapter 6].

5.2.1. Diffusion-weighted image acquisition

Spin-echo echo-planar images were acquired along 30 evenly spaced and non-collinear directions with b=1000 s/mm^2, plus one non-diffusion weighted volume. Other acquisition parameters include TR=5600 ms, TE=89.0 ms, in-plane matrix resolution 102x102, field of view 224x224 mm^2, and a SENSE acceleration factor of 2.

5.2.2. Image preprocessing

All image processing was completed using the diffusion tools within the FSL package (FMRIB's software library) (Smith et al., 2004), version 5.0. Diffusion-weighted images were first corrected for head motion and eddy current artifacts (Jenkinson and Smith, 2001). With the purpose of extracting non-brain tissues from the diffusion images, a brain mask was created for each subject using FSL's brain extraction tool (BET) (Smith, 2002a). Each individual mask was later applied to all diffusion volumes. Using FSL's diffusion toolbox (FDT), the diffusion tensor was estimated in each voxel by linear regression (Basser et al., 1994) and fractional anisotropy (FA) and mean diffusivity MD were derived.

5.2.3. Quality assurance of images

Initially, all FA and MD maps were visually inspected in order to control for quality. For a more careful investigation, diffusion-weighted volumes were inspected using the video mode on the FSLview tool within FSL. Sagittal, coronal and axial views were checked.

During this step, several inconsistencies were found on the dMRI data. These included:

1. Frontal ring artifacts of seemingly insufficient or incorrect fat suppression were found (Fig 5.3) (Tournier et al., 2011);

2. Even though the acquisition resolution was 2.2x2.2x2.2 mm^3, some of the datasets presented resolutions of 0.88x0.88x0.88mm^3, 1.75x1.75x1.75mm^3;

3. Some diffusion volumes had missing axial slices, or significant signal drop on some axial slices (Fig 5.4); and

4. Localised frontal or top of head distortions (Fig 5.4).

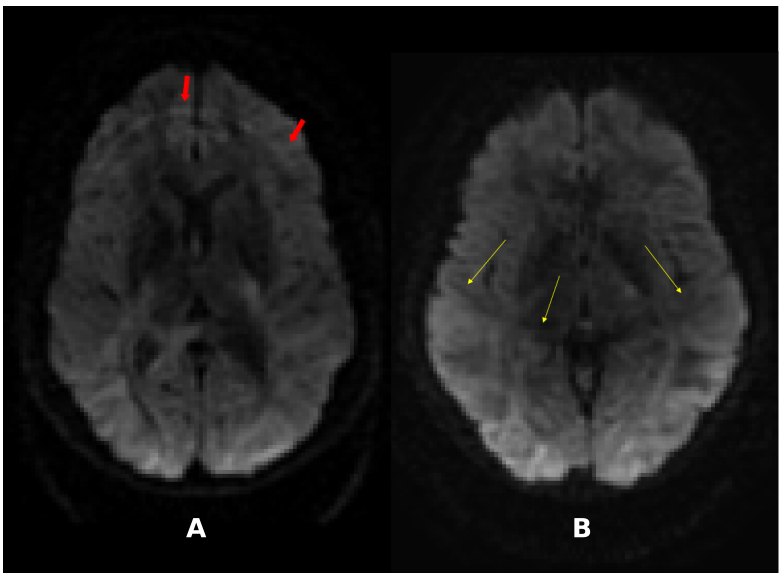


Figure 5.3.: Examples of artifacts encountered in the dMRI dataset during quality control: A. Frontal ring artifact present in some of the datasets (female healthy volunteer, 59 years old); B. Posterior portion of the brain significantly brighter than the rest of the brain (female healthy volunteer, 54 years old).

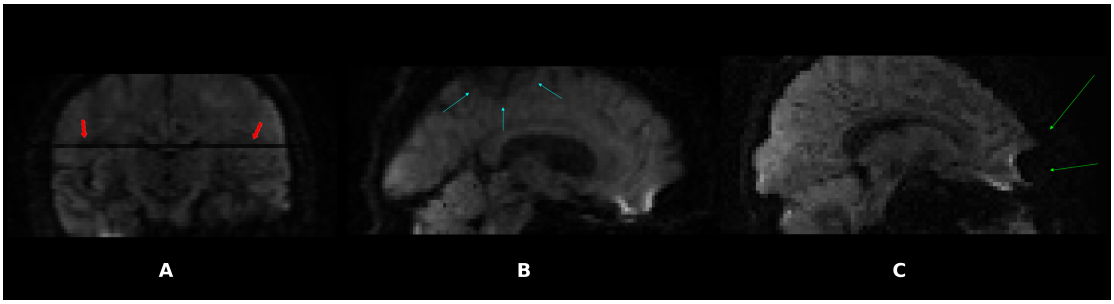


Figure 5.4.: Further examples of artifacts encountered in the dMRI dataset during quality control: A. Missing axial slice; B. top of head distortion; C. Frontal distortion.

Datasets presenting the above described artifacts were excluded from analyses. The criteria used was visual detection of the artifacts described above, as well as inconsistencies in voxel sizes, which are known to affect fibre reconstruction and derived measures (Oouchi et al., 2007). Such variability in resolutions were likely due to inconsistent choice of interpolation methods during scanning, and therefore raw data was sought but found to be unavailable. In order to minimize heterogeneity while keeping numbers to a reasonable sample size, datasets kept for analyses had voxel sizes of either 2.2 x 2.2 x 2.2 mm^3 or

1.75 x 1.75 x 2.2 mm^3, which were the most homogeneous in size.

5.2.4. Image analysis

Tract-based spatial statistics (TBSS) (Smith et al., 2006), a commonly used approach for investigating potential abnormalities in white-matter, was performed.

Individual FA data were first non-linearly aligned to the FMRIB58_FA standard space image, then registered to the MNI152 space (Montreal Neurological Institute) and, ultimately, averaged. The same spatial transformations were applied to MD maps. The resulting mean FA image was thinned to create a mean white matter tract "skeleton", which was then thresholded at FA>0.25, to include only the white-matter voxels representing the centres of all tracts common to the group. This step was performed for the whole group and separately for the OA cohort.

Each subject's aligned FA and MD maps were further projected back onto the mean FA skeleton. The voxels in the subject's FA map which lay perpendicular to the mean skeletal tracts were searched for the maximum FA value, which was therefore assigned to the corresponding skeletal voxel.

Further analysis was performed focused on the thalamus and cingulate cortex. Masks were extracted using *fslmaths* from the Harvard-Oxford cortical atlas within FSL. Results from TBSS analysis were thickened using the *tbss_fill* tool within FSL in order to ease visualisation.

5.2.5. Statistical analysis

The significance of voxel-based comparison between groups as well as correlation of dMRI indices with pain duration and painDETECT scores were tested within the

white-matter skeleton and within the thalami using a permutation-based inference tool for non-parametric statistical thresholding (randomise tool within FSL). The number of permutations was set to n=5000, with age and sex considered as covariates of no interest, given the extensive literature showing the effects these variables have on FA and MD (Pfefferbaum et al., 2000, Hsu et al., 2008). For FA analysis, voxel size was also included as a covariate because of the previously shown effect that different voxel sizes and shapes have on FA values but not on MD (Oouchi et al., 2007). The resulting statistical maps were thresholded at $p<0.05$, corrected for multiple comparisons, at a cluster level using the threshold-free cluster enhancement (TFCE) option. Results were interpreted according to the JHU diffusion-based white-matter atlases within FSLview (Mori et al., 2005, Wakana et al., 2007, Hua et al., 2008).

5.3. Results

Data from 23 right-handed OA patients (9 females, age±SD: 66±8 years, age range: 48 to 81 years) and 23 pain-free participants (14 females, 4 left-handed, age±SD: 61±7, age range: 46 to 72 years) were included in the analysis (Table 6.4). Twelve patients reported having dominant pain on their right knee, while eleven reported the left knee as most affected by pain. A number of datasets were discarded due to poor image quality ($n_{OA} = 2$), presenting frontal ring and signal dropout artifacts, as described and illustrated in the methods section, as well as large discrepancy in voxel sizes and shapes ($n_{OA} = 6$, $n_{HV} = 1$).

Even though negative affect is a common comorbidity of chronic pain, none of the participants reported more than mild to moderate levels of depression, as defined by BDI, with all participants scoring less than 25 (6.5 ± SD: 6). Reported pain duration

ranged from 1 to 30 years, with an average of eight years (mean ± SD: 8.5 ± 7 years). PainDETECT scores ranged from 1 to 25, with only 2 subjects likely presenting features of neuropathic pain, 6 were ambiguous, and the remaining majority (n=16) were classified as predominantly nociceptive.

	OA, mean±SD	HV, mean±SD
Subjects	23	23
Age	66 ± 8 yrs	61 ± 7 yrs
Sex	14 males	9 males
Pain duration	8.5 ± 7 yrs	0
PainDETECT	10.8 ± 5.5	
STAI(T)	33.2 ± 8.5	29.2 ± 6.0
PCS	16.5 ± 11.4	11.4 ± 9.1
PPT remote	260 ± 146	217 ± 90
PPT local	242 ± 118	334 ± 147
VAS	29.0 ± 28.4	-

Table 5.2.: Summary of demographic and psychometric data of participants. OA: osteoarthritis cohort; HV: healthy volunteers cohort; PPT: Pressure pain thresholds; STAI: State Trait Anxiety Inventory; PCS: Pain Catastrophizing Scale; NS: Non-significant; yrs: years.

TBSS analysis of the whole brain white-matter tracts did not detect any abnormalities in FA or MD when contrasting patients to healthy volunteers and considering age and sex as covariates of no-interest. No associations were seen between diffusion metrics and PainDETECT, pressure thresholds or anxiety scores. Voxel-based ROI analysis of the thalamus however detected significant positive correlation between MD values and pain duration within the OA group ($p<0.05$, TFCE, corrected for multiple comparisons),

considering age and sex as covariates of no interest, on the left hemisphere [Fig 5.5]. The longer patients had chronic pain, the higher the MD values within the region.

In order to investigate the subthalamic region displaying such association, the cluster was overlaid on an atlas which maps subthalamic areas according to their anatomical connectivities to cortical regions, as determined by diffusion imaging studies (Behrens et al., 2003). The procedure revealed that the thalamic region presenting elevated MD values with longer pain duration overlaps with those areas projecting to sensory, prefrontal, premotor and posterior parietal regions [Fig 5.5].

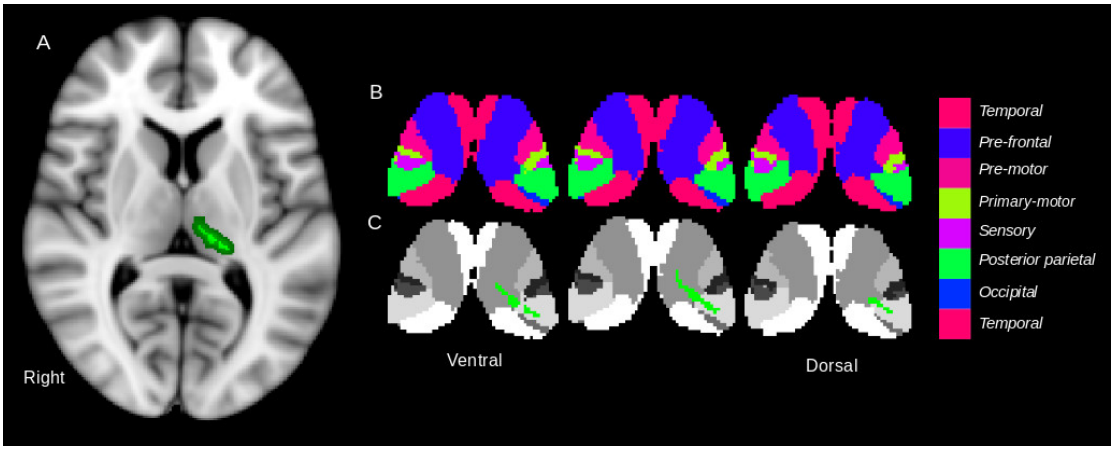


Figure 5.5.: (A) Axial slice displaying the region with positive correlation between MD and pain duration (bright green) and (B) axial slices from the thalamus, including the divisions described by Behrens and colleagues (Behrens et al., 2003), and (C) the overlay (green) of the significant cluster.

However, when plotting the mean MD values from the thalamic cluster with pain duration, it seems like the correlation may have been driven by one subject [Fig 5.6]. Following exclusion of that data point, voxel-based ROI analysis of the left thalamus was performed again and there were no correlations between pain duration and MD values that survived correction for multiple comparisons (TFCE, $p<0.05$).

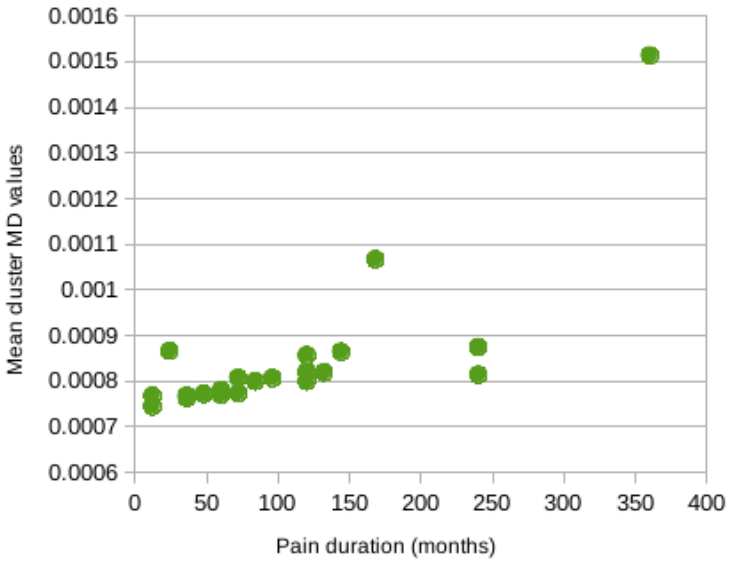


Figure 5.6.: Scatterplot showing relation between mean MD values within the left thalamic cluster displayed in Fig 5.5 with the duration of pain in months, following voxel-wise analysis on small volume.

5.4. Discussion

Using a well accepted and widely used technique for investigating the microstructure of white-matter tracts in the brain, no abnormalities were detected in subjects with chronic knee osteoarthritis pain. Voxel-wise region of interest analysis focused on the whole thalami initially detected a positive interrelation between mean diffusivity and the length of time during which pain has been present. However, following inspection of the results, it became clear that such correlation was driven by an outlier.

Higher mean diffusivity implies in less structural density and therefore the lack of association between MD and longer pain duration suggests that the progression of chronic osteoarthritis pain does not seem to relate to reductions in projections and density of neural fibres. These results go against previously published dMRI literature that reported ultra-structural cerebral fibre abnormalities in chronic pain populations. However, this may be explained by the fact that such studies investigated neuropathic types of chronic

pain (Geha et al., 2008,Ellingson et al., 2013) as opposed to the predominantly nociceptive nature of osteoarthritis pain (Ohtori et al., 2012).

It is necessary to mention however that another reason which may have contributed for non-replication of previous studies in chronic pain is the limited quality of the acquired diffusion images. The quality would have been improved using a longer TR, in order to allow for full recovery of magnetisation and therefore better signal. In addition, only one non diffusion-weighted image (b0) was obtained for each subject, when four would have been recommended for the acquisition along 30 diffusion directions.

After investigation of the problems and artifacts encountered in the data which was excluded from analysis, potential causes and solutions were identified (with support from Maryam Abaei). Firstly, the problem with varied voxel sizes was caused by inconsistent choice of interpolation method by the radiographer. After some tests, the method yielding resolution of 2.2 x 2.2 x 2.2 mm^3 was chosen and clarified to the radiographer. Secondly, the frontal ring artifact was no longer present after changing the GE fat suppression type from "fat" to "fat special" and increasing TR. The distortions were also no longer present after this. Thirdly, the scanner bridge was not well centered which caused it to tremble slightly. This was solved by the GE engineers.

Taking these details in consideration, an improved acquisition protocol was developed (with support from Paul Morgan) for use in future studies. This new protocol consisted of an increased TR, and additional three b0 images. This yielded better images however, given the increase in TR, scan time was also augmented. None of the artifacts mentioned above were found on the images using this new acquisition protocol.

Newly available techniques now allow more sophisticated inferences of microstructure, which future studies should employ. MRI systems equipped with multi-band allow

acquisition of different weighting factors under a short time. This and a higher number of diffusion directions would provide more detailed reconstruction of axonal bundles, with improved detail on crossing fibres, and additionally allow study of neurite orientation dispersion and density imaging (Zhang et al., 2012).

In conclusion, the findings in this study suggest that chronic osteoarthritis pain, a primarily nociceptive condition, is not associated with micro-structural changes. It may be that plasticity changes in this condition can only be detected at the molecular or functional levels. This is addressed in the following chapter, where the molecular level is explored in order to investigate potential associations of osteoarthritis pain and changes in neurotransmission.

6

Is there a neurotransmitter profile in COAP?

Overview

In the previous chapter, the microstructure of the brain in chronic osteoarthritis pain was studied and, differently from other chronic pain conditions such as irritable bowel syndrome, no major markers of structural plasticity were identified in association with chronic osteoarthritis pain. It may be, however, that potential abnormalities associated with such condition occur at the functional and molecular levels. The molecular mechanisms of pain chronification are not well understood and evidence from studies in fibromyalgia points to a role of prefrontal disinhibition.

This chapter presents a mechanistic investigation about the role of γ-aminobutyric

acid levels in chronic osteoarthritis pain. Quantification of GABA is performed within the anterior cingulate cortex, in a population of patients with knee osteoarthritis painand healthy controls.

Contributions of this chapter

- A PRESS sequence optimized for detection of GABA is employed to investigate anterior cingulate cortex GABA in chronic knee osteoarthritis pain;
- Results show that self-reported levels of the intensity of chronic osteoarthritis pain are inversely correlated with GABA;
- No relations were seen for negative affect and pain thresholds. The pain-GABA interrelation remained strong while controlling for depression levels.

Publications

Contributions from this chapter have appeared in the following:

- Reckziegel D, Raschke F, Cottam WJ, Auer DP (2016). Cingulate GABA levels inversely correlate with the intensity of ongoing chronic knee osteoarthritis pain, *Molecular pain*, 12, 1744806916650690.
- Reckziegel D, Raschke F, Cottam WJ, Auer DP (2015). Ongoing pain intensity is associated with cingulate GABA and NAA in osteoarthritis. *Proceedings of the ESMRMB Annual Meeting*, p.528, Edinburgh, Scotland.

6.1. Introduction

Evidence points to a key role of the anterior cingulate cortex (ACC) in the perception of pain, intensity encoding and progression of pain (Büchel et al., 2002, Seminowicz et al., 2009). This brain region is one of the most frequently reported in pain induction experiments (Duerden and Albanese, 2013, Tanasescu et al., 2016). In particular, it appears to encode pain severity in healthy subjects (Favilla et al., 2014, Brügger et al., 2012, Coghill et al., 1999, Büchel et al., 2002). Also, in chronic osteoarthritis pain (COAP), a recent arterial spin labelling study found that cerebral blood flow within the dorsal ACC correlated with reported severity of pain (Cottam et al., 2016). Other similar or glucose metabolism studies reported augmented ACC activity in COAP in contrast with healthy controls (Kulkarni et al., 2007, Howard et al., 2012).

Electrophysiological recordings in animals, in combination with ACC stimulation and electrical stimulation or chemical manipulations, suggest that the ACC exerts powerful modulation of sensory and affective components of pain, through activation of the rostral ventral medulla and periaqueductal grey (Duerden and Albanese, 2013, Apkarian et al., 2009, Senapati et al., 2005). Nevertheless the molecular basis of altered neural activity within the ACC is still to be elucidated (Zhuo et al., 2011, Koga et al., 2015, Zhuo, 2016).

As described in Chapter 4, γ-aminobutyric acid (GABA) is responsible for managing the brain's excitability (Yildiz-Yesiloglu and Ankerst, 2006, Hertz, 2006). GABA receptors are broadly spread across the spinal cord, thalamus and cortex, and are considered to modulate pain (Sivilotti and Nistri, 1991, Gwak et al., 2006, Jasmin et al., 2003). In particular, the GABA-B receptor agonist baclofen has been shown to be an effective analgesic in both preclinical models of acute and chronic pain (Gwak et al., 2006, Cleve et al., 2015), supporting a role of GABA in pain transmission.

As described in Chapter 4, *in-vivo* quantification of GABA requires sophisticated acquisition approaches, which range from spectral editing techniques to J-coupling, or optimised sequences (Mullins et al., 2014, Napolitano et al., 2013, Duncan et al., 2014a).

A search of the literature performed on PubMed and Google scholar indicates that, by February 2016, there were 28 peer reviewed MRS studies published comparing human chronic pain conditions to the healthy state. Table 6.1 summarizes these studies in terms of methods, study samples and regions investigated. Only a small number of such studies have employed appropriate techniques for GABA detection at sufficiently strong magnetic field strengths ($\geq 3T$ or higher) (Foerster et al., 2015, Foerster et al., 2012, Henderson et al., 2013, Petrou et al., 2012). In summary, decreased GABA/Cr has been detected in the thalamus of painful trigeminal neuralgia patients (Henderson et al., 2013), while in fibromyalgia and diabetic neuropathy insular GABA levels have been found to be reduced (Petrou et al., 2012, Foerster et al., 2012). Also in fibromyalgia, GABA has been found interrelated to pain sensitivity in the posterior insula (Foerster et al., 2012) and negatively related with the severity of perceived pain in the ACC in a small group of patients (n=10) (Foerster et al., 2015). Thermal pain has been shown to cause GABA reductions in the occipital cortex but no alterations have been observed in the mACC in healthy volunteers (Cleve et al., 2015). On the other hand, increased GABA has been detected in the rostral ACC in healthy controls (Kupers et al., 2009). Altogether these findings suggest that GABA may play a modulatory role in pain perception, being region- and condition-dependent, and may underpin chronification of pain.

In fact, reduced ACC GABA could potentially be a common mechanism of pain progression across different pain aetiologies. GABA levels have been previously found to predict BOLD responsiveness in a range of brain regions and networks (Duncan et al.,

2014b, Northoff et al., 2007), which may therefore account for enhanced ACC activity in chronic pain conditions.

Here it is hypothesized that low ACC GABA is a molecular marker of severity of COAP. More specifically, it is hypothesized that individuals with chronic osteoarthritis pain present decreased levels of GABA within the ACC, and that these levels are correlated with the perceived intensity of chronic knee osteoarthritis pain.

In order to test this hypothesis, GABA levels in the mACC of individuals with chronic nociceptive knee OA pain and pain-free controls are investigated. An optimised point resolved MR spectroscopy sequence (PRESS) for GABA detection is utilised. This acquisition does not require special editing techniques and is less sensitive to macromolecule contamination than Mescher-Garwood (MEGA) PRESS. It also shows good reproducibility at $3T$ (Napolitano et al., 2013, Raschke et al., 2015).

6.2. Methods and Materials

Similarly to the previous chapter, data presented here has been collected as part of the ImPOA study whose general design and methodology has been described in the preamble of Part III. Beyond the general set-up, specific details regarding MRS methods are further expanded here when necessary.

6.2.1. Participants

There were 39 spectroscopy datasets available, including 20 patients (age 67 ± 9 years, 11 males) and 19 controls (age 59 ± 9 years, 7 males). These groups were not matched for age and therefore age-matched subgroup analyses are additionally

Table 6.1.: Summary of previous MRS studies on chronic pain. Featured in bold are the studies that used specific techniques for GABA detection

First author, year	Journal	Sample	nP per nC	Method	Regions
(Grachev et al., 2000)	Pain	Back pain	12 per 16	1.5T GE STEAM	DLPFC, CC, Ins, OFC, Tha, VC
(Pattany et al., 2002)	AJNR	SCI	7+9 per 10	1.5T STEAM	Thal
(Grachev et al., 2002)	Brain & Cog	CRPS	1 per 10	1.5T GE STEAM	DLPFC, CC, OFC, Thal
(Grachev et al., 2003)	J Neural Trans	Back pain	10 per 10	1.5T GE STEAM	DLPFC, ACC, OFC, Thal
(Fukui et al., 2006)	Mag Res Im	Neuropathic pain?	9 per 14	1.5T GE PRESS	Thal
(Siddall et al., 2006)	Anest & Analgesia	Back pain	9 per 14	1.5T GE STEAM	PFC, ACC, Thal
(Harris et al., 2008)	Arthr & Rheum	Fibromyalgia	10 pre & post	3T GE PRESS	aIns, pIns
(Sorensen et al., 2008)	Diabetes Care	Neuropathic	14+12 per 18	1.5T	DLPFC, ACC, Thal
(Harris et al., 2009b)	Arthr & Rheum	Fibromyalgia	19 per 14	3T GE PRESS	aIns, pIns
(Wood et al., 2009)	J Pain	Fibromyalgia	16 per 8	3T GE PRESS	Hipp
(Prescot et al., 2009)	Mol Pain	Migraine	10 per 8	4T Varian PRESS	ACC, Ins
(Fayed et al., 2010)	Arth Res & Th	Fibromyalgia	10 per 10	1.5T GE PRESS	Hipp, PG, SMC, Thal
(Stanwell et al., 2010)	Neuroimage	SCI	5 + 5 per 10	3T Siemens STEM	ACC, PFC, Thal
(Valdés et al., 2010)	Arth & Rheum	Fibromyalgia	28 per 24	1.5T GE PRESS	amyg, thal, PFC
(Feraco et al., 2011)	AJNR	Fibromyalgia	12 per	3T GE PRESS	VLPFC, Thal
(Niddam et al., 2011)	Am J Gastr	IBS	15 per 15	3T Siemens PRESS	Hipp
(Gussew et al., 2011)	Neuroimage	Back pain	10 per 10	3T Siemens PRESS	ACC, aIns, Thal
(Sharma et al., 2011)	J Pain Res	Back pain	11 per 11	3T Siemens PRESS	SSC
(Gustin et al., 2011)	J Neurosci	TNP, TMD	20+21 per 31+30	3T Philips PRESS	Thal
(Shigemura et al., 2012)	Bone Joint Res	Osteoarthritis	9 per 9	3T GE	Thal
(Sharma et al., 2012)	Brain Sciences	Back pain	19 per 14	3T Siemens PRESS	M1
(Gerstner et al., 2012)	J Dent Res	TMD	11 per 11	3T GE PRESS	pIns
(Petrou et al., 2012)	**Acad Rad**	**Diabetes**	**7 per 7**	**3T Philips MEGAPRESS**	**ACC, aIns, pIns, Thal**
(Foerster et al., 2012)	**Arthr & Rheum**	**Fibromyalgia**	**16 per 17**	**3T Philips MEGAPRESS**	**aIns, pIns, ACC, occ**
(Henderson et al., 2013)	**J Neurosc**	**PTN**	**23 per 43**	**3T Philips MEGAPRESS**	**Thal**
(Widerström-Noga et al., 2013)	Pain	SCI	16+24+14 per 22	3T Siemens PRESS	ACC
(Foerster et al., 2012)	**Arthr & Rheum**	**Fibromyalgia**	**16 per 17**	**3T Philips MEGAPRESS**	**aIns, pIns, ACC, occ**
(Foerster et al., 2015)	**Arthr & Rheum**	**Fibromyalgia**	**12 sham, 12 tDCS**	**3T Philips MEGAPRESS**	**ACC, aIns, pIns, Thal**

performed and reported here.

Pain duration ranged from 1 to 24 years (mean±SD, 7.7±4.9 years). Even though OA pain is largely considered nociceptive, in order to exclude putative neuropathic pain phenotypes, patients with likely neuropathic-like syndromes were excluded based on the PainDETECT screening tool (score 19 or higher) (Freynhagen et al., 2006a).

6.2.2. Data Acquisition

Axial T1-weighted images were obtained using a fast spoiled gradient echo (FSPGR) sequence, with TE= 3.18ms, TR= 8.2ms, in-plane matrix: 256x256, voxel size 1x1x1 mm^3 and a scan time of 4 minute. Spectra from a voxel in the anterior cingulate cortex (ACC) were collected using an optimised PRESS sequence for GABA detection (Napolitano et al., 2013) with TE= 105ms, TE1= 15ms, TR=2.5s, 128 averages, 16 water unsuppressed averages for water scaling and a scan time of 6 minutes.

Spectroscopic voxel was placed in the mACC on the axial and coronal views, parallel to the body of the corpus callosum on the sagittal view [see Fig 6.1 for visualisation of the VOI], with typical volume of 12ml, even though in eight cases the voxel volume varied between 8ml and 10ml. The variation in VOI sizes was accidental and these cases were kept in the analyses as volume did not correlate with metabolite concentrations, nor any of the measures of interest.

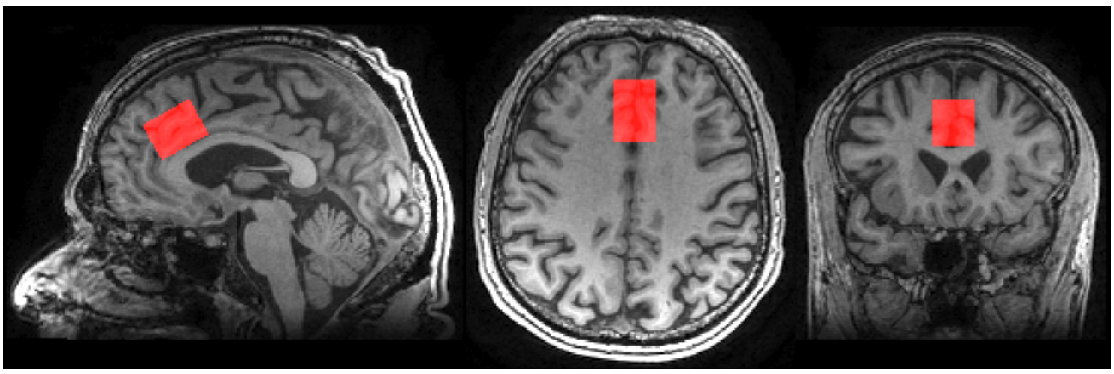


Figure 6.1.: **MRS volume of interest (red) placement in the mACC**, overlaid on anatomical T1-weighted image. *Reproduced from (Reckziegel et al., 2016).*

6.2.3. Data Analysis

Spectroscopy data were processed using MATLAB (MathWorks Inc., Natick, MA, USA) scripts written by Felix Raschke. For each individual, the 128 spectra were averaged by the scanner in sets of 16 spectra, equivalent to eight phase cycles. To correct for motion-induced frequency drift, resulting spectra from each participant were realigned to the N-acetylaspartate (NAA) peak by fitting a Lorenzian line shape.

