Structural and Functional MRI Studies of Pain Behaviour, Selective Attention and Fear of Pain in Pain-Free Volunteers and Chronic Low Back Pain Patients

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By

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Abstract

The aim of the original work presented in this thesis was to investigate morphological and functional differences in clinical and control populations, which may negatively impact the experience of pain. Firstly, morphological differences were investigated between groups of healthy controls and chronic low back pain (CLBP) patients. Included in the latter were subgroups of patients not previously investigated in the current morphological literature, those with and without pain behaviour. We investigated differences in gray matter (GM) volume between groups using an automated whole brain technique, and a manual method applied to two regions of interest, namely prefrontal cortex and insular cortex. A deficit in GM volume of right dorsal prefrontal cortex between CLBP patients and controls was found, with a further deficit in left insular cortex for CLBP patients with concomitant pain behaviour.

Secondly, we conducted two studies using functional magnetic resonance imaging (fMRI) and a task of selective attention. Our initial investigation provided a proof of principle regarding the suitability of a semantic dot probe task within the fMRI environment. Pain-free participants were grouped based upon fear of pain scores. The results indicated differential behavioural and functional results between the groups.

The thesis culminates with an fMRI study investigating selective attention in CLBP patients with pain behaviour. The clinical group were tested prior to and on completion of an intensive multidisciplinary pain management programme (PMP), with the aim of assessing if selective attention is sensitive to cognitive interventions. Selective attentional bias was demonstrated in the clinical group for pain-related trials at both testing sessions, although the direction of attention differed. Similarly, fMRI results showed differences in neural correlates for task performance between groups, with pre-PMP results demonstrating a reliance on semantic and memory processes.

The findings suggest that fear of pain may be a vulnerability factor in the transition from acute to chronic pain and furthermore, CLBP patients with pain behaviour have structural, functional and behavioural differences which may negatively impact their ability to cope with their pain condition.

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List of Acronyms

AB- Attention bias

ACC- Anterior cingulate cortex

ADC- Apparent diffusion coefficient

ANOVA- Analysis of variance

BOLD- Blood oxygen level dependent

CE- Coefficient of error

CPP- Chronic pain patient

CSF- Cerebrospinal fluid

CT- Computed tomography

CTA- Cortical thickness analysis

CV- Coefficient of variance

DLPFC- Dorsolateral prefrontal cortex

DMPFC- Dorsomedial prefrontal cortex

DP- Dot probe task

DTI- Diffusion-Tensor Imaging

EPI- Echo Planar Imaging

FA- Fractional Anisotropy

FABQ- Fear avoidance behavioural questionnaire

FID- Free induction decay

FM- Fibromyalgia

fMRI- Functional magnetic resonance imaging

FOP- Fear of Pain

FOV- Field of view

FPQ- Fear of pain questionnaire

FT- Fourier transformation

GM- Gray matter

GMD-Gray matter density

GMV- Gray matter volume

HADS- Hospital anxiety depression scale

HRF- Haemodynamic response function

IB- Illness behaviour

IC- Insular cortex

ICC- Inter-class correlation coefficient

ISI- Inter-stimulus interval

LFP- Local field potential

MARIARC- Magnetic resonance image analysis and research centre

MDM- Magnetic Dipole Moment

mEFP- Mean extracellular field potential

MR- Magnetic resonance

MRI- Magnetic resonance imaging

MRS- Magnetic resonance spectroscopy

MTL- Medial temporal lobe

mTMD- Myofascial temporomandibular pain

MUA- Multiple-unit spiking activity

NAA- N-acetylaspartate

NMR- Nuclear magnetic resonance

OA- Osteoarthritis

OLPFC- Orbitolateral prefrontal cortex

OMPFC- Orbitomedial prefrontal cortex

PB- Pain behaviour

PCG- Posterior Cingulate Gyrus

PCS- Pain catastrophising scale

PD- Pain disorder

PFC- Prefrontal cortex

PIFP- Persistent idiopathic facial pain

PMP- Pain management programme

PMPAC- Pain management programme assessment clinic

RA- Rheumatoid arthritis

RF-pulse- Radiofrequency pulse

SOA- Stimulus onset asynchronicity

SVC- Subventricular zone

T1- Longitudinal (z direction) magnetisation recovery time constant

T2- Transverse (x-y direction) magnetisation decay time constant

T2*- Transverse decay time constant including magnetic field inhomogeneity effects

TE- Time to echo (from the RF excitation pulse)

TIV- Total intercranial volume

TR- Repetition time

TR- Time for repetition

TSK- Tampa scale of Kinesiophobia

TT- Total volume

VAS- Visual analogue scale

VBM- Voxel based morphometry

WCNN- Walton centre for neurology and neurosurgery

WM- White matter

WS- Waddell signs

CHAPTER ONE
General Introduction

1.1 General Introduction

This thesis investigates, using structural and functional MRI, differences in chronic low back pain patients and pain-free volunteers. The Fear-avoidance model of pain and functional magnetic resonance imaging were used to extend current knowledge of cognitive processing biases, using two of the models components, namely fear of pain and hypervigilance. In addition, morphological differences in chronic low back pain patients were investigated. To ensure homogeneity of the clinical groups, all clinical participants had a common pain condition, chronic low back pain and were assessed using a clinically relevant test known as the Waddell Signs.

The literature review begins by discussing the historical and early contemporary models of pain. Specific focus will be given to the Fear-avoidance model, and specifically two components of the model; hypervigilance and fear of pain. The Waddell Signs, a clinically useful screening tool for psychological factors in chronic low back pain will be discussed. This Chapter outlines the theoretical perspectives which underlie the aims and objectives of the presented work.

1.2 Definition of Pain

The experience of pain has been defined as:

> 'an unpleasant sensory and emotional experience associated with actual tissue damage or expressed in such terms'

International Association for The Study of Pain (IASP, 1979).

This definition of pain has provided much debate within the pain arena for the last 30 years and has been deconstructed by many aiming for a better understanding of the many factors relating to the experience we call pain. As alluded to by the definition, pain is not confined to a physical sensation. It does not have to have a

well-defined organic pathology. Instead the experience of pain is a combination of both physical and emotional factors, which may relate to past or present experiences.

Acute pain is believed to serve three purposes. Firstly, it has a survival value as pain is often the first signal that a potential physical threat is nearby. The pain often occurs prior to significant injury, such as picking up something which is extremely hot. Secondly, we learn from this painful encounter and can generalise to other situations, therefore preventing further injury. Finally, the experience of pain may limit activity forcing us to rest, which allows the healing process to occur, the purpose being recovery and ultimately survival. In comparison, chronic persistent pain, defined as pain which lasts 3 months or longer, lacks the motivational advantages of acute pain, and places a persisting stress on protective and adaptive systems (Apkarian, Hashmi & Baliki, 2011).

1.3 Pain Models

1.3.1 Biomedical Model

Early models of pain were both reductionist, assuming a direct link between disease and physical pathology and exclusionary, as they only considered the physiological aspects of the pain experience and ignored psychological and social ramifications. The Humor hypothesis proposed by Hippocrates and extended by Galen stated that four bodily fluids were responsible for maintaining health and well-being. An adjustment to the levels of the four humors, which consisted of blood, phlegm, yellow bile and black bile, was believed to be responsible for various physical and mental illnesses.

In the 17th Century, Rene Descartes proposed a dualistic theory of pain which claimed that the forces governing the operation of the body were different to those that governed the mind (Descartes, 1664). Whilst Descartes did recognise humans as having a soul, he believed that it was separable from the body, which he likened to a machine. Pain was the direct result of an injury being detected in the periphery, resulting in a signal being transmitted to a pain centre in the brain via a series of threads. The model proposed that the amount of pain experienced, was directly proportional to the amount of tissue damage. The pain system was therefore a one-way un-modifiable system. The theory was adapted somewhat over several centuries, yet the main aspects of the theory were maintained, persisting well into the 20th century, as the Biomedical Model.

Irrespective of any revisions that the model may have undergone, it remained both reductionist and exclusionary. The Biomedical model views pain as a direct transmission of impulses from the periphery to structures within the central nervous system. The only phenomena of interest are isolated causal chains, which are believed to be predictable and universal. Only the physical aspect of pain is investigated, with the patient's own beliefs and experiences regarding their condition, being excluded. This has led critics of the Biomedical model to claim that the many personal aspects of illness are reduced to a depersonalised expression of a disease, whereby individual aspects of the condition are ignored (Quintner, Cohen, Buchanan, Katz, & Williamson, 2008). The theory may be more suited to explanations surrounding acute pain, whereby there is often a determinable pathology to the pain experience, which may be alleviated through efforts focused solely on the somatic. However, chronic persistent pain frequently has no obvious pathology. As scientific and clinical investigations into the pain experience continued, the limitations of the model became more apparent.

The Biomedical model fails to adequately explain the idiosyncratic nature of pain, similar to that documented by Beecher (1956), who observed the pain responses of soldiers injured on the battlefield during the Second World War. He observed discrepancies between the extent of the injuries and the associated pain. Some soldiers complained of severe pain when their injuries appeared to be only minor, whilst more seriously injured soldiers either denied experiencing any pain, or claimed it was so minor they did not require medication. Only one in three soldiers complained of pain severe enough to warrant morphine.

The observations made by Beecher indicated that there is not always a linear relationship between the extent of injury and the amount of pain experienced. Whilst this relationship may be proportional, this is not always the case. Pain perception can be different between injuries of a similar nature if they occur at different times, in different social settings, even within the same individual. Therefore, differences in pain perception may be even greater when they are considered between individuals.

Sensory accounts of pain don't explain many of the incidents of pain, such as pain in absence of pathology, pathology in the absence of pain, individuals producing different results to the same treatments, phantom limb pain, placebo and the low association between impairment and disability. Low back pain, for example, is a very common pain condition experienced by as many as 7 out of 10 people at some point in their lives (McCracken & Turk, 2002). Every structure including muscles, nerves,

joints, bones and discs have been used to explain the pain phenomena, but as many as 70% of cases still have no known organic pathology (Melzack & Wall, 1996). Similarly, although there are a significant number of individuals who experience pain without organic pathology, there are also a significant number of individuals with pathology on CT or MRI scans who are and remain asymptomatic. It has been posited that as many as 35% of scans reporting significant pathology belong to an asymptomatic individual (Turk & Flor, 1999). Proponents of the Biomedical model have suggested that as technology advances, some of the previous discrepancies between pathology and pain experiences will be resolved (Andrasik, Flor & Turk, 2005). However, this suggestion remains speculative.

In response to the limitations of the Biomedical model, pain models evolved from dualistic thinking to progressively more complex and comprehensive models, that placed an emphasis upon the idiosyncratic experience of pain. Psychological factors which had previously been ignored, were included as important components of the pain experience.

1.3.2 Gate Control Theory of Pain

In 1965 Melzack and Wall proposed the Gate Control theory of pain, which was one of the first theories to integrate the Biomedical model with psychological mechanisms (Melzack & Wall, 1965). As such it offered an explanation for within-person and between-person differences of the pain experience. In brief, the theory postulated that nociceptive information from the periphery is routed through a hypothetical gating mechanism located in the dorsal horn of the spinal cord. The gating mechanism, which modulates the intensity of the ascending transmission, can be influenced by several factors, including descending transmission regarding current cognitive and affective states. The experience of pain can be jointly determined by physiologic, motivational, cognitive and emotional factors. In 1968, Melzack and Casey further categorised these factors into three subsystems; sensory-discriminative, cognitive-evaluative and motivational-affective. Due to the influence of multiple systems, there is great potential for individual shaping of the pain experience (Melzack & Casey, 1968).

The Gate Control theory of pain revolutionised pain research, as the theory proposed a multi-dimensional concept of pain that included, previously ignored, psychological factors. Furthermore, the theory highlighted that processes mediated by the central nervous system, such as cognition and affect, were essential

components of pain processing and pain perception. The previously exclusionary and reductionist theories of pain were being modified. However, whilst acknowledging the contribution of psychological factors to the pain experience, the model failed to address the nature of these psychological factors (Turk and Flor, 1999).

In conclusion, the IASP definition of pain states that pain does not have to have a well-defined organic pathology. However, the theoretical underpinnings of both the Biomedical model and the Gate Control theory lack clarity in describing situations where pain levels are not associated with the extent of injury, such as when pain persists after damage has apparently healed, or when pain is experienced in the lack of any organic pathology. Proponents of the Biomedical model have claimed that technical advancements within the medical field will allow for these discrepancies to be resolved. However, whilst a somatic explanation may be revealed in time, this does not exclude the need for current pain management. The growing literature on the impact that psychological and sociological factors can have on the maintenance and exacerbation of the pain experience cannot and should not be ignored in favour of ideological preferences. Due to the inclusive nature of the model, the Biopsychosocial model reviewed below supersedes previous exclusionary theories of persistent chronic pain. This is because whilst the Biopsychosocial model of pain may reject the reductionist philosophy of the Biomedical model, it acknowledges the contribution to understanding and treating chronic pain that this discipline makes.

1.3.3 Biopsychosocial Model

The first person to upgrade pain from a simple sensation, to one accompanied by an emotional element was Cannon (1915). He believed that pain perception involves two basic components, a sensory component and an emotional component. The emotional component can be further divided into the pain experience, pain behaviour and physiological responses to pain. If one component becomes dysfunctional, this will impact the other, resulting in a negative impact upon the pain experience.

In 1977 George Engel proposed the Biopsychosocial model of pain, as a response to what he believed to be the inadequacies of the Biomedical model (Engel, 1977). The Biopsychosocial model is not a model in the traditional sense. Unlike both the Biomedical model and the Gate Control Theory of pain, the Biopsychosocial

model is more a philosophy of clinical care, than either a treatment route, or description of the pain experience (Borrell-Carrio, Suchman, & Epstein, 2004). Engel stated that the Biomedical model assumes that disease can be fully accounted for by a physical explanation. However, as the physical aspects of the disease remain the focus, social, psychological and behavioural dimensions of illness are largely ignored. By adopting a more inclusionary process of health care, Engel believed that the processes he alleged were dehumanising care would be overcome. Specifically, (i) dualism; the separation of mind and body, (ii) reductionism; anything other than somatic explanations are excluded and (iii) the patient/physician relationship; whereby the individuals account of illness is disqualified (Engel, 1977).

His work was based on General Systems Theory as proposed by biologists in the mid-20th century. Paul Weiss (1951) and Ludwig von Bertalanffy (1967) independently argued that biological systems do not exist in isolation, but are influenced by the configuration of their surrounding environment. A hierarchical relationship is created by larger and more complex systems, extending from the subatomic particle to the biosphere. Any change to one part of the system, might produce changes to all or some of the other systems in the hierarchical structure. By applying this theory to clinical care, Engel proposed that health and behaviour should be treated using biological, psychological and social perspectives simultaneously. This resulted in a dynamic, interactional and dualistic view of the human experience, whereby mind and body had a mutual influence. The model was not a paradigm shift, with new information replacing previous knowledge, but rather the expanded application of existing knowledge.

The Biomedical model is criticised for being reductionist and exclusionary in its approach to pain, but this criticism is not unique to this profession. Whilst the strength of the Biopsychosocial model lies in its multidiscipline approach to chronic pain, Engel argued against disciplinary reductionism, whereby one school of thought is favoured over another. The underlying philosophy of the Biopsychosocial model is that no one discipline can explain everything. Instead, there needs to be a balance between different disciplines to understand and treat chronic pain.

The Biopsychosocial model recognises that individual variability is the result of complex interrelationships among biological, psychological and sociologic characteristics, and that as pain becomes chronic, these variables play an increasingly dominant role in the maintenance of pain behaviour and suffering. However, one criticism of the model is that as with all open systems, it is impossible to know all the

contributors to an influence. Proponents of the model counter that instead of proposing a one size fits all approach to chronic pain, and by understanding the hierarchy of the way the pain system is organised, guideposts will be provided to inform clinician's actions. Furthermore, in time it may be possible to individualise the hierarchy of the pain experience, resulting in patient pain profiles, leading to better and more effective treatments (Keefe, 2011). It is this complexity and multifactorial account of pain that is the strength of the Biopsychosocial model. By treating the whole person, rather than reducing the pain experience to the somatic, the Biopsychosocial model has advanced beyond the Biomedical model.

1.4 The Fear-avoidance Model

The theoretical underpinnings of the Biopsychosocial model, which suggests that complex combinations of psychological, sociological and neurophysiological factors impact the pain experience, resulted in models of pain which focused on factors beyond the purely physical. Early Fear-avoidance models conceptualised the role of fear of pain (FOP) and avoidance behaviour as distinct from the physical component of pain (Lethem, Slade, Troup & Bentley, 1983; Philips, 1987; Waddell, Newton, Henderson, Somerville & Main, 1993). Prior to the model proposed by Lethem et al (1983), psychological contributions to the investigation of the pain experience fell into three categories. Firstly, empirical studies were conducted on patients to correlate clinical features of pain to personality and social factors. Secondly, theories of pain perception began to include the role of psychological variables. Finally, psychologically based treatments for chronic pain were utilised. However, there were few attempts to integrate these contributions. The Fear-avoidance model originally attempted to explain the transition from acute pain to chronic persistent pain in chronic low back pain (CLBP), through emphasising the relationship between patient characteristics and psychological factors with the aim of producing greater understanding and better treatments for chronic pain patients (CPP). In doing so the model provides an explanation of why some chronic pain sufferers develop problems associated with disability and coping ability; through the interaction of affective states (mood) and cognitive-interpretative processes (thought), (Turk & Wilson, 2010).

Vlaeyen and Linton, (2000) proposed a model which not only expanded the cognitive and affective constructs of preceding models, but also integrated contemporary empirical findings. This resulted in a Fear-avoidance model which is a

cognitively oriented model of pain-related fear, including both behavioural and physiological aspects of the pain experience. The model is a heuristic for which much empirical research and practical applications have been conducted, resulting in increasing modification through empirical investigation (Pincus, Vogel, Burton, Santos, & Field, 2006; Leeuw, Goosens, Linton, Crombez, Boersma & Vlaeyen, 2007; Norton & Asmundson, 2003).

Regardless of the refinements made, the central tenet of the model is the way a painful experience, either acute or chronic, is interpreted in relation to the idiosyncrasies of the individual. These will determine to what extent the pain is regarded as threatening or harmful. Beecher (1956) emphasised the importance of cognitive processes in the pain experience, as pain lacks an external standard of reference thus allowing considerable room for interpretation. According to the Fear-avoidance model, when a painful sensory experience is perceived, the sufferer will place a judgement on the meaning of the pain. This judgement will determine which of the two behavioural pathways will be taken, either confrontation or avoidance.

The majority of individuals will define the pain experience as unpleasant and undesirable, but the painful episode will not be accompanied by or associated with fear. Due to the non-threatening perception of the pain experience, the individual will engage in appropriate behaviour restriction, with a gradual increase in daily activities (confrontation), until healing has occurred. Lethem et al, (1983) regarded this behaviour as synchronous, with both the sensory and affective components of pain responding in a similar manner, leading to an adaptive type of pain response and ultimately successful recovery. Those who take an adaptive rather than passive coping style to pain, cope better when faced with acute pain episodes (Slade, Troup, Lethem, & Bentley, 1983).

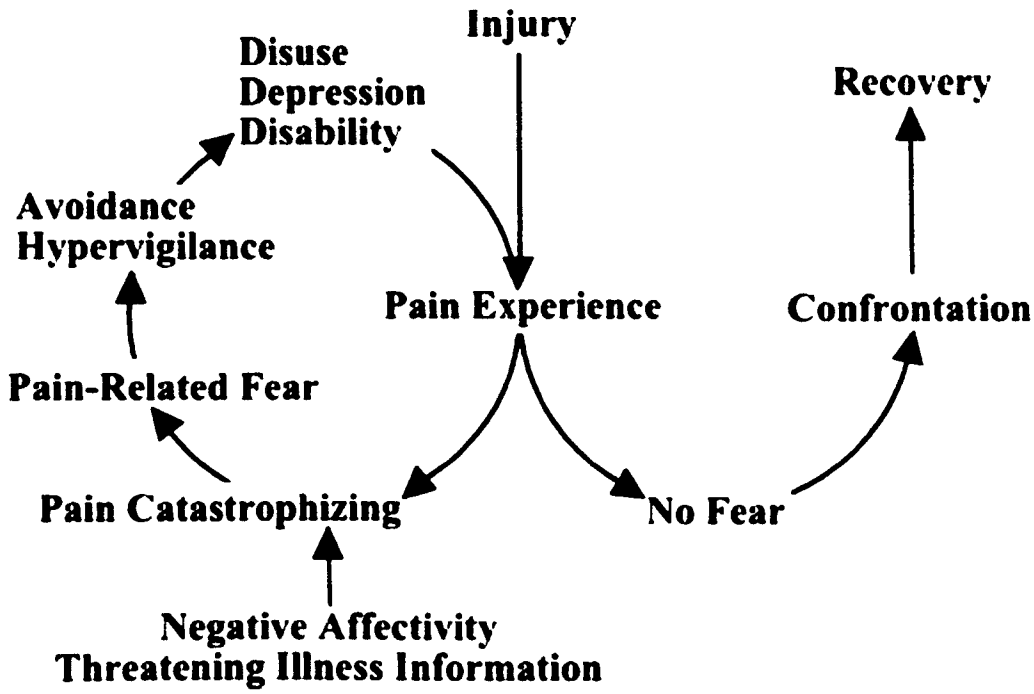
However, for a significant minority of individuals an extremely negative or catastrophic (mis)interpretation of the pain occurs. This response to the pain experience induces physiological, cognitive and behavioural fear responses (Turk & Wilson, 2010).These fear responses are accompanied by cognitive adjustments, which may lead to pain-related fear, avoidance and hypervigilant behaviours. In a chronic pain state, this behaviour may lead to a vicious and self-perpetuating fear-avoidance cycle which promotes and maintains activity limitations, pain and disability. An exaggeration of pain perception occurs as the sensory and emotional components of pain become misaligned. The emotional component increases, regardless of an increase or decrease within the sensory component. Therefore, fear-

avoidance has been put forward as a central mechanism in the development of long term back pain problems (Lethem, et al, 1983; Vlaeyen, et al, 2000). A schematic representation of the model is illustrated in Figure 1.1.

The original work contained within this thesis, has investigated pain-related fear in both clinical and pain-free populations. Specifically, two studies investigated the relationship between pain-fearfulness and selective attention, in combination with functional magnetic resonance (fMRI) scanning. An additional study investigated morphological differences between controls and a clinical population, which contained a subgroup of patients with increased pain-related fear. To reflect the focus of the thesis, emphasis is placed upon pain-related fear as a component of the Fear-avoidance model.

However, the Fear-avoidance model posits that catastrophising is a preceding characteristic of pain-related fear. Although pain-related fear is the focus of investigation within the presented thesis, there is an interrelatedness of fear of pain and catastrophising. To reflect this relationship, the discussion will begin with a brief overview of the catastrophising component of the Fear-avoidance model, before embarking upon a discussion on pain-related fear.

Figure 2.1 Schematic Representation of the Fear-avoidance Model



Reproduced from Vlaeyen & Linton, (2000)

1.4.1 Catastrophising

The term catastrophising was formally introduced by Albert Ellis and adapted by Beck, Emery & Greenberg, (2005) to describe a maladaptive cognitive style employed by patients with anxiety and depressive disorders. Catastrophising is generally assessed as a trait-like or dispositional characteristic, although some investigations have examined if state-like qualities exist (Quartana, Cambell & Edwards, 2009). Pain-related catastrophising is a tendency to use negative interpretations about pain. Specifically it is characterised by the tendency to magnify the threat value of pain (magnification), to feel helpless in its presence (helplessness) and to be unable to inhibit pain related thoughts in anticipation of, during or following a painful encounter (rumination), (Sullivan, Bishop, & Pivik, 1995).

The contemporary Fear-avoidance model posits that catastrophising beliefs precipitate pain-related fear, which in turn mediates avoidance and hypervigilance, followed by disuse, disability and depression. Prospective studies have provided support for the Fear-avoidance model components and have inferred a causal nature for catastrophising, (Burton, Tillotson, Main & Hollis, 1995; Crombez, Eccleston, Baeyens, & Eelen, 1998). To help delineate if catastrophising is a precursor to pain-

related fear, Severijns, van den Hout & Vlaeyen, (2005) conducted a study which experimentally induced catastrophising in pain-free volunteers, completing a cold-pressor task. A linear relationship was predicted between catastrophising and both expected and experienced pain. This prediction was not supported, even though the manipulation of catastrophising levels was successful. Furthermore, no effect was found between pre-experimental catastrophising levels and outcome measures.

A more recent prospective study aimed to examine the sequential relationships of the fear-avoidance components, namely catastrophising and pain-related fear (Widemann, Adams & Sullivan, 2009). Specifically, they investigated if early change in catastrophising predicted late change in fear of movement, and furthermore if these factors influence return to work. The sample consisted of 121 participants with work-related musculoskeletal injury, high catastrophising and fear of movement scores, who completed a 10-week community based disability management intervention. Measures were completed at pre-, mid- and post-treatment. Although return to work was predicted by changes in both fear and catastrophising scores, no sequential relationships between these components were established. The authors suggested study limitations regarding the self-selection of the participants and the time between testing. A decrease between time intervals was suggested to improve the detection of sequential cognitive, affective and behavioural components. To date, while there is growing evidence that both catastrophising and FOP can be indicative of characteristics of the pain experience such as return to work, the causal relationship between FOP and catastrophising as posited by the Fear-avoidance model, remains elusive.

1.4.2 Pain-Related Fear

'fear of pain and what we do about it may be more disabling than pain itself' Waddell, 1993 p.9.

Fear is a multifaceted phenomenon, containing three dimensions along which fear is expressed, (Lang, 1968). The cognitive dimension is characterised by involuntary thoughts of danger, threat or even death. Cognitive appraisal results in an ability to increase attention towards the object of threat and away from irrelevant distracters. The degree of fear experienced may be in proportion to the perceived ability to cope with the threat. The second dimension of fear is physiological, which

is characterised by the activation of the sympathetic nervous system. Physiological arousal is increased and non-essential functions decreased to maximise any physical needs. Finally, defensive behavioural responses are the final dimension whereby individuals may engage in other coping behaviours, such as immobility or washing, when they cannot directly escape or avoid the threat (Asmundson, Vlaeyen & Crombez, 2004).

Therefore, pain-related fear may contribute to disability. Fight or flight behaviours which urge escape may prevent other daily activities from being undertaken; interfering with cognitive functioning by attending more to threat signals and an inability to disengage from pain-related material; avoidance behaviours means expectancies are not challenged and maladaptive beliefs not disconfirmed (Crombez, Vlaeyen, Heuts, & Lysens, 1999).

The Fear-avoidance model posits that pain-related fear is another component associated with increased risk of developing persistent pain. Although the model asserts a sequential relationship between catastrophising and pain-related fear, the interrelatedness of these processes means it has been hard to establish this relationship (Cook, Brawer & Vowles, 2006; Vlaeyen, Kole-Snijders, Boeren & van Eek, 1995; Wideman et al, 2009). One explanation for the strong association between fear of pain and catastrophising is that catastrophising is an expression of pain-related fear on the cognitive level, (Hasenbring & Verbunt, 2010).

The aim of pain-related fear is to protect the individual from an identifiable and immediate threat. However, with chronic pain conditions the threat is ever present, so the focus of this fear remains unclear and uncertain. Patients with persistent pain are more likely to fear stimuli or experiences not directly related to the pain experience, but it is uncertain if they generalise these fears, whereby non-pain fears arise as a result of CLBP (Vlaeyen et al, 1995).

The pain experience is both a complex and subjective phenomenon, which is impacted upon by the individuals' beliefs, which in turn influence the individual's ability to cope. As such pain-related fear represents the current concerns of the patient and can reflect any number of multiple concerns. To address the broad range of concerns, pain-related fear is often assessed by self-report measures, which focus on one aspect on the pain experience. For example the Tampa Scale of Kinesiophobia (TSK; Woby, Roach, Urmston, & Watson, 2005), examines the fear of movement and (re)injury, the Fear-avoidance Beliefs Questionnaire (FABQ; Waddell et al, 1993), measures beliefs about work, the Pain Anxiety Symptom Scale,

(PASS; McCracken, Zayfert, & Gross, 1992), assess behaviours related to fear of pain and cognitive-anxiety, and finally the Fear of Pain Questionnaire (FPQ; McNeil & Rainwater, 1998), assess fear in a number of different environmental contexts.