Metabolite quantities were estimated using LCModel v6.3 (Provencher, 1993, Provencher, 2011) and a simulated basis dataset (Napolitano et al., 2013). The unsuppressed water signal was used for quantification. The minimal standard deviation of the fitting was estimated using the Cramér-Rao Lower Bound, $CRLB$, expressed as the percentage of estimated metabolite levels.

Despite $CRLB < 20\%$ being a common criterion for quality control of MR spectroscopy data, it has been recently argued that such filtering may lead to erroneous statistical findings, particularly when examining conditions associated with reduced GABA (Tisell et al., 2013, Kreis, 2016). Thus, instead of employing

this criterion, the full width at half maximum (FWHM) of the water peak was used. Datasets not meeting $FWHM < 0.078ppm$ ($< 10Hz$) were not included in the analysis.

LCModel metabolite quantifications were corrected according to water concentrations in gray matter (GM), white matter (WM) and cerebral spinal fluid (CSF) (Ernst et al., 1993), using the following procedure. SPM8 (Statistical Parametric Mapping version 8, London, UK) was used to segment the structural images into GM, WM and CSF; a mask corresponding to the volume of interest (VOI) was created using an in-house Matlab script, so that the fractions corresponding to GM (f_{gm}), WM (f_{wm}) and CSF (f_{csf}) could be computed; Based on this, the corrected water concentration ($WCONC_{corr}$) for each individual VOI was estimated according to the following relation (Provencher, 2011)

$$WCONC_{corr} = 43300 f_{gm} + 35880 f_{wm} + 55556 f_{csf}$$

Metabolite concentrations can be assumed null within CSF (McLean et al., 2000), and therefore a partial volume correction factor can be added to the above equation:

$$WCONC_{corr} = \frac{43300 f_{gm} + 35880 f_{wm} + 55556 f_{csf}}{(1 - f_{csf})}$$

By default, LCModel sets the water concentration reference to $35880mM$, which is equivalent to pure WM. For that reason, default LCModel metabolite outputs

M_{LCM} were corrected for the newly calculated $WCONC_{corr}$ as

$$M_{corr} = \frac{M_{LCM}.WCONC_{corr}}{35880}$$

No correction for relaxation times was performed, thus metabolite levels are reported in arbitrary units. See Figure 6.2 for an example of spectrum fitting for a healthy subject.

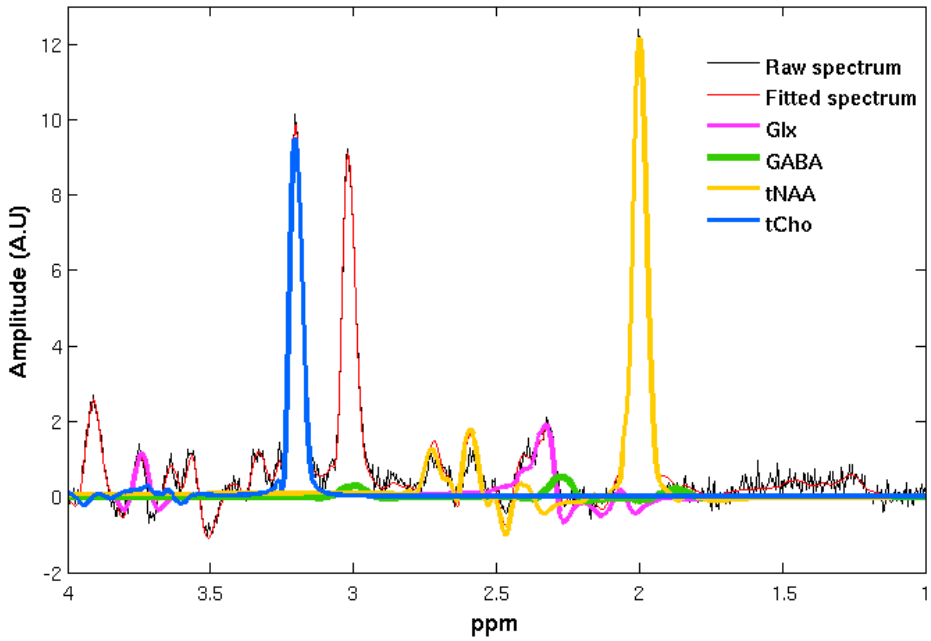


Figure 6.2.: **LCModel spectrum fit from the ACC of a healthy subject.** In highlight are some of the major metabolites: tNAA, total N-acetyl-asparatate (yellow); GABA (green); tCho, total choline-containing moieties (blue); Glx, total glutamate and glutamine (purple). AU, arbitrary units; ppm, parts per million. Adapted from (Reckziegel et al., 2016).

6.3. Statistical analysis

Statistical tests were carried out with IBM SPSS Statistics 22. Independent samples t-tests ($p < 0.05$ set a priori as significant) were used to test for differences in neurochemical levels between patients and controls. A second such test was undertaken after exclusion of three patients and three healthy participants in order to improve age matching ($n = 14$ vs. 14), despite the previously shown lack of age and sex differences in ACC GABA (Aufhaus et al., 2013). To test for correlations, Pearson's product moment correlation coefficient, r, was used for continuous, approximately normally distributed variables. Spearman's rank correlation method, ρ, was used otherwise, with values of $p < 0.05$ considered statistically significant. For potential confounding factors, partial correlation tests were additionally performed.

6.4. Results

A total of five datasets were excluded from analysis due to the following reasons. Two did not meet the FWHM<10Hz criterion; three were corrupted by movement, presenting major deviation of the VOI from planned location relative to structural scan. Data from 17 COAP individuals (11 males, age range: 48 to 83 years) and 17 healthy controls (7 males, age range: 43 to 72 years) were kept for analyses.

Subjects included all presented $CRLB$ below 25% for GABA, NAA, NAA plus N-acetylaspartylglutamate (NAA plus NAAG), glutamate (Glu), Glx (Glu and glutamine combined), myo - inositol (Ins), and total choline (tCho). No differences

in VOI tissue proportions were seen between patients and controls (main group and age-matched subgroups). Additionally, GABA did not correlate with tissue percentages.

Patients presented higher depression scores than healthy controls, as measured by BDI ($t = 3.1, p < 0.005$). Anxiety (state and trait), pain catastrophising, and pressure pain thresholds (PPT) did not differ between groups. COAP severity ratings measured by VAS, just before the scan, ranged from 0 to 80 in patients (mean±SD: 31 ± 28).

Cingulate GABA did not differ between groups (mean±SD, 4.13 ± 0.75 arbitrary units (a.u.) for patients, 4.25 ± 1.16 a.u. for controls), nor did Glx (mean ± SD, 16.46 ± 2.8 a.u. for patients, 16.99 ± 1.96 a.u. for controls), even after age matching of groups through exclusion of three patients (n=14, age range 48 to 77 years, mean: 64 years) and three controls (n=14, age range 51 to 72 years, mean: 62 years) (Table 6.4). Similarly, there were no group differences in other test metabolite levels (Table 6.4).

	OA, mean±SD	HV, mean±SD	t-test
Subjects	14	14	-
Age	64.1 ± 7.4	62.0 ± 6.6	NS
Sex	9 males	5 males	-
Pain duration	7.7 ± 5.1 yrs	0	-
BDI	6.2 ± 5.0	1.3 ± 1.6	$t = 3.4, p < 0.005$
STAI(S)	31.1 ± 9.7	25.0 ± 6.4	NS
STAI(T)	33.2 ± 8.5	29.2 ± 6.0	NS
PCS	16.5 ± 11.4	11.4 ± 9.1	NS
PPT remote	260 ± 146	217 ± 90	NS
PPT local	242 ± 118	334 ± 147	NS
VAS	29.0 ± 28.4	-	-

Table 6.2.: Demographic and psychometric data of participants. [PPT]=KPa; OA: osteoarthritis cohort; HV: healthy volunteers cohort; BDI: Beck Depression Inventory II; PPT: Pressure pain thresholds; STAI: State Trait Anxiety Inventory; PCS: Pain Catastrophising Scale; NS: Non-significant; yrs: years.

Metabolite	OA, mean±SD	HV, mean±SD	t-test
GABA	4.26 ± 0.76	4.28 ± 1.21	NS
Glx	17.11 ± 2.70	17.08 ± 2.11	NS
NAA	25.87 ± 2.67	26.78 ± 3.04	NS
tCho	5.99 ± 1.23	5.91 ± 0.73	NS
mI	11.67 ± 2.02	12.01 ± 1.4	NS

Table 6.3.: Metabolite levels per group, given in arbitrary units. OA: osteoarthritis cohort; HV: healthy volunteers cohort.

There was a strong negative association of COAP severity ratings with mACC GABA ($r = -0.758, p < 0.001$) (Figure 6.3), as opposed to Glx (Table 6.4). Depression, catastrophising, and anxiety scores, as well as PPT, did not relate to GABA (Table 6.4). The GABA-severity correlation was further verified with Spearman correlation ($\rho = -0.708, p < 0.005$), as COAP severity scores were only approximately normally distributed.

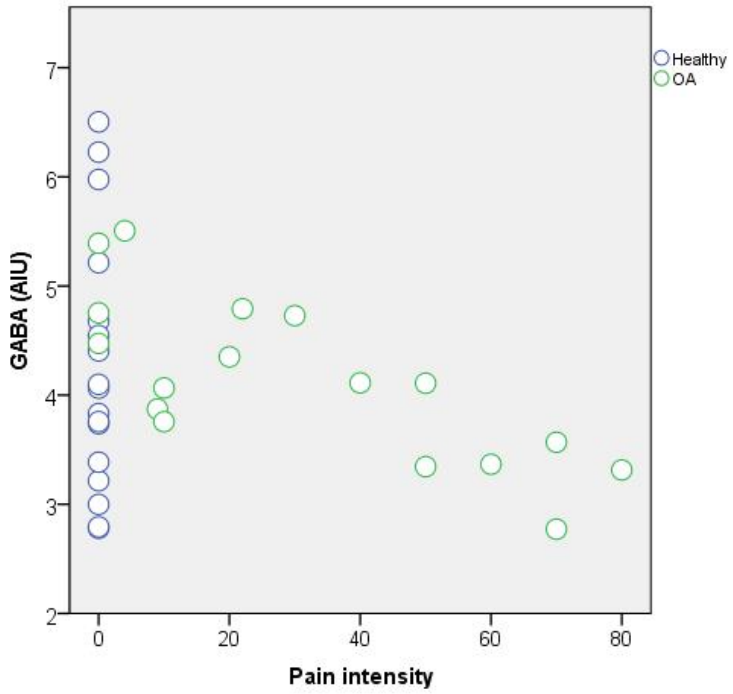


Figure 6.3.: **mACC GABA levels relation to reported severity of chronic osteoarthritis pain. Osteoarthritis group (green) and healthy controls (blue).** AIU, arbitrary units

In order to verify potential confounds, GABA correlation with age was checked but no significant relation was seen. Age and gender also did not significantly affect the GABA correlation with COAP severity ($r = -0.711, p < 0.005$). Similarly, the correlation was kept when controlling for depression scores ($r = -0.820, p < 0.001$)

(Table 6.4).

Additionally, given that the variance in GABA levels was higher in the control group, we further investigated potential contributions from variation in voxel sizes. However, voxel volume did not relate to GABA levels in any of the two groups, while both groups contained variations in voxel sizes: 3 control cases and 5 OA. Voxel size was also not associated with pain intensity and did not affect the GABA-pain correlation when included as a nuisance variable (Table 6.4).

Variable 1	Variable 2	Controlling for	Corr (OA)	Corr (HV)	Corr (all)
GABA	VAS	-	$r = -0.758, p < 0.001$	-	-
GABA	VAS	Volume	$r = -0.739, p < 0.005$	-	-
GABA	VAS	BDI	$r = -0.820, p < 0.001$	-	-
GABA	VAS	Age	$r = -0.712, p < 0.005$	-	-
GABA	VAS	Sex	$r = -0.757, p < 0.005$	-	-
GABA	VAS	Age, sex	$r = -0.711, p < 0.005$	-	-
Glx	VAS	-	NS	-	-
Glx	VAS	-	NS	-	-
Glx	VAS	-	NS	-	-
GABA	PCS	-	NS	NS	NS
GABA	PPT	-	NS	NS	NS
GABA	BDI	-	NS	NS	NS
GABA	STAI	-	NS	NS	NS

Table 6.4.: Correlation tests for OA ($n = 17$) and HV ($n = 17$) cohorts and all ($n = 34$).

6.5. Discussion

Here it was found that GABA levels in the mid-ACC were negatively correlated with perceived clinical pain intensity in chronic knee osteoarthritis pain. This association was unrelated to negative affect.

COAP individuals reporting higher intensity of knee pain presented the lower levels of mACC GABA. This is indicative of more ACC disinhibition in patients with more severe levels of pain. Given that the mACC constitutes a hub of the

salience network (Seeley et al., 2007), low GABA tone would yield augmented salience network activity. This could additionally explain enhanced pain perception, as previously shown in COAP (Suokas et al., 2012), and could offer a molecular mechanism of pain progression.

Such mACC GABA relation with reported pain intensity has been also recently seen in individuals with fibromyalgia (Foerster et al., 2015). Fibromyalgia pain and COAP however are described distinctively. While fibromyalgia is considered primarily a central pain disorder, with common comorbidities and arguable classification as neuropathic pain (Üçeyler et al., 2013, Martinez-Lavin, 2013), COAP is classified as predominant nociceptive pain disorder in spite of considerable evidence for development of central pain components and neuropathic features in a subgroup of around 5% of patients (Ohtori et al., 2012). In order to account for this, patients with neuropathic features, based on PainDETECT (Freynhagen et al., 2006b), were excluded from analysis, and an additional exploration on whether negative affect was modulating the observed association of GABA and pain severity, was undertaken. Controlling for depression levels did not significantly affect the correlation, but resulted in augmented mutually explained variance (67%). Therefore, the independent and strong association points out to it being not a reflection of comorbid negative affect but more likely a feature of central pain augmentation. Additionally, the close similarity with findings in fibromyalgia suggests shared mechanisms between a predominately nociceptive and a primarily central pain disorder.

The results found here further support fMRI and PET literature pointing out

to certain parts of the ACC as encoding pain severity during pain stimulation in healthy volunteers (Büchel et al., 2002, Coghill et al., 1999, Brügger et al., 2012, Favilla et al., 2014, Straube et al., 2009). Specifically in COAP, it has been demonstrated that blood flow within the dorsal ACC was related to the levels of reported intensity of ongoing pain (Cottam et al., 2016). Collectively, these findings suggest the possibility of mACC GABA levels indexing the pain state in inverse relation.

The methods employed in this study do not allow identification of the precise responsible mechanisms for the GABA association with pain intensity seen in COAP, which could be a result of changes in presynaptic terminals, synaptic vesicles, or in GABA uptake mechanisms (Stagg et al., 2011). Nevertheless, the association is in accordance with functional neuroimaging evidence in OA.

Previous studies have found inverse correlations of GABA levels with BOLD activity, both during task and rest conditions, locally and at network level (Duncan et al., 2014b). One such study, in particular, found that both GABA and Glu in the posteromedial cortex, which constitutes a node of the default mode network, predicted intrinsic functional connectivity of the network (positive and negative correlations with GABA and Glu, respectively) (Kapogiannis et al., 2013). Given that activity within the default-mode and salience networks are mostly anticorrelated, low GABA may be related with upregulated salience network activity. Supporting this idea, several functional studies indicate aberrant salience network activity in chronic pain conditions (Borsook et al., 2013). Further investigation is required to elucidate whether mACC GABA predicts salience network activity

and if such network upregulation may mediate the association with chronic pain.

It is noteworthy that no alterations, nor associations with pain characteristics, were seen with Glx. Given that neural activity may be modulated by both GABA and glutamate, findings from functional studies showing augmented ACC activity in chronic pain conditions could be mediated by not only inhibitory but excitatory neurotransmission. However, the lack of Glx associations with pain intensity found here is indicative that pain augmentation in nociceptive chronic knee osteoarthritis pain is predominantly related to GABAergic disinhibition. This would be in partial disagreement with the literature on animal research, where glutamatergic neurotransmission has a established role in the development of neuropathic pain (Latremoliere and Woolf, 2009). Additionally, pain-like behaviour in animals was inhibited following blockage of excitatory neurotransmission in the ACC (Wei et al., 2002). In human research, there is also some recent evidence of augmented glutamate levels within the healthy ACC in response to thermal stimulation (Cleve et al., 2015).

In line with this, a study using transcranial direct current stimulation (tDCS) in fibromyalgia patients detected ACC Glx modulation following active tDCS. The same study also found association between ACC Glx levels and pain response (Foerster et al., 2015), even though abnormalities of ACC Glx levels have not been reported in fibromyalgia (Foerster et al., 2012). Notably, a number of studies in chronic pain conditions also found concomitant inverse relation in insular Glx and GABA concentrations, including Petrou et al in diabetic neuropathy (Petrou et al., 2012), or only decreased insular GABA, as found in fibromyalgia

individuals (Foerster et al., 2012). Collectively, these findings raise the possibility of augmented insular Glx/GABA ratio as an underlying mechanism in some clinical pain conditions (Harris and Clauw, 2012). In the COAP cohort here investigated, no Glx/GABA abnormalities were seen in the mACC, suggesting that such ratio imbalance is not present in COAP. This is indicative of a less prominent role of mACC excitation in nociceptive pain augmentation.

Taken together, findings here are suggestive of a predominant role of GABA induced disinhibition and apparent lack of enhanced excitatory activity, as indexed by combined levels of glutamate and glutamine. This indicates a promising role for mACC GABA as a mechanistic biomarker of nociceptive COAP. The rising availability of 3T MRI scanners with dedicated GABA detection sequences underline the potential of mACC GABA as novel molecular pain biomarker, with particular relevance for evaluation of GABAergic modulation in pain relief. Furthermore, the feasibility to non-invasively measure mACC GABA provides unique opportunities to re-evaluate the mechanistic efficacy of pharmacological GABA modulation in the search of novel, more potent analgesics.

There are a number of limitations to the methodology of the present experiment that should be considered. First, a relatively small sample size was investigated, even though it is larger than previously published GABA investigations of pain. Second, the PRESS sequence utilised here is an unedited sequence and therefore it is not as specific as MEGA-PRESS (Mullins et al., 2014). Additionally, the optimised PRESS sequence is not the standard choice of the research community, which tends to use MEGA-PRESS instead, making quantitative comparisons with

the GABA literature less straightforward. However, the present method provides GABA measurements with less contributions from macromolecules, requires less acquisition time and is less susceptible to subject motion (Napolitano et al., 2013, Raschke et al., 2015). Importantly, the healthy group presented higher variance in GABA than the patient population. This was likely due to additional uncontrolled factors in healthy controls, due to the small sample size, as it was unrelated to the variation in VOI sizes. Finally, the large VOI used may have hidden potential group differences. Further studies, preferably performed at higher field strengths such as 7T, may be advantageous, not only to allow reduction of voxel sizes, given the increased signal to noise ratio and, therefore, allow disclosure of more region-specific neurochemical information, but also to allow measurements of multiple voxels.

In summary, findings here further support the role of the mACC in chronic pain, highlighting low GABA as an underlying molecular mechanism underpinning chronic pain. The strong inverse association of GABA with COAP intensity was not dependent on depression nor anxiety levels, and supports the use of GABA as a biomarker for mechanistic evaluation of GABAergic modulation for pain relief in COAP.

Part IV.

Mechanistic functional brain imaging of pain relief in osteoarthritis

7

Trial design and methods development

Overview

Despite substantial advances in elucidating the underlying neurobiology of chronic pain in the past two decades since neuroimaging of pain started, there is still a major gap in translating these efforts into more meaningful treatments. Studies, such as the work presented in the previous chapters, are limited by their cross-sectional setting. This means that one can only infer associations between measurements and chronic pain, without information on causal relations. In order to capture causal mechanisms and validate biomarkers, intervention studies are necessary.

It is still unclear why some patients respond to certain drugs while others do not. Pinpointing specific mechanisms involved in analgesia while segregating those from other drug effects, may provide valuable information to the understanding of pain relief mechanisms. This has the potential to bring the field of pain research closer to having a significant impact on alleviation of chronic pain.

Duloxetine is one such drug from which only around $50 - 60\%$ of individuals with COAP receive satisfactory pain relief (Chappell et al., 2011). The fact that around half of the patients receive analgesia and half do not, makes this novel antidepressant a particularly ideal candidate for study of mechanisms of pain alleviation as it allows direct comparison between nearly equal numbers of responders and non-responders. Moreover, it has less strong side effects than most drugs used to treat chronic pain. The study presented here has two main objectives. The first consists of identifying functional changes in brain signature indexing the analgesic mechanisms of duloxetine. The second concerns identification of brain imaging and psychophysical markers that predict the response to duloxetine treatment.

The initial general proposal was suggested by Dorothee Auer, and discussed with David Walsh as a cross-over placebo controlled study involving 30 individuals with COAP, and 30 healthy volunteers for baseline comparison. With some initial support from Naranjargal Dashdorj (who reviewed the literature), that was the start point of the piece of work presented here. Following advice from external review by the scientific advisory board of the Arthritis Research Pain Centre, an opioid agonist was requested to be introduced to the study and the design was

reshaped a number of times. Due to addiction issues related to opioid use, the short acting opioid agonist remifentanil was chosen because a short intra-venous infusion of the drug would be sufficient for investigation of its neural effects. Upon numerous discussions and review of literature between Dorothee Auer and myself, the research design was slowly taking shape. Support was sought from a specialists, including a clinical research pharmacist (Roger Knaggs), an anaesthetist (Ravi Mahajan) and a statistician (Christopher Tench). All these collaborators provided important input on their area of expertise, which contributed to the final research protocol.

This chapter presents the complex methods-development, setup and trial governance required in order to run the *Imaging Pain Relief in Osteoarthritis* study. The work described here was led and performed by me. A team of researchers provided support with recruitment, data collection and communication with participants and these are respectively acknowledged throughout the chapter. The trial has been registered on clinicaltrials.gov (identifier: NCT02208778) prior to recruitment.

Contributions of this chapter

- New state-of-the-art psychophysical testing protocols were developed, which now have been transferred and integrated into numerous further studies from the Arthritis Research UK (ARUK) Pain Centre;
- Pain induction fMRI protocols were developed and translated onto further studies;

- Two analgesia studies using the setting of randomised controlled trials were established;
- New recruitment routes were set, and a database was created containing contact details of people interested in taking part in future studies within the Arthritis Research UK Pain Centre;
- A Patient and Public Involvement group was formed.

Publications

Contributions from this chapter have appeared in the following:

- D Reckziegel, HS Bailey, WJ Cottam, CR Tench, RP Mahajan, DA Walsh, RD Knaggs, DP Auer (2017). Imaging Pain Relief in Osteoarthritis (IPRO): protocol of a double blind randomised controlled mechanistic study assessing pain relief and prediction of duloxetine treatment outcome, *BMJopen*. Accepted.

Press release

- Clinical trial explores new ways to help chronic pain sufferers - NUH communications, Arthritis UK and local newspapers.

7.1. Introduction

Current treatment options for chronic osteoarthritis pain consist of a combination of pharmacological and non-pharmacological interventions (McAlindon et al., 2014). Non-pharmacological options range from moderate exercise, physiotherapy and cognitive behavioural therapy (CBT) to joint replacement surgery. Pharmacological options include non-steroidal anti-inflammatory drugs (NSAID), opioid analgesics, antidepressants or intra-articular injection of corticosteroids with local anaesthetics.

Despite these options, around 20% of patients do not get pain relief, even after undergoing arthroplasty. In addition, there is currently no protocol to predict which treatment may work for a given patient. This leads to a frustrating trial and error approach where patients try numerous treatment options until they potentially find one that suits them. This is a reflection of the complexity of chronic pain, and the lack of understanding about the mechanisms of chronic pain relief. As described in the previous chapters, converging evidence points to the central nervous system's contribution to the maintenance of pain, including in osteoarthritis pain.

Advances of both human and animal neuroimaging tools over the last two decades permitted a significant improvement in the understanding of the multifaceted nature of chronic pain. It also allowed more objective measurements of the effects of different types of treatment, as identified in longitudinal studies (Sanders et al., 2015, Petzke et al., 2013, Giménez et al., 2014). One promising use of advanced neuroimaging techniques lies in improving the phenotyping of clinical pain patients and through that, identifying brain signatures that may predict

response to treatments (Tracey, 2011, Tracey and Dickenson, 2012, Wager et al., 2013b, Duff et al., 2015). Therefore neuroimaging techniques have the potential to pave the way to personalized pain management.

In particular, such techniques have recently started to be used to investigate the effects of antidepressants drugs, such as serotonin and norepinephrine reuptake inhibitors (SNRI), in healthy volunteers and in depression populations (Van Marle et al., 2011, Ossewaarde et al., 2011). Imbalance of serotonin and norepinephrine systems within central pain pathways have been implicated in the development and maintenance of central sensitization and associated chronic pain states (Fields et al., 1991). Chronic OA pain is known to involve dysfunction of central pain pathways (Kidd, 2006) and SNRI drugs have been shown to effectively alleviate chronic OA pain (Chappell et al., 2011, Chappell et al., 2009).

One example of SNRI is duloxetine, which is currently used to treat chronic OA pain. However, the precise mechanisms by which duloxetine act to relieve pain are unclear and only around $50-60\%$ of OA patients have sufficient pain relief (50-30% from baseline) after duloxetine intake (Chappell et al., 2009). Despite this, there is currently no way of predicting whether a patient is likely to gain satisfactory pain relief by undergoing treatment with the drug (Chappell et al., 2011, Chappell et al., 2009).

Another class of medication sometimes used to treat chronic OA pain are opioids. These are the most powerful and effective analgesics available and are well established for the treatment of severe acute (Wu and Raja, 2011), surgical and cancer pain (Portenoy, 2011). However their use in non-malignant chronic

pain remains controversial due to associated issues such as physical dependence, addiction, respiratory depression and tolerance during chronic use. Nevertheless clinicians still consider that long-term opioid treatment of pain can be helpful with appropriate patient and opioid selection (Chou et al., 2009, Kahan et al., 2011, Kahan et al., 2011).

Neuroimaging studies may improve our understanding of the central processes through which SNRIs and opioids operate to provide pain relief, and further, may help identify predictors of treatment response. Most human neuroimaging research on opioids has focused on the acute effects of opioids in healthy participants, which does not reflect a chronic pain state, as its neurobiological basis in OA pain patients is likely to differ from that in healthy and psychiatric patients. Similar studies investigating the effects of duloxetine are only available on psychiatric disorders and healthy populations but not on chronic pain itself (Fu et al., 2015, van Wingen et al., 2014, Ossewaarde et al., 2011).

The main aim of this study is to investigate the neural mechanisms of pain relief in chronic osteoarthritis pain (COAP) following treatment with duloxetine, and to establish predictors of treatment response. A secondary aim seeks to achieve a better understanding of how the opiate remifentanil acts in the brain of this patient population to provide pain relief.

Remifentanil is a short-acting μ-opioidergic agonist and was chosen for this study for the following reasons. It has very rapid onset and offset of action, which makes it well suited for MRI investigations. Instead of undergoing chronic treatment to measure the drug effects, an intravenous infusion can be applied during the

scan session, in a well controlled environment. This was also a way of avoiding potential problems of opioid addiction. In addition, the use of remifentanil in fMRI studies has been previously validated in young, healthy individuals (Leppä et al., 2006, Wise et al., 2002, Wise et al., 2004).

It is hypothesized that the duloxetine responding and non-responding sub-groups of OA patients differ in their limbic brain signatures and may show differences in negative affect and/or sensory pain phenotypes. Additionally, it is hypothesized that duloxetine induces brain network and activation changes that partially overlap with antidepressant mechanisms, in particular by improving top down emotional control. For the opiate investigation it is hypothesized that activity in pain-related brain regions with high opiate receptor densities and shown to be downregulated by opiates in healthy subjects, is reduced after remifentanil. High opiate receptor regions include the ACC, thalamus, insula and basal ganglia (Baumgärtner et al., 2006).

Details of the rationale and protocol development of the IPRO study are described below.

7.2. Primary objectives

The primary objectives are:

- To identify a functional change in brain signature indexing the analgesic mechanism of duloxetine.
- To identify brain imaging and psychophysical markers that predict the

response to duloxetine treatment.

7.3. General Hypotheses

- Analgesic response to duloxetine treatment can be predicted using a range of baseline brain imaging markers and QST.
- Analgesic response to duloxetine is mediated by modulation of neural networks underpinning emotional control.
- Duloxetine-induced changes in brain activation differ between responders and non-responders.
- Activity in brain regions with high opiate receptor densities, and shown to be downregulated by opiates in healthy subjects, are modulated by remifentanil in COAP.

7.4. Design and Methods

In order to address the above hypotheses, a single centre double-blinded placebo controlled design was proposed, except that the remifentanil component of the study was single-blind. The single blinded was required as an anaesthetist was needed to prepare and apply the intravenous opioid (or saline) infusion. Notably, the anaesthetist was the only researcher aware of the acute treatment allocation.

After telephone screening (done by Nadia Frowd and Helen Bailey, with some help from William Cottam) and medical notes checks (by Roger Knaggs and I, with

initial help from Helen Bailey), eligible subjects visited the university premises in order to be consented into the study and undergo baseline data collection. Data collected includes a range of questionnaires, multi-modal MRI, blood sampling, knee examination and psychophysical testing 7.1

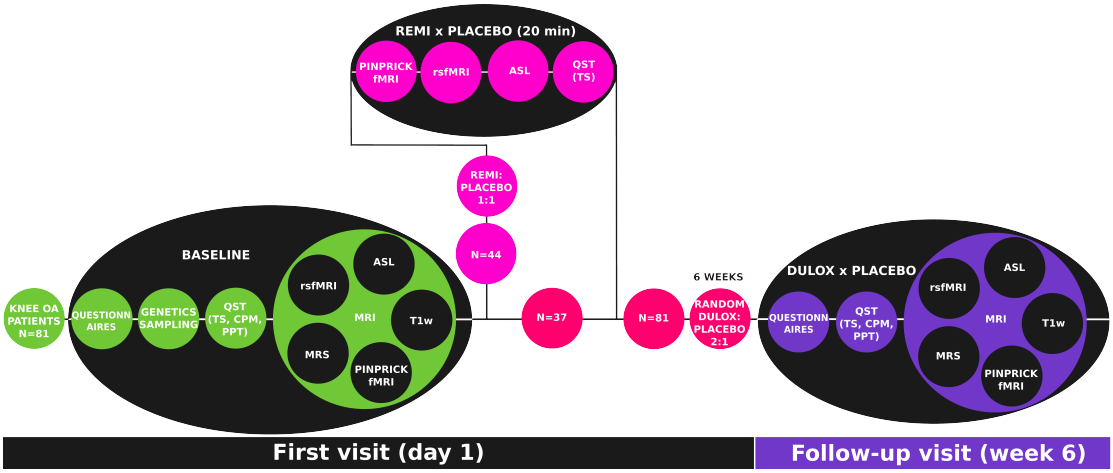


Figure 7.1.: **Illustration of the general IPRO design.** Eighty-one subjects with chronic knee osteoarthritis was the recruitment target. These were randomised between duloxetine or placebo equivalent (2:1 ratio) treatment with tablets intake during six weeks. Forty-four of these patients were estimated to take part in the additional opioid study. The latter were additionally and independently randomised between remifentanil and saline infusions (1:1 ratio). Taking part on the opioid study meant that participants would, after having all baseline scans collected, repeat some of the functional scans while receiving an intravenous infusion.

The study population was composed of people with COAP living around Nottinghamshire, England, who met the inclusion and exclusion criteria described below.

Inclusion criteria

- Radiographically defined osteoarthritis changes in the knee;
- Primary pain in the knee (right or left);

- Chronic knee pain, defined as pain present during most of the day, for more than fourteen days per month, during the last three months;
- Able to give informed consent;
- Over 35 years old.

Exclusion criteria

The exclusion criteria was based on MRI contraindications, drug interactions, neurological and psychiatric conditions and duloxetine and remifentanil contraindications.

MRI related:

- Current pregnancy or breastfeeding;
- Metals in the body (such as pacemaker, shrapnel, iron filings, etc);
- Claustrophobia;
- Large tattoos in the head, neck or shoulder regions;

Medical conditions:

- Uncontrolled hypertension;
- Liver disease;
- Renal implant (Creatinine clearance $\geq$30ml/min);
- Insulin dependent diabetes;
- Parkinson's disease;

- Epilepsy and taking medication for it;
- CVA/Stroke (TIA only can be included in the study);
- Multiple sclerosis;
- Active cancer;
- Chemo-induced neuropathy;
- Major depressive disorder;
- Mania;
- Schizophrenia;
- Uncontrolled narrow-angle glaucoma;

Current and past treatment:

- Previous or current exposure to Duloxetine;
- Benzodiazepines such as Diazepam, Midazolam or Zopiclone;
- Beta-blockers such as Atenolol, Bisoprolol or Propanolol;
- Betahistine;
- Calcium channel blockers such as Amlodipine or Felodopine;
- Chemotherapy;
- Chemotherapy;
- Clopidogrel;

- Dipyridamole;
- Enoxacin;
- Monoamine oxidase inhibitors such as Isoniazid;
- Morphine;
- Potent inhibitors of CYP1A2 such as Fluvoxamine;
- Radiotherapy;
- Remifentanil;
- Sedatives;
- Serotonin-norepinephrine reuptake inhibitors such as Venlafaxine;
- SSRIs such as Citalopram, Fluoxetine or Sertraline;
- Tramadol;
- Tricyclic antidepressants such as Amitriptyline;
- Hypericum perforatum;
- More than 60mg Codeine four times a day;
- More than Dihydrocodeine 30mg four times a day;
- More than 5mcg/h or 10mcg/h Buprenorphine transdermal patches for seven days;

Other:

- Refusal by participant to GP being informed;
- Unwillingness to take caution in relation to use of other centrally active substances such as alcohol and sedative drugs;
- Known hypersensitivity, allergy or intolerance to one of duloxetine's components;

Additional criteria uniquely for participants taking part in the remifentanil component:

- Known hypersensitivity, allergy or intolerance to one of remifentanil's components or other fentanyl analogues;
- Current treatment with cardiac depressant drugs such as beta-blockers and calcium channel blocking agents unless the medications are solely for the treatment of hypertension and have been commenced more than three months ago.

7.5. Randomization and blinding

Participants were randomized twice if taking part in both interventions. Once for the acute treatment allocation (remifentanil and placebo), in which 44 participants were estimated take part: 22 to receive remifentanil and 22, saline. And again, independently of the first randomization, 81 participants were set to be randomized on a 2:1 ratio (n=54, duloxetine and n=27, placebo) given that around half of the population undergoing duloxetine treatment was expected to have satisfactory

pain relief, leading to approximately equal numbers between groups of placebo intakes, duloxetine responders and non-responders.

A block randomization, with random block sizes, was utilized. It was generated by the Clinical Trials Pharmacy (CTP) at Nottingham University Hospitals (NHS Trust) using a pseudo-random algorithm at http://www.randomization.com. The allocation list was kept in a password-protected file, by the CTP and by one of the members of the research team (Nadia Frowd) who is not involved in data acquisition nor analyses. It was possible for the investigators to identify participant's treatment via request to the CTP or Nadia Frowd. However, during treatment, this breaking the code procedure could only be done in the event of a medical emergency, when treatment was dependent on knowledge of the actual drug received. For the case of the treatment code for a participant being broken or other actions, such as recording date or reasons for breaking the blind, discontinuation of trial treatment was to be reported to the sponsor (University of Nottingham).

On the sub-chronic treatment (duloxetine/placebo), participants received six weeks (plus two weeks dose reduction for those taking active drug, for minimizing withdrawal effects) treatment with either active medication or placebo. Duloxetine dose consisted of 30mg daily on the initial two weeks and 60mg daily during the following four weeks. Both participants and investigators were blinded to the type of sub-chronic treatment. For the acute treatment (remifentanil/placebo) however, the anaesthetist was not blinded but participants and other investigators were.

Individual treatment allocation was disclosed to the participant at the end of the follow-up visit by one of the staff members from the CTP, who were not involved

in any data acquisition nor analyses.

Even though the study is not classed as a clinical trial of an investigational medical product, which would require stricter control over randomisation and blinding, by a statistician, substantial advice was received from a statistician (Christopher Tench).

7.6. Experimental testing

The experimental testing was primarily based on multi-modal neuroimaging and QST, in conjunction with questionnaire assessments, genetics and a clinical exam of the knee. This should provide a broad evaluation of the multi-faceted nature of pain, assessing both central and peripheral mechanisms, as well as psychological traits, among others, which should allow pinpointing precise phenotyping of patients into distinct groups.

Given that duloxetine is an SNRI and therefore expected to act on the descending modulatory control system, changes in brain function rather than structure are primarily expected in response to treatment. Therefore the chosen imaging modalities were predominantly functional. Resting-state BOLD fMRI was chosen in order to investigate whole-brain network measures, based on low-amplitude fluctuations of BOLD, measured at rest. Arterial Spin Labelling, also measured at rest, allows mapping of cerebral blood flow, an indirect measure of brain activity, which is independent of the BOLD contrast and therefore was chosen for complimentary functional information, as it allows direct correlations of blood flow with

behavioural information. A task-related fMRI scan was included in order to treatment effects on response to nociceptive stimulation and anticipation. A structural T1-weighted scan was included for registration purposes. See section 7.18.2 for detailed information on the MRI acquisition.

Individuals who met all inclusion and exclusion criteria were asked to refrain from changing their current medication treatment, if any was being taken. Further details about the data collected are given below and a copy of each questionnaire is given in Appendix A (for questionnaires also used as part of ImPOA) and Appendix B (for questionnaires used as part of IPRO, exclusively).

MR-scan:

- Arterial Spin Labelling (ASL) (baseline and follow-up)
- Pinprick BOLD functional MRI (fMRI) (baseline and follow-up)
- Non-task related BOLD fMRI (rsfMRI) (baseline and follow-up)
- T1-weighted (baseline and follow-up)

QST and clinical exam

- Conditioned Pain Modulation (CPM) (baseline and follow-up)
- Temporal Summation of Pain (TSP) (baseline and follow-up)
- Pressure Pain Thresholds (PPT) of the most affected knee and sternum (baseline and follow-up)
- Clinical exam of the knee (baseline and follow-up)

Questionnaires:

- PainDetect (Freynhagen et al., 2006b) (baseline)
- Pain Catastrophizing Scale (PCS) (Sullivan et al., 1995) (baseline)
- State-Trait Anxiety Inventory (STAI) (Spielberger, 2010) (baseline)
- Beck Depression Inventory II (Beck et al., 1996) (baseline)
- The intermittent and constant pain score (ICOAP) - a questionnaire to characterise pain in individuals with osteoarthritis pain. It gives two sub-scales relating to intermittent and constant experience of pain (Hawker et al., 2011) (baseline and follow-up)
- The pain sleep questionnaire - a 5-item tool to assess the impact of pain on sleep (Ayearst et al., 2012) (baseline)
- Brief Pain Inventory - a 9-item questionnaire for measuring the severity of pain and the impact of this pain on daily functioning. The severity sub-scale is used to define the response definition (Cleeland and Ryan, 1994) (daily)
- Mini-Cog - contains a 3-item recall test and a clock drawing test to screen cognitive impairment (Borson et al., 2000) (baseline)
- Educational Level (baseline)
- Questions on belief - four questions to assess an individual's expectations on treatment and medical care (Pouli et al., 2014) (baseline)

Participant general details:

- Age
- Sex
- Pain intensity
- Pain duration
- Caffeine intake (last 24h)
- Average daily caffeine intake
- Average weekly alcohol intake
- Medication

Other:

- 10ml blood samples
- Analgesia diary (daily)

7.7. Response definition

Response to duloxetine is defined as a 50% reduction in the severity of ongoing pain from baseline to end of treatment.

7.8. Primary outcomes

1. Reduction in nociceptive brain response after duloxetine;
2. Neural network change (resting condition) induced by duloxetine ;

3. Differences in brain response and network change between responders and non-responders;

4. Predicting response to duloxetine from baseline fMRI metrics using univariate and multivariate analysis.

7.9. Secondary outcomes

1. Reduction in nociceptive brain response in regions with high opiate receptor densities after remifentanil;

2. Neural network change (resting condition) induced by remifentanil;

3. Correlation between baseline CPM and TS with brain activity and connectivity changes;

4. Identification of QST and questionnaire parameters that predict response to duloxetine;

5. Group differences in brain activity and structure in pain processing, limbic and modulatory pathways changes following duloxetine treatment in comparison to placebo.

7.10. Other outcomes

Exploratory outcomes include, among others: determination of gene variations that can be linked with duloxetine treatment response; GABA modulation according to

changes in pain severity; pooling of baseline neuroimaging data with other studies where participants consented to this.

7.11. Analyses of data

Parametric maps will be derived for functional and structural parameters and regional activation associated with the functional BOLD changes, along with functional connectivity maps for regions of interest (ACC, insula, brainstem, thalamus and limbic system) and networks. Image processing and statistical analyses will be performed using the latest versions of statistical and image analysis software such as SPSS (IBM), FSL (FMRIB) (Jenkinson et al., 2012), PyMVPA (Hanke et al., 2015), SPM (Statistical Parametric Mapping version 8, London, UK), REST (Song et al., 2011), GRETNA (Wang et al., 2015) and Brain Connectivity Toolbox (Rubinov and Sporns, 2010), and LCmodel (Provencher, 1993) for MRS data.

7.12. Sample size calculation

Sample size calculation was performed according to the guidelines by Lenth and colleagues (Lenth, 2001). Estimates of change in functional activation were deduced from a duloxetine invervention study (Van Marle et al., 2011).

Using the Java applets for power and sample size (Lenth, 2006), it was estimated that a one-sample, one-tailed, t-test would have 80% power at alpha of < 0.005 (to allow for ten multiple independent tests) with approximately 19 participants per

group: responders to duloxetine treatment, non-responders, and placebo groups.

Based on previous experience of non-usable imaging data due to patient and/or technical factors in 10% of cases, a sample size of at least 22 participants per group was required. In addition, a further allowance for 20% dropout due to duloxetine / placebo gives 27 participants in each group resulting in a recruitment target of 81 participants.

Following similar procedure, based on a previous study of remifentanil (Atlas et al., 2012), roughly 44 participants were estimated to be enrolled in the opioid investigation. No drug side-effect dropouts were expected.

7.13. Concomitant medication

Participants were instructed not to change their regular pain treatment while taking part in the study. Rescue pain medication, taken on an "as needed basis", was accepted during the trial period and its use was documented in a daily pain diary.

7.14. Criteria for withdrawal

Participation in this study was voluntary and participants were free to withdraw from the trial at any time, without giving a reason. However they were also informed to seek advice from the researchers before withdrawing, as discontinuing duloxetine treatment suddenly may cause drug withdrawal effects. Involvement could be also discontinued by the investigators in case of any significant adverse

effect of duloxetine/placebo treatment, or when subjects required major changes to their ongoing pain management, during the course of the study.

7.15. Adverse events and data safety monitoring

All observed or reported adverse events (AEs) were recorded by the investigators. No AEs were expected in relation to the MRI scanning. Potential AEs were anticipated to be only related to drug intake. All adverse events were recorded and monitored until resolution, stabilisation, or until it was shown that the study intervention was not the cause. Any serious adverse event (SAE) was to be recorded and reported to the research ethics committee (REC) as part of the annual reports.

7.16. Handling and storage of data and documents

Personal data was handled confidentially. Imaging, sample and questionnaire data were anonymised by assigning a unique participant identification code, containing the abbreviation of the study's name followed by a sequence number. The link between study identification code and individual's personal details was kept on the consent and MR screening forms.

In the following sections, the process of obtaining initial approvals to run the study is described.

7.17. Research governance and regulation

Before a research study involving human subjects takes place, a number of approvals are necessary to be in place. The process of obtaining the required approvals for the IPRO study is summarised below and was fully driven by me.

7.17.1. Integrated Research Application System

The Integrated Research Application System (IRAS) is a web-based system for composing governance and regulatory applications for health research. Applications to Research Ethics Committees (REC), Research and Development (R&D) offices and Medicines and Healthcare products Regulatory Agency (MHRA), among others, may all be prepared through IRAS. This system was utilised to create the REC, R&D and CRN Portfolio application forms of the IPRO study.

7.17.2. Medicines and Healthcare products Regulatory Agency

Consultation with the MHRA was necessary for the REC application in order to have an official classification of the study. After initial consultation, the study was controversially classed as a clinical trial of an investigational medical product (CTIMP). However, after clarification on the research protocol, the MHRA agreed with our view that the study was assessing physiology of pain, and therefore would not be classified as a CTIMP (conversation with MHRS: Appendix C). That also meant it would no longer need MHRA clinical trial authorisation (CTA).

7.17.3. NIHR CRN Portfolio

The National Institute for Health Research (NIHR) is a national organisation within the National Health System (NHS) to improve the health and wealth of the nation through research. Part of it is the Clinical Research Network (CRN) which is responsible for the Portfolio, which is a collection of high quality clinical studies that receive support from the CRN. The IPRO study was eligible for consideration due to being funded by Arthritis Research UK, and has been granted Portfolio status, thus receiving some support on recruitment of participants and other costs.

7.17.4. Ethical review

Any research involving human subjects has to be in accordance with the Declaration of Helsinki (Association et al., 2001). After the documentation, including REC forms, research protocol, patient information sheets (Appendix D), consent forms, invitation letters, recruitment adverts, GP and appointment letters and etc., were ready and approved by the sponsor (University of Nottingham), it was submitted to the REC Nottingham I, and considered valid in December 2013. The REC normally has around sixty days to give an ethical opinion, after the application has been validated. The study was then discussed during a REC meeting, to which I attended representing the research team. The REC did not approve the study at this time as there were concerns from the REC, mainly regarding screening of patients. Besides, the REC asked that a further submission should be done to a different REC, one that deals with clinical trials, as the members would be more prepared to review this type of study.

After editing the documentation in order to address any REC concerns, a new application was submitted to a different committee, the REC Nottingham II. The new application was discussed in a meeting in May 2014, to which Prof Auer, Prof Ravi Mahajan and I attended, in order to address any potential concerns. After obtaining a conditional approval, full approval was gained in August 2014 (REC reference: 14/EM/0189).

7.17.5. Research and Development offices review

In addition to REC approval, any research involving NHS patients requires NHS R&D permission. However, given that there is no formal deadline for the R&D offices to give an opinion, it is important to start this application as soon as possible and not wait for the REC approval to start the R&D approval process.

The IPRO R&D application started to be discussed in December 2013 and after numerous discussions, full approval was received in November 2014.

7.18. Methods development

In this section, some of the protocols which have been established for the IPRO study are described.

7.18.1. Quantitative Sensory Testing

QST is a physiological technique applied to patients in order to define individual somatosensory characteristics. It is a reliable way of assessing large and small

sensory nerve fibre function by use of temperature, vibration and pressure. Used in conjunction with other methods, it helps with stratification of patients according to different pain phenotypes and has been previously shown to help predicting treatment outcomes (Petersen et al., 2015, Phillips, 2015, Yarnitsky et al., 2012). The German Research Network on Neuropathic Pain (DFNS) was founded in 2002 and focuses on the idea of a mechanism-based and symptom-orientated approach to neuropathic pain. They have developed standardised comprehensive QST protocols that are now employed by numerous research groups worldwide.

For the IPRO study, in particular, physiological measures such as conditioned pain modulation, temporal summation and pressure pain thresholds are of interest given that abnormal levels of these measures have been previously reported to be predictive of treatment response to different types of treatment and/or other chronic pain conditions. Since IPRO participant visits had to be kept under a limit of three hours, time length was a major concern. Therefore some of the standard protocols as used by the German QST network (Rolke et al., 2006) have been shortened. The ARUKPC has been using certain QST modalities for several years and provided training on the pressure pain thresholds to all researchers involved in the IPRO study. Further advice was obtained from the University of Oxford and from Somedic SenseLab. A description of each QST protocol is given below, with details of any deviation of the standardised guidelines.

Pain pressure thresholds

A PPT protocol was already being used by the ARUKPC. The equipment consists of a hand-held pressure algometer (Somedic SenseLab AB, Sweden) with LED indicators of the pressure being applied (Figure 7.2). The pressure applied should start from 0 and linearly increase with time, until the patient being examined presses a button indicating the first sign of pain.

The previously used protocol have been reshaped in order to make it more reliable and comparable to the standard test by the DFNS as well as to save time (Rolke et al., 2006). The modifications employed were the following:

- On the previous protocol, thresholds were being measured three times at each body site (knee, sternum and finger) before moving on to the next one. However, this required some rest time upon each measurement in order to avoid sensitisation. For the IPRO study, it has been decided to do one measurement at each site then move back to the first site and start measuring for the second time. Besides being in accordance with the DFNS protocol, this makes the process less time-consuming and sensitisation is avoided, resulting in more reliable measurements;

- Based on the ImPOA study, it became clear that a third measure for each site was not necessary as the repeated measures were consistent. Therefore it has been decided to only perform two measurements per site, which also resulted in a shorter procedure;

- The third modified aspect from both the DFNS and previous protocol was

the reduction from three to two sites (knee and sternum).

A copy of the finalised IPRO PPT protocol may be found in Appendix E, together with conditioned pain modulation test described in the following paragraphs.

Conditioned pain modulation

CPM is a test paradigm that measures an individual's ability to modulate pain centrally and is considered to represent the supraspinal level of the CNS. It is indicative of abnormalities in central descending modulatory mechanisms of pain *i.e.* diffuse noxious inhibitory control (DNIC), measured through the ability of a conditioning pain stimulus to dampen perceived intensity of a test pain stimulus in an anatomically different location from the conditioning pain (Backonja et al., 2013, Yarnitsky et al., 2015).

Upon reviewing the literature (Lewis et al., 2012, Yarnitsky et al., 2012) with the aim of formulating a cheap and robust CPM protocol, that would not be too prolonged, it was decided to use the existing pressure algometer (Figure 7.2) as test stimulus and a pressure cuff as conditioning stimulus. More specifically, after measuring PPT (test stimulus), this measurement was repeated twice on the most affected knee, while applying the pressure cuff (conditioning stimulus) on the contralateral arm. The intensity of the conditioning stimulus was individualised, at around $VAS = 40$ out of 100 on a numeric rating scale (Appendix F). The CPM effect was quantified subtracting the local PPT from the local conditioned pressure

pain threshold (CPPT):

$$CPM = CPPT - PPT$$

Results denoting pain inhibition are therefore reported by a negative value, and those denoting pain facilitation are reported by a positive value, according to the recommendations on practice of CPM testing (Yarnitsky et al., 2015). A complete description of this protocol is given in Appendix E and has been developed in collaboration with Helen Bailey.



Figure 7.2.: **Pressure algometer (Somedic SenseLab AB, Sweden) utilised in both pressure pain thresholds and conditioned pain modulation protocols** (Appendix E)

Temporal summation of pain and mechanical pain thresholds

TSP is a test paradigm that is considered to represent the spinal level of the central nervous system. TSP constitutes an increased pain response to repetitive (0.3 Hz) or prolonged nociceptive stimulation at C-fibre activating intensity. It is thought to be the experimental correlate of the electrophysiological "wind-up" phenomenon

in the superficial dorsal horn neurons of the spinal cord (Suzan et al., 2013).

Since the DFNS guidelines require mechanical pain stimulators for TSP, a set of pinprick devices was purchased from MRC (Germany) (Figure 7.3). In order to establish mechanical pain thresholds (MPT) and TSP protocols, three sources of information were used. The main sources were the instructions from the DFNS, as described in (Rolke et al., 2006) and the instructions manual of the pinprick kit provided by MRC (Germany). Given that there was substantial unclarity within the manual and paper, a visit to the imaging research facilities at the University of Oxford was necessary in order to learn about their experience using the kit.

The main issues were potential problems with skin perforation. And only after several discussions regarding disinfection, sterilisation and timings, SOP for TSP and MPT were established. The MPT protocol was relevant for deciding what pinprick weight to use for each individual during the task-fMRI paradigm (described later in this section). However it was later discarded as it proved excessively time consuming. The TSP procedure takes less than two minutes to be performed and the protocol can be found in Appendix G, including disinfection instructions. This protocol had some contributions from Hafiz Hassan.



Figure 7.3.: **Illustrative example of pinprick device, used for measuring TSP.** In the TSP protocol (Appendix G) the 256mN weight is utilised. TSP, temporal summation of pain

7.18.2. MR protocol

MR data was acquired on a clinical 3T GE (General Electric, Milwaukee, WI) MR Discovery 750 Whole Body scanner, equipped with a 32 channel head coil. The MR sequences of interest had already been optimised in previous studies and found to be reproducible. However, there has been a slight change on the BOLD fMRI protocol, regarding resolution and echo time. The previous sequence yielded anisotropic resolution and was not tailored at any specific region of the brain. Given the key role of the brainstem in descending pain modulation, TE was changed to maximise signal coming from that specific region. In addition, given the small size of the structures of interest within the brainstem, spatial resolution was decreased slightly. Previous studies at 3T had optimised BOLD acquisition for the brainstem and the modifications performed here were based on their acquisition parameters and recommendations (Beissner et al., 2014, Harvey et al., 2008, Beissner and Baudrexel, 2014). Details on the acquisition sequences utilised are described below.

- **Pinprick fMRI:** T2* weighted images, TE= 30ms, TR= 2s, flip angle= 90°, field of view=192x192mm^2, slice thickness= 3mm, gap between slices= 0.5mm, 37 slices, in-plane matrix=64x64 and resolution=3x3mm, scan time: 5.5 minute (including 5 dummy volumes). Slices were acquired parallel to the anterior-posterior commissure plane. Here a gap between slices was chosen in order to allow full brain coverage without extending scan time. Flip angle and TR were kept according to optimisation and reproducibility tests that were performed as part of previous studies.

- **rsfMRI:** similar parameters to fMRI pinprick described above was used. Participants were instructed to keep their eyes closed.

- **ASL:** Pulse-continuous ASL labelling with 3D spiral read-out was used. TE = 10.5ms, TR = 4.6s, flip angle = 111°, FOV = 240mm, slice thickness = 4mm, number of slices = 36, in-plane matrix = 128 x 128, slice gap = 4mm, labelling duration = 1450ms, post-labelling duration = 1525ms, echo train length = 1, number of excitations = 3. An M0 image was collected for image quantification and background suppression was performed. Scan time: 4.5 minutes.

- **T1-weighted:** Axial T1-weighted images were obtained using fast spoiled gradient echo (FSPGR), acquired parallel to the anterior-posterior commissure plane, with TE= 3.18ms, TR= 8.2ms, in-plane matrix: 256x256, voxel size 1x1x1 mm^3, scan time: 4 minute. This acquisition is similar to the one used in Chapter 6.

- **MRS:** Single voxel spectra from the anterior cingulate cortex (ACC) were collected using a PRESS sequence optimised for GABA detection (Napolitano et al., 2013) with TE= 105ms, TE1= 15ms, TR=2.5s, 128 averages, 16 water unsuppressed averages for water scaling, scan time: 6 minute. Similar acquisition was employed in Chapter 6.

A software named Spike2 (Cambridge Electronic Design Limited) was used to record physiological readings from respiratory belt and oximeter, during 4D fMRI. A volume trigger was used in order to have a mark on the physiological readings

corresponding to the start of the acquisition of each volume.

Pinprick fMRI

The idea of this protocol was to have a short and simple design that would allow imaging of nociception, anticipation and rating of each stimulation. A similar paradigm to the one used by Lui and colleagues (Lui et al., 2008) was followed (Figure 7.4) and repeated eight times. E-Prime was used to give instructions to the participant and to display a numeric rating scale which ranged from 0 to 100, where 0 corresponded to no pain and 100 meant excruciating pain. In order to synchronise scanner acquisition with E-prime presentation, it was necessary to set-up a signal coming from the scanner which triggered E-prime. Each stimulation was followed by a numeric rating scale where subjects were asked to use a set of response buttons to rate each stimulus (Figure 7.4).

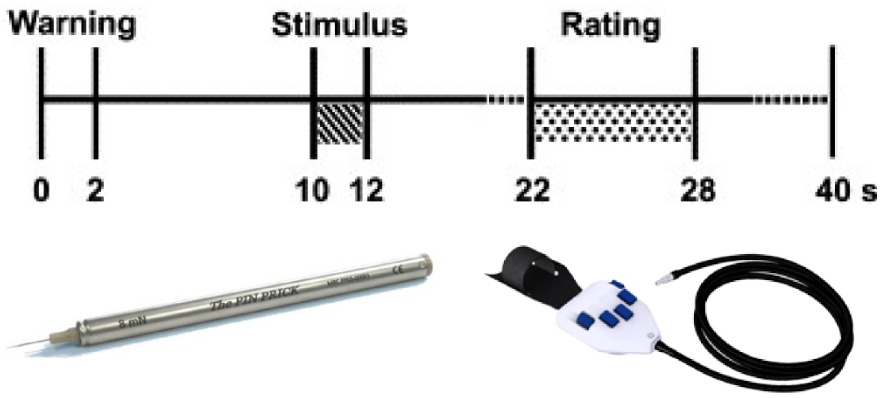


Figure 7.4.: **Pinprick paradigm utilised during fMRI, following the design proposed in** (Lui et al., 2008). The figure also shows an MR-compatible pinprick device and the *PST Fiber Optic Response Glove* used during pinprick fMRI to rate the intensity of stimulus on a numeric rating scale displayed in front of the scanner. Total scan time: 5.5 minutes, including 10s of dummy scans at the start

CPM fMRI

Taking a step further from the pinprick fMRI, once the protocol for measuring CPM was set, as described in subsection E above, a different one was necessary for performing CPM inside the scanner, given that neither the pressure algometer nor the pressure cuff were MR-compatible. A commonly used test stimulus is hot temperature applied with a peltier device (Somedic AB, Sweden), which was already available at the ARUK Pain Centre. It was decided to make use of an approximately similar design to the one used by Sprenger and colleagues (Sprenger et al., 2011). In this paradigm, a hot stimulus applied to the forearm was used as the test pain, while two bags with ice and water mixture at 0^oC was utilised as the conditioning stimulus.

In summary, thermal stimuli were initially applied to the participant's forearm in order to determine the temperature that would yield a NRS rating of 60 out of 100. This was done following a specific algorithm, based on the work in (Granot et al., 2008, Yarnitsky et al., 2012). The temperature that yielded a rating of 60 was later used as test stimulus during the scan session. The procedure was performed on the scanner bed, in order to keep similar conditions to the actual scanning session, such as ambient temperature and light. The full instructions and details about the test may be found in Appendix H.

It was considered whether the pressure CPM testing could have been removed from the study protocol given that CPM could be now measured inside the scanner, while imaging its neural correlates. However, the MRI-compatible test was less robust, as the Peltier device would sometimes be faulty. In addition, the standard

recommendation is that ideally, both thermal and mechanical CPM tests should be performed in order to improve comparability with other studies (Yarnitsky et al., 2015). Even though, it was decided to keep both tests in the protocol, the peltier device proved to be highly unreliable to the point of not working at all. The equipment had to be sent back to the manufacturer where it was kept for some months in order to fix a manufacturer fault. Once it was returned to the researchers it became faulty again and therefore this protocol was abandoned.