Pain-related fear as a maintaining factor

The Fear-avoidance model considers pain-related fear as a maintaining factor in a chronic pain condition. The way this is achieved is through safety behaviours, specifically avoidance and hypervigilance, which is when individuals scan their environments for potential signs of pain, and maintain attention at the location of perceived threat (Leeuw et al, 2007). The role of hypervigilance in chronic pain conditions is investigated in Chapters Five and Six, and therefore is critically evaluated in greater detail in Chapter 1.7.

The second behavioural consequence of pain-related fear, as proposed by the model, is avoidance. Whilst the term fear-avoidance was first applied to the field of pain by Lethem et al, avoidance had been previously recognised as a spontaneous and adaptive response to acute injury (Wall, 1978). In an acute pain episode, avoidant behaviours may allow healing to occur, prior to confrontation. However, if the individual maintains an avoidant behavioural response beyond the healing process, this creates a vicious cycle whereby limitations in activity over time lead to disability and a preoccupation with somatic perceptions, further fuelling avoidant behaviour (Asmundson, Norton & Norton, 1999). Avoidance behaviour may be extended to other pain-free situations establishing a pattern of pain-related associations to a wider context.

To be successful, avoidance behaviour must occur prior to the painful event and once established is extremely resistant to extinction, as successful avoidance prevents the individual from interacting with the perceived threat, resulting in a lack of disconfirming evidence (Leeuw et al, 2007). Individuals display a belief bias, whereby false assumptions are reinforced due to a propensity to confirm their own beliefs. A study investigating thought processes in hypochondriacs found that they displayed immunity for disconfirmatory information, instead adopting a 'better safe than sorry' threat-confirming attitude (Smeets, de Jong, & Mayer, 2000).

In chronic pain populations avoidance behaviour, can lead to both over and under predictions of pain. Low fear levels may lead to an underestimation of pain, resulting in inappropriate behaviour, whereas over predictions of pain may result in inappropriate avoidance by terminating pain inducing activities more quickly. High

levels of pain-related fear have been associated with impairments in performance with; decreased speed of walking in CLBP patients (Al-Obaidi, Al-Zoabi, Al-Shuwaie, Al-Zabbie & Nelson, 2003), reduced peak performance of trunk extensions in CLBP (Thomas, France, Sha & Wiele, 2008) and a reduced performance on physical tasks (Geisser, Haig & Theisen, 2000).

Pain-related fear as a risk factor

In CLBP cohorts pain-related fear has been associated with greater perceived disability (Gheldof, Vinck, Van Den Bussche, Vlaeyen, Hidding & Crombez, 2006; Crombez, Vlaeyen, Heuts & Lysens, 1999), increase in sick leave (Dawson, Schluter, Hodges, Stewart & Turner, 2011), an over prediction of pain (Huijnen, Verbunt, Peters & Seelen, 2010) and a reduction in activities of daily living (Buer & Linton, 2002). However, these studies tend to be cross sectional. Although they provide commentary on the chronic pain condition, they fail to distinguish between factors which serve as a risk factor to pain, and those factors which are a response to a persistent pain condition.

A more efficacious approach is to use prospective studies, whereby individuals are followed during a new pain episode (baseline), with follow-up assessments after a previously defined period of time, such as six months to one year, with the aim of establishing relationships between baseline measures and outcome measures at follow up. In one of the first prospective studies investigating pain-related fear in a population-based cohort, Picavet, Vlaeyen & Schouten, (2002) showed that increased levels of pain-related fear at baseline were predictive of future back pain and disability. Similarly, Swinkels-Meewisse, Roelofs, Schouten, Verbeek, Oostendorp, & Vlaeyen, (2006) investigated the relationship between pain-related fear and disability in patients with acute LBP. Increased fear levels at baseline, were predictive of disability and to a lesser extent, participation in daily activities, at a 6 month follow-up. Additionally, elevated pain-related fear during baseline has been predictive of self-reported disability 4 weeks later (Fritz, George & Delitto, 2001), 8 weeks later (Klenerman, Slade, Stanley, Pennie & Reilly, 1995), and 12 months later (Sieben, Vlaeyen, Tuerlinckx & Portegijs, 2002). Heightened levels of pain-related fear at baseline have been related to several aspects of the working environment, including the probability of returning to work, of being on sick leave or disability four weeks later (Boersma & Linton, 2005; Fritz et al, 2001; Storheim, Brox, Holm & Bo, 2005).

Several systematic reviews have investigated the predictive value of pain-related fear to measures, such as pain and disability. Linton (2000) concluded that cognitive variables including fear-avoidance beliefs were significantly associated with the increased risk of long term pain problems, such as disability and pain intensity. More recently Ramond and colleagues (Ramond, Bouton, Richard, Roquelaure, Baufreton et al, 2011) reported that amongst many psychological and sociological factors investigated, pain-related fear had a significant prognostic value in the transition from acute to chronic pain, in half the studies it was included in. Chou & Shekelle, (2010) reported that the most useful items for predicting recovery from LBP at 12 months, was lower levels of pain-related fear. However, two systematic reviews by Pincus and colleagues found only limited support for pain-related fear as a risk factor to long term pain (Pincus, Burton, Vogel, & Field, 2002; Pincus, Vogel, Burton, Santos & Field, 2006). Methodological differences can explain the different findings in systematic reviews such as differences in study inclusion/exclusion criteria, different pain conditions and different outcomes measures used. It would appear that heightened levels of pain-related fear are a risk factor for the development of chronic low back pain, although this relationship is not always consistent.

Pain-related fear as a vulnerability factor

As virtually all individuals have some previous pain experiences, regardless of the pathology, they can transfer these beliefs onto the current situation. Therefore, it can be assumed that fear-avoidance beliefs, and specifically pain-related fears, also exist within a pain-free population. Houben, Leeuw, Vlaeyen, Goubert & Picavet, (2005) investigated fear of movement beliefs in the Dutch population, in individuals with and without experiences of back pain in the preceding 12 months. Results showed pain-free respondents had pain-related fear scores comparable to acute and CLBP patients. Additionally, misconceptions regarding low back pain are prevalent in the general population, with individuals believing that back pain is indicative of tissue damage (Leeuw et al, 2007). These beliefs may act as a vulnerability factor when a new pain episode occurs, leading to the misinterpretation of ambiguous physical sensations and increasing the likelihood of a transition from acute to more persistent pain (Pincus & Morely, 2001).

Empirical studies investigating pain-related fear as a vulnerability factor, have explored if these beliefs can be predictive of responses to experimental pain.

Although not strictly comparable to an unpredictable acute pain experience, the use of experiential pain allows for psychological components related to the pain experience, to be collected prior to, during and after the pain experience. Using a cold pressor task George, Dannecker & Robinson, (2006) collected fear scores prior to testing and found them to be predictive of pain threshold and intensity ratings, suggesting predispositional pain-related fear strongly influences responses to an acute pain experience. A recent follow-up study conducted by Hirsch, George, Bialosky & Robinson, (2008), found pain-related fear was consistently the strongest predictor of pain, in agreement with their previous study. A prospective study in young pain-free healthcare or distribution workers, found that pain-related fear at baseline was as predictive as other work-related factors such as posture, of low back pain episodes lasting beyond seven days, at a 12 month follow up (Van Nieuwenhuyse, Somville, Crombez, Burdorf, Verbeke, Johannik & et al, 2006). These results suggest that pain-related fear may predispose individuals to engage in behaviour making it a vulnerability factor for the development of persistent pain.

Fear-avoidance beliefs held by healthcare professionals may indirectly encourage pain-related fear within their patients. A recent systematic review conducted by Darlow, Fullen, Dean, Hurley, Baxter & Dowell (2011) investigated the attitudes and general beliefs of healthcare providers, such as general practitioners, physiotherapists and chiropractors. The findings suggested a strong association between healthcare practitioners' beliefs and those of their patients. Furthermore, healthcare practitioners with fear-avoidance beliefs were more likely to advise a limitation on work and physical activity and were less likely to adhere to treatment guidelines, thus reducing the opportunities to provide disconfirming evidence for the patient's own fear-avoidance beliefs (Linton, Vlaeyen, & Ostelo, 2002; Coudeyre, Rannou, Tubach, Baron, Coriat & et al, 2006). Therefore, the beliefs of the patient and their healthcare providers may interact in a mutually reinforcing way. If a patient already holds elevated pain-related fear, which is reinforced by their healthcare profession and the treatment which they then receive, this may make them more vulnerable to fear-avoidance beliefs, catastrophic thinking and a greater risk of developing a persistent pain condition.

1.5 Pain Behaviour

As previously discussed, empirical investigations in chronic pain conditions are beginning to include biological, psychological and social factors, which may impact the pain state. The components of the Fear-avoidance model have highlighted the subjective nature of the pain experience. Although patients can be broadly classified into different groups on a variety of measures, such as catastrophising and pain-related fear, these measures may not be easily adopted within a clinical setting. Clinical healthcare professionals may be either unfamiliar or simply less inclined to use psychological measures within their practice. However, this does not circumvent the need for subgrouping patients, thus allowing for optimal care, as interventions can be targeted to their individual needs (Apeldoorn, Bosselaar, Ostelo, Blom-Luberti, van der Ploeg, Fritz & et al, 2011).

Similarly to the Fear-avoidance model, whereby individual characteristics are believed to be important in the development and maintenance of chronic pain, excessive pain behaviour (PB) has also been posited as negatively impacting the pain state. Pain behaviour is a normal response to persistent pain. However, a subgroup of low back pain patients may develop disproportionate and exaggerated responses to their physical condition. In 1980 Waddell, McCulloch, Kummel & Venner, developed a screening tool to identify patients who required more detailed psychological assessment. Named the Waddell Signs (WS), the screening tool was developed to distinguish between medically incongruent behaviour from clinical signs and symptoms which represent the physical pathology of chronic low back pain. The WS consist of 8 standardised physical manoeuvres, which are subdivided into 5 categories. A positive score is given for a category, if at least one test is scored positive. If three or more categories are scored positive, the individual is regarded as demonstrating PB. (A detailed discussion about the manoeuvres used for the categories can be found in Chapter 3.5).

The WS are primarily used within a clinical setting as a quick and useful tool to investigate if the patient displays behaviour which may impact upon their pain condition. This is especially useful within clinical situations were other psychometric evaluations are not readily available. The aims of the WS cannot easily be interpreted by the patients, and may therefore be less susceptible to presentational bias compared to other self-report measures. Of more importance is that the signs are not to be misinterpreted as a way of establishing between patients with 'real' or 'fake' pain (Main & Waddell, 1998). Pain behaviour does not exclude an organic basis of pain;

the validity of the pain condition is not being questioned. Instead the WS should be considered as an indicator of distress and as a useful measure of integrating physical assessment with psychological functioning. Furthermore, the reliability of the WS has been established for intra-observer scores and diagnostic agreement (Apeldoorn, Bosselaar, Blom-Luberti, Twisk, & Lankhorst, 2008; Waddell et al, 1980).

Although frequently used within clinical practice, there is a paucity of empirical enquiry into the WS. An evidence based review conducted by Fishbain and colleagues (Fishbain, Cole, Cutler, Lewis, Rosomoff, & Rosomoff, 2003) claimed that there existed little evidence that the WS were representative of psychological distress, that nonorganic signs may still be indicative of an organic condition, that WS do not discriminate between organic and nonorganic problems. However the review also highlighted that the WS are regarded as indicative of greater pain levels, associated with poorer treatment outcome and that the score is not representative of malingering or secondary gain. More recent investigations have expanded the relationship between WS and other aspects of the pain experience. A systematic review conducted by Chou & Shekelle, (2010) into individual risk factors for identifying patients more likely to develop chronic low back pain, found that baseline nonorganic signs were one of several factors most useful in predicting outcome at 1 year.

A study conducted by Carleton, Kachur, Abrams & Asmundson, (2009) investigated the discrepancy in the literature regarding the association between higher numbers of positive scores being indicative of poorer prognosis. Endorsement of three or more categories was believed to represent PB, although this had not been empirically supported (Fishbain et al, 2003). However, Carleton et al (2009), investigated if a positive score on 2 categories was sufficient to indicate psychological distress. Patients who scored more than two categories reported higher levels of psychological distress, perceived disability, pain intensity, and pain duration. Furthermore, these patients were less likely to return to work. Similar findings were reported by Olaya-Contreras & Styf, (2009) for patients with chronic musculoskeletal pain. Patients with PB reported greater pain levels and suffered from greater levels of depression, than patients without PB.

Finally, the assertion that the WS are indicative of the need for additional psychological assessment was investigated by Apeldoorn et al, (2011). CLBP patients were assessed using two differing methods; an interview with a clinical psychologist, who indicated if the patient had any psychological disturbance, and

several other methods, such as self-report measures and physical activities. Results indicated that WS were one of four variables that could be used as a screening tool for additional psychological disturbance.

In summary, the WS are often conducted in clinical practice to indicate if the patients' pain experience contains an additional psychological component. The few empirical studies conducted on the WS support this view, although the nature of the psychological disturbance remains unidentified.

In conclusion the evidence suggests that pain-related fear negatively impacts the pain experience, through either maintaining or exacerbating persistent pain. However, one criticism of the current literature is that unless pain-related fear, or other psychological variables, relate to the specific aims of the investigation, empirical research sometimes neglects the homogeneity of the clinical population. Similarly, often diverging pain conditions may be used under umbrella terms, such as musculoskeletal pain, which may further dilute any condition specific effects. To counter this argument, the original work contained within this thesis used stringent inclusion/exclusion criteria for the assessment of CLBP patients. Furthermore, we investigated a subgroup of patients who were assessed on levels of pain behaviour. In addition, pain-free volunteers were assigned to empirical groups according to their self-reported level of pain-related fear.

1.6 Attention to Pain

From an evolutionary perspective, the experience of pain is critical in promoting survival, thus our attention may be primed to process painful stimuli at the expense of other attentional demands, whereby pain interrupts on-going activity and urges escape (Asmundson et al, 1999). However, as pain demands more attention, this interruption may become dysfunctional which may lead to difficulties with cognitive functioning, such as memory and concentration. There are several proposed theories of attention, the scope of which is beyond the current thesis.

One of the most detailed accounts of visual attention has been proposed by Allport (1989). According to this theory, the primary purpose of any attentional system is to ensure coherence of behaviour, regardless of any competing demands. An attentional set is created through the prioritising and coordination of several sub-processes, (motivational, cognitive, motor and sensory). For these goals to be

achieved the attentional set must be maintained. However, a sophisticated attentional system must also allow for the interruption of attentional engagement due to changing internal or external events. The critical problem for an attentional system is how to satisfy these two conflicting requirements. Allport argued that normal selective attention exists between these two extremes, containing the ability to evaluate potential threats outside of current attentional engagement, whilst maintaining current goal oriented behaviour, without behavioural disruption. Therefore, attention is a dynamic mechanism of selection for action.

The primary task paradigm has been used to investigate the qualities of a painful stimulus needed to interrupt attentional processes. The task requires participants to perform an attentionally demanding task, with a painful stimulation being delivered at some point during task completion. The rationale is that if selective attention of pain occurs at the expense of other cognitive demands a decrease in task performance will be observed from pre-stimulation levels. The primary task paradigm has been used to identify characteristics of the pain stimulus which amplify attentional disruption namely, novelty, intensity and threat. The original work contained within this thesis, whilst investigating the chronic pain state, did not require the application of any experimental sensory pain stimulus. Rather both fMRI studies presented here utilised cognitive tasks. As such discussion will be confined to how the perception of threat interrupts attentional performance.

Threat

A cognitive-affective model of the interruptive function of pain was proposed by Eccleston & Crombez (1999). When pain is experienced, a primitive defence system is activated, which has an overriding priority for attentional engagement. The organism must respond promptly to the perceived source of threat regardless of the other attentional demands placed upon the individual. For pain to enter awareness it must interrupt other cognitive functions, which occurs at the expense of other attentional demands within the environment. Attentional interruption may be facilitated by the characteristics of both the painful event and the environment in which it occurs. The cognitive-affective model has focused on the qualities of the stimulus needed to produce an interruptive effect suggesting that the interruptive function of pain is not mediated solely by its sensory characteristics, but also by its affective characteristics, such as its threat value.

The threat of pain is not an emotionally neutral encounter, as the threat of impending pain may be a source of distress and increased arousal. Preparatory responses will be activated in anticipation of the pain stimulus, which in turn increases awareness to objects outside of current goal oriented behaviour, thus interrupting attentional processes. When empirical investigations manipulate the threat value of a stimulus, such as inducing a belief that a stimulus will be delivered when it will not, have reported that stimuli deemed as highly threatening result in the greatest disruption of attentional processes (Crombez, Eccleston, Baeyens & Eelen, 1998a; Koster, Crombez, Van Damme, Verschuere & De Houwer, 2004a; Van Damme, Crombez & Lorenz, 2007).

The Fear-avoidance model posits that the level of threat assigned to a stimulus is mediated by individual characteristics, such as pain-related fear. Therefore, empirical studies have investigated the level of disruption the threat of pain produces. Eccleston, Crombez, Aldrich & Stannard, (1997) used the primary task paradigm to assess attentional disruption in CPP with high and low levels of somatic awareness (a pain fearfulness measure). The greatest attentional degradation was found for patients with high levels of fear, suggesting they perceived the pain as more threatening. However, CPP may already display a reduction in attentional capacity. Therefore, the effects of the threat of pain have been examined in pain-free volunteers. In a replication of a previous study Crombez, Eccleston, Baeyens & Eelen, (1998b) threatened participants with a high-intensity pain stimulus, whilst only administering a low-intensity stimulus. A more pronounced disruption of the primary task was demonstrated in participants who displayed greater levels of pain-related fear and catastrophic thinking about pain.

Within the Fear-avoidance model, the mechanisms driving attention to pain become dysfunctional resulting in a hypervigilant state. This in turn is regarded as a contributing factor in the maintenance of a vicious cycle of maladaptive pain behaviour. Although both the intensity and novelty values of a painful stimulus may interrupt attentional processes, these effects may be magnified within chronic pain populations by levels of pain-related fear. Fearful individuals will attend more to possible signals of threat (hypervigilance) and will be less able to shift attention away from pain-related information (disengagement). This will be at the expense of other tasks.

1.7 Attentional Bias

Cognitive bias may be considered an umbrella term for the preferential treatment of a certain class of information, which includes attentional, memory and interpretational processes. The Fear-avoidance model asserts that a significant minority of individuals have a negative or catastrophic (mis)interpretation of their pain. In turn, this evaluation of the pain experience is accompanied by cognitive adjustments which lead to avoidance and hypervigilant behaviours, both of which are posited as maintaining or exacerbating the pain condition. The focus of the work presented in this thesis was on attentional processing of pain-related information. Although the term hypervigilance refers to a focus towards the object of attention, several other components of attention may be related to hypervigilance, such as avoidance or disengagement. As such, the broader term 'attentional bias' is used as an inclusive term for different attentional processes.

1.7.1 Theories of Attentional Bias

Attentional biases are demonstrated by the preferential selective processing of a specific type of stimulus over other competing demands on attention. Biases for the selective processing of threat-related information have been discussed at great length within the anxiety literature. Similarly to the Fear-avoidance model, cognitive models of threat and anxiety have regarded cognitive biases as having a detrimental effect upon the anxiety disorder. Therefore, a discussion relating to the theories of attentional bias in anxiety disorders will be relevant to the discussion of selective attentional processing in chronic pain conditions.

Eysenck's Hypervigilance Theory

One of the first hypervigilance theories was proposed by Eysenck, (1992) who claimed that hypervigilance is the crucial characteristic of anxious individuals who display a greater vigilance towards threat-related information at all levels of cognition. According to Eysenck, attentional bias in individuals high in trait anxiety may manifest itself in a variety of ways; (i) continuous scanning of the environment for threat, (ii) greater distractibility by environmental stimuli, even to threat-irrelevant stimuli, (iii) specific hypervigilance, a propensity to attend to threat-related stimuli and (iv) a narrowing of attention to other stimuli when exposed to a threat-related stimulus.

Hypervigilance is the product of learning processes. Therefore it reflects the effects of past experience on present perception. In relation to chronic pain conditions, some individuals may develop perceptual habits of vigilance for pain sensations or a narrowing of focus (specific hypervigilance). This specificity is focused on cues which are thematically related to the current concerns of the individual. In CPP, this has been demonstrated as being pain-related information (Pearce & Morley, 1989).

The attentional mechanisms underlying the processes of hypervigilance are the same as those used for any other normal attentional processing. In essence hypervigilance can be viewed as a deviation from normal attention (Van Damme, Crombez, Eccleston, & Roelofs, 2004). It is the result of normal mechanisms working within an abnormal situation that occurs when threat value of pain is high, a fear system is activated and the individuals' current concern is to escape and avoid pain. Whilst the attentional system is maintained, the pain sufferer may rely more heavily on specific processes and be less able to execute others, such as disengagement. As such, CPP may rely on avoidance behaviours rather than preventative behaviours, which are effective in the short term but may be increasingly maladaptive for long term pain conditions.

Eysenck's hypervigilance theory assumed that only individuals with trait anxiety are vulnerable to hypervigilance to threat. Low trait individuals were believed to become avoidant and orient attention away from threat. From an evolutionary perspective, there is no survival advantage in ignoring threat. Instead Mogg and Bradley (1998) proposed a threshold explanation relating to hypervigilance and attention to threat. They believed that a non-linear relationship exists between the level of threat and the attentional bias. If threat is only of a low level, it will be ignored. However, as the threat level increases it is attenuated to and acted upon.

Williams' Integrative Account

The central theoretical distinction for this account was between automatic and strategic information processing (Williams, Watts & MacLeod, 1988). Automatic processing is when the threat value of a stimulus is assessed at a pre-attentive stage. Once the source has been assessed as threatening, strategic processing directs attention towards the perceived threat. Similarly to Eysenck's theory, the integrative account proposed that high trait individuals selectively attend to threat-related stimuli, creating a greater vulnerability in developing and maintaining specific

disorders, as an attentional bias towards sources of threat would make the environment seem more threatening, raising the individuals state anxiety levels. Through avoiding other potential sources of threat, the individual never has the opportunity to disconfirm their current beliefs. In contrast non-anxious individuals selectively attend away from threat-related stimuli.

Beck's Schema Model

By far the most influential model relating to cognition and anxiety is Beck's Schema Model (Beck et al, 2005) which has been extended and specifically applied to the experience of pain by Pincus and Morley (2001). This model assumes that when focused with a threat, the individual does not have to reappraise the situation anew. Instead past experiences can be recalled and used to guide the individual in how to react. This advance preparation involves the activation of cognitive constellations called schemas, which allow the individual to select and recall relevant information when needed. The first systematic schema theory was developed by Bartlett (1932) who regarded schema as a body of knowledge stored within long-term memory.

A schema may be narrow of focus and based on specifically concrete items, such as shoes, or it may be broad focused and based on abstract concepts, such as justice. A cognitive set occurs when a constellation of schemas are activated. This set is exclusionary in nature, by blocking all which does not relate directly to the content of the schema. This focus on attention achieved through the cognitive set, results in an influence over an individual's perceptual, interpretational, association and memory processes. When ambiguous information is encountered, the cognitive set will determine the meaning assigned to the stimulus.

In addition to schemas, there is an organising principle known as a mode. A mode contains rules and concepts which are organised into themes. A dominant mode will determine the aspect of a schema that is activated and is believed to represent the focus of current concern of the individual. The activities of the modes are reflected in the thinking disorder characteristic of anxiety, depression and other related disorders. An overly fearful individual may have a dominant mode of vulnerability and danger guiding the activation of schema. As such the individual may make conceptual errors such as misinterpretation, overgeneralization, and exaggeration in relation to threat. Cognitive bias results from the activation of

schemas relevant to the mode and from the deactivation of schemas inconsistent with it.

In anxious individuals or pain fearful patients, maladaptive schemas may develop which involve an enhanced detection of threat leading to a state of constant vulnerability (Eysenck, 1992). Schemata direct processing resources to aspects of the internal and external environment which reflect the current concerns of the individual. Therefore, for anxious patients anything seen as a threat will be processed as threatening, ambiguous stimuli will be interpreted as threatening and threatening information will be retrieved from memory. Prolonged activation of fear schemas can lead to the variety of symptoms associated with anxiety such as distress, inhibitions, disturbance of sleep, appetite and tremors. In relation to anxiety, Beck believed that anxiety was maintained until an all-clear signal was given. However, for pain-fearful pain patients, the pain condition is not resolved. As such danger and threat are always assumed to be present and imminent. Schemas used for processing pain-related information become hypervalent, while schemas related to safety remain relatively inactive.

The Schema Enmeshment Model of Pain

Pincus and Morley (2001) applied schema theory to the experience of pain, known as the Schema Enmeshment Model of Pain. Schemas are viewed as being relatively stable over time, but as certain information is activated repeatedly, and irrelevant elements are inactivated, a process of enmeshment may occur. This is when the repeated activations from several schemas become associated with each other, with elements eventually being incorporated into one another. The consequence of this is that after enmeshment occurs, events that previously only activated one schema may now also activate other possibly unwanted schemas. The model posits that attentional biases demonstrated for pain-related information in pain patients is the result of the enmeshment of three schemas representing pain, illness and self.

The pain schema represents the sensory, spatial and temporal features of pain and is associated with an interruption of on-going behaviour and the initiation of protective behaviours. The illness schema contains information regarding affective and behavioural consequences of illness, essentially quality of life views. The illness schema and pain schema are separated concepts as there are some illnesses which do not elicit pain and pains that are not the signal of illness.

The third and final schema is related to the concept of the self. At any one time individuals can hold many distinct concepts of the self, this can be situational; work or home self, or related to the relationships with other people; parent or child self, or be more abstract; the ideal self, which may be the attributes the individual believes will make them perfect. Each concept of the self can represent the thoughts, behaviour and emotions related to that character. The schema enmeshment model proposes that the level of enmeshment between the schema is determined by how disrupted the self-schema has been due to the pain experience.

The three schemas are never independent. However, the critical feature of the model is the extent to which the schemas overlap and the context which they overlap in. According to the model, a healthy person will demonstrate a partial overlap between all three schemas, a chronic pain sufferer who is an adaptive coper will demonstrate a partial enmeshment of pain and illness whilst the self-schema remains separate. An experience of acute pain will result in a partial enmeshment of pain and self-schema, with a separate illness schema. Finally, a complete enmeshment of all three schemas is evident in a chronic pain condition. The critical feature is the extent to which the pain schema is linked to both the illness and self-schema.

The enmeshment model posits that the biases in information processing demonstrated by CPP are the result of this overlap. Specifically, self-relevant information is always prioritised and information congruent with the self receives preferential processing. In CPP, it is suggested that the three schemas are completely enmeshed. As such, pain or illness-related information is given preferential processing, as it has become so entwined with the concept of the self. This results in attentional biases, whereby pain and illness-related information, as part of the self, is always given preferential processing.

The models of cognitive biases summarised here, may take different perspectives upon the mechanisms which either cause or maintain selective attentional processing within anxious individuals or CPP. However, there is a theoretical consensus that attentional biases can impact a wide variety of conditions including anxiety and chronic pain conditions.

1.7.2 Experimental Tasks

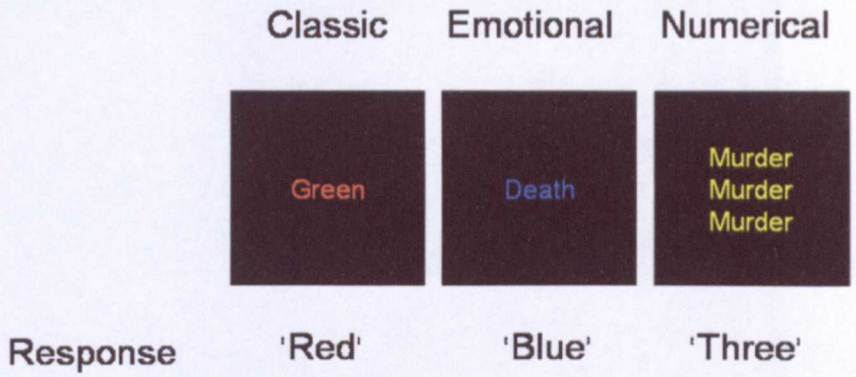
Attentional biases have been observed using several different tasks, suggesting that the phenomenon is not an artefact of a particular experimental task. The general concept is that individuals display attentional biases to stimuli that they perceive as threatening. The object of threat is often symptom specific and with a few exceptions, the empirical evidence is supportive of this view (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Eysenck, 1992; Williams, Mathews, & MacLeod, 1996). The wealth of data on attentional biases allows for a global prediction that if threatening and neutral stimuli occur together, the attention of an anxious individual will be biased in relation to the threat. Empirical investigations of pain-related attentional biases have adopted a number of different experimental paradigms, such as the stroop task, the dot probe task and the exogenous cueing task. The majority of the literature employs the stroop and dot probe tasks and therefore a brief discussion pertaining to both will be presented. However, the work contained within this thesis has utilised the dot probe task only.