Instead, based on results from the recently performed GABA investigation (presented in Chapter 6), it was decided to replace the CPM fMRI acquisition for an MRS protocol to specifically look at whether the inverse correlation between GABA and pain severity in the ACC is kept following treatment analgesia with duloxetine.

7.19. Recruitment

Identification of potential participants was initially performed by screening consent forms and contacting people who have previously participated in ARUKPC studies and have agreed to be contacted again regarding future research.

Primary care recruitment through GP practices was set-up at a later stage, which was supported by the CRN. In addition, posters calling for participants were displayed in public spaces such as leisure centres, surgeries and Nottingham University Hospitals (NUH) (poster can be seen in Appendix I).

Due to difficulties with identification of potential participants, further alternative

routes were sought started to be set-up in late 2015. These included recruitment through physiotherapy clinics and the use of local press. An article regarding the IPRO study was published in some local newspapers and online newsletters.

7.20. Discussion

A number of advanced pain induction methods for concurrent and parallel studies such as pinprick fMRI, TSP fMRI, CPM and TSP protocols have been successfully established. New recruitment pathways within the ARUK Pain Centre have been devised, including primary care recruitment through physiotherapy clinics and a database containing contact details of individuals interested in being informed about future studies.

Given the complexity and requirements for achieving the study's aims, support was required from a wide range of specialists, including experienced professionals to exclude contraindications for opioids, and anaesthetists to safely deliver remifentanil intravenously, specialists in neuroimaging and spatial statistics, clinical research assistants and recruitment support.

By closely working with the Nottingham University Hospitals Clinical Trials Pharmacy (NHS Trust), who was responsible for the randomisation, it was possible to ensure coordination of staff and tasks around manufacture and release of the drugs. In addition, since early stages of protocol development, the study and key documentation have been extensively discussed with a focus group composed by representatives of patients and the public. The group helped shaping the study and

positively contributed with modifications to the study's documentation. Besides all the complexity, it was possible to gather the necessary expertise and co-ordinate participants' visits with the availability of scanner, radiographer, anaesthetist, research assistants and clinical staff.

Several innovative steps were taken as part of IPRO. New state-of-the-art psychophysiological testing protocols were developed, including CPM and TSP. Novel MRI acquisition protocols were developed, including a pain induction fMRI design, which involved synchronised pinprick stimulation with image acquisition, allowing simultaneous pain ratings and visual cues. A CPM fMRI protocol was also developed, however it was later withdrawn due to repeated technical failures of the Peltier device. Most of these novel protocols are now also being utilised in other research studies within the ARUK pain centre, and especially as part of an MRC funded studentship (Thomas Kurien), to investigate the neural changes underpinning abnormal TSP in patients before and after total knee replacement.

Recruitment for the combined study has been more challenging than individual studies would have been but was necessary to make the studies financially viable, and will allow further important scientific conclusions to be drawn from direct comparisons once the duloxetine arm is completed. This led to a long list of exclusion criteria, composed of interactions and contraindications to duloxetine and opiates, together with MRI safety requirements. New recruitment routes not yet established in the ARUK Pain Centre had to be set-up, including recruitment through local press and physiotherapy practices. Some support has been received from the CRN, as part of the portfolio adoption. Most participants were recruited

via GP surgeries and previous studies. And those who were ineligible but happy to know about further studies were included in the newly established database.

The setting of the IPRO study has a number of limitations. Firstly, ideally a cross-over design should be used for placebo-controlled studies, allowing for randomisation of active/non-active treatment order. However, following such design would have significantly increased the minimum amount of time participants would need to be engaged in the study: it would have doubled the study involvement time as every participant would undergo both active and non-active treatments and a wash-out period would have been needed in between interventions to allow drug effects to wash out. Therefore instead of six weeks, participant involvement in the study would have been for a minimum sixteen weeks. Given the difficulties with recruitment in previous studies and in an effort to minimise costs, burden to participants and dropout rates, the shorter design option was chosen.

Secondly, although it would have been useful to use a block randomization scheme with stratification for factors such as sex, age and depression levels, that was not possible given that the population to be recruited was unknown and the need for having approximately similar numbers in each group (responders, non-responders and placebo). Therefore no stratification was used, but instead, a block randomization with varying sizes of blocks.

Thirdly, the pinprick paradigm employed here is not ideal for a number of reasons. Delays of random duration should have been used between stimuli (a process known as jittering). That would have avoided prediction by the participant on when the next stimulus would be applied and, importantly, it would have

allowed unique estimates of the parameters of the statistical models that are used to analyse fMRI data (Ashby, 2011). Also, pinprick stimuli were applied manually, which means the timings are not perfect. And finally, stimulus intensity was not subjective but constant to all patients. However, given the number of issues which had to be overcome in order to just get this simple paradigm to work, together with the time constraints due to funding and in order to keep patient visits to a reasonable duration, it was necessary to simplify procedures. Nevertheless, for the first time in this research group, a pain induction fMRI protocol has been successfully setup and this paradigm has already served as a basis for other studies [see for example (Kurien et al., 2016)].

And finally, with regards to the BOLD acquisition, the decrease in voxel size came at a cost of a reduction in SNR which should have ideally been compensated by, for instance, longer acquisition time. However, due to time limitations, the acquisition time was kept to 5.5 minutes.

In summary, the main work towards the development of the IPRO study has been described here. Innovative steps were undertaken and state of the art protocols for pain research have been established, which have been transferred onto further studies. The rich data collection system put in place should allow addressing important questions regarding analgesia mechanisms of two major classes of drugs and placebo in chronic pain, as well as further investigation of biomarkers of chronic knee osteoarthritis pain. Additionally the establishment of these first intervention studies, using the setting of randomised controlled trials, in this pain research group should ease the development of future, improved, study protocols of this

kind. In the next chapter, some of the general results of study participation and demographics are given, which will be followed by two interim analyses of the remifentanil and duloxetine components of IPRO.

8

IPRO: General participation characteristics

Overview

Following the developmental work described in Chapter 7, here some general information on recruitment and study participation in IPRO is presented. Some information on enrolment and discontinuation is described here whereas further, more specific details will be discussed in Chapters 9 and 10.

Contributions of this chapter

- A middle sized multi-modal dataset was acquired, including two analgesia studies, and baseline data for cross-sectional investigations of brain plasticity and biomarkers in chronic pain;
- Contact details of 190 individuals with osteoarthritis pain, who interested in taking part in future studies, were added to the created database.

8.1. Recruitment and participation

Recruitment started in December 2014 and the last participant was recruited in July 2016. A total of 2202 people were contacted initially by Nadia Frowd (with contributions from Helen Bailey). Around 230 completed preliminary phone screening. Following medical notes checks, 77 subjects were finally consented into the study (Figure 8.1). Most of the participants originated via primary care recruitment through general practitioners (nine clinics). Smaller contributions to recruitment came from previous studies within the ARUK pain centre, posters and local press. Around 260 people with CKOAP who could not take part in IPRO, and those who participated in IPRO and confirmed that were happy to be informed about future studies, were included in a database for recruitment into future studies (REC reference 13/EM/0234).

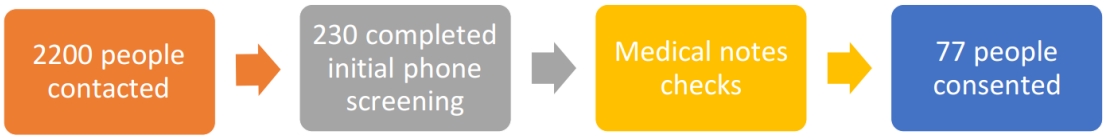


Figure 8.1.: **General summary of IPRO recruitment.**

Screened	Screening failed	Consented	Discontinued	Not randomized	Completed
230	around 140	77	20	1	56

Table 8.1.: **Participation to date.**

8.2. Participant disposition and demographics

Seventy-seven subjects who met inclusion and exclusion criteria were recruited (Table 8.1). 34 participants were randomized into the remifentanil study and 76 were randomized between duloxetine and placebo (Table 8.2). 13 dropped out as they did not tolerate side effects of sub-chronic treatment, 7 discontinued participation for other reasons, as specified in Table 8.2. General demographics of study participants are given in Table 8.2, Table 8.2, Table 8.2. Demographic, sensory and questionnaire data were digitised and scored by me, Nadia Frowd and Marianne Drabek, with contributions from Amy Hardie, Oliver Hacking, Ailish Byrne and Zuzanna Ludzik. Key data was double checked in at least 70%.

Participation	Target no	Actual
Total recruited	81	77
DLXPlac completed	56	56
RemiSal completed	44	34
DLX	38	34
Plac (DLX)	19	22
Remi	22	20
Sal (Remi)	22	14
SE dropout	15 (estimated)	13
Other dropout	9 (estimated)	5 + 2
DLX SE dropout	10 (estimated)	10
Plac (DLX) SE do group dropout	5 (estimated)	3
DLX other dropout	6 (estimated)	6
Plac (DLX) other dropout	3 (estimated)	0

Table 8.2.: **Accrual breakdown of general participation on the IPRO study.** Other dropouts refer to subjects who were MRI unfit (n=3), became ill or decided to withdraw for other reasons than side effects. SE, side effects; DLX, duloxetine; Remi, remifentanil; Plac, placebo; Sal, saline.

Reason	N	%
Side effects	13	17%
LTF	0	NA
Claustrophobia	1	1%
MRI incompatibility	2	3%
Non-compliance with study medications	0	NA
Became ill (unrelated study drugs)	2	3%
Protocol deviation	0	NA
Withdrew	2	3%

Table 8.3.: **Subject status, for discontinued participation ($n = 20$).** Percentages are relative to the full cohort recruited ($n = 77$). The first patient recruited was not randomised to treatment. LTF, lost to follow

Female	$n = 25$	45%
Male	$n = 31$	55%
age (mean±SD)	63.6±10.5 yrs	NA
age (median, mean, max)	64.5, 63.6, 86 yrs	NA

Table 8.4.: **Demographics of full group who completed sub-chronic treatment with duloxetine or placebo equivalent ($n = 56$).**

Female	$n = 7$	54%
Male	$n = 6$	46%
age (mean±SD)	64.5±7.5 yrs	NA
age (median, mean, max)	65, 64.5, 81 yrs	NA

Table 8.5.: **Demographics of subjects who discontinued participation due to side effects of sub-chronic treatment. Percentages are relative to the full number of drop-outs due to side effects ($n = 13$).**

Female	$n = 16$	47%
Male	$n = 18$	53%
age (mean±SD)	65.6±9.9 yrs	NA
age (median, mean, max)	66.5, 65.6, 89 yrs	NA

Table 8.6.: **Demographics of participants who completed acute intervention with remifentanil or saline equivalent ($n = 34$).**

8.3. Discussion

During the twenty-one months of data collection, a rich dataset was generated. This data is currently being analysed and will not only provide answers regarding two major classes of drugs and placebo in chronic pain, but baseline data will also allow further investigation of brain plasticity and biomarkers of chronic osteoarthritis pain. The following two chapters present two interim analyses of the remifentanil and duloxetine studies, respectively.

9

Remifentanil effects on pinprick related brain activity in COAP

Overview

Following the developmental work described in chapter 7, an interim analysis of the opioid component of the IPRO study is presented here. More specifically, the analysis involves the pinprick fMRI paradigm, quantitative sensory testing and psychometric data collected until January 2016, involving a total of 28 subjects.

Contributions of this chapter

- BOLD fMRI was used to investigate mechanisms of opioid action during pinprick stimulation in chronic knee osteoarthritis pain;
- Results from this interim analysis suggest that the analgesic effect of remifentanil, in chronic knee osteoarthritis pain patients with impaired conditioned pain modulation, is associated with reduced ACC activity

Publications

Contributions from this chapter have appeared in the following:

- Reckziegel D, Bailey H, Chana A, Cottam WJ, Tench CR, Mahajan RP, Walsh DA, Knaggs R, Auer DP (2016). Opioid reduces nociceptive activation of the anterior cingulum preferentially in patients with impaired conditioned pain modulation: a BOLD fMRI study in chronic osteoarthritis pain. *Proceedings of the World Congress on Pain*, Yokohama, Japan.

9.1. Introduction

Opiates are the most powerful and effective analgesics available and are well established for treatment of severe acute (Wu and Raja, 2011), surgical and cancer pain (Portenoy, 2011). However their use in non-malignant chronic pain remains controversial due to associated issues such as addiction, tolerance and side effects of chronic use (Chou et al., 2009). Importantly, chronic opioid therapy can induce

hyperalgesia, a paradoxical state of increased sensitivity to pain (Trang et al., 2015). Nevertheless, clinicians still consider that long-term opioid treatment of pain can be helpful, or at least safe with appropriate patient selection (Chou et al., 2009, Lee et al., 2014).

Pharmacological fMRI studies may improve our understanding of the central processes through which opioids operate to provide pain relief and possibly to identify to whom they are more likely to be beneficial. Most human neuroimaging research on such drugs has focused on their acute effects in healthy participants, either at rest or during experimental noxious stimulation. A search of the literature performed on PubMed and Google scholar in August 2016 confirmed that opioid neuroimaging research focused on the healthy brain. There has been only one published fMRI study investigating the effects of opioids in chronic pain participants (chronic regional pain syndrome) (Gustin et al., 2010). Thirteen studies employed an experimental noxious paradigm in healthy volunteers and nearly consistently reported modulation of activity in brain regions known to be involved in processing nociceptive stimuli (the insula, thalamus, sensory and anterior cingulate cortices (Bingel et al., 2011, Petrovic et al., 2002, Wagner et al., 2007, Wanigasekera et al., 2012, Wise et al., 2002, Wise et al., 2004). Table 9.1 summarizes the literature in healthy populations. However these studies do not reflect a chronic pain state and did not focus on subject-to-subject variation.

Here the aim is to study neural correlates of the analgesic effects of the short-acting μ-opioidergic agonist, remifentanil, in people with chronic knee osteoarthritis pain (CKOAP). The secondary aim is to explore whether pain phenotyping, based

on questionnaires and psychophysiological testing, allows prediction of the extent of opioid response.

It was hypothesized that nociceptive activity in pain-related brain regions with high opioid receptor densities (Baumgärtner et al., 2006), and shown to be down-regulated by opioids in healthy subjects, would be modulated by remifentanil in CKOAP. In addition, it was hypothesized that opioid action would be related to levels of impairment in pain modulatory systems. Central pain modulation refers to neural mechanisms that allow a person to downregulate pain perception and underlies widely known observations where, for instance, high levels of stress in an acute injury prevent the victim to 'feel' the pain immediately.

9.2. Materials and methods

This is a randomised, baseline and saline controlled study. Block randomisation was used to allocate subjects to receive remifentanil or saline intravenously on a 1:1 ratio, to which both researchers and participants were blinded, apart from the anaesthetist administering the infusion. Participants had a cannula inserted, just before being scanned, on the hand ipsilateral to the most affected knee, and were advised that at some point during the scan they would receive an infusion with either remifentanil or saline. They were not given any hints when the infusion started to take place. Drug was delivered using a target controlled infusion pump, with maximum estimated plasma target of 1.0 ng/ml. The methodology described in this chapter consists of a subset from the IPRO study and only the relevant

Table 9.1.: **Summary of previous task-related neuroimaging studies in healthy volunteers investigating the effects of opioids.** nP, number of patients; NHV, number of healthy volunteers. When not otherwise stated, bilateral regions were found significant. SMA, supplementary motor area; AIns, anterior insula; PIns, posterior insula; BG, basal ganglia; DLPFC, dorso-lateral prefrontal cortex; CC, cingulate cortex; CN, caudate nucleus; Thal, thalamus; DFMN, default mode network; Pc, precuneus; amy, amygdala.

Study	Journal	Sample	Method	Drug	Increased activity	Decreased activity
(Wise et al., 2002)	NeuroImage	9	BOLD	remifentanil	-	Contralateral Ins
(Petrovic et al., 2002)	Sciences	9	PET	remifentanil	-	Ins
(Wise et al., 2004)	N.psychopharmacology	9	BOLD	remifentanil	-	Contralateral Ins
(Wagner et al., 2007)	Anesthesiology	7	PET	remifentanil	PAG, cingulofrontal and occipital cortex	Thal, parahipp, SII, BG, PFC, AIns, OCC
(Bingel et al., 2011)	Sci Trans Med	26	BOLD	remifentanil	-	S1, S2, DLPC, Ins, L Thal, putamen, CN, cerebelum.
(Wanigasekera et al., 2012)	PNAS	25	BOLD	remifentanil	-	Ins, BG
(Adler et al., 1997)	Anesthesia&Analgesia	9	PET	fentanyl	SMA, L inf fron cort	-
(Casey et al., 2000)	J N.physiology	20	PET	fentanyl	contralateral ACC	S1, Thal
(Hoffman et al., 2007)	Anesthesia&Analgesia	9	BOLD	hydromorphone	-	Ins, Thal
(Oertel et al., 2008)	Clinical Pharmacology & Therapeutics	BOLD	alfentanil	-	L S1, S2, Ins	
(Upadhyay et al., 2011)	J N.physiology	24	BOLD	buprenorphine	DMN, ACC, PCC, mPFC, parietal cortex, Pc; striatum, amy	Contralateral S1, PIns, SMA
(Upadhyay et al., 2012)	NeuroImage	36	BOLD	buprenorphine	ACC, PCC, Thal, putamen/globus palidus	S1, S2, PIns, SMA, R Thal, R mid-CC, sup temp
(Hansen et al., 2015)	BCPT	20	BOLD	morphine	-	R Ins, ACC, inf parietal cortex

components to the opioid investigation of brain activity during nociception will be included here. For a full description of materials and methods, the reader may refer to Chapter 7.

BOLD fMRI was acquired during a pinprick task paradigm involving visual cues ("pinprick starting"), response to pinprick stimulus (256mN), followed by rating, in an event-related design Figure 9.2. Volunteers were familiarized with the NRS and response buttons and underwent a simulation prior to the experiment. Participants received a total of eight pinprick stimuli during each scan session, which were applied to 2cm distal to the infero-medial aspect of patella of the most affected knee. Each stimulus was rated on a 0-100 numeric rating scale (Figure 9.1. Further details can be found in Chapter 7. This paradigm was performed both before (baseline) and during infusion with remifentanil or saline equivalent (treatment).

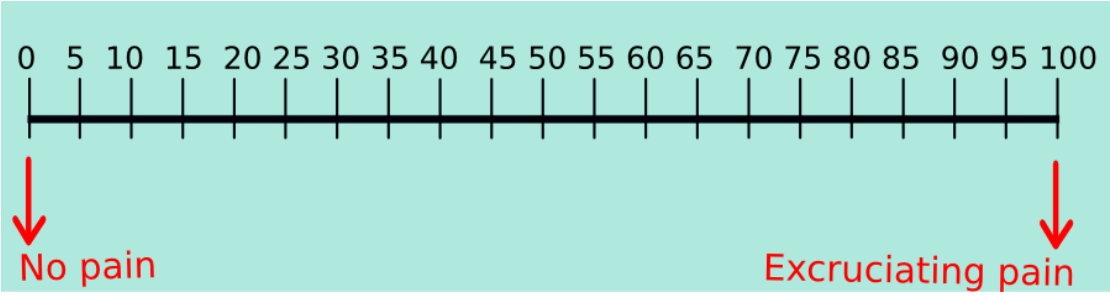


Figure 9.1.: **Numeric rating scale used to rate pinprick stimulations during BOLD fMRI.** Participants had 6s for each rating

Movement was monitored during fMRI and whenever significant movement was detected, the acquisition was re-started. A T1-weighted volume was acquired at baseline only and used for registration purposes. Acquisition parameters for pinprick fMRI and T1-weighted images have been described in Chapter 7. Example

volumes of the BOLD images can be seen in Figure 9.3.

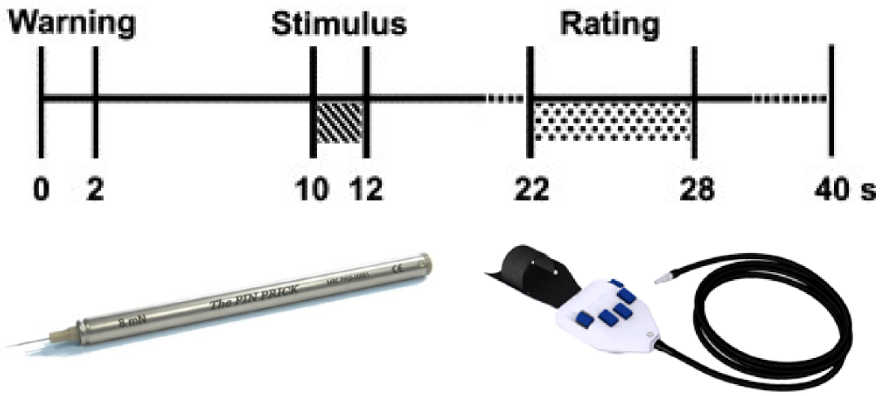


Figure 9.2.: **Pinprick fMRI paradigm.** A pinprick device was used to deliver the stimulus and response buttons were used to rate the intensity of each stimulus. Both pinprick and button devices are illustrated here. Further details have been described in Chapter 7

Psychophysical tests performed prior to scanning included pressure pain thresholds (PPT) and conditioned pain modulation (CPM) (Appendix E), together with temporal summation of pain (TSP). As described in Chapter 7, CPM measures the reduction of pain perception when inflicting a second type of pain, while TSP correspond to the wind-up of pain perception. A pressure algometer was used to measure PPT in two sites: 2cm distal to the infero-medial aspect of patella of the most affected knee and 3cm below the sternum notch. CPM testing consisted of repeating measurements of PPT on the most affected knee, while inflicting pressure pain ($NRS \sim 40$) on the contralateral arm (conditioning stimulus). Levels of TSP were measured using a pinprick device (256mN) and measurement was repeated after infusion.

A set of questionnaire data on negative affect, including Beck Depression Inventory II, State-Trait Anxiety Inventory and Pain Catastrophizing Scale, were also

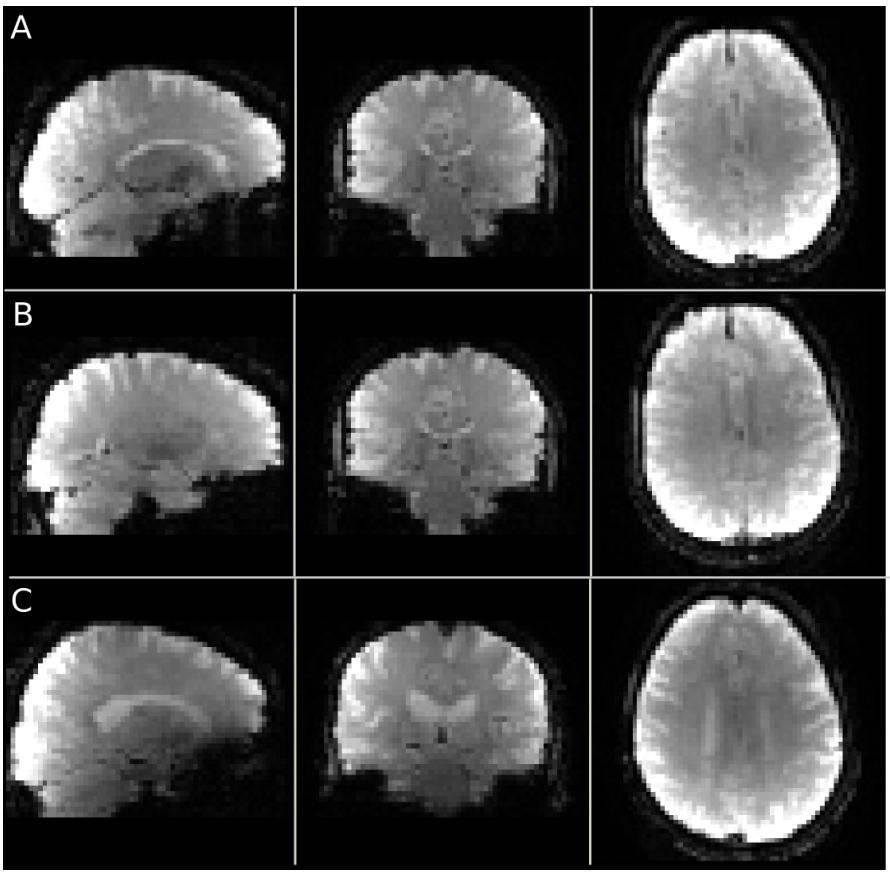


Figure 9.3.: **Example volumes of the BOLD acquisition. A. Subject 1, volume 1. B. Subject 1, volume 51. C. Subject 2, volume 85.**

collected.

9.2.1. Handling of imaging data and preprocessing

DICOM data was transferred onto CDs and uploaded to one of the Radiological Sciences servers. Data was converted from DICOM to ANALYZE format using *dtoa*[1]. A Bash script was then used to sort out data and rename it according to acquisition and participant identification code.

Before any pre-processing took place, all structural and functional images were

[1]dtoa was developed by Paul Morgan http://www.nottingham.ac.uk/ msapm1/software/dtoa.html

visually inspected. Those presenting strong movement or other artifacts were excluded.

All image processing was performed using FSL version 6.0 (FMRIB's software library, www.fmrib.ox.ac.uk/fsl). BET was used to remove non-brain tissues from the structural volumes. Functional volumes were then co-registered to the corresponding individual brain-extracted structural images. Preprocessing steps included motion correction using MCFLIRT (Jenkinson et al., 2002), slice-timing correction using Fourier-space time-series phase-shifting, non-brain removal using BET (Smith, 2002b). Data were also spatially smoothed using a Gaussian kernel of $FWHM = 5mm$, had grand-mean intensity normalisation of the entire 4D image by a single multiplicative factor, and had high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with $\sigma = 50.0s$).

Melodic (Beckmann and Smith, 2004) was used for multivariate exploratory decomposition into independent components, in order to investigate potential artifacts or unexpected activation. Components from 10 datasets were manually checked and classified into signal, noise or unsure. The selected noise components were used to create a training file with FIX (Salimi-Khorshidi et al., 2014). This training file was later employed to classify and remove noise components from the whole dataset. The automatically removed components were verified for coherence in at least two subjects.

9.2.2. Statistical analysis

Statistical maps were generated to identify brain activity evoked by pinprick in each individual scan. For higher level analysis, a mixed effects model, FLAME, was employed and a cluster-based correction for multiple comparisons were used ($Z > 2.3$, cluster significance $p < 0.05$). Similar analysis was carried out for the anticipation period, preceding pinprick stimulation. *Post-hoc* analysis was performed using Featquery to extract Z-scores from regions of interest (ROI), based on group activation maps during pinprick stimulation, and previous studies (Table 9.1). The following procedure was conducted in order to create these ROI masks: first, anterior cingulate (ACC), insula and thalamic cortices, brainstem and precuneus masks were separately extracted from the 50% probability Harvard-Oxford cortical and subcortical structural atlases. The intersections between these masks and the baseline activation during pinprick maps were computed using *fslmaths* and registered to each individual functional native space.

SPSS21 was utilised for further statistical tests. Paired t-tests were used for normally distributed data, comparing the ROI means between infusion and baseline. Non-parametric Wilcoxon signed-rank test was used for non-normally distributed data.

9.3. Results

From the 28 subjects recruited into this acute intervention study by January 2016, four were excluded from analysis: one did not undergo pinprick fMRI due to

inability to see the screen at the front; one did not have a structural scan; one dataset was acquired in the wrong orientation (sagittal instead of axial); and one dataset presented significant signal drop. Therefore, 24 datasets passed quality control, 13 of which corresponded to remifentanil (6 females, mean age ± SD: 64.6 ± 8.5 years) and 11 to saline (5 females, mean age ± SD: 68.8 ± 11.2 years) (Table 9.3).

	Remifentanil, mean±SD	Saline, mean±SD
Subjects	13	11
Age	64.6 ± 8.5 yrs	68.8 ± 11.2 yrs
Sex	7 males	6 males
Pain duration	7.2 yrs	11.6
NRS	20.7	40.4

Table 9.2.: Summary of demographic and pain data of participants. NRS, numeric rating scale for pain severity; yrs, years.

Baseline whole group activation map displays pinprick-related brain activity including the known pain processing areas such as insula, anterior cingulate cortex, thalamus, brainstem, amygdala, putamen and primary somatosensory cortex (S1) Figure 9.4. Areas of decreased activity during pinprick include posterior default-mode network regions Figure 9.4.