Emotional Stroop

The emotional stroop task (sometimes referred to as the modified stroop), is an adapted version of the classic colour-naming stroop interference paradigm developed in 1935 (Stroop). In the classic version, colour words are presented in coloured ink. The aim of the task is to ignore the semantic content of the word and name the ink colour. Trials are either congruent, (the word 'green' printed in green ink) or incongruent (the word 'green' printed in blue ink).

The emotional stroop manipulates the valence of the words to gain an interference effect. This can be achieved in one of two ways. Firstly, some tasks require the colour of the ink to be named, with trials being either of a neutral valence, e.g. the word 'curtain', or of a threatening nature e.g. the word 'death'. Secondly, some tasks have multiple words presented on the screen. The task requires the respondent to indicate the number of presented words, (For different manipulations of the stroop task see Figure 1.2). Regardless of the method of modification, the task still infers selective attentional bias through the differences in response latencies for neutral trials compared to emotional trials. Longer latencies for threat categories are indicative of a threat-related bias.

Figure 1.2 The Different Manipulations of the Stroop Task



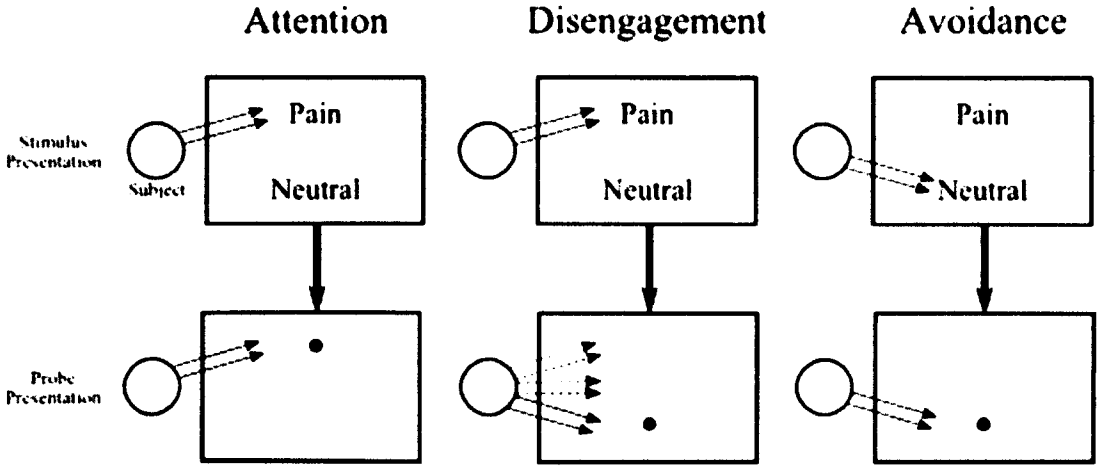
The modified stroop task was initially the most widely used experimental paradigm for investigating attentional biases within emotional disorders (Bar-Haim et al, 2007) and was the first paradigm used to investigate pain-related attentional biases (Pearce & Morley, 1989). However, several criticisms of the task have been made, namely that the task may be measuring additional processing as well as attention, including an interference with response selection, that biases may reflect information processing differences or differences in verbal response ability (Asmundson, Kuperos & Norton, 1997; Liossi, Schoth, Bradley, & Mogg, 2008).

The dot probe task

To overcome some of the criticisms of the emotional stroop task, the dot-probe task was developed and has become the optimal experimental paradigm for the study of pain-related selective attentional biases (MacLeod, Mathews, & Tata, 1986). The paradigm includes two components. Firstly, participants are presented with a word pair, with one word appearing above the other, but remaining within the visual field. The second component involves the detection of a visual probe, usually a small dot, which will replace one of the words after a predetermined stimulus presentation time. Respondents indicate via a button press if the probe occurred in the upper or lower position on the screen. The critical manipulation of the task is that on some trials one of the words will be a pain-related word, paired with an emotionally neutral word. The detection latency for the dot probe is an indicator of whether the individuals' visual attention is oriented towards the stimuli or away from the stimuli. Participants who possess a selective attention bias should have shorter detection latencies for when the dot replaces the pain-related word, compared to when it

replaces the paired neutral word. A discussion of the method used in the two dot probe studies contained within this thesis can be found in Chapter 3.4.

Figure 1.3 The Attentional Measures of the Dot Probe Task; Attention, Disengagement and Avoidance.



Reproduced from Frewen, Dozois, Joanisse & Neufield (2008)

The task is considered a more direct measure of attention than the stroop task. Firstly, the task allows for an investigation into how visual attention is allocated, by examining both attentional engagement and avoidance of threat stimuli (Figure 1.3) Secondly, the task requires a neutral response (button press) to a neutral stimuli (the visual probe), thus overcoming methodological issues relating to response biases. The dot probe task is a very flexible task and has been used for both linguistic and pictorial stimuli (Khatibi, Dehghani, Sharpe, Asmundson, & Pouretemad, 2009), with varying presentation rates (Liossi et al, 2008; Roelofs, Peters, van der Zijden, Thielen, & Vlaeyen, 2003a) and different paradigms for responding (detection versus discrimination).

Asmundson, Wright & Hadjistavropoulos, (2005a) compared performance on the dot probe task and a modified stroop task in CPP and controls. Although both groups displayed a disengagement effect to threat words, there was a lack of consistency in findings between the two paradigms. This had led to speculation that the tasks are measuring different aspects of selective attention.

Although the dot probe task was originally believed to overcome some of the methodological criticisms relating to the emotional stroop, specifically in relation to

the production of responses (Asmundson et al, 1997) the task is not without its criticisms. Schmukle (2005) questioned the test-retest reliability within a non-clinical population, for the semantic and pictorial versions of the task. The different versions were administered twice, with a one week retest interval. The study was not without its own methodological confound, specifically relating to practice effects. The same stimuli were used within each testing session. Dividing the stimuli and counterbalancing between experimental sessions could have overcome this problem, resulting in more robust experimental design. Furthermore, concern was expressed regarding which attentional process was being demonstrated using the bias index. Selective attention towards threat can reflect quick orientation towards threatening stimuli and/or difficulty in disengaging attention away from threatening stimuli (Legestee, Tullen, Kallen, Dielman, Treffers & Verhulst, 2009). However, to overcome this problem, the inclusion of a suitable neutral task allows for congruency and incongruency indexes to be calculated, (Koster, Crombez, Verschuere, & De Houwer, 2004b).

1.7.3 Chronic Pain and Attentional Bias

In comparison to the extensive number of studies examining selective attentional biases in anxious individuals; (a recent meta-analysis by Bar-Haim et al, 2007 included 172 studies), empirical investigations into pain-related selective attention using the modified stroop and dot-probe tasks are limited in comparison. This may not be a reflection of the interest in pain-related attentional biases, but in contrast to the consistent findings regarding pain-related memory and interpretational biases, empirical investigations of pain-related attentional biases have produced mixed results. (A summary table for both dot probe and stroop studies investigating pain-related attentional biases can be found in Table 1.1).

The Fear-avoidance model stresses pain-related fear as a determining characteristic leading to hypervigilance of pain-related stimuli. Therefore, pain-related fear has been investigated in relation to attentional biases, in both pain-free and clinical populations. In some studies group membership has been assigned based on pre-testing FOP scores or post hoc analyses of scores. Asmundson & Hadjistavropoulos (2007) used several post hoc strategies and different representations of pain-related fear to re-analyse a previously published dot probe study (Asmundson, Kuperos & Norton, 1997). The strategies were tertiles based on

ASI and PASS scores and a cluster analysis on the ASI and PASS scores. Cluster analysis identifies homogenous groups based upon a selected characteristic. Regardless of the method employed 48% of the participants were consistently allocated to the same group, with CPP being consistently assigned to the high FOP groups. Therefore, although a variety of methods have been used within the literature to allocate individuals to groups based upon levels of pain-related fear, it would appear that there is some consistency in group membership, regardless of the method used.

Table 1.1 A Review of Pain Related Dot Probe and Stroop Studies

Task	Author	Year	Sample	*N*	Bias	Overview of Findings
DP	Asmundson	1997	CPP/HC	19/22	Sensory	Findings related to anxiety sensitivity-low FOP show avoidance
	Keogh	2001	HC	74	Sensory	HC separated on FOP scores
	Keogh	2002	HC	100	X	Interpretive bias for high AS group-bias not sig.
	Keogh	2003	HC	81	Sensory	Unmasked trials-low FOP orient away-reversed for masked trials
	Dehghani	2003	CPP	169	Sensory	High FOP –slow responding compared to low FOP
	Roelofs	2003	HC	90	X	HC separated on FOP scores
	Roelofs	2003	HC	120	X	Varied presentation rate, 500ms, 750ms, 1000ms
	Dehghani	2004	CPP	42	Sensory	CPP tested pre/post and one month follow up after PMP
	Roelofs	2005	CLBP/HC	31/40	X	Word/pictorial versions-CPP difficulty disengaging from pictures
	Asmundson	2005	H-A/HC	30/19	Sensory	Relationship between bias index and AS
	Boston	2005	HC	100	Sensory, affective	Varied threat expectancy/coping strategies-threat related to affective
	Hunt	2006	HC	124	Anxiety	High AS associated with vigilance in both masked/unmasked trials
	Asmundson	2007	CPP/HC	36/29	Sensory, affective, health	Effects only significant for high FOP-reanalysis of 2005 study
	Liossi	2008	H-A/HC	15/18	Sensory	Varied presentation rates-att. bias for CPP at 1250ms
	Sharp	2009	RA	100	Sensory	Disengagement effects
	Khatibi	2009	CPP/HC	170/40	Painful faces	High FOP CPP-att. towards painful faces
	Schoth	2010	H-A/HC	17/21	Painful faces	Varied presentation rate-CPP orient towards headache pictures
	Haggmann	2010	CPP/HC/APP	107/50/51	Sensory	Low FOP-orient towards sensory pain words, including acute patients
Stroop	Pearce	1989	CPP/HC	16/16	X	MPQ descriptors
	Duckworth	1997	CPP/HC	19/10	Sensory, depression,	CPP divided on somatic complaints- higher no. of complaints greater interference
	Pincus	1998	CPP/HC	20/20	X	Memory bias demonstrated
	Pincus	1998	CPP/HC	17/17	X	RT related to anxiety and depression score not pain intensity
	Snider	2000	CPP/HC	33/33	Sensory, affective	Masked/unmasked conditions-effect unmasked condition only
	Crombez	2000	CLBP	25	Sensory	Results linked to current pain intensity
	Roelofs	2002	HC	80	X	Experimentally induced fear and pain
	Roelofs	2002	HC	40	X	Experimentally induced pain
	Andersson	2003	CPP/HC	20/20	Sensory	CPP slower on pain words-but not significantly diff to controls
	Roelofs	2005	CLBP	30	X	Examined personal relevance of words
	Gonzalez	2010	FM/HC	25/24	X	FM-greater non sig. interference on neutral, symptom & negative words
Both	Asmundson	2005	CPP/HC	36/29		Lack of consistency across tasks

APP-Acute pain patients, CPP-chronic musculoskeletal pain, CLBP-chronic low back pain, FM-Fibromyalgia, H-A-Headache, RA-rheumatoid arthritis. *N*- participant numbers (patients/controls). Sensory-pain related words. FOP-fear of pain, AS-anxiety sensitivity, att.-attention, PMP-Pain Management Program.

If pain-related fear is a vulnerability factor, as proposed by the Fear-avoidance model, one may expect currently pain-free individuals, high in pain fearfulness, to demonstrate attentional biases to pain-related stimuli. Keogh, Ellery, Hunt & Hannent (2001a) used a dot probe task to investigate attentional biases in pain-free participants separated on FPQ scores. Participants with high pain fearfulness were found to orient towards pain words, compared to threat or positive stimuli. In contrast participants low in pain-related fear shifted their attention away from pain-related words. A follow-up study used masked and unmasked trials, to investigate if these biases are automatic or strategic in nature. An attentional effect was only found for the unmasked trials, suggesting that biases are strategic in nature. Additionally, the study only provided partial support for the previous study, with the low FOP group found to orient attention away from the pain words, but not attentional effect being demonstrated by the group with higher levels of pain fearfulness. Roelofs et al, (2003) conducted a replication of the Keogh et al, (2001a) study which failed to find any attentional bias effects for pain-related words irrespective of FOP levels. However, one caveat was their participants had lower FOP levels than those of the Keogh et al, (2001a) study.

One explanation for the inconsistency in findings is that attentional biases may become evident in individuals with high levels of pain-related fear, as a response to an acute pain experience. To investigate this further, Boston & Sharpe, (2003) used a cold pressor task to provide an experimental pain stimulus, with different groups receiving either threatening or reassuring information regarding the task, to manipulate fear levels. Attentional effects were demonstrated depending on threat levels, with participants with high levels of threat expectancy being hypervigilant to affective words (e.g. miserable, unbearable and cruel) and participants in the low threat condition orienting towards pain words. Roelofs, Peters & Vlaeyen, (2002) administered an ischemic pain test to investigate if acute pain can create an interference effect on a concurrently performed stroop task. Fear levels were manipulated by informing some participants that they would receive an electric shock during task completion, which was not administered. Irrespective of pain and fear manipulations, all groups demonstrated an interference effect for naming the colour of pain words compared to neutral words, which was not associated with FPQ scores. However, the pain only group did demonstrate a significant interference of pain words, compared to the control group. A follow-up experiment which omitted

the fear manipulation to investigate attentional bias and pain levels only, reported no significant interference effects (Roelofs et al, 2002).

The results of the presented studies demonstrate the mixed findings relating to pain-related fear and attentional biases within pain-free populations. Although there is some evidence for attentional biases related to pain-fearfulness in this cohort, the evidence is not unequivocal. The fear avoidance model proposes that both pain-related fear and the resulting hypervigilance can both exacerbate and maintain a chronic pain experience. Therefore, selective attentional biases should be more evident within chronic pain populations who have high levels of pain fearfulness. Several dot probe studies have investigated attentional biases in pain patients suffering from a variety of chronic conditions. In one of the largest pain-related attentional bias studies, Dehghani, Sharpe, & Nicholas, (2003) investigated attentional biases in 169 musculoskeletal pain patients. Patients were separated into low, medium and high FOP groups based upon FPQ scores. Those participants with high levels of pain-related fear demonstrated a selective attentional bias, although contrary to expectations, this resulted in avoidance to pain words. In comparison patients with low FOP demonstrated a hypervigilant effect for pain words. In a reanalysis of a previous study Asmundson et al (2007) investigated FOP and attentional bias in both musculoskeletal pain patients and controls. Post-hoc analyses assigned participants into groups of high, medium and low level pain fearfulness. Compared to the low FOP group, CPP with high levels of pain fearfulness demonstrated hypervigilance for all word types.

In a longitudinal study investigating the modification of attentional biases in CPP, Dehghani, Sharpe, & Nicholas, (2004) tested musculoskeletal patients before they commenced a pain management programme (PMP), when they initially completed the programme and at a one-month follow up. A hypervigilance for pain words was demonstrated at both pre and post testing sessions, but this was not evident at follow up. Changes in attentional biases were predicted by changes in pain fearfulness measured by TSK scores. A recent study conducted by Khatibi et al, (2009) extended findings from pain-related verbal stimuli to pictorial stimuli. Photographs depicting painful, happy and neutral facial expressions were presented in a dot-probe paradigm. Chronic pain patients with low levels of pain-related fear were faster at responding to probes which replaced neutral facial expressions, whereas patients with high levels of fear were faster at responding to probes replacing faces with expressions of pain.

Another aspect of fear that has been investigated in relation to pain-related attentional biases is anxiety sensitivity (AS), which is defined as the fear of anxiety-related sensations (Turk & Wilson, 2011). Individuals with high AS interpret unpleasant physical sensations, such as a rapid heartbeat, as signals of danger. Self-report measures of AS have been correlated with selective attentional processing. Asmundson, Carleton & Ekong, (2005b) found significant correlations between scores on the Anxiety Sensitivity Index and the attentional bias index for sensory words for both pain and control groups. Keogh, Dillon, Georgiou & Hunt, (2001b) investigated attentional biases in pain-free controls separated on high and low levels of AS. Similarly to the findings of pain-related fear, individuals with high levels of AS were found to demonstrate an attentional bias towards physically threatening material, whereas individuals with low AS shifted attention away.

In conclusion, there does appear to be an association between pain fearfulness and selective attentional biases, although this relationship is not always consistently found. However, other pain-related cognitive biases have been demonstrated. Memory and interpretational biases have been demonstrated more consistently within the literature, indicating that there is some differential processing of pain-related stimuli. Possible explanations for the inconsistency relate to methodological issues relating to differing levels of pain-related fear across studies, and the heterogeneity of the clinical groups.

1.8 Aims and Objectives

These are; using MRI to investigate morphological and functional differences in a subgroup of chronic low back pain patients; to investigate a prospective characteristic in relation to cognitive bias; to perform a proof of principle that a semantic dot probe task can be adapted for an fMRI environment; to investigate if cognitive biases exist within CLBP patients and if they are altered by the completion of a pain management programme.

Chapter 2 gives a detailed overview of the literature relating to MRI and pain. The chapter focuses upon investigations of brain differences in chronic pain populations, with additional discussions relating to methodological issue surrounding the findings and the possible mechanisms underlying these differences.

Chapter 3 describes in detail the methods and measures used within the presented research.

Chapter 4 comprises an investigation into morphological differences in the brains of chronic low back pain patients and healthy controls. To address criticism of the current literature regarding the heterogeneity of previous cohorts, the participants were further divided into two groups, based upon levels of pain behaviour.

Chapters 5 and 6 describe two studies which investigated selective attentional bias using fMRI. Chapter 5 examines a proof of principle that a dot probe task could be successfully adapted for the fMRI environment. Furthermore, the study investigated a factor which the literature has presented as a predisposing vulnerability to the development of chronic pain, namely fear of pain. Chapter 6 examines a study which extended the findings of the previous chapter, to investigate selective attentional bias in a chronic pain population. Behavioural and cerebral differences were investigated in chronic low back pain patients. To investigate treatment effects patients were tested twice, prior to and completion of a pain management programme.

The thesis concludes with Chapter 7 and discusses the original research findings from Chapters 4-6, with further discussion and interpretation in relation to the wider literature. Future research directions are discussed.

CHAPTER TWO
Magnetic Resonance Imaging and Pain

In recent years the availability of MRI methods of enquiry has resulted in two convergent analysis methods being adopted. Functional magnetic resonance imaging (fMRI) has identified a network of brain areas which are consistently activated to experimental pain stimuli. This network known as the 'pain matrix' will briefly be discussed in Section 2.1.

In comparison, morphological analyses have used T1-weighted anatomical images to investigate underlying morphological differences within the brains of chronic pain patients. The chapter will present an overview of the morphological studies investigating pain states. Section 2.2 will present an initial discussion of MRI and brain changes. Techniques of analysis will be presented in Section 2.3, with their application to specific conditions being discussed in Section 2.4. Methodological considerations relating to the presented studies will be found in Section 2.5. The chapter concludes with a discussion of the underlying mechanisms proposed for morphological changes, Section 2.6.

2.1 Functional MRI and the Pain Matrix

Recent neuroimaging techniques of positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have identified a nociceptive system that is consistently activated to noxious stimuli. This network is commonly referred to as the 'Pain Matrix' taking its name from the 'Neuromatrix' originally proposed by Ronald Melzack in 1989.

The pain matrix as a global system is comprised of the primary and secondary somatosensory cortices (SI and SII), insular cortex, the anterior cingulate cortex (ACC), posterior parietal cortex, the prefrontal cortex (PFC), thalamus, amygdala, basal ganglia, cerebellum, the periaqueductal grey (PAG) and primary motor and premotor cortices (Peyron, Laurent, & Garcia-Larrea, 2000). The six most commonly reported areas of activation in relation to experimental sensory stimulation are ACC, SI, SII, insula, thalamus and the PFC (Apkarian, Bushnell, Treede, & Zubieta, 2005).

Melzack and Casey (1968) and more recently Melzack and Katz (1994) identified a three-dimensional pain experience consisting of sensory-discriminative, affective-motivational and cognitive dimensions. Each of the structures contained within the pain matrix is representative of at least one of the dimensions of the pain experience, but it would be unwise to assume that this relationship was mutually exclusive.

Originally, investigations into the pain matrix compared global brain activity relating to noxious stimuli to periods of no stimulation in healthy controls. In time, neuroimaging studies became more sophisticated at manipulating various demands placed upon the individual during the delivery of noxious stimulation. In recent years these studies have demonstrated that the pain matrix as a global concept may be out-dated and somewhat over simplistic (Apkarian, et al, 2011; Mouraux, Diukova, Lee, Wise & Iannetti, 2011; Neugebauer, Galhardo, Maione, & Mackey, 2009). Although a network of regions is consistently activated in healthy participants to acute experimental pain, other unessential processes may also be included such as attentional, anxiety, fear or autonomic (Apkarian et al, 2011). As such, the roles assigned to specific structures are gradually being updated. Areas, defined as playing an emotive role in pain processing, such as the insula, are now being parcellated into anterior and posterior regions, based partly on anatomy and partly of functional specialisation (Brooks, Zambreanu, Godinez, Craig & Tracey, 2005; Schweinhardt, Lee & Tracey, 2006). As neuroimaging investigations continue, a greater understanding of the network known as the pain matrix will be unravelled.

In conclusion the pain matrix is a collection of structures that are commonly activated in imaging studies that contain a physically painful paradigm. However, as a whole brain system the pain matrix does not provide explicit information about the subjective experience of pain. Through a more detailed dissociation of the structures and the subprocesses that they perform, a greater understanding of the pain experience can be gained.

2.2 Morphological Differences in Chronic Pain

The emergence of magnetic resonance imaging for investigating the functional mechanisms underlying chronic pain conditions has proved to be fruitful. As well as investigating the cerebral processes involved in the response to sensory painful stimuli, the use of fMRI has also led to an understanding of functional

reorganisation, specifically within back pain and phantom limb pain (Flor, Nikolajsen, & Staehelin Jensen, 2006; MacIver, Lloyd, Kelly, Roberts, & Nurmikko, 2008). Initially chronic pain states were assumed to be functional changes within a normal brain structure. The reorganisation indicated either by an increase in cortical activity or a shifting of cortical representations within the brain has led to other methods of enquiry to be considered.

Prior to the availability of MRI, underlying morphology was investigated by the use of post mortem data. However, MRI allows for the study of the human brain *in vivo*, thus enabling investigations into brain structure to be conducted. Morphometry compares specific parameters between two or more groups and assumes that the characteristics which distinguish the groups are associated with the disorder in question. Furthermore, the non-invasive technique permits longitudinal study design. Repeated data collection is acquired over different time points during an intervention, thus examining learning related morphological changes.

Plasticity can refer to either functional or structural changes which occur within the brain as an adjustment to either internal or external environments. It has been suggested that the extent of reorganisation correlates with amount of behaviour change (Draganski, Gaser, Kempermann, Kuhn, Winkler, Buchel & et al, 2006). The behavioural consequences of cerebral reorganisation can either result in beneficial or maladaptive consequences. Beneficial consequences are often seen to underlie the learning of new skills, which allow for a proficiency in the skill (Draganski, Gaser, Busch, Schuierer, Bogdahn, & May, 2004). However, maladaptive consequences may be just as likely to occur when excessive demands are placed upon the organism, resulting in a response in plasticity which favours behavioural loss or the development of disease symptom, which may then be maintained.

2.2.1 Brain Morphometry Using MRI

Structural MRI produces three-dimensional images which are used in volumetric calculations of brain volume. These calculations can be of total brain volume, the volume of tissue components, such as gray matter (GM), white matter (WM) or cerebrospinal fluid (CSF), or the volume of specific regions of interest, such as prefrontal cortex or amygdala. There are several methods of enquiry used to investigate morphological changes within a variety of normal and clinical populations, such as, cortical thickness (DaSilva, Becerra, Pendse, Chizh, Tully, & Borsook, 2008), pixel counting (Maguire, Gadian, Johnsrude, Good, Ashburner,

Frackowiak & et al, 2000), manual tracing (Wilke, de Haan, Juenger & Karnath, 2011), stereology (Howard, Roberts, Garcia-Finana, & Cowell, 2003) and finally voxel based morphometry (VBM; Ashburner & Friston, 2000). In relation to the pain literature, VBM remains the most commonly used method of morphological analysis.

2.3 Analysis Techniques

2.3.1 Voxel Based Morphometry

Voxel based morphometry is an automated whole brain method which performs statistical analyses of variance on T1-weighted MRI images. To enable this method, brain matter is transposed into a three dimensional unit typically measured as $1mm^3$. The volume of brain tissue is calculated by type (GM/WM/CSF) to examine regional differences, for example the proportion of tissue in a given voxel or the proportion of these voxels found within an anatomical location. Furthermore, the segmentation process allows for the quantification of the volume of gray matter, white matter and cerebrospinal fluid, which allows for a total brain volume to be calculated.

The MRI images undergo several types of preprocessing to make them suitable for the statistical analyses to be performed namely spatial normalisation, segmentation and smoothing.

Spatial normalisation; Due to the natural variations in brain size, spatial normalisation warps the images into a common stereotactic space. This ensures that the voxels within the same anatomical location have the same spatial coordinates. Two different approaches can be used which produce two different units of analysis, either gray matter volume (GMV) or gray matter density or concentration (GMD). Gray matter volume is derived from images which are 'modulated', whereby the amount of displacement a voxel undergoes during normalisation, is maintained and added to the voxels numerical value. Therefore, voxels which are stretched or shrunk during image warping have their numerical value subsequently expanded or reduced in proportion to this alteration. Alternatively, gray matter density or concentrate is derived from images which are 'unmodulated' and have not undergone this process. Therefore, the numerical values of the voxels are preserved during normalisation.

Segmentation; Voxels are assigned a value between 0 and 1 which determines the probability of finding a specific tissue type within that voxel or of a specific voxel belonging to a particular tissue type.

Smoothing; The final preprocessing step serves several purposes. Firstly, it renders the data more normally distributed, therefore allowing for parametric statistical tests to be conducted. Secondly, it reduces the effects of between subject differences in the exact location of gyri and sulci. Thirdly, it reduces the number of effective statistical comparisons, thus making the correction for multiple comparisons less severe. Finally, smoothing increases the detection of group differences whose spatial extent matches the width of the smoothing kernel (Schweinhardt, Kuchinad, Pukall, & Bushnell, 2008).

Once the preprocessing steps are completed statistical analyses are then performed using the general linear model. Voxel based morphometry is a mass univariate approach, whereby a statistical test is performed for each voxel. Voxels which survive significance thresholds are then superimposed onto a normalised brain. The data from several participants is then grouped together, with any differences found between groups being attributed to the disease process which is being evaluated.

2.3.2 Cortical Thickness Analysis

The whole brain has an average cortical thickness of between 2.5 and 3mm. The thinnest area is typically the calcarine cortex with a width of approximately 2mm. The superior frontal lobes, superior temporal lobes and the precentral gyrus are typically 4mm in width, making them the thicker regions (Zilles, 1990). The cortex tends to be thicker at the gyral ridges and thinner at the fundi of the sulci. Changes in gray matter associated with the pain state, may result in either a thinning or thickening of the cortex.