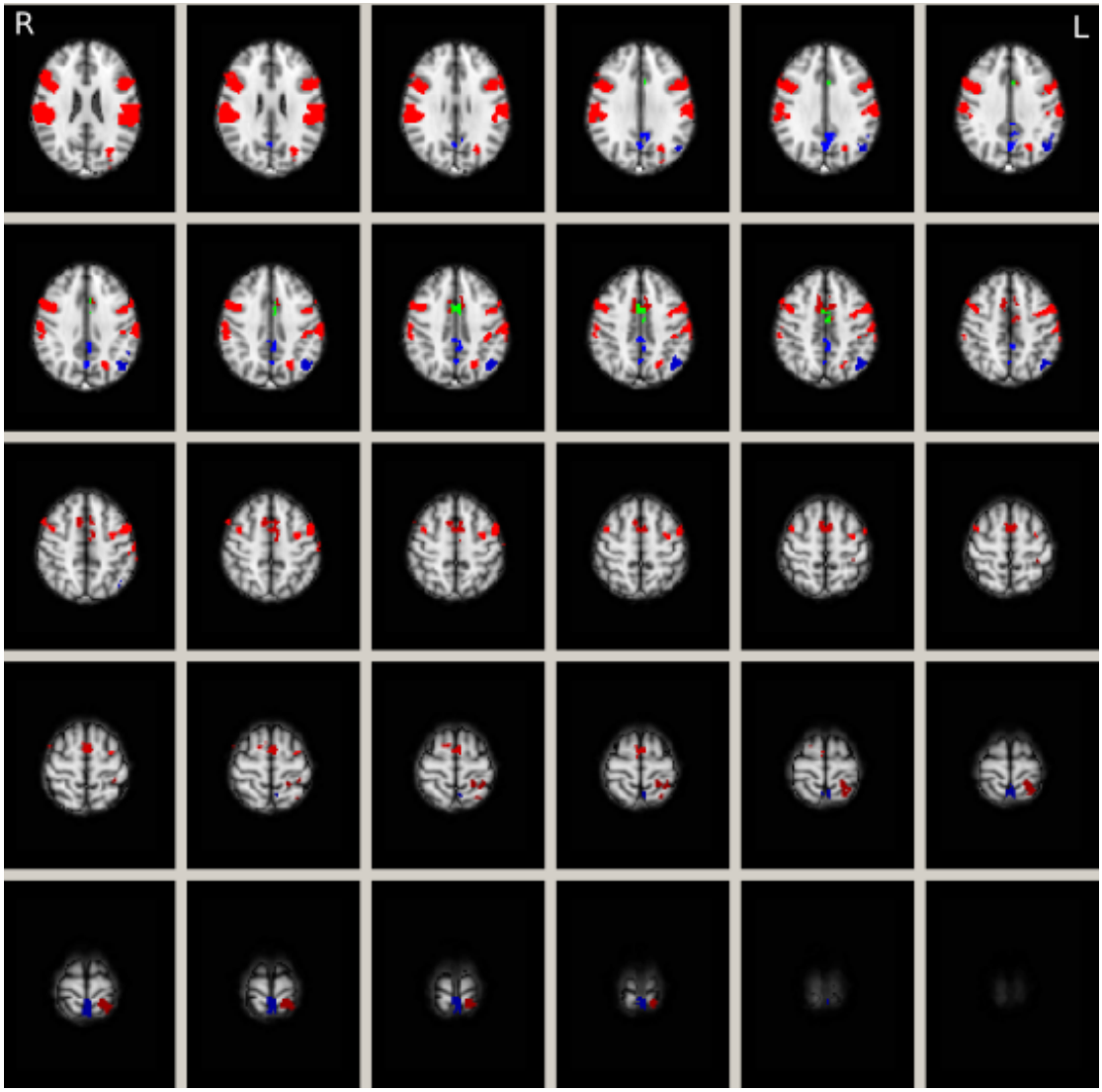
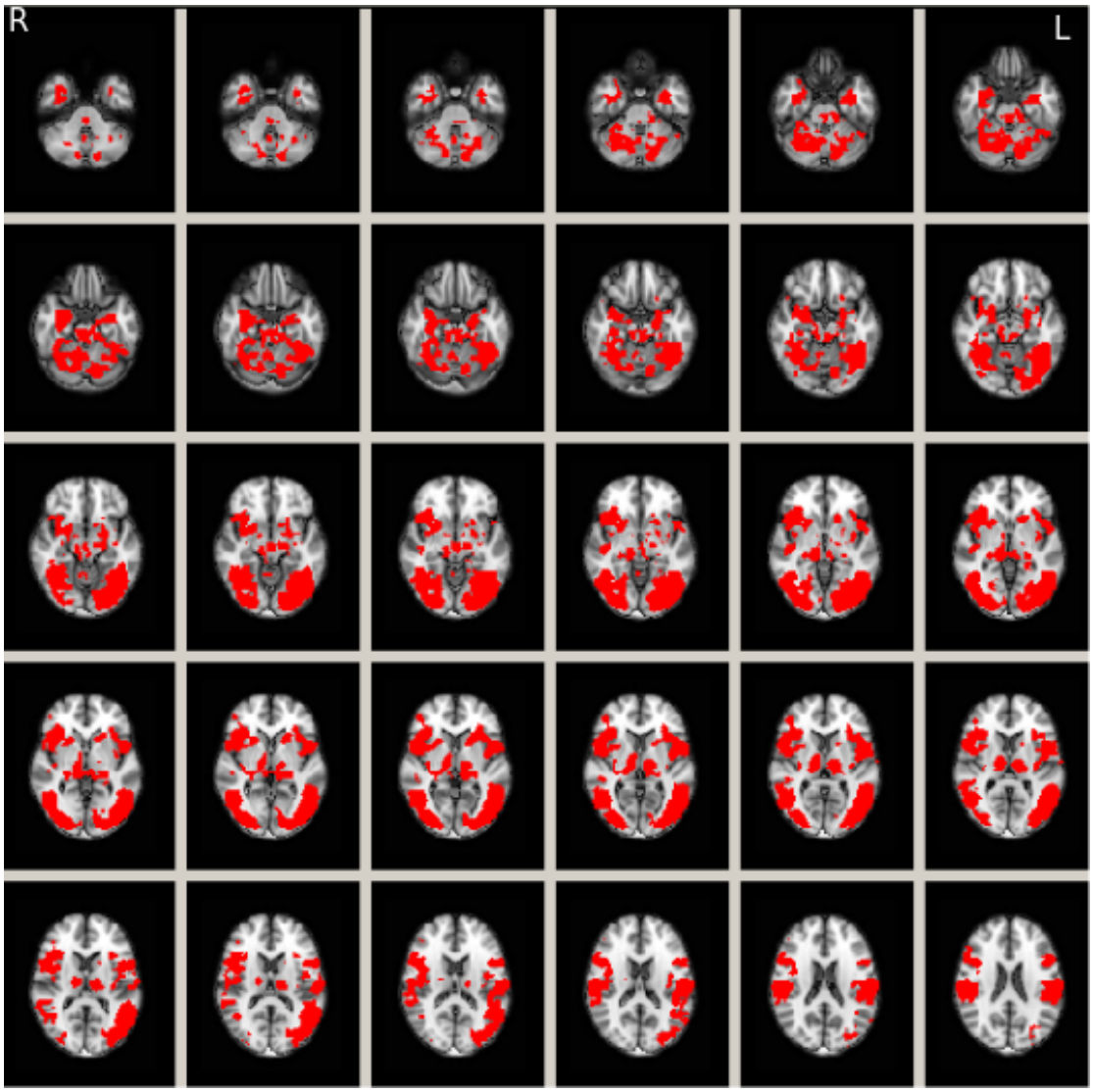


Figure 9.4.: **Areas of increased (red) and decreased (blue) BOLD-related activity during baseline pinprick stimulation** (n=24, mixed effects, $Z > 2.7$, cluster threshold $p < 0.05$). Green represents region with activity modulation by remifentanil ($Z = -2.5$, $p = 0.013$, $n = 13$). Regions of interest for effect of remifentanil were constrained to the areas of increased BOLD-response during pinprick at baseline. (See next page for further brain slices)

No differences were found between baseline and infusion during pinprick with whole brain voxel-based analysis. Post-hoc ROI analyses using Wilcoxon signed-rank test detected remifentanil-induced activity decrease within the mid-ACC

($Z = -2.5, p = 0.013$) Figure 9.4. No other ROI changes were detected between baseline and saline nor baseline and remifentanil.

Moreover, remifentanil-induced modulation of nociceptive response in the ACC was correlated to CPM measurements ($p < 0.005, \rho = 0.778$). Patients with more impaired CPM showed larger decreases in ACC activation.



Continuation of Figure 9.4 (previous page).

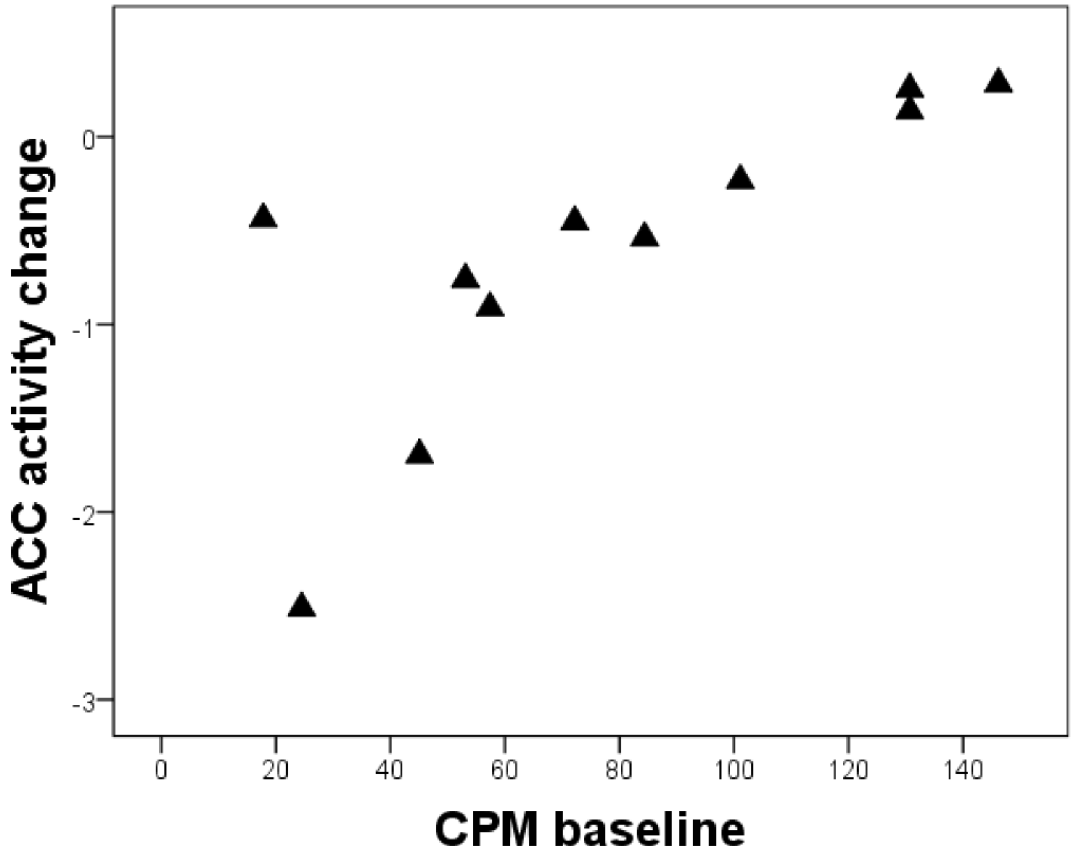


Figure 9.5.: **Drug-induced modulation in the mid-ACC cluster during pinprick stimulation in relation to levels of CPM prior to infusion**. [CPM]=kPa.

9.4. Discussion

By employing a pain-induction fMRI protocol in CKOAP, it was found that, at baseline, pinprick elicited brain activity in the regions commonly found to be activated during nociception. Pinprick-related activity in the mid-ACC was reduced during remifentanil but not saline infusion. Interestingly, patients with more CPM impairment had stronger opioid modulation in the region, suggesting that CPM

impairment may be opioid-responsive. It has been previously found that CPM variability was associated with drug treatment response, where healthier CPM was related with improved pain relief following nonsteroidal anti-inflammatory (NSAID) treatment in osteoarthritis patients (Edwards et al., 2016). Conversely, in diabetic neuropathy, more impaired CPM was found to be related with improved response to a serotonin-norepinephrine reuptake inhibitor (Yarnitsky et al., 2012). Therefore CPM might allow prediction of non-response to NSAID's and the need for centrally acting drugs.

The ACC is a key region for pain and a hub of the salience network. It has been previously shown to play a role in pain perception, intensity encoding and aversiveness. Moreover, it is suggested to have high densities of opioid receptors and to mediate cortical anti-nociceptive effects of opioid (Baumgärtner et al., 2006). Recently, the ACC was also shown to be one of the few regions having modulation of pain-response by analgesics, in a multi-study fMRI investigation (Duff et al., 2015).

The majority of previous human neuroimaging studies of opioids involved healthy and young participants (Lee et al., 2014) only and the ACC was one of the most commonly reported regions to undergo modulation by opioids. One investigation using morphine in chronic regional pain syndrome found that the drug modulated nociception-related activity within the ACC only (Gustin et al., 2010). Taken together, the literature and our findings suggest shared neural mechanisms of opioidergic analgesia between chronic pain states and the healthy state.

We did not find opioid-driven modulation in other areas such as insula and

thalamus, which have been frequently reported in previous studies. This may be due to the different biological mechanisms in chronic pain as opposed to the healthy brain. Additionally previous studies included younger individuals, which may also be a contributor for such differences. It is however important to state that the sample size used in the current interim analysis is small and therefore some of the activity changes may have passed undetected.

Apart from the small sample size, another limitation is the constant stimulus weight used for all participants. Some subjects classed the severity of the pinprick stimulus as 70 out of 100, while others rated them as 0. In addition, most participants had very mild osteoarthritis pain while lying in the scanner ($NRS = 0$) and therefore it wasn't possible to measure analgesia effect on the ongoing knee pain. Ideally in future studies involving pain relief, there should be a higher minimum level of ongoing pain as criteria for entering the study. It could not be determined whether remifentanil affects or disrupts CPM, as CPM test was only collected at baseline due to time constraints. Also, as numerous CKOAP only feel knee pain when exercising, upright clinical MR scanners or a paradigm that involves exercising would be particularly advantageous.

In conclusion, findings from the interim analysis presented here suggest that the analgesic effect of remifentanil in CKOAP with impaired CPM is associated with reduced ACC activity.

10

Analgesia mechanisms of duloxetine in COAP

Overview

Following the developmental work described in Chapter 7 and the analysis of the effects of remifentanil on brain activity during nociception (Chapter 9), here an investigation of both the effects of, and predisposition to, duloxetine are presented. In particular, similar to the investigation presented in the previous chapter, an analysis of the available pinprick fMRI data of subjects who completed the study by January 2016 (n=29) is presented. Additionally, the quantitative sensory testing and psychometric data from the 56 individuals who completed the duloxetine

intervention study is also described.

Contributions of this chapter

- BOLD fMRI was used to investigate mechanisms of SNRI action during pinprick stimulation in chronic knee osteoarthritis pain;
- Effects and potential predictors of duloxetine on pinprick-related brain activity are evaluated;
- The extent of improvement in chronic knee osteoarthritis pain scores following treatment with duloxetine was associated with baseline activity within the bilateral anterior insular cortices.

10.1. Introduction

As introduced in Chapter 7, the serotonin-norepinephrine reuptake inhibitor (SNRI) duloxetine has recently started to be used to treat a number of pain conditions, including COAP. However, little is known about the neurobiology that mediates its analgesic effects and only around $50-60\%$ of OA patients gain satisfactory pain relief (Chappell et al., 2009) after duloxetine intake.

Neuroimaging tools may improve current understanding of such mechanisms and help identifying predictors of treatment response. In healthy participants, duloxetine was found to attenuate activity in affect processing areas (amygdala, thalamus, insula and anterior cingulate cortex) while simultaneously increasing functional

coupling between the amygdala and the anterior insula, during an emotional face matching task (Van Marle et al., 2011). After duloxetine intake but not placebo, major depression disorder patients presented augmented connectivity in the anterior default mode network (DMN), a core network linked to self-referential brain function with distinct abnormalities in major depression (Fu et al., 2015, Sheline et al., 2009). Importantly, reduced connectivity within the orbitofrontal component of the DMN, especially the subgenual cingulate, at baseline was predictive of clinical antidepressant response (Fu et al., 2015).

Mood disorders and pain conditions are often comorbid, as it is the case with COAP (Bair et al., 2008). They also show strong similarities in their neurobiological mechanisms and both are known to critically involve limbic neurocircuits (Baliki and Apkarian, 2015, López-Solà et al., 2010). In fact, previously reported brain activity linked to perceived severity of clinical pain may be partially explained by anxiety (Cottam et al., 2016). This may be specific to reported intensity of perceived COAP, as experimental responses to nociceptive stimuli were not modulated by negative mood (depressive symptoms, anxiety or catastrophizing) in fibromyalgia (Jensen et al., 2010). In neuropathic pain, the analgesic efficacy of duloxetine treatment was also independent of depression, and was instead predicted by psychophysical measures of impaired conditioned pain modulation (CPM), but not temporal summation (TSP) (Yarnitsky et al., 2012). Increased activity in the right pre-frontal cortex may predict the analgesic effect of duloxetine as found in a small pain fMRI study in 13 depressed patients (López-Solà et al., 2010).

The aim of this chapter is to investigate the mechanisms of pain relief in

chronic OA pain following treatment with duloxetine, and to establish predictors of differential outcome to treatment, based on an fMRI paradigm involving pinprick stimulation, quantitative sensory testing and questionnaires. This aim partially fulfills the overall primary objectives of the IPRO study, described in chapter 7.

It is hypothesized that duloxetine will induce reduction in nociception-related brain response and that responding and non-responding sub-groups of COAP patients differ in their limbic brain signatures. Additionally, these two groups may show differences in negative affect (such as anxiety, depression, pain catastrophising) and/or sensory pain phenotypes. More specifically, for responders to either treatment (duloxetine or placebo) it is hypothesised that pain intensity encoding regions such as the anterior insula and the mid anterior cingulate cortex are down regulated after treatment. Duloxetine responders in particular, are expected to also present treatment-induced modulation of activity within limbic regions including the amygdala and putamen, as opposed to placebo responders. Additional activity modulation or enhancement after duloxetine treatment is expected as direct drug effect. And finally, assessment of similarity to available studies predicting antidepressant response will be performed (McGrath et al., 2013).

10.2. Materials and methods

Only the relevant methods to this sub-analysis will be described here, whilst a more extensive description has been given in Chapter 7. The duloxetine component of IPRO is illustrated in isolation in Figure 10.1 below. Here the focus is on the

pinprick fMRI and associated psychometric and quantitative sensory testing.

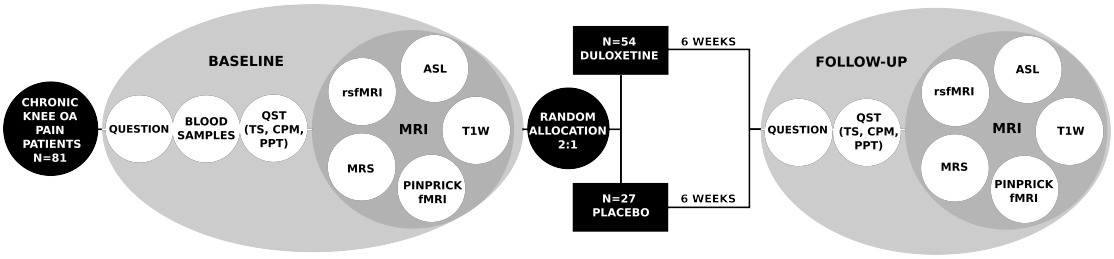


Figure 10.1.: Duloxetine component of the IPRO investigation.

10.2.1. Study design

As described previously (Chapter 7) this component of the IPRO investigation consists of a double blind randomised baseline and placebo controlled mechanistic study. A block randomisation was used to allocate subjects to receive oral duloxetine tablets or placebo equivalent on a 2:1 ratio, as approximately 50% response rate was expected on the duloxetine arm (Chappell et al., 2011). Treatment consisted of two initial weeks of daily $30mg$ duloxetine (one tablet) intake, followed by four weeks of $60mg$ duloxetine (two tablets) intake. At end of week six, participants on the active drug arm underwent a dose-reduction period, taking $30mg$ duloxetine once daily for an extra two weeks, in order to minimise drug withdrawal effects, while placebo intakers did not. A schematic timeline of study participation is given in Figure 10.2. Participants undergoing treatment as part of their medical care were asked to keep medication constant.

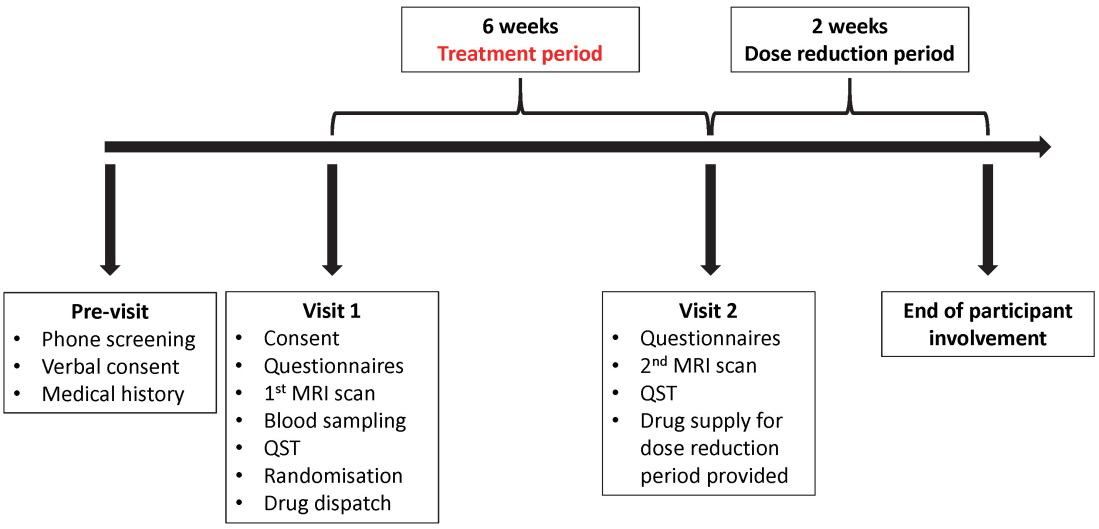


Figure 10.2.: **Timeline of participation in the study.** QST, quantitative sensory testing. *Reproduced from accepted manuscript at BMJ Open.*

10.2.2. Questionnaire and psychophysical testing

From the full questionnaire and sensory testing battery described in Chapter 7, only the relevant subset necessary to test the hypotheses above is taken into consideration here. This includes the Brief Pain Inventory (BPI, completed daily), Intermittent and Constant Osteoarthritis Pain Scale (ICOAP, completed both at baseline and post-treatment), Pain Catastrophising Scale (PCS, completed at baseline), Beck Depression Inventory (BDI, completed at baseline), State-Trait Anxiety (STAI, completed at baseline), Pain Sleep Questionnaire (PSQ, completed at baseline) and Quantitative Sensory Testing (QST, completed both at baseline and follow-up).

10.2.3. Response criteria

Participants undergoing a 50% reduction in reported severity of COAP from baseline to endpoint, based on the BPI 24h-average severity sub-scale, were considered responders. The 50% threshold was defined based of previously published randomised controlled trials of duloxetine in COAP (Chappell et al., 2011).

10.2.4. Image acquisition

From the full MR protocol acquisition, pinprick fMRI and T1-weighted volumes acquired at baseline and following duloxetine or placebo treatment completion are considered here. Image acquisition parameters and fMRI paradigm were similar to the description given in Chapter 9.

10.2.5. Image processing and statistics

An identical approach to the preprocessing steps taken in Chapter 9 was followed, as well for generating statistical maps. Using a mixed effects model within FSL 6.0 (FMRIB's software library, www.fmrib.ox.ac.uk/fsl), FLAME, and a cluster-based correction for multiple comparisons were used ($Z > 2.3$, cluster significance $p < 0.05$). Featquery was utilised in order to extract Z-scores from regions of interest (ROI). Chosen regions for this analysis were defined *a priori* based on results from meta-analysis of neuroimaging studies of pain (Tanasescu et al., 2016), emotion regulatory, and salience network including anterior cingulate cortex (ACC), anterior and posterior insula, amygdala, periaqueductal grey (PAG), thalamus and putamen. In order to create masks for these regions, the FSL 50% probability

Harvard-Oxford cortical and subcortical structural atlases were used to extract masks for the ACC, amygdala, insula and thalamic cortices. For the PAG, a spherical masks with 6mm of diameter was generated with centre at the following MNI coordinates: x=1, y=-29, z=-12 (Linnman et al., 2012). The intersection between each of these masks and the mean baseline activation map during pinprick stimulation were then computed using *fslmaths*. This was done by multiplying the ROI masks by the mean baseline activation map, so that only the voxels with significantly increased activity during pinprick stimulation were included in the new binary masks. Each of these masks were next registered to each individual functional native space for Z-scores extraction with *featquery*.

SPSS21 was used for the following statistical tests: paired t-tests, to evaluate treatment effects; independent samples t-tests, to check differences measurements between responders and non-responders; Pearson's correlation analysis to determine associations with psychophysical and psychometric measurements. Where data was non-normally distributed, non-parametric Wilcoxon signed-rank, Mann Whiney and Spearman's rank correlation tests were respectively used.

As described in Chapter 7, the sample size calculation was based on ROI activity reduction from baseline to post-duloxetine at $\alpha < 0.05$ after Bonferroni correction for multiple comparisons. Given that the present neuroimaging analysis only involves around half of the estimated sample size, it is anticipated that potential findings will likely not survive correction for multiple tests.

In order to keep the blind of the data analyst, a spreadsheet was created containing questionnaire scores, demographics, ROI and psycophysical test information,

which was given to Nadia Frowd who keeps the randomisation list. A column was added by Nadia Frowd indicating treatment allocation. Participants identification numbers were removed and the data was sub-sequentially randomised by Marianne Drabek, before returning the spreadsheet to the data analyst.

10.3. Results

10.3.1. Participant characteristics and randomisation

From the seventy-seven recruited individuals with chronic knee osteoarthritis pain who satisfied inclusion and exclusion criteria (Chapter 7), fifty-six completed participation in this component of IPRO. Participants who completed the study had knee pain for a minimum of one year and a maximum of fifty years. Thirty-four underwent treatment with duloxetine, while 22 received placebo. There were no statistical differences in age, sex and pain characteristics (Table 10.3.1).

	Duloxetine, mean±SD	Placebo, mean±SD
Subjects	34	22
Age	64.7 ± 10.2 yrs	61.8 ± 10.9 yrs
Sex	19 males	12 males
Pain duration	11.3 ± 10.6 yrs	10.4 ± 10.9
ICOAP constant	33.8 ± 21.6	41.2 ± 24.1
ICOAP intermittent	37.2 ± 14.1	45.4 ± 21.3
PainDetect	10.9 ± 6.7	11.7 ± 7.3

Table 10.1.: **Summary of demographic and pain characteristics of both duloxetine and placebo groups**. Five subjects on the duloxetine and one on the placebo arm had missing ICOAP data. ICOAP, intermittent and constant osteoarthritis pain scale; yrs, years.

10.3.2. Treatment response

The duloxetine group presented a significant improvement in their general ICOAP scores ($p < 0.0005$), a measure of COAP intensity and its effect on quality of life, as opposed to the placebo group.

18 (53%) duloxetine intakers had at least 50% reduction in their 24h average perceived pain severity scores (from BPI) and therefore were classified as responders, against 8 (36%) of those who received placebo. Baseline levels of depression and trait anxiety did not differ between responders and non-responders to duloxetine (Table 10.3.2), while responders presented increased rumination (a sub-scale measure of catastrophising). Psychophysical measures did not differ between responders

and non-responders (Table 10.3.2).

Most participants were taking at least one type of drug treatment as part of their medical care, which they were asked to keep constant throughout the duration of study participation. Common concomitant medications included treatment for high blood pressure, nonsteroidal anti-inflammatory drugs, thyroid medication and proton-pump inhibitors (see Appendix J for a list of medications per participant).

	DLX-R (mean±SD)	DLX-NR (mean±SD)	t-test
Subjects	18	16	-
Age	65.4 ± 10.8 yrs	64.0 ± 9.8 yrs	NS
Sex	10 males	9 males	-
Pain duration	10.4 ± 9.21 yrs	12.2 ± 12.3yrs	NS
ICOAP constant	32.5 ± 21.1	35.0 ± 22.7	NS
ICOAP intermittent	36.7 ± 16.6	37.6 ± 11.9	NS
PainDetect	8.8 ± 6.0	12.7 ± 6.9	NS
BDI	5.1 ± 4.0	5.3 ± 5.1	NS
STAI(T)	28.2 ± 6.5	28.8 ± 7.6	NS
PCS	12.2 ± 9.8	6.8 ± 7.4	NS
PPT knee	378.5 ± 249.5 kPA	434.8 ± 319.3kPa	NS
CPM	65.3 ± 49.3 kPA	55.11 ± 132.2kPA	NS
TSP	13.5 ± 18.0	8.0 ± 14.9	NS

Table 10.2.: **Demographic, psychometric and psychophysical data of participants who underwent treatment with duloxetine.** DLX-R: responders to duloxetine; DLX-NR: non-responders to duloxetine; BDI: Beck Depression Inventory II; STAI(T): State Trait Anxiety Inventory - trait sub-scale; PCS: Pain Catastrophizing Scale; PPT: Pressure pain thresholds; NS: Non-significant; yrs: years.

	PLAC-R (mean±SD)	PLAC-NR (mean±SD)	t-test
Subjects	8	14	-
Age	66.2 ± 10.7 yrs	59.2 ± 10.7 yrs	NS
Sex	4 males	8 males	-
Pain duration	10.3 ± 5.2 yrs	10.5 ± 13.3	NS
ICOAP constant	50.0 ± 24.2	23.6 ± 11.4	NS
ICOAP intermittent	50.0 ± 24.4	34.7 ± 10.4	NS
PainDetect	13.5 ± 7.9	8.6 ± 5.0	NS
BDI	6.6 ± 6.6	8.7 ± 6.3	NS
STAI(T)	34.4 ± 15.3	36.3 ± 10.7	NS
PCS	6.7 ± 6.0	18.5 ± 14.9	NS
PPT knee	352.9 ± 149.2 kPA	408.4 ± 250.4 kPa	NS
CPM	70.0 ± 51.3 kPA	63.3 ± 133.3 kPA	NS
TSP	16.6 ± 9.4	16.6 ± 9.4	NS

Table 10.3.: **Demographic, psychometric and psychophysical data of participants who underwent treatment with placebo.** PLAC-R: responders to duloxetine; PLAC-NR: non-responders to duloxetine; BDI: Beck Depression Inventory II; STAI(T): State Trait Anxiety Inventory - trait sub-scale; PCS: Pain Catastrophizing Scale; PPT: Pressure pain thresholds; NS: Non-significant; yrs: years.