Cortical thickness analysis is an automated whole brain analysis. Similarly to VBM analysis, some operator input may be needed to ensure maximum results. Prior to analysis, the individual MRI scans are segmented to identify the gray and white matter boundaries. Each individual segmented brain is then aligned to either a standardised template, such as the Talairach template (Blankstein, Chen, Diamant & Davis, 2010), or to a group average brain (Davis, Pope, Chen, Kwan, Crawley, & Diamant, 2008). Although there may be variations to the methodology undertaken, in general there are two approaches to the analysis; a surface-based approach and a voxel based approach. The surfaced-based approach typically involves an extraction of the surface between gray and white matter, with a volume of thickness being determined by calculating the distance between the extracted surfaces. To compare

groups of participants, corresponding cortical surfaces are matched. Voxel-based analysis uses whole voxel information to define gray and white matter boundaries. Cortical thickness is then calculated whereby each voxel of gray matter is assigned a cortical thickness value. Similarly to VBM analysis, differences between groups can be calculated on a voxel by voxel basis.

Cortical thickness analysis (CTA) has been used in conjunction with other methods of analysis to investigate brain differences in chronic pain patients. To date CTA combined with VBM in chronic pain patients has been primarily used in studies investigating structural differences in patients with irritable bowel syndrome (Davis, et al, 2008; Blankstein et al, 2010; Seminowicz, Labus, Bueller, Tillisch, Naliboff, Bushnell & et al, 2010). Although a recent study has combined both CTA and fMRI to investigate brain changes in CLBP patients (See Section 2.4 below for detailed discussion).

2.3.3 Manual Methods

The manual methods used to quantify brain volume tend to be either manual tracing (planimetry) or stereology, which is the method used for data analysis contained within this thesis. Whilst the exact procedures of the techniques vary, manual methods share several commonalities. Firstly, they are semi-automated relying on both computer software and operator skill to produce accurate findings. Secondly, the T1-weighted image is often preprocessed to achieve a clearer image resolution or to place within a specific unit of volume. However, this is not a requirement for analysis, as manual tracing used within a clinical setting may be performed upon the MRI or computed tomography (CT) image in its native space. Depending upon the region being studied, demarcation of the region of interest using *a priori* anatomical landmarks may be performed. For planimetry, the border of the area is traced around using the computers mouse. The software then automatically 'fills' this area creating a three-dimensional volume of interest. It is this volume upon which statistical analysis will be performed.

Stereological methods vary slightly as tracing is replaced by the placement of a stereological grid system over the image. The grid contains points which are representative of a specific volume size. Points which are contained within the area of interest are counted, thus producing a computed volume for the region of interest. These values are used in statistical analysis. (Please refer to Chapter 3.2 for an in-depth discussion of the stereological technique used within this thesis).

2.3.4 Comparison of Techniques

There are several factors which may influence the choice of analysis technique used. Any perceived strengths or weaknesses inherent in the techniques are relative to issues such as time, software or personnel that may be available for the length of the study.

Whole brain ROI Analysis

In comparison to whole brain techniques such as VBM and CTA, manual methods are used for region of interest analyses. Not only does this make them more time consuming, it may potentially bias the results. Morphological differences may exist between cohorts but may go undetected, as manual methods rely upon *a priori* hypotheses. However, whilst whole brain techniques may avoid this potential bias, they are more susceptible to false positives, as 'chance' findings are more likely to be reported.

Voxel/Volume of Interest

Automated techniques work on a voxel by voxel basis, with a voxel often being a $1mm^3$ unit of measurement. They are therefore able to quantify brain tissue, such as gray matter and white matter, as well as giving two different units of analysis, GMV/GMD. Furthermore, VBM can overcome the possibility of edge effects by using a numerical matter value between 0-1 and excluding voxels that fall below an arbitrary value.

Manual methods cannot replicate a voxel wise analysis. Instead a volume of interest measurement is recorded, which may contain both gray and white matter. Similarly, boundaries of structures can be difficult to define, even with demarcation of a region using anatomical landmarks. On occasion as a way of overcoming boundary issues, the volume of interest will be smaller than the actual structure. Consistency of analysis is used as a way of overcoming boundary issues.

Operator Skill

The skill of the operator may also determine the results. Whereby VBM is relatively simple to use, manual tracing is much more labour intensive and requires some training to become a skilled operator. Observer interpretation of the volume of interest may impact the volume estimation gained. Often inter and intra-rater reliability studies are performed to overcome any under or over estimation of volume which may occur. Similarly, cortical thickness analysis requires the alignment of segmented images to a template. The operator must ensure that this is performed

accurately otherwise misaligned images may produce false positives or obfuscate small scale effects.

Preprocessing of Data

Whilst manual methods are more susceptible to operator bias, the results gained from automated techniques can be influenced by the decisions of the investigator when preprocessing the data. In the case of VBM, there are several steps which require decisions which may impact the results, such as the size of smoothing kernel, modulation, or template used for warping. Furthermore, the extensive preprocessing that VBM datasets undergo may obliterate any small effects that exist within the dataset. Manual methods, which undergo much less preprocessing, if any, may be better suited to detect small scale effects.

Voxel based morphometry and cortical thickness analyses are most widely used for research purposes (May, 2008), unlike stereological and tracing methods which can be used in both research settings and clinical practice (Acer, Ugurlu, Uysal, Unur, Turgut & et al, 2010; Eriksen, Rostrup, Andersen, Lauritzen, Fabricius, Larsen & et al, 2010; Roberts, Puddephat, & McMulty, 2000). One explanation for this is that VBM needs a relatively large sample size (N=20) for smaller effects to be detected (Wood, 2010). Stereology and manual tracing however, can be used in much smaller sample sizes. These techniques can be used in longitudinal studies of an individual patient. As such even with some of the technical weaknesses compared to automated methods, manual methods are considered the gold standard for investigating morphological differences in clinical settings.

2.4 Condition Specific Changes

In recent years there has been an increase in the number of studies investigating possible brain differences in chronic pain patients when compared to pain-free controls. Several studies have examined structural reorganisation in headache and migraine syndromes, but due to the issue relating to chronicity status of these conditions, they will not be discussed any further (Kim, Suh, Seol, Oh, Seo, Yu & et al, 2008; Matharu, Good, May, Bahra, & Goadsby, 2003; Schmidt-Wilcke, Ganssbauer, Neuner, Bogdahn, & May, 2008a; Schmitz, Arkink, Mulder, Rubia, Admiraal-Behloul, Schoonman & et al, 2008). Three additional standalone studies

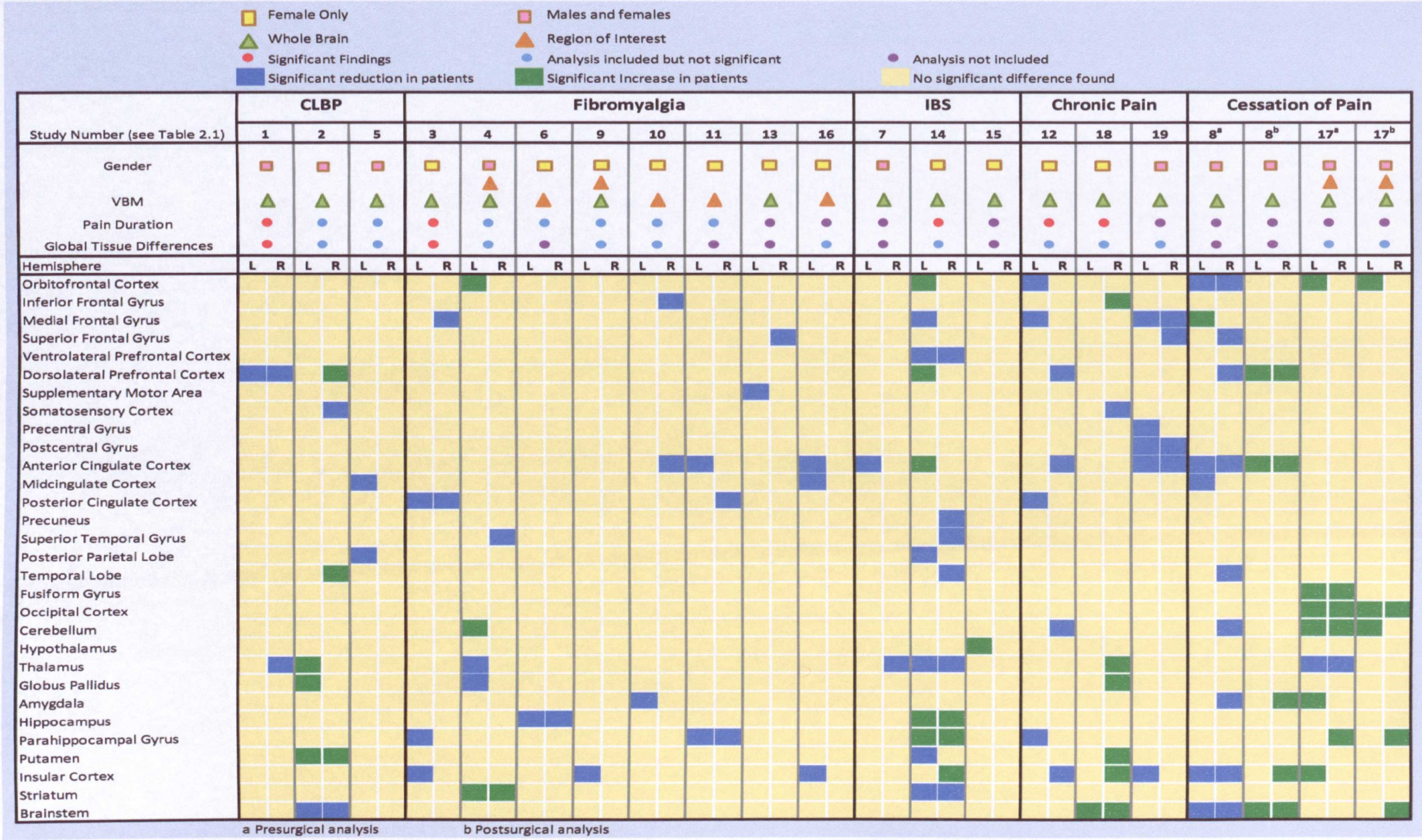
investigated chronic vulvar pain (Schweinhardt et al, 2008), menstrual pain (Tu, Niddam, Chao, Chen, Chen & et al, 2010) and finally chronic tension type headache (Schmidt-Wilcke, Leinisch, Straube, Kampfe, Draganski & et al, 2005). All studies indicate gray matter loss in the patient groups, but similarly to the investigations of headache and migraine, the chronic status of the condition may confound findings. The remaining studies presented here have examined morphological changes relating to chronic pain states and these will be discussed in greater detail. To accompany the detailed summary of the morphometric studies, Table 2.1 and Figure 2.1 respectively, summarise the technical specifications of the VBM studies and provide an overview of findings.

Table 2.1 Technical Specifications of VBM Studies (See Overleaf)
[a] -Presurgical / [b] -postsurgical; **HC**-Healthy Control; **CLBP**-Chronic Low Back Pain; **FM**-Fibromyalgia; **AD**-Affective Disorder; **OA**-Osteoarthritis; **PD**-Pain Disorder; **F**-Fatigue; **IBS**-Irritable Bowel Disorder; **mTMD**-Myofascial Temporomandibular Pain; **PIFP**-Persistent Idiopathic Facial pain; **GMC**-Gray Matter Concentrate; **GMV**-Gray Matter Volume; **n.s**-Not Specified; **Sig**-Significance.

Table 2.1 Technical Specifications of VBM Studies

Study Number	Study	Participants	MRI	Analysis Software	Tissue Measure	Smoothing Kernel	Basis for Sig
1	Apkarian et al, 2004a	26 CLBP 26 HC	1.5T	SPM99	GMV	12mm	Cluster
2	Schmidt-Wilke et al, 2006	18 CLBP 18 HC	1.5T	SPM99	GMD	10mm	Cluster
3	Kuchinad et al, 2007	10 FM 10 HC	1.5T	n.s	GMD	10mm	Voxel/ Cluster
4	Schmidt-Wilke et al, 2007	20 FM 22 HC	1.5T	SPM2	GMD	10mm	Cluster
5	Buckalew et al, 2008	8 CLBP 8 HC	3T	SPM2	GMD	12mm	Voxel
6	Lutz et al, 2008	30 FM 30 HC	1.5T	SPM2	GMV	n.s	Voxel
7	Davis et al, 2008	9 IBS 11 HC	1.5T	SPM2	GMV	10mm	Voxel
8[a]	Rodriguez-Raecke et al, 2009	32 OA 32 HC	3T	SPM2	GMC	10mm	Voxel
8[b]	Rodriguez-Raecke et al, 2009	10 OA 3 timepoints	3T	SPM2	GMD	10mm	Voxel
9	Hsu et al, 2009	29 FM+AD 29 FM-AD 29 HC	3T	SPM5	GMV	10mm	Voxel
10	Burgmer et al, 2009	14 FM 14 HC	3T	SPM5	GMV	n.s	Cluster
11	Wood et al, 2009	30 FM 20 HC	1.5T	SPM2	GMD	12mm	Voxel
12	Valet et al, 2009	14 PD 25 HC	1.5T	SPM2	GMD	8mm	Voxel
13	Puri et al, 2010	5 FM+F 5 HC	3T	FSL-VBM	GMD	3mm	Cluster
14	Seminowicz et al, 2010	55 IBS 48 HC	3T	CIVET	GMD	8mm	Cluster
15	Blankstein et al, 2010	11 IBS 16 HC	3T	SPM5	GMV	10mm	Cluster
16	Robinson et al, 2010	14 FM 11 HC	3T	Brain-voyager	GMV	n.s	Cluster
17	Gwilym et al, 2010	16 OA 16 HC	3T	FSL-VBM	GMV	7mm	Voxel
18	Younger et al, 2010	14 mTMD 15 HC	3T	SPM8	GMV	8mm	Voxel/ Cluster
19	Schmidt-Wilke et al, 2010	11 PIFP 11 HC	1.5T	SPM5	GMV	8mm	Cluster

Figure 2.1 An Overview of VBM Findings



2.4.1 Fibromyalgia

Fibromyalgia (FM) is a chronic widespread musculoskeletal pain disorder. Clinical symptoms include pain, stiffness, subjective weakness and muscle fatigue. To date, the study of brain changes in chronic pain states have focussed primarily on FM patients, with a growing body of evidence indicating brain changes in FM patients.

The study conducted by Kuchinad, Schweinhardt, Seminowicz, Wood, Chizh, & Bushnell, (2007) compared morphological differences in 10 female FM patients and 10 age-matched female controls. Age related reductions in both gray matter volume and whole brain volume were demonstrated in the clinical group in comparison to the controls. Other regional decreases in gray matter were demonstrated in left parahippocampal gyrus, left insula, right medial frontal cortex and bilateral mid/posterior cingulate gyrus. Interestingly these findings were negatively correlated with time since diagnosis, indicating the results may be the result of the condition, rather than a preceding factor.

Schmidt-Wilcke and colleagues examined structural changes in 20 FM patients and 22 matched controls (Schmidt-Wilcke, Luerding, Weigand, Jurgens, Schuierer, Leinisch, & Bogdahn, 2007). Whilst no whole brain effect was found, patients did display a reduction in the right superior temporal gyrus and left dorsal thalamus. Additionally an increase in gray matter density was demonstrated by the patient group in the left cerebellum, left orbitofrontal cortex and bilateral striatum. In contrast to the findings of Kuchinad et al, (2007) no significant correlation was found between pain duration and gray matter density within these regions. A positive correlation was found between sensory pain scores and GMD in the orbitofrontal cortex. No significant correlations were demonstrated between affective pain scores and GMC. However, when controlling for the severity of depressive symptoms, observed differences in cerebellum, PFC, superior temporal gyrus and thalamus were no longer evident.

More recently Burgmer and colleagues (2009) compared morphological differences in 14 fibromyalgia patients with an equal number of controls (Burgmer, Gaubitz, Konrad, Wrenger, Hilgart & et al, 2009). A decrease in gray matter volume in the right anterior cingulate cortex, right inferior frontal gyrus and left amygdala were demonstrated for the FM patients. No increases in gray matter were detected. Interestingly, correlational analyses revealed a significant positive relationship between medication intake and right ACC volume, whereby longer medication use

correlated with greater gray matter volume within this area. No significant relationship between pain duration or pain severity was found.

Voxel based morphology has also been combined with diffusor-tensor imaging (DTI) to investigate morphological changes in fibromyalgia. Lutz and colleagues used both techniques to assess gray and white matter changes in 30 female fibromyalgia patients and 30 matched controls (Lutz, Jager, de Quervain, Krauseneck, Padberg & et al, 2008). The VBM analysis was performed on several *a priori* regions of interest. Compared to controls, patients had a decrease of gray matter volume of the hippocampus bilaterally and a non-significant trend to lower GMV in the bilateral amygdala. Microstructural changes showed a decrease in FA in bilateral thalamus, and insular regions in patients compared to controls. Significantly higher gray matter FA values were found in amygdala, hippocampus, ACC, SFG and postcentral gyrus. The white matter of the ACC and SFG also demonstrated significant increases in FA. The results of the DTI analysis were significantly correlated with several self-report measures examining the symptoms of fibromyalgia, such as pain intensity, fatigue and anxiety.

A further study which combined experimental methods to investigate morphological changes in FM patients was conducted by Robinson, Craggs, Price, Perlstein & Staud, (2010). The authors had conducted a previous fMRI study investigating cerebral activation to windup pain, which occurs when a stimulus of low intensity is repeatedly applied to produce a painful response (Staud, Craggs, Perlstein, Robinson & Price, 2008). The results indicated 19 brain areas had become activated during the study. Robinson et al, (2010) extended these findings to investigate if gray matter volumes of these regions differed between 14 FM patient and 11 controls. Results indicated that only 3 of the 19 areas examined showed significantly lower gray matter volumes in the FM patients compared to healthy controls. Specifically reduced gray matter was left lateralised and was found in insula, ACC and midcingulate cortex. No whole brain effect was demonstrated. Although the patient group had significantly higher scores on negative affect measures, such as depression and fear, no relationship between these measures and gray matter volumes was demonstrated.

A small number of studies have been extending the idea of FM to include patient characteristics which may have an impact on morphological changes. Hsu Harris, Sundgren, Welsh, Fernandes, Clauw & et al, (2009) investigated gray matter volume in 29 female FM patients with affective disorder, 29 FM patients without

affective disorder and 29 healthy controls. Affective disorder was characterised as major depressive disorder, bipolar disorder, dysthymia and general anxiety disorder, although severe psychiatric illness such as major depression with suicidal ideation was an exclusion criteria for all groups. There were no significant differences detected among the three groups for either whole brain volume or gray matter volume. Similarly, no significant correlations were found among patients between the severity of depressive symptoms or pain duration and GMV. However, a significant negative correlation between gray matter volume of left anterior insula and trait anxiety was found between healthy controls and FM patients with affective disorder. This did not extend to the other patient group, indicating a relationship between GM volume and trait scores.

A second study investigating FM patients with disorder specific characteristics was conducted by Puri and colleagues who compared gray matter density in 5 FM patients with marked fatigue and 5 healthy controls (Puri, Agour, Gunatilake, Fernando, Gurusinghe & Treasaden, 2010). Marked fatigue had to be experienced whilst at rest. In comparison to healthy controls, FM patients were found to have a reduction in gray matter concentrate in the left supplementary motor area and the right superior frontal gyrus.

Wood, Glabus, Simpson & Patterson, (2009) investigated both brain morphology and dopamine metabolism in FM patients. Thirty female patients and 20 age and gender matched controls were investigated. Significant reduction of GMD was found in bilateral parahippocampal gyri, right posterior cingulate cortex, and left anterior cingulate cortex, with additional differences in cingulate cortex failing to reach significance. A significant positive correlation between dopamine metabolism and gray matter density was demonstrated in the bilateral parahippocampal gyri and left peregenual cortex in a subgroup of participants (n=14; 6 patients, 8 controls). No significant relationship was demonstrated between pain duration and gray matter density. The authors interpreted their findings to suggest that changes in dopamine metabolism contribute to the GM changes demonstrated within FM.

2.4.2 Chronic Low Back Pain

VBM studies investigating morphological reorganisation within fibromyalgia have provided evidence that fibromyalgia patients demonstrate morphological differences compared to healthy controls. However, although some consistencies are observed, the regions associated with these changes often vary from one study to

another. This is also true for the studies investigating morphological changes in chronic low back pain patients.

The first paper to examine morphological changes in chronic low back pain was conducted by Apkarian, Sosa, Sonty, Levy, Harden et al, (2004). Twenty six chronic back pain patients were compared with 26 age and gender matched controls. Patients comprised of 15 with musculoskeletal pain, 5 with radiculopathic pain and 6 with a mixture of both. Whole brain analysis of neocortical gray matter volume indicated that compared to the healthy controls, the pain patients demonstrated a 5-11% loss, the equivalent of 10-20 years of normal aging. Although the effects correlated with pain duration, this accounted for only 18% of the variance. Regional analysis revealed reduced gray matter density within bilateral dorsolateral prefrontal cortex and right thalamus. When patients were further subdivided into neuropathic and non-neuropathic groups, pain duration explained 40% and 80% of the DLPFC reduction in neuropathic and non-neuropathic patients respectively. The results indicate that the pathophysiology of chronic pain includes thalamo-cortical processes.

The second study to examine morphological changes within chronic back pain patients was Schmidt-Wilcke and colleagues (2006) who investigated pain-related changes in morphology in 18 patients with chronic back pain and 18 age and gender matched controls (Schmidt-Wilcke, Leinisch, Ganssbauer, Draganski, Bogdahn, Altmeppen & et al, 2006). In contrast to the findings of Apkarian et al, (2004a) no significant between group differences in whole brain volume was found. In further contrast to the previous study, VBM analysis showed decreases in both brainstem and right somatosensory cortex and increases in gray matter volume were detected within bilateral basal ganglia and left thalamus. Further uncorrected analyses revealed gray matter increases in the right DLPFC and temporal lobe, but should be treated with caution. There was no significant correlation between morphology and pain duration. A significant negative correlation was established between pain intensity, pain unpleasantness and brainstem and left somatosensory cortex findings. In contrast the same unpleasantness and pain intensity ratings were significantly positively correlated with the left thalamus and left putamen. The reduction of gray matter in regions involved in pain suppression could lead to a loss of effective antinociception. Prolonged nociceptive input leads to reorganisation which in turn may contribute to the pain experience becoming a chronic condition.

The final study examining morphological changes in chronic back pain patients was conducted by Buckalew and colleagues (Buckalew, Haut, Morrow, & Weiner, 2008). This study diverged from the previous studies as it examined both structural changes and neuropsychological performance in older controls (>65 years of age). Eight adults with chronic back pain and eight age matched healthy controls completed a number of tests of cognitive performance, such as digit span and trail making tasks. Both VBM and manual tracing were performed on the MRI scan. No significant differences for whole brain volume gray matter or white matter volumes were found. The planimetry analysis found a non-significant trend for a reduced corpus callosum in the patient population. This finding did not extend to either the prefrontal cortex or thalamic volume analyses. However, the VBM analysis did indicate decreased GM volume within the left posterior parietal cortex and decreased WM volume in the left mid cingulate. These findings did not correlate with either pain severity or pain duration. In addition to morphological findings, the pain participants also displayed a decrease in performance on the forward digit span indicating impairment in attention and mental flexibility. The authors interpreted their findings to indicate that the reduction of the mid cingulate, which is associated with attentional processes, is reflected in cognitive deficits.

2.4.3 Irritable Bowel Syndrome

To date there have been three studies examining morphometric changes associated with irritable bowel syndrome (IBS). All three studies combined VBM with cortical thickness measures. Davis et al, (2008) used both methods to compare 9 IBS patients with 11 controls. In both analyses the IBS group displayed lower values of brain tissue compared to the control group. The VBM analysis revealed a reduction in gray matter volume in the right thalamus and the left ACC. Cortical thickness analysis (CTA) found the right ACC and the bilateral anterior insular cortex, to be reduced for the patient group.

Similarly Blankstein, Chen, Diamant & Davis (2010) used VBM and cortical thickness when comparing 11 IBS patients and 16 age and gender matched controls. The only significant difference in gray matter volume was an increase in the hypothalamus in IBS patients compared to controls. Cortical thickness analyses revealed several significant findings. Cortical thinning was found in both the midcingulate cortex and the anterior insula. Interestingly, only patients with short-term IBS demonstrated cortical thinning of the insular cortex. Insular thickness in

long-term IBS patients was comparable with the controls. The authors interpreted the thinning of the anterior insula as possible evidence for a predisposing factor in the development of IBS. Long-term IBS might produce adaptive changes in gray matter which results in a movement towards normal thickness values. Further to the positive correlation between insular cortex thickness and pain duration, a significant negative correlation between dorsolateral prefrontal cortex thickness and pain catastrophising was demonstrated in the IBS group. The control group showed an opposite trend.

In one of the largest VBM studies investigating morphological changes in a pain population, Seminowicz et al, (2010) investigated a cohort of 55 IBS patients, with a similarly large control group (N=48). In the IBS groups, gray matter density was found to be decreased in medial prefrontal cortex, ventrolateral prefrontal cortex, posterior parietal cortex and precuneus, superior temporal gyrus, ventral striatum, putamen and thalamus. In contrast to the majority of VBM analyses, several brain areas demonstrated increases in gray matter density in the patient group. Compared to controls, IBS patients had GMD increases in ACC, posterior insula/somatosensory cortex, (para)hippocampus, orbitofrontal cortex and DLPFC. Once anxiety and depression were controlled for, only reduced GMD in prefrontal and posterior parietal cortex remained. The cortical thickness analysis produced no significant findings.

2.4.4 Regional Pain Disorders

Valet and colleagues (2009) investigated morphological changes in 14 patients with pain disorder. When compared to 25 healthy controls, VBM analysis demonstrated significant gray matter reduction in prefrontal (orbito, medial and ventromedial frontal regions), cingulate (anterior and posterior), insular cortex, parahippocampal regions and the cerebellum. No significant global gray matter reduction was found between the participant groups. However, when gray matter density was correlated with pain duration, a significant negative correlation was found in left parahippocampal cortex whilst the right thalamus demonstrated a significant positive correlation.

More recently there have been two studies investigating morphological changes in patients with facial pain. Schmidt-Wilke, Hierlmeier & Leinisch, (2010) used VBM to compare 11 patients with persistent idiopathic facial pain (PIFP), with 11 healthy controls. A decrease in gray matter was found in several brain regions, which increased in number when images were flipped to ensure that there was

consistency in the lateralisation of pain. When compared to healthy controls, patients demonstrated a bilateral reduction in gray matter volume in the ACC, medial frontal gyrus (extending to the right superior frontal gyrus) and postcentral gyrus. Further left lateralised gray matter reductions were found in the insular cortex and the precentral gyrus.

Younger, Shen, Goddard & Mackey, (2010) studied gray matter differences in 15 healthy controls compared to 15 patients with myofascial temporomandibular pain (mTMD). In contrast to the findings of Schmidt-Wilke et al, (2010) and most of the chronic pain studies cited, mTMD patients demonstrated a significant increase in gray matter volume in several regions compared to their matched controls. These increases were right lateralised in the anterior insular cortex, inferior frontal gyrus, putamen, thalamus and globus pallidus, with bilateral increases being found in trigeminal sensory/motor regions and the middle cerebellar peduncle. The only significant reduction in gray matter volume was in right somatosensory cortex. Several characteristics of disease severity were correlated with gray matter volume. Illness duration was positively correlated with gray matter volume in the posterior cingulate cortex, hippocampus, midbrain and cerebellum, indicating greater volumes with longer pain duration. Four areas were negatively associated with jaw pain, right lateralised ACC, posterior cingulate cortex and superior frontal gyrus, with left superior temporal gyrus. The divergence of results from previous findings was partly attributed to the patients having shorter pain duration than previous chronic pain cohorts.

2.4.5 Cessation of Pain

A criticism of the morphology literature is that most studies are cross sectional in nature and do not investigate longitudinal disease processes.
For the majority of pain conditions, once they have become chronic in nature (>6months) they are merely managed but never 'cured'. However, osteoarthritis (OA) of the hip is one exception to this. After surgical intervention OA can have a pain free success rate of 81%. Two studies have investigated structural brain changes in patients with OA prior to (pain state) and after successful hip replacement surgery (pain free state).