Available pinprick fMRI data for participants who completed study by early January 2016 consisted of twenty-nine datasets. Following quality inspection, data from one subject was excluded from analysis due to significant poor signal to noise ratio which did not allow registration to their corresponding T1-weighted images nor to standard space. One participant was not able to see the screen during scanning but was kept in the analysis. Finally, datasets from 28 subjects with baseline and post-treatment images were included in the analysis, 17 of which received duloxetine (9 females, mean age ± SD: 66.0 ± 10.8 years) while 11 were on the placebo arm (6 females, mean age ± SD: 60.7 ± 11.5 years) (Table 10.3.2).

	Duloxetine, mean±SD	Placebo, mean±SD
Subjects	17	11
Age	66.0 ± 10.8 yrs	60.7 ± 11.5 yrs
Sex	8 males	5 males
Pain duration	9.2 ± 7.7 yrs	7.0 ± 4.3
ICOAP constant	37.0 ± 22.2	36.5 ± 22.7
ICOAP intermittent	44.3 ± 12.6	47.7 ± 21.5
PainDetect	11.7 ± 6.9	11.1 ± 2.6

Table 10.4.: **Summary of demographic and pain data of sub-group of participants with available pinprick fMRI data who completed sub-chronic treatment by early January 2016**. ICOAP, intermittent and constant osteoarthritis pain scale; yrs, years. There were three ICOAP scores missing.

Baseline whole group mean activation map show pinprick-related brain activity in known pain-related areas such as insula, anterior cingulate cortex, thalamus, brainstem, amygdala, putamen and primary somatosensory cortex, whereas deactivation maps correspond to default-mode network regions (Figure 10.3).

Duloxetine treatment was not associated with significant changes, from baseline to endpoint, in mean brain activity within the regions of interest analysed when testing the whole duloxetine group ($n = 17$), nor when evaluating responders ($n = 9$) and non-responders ($n = 8$) separately (Figure 10.4). Following placebo treatment, participants ($n = 11$) presented activity reduction within both right ($p < 0.005$) and left thalami ($p < 0.05$) from baseline.

Baseline activity within both right and left anterior insular cortices were associated with treatment response following duloxetine treatment (Figure 10.5), as detected by Pearson's correlation with changes from baseline to endpoint in ICOAP intermittent pain scores ($p < 0.05, R = -0.67$ and $p < 0.05, R = -0.61$ for right and left respectively). This association was not seen in individuals undergoing placebo treatment.

Regardless of treatment received, responders had a tendency ($p = 0.059$) for lower ACC activity at baseline (mean $\pm$ SD: 0.35 ± 0.57) as compared to non-responders (mean $\pm$ SD: 1.05 ± 1.16).

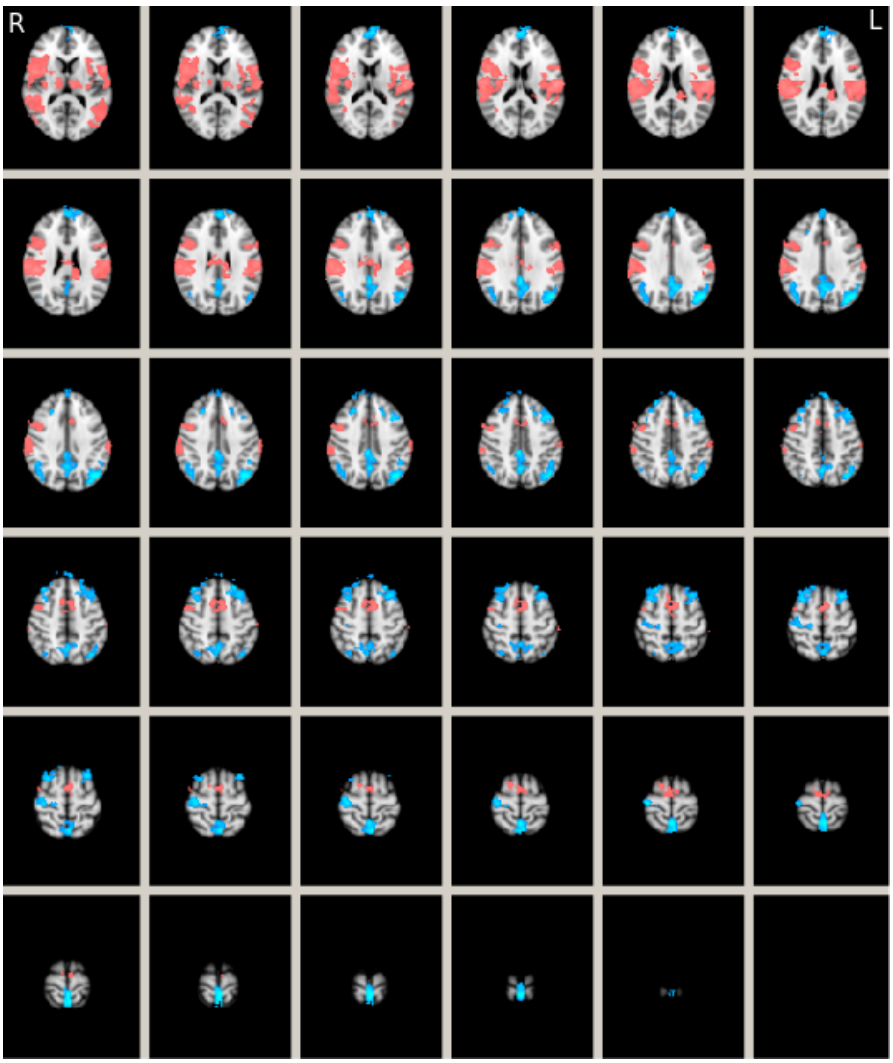
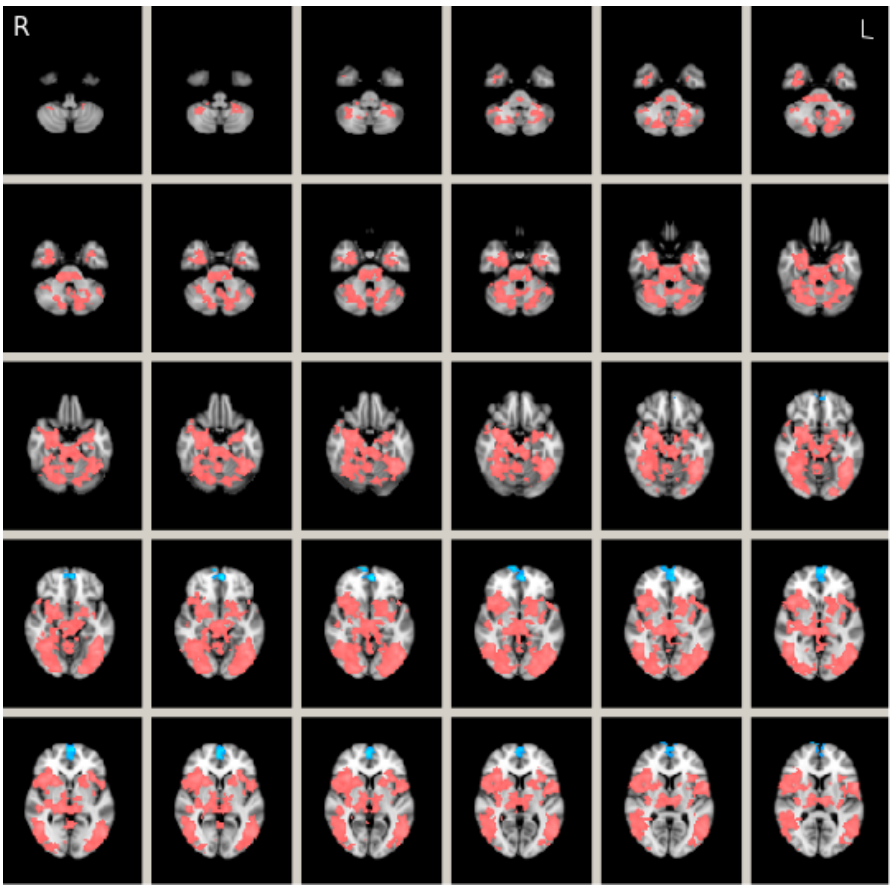


Figure 10.3.: **Pinprick-related activity at baseline.** Statistical map for increased (pink) and decreased (blue) BOLD-related activity during baseline pinprick stimulation (n=28, mixed effects, $Z > 2.3$, cluster threshold $p < 0.05$). For further slices, see continuation of figure on the next page.



Continuation of Figure 10.3 (previous page).

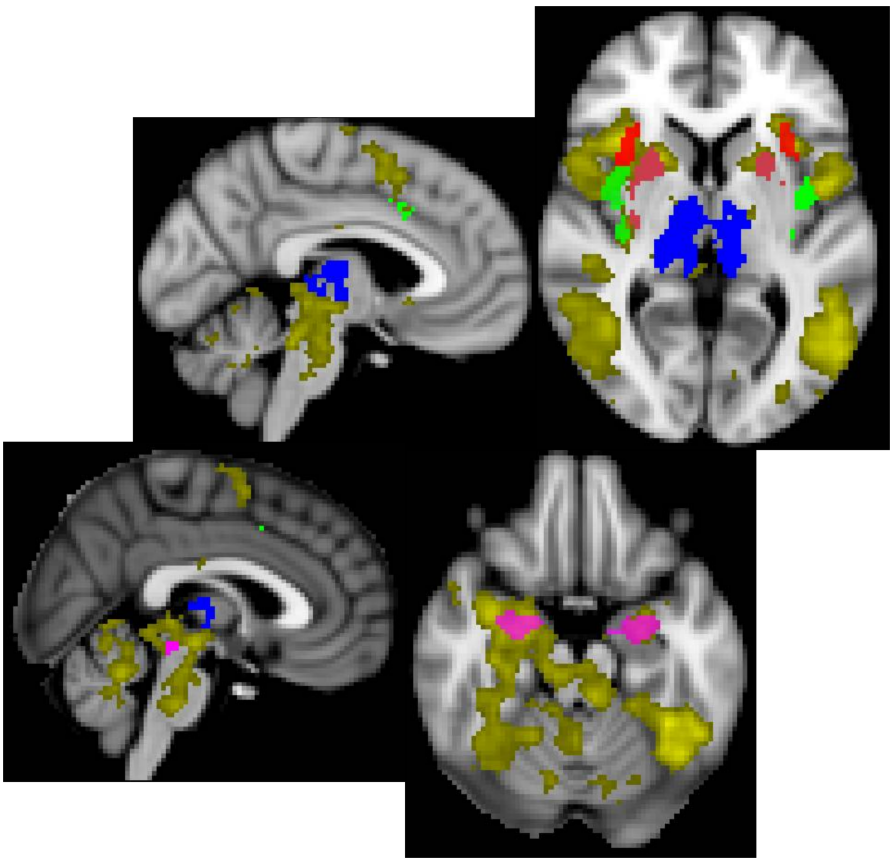


Figure 10.4.: **Regions of interest that were investigated.** Dark yellow represents statistical map for increased BOLD-related activity during baseline pinprick stimulation (n=28, mixed effects, $Z > 2.7$, cluster threshold $p < 0.05$), which was used to define the regions of interest investigated. These included the anterior insula (red), posterior insula (green), thalamus (blue), middle anterior cingulate cortex (green), amygdala (pink), brainstem (red) and putamen (dark pink).

10.4. Discussion

Using the setting of a randomised controlled trial, the analgesic mechanisms and predisposition to duloxetine were evaluated in individuals with chronic knee osteoarthritis pain following a 6-week treatment period.

The primary hypothesis that duloxetine would induce attenuation of pinprick-related brain activity could not be demonstrated since no significant changes were

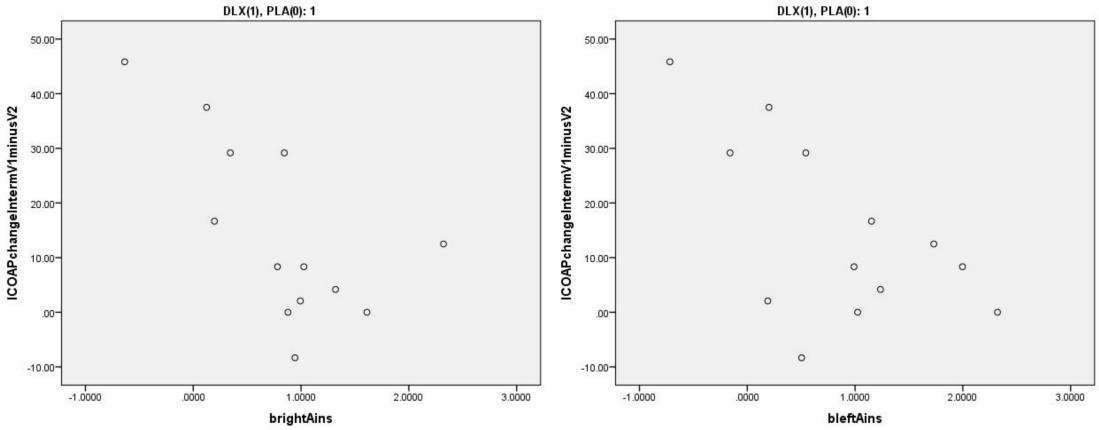


Figure 10.5.: **Change in ICOAP scores following treatment with duloxetine in relation to baseline activity within the right and left anterior insula.** Lower activity levels were associated with larger improvement in severity of chronic osteoarthritis pain, as measured by the ICOAP intermittent scale.

detected in the regions of interest investigated. However, it could also not be rejected given the reduced sample size with available imaging data and consequent lack of statistical power of the imaging sub-sample analysed here. Similarly, and even further affected by lack of power was the group comparison of ROI baseline activity between duloxetine responders and non-responders which did not detect group differences. Correlation tests, which were likely less affected by the small sample size, show association of baseline bilateral anterior insula activity with changes in ICOAP intermittent sub-scale scores, a measure of both pain intensity and the effect of osteoarthritis pain on quality of life, from baseline to endpoint.

It is well established that, among numerous functions, the anterior insula plays a key role in pain. The insula is considered to be related to interoception and

homeostasis / distress (Pollatos et al., 2016, Critchley et al., 2004) and serves important cognitive control functions, being a key region of the salience network (Seeley et al., 2007). It is not only considered to encode sensory aspects of pain but also to integrate limbic and salience information into perceptual decisions about pain (Wiech et al., 2010). Findings of baseline anterior insula activity association with duloxetine treatment outcomes and specifically the direction of such associations: lower baseline activity during pinprick stimulation relation with larger analgesia effect, may indicate that individuals with less salient activity during pinprick *i.e.* subjects less aroused by pinprick stimulation, are more likely to respond to the drug.

Alternatively, given the contrast metric (activity during pinprick against baseline activity) analysed here, reduced pinprick activity may be a consequence of ceiling effect, as a result of high baseline activity masking further stimulus-induced changes. In fact, the pinprick paradigm employed here, involved anticipation cues and the anterior insula has been previously found to be involved in the anticipation of pain in healthy volunteers (Wiech et al., 2010). Other measures such as the acquired arterial spin labelling data may provide further clarification. Nevertheless, given the anterior insula connections with various frontal, limbic and brainstem regions, including the amygdala and anterior cingulate cortex (Augustine, 1996), its involvement in duloxetine response prediction may additionally be related to the insula connections with descending inhibitory control regions such as the brainstem, which are rich in serotonin and noradrenaline, thus potentially reflecting the pharmacology of action of duloxetine. This is further supported by published

literature which found anterior insula activity involvement following duloxetine treatment in healthy volunteers (Van Marle et al., 2011). Also, in individuals with major depression, metabolism was found to differentially predict treatment response to a serotonin reuptake inhibitor drug and psychotherapy (McGrath et al., 2013).

Differently from diabetes neuropathy pain (Yarnitsky et al., 2012), conditioned pain modulation was not related with duloxetine efficacy. Nevertheless similarly to diabetes neuropathy (Yarnitsky et al., 2012), efficacy was not associated with temporal summation of pain.

The findings regarding a tendency for low ACC activity in responders to either placebo or active drug are interesting. Importantly, it has been hypothesized that aberrant functioning of the brain circuits which assign salience values to stimuli may contribute to chronic pain (Borsook et al., 2013). This may be reflecting that people who assign less salience to pain may be more likely to respond to placebo and other drugs. This will be followed on future analysis of the full cohort.

Psychometric and psychophysical data from the full group indicate that there were no differences in depression, anxiety, nor psychophysical scores between responders and non-responders to duloxetine. Responders however were found to ruminate more about their pain, according to a subscale of the PCS. According to the author of the scale, rumination corresponds to "I can't stop thinking about how much it hurts". This will be closely followed in the planned future analysis.

The interim analysis of imaging data presented here has an obvious major limitation which is the lack of statistical power for group comparisons. This likely

did not allow detection of potential duloxetine treatment effects on responders and non-responders groups, leading to potential false negative findings. Other factors such as the two protocol variations cases (no screen during pinprick and the missed dose increase to $60mg$) may have introduced further variance to the data.

Additionally, the approach of mean regional z-cores analysis may not be sensitive enough. Even if a brain area is significantly active, the activation may possibly only occur in a small proportion of voxels within the ROI, and therefore simply averaging across the entire region could swamp the signal from this small number of voxels with noise from the remaining non-activated voxels. Notably, not all candidate regions to prediction of duloxetine response were investigated here, including the subgenual cingulate and anterior default mode network regions such as the medial prefrontal cortex. However, these will be likely best captured by the resting state neuroimaging data. Also, certain processes that may be affected by duloxetine such as synchrony between different regions as found by previous studies in depression patients (Van Marle et al., 2011) were not examined here. Smaller masks and other methodology such as functional connectivity between regions, network analysis and multi-voxel pattern analysis may be more sensitive to capture neural patterns that may be affected by duloxetine or predict treatment responses. Moreover most of the findings here did not survive multiple test correction as the 95% confidence intervals defined at power calculation analysis of the IPRO study (Chapter 7) were based on Bonferroni correction.

Further limitations concern the general study set-up. For instance, two participants scored pain severity as little as 1.5 out of 10 at baseline. Ideally there would

have been a set threshold of minimum perceived pain intensity. However, based on experience with previous studies, this would have not been practical in terms of recruitment. Yet the approach utilised here is more representative of the general population with COAP. Additionally, TSP and CPM protocols were not subject to reproducibility tests and therefore must be interpreted with caution. A small number of controversial scores (potentially reflecting adaptation or sensitisation) were encountered which were considered as missing data in the present analysis. The ARUK Pain Centre plans to run further investigations on this, including reproducibility tests which should provide clarification. One pertaining question is nevertheless regarding the criteria for response which was considered as 50% reduction in pain severity. Perhaps a different criteria should have been considered for those individuals with low levels of baseline severity, such as full recovery. An additional threshold (30% improvement) should be additionally investigated in future analysis, as did past clinical trials of duloxetine in COAP (Chappell et al., 2011). Finally, one may argue that a higher dose of duloxetine could have been used in order to potentially increase effects of the drug, however the maximum recommended dose ($60mg$) to treat osteoarthritis was chosen (Citrome and Weiss-Citrome, 2012).

Given the limited available dataset at the time when the interim analysis presented here took place, it is not possible to draw firm conclusions regarding the neural mechanisms underlying duloxetine analgesia. However results indicate a role of the anterior insula for determining the likelihood of clinically meaningful duloxetine treatment in chronic knee osteoarthritis pain. The full dataset is now

available and will be combined with additional resting state BOLD and analysis of cerebral blood flow in order to infer firmer conclusions on the underlying neurofunctional mechanisms of duloxetine. Once such mechanisms become clearer, it is ultimately hoped to build a model of response prediction considering multi-dimensional contributions from neuroimaging, questionnaires and psychophysical data.

Part V.

General discussion

11

Discussion

11.1. Summary of findings

In this dissertation, the overall purpose was to improve understanding of central components of chronic knee osteoarthritis pain and mechanisms of centrally active analgesic treatment. Here it was shown that, differently from other chronic pain conditions such as back pain, and irritable bowel and chronic regional pain syndromes, chronic knee osteoarthritis pain does not seem to be associated with major structural reorganisation. It was also found that perceived intensity of chronic knee osteoarthritis pain was inversely correlated with γ-aminobutyric acid levels within the anterior cingulate cortex, which explained nearly 60% of the variance. The GABA relationship with pain severity was not dependent on

negative affect (depression and anxiety), nor was it dependent on pressure pain thresholds, suggesting a direct link to nociception rather than affective modulation.

Further, two intervention studies using the setting of RCT were established, and new investigation methodologies were developed. Interim analysis of the opioid study characterised pinprick-related BOLD activity in chronic knee osteoarthritis pain individuals, and found that pinprick-related activity in the mid-ACC was attenuated during infusion with the μ-opioid agonist remifentanil but not during saline infusion, in contrast with baseline measures. Additionally, patients with more impaired conditioned pain modulation presented stronger opioid modulation in the region. Differently from remifentanil, pinprick-related activity was not modulated by duloxetine, based on interim analysis of a sub-cohort of recruited participants with chronic knee osteoarthritis pain. Pinprick-related brain activity within bilateral anterior insular cortices was associated with the extent of chronic pain relief following duloxetine treatment. This suggests a potential role of the salience network in prediction of treatment response. In this case, subjects less aroused by pinprick stimulation would more likely to respond to the duloxetine. Interestingly, responders to either placebo or duloxetine presented a tendency for lower pinprick-related ACC activity at baseline as compared to non-responders.

The aim was achieved as novel evidence was provided for a clearer understanding of central mechanisms of COAP and its analgesia. Even though analyses of the opioid and antidepressant studies were based on a sub sample of the full dataset acquired, as recruitment was still ongoing. Both RCTs, which were put in place during the PhD programme, have now been completed, and analyses of the entire

dataset is ongoing. Despite this, the work presented here provides some important contributions to both the ARUK Pain Centre and the field of chronic pain research.

11.2. Scientific impact and future directions

Evidence from the cross-sectional studies presented here suggests that the major structural and neurochemical brain reorganisation seen in other chronic pain conditions are likely pain-type specific. This is relevant as it indicates that certain drugs that are effective for one given condition may not work for another. Similarly, the partial overlap of GABA encoding pain intensity observed in the nociceptive COAP cohort studied, with fibromyalgia, suggests that certain mechanism-based drugs may be useful for both COAP and other pain conditions. Additionally, the inhibitory neurotransmission findings, which were independent of negative affect, suggest GABA as a mechanistic biomarker of COAP.

The putative predictive association of baseline insular and ACC activity with pain relief, together with the molecular findings within the ACC, provides further evidence for the role of the salience network in chronic pain, both on the molecular and functional levels. To our knowledge, this is the first observation of a potential predictive value of salience activity in chronic pain. Convergent evidence from functional studies supports the contention of chronic pain being a condition of altered responsive salience (Borsook et al., 2013). The GABA study suggests more ACC dishinibition with higher levels of pain. Mechanistic or dynamic GABA measurements would be needed to validate these findings. If validated, they would

be especially relevant for evaluation of therapeutic interventions. GABAergic modulation within the ACC could be measured and potentially prove to be a more useful guide to novel treatment discovery than the classical approaches, which seem to rely primarily on particular receptor systems of drugs, with a specific mechanism of action. Additionally, the strong negative interrelation of GABA and pain intensity represents an advance that may beneficially impact evaluation of personalised treatments for chronic pain. For instance, individuals with high levels of COAP may be more responsive to treatments known to enhance GABAergic neurotransmission, such as gabapentin and transcranial magnetic stimulation (Stagg et al., 2011, Kuzniecky et al., 2002).

Further work is required in order to confirm whether mACC GABA predicts salience network activity, and if up regulation of such network may mediate the association with chronic pain. In this direction, the work presented here has already influenced research within the ARUK pain centre, in at least two ways. First, pooled analysis of data from multiple studies is being carried out as an investigation of whether GABA within the mACC is related to changes in salience network activity (based BOLD fMRI data at rest), or fractional amplitude of low frequency fluctuations. Second, it was decided to include spectroscopy as part of the duloxetine study in order to verify whether mACC GABA would indeed increase with pain reduction, following duloxetine treatment. This dataset has been processed and quality controlled by Andrew Cooper and I, and is ready for statistical inferences. Moreover, plans for further study of ACC GABA and glutamate at 7T in pain are currently being discussed and would allow increased

sensitivity to observe GABA changes during nociceptive stimulation.

The two studies of central analgesia established here are novel in terms of their advancements in mechanistic understanding of pain relief in chronic pain. They make a crucial step towards understanding of mechanisms of central analgesia for a number of reasons. Most previous neuroimaging studies focused on studying the effects of analgesics in the healthy brain, while applying nociceptive stimulation, sometimes under the idea that such stimulus would mimic pain, and potentially the brain in chronic pain. As previously discussed, there is extensive evidence indicating that the healthy brain likely does not mimic the brain in chronic pain. Also, most of these studies only looked at the effects of acute interventions rather than sub-chronic (Duff et al., 2015). The two drug studies presented here focus on a chronic pain population, and involve an acute and a sub-chronic intervention. Additionally, they add novelty to the field by its methodological innovations, where multi-modal MRI, together with psychometric and psychophysical testing were performed in order to evaluate contributions from multiple factors, which have been previously shown to play a role in chronic pain.

The opioid study results (study three), when contrasted with the published literature of central analgesia, suggest shared neural mechanisms of opioidergic analgesia between chronic pain conditions and the healthy state. Collectively, they also reveal that conditioned pain modulation (CPM) might allow prediction of the need for centrally acting drugs. CPM was however not related with differential treatment outcomes to duloxetine treatment (study four). Instead, the duloxetine findings are indicative of a putative role of the anterior insula, in determining

the likelihood of clinically meaningful response to duloxetine treatment. However, as emphasized a number of times, these are only preliminary investigations and represent only a small proportion of the planned analyses. In order to fully address the pre-defined hypotheses (Chapter 7), further neuroimaging methods will be used, including network analysis (of both functional and structural data), and functional and effective connectivity of functional data. Machine learning approaches will be then used for prediction of treatment outcomes. Ultimately it is hoped that a multivariate model for prediction of treatment outcome will become available. Altogether, these analgesic studies may allow pinpointing precise phenotyping of patients into distinct groups, providing potential for personalised treatment optimisation through individual prediction.

Finally, a number of exploratory outcomes will also be investigated. These include: the study of placebo mechanisms; prediction of likelihood of a subject to develop side effects to duloxetine; group level relations between remifentanil and duloxetine to see whether duloxetine response can be related to specific features in the observed effects of a single acute opioid infusion. Athough of exploratory nature, these questions may provide preliminary information for future investigations.

11.3. Limitations

Adding to the limitations already discussed in the study-specific chapters, a number of methodological issues need to be additionally considered or emphasized here, as some of them could potentially impact on the validity of results and warrant

further investigation.

The ImPOA data considered in Chapter 6 and Chapter 5 had a number of inconsistencies in data acquisition. Since there are no clear valid way of dealing with large variations in voxel sizes of diffusion data, a number of datasets had to be excluded from analysis. Also, even though the variation in the spectroscopy volume of interest across subjects was not associated with metabolite levels, they may have masked potential associations or group differences.

Recently, in an attempt to validate common statistical methods for analysis of fMRI data, it was found that, for a family wise error rate of 5%, parametric methods with cluster-based inference have inflated false positive rates (Eklund et al., 2016). In both remifentanil (Chapter 9) and duloxetine (Chapter 10) analyses, the widely used FLAME tool within FSL was used to generate brain activity maps representing response to pinprick stimulation. FLAME is one such parametric test and, since cluster-based inferences were used, they may have yielded increased false positive results. However, since the group differences were all based on average scores within region of interest, these results were not directly affected. Even so, on the planned analyses of the full IPRO cohorts, either a non-parametric testing (such as the *randomise* tool within FSL) or a parametric voxel-based inference method will be used, both of which have been shown to be valid (Eklund et al., 2016).

Notably, the recruitment target of 81 participants on the duloxetine study was not achieved, which was supposed to yield 19 datasets per group of responders and non-responders to duloxetine. This was due to the unexpectedly high drop

out rates at the latest stage of recruitment, which also coincided with scanner closure due to building work within the MRI unit. Given that production of placebo and encapsulation of duloxetine medication takes around six weeks, it was not feasible to order a new batch at such late stage to account for the increased drop out rates. Numbers are however very close to the target on the duloxetine arm 18 responders and 16 non-responders, and passed the target on the placebo arm, with 22 completed. Also, the sample size is significantly larger than other published mechanistic studies using duloxetine (13 patients, 20 controls (López-Solà et al., 2010); 26 controls (Van Marle et al., 2011)). They are higher than a recent predictive neuroimaging study of duloxetine in COAP, where responders and non-responders to duloxetine constituted a total of 19 participants, against 20 controls (Tétreault et al., 2016). Finally, as mentioned above, there are concrete plans for pooled analysis of the duloxetine RCT data presented by Tetrealt and colleagues (Tétreault et al., 2016), in collaboration with the Apkarian Lab at Northwestern University.

A major drawback from the two interim analyses of analgesia is obviously the small sample size and consequent lack of statistical power. This was necessary as the challenging recruitment of participants was not completed before the end of the third year of the PhD programme. However thanks to major efforts from the team of researchers, and despite the extensive exclusion criteria, recruitment performed significantly better than previous studies. Also, it is not best practice to perform interim analyses in blinded trials, as this may introduce bias to further data collection. However, the data analyst was kept blinded. Moreover, a consequence

of the lack of statistical power on the interim analyses presented here, was that it did not allow evaluation of predictive models of treatment response. However, with the ongoing analyses of the full cohort data, it will be possible to evaluate such predictive models, involving multidimensional variables. Mechanisms of placebo response and prediction will be additionally investigated, taking into account the recently published predictive model of placebo response proposed by Tetrealt and colleagues (Tétreault et al., 2016).

A further limitation from IPRO was that questionnaires were completed in paper, requiring manual digitisation and scoring. This system is prone to human error, however most digitisation and scoring of key data was double-checked by a second person. Currently, process of full checks of key data is ongoing. Future studies should nevertheless aim to provide electronic alternatives via, for example, the "Research electronic data capture" (REDcap) (Harris et al., 2009a).