Rodriguez-Raecke, Niemeier, Ihle, Ruether, & May, (2009) investigated morphological changes in 32 patients with primary hip osteoarthritis compared to 32 age and gender matched controls. Patients were scanned prior to total hip

replacement surgery, with a subset of patients (N=10) being scanned two further times, at 6 weeks and four months postoperatively. When compared to healthy controls, preoperatively the chronic pain patients demonstrated a widespread reduction of gray matter density. The areas found to have a significant GM reduction were ACC, orbitofrontal cortex, right insular cortex and operculum, right midorbital gyrus, left superior medial gyrus, cerebellum, DLPFC, amygdala, temporal lobe and brainstem. The only significant increase in gray matter density was found in the left middle frontal gyrus of patients compared to controls.

The subset of patients who were followed longitudinally showed no significant gray matter changes between scan time 1 (preoperative) and scan time 2 (6-8 weeks postoperative). When these scans were combined and compared to the scans acquired at timepoint 3 (16-18 weeks postoperative) significant increases in gray matter volume were found in ACC, DLPFC, amygdala, brainstem and right insular cortex. The authors claimed that in these patients at least, gray matter decreases associated with chronic pain may be partly reversible and as such the gray matter changes found in chronic pain patients do not reflect brain damage. Instead these changes should be considered a reversible consequence of nociceptive transmission, which is reversed once pain is adequately treated.

The second study investigating gray matter change prior to and after surgical intervention was conducted by Gwilym, Filippini, Douaud, Carr & Tracey, (2010). Sixteen osteoarthritis patients were studied four weeks before and nine months after hip arthroplasty, with their results being compared to 16 healthy controls. In contrast to the previous study, VBM analysis indicated prior to surgery patients had an increase in gray matter volume in several brain areas. Specifically, increases were left lateralised to anterior insular cortex, amygdala and orbitofrontal cortex, with the only right sided increase occurring in the parahippocampal gyrus. Bilateral increases in gray matter volume were found in fusiform cortex, occipital cortex and cerebellum. The only decrease in gray matter volume for patients was found in bilateral thalamus.

Further increases in gray matter volume were found after surgery in left orbitofrontal cortex, left cerebellum, right parahippocampal gyrus, right brainstem and bilateral occipital cortex. However, these results should be treated with caution as whole brain results were uncorrected for multiple comparisons and findings include very small clusters (<10). Whilst conceding that these limitations can lead to

false positives, the authors stated that the inclusion of such findings is useful in hypothesis generation and the wider interpretation of results from the literature.

Whilst the evidence for the irreversible nature of gray matter changes associated with a chronic pain state might appear persuasive, the patient population used within the above two studies, may provide some influence over the findings. Patients with osteoarthritis have an identifiable condition, which has a successful method of treatment and often have surgical intervention in a relatively short time period. In comparison, chronic pain states such as FM, IBS and CLBP not only have a high rate of co-morbidity, they are often idiopathic in nature, are managed but unfortunately not treated, and the sufferer may endure their pain condition for several decades. Furthermore, functional conditions, such as FM, IBS and CLBP, are believed to have strong psychological components impacting the condition, which may be lacking in OA populations.

A longitudinal study conducted by Seminowicz and colleagues (2011) addresses several of these issues (Seminowicz, Wideman, Naso, Hatami-Khoroushahi, Fallatah, Ware & et al, 2011). They compared cortical thickness in 16 controls and 18 CLBP patients, before and 6 months after treatment. The CLBP patient group, who had a pain duration ranging between 1-20 years, were treated with either spinal surgery (N=8 post-treatment group) or facet joint injections (N=6 post-treatment group). Treatment was considered successful if any reduction in either/or pain levels or pain-related disability was reported (N=14). Prior to treatment, CLBP patients had a cortical thinning of the left DLPFC, compared to controls. After treatment, those who responded to treatment demonstrated an increase in cortical thickness in left DLPFC, which correlated with the pain and physical disability scores. In comparison, non-responders to treatment, showed decreases in CT in the same region. Furthermore, reduced levels of physical disability and pain were correlated with increases in cortical thickness in primary motor cortex and right insular cortex respectively. The authors suggested that pain-related differences in brain regions are reversible after successful treatment.

The study conducted by Seminowicz et al, (2011) provides some compelling evidence that structural brain changes associated with chronic low back pain are reversible after successful treatment. The study does have some limitations. Due to the small sample sizes, the CLBP patients were not separated based upon pain type, neuropathic/ non-neuropathic. Similarly, the type of treatment received may have

had an impact upon the findings, with one intervention providing greater CT changes than another.

The definition of treatment success may be open to question. Only one patient appeared to be pain-free at follow-up. Furthermore, the majority of patients were still taking medication at 6 months post-treatment. Only one patient had stopped taking medication altogether, with a further patient increasing their medication intake. The ranges of scores from the self-report measures demonstrate some reduction, but CLBP as a group were mixed in their scoring. For example, pain levels measured by the short-form McGill Pain Questionnaire (SFMPQ) indicated a reduction of 39 points for one patient, whilst another had a more modest 1 point reduction. If the treatment is not 'successful' i.e. pain-free at post-treatment, then the question raised must be 'what is underlying the CT changes?'

The correlations conducted by the investigators showed a significant relationship between self-report measures and CT, thus supporting the assumption that it is the treatment that is fuelling the changes in CT. One possibility is that the increase in physical activity in response to a decrease in physical disability may be responsible for these CT changes and not pain per se. The authors themselves concede that either placebo or pain modulation might be underlying the effects. The study's conclusions however, are strengthened by the inclusion of fMRI testing during a cognitive task, which showed increased cortical activation of the left DLPFC post-treatment. Overall, this is the first longitudinal study to investigate structural brain changes in CLBP patients after surgical treatment and the findings for the reversibility of pain-related changes are persuasive.

2.5 Methodological Considerations

The literature investigating brain morphology and chronic pain states is growing yearly. To date, quantifiable differences in brain gray matter have been demonstrated in a variety of chronic pain conditions. Previously there has been a suggestion that chronic pain conditions may have their own 'common brain signature', whereby decreases in GM overlapped for a variety of pain conditions (May, 2008). Specifically changes in cingulate cortex, insula, dorsal pons and the orbitofrontal cortex were posited as being the brain regions most associated with chronic pain state. However, as the literature has grown, there appear to be as many inconsistencies as similarities demonstrated. Some studies have been able to establish a significant relationship with the pain condition, whilst other studies have failed in

this endeavour. These differences in results are not easy to interpret. Methodological inconsistencies may help to explain the variability in findings.

Firstly, in relation to the studies examining low back pain, the patient group may have been too heterogeneous to exhibit consistent findings. The inclusion of patients with and without neuropathic pain in Apkarian et al's study (2004), demonstrated how different underlying pathology of a similar condition may have effects on the results. Similarly, for FM patients, the trait characteristics of the participants may be underlying changes. As demonstrated by Hsu et al, (2009) trait scores were correlated with gray matter volume of left anterior insula, in FM patients with affective disorder. Whilst it is improbable that patient characteristics alone are responsible for the inconsistency of results, investigating homogenous patient groups with measures of trait characteristics being included, may provide more confidence in the findings.

Other patient characteristics which may be influencing the results obtained to date are the ages and gender of patients. Studies investigating FM patient groups often use female-only cohorts (Burgmer et al, 2009; Kuchinad et al, 2007; Lutz et al, 2008). Whilst this may provide consistency for studies in FM, it may impact the findings when being compared to non-female only studies, both within FM and other pain states (Davis et al, 2008; Schmidt-Wilke et al, 2007). The ages of participants across the literature varies, which may be impacting results, as age-related gray matter changes have been identified previously (Ge, Grossman, Babb, Rabin, Mannon, & Kolson, 2002; Hutton, Draganski, Ashburner, & Weiskopf, 2009). Although most studies have included this as a covariate or age match their controls (Apkarian et al, 2004a; Blankstein et al, 2010), if ages differ significantly across studies, some age-related effects may remain.

The use of medication by patient groups has also been considered a factor that may contribute to inconsistent findings. A recent study conducted by Younger and colleagues (2011) demonstrated that one month of taking oral morphine, was enough to produce widespread gray matter change, both increases and decreases (Younger, Chu, D'Arcy, Trott, Jastrzab & Mackey, 2011). Furthermore, follow-up scans performed 4-5 months later suggested that some of these changes were persisting. Although most studies have exclusion criteria for the type of medication currently being used by patients, this does not control for previous medication use. Also it would be unethical to ask for a washout period, especially as the study

conducted by Younger et al, (2011) suggests this period could be as long as several months.

The number of participants contained within the studies cited may also be responsible for the inconsistency of findings. By far the largest study was conducted by Seminowicz et al, (2010) who had 103 participants in their study, with the smallest study cited being by Puri et al, (2010), which contained a much more modest 10 participants. The reliability and reproducibility of findings will undoubtedly be impacted by such large variations in participant numbers. Furthermore, most of the studies cited use VBM as their primary method of analysis. However, for optimum results groups need to contain a minimum of twenty participants per group (Wood, 2010). Of the 19 studies reviewed here, only 7 studies have participant numbers greater than 20 per group.

The results may have further been influenced by image acquisition and subsequent analysis (See Table 2.1 for technical details regarding the studies cited). Initially studies used scan strengths of 1.5T, but in recent years this has been replaced by more studies using 3T magnetic strength. The difference in scan strengths may mean that subtle differences in cohort studies may be better detected at greater field strengths. Similarly, the use of different scanners and scan parameters may produce small scale differences in the acquired image, which may result in slight differences being achieved in the preprocessing steps, not related to pain state. This is most notable in areas close to sinus cavities which can produce slight distortion within the acquired image. This could impact the results of both normalisation and segmentation, as blurred boundaries could result in slight misalignment to the template image or inaccurate tissue classification. A recent study conducted by Focke and colleagues, (2011), investigating multi-site VBM, found significant differences when the same 18 controls were scanned at different sites (Focke, Helms, Kaspar, Diederich, Toth, Dechent & et al, 2011).

Studies have used different criteria for analysis. Voxel based morphometry allows for both whole brain analysis, as well as region of interest analysis or a combination of the two. Similarly to manual methods, region of interest analysis may miss other significant findings, if they are contained outside the ROI. Whole brain analysis is more open to chance findings. These problems may be further compounded by studies which do not correct for multiple comparisons (Gwilym et al, 2010). Although they often use a more stringent significance threshold, false positives are more likely to be found.

As discussed previously VBM analysis contains two different approaches, which produces two different units of analysis. Gray matter volume (GMV) is the proportion of voxels found within an anatomical location. Gray matter density or concentration (GMD) is the proportion of tissue in a given voxel. Therefore VBM studies of chronic pain states, although cited together, may be analysing different underlying mechanisms of change. Although the data does illustrate on a macroscopic level, that there exists gray matter differences between patient groups and healthy controls, the mechanisms which are responsible for these changes remain elusive.

2.6 Underlying Mechanisms of Morphological Changes

Several suggestions have been posited regarding the underlying mechanism of these differences in GM, such as changes in blood or water volume, increases in the size or number of neurons or glia, rearrangements on a cellular level and neuronal loss, (Apkarian et al, 2011; May, 2008; Schmidt-Wilke, 2008; Wood, 2010). The GM differences reported might be pre-existing, suggesting vulnerability in developing persistent pain, or are a consequence of long term nociceptive stimulation. Understanding if the GM changes are a cause or consequence of pain would allow for preventative treatments being targeted at engaging cerebral regions known to interact with the pain condition. Support for structural differences being secondary to pain has been demonstrated in GM changes due to amputation (Draganski, Moser, Lummel, Ganssbauer, Bogdahn, Haas & et al, 2006b) and spinal cord injury (Wrigley, Gustin, Macey, Nash, Gandevia, Macefield & et al, 2009). However, analysis performed on MRI images, regardless of the method employed, can only present macroscopic findings. Consequently the neurobiological basis underlying the differences relating to chronic pain states, are still being debated and intensively researched.

For instance, authors who have suggested a relationship between pain duration and the extent of GM differences, discuss their findings in terms of neurodegenerative processes, specifically neuronal loss (Apkarian et al, 2004a). This suspected atrophy is placed within a framework of normal ageing, with the insinuation that structural and functional impairments of CPP are the result of premature ageing of the brain (Apkarian et al, 2004a; Kuchinad et al, 2007). Park, Glass, Minear & Crofford, (2001) investigated cognitive functioning in FM patients

with a control group age matched 20 years older. With the exception of information processing speed, the FM patients' performance was equivalent to the older group, which was interpreted as indicating cognitive ageing within the CPP. However, whilst GM differences might be the result of neuronal loss, the association with a rapid ageing process, might be misleading. Recent research has demonstrated that neuronal loss, as part of the normal ageing process, is more discreet than previously thought (Mora, Segovia & del Arco, 2007). Instead the post-mortem decrease in GM volume is believed to be a complex set of changes at the subcellular level of analysis (Anderson, 2011). Therefore, the structural and functional deficits demonstrated by CPP may be representative of GM atrophy, but this does not automatically infer an accelerated ageing process.

Investigations of neurochemical profiles, a complimentary technique to morphological studies, have provided support for the premise of GM atrophy related to the pain state (Apkarian et al, 2011). Grachev, Fredrickson, & Apkarian, (2000) used magnetic resonance spectroscopy (MRS) to investigate the chemical profile of DLPFC in CLBP sufferers. The patient group demonstrated a 6.5% reduction of total chemical concentrate in this region, with N-acetylaspartate (NAA) and glucose showing reductions of 7.8% and 17.2% respectively. As the duration and intensity of pain increases, so does the magnitude of the shift from normative data (Grachev et a, 2000). Similarly, larger changes in NAA profile have been associated with co-morbid anxiety (Grachev, Fredrickson, & Apkarian, 2001; Grachev, Fredrickson, & Apkarian, 2002) and depression (Grachev, Ramachandran, Thomas, Szeverenyi, Fredrickson et al, 2003). Siddall and colleagues (2006) demonstrated that the magnitude of shifts in neurochemical profile in ACC, PFC and thalamus can differentiate between those with CLBP and healthy controls with an accuracy rate between 97-100% (Siddall, Stanwell, Woodhouse, Somorjai, Dolenko, Nikulin, Bourne & et al, 2006). Similar changes have been noted from studies investigating neurodegenerative conditions such as Alzheimer's disease (AD), stroke, and multiple sclerosis (MS). As NAA is located within neurons and is interpreted as a marker for axonal and synaptic integrity (Miller, 1991), decreases in NAA within chronic pain subgroups, have supported the suggestion that there is a link between chronic pain and neuronal loss and degeneration.

In contrast, recent studies investigating morphological changes resulting from a cessation of pain, have questioned the assumption that GM decreases are the result of neuronal atrophy. As discussed previously, Gwilym et al, (2010), Rodriguez-

Roecke et al, (2009) and Seminowicz et al, (2011) investigated morphological differences in OA and CLBP patients, before and after surgical intervention. All studies found post-treatment GM increase, suggesting that some of the pre-treatment GM differences are the direct consequence of nociception. The cessation of pain in a non-clinical cohort was conducted by Ruscheweyh and colleagues (2011) who investigated GM changes in 45 patients with on-going pain, 29 participants with past-pain experience (who had been pain free for at least 12 months) and 31 pain-free controls (Ruscheweyh, Deppe, Lohmann, Stehling, Flöel, Ringelstein & et al, 2011). Relative to controls, the on-going pain group demonstrated a global GM reduction of 3.3%, with regional decreases being found in ACC, prefrontal and motor/premotor regions. No significant GM differences were demonstrated for the past-pain group. These findings question the irreversibility of pain-related GM changes, specifically those associated with neuronal loss or atrophy as they suggest that upon the cessation of pain, GM returns to pre-pain levels. However, there are some caveats to this view. Firstly, increases in GM volume did not correspond to the presurgical decreases (see Figure 2.1). Secondly, not all patients were pain-free after treatment (Seminowicz et al, 2011) indicating that the GM decreases might not only be related to pain changes. Finally, as the results of Ruscheweyh et al, (2011) are cross sectional, there is no evidence that the past-pain group had any GM differences during their pain experience and furthermore, if reduced GM is a predisposing factor to developing chronic pain, as some have suggested, the lack of GM differences in this group could be used to explain their current pain-free status.

With the exception of the dentate gyrus of the hippocampus and the subventricular zone (SVC) abutting the lateral ventricles, neuronal quantity remains fairly stable across the human lifespan and therefore it is unlikely that the observed GM increases are the result of neurogenesis (Aimone, Deng & Gage, 2010; Nowakowski, 2006). Instead the increases in GM may be similar to those that we see in training induced plasticity, whereby GM increases are detected in brain regions ascribable with the task (Draganski Gaser, Busch, Schuierer, Bogdahn, & May, 2004) and similarly with exercise and learning (Gaser, & Schlaug, 2003; Draganski et al, 2006a).

However, GM decreases tend to dominate the chronic pain literature and have been located in areas described as important for pain processing or pain modulation (see Section 2.1 regarding pain processing areas). That we do not observe GM increases in the somatosensory area of chronic pain patients has been debated.

Teusch and colleagues (2008) investigated if exposure to repeated noxious stimulation for an eight day period resulted in GM changes in pain-free volunteers (Teusch, Herken, Bingel, Schoell & May, 2008). MRI was conducted on days 1, 8, 22 and after a one year period. Increases in GM were found in somatosensory cortex, mid-cingulate cortex and the parietal lobe when comparing day 1 to day 8 or 22. VBM analysis performed on scans collected after 1 year indicated that GM had returned to baseline levels. When discussing their findings, the authors posited that the lack of GM increases in somatosensory areas in CPP relates to pain sufferers no longer experiencing a noxious input. Instead, the pain experience is being driven by changes that have occurred within the brain itself. An alternative proposition is that GM changes are only evident until a task is learned, with the brain returning to baseline levels once this has been achieved. In relation to pain conditions, acute pain may still result in GM increases which may be diminished as the condition progresses and becomes chronic.

When investigating GM changes in chronic pain populations, some increases have been demonstrated in CLBP (Schmidt-Wilke, et al, 2006), FM (Schmidt-Wilke, et al, 2007), IBS (Seminowicz, et al, 2010) and OA (Gwilym, et al, 2010; Rodriguez-Roecke, et al, 2009). Animal studies investigating enriched environments in rodents have found similar increases in GM volume (Anderson, 2011). Environmental enrichment is an experimental model that allows a controlled increase in sensory and social stimulation, for the study of plastic changes. Unlike human studies, post-mortem analysis allows for a detailed investigation into the mechanism underlying the observed volumetric change. It was noted that enrichment resulted in an increase in the number of synapses per neuron, as well as an increase in dendritic length. These changes in turn resulted in an increase in GM volume. Once enrichment was stopped, a reduction in cell density was found. However, these processes occurred without a significant change in cell numbers, either by atrophy or neurogenesis. In relation to chronic pain, it may be inferred that due to the constraints of the condition, chronic pain sufferers live in a deprived environment, with reduced social interaction, physical exercise and intellectual stimulation, thus resulting in a reduction of cell size. With successful surgical intervention, as reported by Gwilym et al, (2010), Rodriguez-Roecke et al, (2009) and Seminowicz et al, (2011) they experience greater stimulation through returning to the work environment, increases in physical activity, socialising and better quality of life. It is this alteration in lifestyle that may be responsible for changes in GM post-treatment. However, as

with the enrichment studies, the results cannot be used to infer changes in cell numbers, but rather are representative of changes in cell size and shape. GM increases that have been reported without any surgical intervention may be the result of changes in cell size or shape, that are the result of underlying adaptations to the nociceptive system. The chronicity of the condition may result from changes via neuronal plasticity to the nociceptive system, which may be underpinning the results of morphological studies (Schweinhardt & Bushnell, 2010).

Neuronal plasticity is the capacity of neurons to change their function, chemical profile or structure (Woolf & Salter, 2000). In relation to clinical pain and specifically pain hypersensitivity, these changes can be broadly divided into two forms, modulation and modification. Modulation refers to reversible changes in the excitability of primary sensory and central neurones. In contrast, modification refers to long lasting alterations in the structure, connectivity and survival of neurons. This may result in a grossly modified system, which distorts the normal stimulus-response characteristics. Modification is the more plausible link to the transition from acute to chronic pain, leading to central sensitisation (Voscopoulos & Lema, 2010).

Central sensitisation is an increased response to stimulation through an amplification of signals within the central nervous system. The intensity of the stimulation does not need to be of a noxious level for it to recruit mechanisms used to signal a noxious response, as is seen in allodynia. A study conducted by DaSilva et al, (2008) investigated cortical thinning and fMRI activation in relation to allodynia in patients with trigeminal neuralgia. They observed that reduced cortical thickness of sensorimotor cortex was co-localised with fMRI activations achieved through provocation of brush-induced allodynia. The authors suggested that cortical thinning could be a compensatory response to persistent noxious input, resulting in neural instability.

The recent study by Seminowicz et al, (2011) also combined CTA and fMRI. In comparison to the study conducted by DaSilva et al, (2008), a cognitive task was performed during fMRI data collection. The left DLPFC demonstrated both cortical thinning and significant differences in cortical activation in the pre-surgery group compared to controls. After treatment, pain-related differences in the left DLPFC returned towards the control values, for both structural and functional measures. Performance on the behavioural task did not differ between groups or testing sessions indicating that functional data was not related solely to task performance. A small subgroup of patients (N=10) and controls were tested at an intermediate

timepoint (6 weeks after treatment). Only functional activity, and not cortical thickness, demonstrated a change that was related to the findings at the 6 month follow up. These results suggest that the change in brain structure was mediated through the changes to brain function.

Previous fMRI studies have suggested altered cerebral processing in chronic pain patients to both acute experimental pain and the default mode network (DMN) at resting state (Baliki, Geha, Apkarian, & Chialvo, 2008; Giesecke, Gracely, Grant, Nachemson, Petzke & Williams, 2004; Lloyd, Findlay, Roberts, & Nurmikko, 2008; Tagliazucchi, Balenzuelaa, Fraimanb, & Chialvob, 2010). The findings of Seminowicz and DaSilva, suggest that functional changes to the nociceptive system may underlie morphological changes in chronic pain states. It is noted that with few exceptions, primary and secondary somatosensory cortices and thalamus, most areas cited as displaying GM changes in morphological studies, are those areas reported as part of the 'pain matrix'. However, changes are also noted in areas not considered to be related to the sensory aspects of the pain experience, but rather are associated with the affective aspects of pain or rather affect in general (May, 2008). Therefore, changes in synaptic pathways in response to cortical and subcortical reorganisation could be the result of either overuse or disuse of transmission over synaptic pathways (Apkarian et al, 2011) and that these changes are not confined to pain sensation, but may be related to all aspects of the pain experience, including cognitive and affective processing.

As with any discussion regarding the underlying mechanisms responsible for the altered morphology found within chronic pain states, any suggestion remains speculative. Current evidence indicates that morphological changes can be observed at early timepoints from initial injury as well as after long periods, suggesting multiple mechanisms may be involved. The studies investigating cessation of pain suggest that at least some of the observed morphological changes in chronic pain states are, at least partly, a consequence of the presence of constant nociception. It has been suggested that underlying GM changes can be caused by changes in neuronal elements such as dendrites or synapses, glial cells, blood or water content or due to neurodegenerative processes (Schweinhardt et al, 2010). Conventional MRI cannot determine the histopathology underlying GM changes. Furthermore, it cannot distinguish which cell type is affected. It is likely that the extent of involvement of different cellular types will also vary with the type of chronic pain (Apkarian et al, 2004a).

2.7 Conclusion

In conclusion, it would appear that the experience of chronic pain is associated with changes in the structure of an individual's brain. Whether these morphological changes are the result of a persistent activation of the nociceptive, and associated affective and cognitive assemblies', remains to be seen. Furthermore, our understanding of whether these changes are irreversible, or represent a permanent change needs to be examined further. Most studies are cross sectional in design, providing only comparable data. Longitudinal studies, using a triangulation of metabolic, functional and morphometric analyses will begin to unravel the time course of the observed changes. Investigations of the underlying mechanism of change however, will require histopathological investigations which are currently lacking from the literature.

CHAPTER THREE
Materials and Methods

This chapter will discuss the materials and methodology used for the experimental studies presented within this thesis. Firstly, a brief discussion relating to the principles underlying nuclear magnetic resonance (NMR) and its application to magnetic resonance imaging (MRI) will be presented in Section 3.1. A discussion of stereological volume estimation and error prediction is presented in Section 3.2. Functional MRI methodology is discussed in Section 3.3 and is divided into details regarding fMRI experimental designs, fMRI scanning protocol, preprocessing and statistical analysis of acquired fMRI data. The dot probe task design and analysis are described in section 3.4. The chapter concludes with the methods used for participant assessment for pain behaviour in Section 3.5 and self-report measures in section 3.6.

3.1 Principles of Magnetic Resonance Imaging

The discovery of nuclear magnetic resonance (NMR) occurred in the mid 1940's by researchers detecting a resonance phenomenon from samples placed within a magnetic field. Although they both worked independently, Edward Purcell at Harvard University and Felix Bloch at Stanford University shared the 1952 Nobel Prize for Physics for their discoveries (Bloch, Hansen, & Packard, 1946; Purcell, Torrey, & Pound, 1946).

The application of NMR for clinical imaging was first demonstrated in the 1970's and is still currently used within the field of diagnostic radiology. MR lends itself well to clinical studies as it provides high-resolution images of soft tissue. Although labelled nuclear magnetic resonance, the term 'nuclear' was subsequently dropped from use in clinical practice, due to negative connotations and possible misunderstandings of the term. The NMR is a nuclear effect as it is the constituents of the atomic nucleus that resonate, however no ionising radiation is used, thus reducing risks to patients.

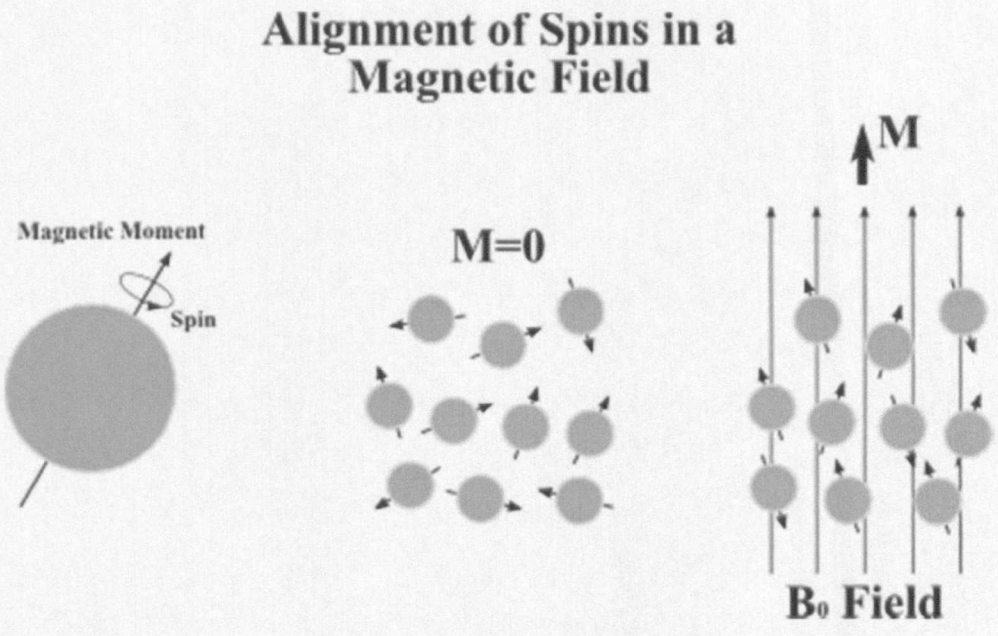
There are four key elements underlying MR data acquisition. Firstly, nuclear spin, secondly, the radiofrequency pulse, thirdly, the NMR signal and relaxation and finally, the transformation of the MR signal into a 3D MRI image using MRI gradients. These four key elements will now be discussed in greater detail.

3.1.1 Nuclear Spin and the Magnetic Field

Magnetic resonance is based upon the interaction between an external magnetic field and a nucleus that possesses spin. Spin is the angular momentum which is a physical property of protons and neutrons. The speed of the spin of a nucleus can be described as the angular frequency. In NMR it is the net spin which is of interest. However, not all nuclei have a net spin and therefore they have no NMR signal. Although the NMR explanation applies to all nuclei which are electrically charged and spin on their axis, hydrogen is the most commonly used in MR imaging. Hydrogen has one of the strongest nuclear moments making it very sensitive to the magnetic field (Jezzard, Matthews, & Smith, 2001).

When electrically charged particles move, they create a local magnetic field or a magnetic dipole moment (MDM). In the absence of an external applied magnetic field (B_0), magnetic dipoles have no preferred orientation. The presence of the B_0 field produces two effects upon the hydrogen nuclei.

Figure 3.3 The Alignment of Spins in a Magnetic Field



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Firstly, a proportion of the hydrogen nuclei align themselves either parallel or anti-parallel to the B_0 field. Nuclei align themselves in opposing directions as they

possess thermal energy. Nuclei can assume either a high energy state (orienting against the applied magnetic field) or a low energy state (aligned with the applied magnetic field). There is only a small imbalance between those in a high and those in a low energy state. At 1.5T only 10 spins of 1 000 000 contribute to the net magnetic moment. The other 999 990 will cancel each other out (Jezzard et al, 2001).

The second effect on the hydrogen nuclei due to the application of the B_0 field is that the nuclei begin to precess. Precession occurs as the spins do not align themselves perfectly to the longitudinal axis of B_0. Instead the nuclei align themselves at a slight angle away from B_0. Therefore the nuclei precess about the direction of the magnetic field.

Both the proportion of the aligned hydrogen nuclei and the rate of precession are determined by strength of the applied B_0 field and results in Equation 3.1:

$$\omega_0 = \gamma \cdot B_0 \qquad \textbf{Equation 3.1}$$

where (ω_0) is the precessional frequency in MHz, (γ) is a constant known as the gyromagnetic ratio in MHz/Tesla and (B_0) is the strength of the applied magnetic field in Tesla. This expression is called the Larmor Equation.

3.1.2 The Radiofrequency Pulse

Energy is transferred to the hydrogen nuclei by a radiofrequency pulse (RF-pulse) or the radio frequency field, also called the B_1 field. The RF-pulse is an electromagnetic wave resulting from a brief application of an alternating current. Each nuclei type precess at their own rate therefore by matching the frequency of the pulse to the frequency of the precession of the MDM's, the B_1 field can be applied selectively.

The applied RF-pulse is always transverse to the main static magnetic field. The length of the pulse determines the angle of the pulse, with longer pulses resulting in an increase in the tip angle and signal. Typically a 90^0 RF-pulse is used to 'tip' the MDM's of the hydrogen nuclei into the x-y plane. After the hydrogen nuclei are 'tipped' the RF-pulse is terminated. This allows for the nuclei to return back to their original orientation. This process is known as relaxation. The RF-pulse places energy into the system, which is released through the relaxation process. It is this release of energy that is the radiofrequency signal that is measured during MRI. During the

normal course of an MRI scan the RF-pulse and measurement of energy released during relaxation, is repeated many times.

3.1.3 Relaxation and the NMR Signal

NMR measurement can be analysed in terms of energy transfer. Relaxation is the process by which protons release the energy absorbed from the applied B_1 field. The process of relaxation differs for different tissue types, but regardless relaxation times are measured for tissue bulk, not individual molecules.

When the RF pulse is switched off, the proton contained within the hydrogen nuclei will return to its original orientation. During this process energy will be emitted at frequency ω_0. If a conductor is placed within the magnetic field, a current is induced. For NMR imaging a receiver coil is placed within the magnet. When the rotating magnetic field passes through the coil, a current is induced in the wire contained within the coil. This is an alternating current (AC) that is the same as the precessional frequency. The signal produced is known as the free induction decay (FID). The FID signal is the basis of the NMR signal. However, it does not produce spatial information.

The release of energy is the basis of MR image contrast. This energy release, which is also known as relaxation, can be manipulated to produce different image effects. Typically two types of relaxation are examined, T1 and T2 relaxation. Two components determine the type of relaxation under investigation. The time to echo (TE) is the time from the RF-pulse to the measurement of the signal. Time to repetition (TR) is the time between two successive RF-pulses. By manipulating the TR and TE times of a scan, the scan is 'weighted' for a particular relaxation effect that provide image contrast.

T1 Relaxation

T1 relaxation occurs through a re-growth along the z-axis. A scan is T1-weighted by having a short TE and a short TR, the values of which differ according to the MRI sequence being applied. T1 relaxation is field strength dependant. The image contrast resulting from a T1-weighted scan is that cerebrospinal fluid appears very dark; white matter appears bright, and grey matter appears at an intermediate intensity. This type of scan is often termed an anatomical scan.

T2 and T2* Relaxation

Unlike T1 relaxation, which is a recovery process, T2 relaxation is caused by decay, whereby magnetisation is lost in the x-y plane. A T2-weighted scan typically has longer TE and TR periods than those used for T1. The T2 signal is insensitive to inhomogenieties in the magnetic field as it occurs at the molecular level. When the RF pulse is applied, the nuclei will experience slight fluctuations at the molecular level that cause the Larmor frequencies to vary, which lead to a loss of signal. A T2-weighted scan produces good contrast between tissue types and as such is often used for pathological investigations.

The signal most commonly used in BOLD fMRI is T2* relaxation. Unlike T2 relaxation which occurs at a molecular level, T2* decay results from large scale variations in the applied magnetic field. Magnetic field inhomogeneity may result in MDM's at different points in the magnetic field precessing at different frequencies. Usually the inhomogeneity of the magnetic field is considered an artefact and attempts are made to make the magnetic field as uniform as possible. However, some small inconsistencies exist within the magnetic fields which are used to measure the BOLD fMRI signal using T2*.

To make the magnetic field as homogenous as possible, 'shimming' is applied at the beginning of the MRI experiment. However, if dephasing occurs due to the inhomogeneity of the magnetic field this can be corrected and this correction allows the T2* signal to be obtained. If an RF-pulse is applied in the opposite direction, (an 180^0 RF-pulse) of the original pulse (90^0 RF-pulse) the direction of rotation of the precession of the MDM's is reversed. When the same amount of time has elapsed from the 180^0 RF-pulse as was given between the 180^0 RF-pulse and 90^0 RF-pulse the MDM's will be in phase again.

3.1.4 MRI Gradients

The processes of nuclear spin, RF-pulse and relaxation demonstrate the creation and detection of the MR signal. However, these processes do not produce spatial information to allow the transformation of the 2D MR signal into a 3D MRI image. Imaging the location of resonating nuclei is made possible with the use of small magnetic field gradients that are superimposed on the larger static magnetic field of the imaging magnet. A magnetic gradient is a change in the strength of the magnetic field over a specified spatial distance. As the spatial distance between two points change, so does the magnetic field between these points. In MRI the magnetic gradients are linear thus allowing for the collection of spatial information. The use of

these three gradients will determine the unique contribution of each part of the brain to the extracted MRI signal.

Slice select gradient (G_z)

This gradient occurs in the longitudinal (z-axis) of the B_0. This results in a less uniform magnetic field along the z-axis, with nuclei precessing at different frequencies along the axis. The slice selection gradient is briefly switched on during the RF-pulse. As precession of the MDM's varies proportionally to the strength of the magnetic field, the frequency of the RF-pulse has to match the frequency of the precessing nuclei. This allows for a 'tipping' of the nuclei across a specified spatial range along the z-axis allowing for the measurement of relaxation in a specific 2D slice of brain. Through increasing the frequency of the RF-pulse, but keeping the range of the frequencies equal, each slice of the brain is selected successively until the entire brain is measured.

There are two means of selecting the thickness of the slice, either the steepness of the gradient or through manipulation of the range of frequencies in the RF-pulse. Thinner slices, which produce less partial volume averaging, are gained by using a larger gradient resulting in less nuclei precessing at that frequency. Alternatively, thicker slices, which provide more signal per voxel, can be achieved by varying the bandwidth of the applied RF-pulse, thus including more nuclei. Slice thickness Δt is given by the Equation 3.2:

$$\Delta t = BW / \gamma \cdot Gz \qquad \textbf{Equation 3.2}$$

where BW is the RF-pulse bandwidth, γ is the gyromagnetic ratio and G_z is the strength of the slice selection gradient. Minimum slice thickness is often determined by technical constraints. For clinical neuroimaging a slice thickness of 3-5mm is commonly used.

After the application of the slice select gradient, two further orthogonal linear gradient fields are introduced. These determine the contribution of each voxel to the image by a combination of phase and frequency.

Frequency encoding gradient (G_x)

The frequency encoding gradient encodes spatial information in the x-axis by altering the precessional frequencies of the nuclei along the gradient. The gradient is switched on just prior to the signal being recorded by the receiver. Prior to the application of the G_x the MDM's precess at the same frequency. However, after application, the magnetic field strength is dependent upon the location within the slice along the x-axis.

Phase encoding gradient (G_y)

The phase encoding gradient is briefly applied across the y axis at a moment in time between the RF-pulse and the measurement of the MRI signal. Phase relates to how the nuclei precess. When the nuclei precess at the same point in their cycle they are said to be in-phase. If they are in different points of their cycle they are said to be out of phase. The application of the RF-pulse 'tipped' the MDM's in the x-y plane and forced them to precess in phase. The application of the G_y gradient varies the Larmor frequency, as nuclei now experience a difference in the magnetic field strength along the y-axis. The resulting effects are that different rows will now precess at different frequencies. When the G_y is terminated the MDM's will start precessing at the same frequency but they will be out of phase. Different hydrogen nuclei are surrounded by different other nuclei which will affect the frequency of precession in the hydrogen nuclei. This is known as spin-spin interaction.

The frequency encoding and phase encoding gradients result in an MRI signal for each voxel being represented by a unique combination of frequency and phase. This produces a *k*-space image, which is the space in which the data is acquired. An inverse Fourier transformation (FT) that simultaneously processes both phase and frequency encoded information is then applied. Fourier analysis involves the transformation of a mathematical function that varies with time into an amplitude function that varies with frequency. The result of the FT is that information regarding the location of the nuclei within the x-y planes is produced.

3.2 Stereological Volume Estimation and Error Prediction

Recent advances in MRI have allowed for non-invasive volumetric measurements of brain structures to be performed, which previously would have only been gained through the use of post mortem data. The Cavalieri method is an unbiased volume estimator technique of modern design based stereology. The method is named after an Italian mathematician, Bonoventura Cavalieri (1598-1647) who made significant advances in the mathematics of numerical integration. Cavalieri asserted that the volume of any object can be estimated from a set of 2D slices provided subject to certain criteria; the images are parallel, separated by a known distance and begin randomly within the object. Magnetic resonance imaging allows for the criteria of the Cavalieri method to be met. Therefore the image of a solid object, such as a brain, can be measured through the analysis of sections placed within it. The advantages of using the Cavalieri method is that it is semi-automated, reliable, provides unbiased measurements and when used in combination with point counting, it has been shown to have excellent reliability (Sheline, Black, Lin, Christensen, Gado, Brunsden & et al, 1996).

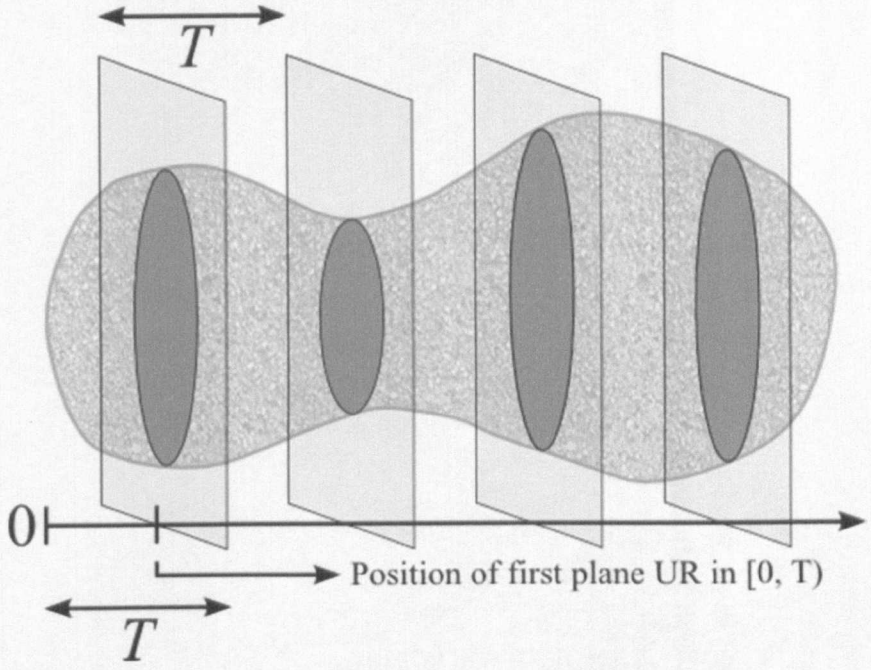
3.2.1 Cavalieri Method

Using the Cavalieri method, the volume of a structure, of arbitrary shape and size, can be estimated without bias. This is possible through several stages. The structure is sectioned with a series of parallel planes which must encompass the object entirely. The planes (or sections) must be at a constant separation, T. The first section must be placed at a uniform and random position. Each area of the section is measured, A, with the section areas being summed and multiplied by the section interval (Gundersen & Jensen, 1987). This gives Equation 3.3;

$$Vest_1 = T \cdot (A_1 + A_2 + ... A_n) = T \cdot \sum_{i=1}^{n} A_i \qquad \textbf{Equation 3.3}$$

where T is the sectioning interval for the n sections, A the area of the sections.

Figure 3.4 The Cavalieri Method of Volume Estimation



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3.2.2. Volume Estimation with 3D MR Images

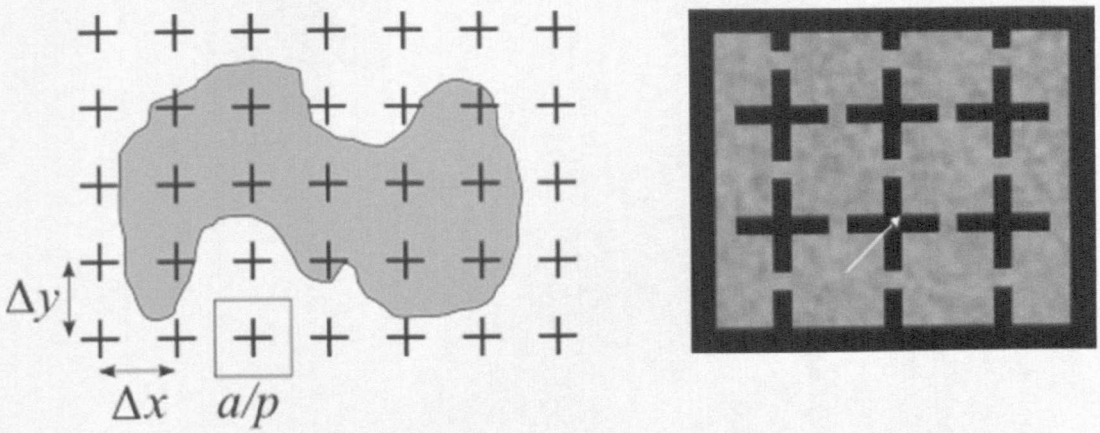
The Cavalieri estimator of volume can be easily applied to high resolution 3D MR images. If section areas cannot be measured exactly, then an unbiased estimate can be obtained by using a well-established point counting technique. The point counting method consists of overlaying each section with a grid consisting of a regular array of test points. To remove bias, the test system should be randomly positioned on each section. The points consist of a cross shape (+). A point is counted if the intersection of the upper right quadrant of the cross is contained within the boundary of the region of interest. The number of points contained within the region of interest is calculated and the unbiased estimator becomes Equation 3.4. This method of volume estimation is both more efficient and precise than manual tracing of intersect areas (Gundersen & Osterby, 1981). The Cavalieri method is only ever truly unbiased when the measured sections are of a zero thickness. However, MRI images have a finite thickness and may therefore be considered as slices rather than planes (McNulty, Cruz-Orive, Roberts, Colin, & Gual-Arnau, 2000).

When point counting is used an unbiased estimator of volume is

$$Vest_2 = T \cdot \frac{a}{p} \cdot (P_1 + P_2 + ...P_n) \qquad \textbf{Equation 3.4}$$

where ($P_1 + P_2 + P_3 ..... + P_n$) denotes the point counts, (a/p) denotes the area associated with each test point. The subscript 2 in V_{est2} indicates that the volume estimator is based on two sampling processes, sectioning the structures of interest and point counting on those sections.

Figure 3.3 Point Counting Methodology



$$\hat{A} = a/p \cdot P = \Delta x \cdot \Delta y \cdot P$$

Left image; demonstrating the grid system for volume estimation. The square around the cross indicates the volume represented by that cross. Right image; arrow indicates the intersection of the upper right quadrant taken as the point. Adapted from Howard & Reed (1997).

3.2.3 Prediction of Coefficient of Error

To evaluate the reliability of the point density of the grids and the sectioning intervals, the associated coefficient of error (CE) is calculated. The CE is a predictive formula which accounts for the systematic nature of the sampling. It is superior to conventional statistical techniques as the MRI sections are sampled systematically and are not independent of one another. As such the sample cannot be assumed to be random and the familiar formula for standard error 'standard deviation divided by the square root of n' is unsuitable.

$$SE = \frac{SD}{\sqrt{n}} \qquad \textbf{Equation 3.5}$$

If the section areas of an object can be measured exactly, the coefficient of error (CE) of the volume estimate of an arbitrary shaped structure can be predicted (Equation 3.6)

$$CE(est_1V) = \left(\sum_{i=1}^{m} A_i^{-1}\right) \cdot \left\{\frac{1}{12}\left(3\sum_{i=1}^{m} A_i^{\,2} + \sum_{i=1}^{m-2} A_i \cdot A_{i+2} - 4\sum_{i=1}^{m-1} A_i \cdot A_{i-1}\right)\right\}^{1/2}$$

Equation 3.6

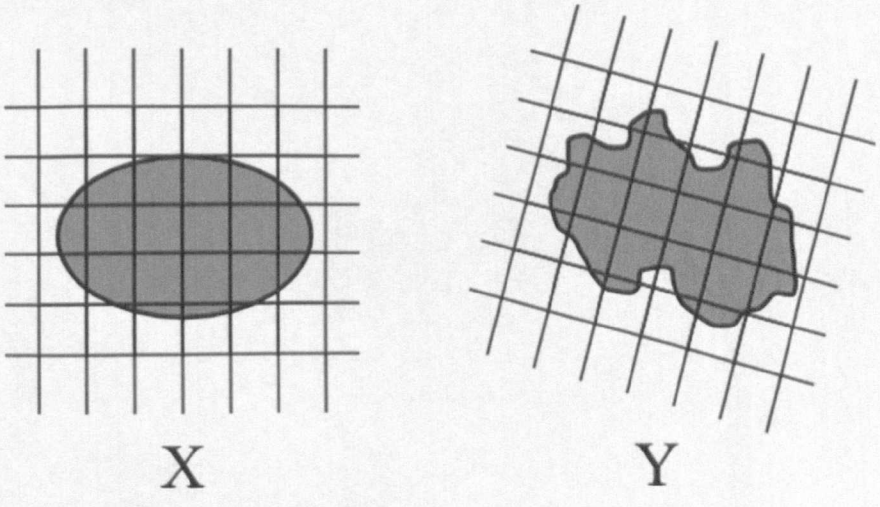
However, if section areas are estimated by point counting then Equation 3.7 applies

$$CE(est_2V) = \left(\sum_{i=1}^{m} P_i^{-1}\right) \cdot \left\{\frac{1}{12}\left(3\sum_{i=1}^{m} P_i^{2} + \sum_{i=1}^{m-2} P_i \cdot P_{i+2} - 4\sum_{i=1}^{m-1} P_i \cdot P_{i+1}\right) + 0.0543\frac{\overline{B}}{\sqrt{\overline{A}}}\left(\sum_{i=1}^{m} P_i\right)^{1/2}\right\}^{1/2}$$

Equation 3.7

The equation includes additional sampling errors due to point counting and the final term in equation 3.7 is known as the "nugget effect" term, which takes into account the complexity of the shape. It involves the dimensionless shape coefficient $\overline{B} / \sqrt{\overline{A}}$ which is equivalent to the mean boundary length $\overline{B}$ of the slices divided by the square root of their mean area $\overline{A}$. Point counting estimates $\overline{A}$ and $\overline{B}$ is estimated by counting intersections, I, between the transect boundaries and an isotropically positioned square grid of test lines. An explanation of shape complexity is demonstrated in Figure 3.4.

Figure 3.4 Shape Complexity



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X and Y share the same area. Due to the random positioning of the grid, and the irregular boundary of Y, there will be a larger variation in the number of points which fall within the area Y than for the area X. The nugget effect term found in Equation 3.7 will be larger for shape Y, which in turn will increase the predicted co-efficient of error. To maintain the same levels of precision, it is necessary to count more points for shape Y than for shape X. (b/ sq root of a) is estimated for individual structures as follows;

The boundary length for each section is estimated using Equation 3.8:

$$est\sum_{i=1}^{m} B_1 = \frac{\pi}{2} \cdot \frac{a}{l} \cdot I \qquad \textbf{Equation 3.8}$$

Where I is the number of intersections between the grid and the shape boundary, a is the area per point, l is the interval between two adjacent points and m the number of sections. If d is the distance between two points, $a = d2$, and $l - 2d$, substituting d in Equation 3.9:

$$est\sum_{i=1}^{m} B_1 = \frac{\pi}{4} \cdot d \cdot I \qquad \textbf{Equation 3.9}$$

Section area A is estimated by point counting, therefore:

$$\frac{B_1}{\sqrt{A_1}} = \frac{\pi \cdot d \cdot I}{4 \cdot \sqrt{d^2 \cdot P}} \qquad \textbf{Equation 3.10}$$

if $\overline{B} = \frac{\sum B}{m} and \overline{A} = \frac{\sum A}{m}$, then :

$$\frac{\overline{B}}{\sqrt{\overline{A}}} = \frac{\pi}{4} \cdot \frac{I}{\sqrt{P}} \cdot \frac{I}{\sqrt{m}} \qquad \textbf{Equation 3.11}$$

The value of $\overline{B} / \sqrt{\overline{A}}$ is calculated for each structure measured during pilot testing, which is then applied to all measures thereafter. Therefore, the nugget effect takes into account the complexity of the structure. The greater the complexity the shape, the higher the error will be in estimating the volume. The CE has a generally accepted limit of 5%.

To obtain a reasonable CE value, the observer may change the number of slices available or the size of the points within the grid. It is generally accepted that a calculation of between 150-200 points for a structure will yield coefficients of error at 5% or less.

3.2.4 Preprocessing and Demarcation of Images

The MR ANALYZE images were imported into BrainVoyager (BVQX 2.1-Brain Innovation, Maastricht, Netherlands; www.Brainvoyager.com) which allows reslicing and resizing of the data according to multiple planes. Datasets were reformatted from 0.781 x 0.781 x 1.6mm to 1mm isotropic images. Images were then resliced for the orientation which provides the best estimation of the volumes. For the PFC subfields this orientation was sagittal. In contrast, insular cortex volumes were estimated on image sections in the coronal orientation.

After resizing images were realigned. Each MR image was realigned to specific criteria, where X is the left-right axis, Y is the dorsal-ventral axis and Z the anterior-posterior axis.

YZ Plane Correction: To correct for anterior to posterior roll a bi-commissural realignment was performed, whereby both the anterior and posterior commissure can be observed upon the same transverse image.

XY Plane Correction: To correct for side-to-side pitch, an orthogonal plane was taken at the most superior aspect of the orbits, when they both appear on the same transverse image.

XZ Plane Correction: The corrected transverse image has a plane taken through the longitudinal fissure. The image is reoriented so that the longitudinal fissure and the plane are aligned. This ensures a standardised sagittal image is generated.

Prefrontal Cortex

The prefrontal cortex was subdivided into four subfields for each hemisphere. The four regions are dorsolateral, dorsomedial, orbitolateral and orbitomedial. The technique for establishing the landmarks for demarcation have been used previously (Howard, Roberts, Garcia-Finana, & Cowell, 2003) and were as follows:

Orbital/Dorsal Demarcation: The bicommissural plane delineates orbital from dorsal regions.

Medial/Lateral Demarcation: Demarcation between medial and lateral subfields is defined on the first slice superior to the olfactory sulcus. The medial most aspect of

gray matter of the arcuate sulcus is used for demarcation between medial and lateral subfields.

Posterior Boundary of the Dorsal Subfields: The anterior most point of the corpus callosum, when viewed within the midline sagittal plane, provides the posterior boundary for the dorsal subfields.

Posterior Boundary of the Orbital Subfields: The posterior boundaries of the orbital subfields are defined by various anatomical landmarks rather than by a demarcation marker. At midline the posterior boundary is defined as the anteroventral-most tip of the corpus callosum. Laterally the boundary followed the anterior-most portion of the caudate nucleus. More laterally, the posterior boundary was defined by the anterior portion of the Sylvian fissure.

The PFC subfields traverse both sulcal and Brodmann based definitions, as indicated in Table 3.1.

Table 3.1 Prefrontal cortex subfields, neuroanatomy and estimated overlap with Brodmann Areas

Subfield	Gyri	Brodmann Areas
DL	Middle & inferior frontal gyri, lateral superior frontal gyrus & frontal pole	8, 9, 10, *44*, 45, 46
DM	Medial superior frontal gyrus, medial frontal pole, anterior cingulate gyrus	8, 9, 10, 24, 32, *6*
OL	Lateral, anterior & posterior orbital gyri, inferior & middle frontal gyri & frontal pole	10, 11, 47
OM	Gyrus rectus, medial orbital gyrus, anterior & posterior orbital gyri, cingulate gyrus, frontal pole	10, 11, 12, 24, 25, 32

Reproduced from Howard et al, (2003)

Insular Cortex

The insular cortex did not require demarcation lines. Instead the volume of the whole of the insular cortex was estimated using the following boundary definitions; anterior-most point being identified at the anterior horizontal ramus of the Sylvian fissure, the posterior-most region began when the circular insular sulcus and Heschl's gyrus can be first visualised. The anterior and posterior insula regions were confined within these boundaries and were separated by the central insula sulcus. Completion of preprocessing led to the creation of two new volume datasets which were imported into Easymeasure Software (MARIARC in-house software) for point counting to be performed. Illustrations for the point counting are presented in Figure 3.5 for dorsal PFC, Figure 3.6 for orbital PFC and Figures 3.7 and 3.8 for anterior and posterior insular cortex respectively.