Under a neuroimaging perspective, there were a number of limitations on the MR data acquisition protocols. For instance, added to the limitations already described in Chapter 5, the diffusion MRI protocol limited the types of analysis, as it had only one weighting factor and was acquired only at thirty directions. This was due to the lack of multi-band on the MRI system, which would have otherwise allowed more sophisticated inferences of the microstructere, using, for example, probabilistic tractography for reconstructions of the axonal bundles or neurite orientation dispersion and density imaging (Zhang et al., 2012). The quality of BOLD acquisition was also limited. For instance a dual echo technique could have been employed in order to correct for noise sources (Bright and Murphy, 2013).

As discussed in Chapter 10, conditioned pain modulation CPM and temporal summation of pain (TSP) findings must be interpreted with caution, because no reproducibility tests were performed. Even though the TSP protocol used here is well established amongst the chronic pain research community, there were some controversial scores, indicating adaptation to the stimuli. Some controversial CPM scores were also encountered and treated as missing variables. Problems with CPM reproducibility have recently been raised at the International Association for the Study of Pain by other groups, and are currently being discussed at the ARUK Pain Centre. The centre has plans for immediate investigation of such issues. In the meantime, efforts have been made for developing user-independent, computer-controlled methodologies for measuring CPM which have shown improved reproducibility compared to other widely used methods (Graven-Nielsen et al., 2017). A learning journey, based on general limitations of the studies presented here, is described in footnote[1]

[1] As mentioned above, the studies presented here contain several limitations, however each of these served as invaluable lessons which were thus attempted to be overcome on the studies that proceeded. Some examples are highlighted below.

Starting with ImPOA diffusion MRI study (study one), considerable amounts of time were spent analysing images which were later found not to be suitable for consideration in the analysis, given the highly heterogeneous acquisition. The importance of regular quality assurance of imaging data became clear. For all the proceeding analyses of neural data, the first step after data acquisition consisted of careful quality inspection of data and definition of clear thresholds for accepted quality.

Similarly to the dMRI data, acquisition of ImPOA spectroscopic datasets (study two) had a number of inconsistencies, such as variations in volume of interest' size or placement, which were immediately detected during quality assurance. Variations in data acquisition result in time-consuming analysis, and potential exclusion of datasets given the additional variance that is introduced to the data by these inconsistencies. It became clear the need for written standard scanner- and study-specific operation protocols (SOP), training of radiographers and having a second trained person supervising MR data acquisition.

All these factors were addressed prior to the start of IPRO data acquisition (studies three and four). Around ninety percent of the IPRO scans were either performed or supervised by me, but all scans were supervised by one of the researchers who had been trained on the specific IPRO acquisition SOP. Quality assurance has been now performed on all structural, functional and spectroscopic data of the duloxetine study and only one operator-related error was found, where the wrong orientation of slices acquisition was used. Given the regular

11.4. Conclusion

We have come a long way from Descartes' concept of pain pathways. Major contribution to the understanding of chronic pain came from neuroimaging research of such conditions. Yet, the field remains young and continues to grow from both a technology point of view and from questions posed by technology.

Having completed the work presented here, which involved direct interaction and conversations with more than a hundred individuals suffering from chronic pain, it becomes clear that chronic pain pain surpasses boundaries of classical individual research fields. Integration from multiple fields seems necessary in order to fully elucidate underlying mechanisms of chronic pain and analgesia, such as genetics, psychology, psychophysics and molecular, functional and structural, investigations. An effort towards this end have been made as part of this PhD, where collaborators from multiple fields were sought, as well as patient representatives, who provided input for shaping this research. Further investigations expanding on

quality assurance checks, it was possible to reschedule the participant to repeat the baseline scan before sub-chronic treatment took place, therefore avoiding data loss.

Additionally, it also became clear the importance of clearly defining specific hypotheses and planned statistical tests for analyses before any data analysis takes place. This is in order to keep validity of statistical tests and avoid mistakenly considering *post-hoc* exploratory, or data-driven analyses, as *a-priori* defined. Given recent findings on lack of reproducibility of scientific studies (Poldrack et al., 2017), it was decided to preregister IPRO on *clinicaltrials.gov* and make a more detailed, in-depth description of the duloxetine study protocol publicly available (Reckziegel et al, accepted with minor revision on BMJ Open), according to proposed guidelines for improving the manner science is performed (Gorgolewski and Poldrack, 2016). Furthermore, for the statistical analyses of the full IPRO cohorts, there are plans to blindly use one real code and two scrambled codes of treatment allocation, in order to minimise the effects of subjective bias in the results.

Together with numerous other teachings from studies conducted as part of this PhD, these lessons trained me towards being an independent researcher. In particular, lessons learned while driving the two analgesia studies, from the very initial general sketch of design to the end of data collection, and (currently ongoing) analyses of data, were immeasurably important towards this end. Thanks to this, I am currently already leading another randomised controlled trial in chronic pain at Northwestern University, since October 2016.

this dissertation's work are already ongoing.

According to Hippocrates, *"divinum opus est sedare dolorem"*, *i.e.*, pain alleviation is a divine act. Thus, it may be rewarding not only for patients but also pain scientists. It remains to be seen whether the proposed multidisciplinary integration of fields will be more successful in delivering more effective therapies for chronic pain. As an early-career scientist I am committed to continue the much required and urgent efforts towards the alleviation of the suffering in individuals with chronic pain.

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Appendix

A

ImPOA questionnaires

BDI®-II

Date:

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today.** Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
- 0 I do not feel sad.
- 1 I feel sad much of the time.
- 2 I am sad all the time.
- 3 I am so sad or unhappy that I can't stand it.

2. Pessimism
- 0 I am not discouraged about my future.
- 1 I feel more discouraged about my future than I used to be.
- 2 I do not expect things to work out for me.
- 3 I feel my future is hopeless and will only get worse.

3. Past Failure
- 0 I do not feel like a failure.
- 1 I have failed more than I should have.
- 2 As I look back, I see a lot of failures.
- 3 I feel I am a total failure as a person.

4. Loss of Pleasure
- 0 I get as much pleasure as I ever did from the things I enjoy.
- 1 I don't enjoy things as much as I used to.
- 2 I get very little pleasure from the things I used to enjoy.
- 3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings
- 0 I don't feel particularly guilty.
- 1 I feel guilty over many things I have done or should have done.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6. Punishment Feelings
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7. Self-Dislike
- 0 I feel the same about myself as ever.
- 1 I have lost confidence in myself.
- 2 I am disappointed in myself.
- 3 I dislike myself.

8. Self-Criticalness
- 0 I don't criticize or blame myself more than usual.
- 1 I am more critical of myself than I used to be.
- 2 I criticize myself for all of my faults.
- 3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10. Crying
- 0 I don't cry any more than I used to.
- 1 I cry more than I used to.
- 2 I cry over every little thing.
- 3 I feel like crying, but I can't.

_____ Subtotal Page 1

Continued on Back

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11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.
1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.
2a I sleep a lot more than usual.
2b I sleep a lot less than usual.
3a I sleep most of the day.
3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.
1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.
2a My appetite is much less than before.
2b My appetite is much greater than usual.
3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

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_____ Subtotal Page 2

_____ Subtotal Page 1

_____ Total Score

painDETECT

PAIN QUESTIONNAIRE

Date: _____

How would you assess your pain **now**, at this moment?

0 1 2 3 4 5 6 7 8 9 10

none max.

How strong was the **strongest** pain during the past 4 weeks?

0 1 2 3 4 5 6 7 8 9 10

none max.

How strong was the pain during the past 4 weeks **on average**?

0 1 2 3 4 5 6 7 8 9 10

none max.

Mark the picture that best describes the course of your pain:



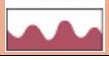
Persistent pain with slight fluctuations ☐



Persistent pain with pain attacks ☐



Pain attacks without pain between them ☐



Pain attacks with pain between them ☐

Please mark your **main area of pain**



Does your pain radiate to other regions of your body? yes ☐ no ☐

If yes, please draw the direction in which the pain radiates.

Do you suffer from a burning sensation (e.g., stinging nettles) in the marked areas?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Do you have a tingling or prickling sensation in the area of your pain (like crawling ants or electrical tingling)?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Is light touching (clothing, a blanket) in this area painful?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Do you have sudden pain attacks in the area of your pain, like electric shocks?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Is cold or heat (bath water) in this area occasionally painful?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Do you suffer from a sensation of numbness in the areas that you marked?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

Does slight pressure in this area, e.g., with a finger, trigger pain?

never ☐ hardly noticed ☐ slightly ☐ moderately ☐ strongly ☐ very strongly ☐

(To be filled out by the physician)

never	hardly noticed	slightly	moderately	strongly	very strongly
☐ x 0 = 0	☐ x 1 = ☐	☐ x 2 = ☐☐	☐ x 3 = ☐☐	☐ x 4 = ☐☐	☐ x 5 = ☐☐

Total score ☐☐ **out of 35**

Development/Reference: R. Freynhagen, R. Baron, U. Gockel, T.R. Tölle / Curr Med Res Opin, Vol.22, No. 10 (2006)

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Michael JL Sullivan

PCS

Date: _____

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures or surgery.

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

0 – not at all **1** – to a slight degree **2** – to a moderate degree **3** – to a great degree **4** – all the time

When I'm in pain …

1. ☐ I worry all the time about whether the pain will end.
2. ☐ I feel I can't go on.
3. ☐ It's terrible and I think it's never going to get any better.
4. ☐ It's awful and I feel that it overwhelms me.
5. ☐ I feel I can't stand it anymore.
6. ☐ I become afraid that the pain will get worse.
7. ☐ I keep thinking of other painful events.
8. ☐ I anxiously want the pain to go away.
9. ☐ I can't seem to keep it out of my mind.
10. ☐ I keep thinking about how much it hurts.
11. ☐ I keep thinking about how badly I want the pain to stop.
12. ☐ There's nothing I can do to reduce the intensity of the pain.
13. ☐ I wonder whether something serious may happen.

… Total

Questionnaire 4: (STAI)

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

DIRECTIONS

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel.

	Almost Never	Sometimes	Often	Almost Always
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool, and collected"	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

B

IPRO questionnaires

STUDY ID #:_____ DO NOT WRITE ABOVE THIS LINE HOSPITAL #: _____

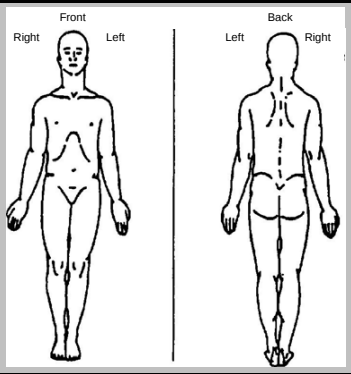
Brief Pain Inventory (Short Form)

Date: ____/ ____/ ____

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these every-day kinds of pain today?

1. Yes 2. No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain by circling the one number that best describes your pain at its worst in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

4. Please rate your pain by circling the one number that best describes your pain at its least in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

5. Please rate your pain by circling the one number that best describes your pain on the average.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

6. Please rate your pain by circling the one number that tells how much pain you have right now.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

STUDY ID #:_____ DO NOT WRITE ABOVE THIS LINE HOSPITAL #: _____

Date: ___/____/____

7. What treatments or medications are you receiving for your pain?

8. In the last 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
No Relief										Complete Relief

9. Circle the one number that describes how, during the past 24 hours, pain has interfered with your:

A. General Activity

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

B. Mood

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

C. Walking Ability

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

D. Normal Work (includes both work outside the home and housework)

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

E. Relations with other people

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

F. Sleep

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

G. Enjoyment of life

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

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{merge ID here}

A Measure of Intermittent and Constant Osteoarthritis Pain, ICOAP: KNEE Version

People have told us that they experience different kinds of pain (including aching or discomfort) in their knee. To get a better sense of the different types of knee pain you may experience, we would like to ask you about any "constant pain" (pain you have all the time) separately from any pain that you may experience less often, that is, "pain that comes and goes". The following questions will ask you about the pain that you have experienced in your knee in the PAST WEEK. Please answer ALL questions.

A) CONSTANT PAIN

For each of the following questions, please select the response that best describes, on average, your *constant knee pain* in the PAST WEEK.

1. In the past week, how intense has your *constant knee pain* been?

□0	□1	□2	□3	□4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

2. In the past week, how much has your *constant knee pain* affected your sleep?

□0	□1	□2	□3	□4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

3. In the past week, how much has your *constant knee pain* affected your overall quality of life?

□0	□1	□2	□3	□4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

4. In the past week, how frustrated or annoyed have you been by your *constant knee pain?*

□0	□1	□2	□3	□4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

5. In the past week, how upset or worried have you been by your *constant knee pain*?

□0	□1	□2	□3	□4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

{merge ID here}

B) PAIN THAT COMES AND GOES

For each of the following questions, please select the response that best describes your *knee pain that comes and goes*, *on average,* in the PAST WEEK.

6. In the past week, how intense has your most severe *knee pain that comes and goes* been?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

7. In the past week, how frequently has this *knee pain that comes and goes* occurred?

☐0	☐1	☐2	☐3	☐4
Never/ No knee pain that comes and goes	Rarely	Sometimes	Often	Very Often

8. In the past week, how much has your *knee pain that comes and goes* affected your sleep?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

9. In the past week, how much has your *knee pain that comes and goes* affected your overall quality of life?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

10. In the past week, how frustrated or annoyed have you been by your *knee pain that comes and goes*?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

11. In the past week, how upset or worried have you been by your *knee pain that comes and goes*?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

THANK YOU!

Educational level

Date: / /

Study ID: .....

Please tick the appropriate box that best describes your educational attainment

(Information will be kept private and confidential)

	Level 1	Higher degree and professional qualifications, Postgraduate degree/qualifications
	Level 2	Undergraduate degree
	Level 3	Diploma, certificate (tertiary qualification below degree level)
	Level 4	Other sub-degree (technical and business qualifications above A-level but below degree level)
	Level 5	A and AS level GCE
	Level 6	Below AS level (O-level GCE/ GCSE, CSE, vocational qualifications below sub-degree level)
	Level 7	Apprenticeship
	Level 8	None

Reference: modified from Muriel Egerton and Killian Mullan (2006)

Pain duration=.....

Body weight=..... Kg

Body height=..... cm

BMI=

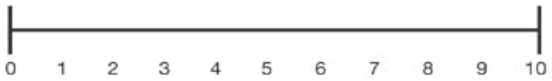
Notes: .....

.....

The Pain Sleep Questionnaire

Please rate the following questions by placing a vertical mark on each of the lines below, where **‘0’** corresponds to **‘never’** and **‘10’** corresponds to **‘always’**.

Q1) ‘How often do you have trouble falling asleep due to pain?’



Q2) ‘How often do you need pain medication to fall asleep?’



Q3) ‘How often do you need sleep medication to fall asleep?’



Q4) ‘How often are you awakened by pain during the night?’



Q5) ‘How often are you awakened by pain in the morning?’



MINI-COG™

Instructions

ADMINISTRATION	SPECIAL INSTRUCTIONS
1. Get patient's attention and ask him or her to remember three unrelated words. Ask patient to repeat the words to ensure the learning was correct.	• Allow patient three tries, then go to next item. • The following word lists have been validated in a clinical study:[1–3] **Version 1** • Banana • Sunrise • Chair **Version 2** • Daughter • Heaven • Mountain **Version 3** • Village • Kitchen • Baby **Version 4** • River • Nation • Finger **Version 5** • Captain • Garden • Picture **Version 6** • Leader • Season • Table
2. Ask patient to draw the face of a clock. After numbers are on the face, ask patient to draw hands to read 10 minutes after 11:00 (or 20 minutes after 8:00).	• Either a blank piece of paper or a preprinted circle (other side) may be used. • A correct response is all numbers placed in approximately the correct positions AND the hands pointing to the 11 and 2 (or the 4 and 8). • These two specific times are more sensitive than others. • A clock should not be visible to the patient during this task. • Refusal to draw a clock is scored abnormal. • Move to next step if clock not complete within three minutes.
3. Ask the patient to recall the three words from Step 1.	Ask the patient to recall the three words you stated in Step 1.

Scoring

3 recalled words	Negative for cognitive impairment
1-2 recalled words + normal CDT	Negative for cognitive impairment
1-2 recalled words + abnormal CDT	Positive for cognitive impairment
0 recalled words	Positive for cognitive impairment

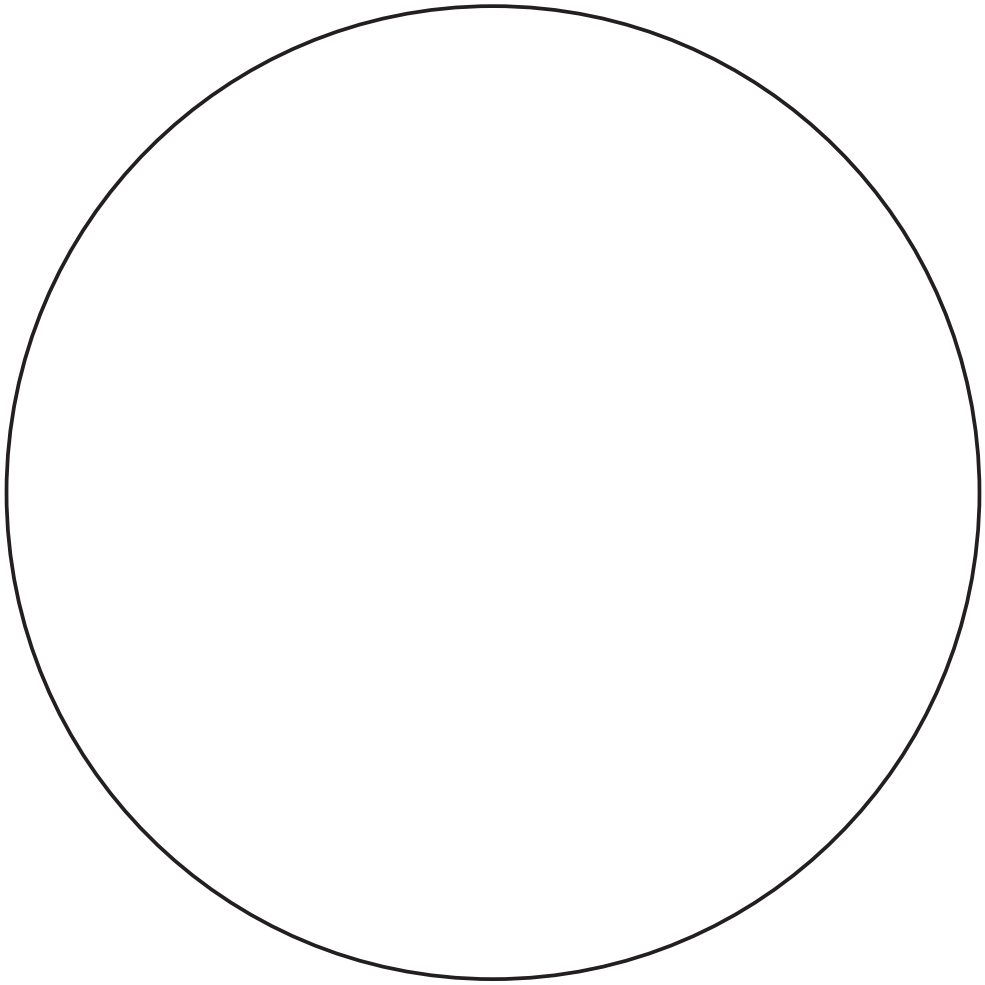
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1. Borson S, Scanlan J, Brush M, Vitaliano P, Dokmak A. The mini-cog: a cognitive "vital signs" measure for dementia screening in multi-lingual elderly. Int J Geriatr Psychiatry. 2000;15(11):1021-1027.
2. Borson S, Scanlan JM, Chen P, Ganguli M. The Mini-Cog as a screen for dementia: validation in a population-based sample. J Am Geriatr Soc. 2003;51(10):1451-1454.
3. McCarten JR, Anderson P Kuskowski MA et al. Finding dementia in primary care: the results of a clinical demonstration project. J Am Geritr Soc. 2012;60(2):210-217.

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CLOCK DRAWING TEST

Date: _____



With regards to the value of the medications you have been taking to treat your pain, please write your answers to the following questions:

Q1) Can you please, describe the effect that treatment had on your condition?

Q2) Can you please, talk to me about what you expect from medical care for your pain/arthritis?

Q3) Can you please, tell me what makes you satisfied with the treatment you receive?

Q4) Can you please, tell me what makes you dissatisfied with treatment you receive?

C

MHRS classification

RE: SCOPE: Protocol Review - Imaging Pain Relief in Osteoarthritis (IPRO)

Clinical Trial Helpline [ctdhelpline@mhra.gsi.gov.uk]

You forwarded this message on 21/11/2013 14:05.

Sent: 21 November 2013 13:14
To: Diane Reckziegel [Diane.Reckziegel@nottingham.ac.uk]

Notification that a Clinical Trial Authorisation (CTA) is not required

Dear Ms Reckziegel

Thank you for your email dated 19 November 2013 and for asking the MHRA to consider your proposed study again.

Following a review of your protocol we consider your proposed trial not to be a Clinical trial of an Investigational Medicinal Product (CTIMP). Based on the information provided, we consider that your study is assessing the physiology of pain relief in knee osteoarthritis, and therefore Clinical Trial Authorisation is not required.

Kind regards

Clinical Trial Helpline
MHRA

-----Original Message-----
From: Diane Reckziegel [mailto:Diane.Reckziegel@nottingham.ac.uk]
Sent: 19 November 2013 18:43
To: Clinical Trial Helpline
Subject: SCOPE: Protocol Review - Imaging Pain Relief in Osteoarthritis (IPRO)

Dear all,

I have contacted you previously with regards to a study involving duloxetine and remifentanil. The study protocol is now ready and has changed significantly since previous consultation. I would like to hear from you with a final opinion as to whether it is classified as a mechanistic study or clinical trial of IMP. Please find the protocol attached.

Many thanks,
Diane

D

Patient Information Sheet

Nottingham University Hospitals NHS Trust | Arthritis Research UK pain centre | The University of Nottingham

PATIENT INFORMATION SHEET
Version 2.4 11/08/15

"Imaging Pain Relief in Osteoarthritis"

Name of Investigators*: Prof. Dorothee Auer, Prof. Ravi Mahajan, Prof. David Walsh, Dr. Neil Nixon, Dr. Roger Knaggs, Mrs Nadia Frowd, Mrs Diane Reckziegel, Mr William Cottam, Dr. Helen Bailey, Dr. Avninder Chana, Miss Marianne Drabek, Dr Thomas Kurien*

You have been invited to take part in a research study which is forming part of a PhD which aims at improving understanding of pain relief mechanisms through brain imaging. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Please do take your time to decide whether you wish to take part or not. If you decide to take part you may keep this leaflet. Thank you for reading this.

Background

Through age and injury, joints (especially the knee) can show signs of degeneration, described as osteoarthritis (OA). Knee OA causes pain that may become chronic over time. The main treatment focus of knee osteoarthritis is pain relief and improvement of function. A variety of treatments to stop the pain (called analgesia treatments) are being used. The most effective ones are typically: invasive (steroid injections into the joint or surgery to remove the diseased joint and replace it by an artificial joint) or drugs (opioids), which cannot be used long-term due to side effects. Individual response to less harmful other analgesic drugs (non-opioid) is varied, and we do not know who would respond best to which treatment. Interestingly, a drug named Duloxetine, originally used to improve mood has been recently shown to be effective in relieving different types of chronic pain, including knee OA pain. However, this drug works in about half of the patients, but we do not know how this drug works to reduce pain, and to whom it may be more useful.

In addition, the exact mechanisms by which opioid agents, the strongest known painkillers, work in chronic pain are also not known.

What is the aim of the research?

By comparing the brains of patients with OA before and after Duloxetine treatment, as well as before and during treatment with the short-acting drug opioid (remifentanil), we hope to achieve a better understanding of the mechanisms of pain relief, whilst helping us select patients who might benefit more from Duloxetine treatment.

The research design involves:

1. Taking capsules of either Duloxetine or placebo (an identical looking capsule that does not contain any active ingredient) by mouth for six weeks.
2. Injecting i.v. (into a blood vessel of the arm) a small concentration of remifentanil or placebo for less than 20 minutes; and assessing the effects through questionnaires, imaging and sensory tests.

Why have you been chosen?
You have been asked to take part because you have either:

- Been diagnosed with OA.
- Previously taken part in research in the Arthritis Research UK Pain Centre at The University of Nottingham.
- Previously taken part in this program of research study.

(We are inviting around 93 participants like you to take part.)

Do I have to take part?

No. It is up to you to make this decision. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. (Even if you decide to take part you are still free to withdraw at any time and without giving a reason.) Your level of ongoing care will not be affected whether you take part or not.
If you do decide to take part, we would like to seek your permission to contact you again in the future regarding other research studies taking place at The University of Nottingham.

What are the benefits of taking part?

Duloxetine has been shown to alleviate pain in people with knee OA, so we expect some participants to have some pain relief. However, it is not clear who will benefit the most or how much the benefit will be. In addition, you will be helping us to have a better understanding of osteoarthritis pain relief which could in turn benefit you, and other osteoarthritis patients, in the future through the development of more effective treatment methods.

The MRI scans are not used for diagnostic purposes in individual cases and are not reviewed for this purpose. However, in the event of an incidental finding upon your MRI scan, we will need permission from you to contact your GP and an appropriate course of action or further tests will be arranged for you.

What will happen to me if I take part?
If you do volunteer, you will be asked to complete some health questionnaires and asked to attend the Queen's Medical Centre/University of Nottingham on two occasions.

We will ask your permission to look at your medical notes prior to your first visit to ensure you have no medical conditions that may prevent you from taking part in the study.

On your first visit you may be asked to:

- Fill out some additional health questionnaires;
- Give a **blood sample**;
- Undergo an **MRI scan** (a non-invasive way of gathering images of your brain and its function). (While having the MRI scan, you may receive an injection of remifentanil or placebo for a period of less than 20 minutes.);
- Undergo quantitative sensory tests (QST) to examine your threshold to different temperatures, pin pressure and/or other pressure sensations.

- (You will be given instructions for taking the capsules and supplied with either Duloxetine or placebo.) Upon your first visit you will be asked to take the given capsules once a day for six weeks.
- Keep a diary of the painkillers and doses you take each day.

The treatment with Duloxetine will consist of two initial weeks with a low dose (one capsule), four weeks with normal dose (two capsules), then two weeks with decreased dose (one capsule). This is in order to avoid any adverse effects from stopping taking the drug.

The treatment with placebo will consist of six weeks of taking mock tablets not containing any active substance, and will follow the same pattern (apart from dose reduction period).

You will be asked to come back at the end of week six for your last visit.

During this second and last visit you may be asked to:

- Fill out some of the health questionnaires again;
- Undergo an MRI scan of your brain;
- Undergo quantitative sensory tests (QST) to examine your threshold to different temperatures, pin pressure and/or pressure sensations as in the first session.

At the end of the visit you will be informed as to whether or not you were taking Duloxetine and, if you were, will be given the reduced drug dose to wean you off over the next two weeks to avoid withdrawal symptoms.

Each of the two visits will last between two and three hours and you will be involved in the study for up to two and a half months. However, it may be that we delay the start of the medication intake in order to accommodate your availability as well as the MR scanner availability. During six weeks of Duloxetine/placebo treatment you will be asked to fill out the Brief Pain Inventory Questionnaire daily which will take less than five minutes of your time. If you are taking any strong painkiller medications, you may be asked to keep taking those medications until you come for your second visit, or let us know about any treatment changes during this time.

Visit schedule	
First visit	**Second visit**
Screening (study explanation, consent taking, medical records check, suitability for drug treatment check)	
Complete set of health questionnaires	Complete set of health questionnaires
QST	QST
Blood sample collection	
MRI scan	MRI scan
I.V. infusion with Remifentanil/placebo	
	Revealing whether participant has taken Duloxetine or placebo
Supplied with Duloxetine/placebo tablets	Supplied with Duloxetine for dose reduction

Please note that the remifentanil/placebo intervention may only happen once, and during the first visit. Some participants will receive both remifentanil/placebo and Duloxetine/placebo interventions, while others will be only given Duloxetine/placebo. Those receiving both interventions will be randomised twice, independently.

The quantity of blood required per participant will be no more than 14ml, which will be identified only with the study ID. Part will be stored in a freezer for genetic testing in

future studies linked to understanding pain and the other part may be collected for a blood test to further check suitability to undergo Duloxetine treatment. Genetic testing as well as storage for use in future studies is optional.

Another optional test measures how susceptible people are to hypnosis. This test involves induction of hypnosis and lasts around twenty minutes. If you take part in the study you may choose whether or not to participate in this test.

Should it not be possible to collect all the data during the two visits we may invite you for a third visit or send you extra questionnaires via post within twelve months after informed consent has been obtained.

What are the possible disadvantages and risks of taking part?

a. Drug side effects

Duloxetine and remifentanil intake may cause side effects in some people and these are discussed in detail in the Duloxetine and remifentanil leaflets you will receive. The main possible side effects of Duloxetine and remifentanil are given below.

Duloxetine side effects:

- Affecting more than 1 user in 10: headache, feeling sleepy; nausea, dry mouth.
- Affecting 1 to 10 users in 100: lack of appetite; trouble sleeping; feeling agitated; less sex drive; anxiety; difficulty or failure to experience orgasm; unusual dreams; dizziness, feeling sluggish, tremor, numbness, pricking or tingling of the skin; burred eyesight; hearing sound in the ear when there is no external sound; feeling the heart pumping in the chest; increased blood pressure, flushing; increased yawning; constipation, diarrhoea, stomach pain, vomiting, indigestion, breaking wind; increased sweating; muscle pain, muscle spasm; painful urination; problems getting an erection, changes in ejaculation; fatigue; weight loss.