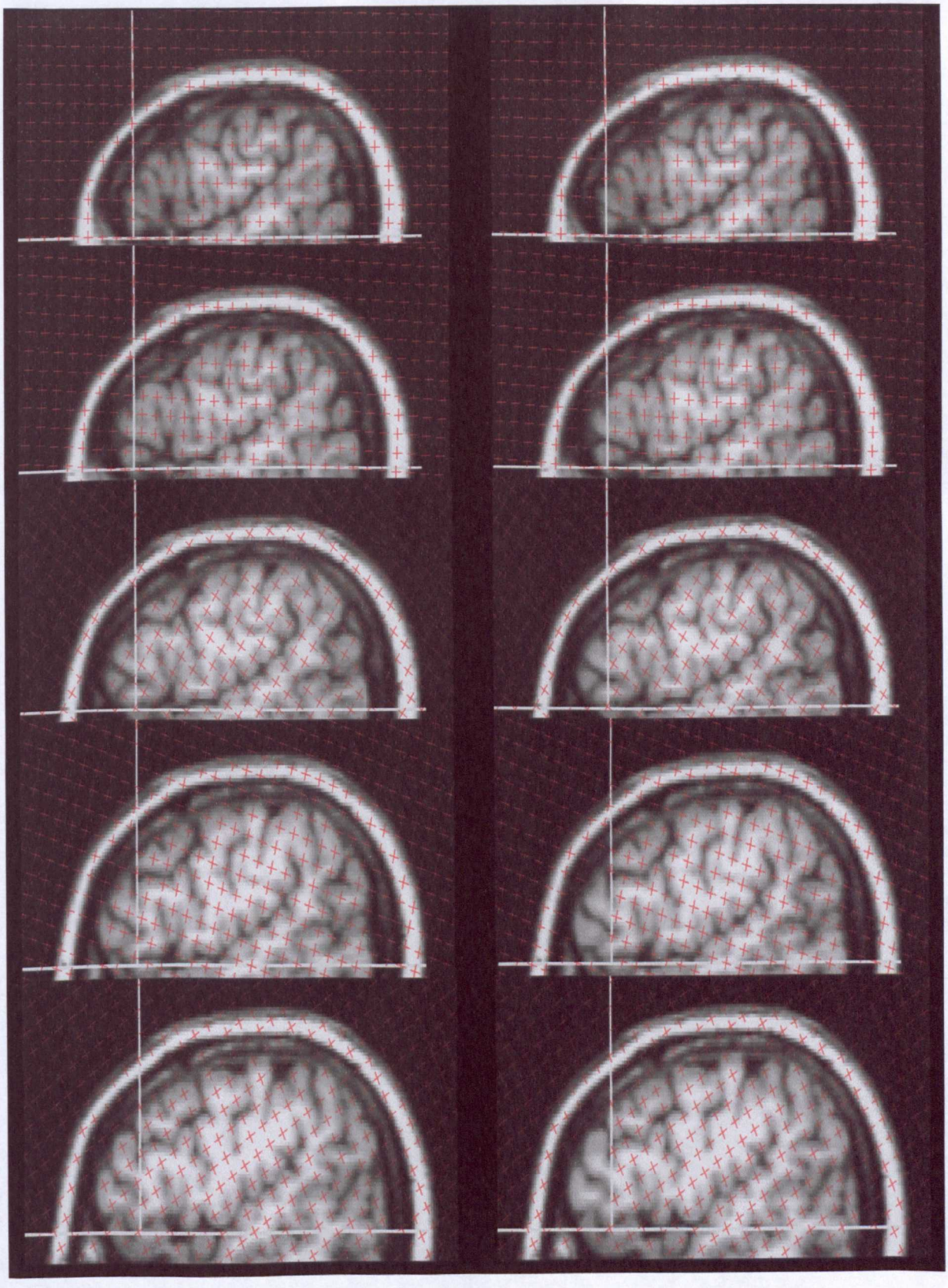


Figure 3.5 Paired Images Illustrating Point Counting for Dorsal PFC (Before; left and after; right).

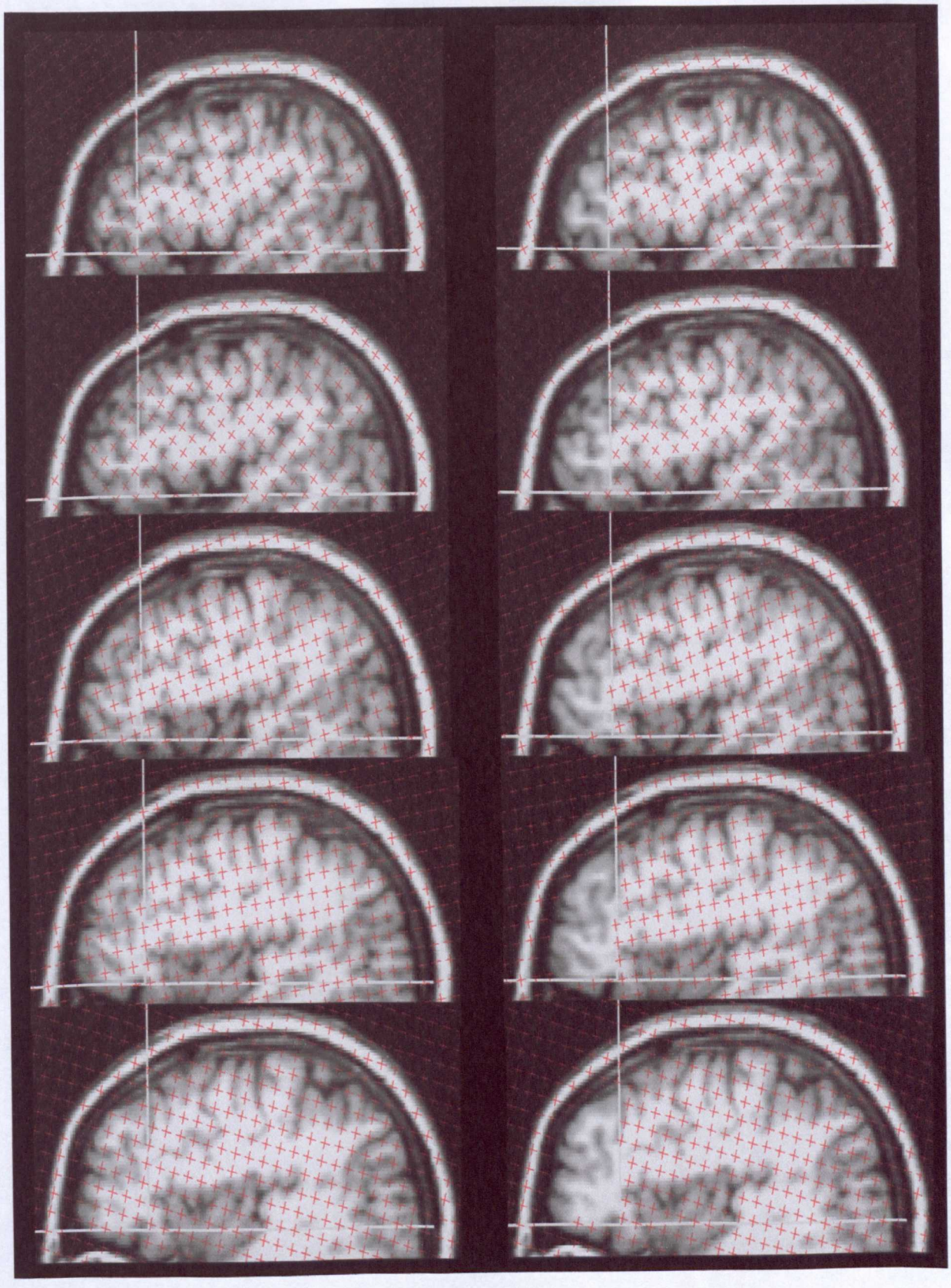


Figure 3.5 Paired Images Illustrating Point Counting for Dorsal PFC (Before; left and after; right).

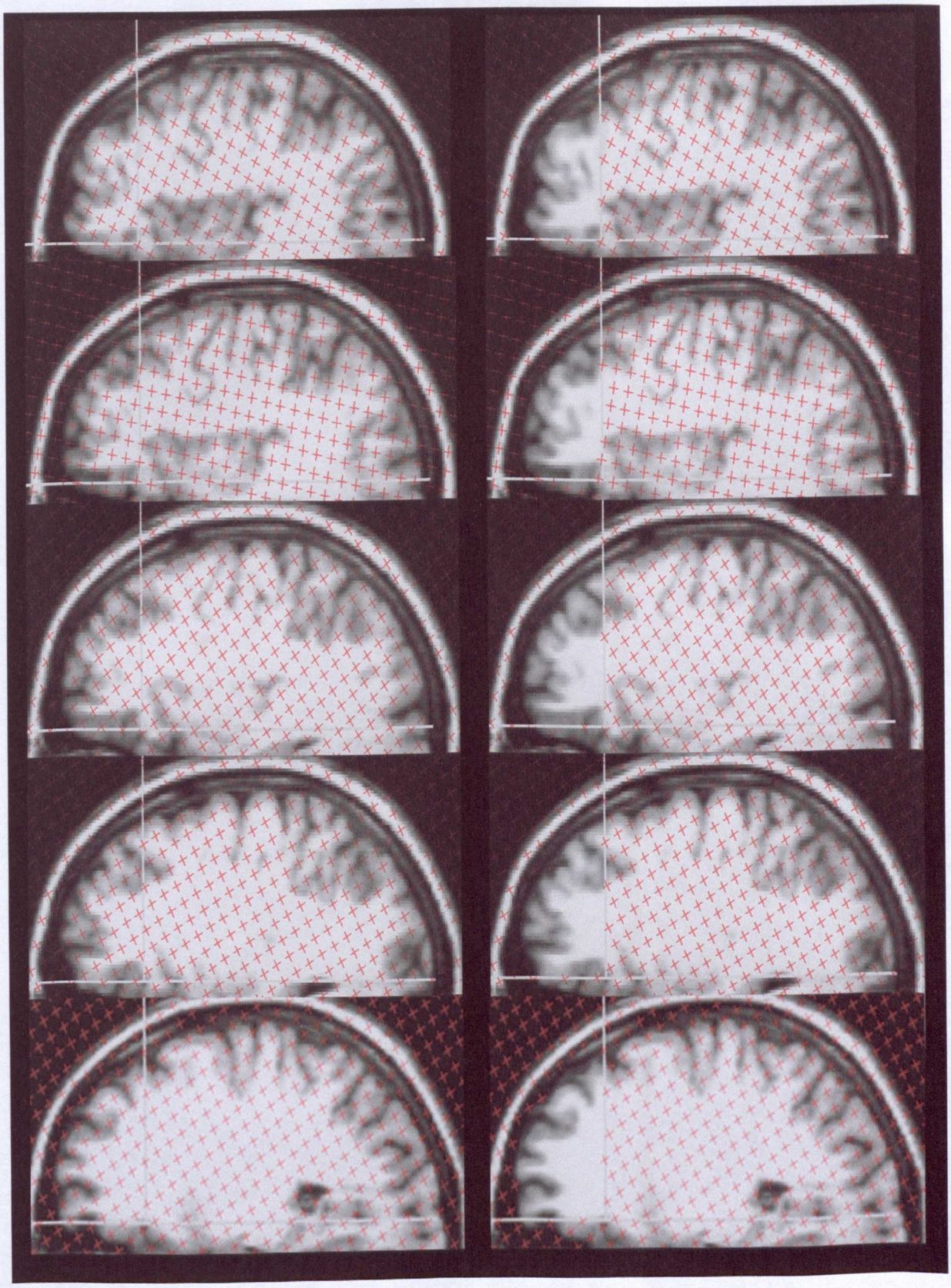


Figure 3.5 Paired Images Illustrating Point Counting for Dorsal PFC (Before; left and after; right).

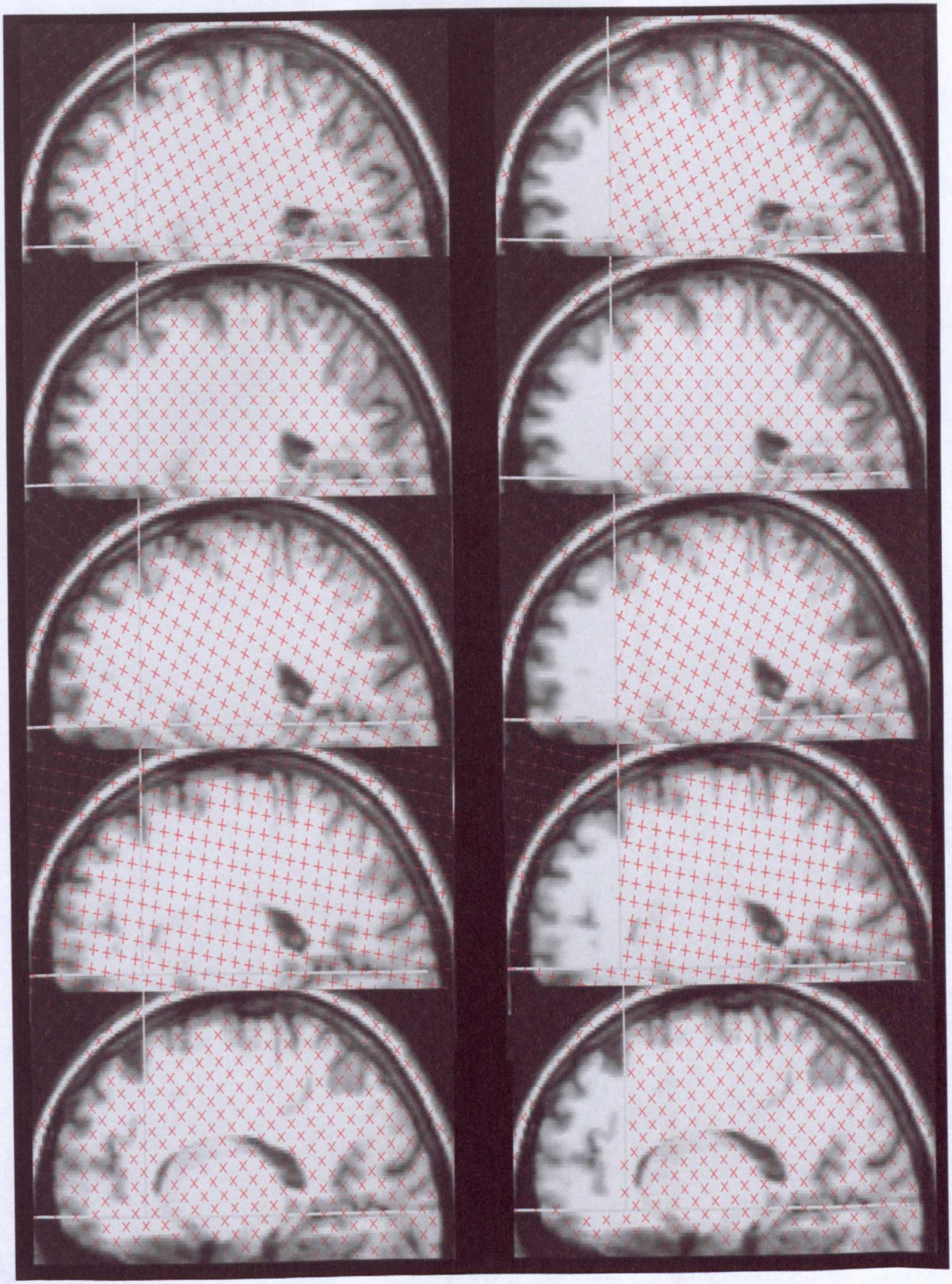


Figure 3.5 Paired Images Illustrating Point Counting for Dorsal PFC (Before; left and after; right).

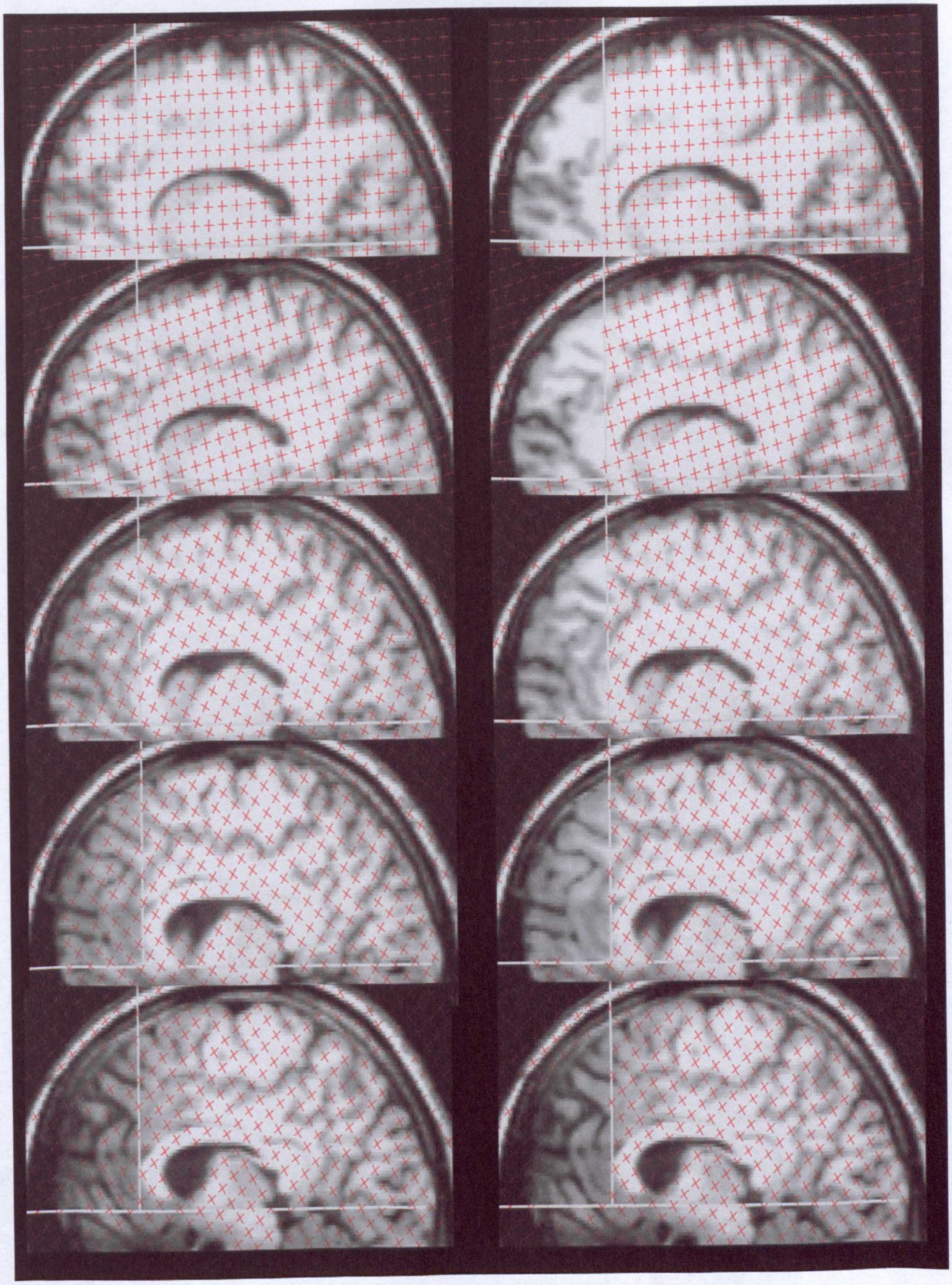


Figure 3.5 Paired Images Illustrating Point Counting for Dorsal PFC (Before; left and after; right).

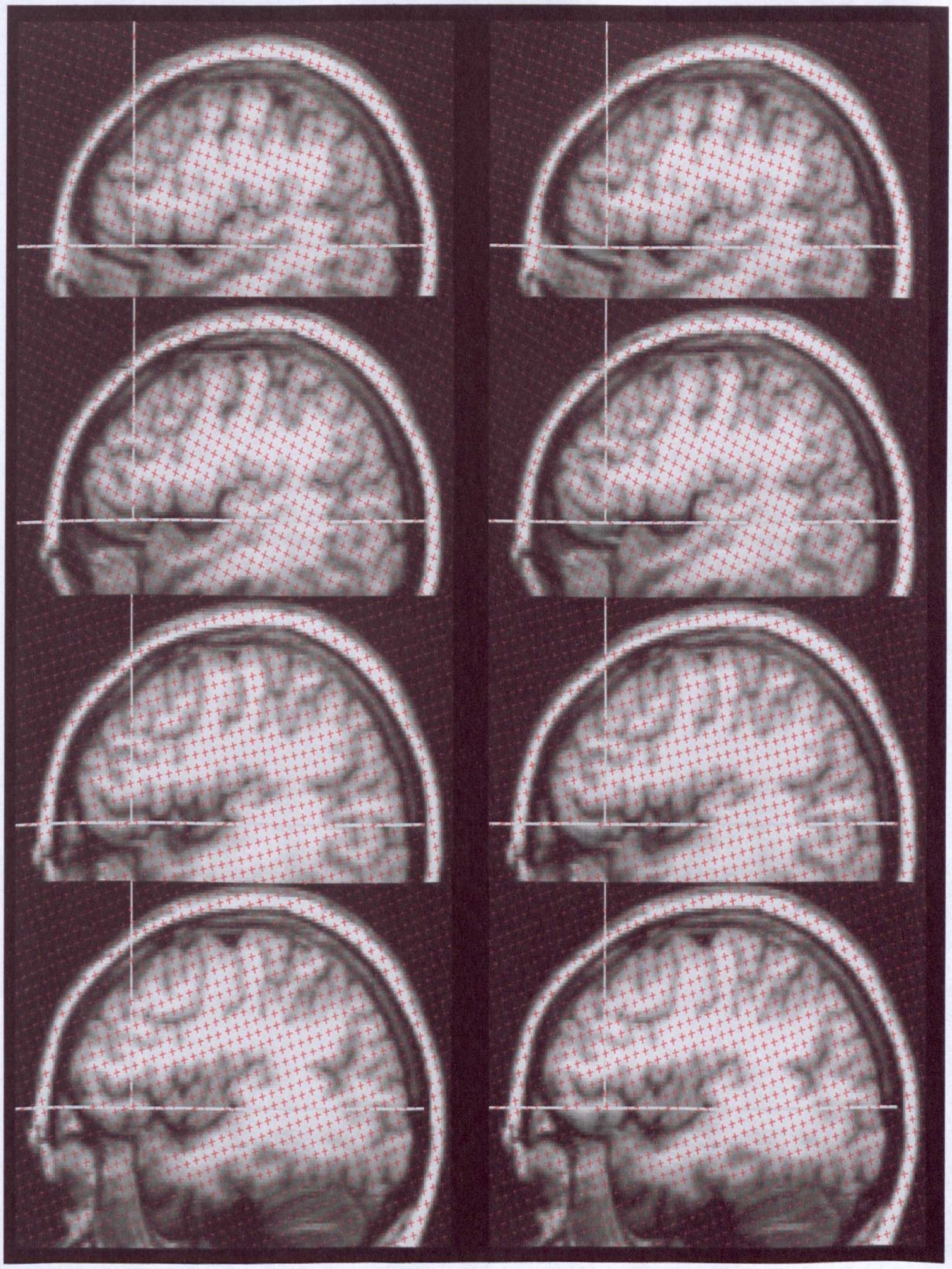


Figure 3.6 Paired Images Illustrating Point Counting for Orbital PFC (Before; left and after; right).

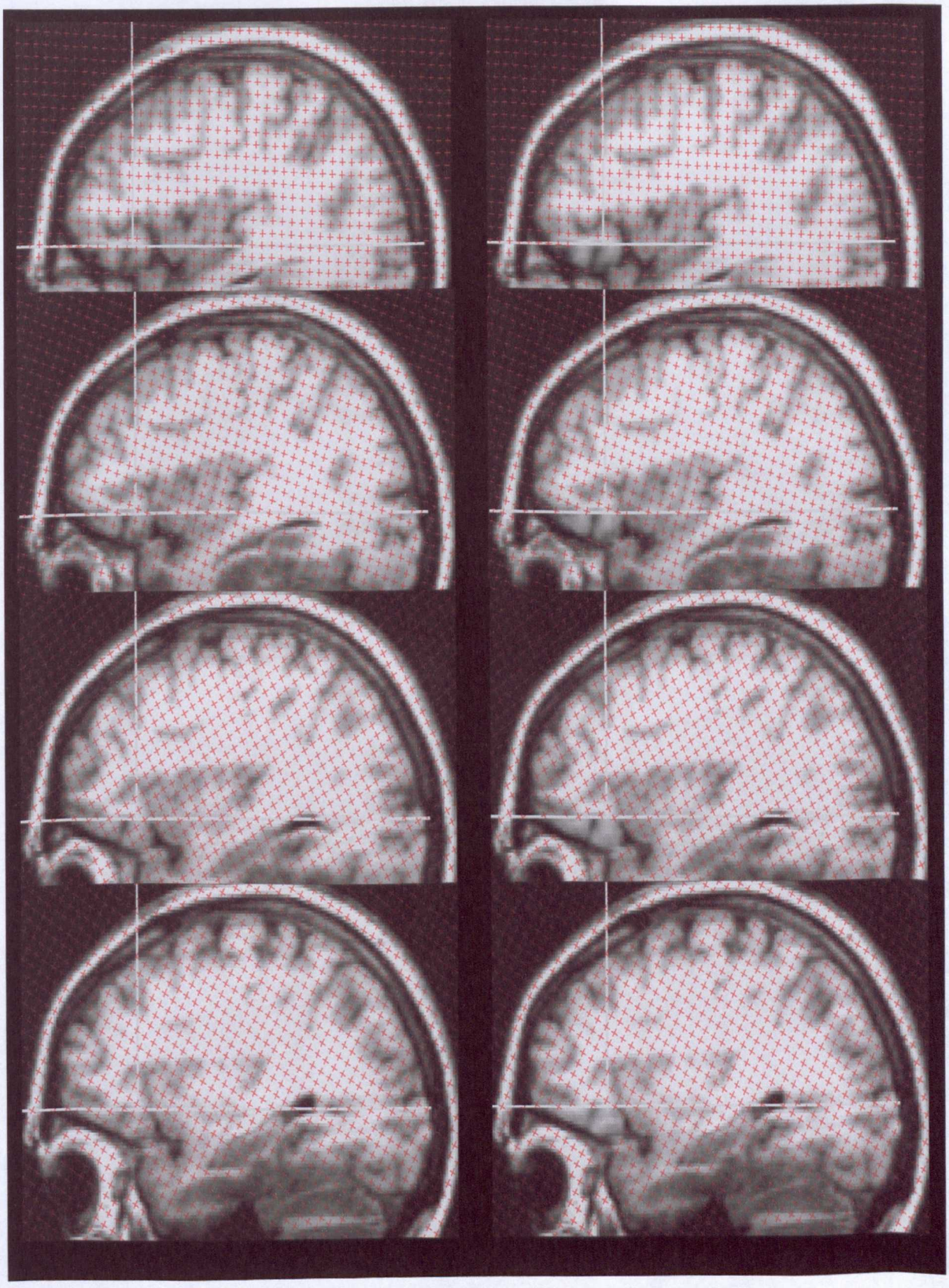


Figure 3.6 Paired Images Illustrating Point Counting for Orbital PFC (Before; left and after; right).

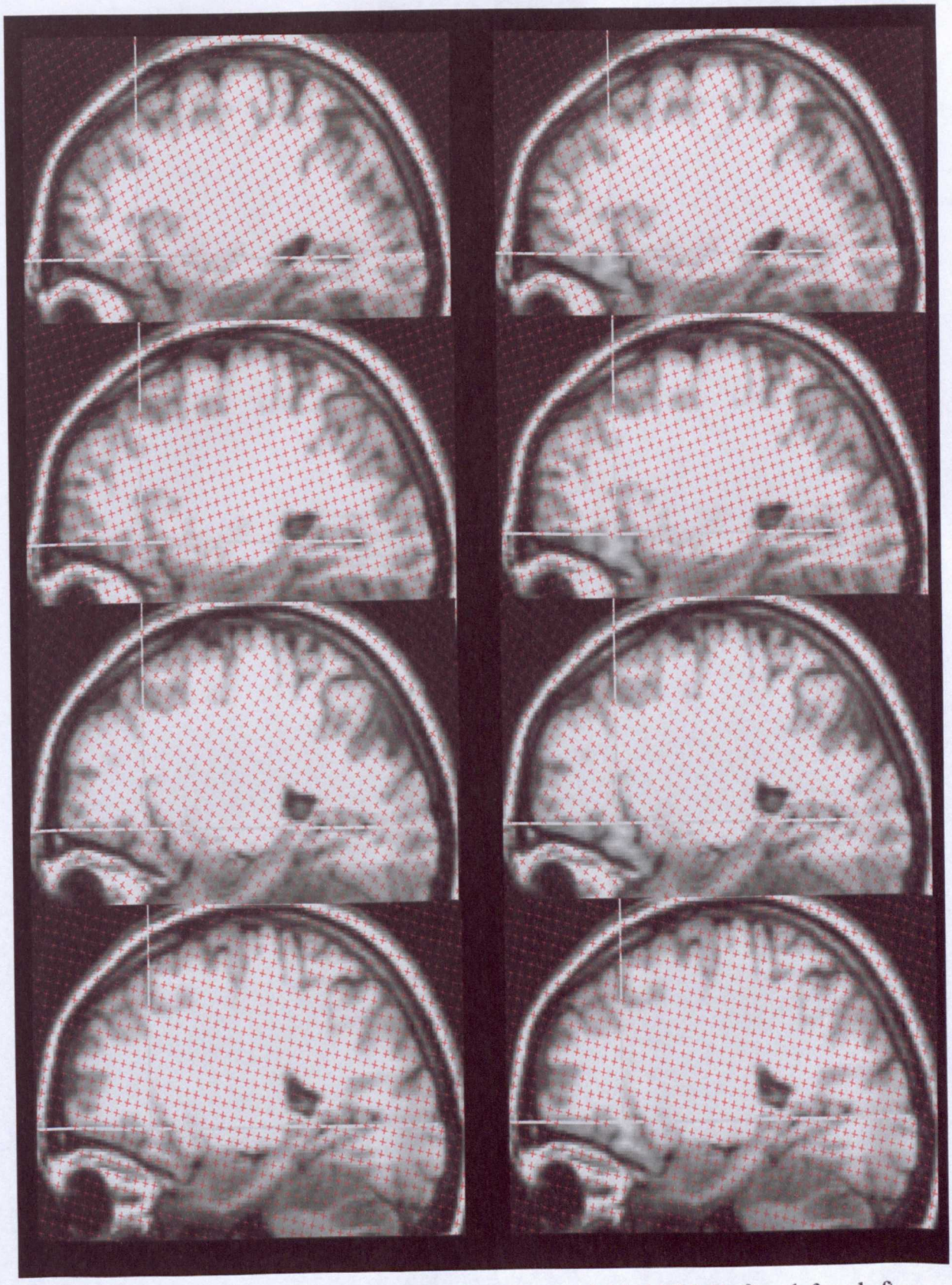


Figure 3.6 Paired Images Illustrating Point Counting for Orbital PFC (Before; left and after; right).