Remifentanil side effects:

- Affecting more than 1 user in 10: muscle stiffness; nausea; vomiting; low blood pressure.
- Affecting 1 to 10 users in 100: slow heart beat; respiratory depression; breathing stops; itching.

The remifentanil side effects, if they occur, are short-lived and wear off after stopping the infusion.

In addition, the doses of both drugs utilised in this study are lower than in usual prescription, so the chance of experiencing side effects should be lower.

After the first visit you will be asked not to drive or make use of heavy machinery until the next day, as you may have been given remifentanil, a powerful morphine related painkiller, and therefore may be not be alert enough to drive.

b. QST

Part of the QST procedure uses a small device (called a Peltier Thermode) applied to the skin which will become warm and cool. Another small pen-like device with a flat-tip probe may also be used to apply a slight pin pressure to the skin and part of the procedure will involve some pressure against your knee (so called Pressure Algometer). Sports ice bags may also applied to your skin during the QST procedures. These may initially feel a little

uncomfortable for some people, with pain levels not exceeding moderately severe degrees.

The application of the pin pressure stimulators is a non-invasive procedure. These are designed in a way that penetration of the skin should not occur even with the heaviest weight. However, there is a small residual risk of penetration through the skin. For this reason, the devices are always disinfected before application.

As described above, some QST will also be carried out during your scans. You can request that we stop at any time.

c. Pregnancy and metallic implants

Pregnant women, or women who plan to become pregnant during the study period, must not take part in this study as we cannot guarantee that the MRI scan itself, or any of the drugs given during the study, will not harm the unborn baby. Women who are at risk of pregnancy may be asked to have a pregnancy test before taking part in order to exclude the possibility of pregnancy. Women who could become pregnant must use an effective contraceptive during the course of this study. Any woman who finds that she has become pregnant while taking part in the study should ***immediately*** tell the research doctor.

As the brain scan involves the use of a magnetic field, you should not take part in this study if you have any metallic foreign bodies in your body (e.g. shrapnel, surgical pins, other metal fragments). Body piercings are allowed providing they can be removed.

d. Other

Taking part will also mean taking time out of your normal activities, which may be for a few hours. This may inconvenience you, though we will be as flexible as we can with arranging a suitable time and day, and we will reimburse travel costs (30p per mile if you have driven or had a lift, and full reimbursement of public transport travel upon production of the appropriate receipts). Also, during your visits, you may be offered tea and biscuits.

Please note that, if you have a restricted diet, you may want to consider the fact that the drug capsule shell contains gelatine which is produced from bovine material (*i.e.*, derived from cattle).

In the event of discovering any abnormal findings on your brain scans we will disclose this information to either your GP or referring doctor, who will then discuss it with you.

To try and determine whether the drug we have given you has altered your symptoms, we will ask you to continue taking any painkillers you are on at the start of the study and not deviate from your normal dose and routine.

What if I don’t want to carry on with the study?

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and may still be used in the project analysis.

Involvement of your General Practitioner/Family doctor (GP)

We will inform your GP of your participation in the study. In addition, should any abnormal findings be detected on your MRI brain scans, questionnaires or blood test, we would seek your permission to inform either your GP/referring clinician who will then inform you of the findings, rather than the research clinician.

What if something goes wrong?

In the unlikely event that you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Please contact Professor Dorothee Auer in the first instance, telephone 0115 823 1178. If you remain unhappy and wish to complain formally, you can do this through NHS complaints. Details can be obtained from the Patient Advice and Liaison Service (PALS) at QMC by calling 0800 1830204

Will my taking part in this study be kept confidential?

We will follow best ethical and legal practice according to good clinical practice guidelines (GCP). Therefore all information about you will be handled in confidence and only by research staff and students with GCP training.

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. The researchers have a duty of confidentiality to you as a participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for 6-12 months after the end of the study so that we are able to contact you about the findings of the study *and possible follow-up studies* (unless you advise us that you do not wish to be contacted). All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

What will happen to the results of the research study?

The results will be published in medical journals and will be submitted as part of a PhD thesis. You will not be identified in any way in any report or publication. If you ask, we can send you a summary of our findings when the project finishes.

Who is organising and funding the research?

This work is supported by the University of Nottingham and Arthritis Research UK.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the East Midlands – Nottingham Research Ethics Committee 2.

Expenses & Payments

Your travel expenses may be reimbursed upon production of a receipt/ticket. Alternatively, if you have travelled to your appointment using your own vehicle, your travel expenses may be reimbursed at 30p per mile.

Contact for Further Information
If you have any questions please feel free to contact:

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E

PPT and CPM protocols

PPT CPM

Equipment

- From Somedic SENSEbox System:
  - **DCU** Data Collection Unit
  - **ALG** Deep Tissue pain transducer
  - **PPB** Patient push button
- Numerical Rating Scale
- Manual Sphygmomanometer + arm blood pressure cuff.
- Skin marker
- Stopwatch

Equipment complies with relevant IEC standards(IEC 60950 for IT equipment and IEC 60601-1 series for medical equipment, and has been verified to meet the EMC requirements of the MDD 93/42/EEC

The SENSEbox system should not be placed adjacent to or stacked with other equipment.

The SENSEbox is not designed to be used where flammable anaesthetic gases are used.

The SENSEbox is connected by USB to a laptop computer. The SENSEbox software is preloaded.

Excel is installed on the computer for report data, but other software installations are discouraged.

The computer is connected to external mains power supply to charge the battery. With the new software it can be kept plugged in during data collection. For any maintenance, adjustment or repair, it should be disconnected. The battery has a 4 hour capacity.

Use only the supplied cable and do not use any type of extender cable.

The Algometer (Mechanical pain threshold) and patient push button are connected to the SENSEbox using leads provided and can remain attached. The algometer connects to point 1 and the push button to point 2 or 3. They cannot be accidentally placed.

The parts of the transducers that are in contact with the patient are made from PEEK and should be disinfected before each use. PEEK is compatible with most common disinfectant used in the medical field, e.g. alcohol based solutions.

The mechanical stimuli are given to the intact skin by the hand held deep tissue pain transducer (ALG) or algometer.

Participant Preparation

1. Confirm that volunteer meets criteria and has read and understands the PIS.
2. Explain the study and obtain consent.
3. Explain the QST procedure. (Standard for each patient)

"The idea of this test is to look at pain thresholds in people with Osteoarthritis. We aren't looking at how much pain you can tolerate, simply at what point you start to feel pain. The pain you feel will only be fleeting, as the test will be stopped as soon as you indicate that you have started to feel pain. You will hold this push button in your dominant hand and I will start to apply a graded pressure to your sternum (breastbone). You will feel pressure as the probe is pressed down and the pressure will be gradually increased. As soon as the pressure starts to change to pain, you should press the button and I will withdraw the probe."

"The two tests are on the sternum and your most painful knee and are just a practice to let you know how it feels. I will then do the same 3 times each on your sternum and knee. There will be a few seconds in between each test."

"The second part of the test involves simultaneously applying pressure to your upper arm via a blood pressure cuff and repeating the pain threshold test on your knee."

"The computer records your pain threshold of each test and this will be compared with other volunteers who also have osteoarthritis in their knee. If, for any reason, you want to stop, let me know straight away."

"Do you have any questions?"

QST Procedure

Ensure patient is not experiencing more than their normal level of pain before applying stimuli. Ask participant to close their eyes during testing.

1. Begin Test Sites:
    a. Sternum (3cm from sternum notch)
    b. 2cm distal to Infero-medial aspect of patella (most painful knee)

2. Repeat as above, alternating between sites. Record 3 measurements for each site.

Apply the probe perpendicular to the skin and start the force application. Apply graded pressure following the probe light, keeping the colour of the indicator on green (the centre light on the probe).

(Red means the pressure is too hard and blue means not enough pressure).
When the patient starts to feel that the pressure has changed to pain, he/she presses the button. This gives an audible indication and the lights on the probe go out. At this point the probe is withdrawn and the pain threshold recorded.

Manually record PPT level as well as via automated data collection unit.

PPT CPM Verbal Instructions:

"I am now going to apply continuous pressure to your arm with the aid of a blood pressure cuff and ask you to repeat some hand grip exercises. I would like you to rate the sensation you perceive on a scale from 0-100 where 0 = no pain and 100 = worst pain imaginable." (***Show patient NRS***).

"I will then re-test your pain threshold on your knee with the cuff still inflated. As before, as soon as the pressure starts to change the pain, press the button and I will withdraw the probe, the cuff will also deflate."
"There will then be a short break and we will repeat the test".

"Do you have any questions?"

Operator Instructions:

- Read out verbal instructions.
- NRS target ≥40.
- Wrap 7.5cm wide tourniquet cuff around the contralateral arm to the knee being tested.
- Lower rim of tourniquet cuff should be 3cm proximal to cubital fossa.
- Set systolic pressure to 270mmHg. After target pressure is reached, ask participant to rate sensation in arm from 0-10.
- Repeat hand grip ≥10 times until 4 is reached on NRS. Ask for NRS rating every 5 hand grips.
- Once NRS 4 achieved, apply probe in same manner as before to knee test site.
- Once participant presses button, withdraw probe and release cuff.
- Wait until cuff evoked pain has subsided before re-test, minimum 1 minute.
- Repeat test.
- Calculate CPM effect: difference in average pain scores of the 2 test stimuli (with conditioning – without conditioning). Negative value = efficient CPM.

N.B

Test stimulus – PPT
Conditioning Stimulus – Cuff evoked pain.

Instructions for use DASYlab 12.0 operating instructions

Laptop Password: Somedic/2010

- Click on DASYLab 12.0 Shortcut icon on desktop (or if using the City Hospital kit, click on Dasylab icon, go to "file" open and click on DasySens20)

Press 'Start' - This will bring up start up dialogue box.

Type in:

- Study name (IPRO)
- Sites - meas 1-3: Sternum, Infero-medial knee, CPM
- Subject ID
- On second page enter operator's initials
- Press start

Calibration
To calibrate, click on the button 'Settings'

Hold the algometer on the desk with the pressure pad standing vertical.

Press "set 0", add 10 kp weight and press "set 10". Remove weight.

N.B. The pressure will naturally fluctuate a little after calibration e.g. between -1 and +1 kPa. However, if the resting pressure after removing the 10N weight is inappropriate e.g. -15N then simply repeat the previous step until acceptable.

Press patient button and PPB circle will go from red to green. Also gives audible signal.

The default settings are: Start 10, Ref 50, Plateau 1600, LED Gain 0.10 and Probe Size 1.0cm2.

Once fully calibrated, return to programme by clicking 'collection'

Before testing Sterilise probe head (prior to each patient)

To start testing, check that the correct anatomical site and occasion 1, 2 or 3 are highlighted in green

Press test START

To save reading press "store, shift, start" and this will automatically move on to the next site. If doing multiple tests at each site, you will need to click back on the required site and also occasion 1, 2 or 3.

If you don't want to save that run, just press "start" again (Equivalent of the reset button on the old programme).

When finished press 'Stop'

To exit click on 'quit program'

Click 'Start' which brings up dialogue box. Click on 'Start' in the dialogue box and this will close the application.

To access data

Open '**Data-Shortcut'** on the Desktop (Files are stored in: Computer/Local Disk (C:)/Data).

DASYLab automatically creates a directory based on the study name you entered earlier (such as 'main pain study'). This contains a separate file for each subject ID entered in that study.

Files are saved as an 'ASC' file type. This can be opened as a spreadsheet (in Excel for example) or as a regular text file in notepad. The layout will be the same in both.

If there are problems with the equipment the contact emails are:

bo.johansson@somedic.com

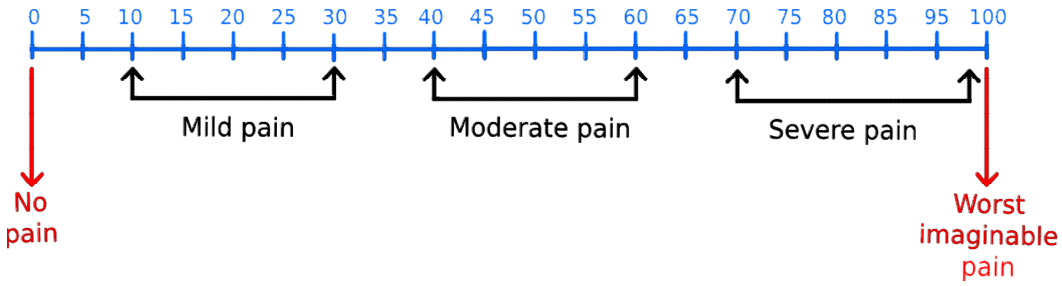
or:

torgny.karlsson@somedic.com

F

Numerical Rating Scale

Numerical Rating Scale



G

Temporal Summation protocol

Updated 01/04/15

Temporal Summation (Wind-up Ratio) Punctate "Pinprick" Protocol for IPRO

Equipment:

- Pinprick stimulators set (should be disinfected before use)*
- Ruler
- Pen for marking test sites
- Alcohol swabs

**Instructions for disinfection of the stimulators can be found at the end of this document.*

Participant preparation:

- Ensure the participant has given informed consent
- Explain the procedure to the participant:

  "This is a test of your ability to detect a sensation of 'Sharpness' or 'stinging'. For this test we will use a weighted blunt needle (pinprick stimulator). This stimulator will be pressed gently against your skin on your knee. The stimulator is only applied for 2 seconds, so the sensation of sharpness will be temporary. The pinprick stimulator is designed not to puncture your skin and is disinfected before we use it on every participant."

- Participant should close their eyes during definitive testing
- Ensure the participant is lying comfortably on the couch in a quiet room with both knees exposed.
- Identify and mark test sites.

Test site:

- Knee: Infero-medial aspect of patella

Training session

- Demonstrate how the equipment works, read out the participant instruction and check the participant understands what they need to do.
- Perform one measure over the ***least affected*** knee for training purposes only. Ask participant to rate the pain or sharpness they experience from 0-100 where 0 indicates no pain or sharpness and 100 indicates the most intense pain or sharpness imaginable. Use visual NRS sheet with verbal descriptors.

Temporal Summation (Wind-up Ratio):

"Once the experiment starts, I will apply a stimulator to your knee in the same place as before, but this time on your worst affected knee. Please rate the pain or sharpness you experience from 0-100 where 0 indicates no pain or sharpness and 100 indicates the most intense pain or sharpness imaginable. I will then apply the same stimulator at the same site 10 times repeatedly at a rate of 1/second. After completing the 10 pinpricks, please rate the overall pain or sharpness you experience, from 0-100 where 0 indicates no pain or sharpness and 100 indicates the most intense pain or sharpness imaginable. I will repeat the procedure twice.

Operator instruction:

A- Read out the participant instruction

B- Perform the following steps**:**

1- Apply the 256mN weighted pinprick stimulator perpendicular to the skin of infero-medial aspect of the patella of the worst affected knee.
2- Ask the participant to rate the pain or sharpness they experience from 0-100 where 0 indicates no pain or sharpness and 100 indicates the most intense pain or sharpness imaginable. Use NRS with verbal descriptors
3- Record the rating.
4- Apply the same stimulator at the same site 10 times repeatedly at a rate of 1/second. The size of this area should be around 1 cm square.
5- After completing the 10 times pinprick test, ask the participant to rate the pain or sharpness they experience overall, i.e. the series of 10 should be interpreted as a single pinprick. From 0-100 where 0 indicates no pain or sharpness and 100 indicates the most intense pain or sharpness imaginable.
6- Repeat the procedure twice.

7- The wind-up ratio (WUR) will be calculated as the mean pain rating of both series of repetitive pinprick stimuli divided by the mean pain rating of both single stimuli.

Disinfection

Before using the pinprick devices on a patient, they should be always disinfected.

Check if all necessary materials are available:

- Deconex (disinfectant)
- 200 ml beaker
- 10 ml measuring cylinder
- Disinfection unit
- Water
- Wa**tch**

Steps for disinfection

(a) By using the beaker and the measuring cylinder, a solution of water and Deconex should be prepared mixing 120ml of water and 5ml of Deconex;

(b) This should be placed inside the glass component of the disinfection unit;

(c) The white component of the disinfection unit should be placed on top of the glass component containing the solution;

(d) Now the seven pinpricks can be carefully placed inside each of the holes of the disinfection unit, in upward orientation so that the needles are immersed in the disinfection solution;

(e) Leave the devices immersed in the disinfection unit for 30 minutes;

(f) After 30 minutes, throw away the solution and leave the needles to dry for a few minutes.

(g) Carefully place the stimulators in order (according to weight) in the woden box, leaving the needles out of the devices if they are not dry.

The needle should already be in the outward position before the pinprick stimulators are applied onto the skin. Therefore, move the pinprick stimulators in the upright position before the application. Move them in your hand so that the weight slides up and down in order to release any resistance.

The stimulators should be applied to the skin in upright orientation and orthogonal direction to the skin. The stimulators should be placed in a smooth movement onto the respective area of the skin.

H

CPM fMRI

CPM protocol: Thermode + Cold Pressor

Equipment

- Digital Thermal Calibration Kit, MRI compatible.
- Velcro strap.
- 2x MRI compatible 23cm ice packs (***Kool Pak)*** each containing 500ml ice (300g) + 125ml water.
- Numerical Rating Scale with verbal descriptors
- Thermometer x1
- Stopwatch

Participant Preparation

· Ensure participant has given informed consent.
· Explain the procedure to the participant:

Verbal instructions:

"A temperature test of your skin will be performed using a special device that cools or warms your skin. The temperature will increase to warm and then a few moments later to hot, but each stimulus will only be applied for a few seconds. The temperatures will only differ slightly from each other.

I would like you to rate this sensation on a scale from 0 – 100, where 0 = no pain and 100 = worst pain you can imagine (***show participant NRS***). Some stimuli in the beginning might be higher than what you get to experience later on but at no point will you experience a temperature that you feel is intolerable, you can stop the device anytime by squeezing the buzzer.

The 0-100 scale will be used throughout the test, including when you are in the MRI scanner. There will be a short training exercise to let you become familiar with everything. In the second part of the test, we will simultaneously apply the temperature to your forearm whilst I place your foot into an ice/water shoe and ask you to rate the sensation in your foot.

Do you have any questions?"

· During definitive testing, participants should be asked to attend to the stimulus by closing their eyes.

Test Sites

- **Test stimulus:** Opposite forearm to foot (if possible).

- **Conditioning stimulus:** Foot - same side as least affected knee.

Training Session

Verbal instructions:

"This is the training exercise. The temperature device will deliver 2 different temperatures to your forearm, after each one I will ask you to rate the sensation from 0 – 100 on the scale.

I will then place your foot into the ice/water shoe and again prompt you to rate the intensity from 0-100 during 30 seconds. There will then be a short break.

At no point will you experience a temperature that you feel is intolerable, you can stop the device anytime by squeezing the buzzer.

Do you have any questions?"

Operator instructions:

- Read out verbal instructions.
- Identify test sites: dorsal aspect of foot – same side as least affected knee and volar aspect of ipsilateral forearm. Ensure patient is not experiencing more than their normal level of pain before applying stimuli.
- Demonstrate how the equipment works; check understanding of participant before proceeding to test.
- Training exercise 1 for test stimulus:
  - Attach thermode to volar aspect of pre-determined forearm with Velcro strap.
  - Ensure baseline temperature is set at 32°C.
  - Ask participant to attend to the stimulus by closing their eyes.
  - Apply two separate heat stimuli to forearm, each of 7 seconds duration ***from when thermode reaches desired temperature***.
  - Temperature values should be set at 43°C and 45°C and received in same order (43°C, 45°C)
  - Increase from baseline at 5°C/s. Decrease from peak temperature at 5˚C/s.
  - Ask participant to rate level of intensity from 0 - 100 on NRS once device has delivered desired temperature for 7 seconds.
  - Ensure any residual discomfort has gone before applying the next stimulus in the sequence, minimum inter-stimulus interval 1 minute.

- Training exercise 2 for conditioning stimulus:
  - Ask participant to attend to the stimulus (ice/water shoe), by closing their eyes.
  - Place participant's foot in-between ice bags for 40 seconds, ensuring contact area is predominantly over dorsal aspect.
  - Ask participants to measure intensity from 0-100 at 10s, 20s, 30s and 40s. Eyes closed the entire time; participant should be familiar with verbal descriptors on NRS before testing.

- When participant and operator are satisfied instructions have been understood and training exercises have been completed proceed to testing.
- Before testing ensure correct positioning and check patient is comfortable. Keep test sites exposed – forearm + hand and below knee.

Determining Test Stimulus Intensity (Pain-60).

Verbal instructions:

“During this experiment, the device will deliver different temperatures to your forearm. After each one, I would like you to rate the intensity you perceive on a scale from 0-100. Where 0 = no pain and 100 = worst pain you can imagine. Again, nothing will be intolerable, but squeeze the buzzer if anything is too uncomfortable and the device will cool down immediately. There will be a short break between each stimulus.”

“Do you have any questions?”

Operator instructions:

- ***Move thermode to volar aspect of opposite forearm, (opposite side to foot being used) to avoid sensitisation.***
- Read our participant instruction.
- Ensure thermode is correctly attached to forearm and set at a baseline temperature of 32°C, increase from baseline at 5°C/s, decrease from peak temperature at 5˚C/s.
- There should be a minimum 1 minute inter-stimulus interval.
- Ask participant to attend to the stimulus, by closing their eyes.
- Follow the algorithm to determine Pain-60:
- Once participant scores pain ≥60 stop algorithm.

Pain-60 Algorithm

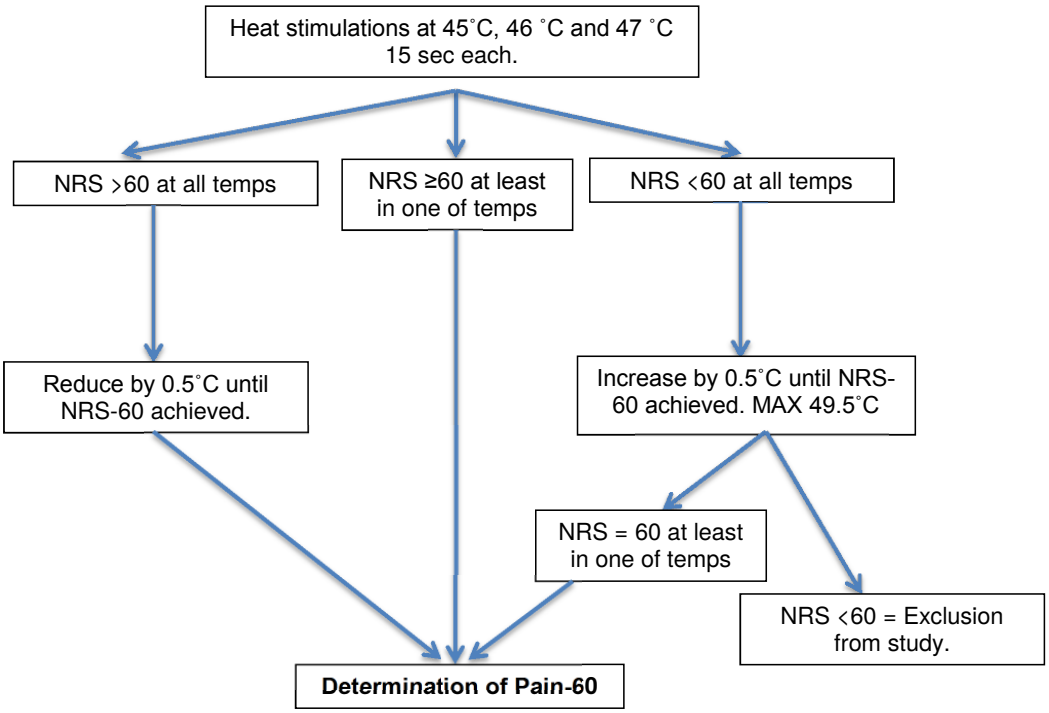


Fig 1: Flow Diagram for determination of pain–60. Adapted from original by Granot et al.[1]

Re-apply test stimulus scored at Pain-60 outside of scanner for 15 seconds to verify result before moving onto CPM.

[1] Granot M, et al 2007. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: Do conditioning stimulus painfulness, gender and personality variables matter? *Pain*, 136, 142-149

Applying Test Stimulus (fMRI)

Verbal instructions:

"During this experiment, I will apply a temperature to your forearm and ask you to score the sensation you perceive several times during the MR scan using the buttons. Again, nothing will be intolerable, but press the button if anything is too uncomfortable and the device will cool down immediately."

"Do you have any questions?"

Operator instructions:

- Read out verbal instructions.
- Ensure thermode is correctly attached to non-dominant forearm and set at a baseline temperature of 32˚C, increase from baseline at 5˚C/. Decrease from peak temp, at 5˚C/s
- Ask participant to attend to stimulus, by closing their eyes.
- Deliver temperature that participant scored at Pain-60 during fMRI.

CPM

- **Ensure 5 minutes has elapsed from giving first test stimulus.**

Verbal instruction:
"I am now going to apply a temperature to your forearm and apply the cold stimulus to your foot at the same time. Please try and keep your foot as still as possible. You will be prompted to score the sensation you perceive several times during the MR scan using the buttons. Again, nothing will be intolerable, but press the button if anything is too uncomfortable and the device will cool down immediately."

"Do you have any questions?"

Operator instructions:

- Read out verbal instructions.
- Ensure Thermode baseline temperature is at 32°C. Increase from baseline at 5˚C/s, decrease from peak temperature at 5˚C/s.
- Ensure ice/water shoe contains specified quantities of ice and water.
- Ask participant to attend to stimulus by closing their eyes.
- Apply temperature via thermode at pain-60 at pre-defined times and apply cold stimulus to foot at pre-determined times.

Recruitment poster

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

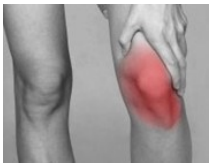
Nottingham University Hospitals NHS Trust

Arthritis Research UK

pain centre

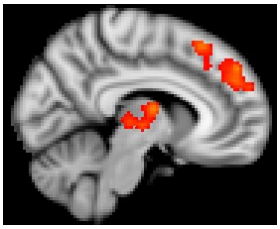
KNEE OSTEOARTHRITIS PATIENTS REQUIRED

Imaging pain relief in osteoarthritis



We are looking for patients with knee Osteoarthritis to take part in a brain imaging study aimed at better understanding mechanisms of pain relief in patients with OA.

You will be required to attend the QMC on two occasion and undergo drug treatment during 6 or 8 weeks. You will be asked to complete health questionnaires and undergo MRI scans of your brain.



YOU MUST BE:

- **Over 35 years old;**
- **Not Pregnant;**
- **Have no significant medical conditions;**
- **Have no metal in your body (Eg. pacemaker, aneurysm clips).**

Version 1.5 - 17.042014

PLEASE CONTACT: Nadia Frowd (0115 8231676)

nadia.frowd@nottingham.ac.uk

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J

Medication list

Participants who completed duloxetine study

ID	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
1	Paracetamol	Ibuprofen							
2	Lansoprazole	Calcium tablets	Ibuprofen	Paracetamol	Bladder meds				
3	Amlodipine	Tamsulosin							
4	Paracetamol	Aspirin							
5	Ibuprofen	Paracetamol	Alendronic acid	Vitamin D	Levothyroxine	Flexiseq			
6	Naproxen	Accupuncture	Cupping						
7	Ibuprofen	Levothyroxine	Naproxen						
8	Pravastatin	Levothyroxine	Paracetamol						
9	Diclofenac	Paracetamol							
10	Anastrozole	Metronidazole	Ibuprofen						
11	Paracetamol								
12	Bendroflumethazide	Tamsulosin	Statin	Ibuprofen	Paracetamol	Glucosamine	Garlic	Omega 3	Hydrocortisone
13	Metformin	Ramipril	Gliclazide						
14	Atenolol	Amlodipine	Perindopril	Aspirin	Co-codamol	Ibuprofen	Lansoprazole	Simvastatin	
15	Aspirin	Doxazosin	Finasteride	Ramipril	Simvastatin	Ibuprofen			
16	Cetrizine	Cimetidine	Enalapril	Oilatum	Paracetamol	Prochlorperazine			
17	Codiene phosphate	Amlodipine	Simvastatin	Paracetamol					
18	Naproxen								
19	short term antibiotics								
20	none								
21	Ibuprofen	Metformin	Zapain	Gliclazide	Atrovastatin	Co-codamol			
22	Lansoprazole	Naproxen	Dehydrocodiene						
23	Paracetamol								
24	Ibuprofen gel								
25	Amlodipine	Atorvastatin	Fultium - D3	Levothyroxine					
26	Ramipril								
27	Lansoprazole								
28	Ibuprofen	Levothyroxin	Ventolin						

29	Amlodipine	Atorvastatin	Furosemide	Ibugel Forte	Levothyroxine	Ramipril						
30	Bendroflumethazide	Adcal - D3	Ibuprofen									
31	Candesartan	Atenolol	Aspirin									
32	Aspirin	Gaviscon	Omperazole	Co-dydramol								
33	Ibuprofen											
34	none											
35	none											
36	Bendroflurazide	Candesartan	Paracetamol									
37	Amlodipine	Omeprazole	Ramipril	Paracetamol	Ibuprofen							
38	Amlodipine	Atorvastatin										
39	none											
40	Glucosamine	Chondroitin										
41	none											
42	none	Diclofenac										
43	none											
44	Ibuprofen											
45	Ramipril	Aspirin	Atrovastatin	Naprosyn								
46	Paracetamol	Lansoprazole	Amlodipine	Clopidogrel	Simvastatin							
47	none											
48	Travoprost	Brinzolamide										
49	none											
50	Amlodipine	Atrovastatin	Aspirin	Losartan								
51	Ramipril	Amlodipine	Doxazosin	Bendroflumethazide	Simvastatin	Clopidogrel	Omeprazole	Humalog mix 50	Paracetamol			
52	Aspirin	Atenolol	Amlodipine	Losartan	Calcium	Zapain						
53	none											
54	none											
55	Mebeverine	Nefopam	Omeprazole	Prochlorperazine								
56	Naproxen	Ranitidine										