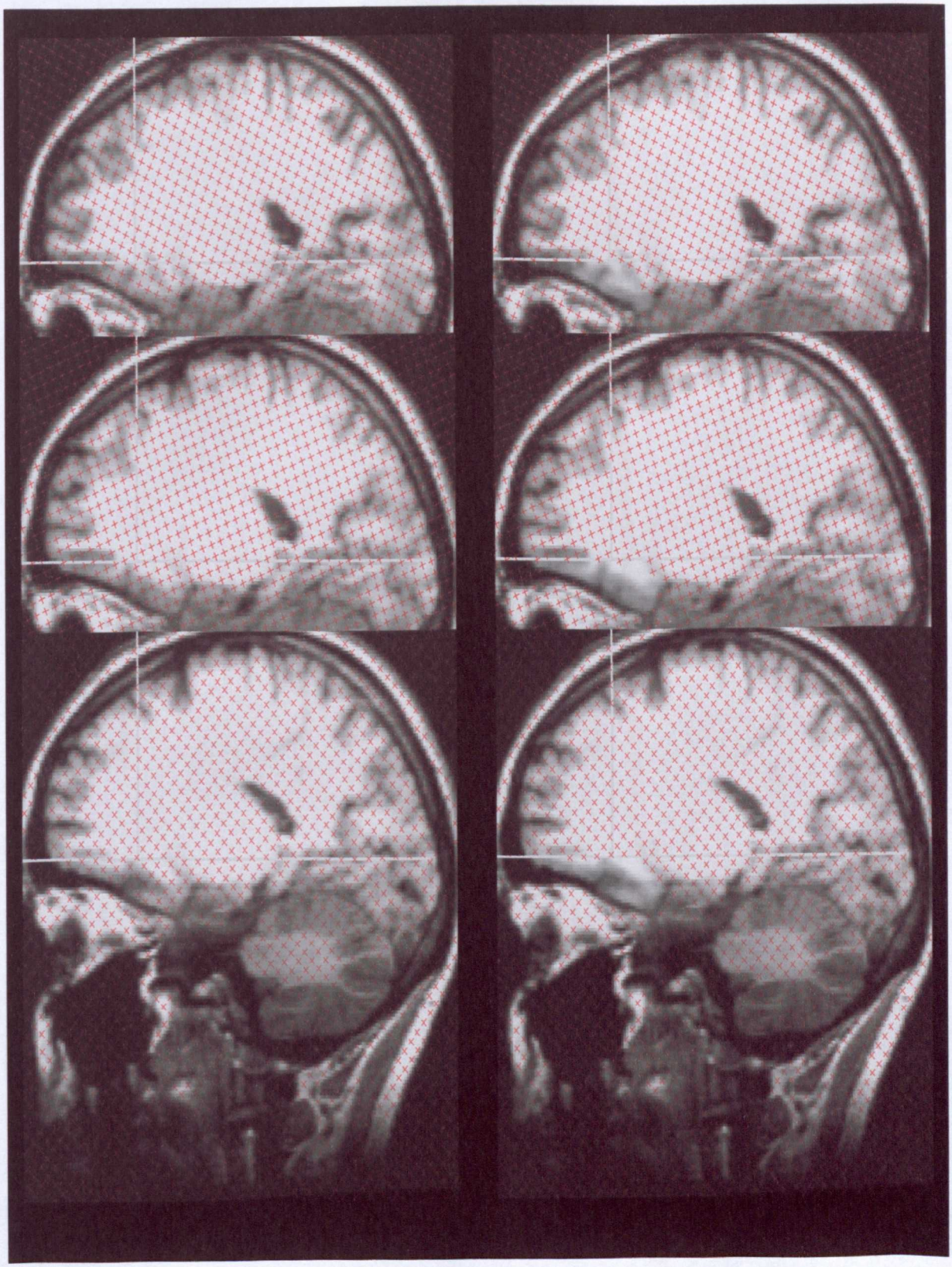


Figure 3.6 Paired Images Illustrating Point Counting for Orbital PFC (Before; left and after; right).

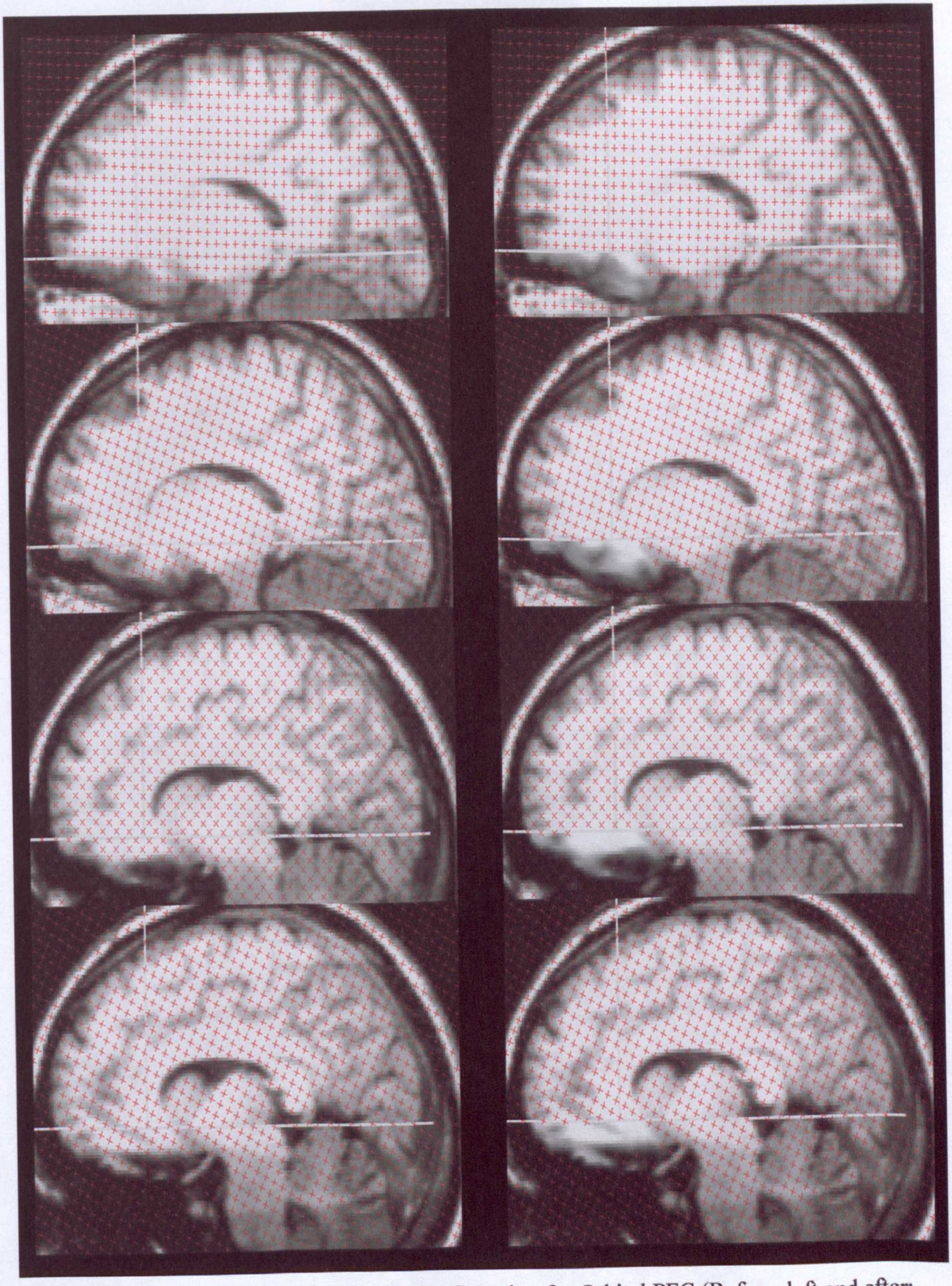


Figure 3.6 Paired Images Illustrating Point Counting for Orbital PFC (Before; left and after; right).

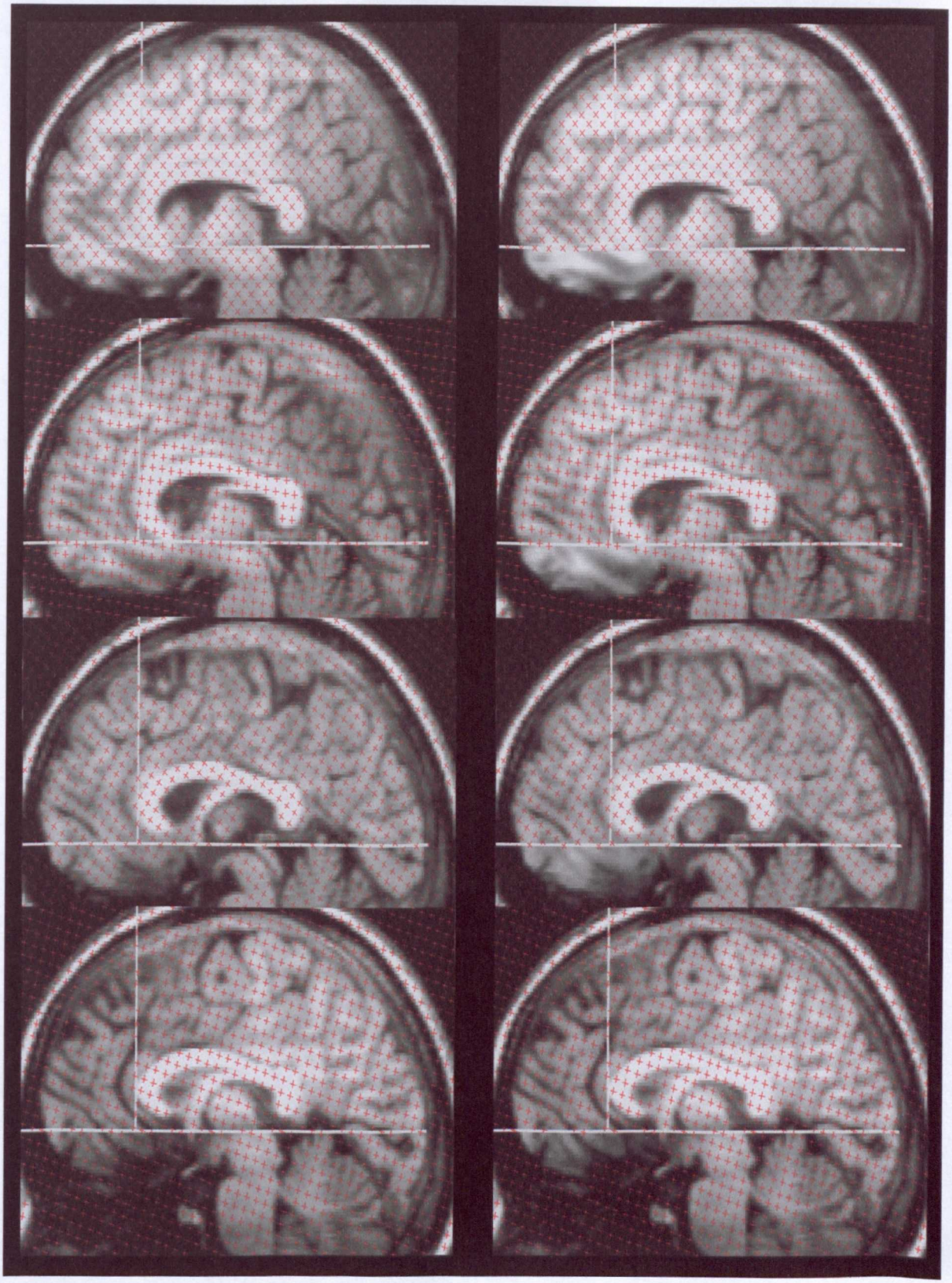


Figure 3.6 Paired Images Illustrating Point Counting for Orbital PFC (Before; left and after; right).

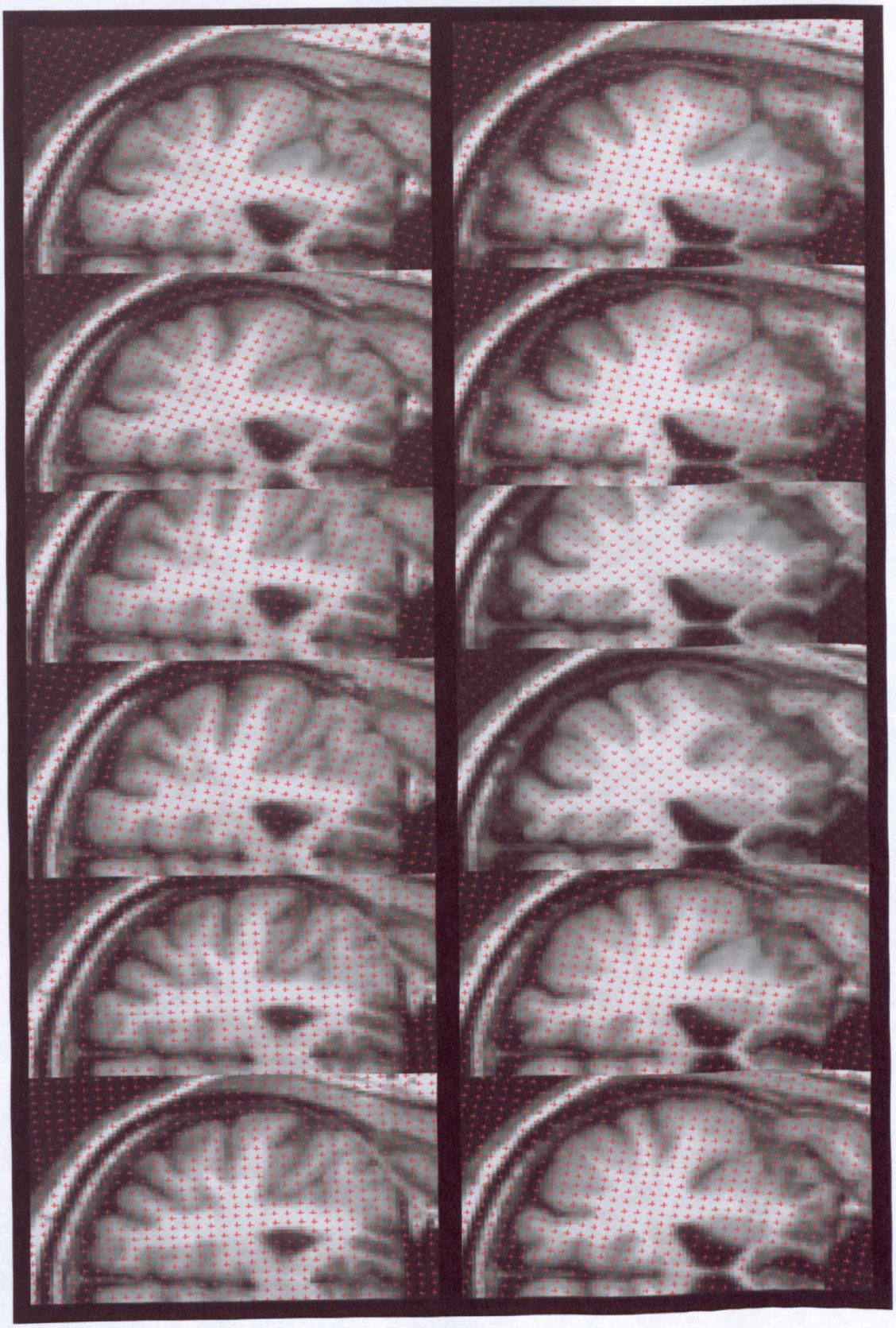


Figure 3.7 Paired Images Illustrating Point Counting For Anterior Insular Cortex (Before; left and after; right).

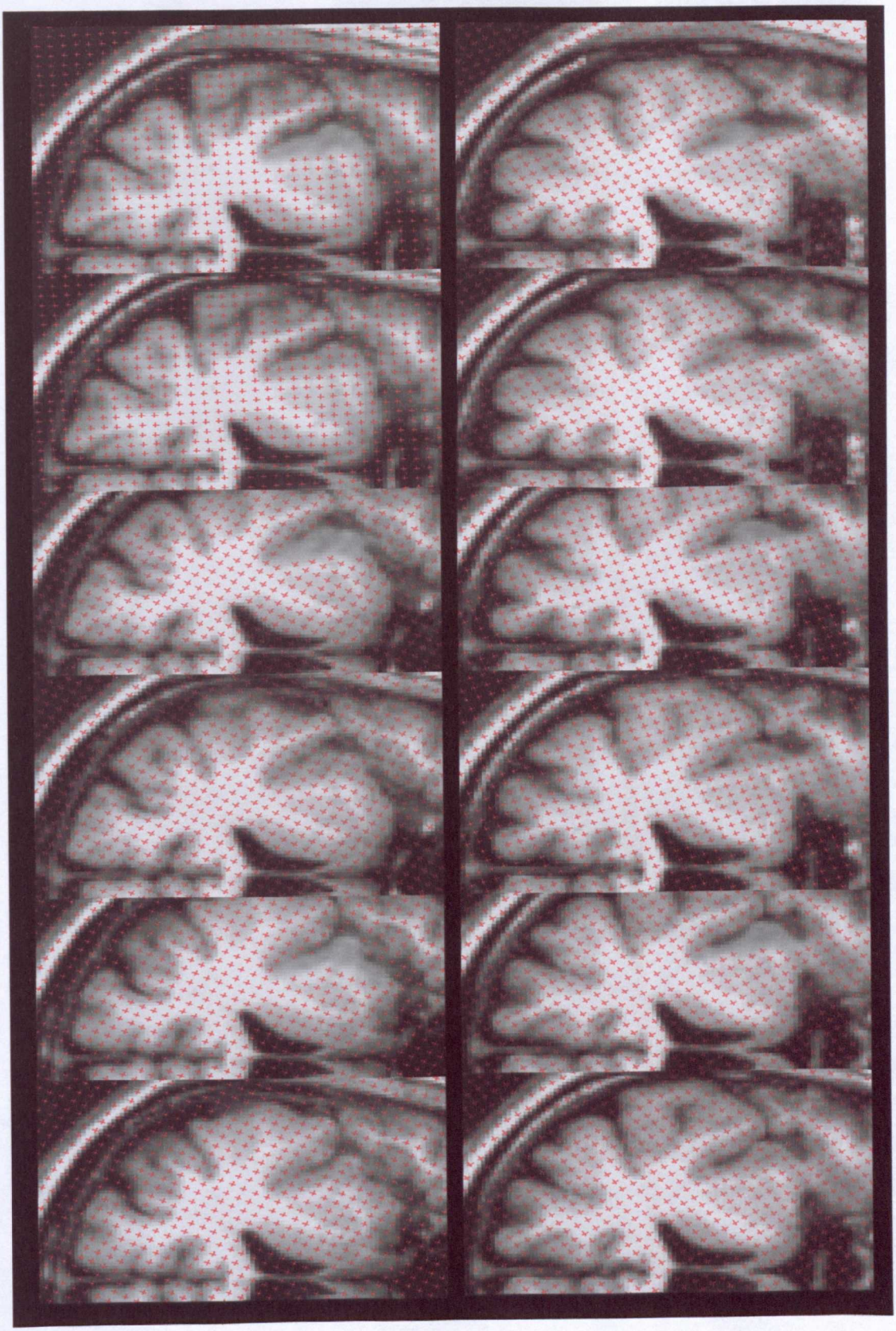


Figure 3.7 Paired Images Illustrating Point Counting For Anterior Insular Cortex (Before; left and after; right).

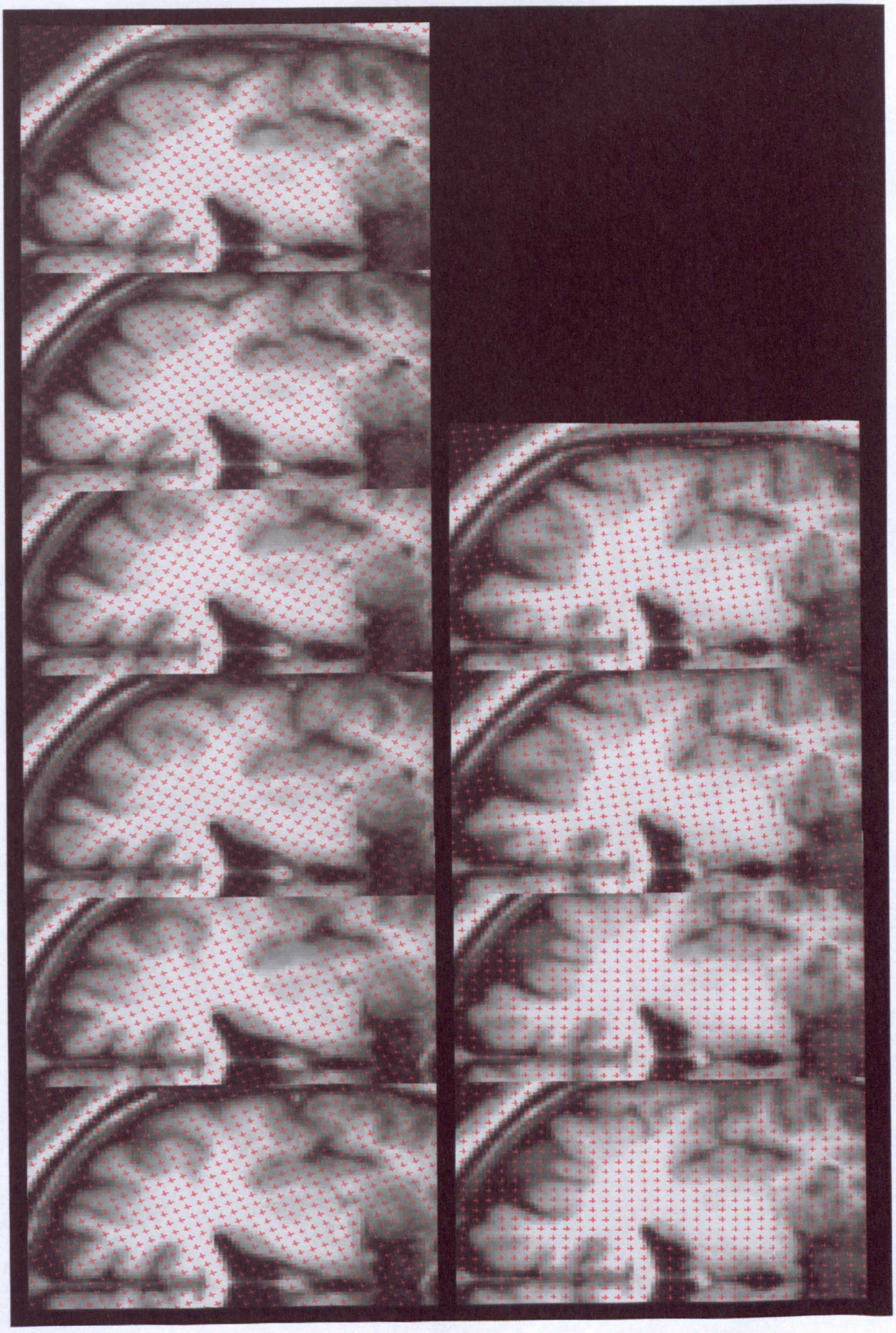


Figure 3.7 Paired Images Illustrating Point Counting For Anterior Insular Cortex (Before; left and after; right).

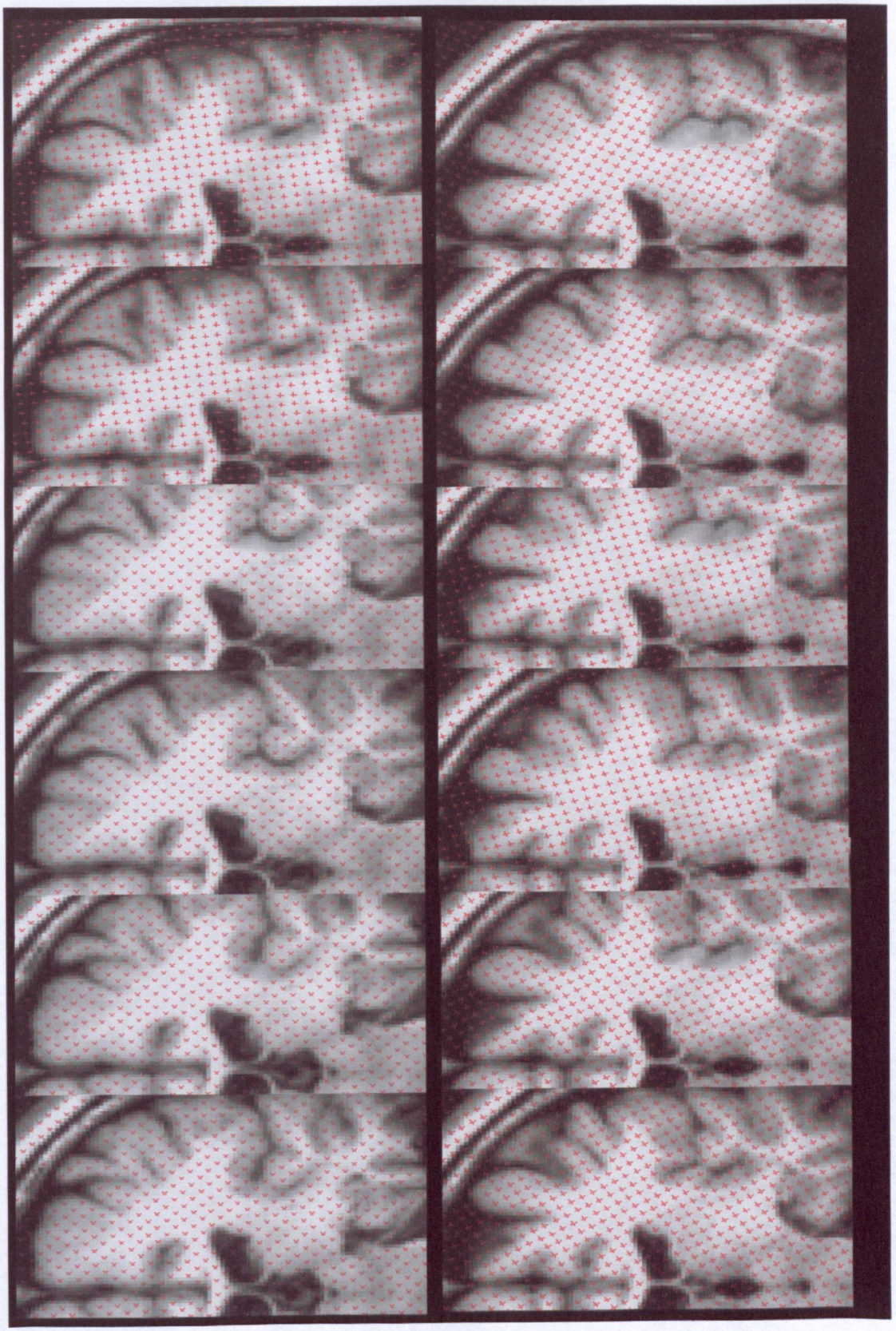


Figure 3.8 Paired Images Illustrating Point Counting For Posterior Insular Cortex (Before; left and after; right).

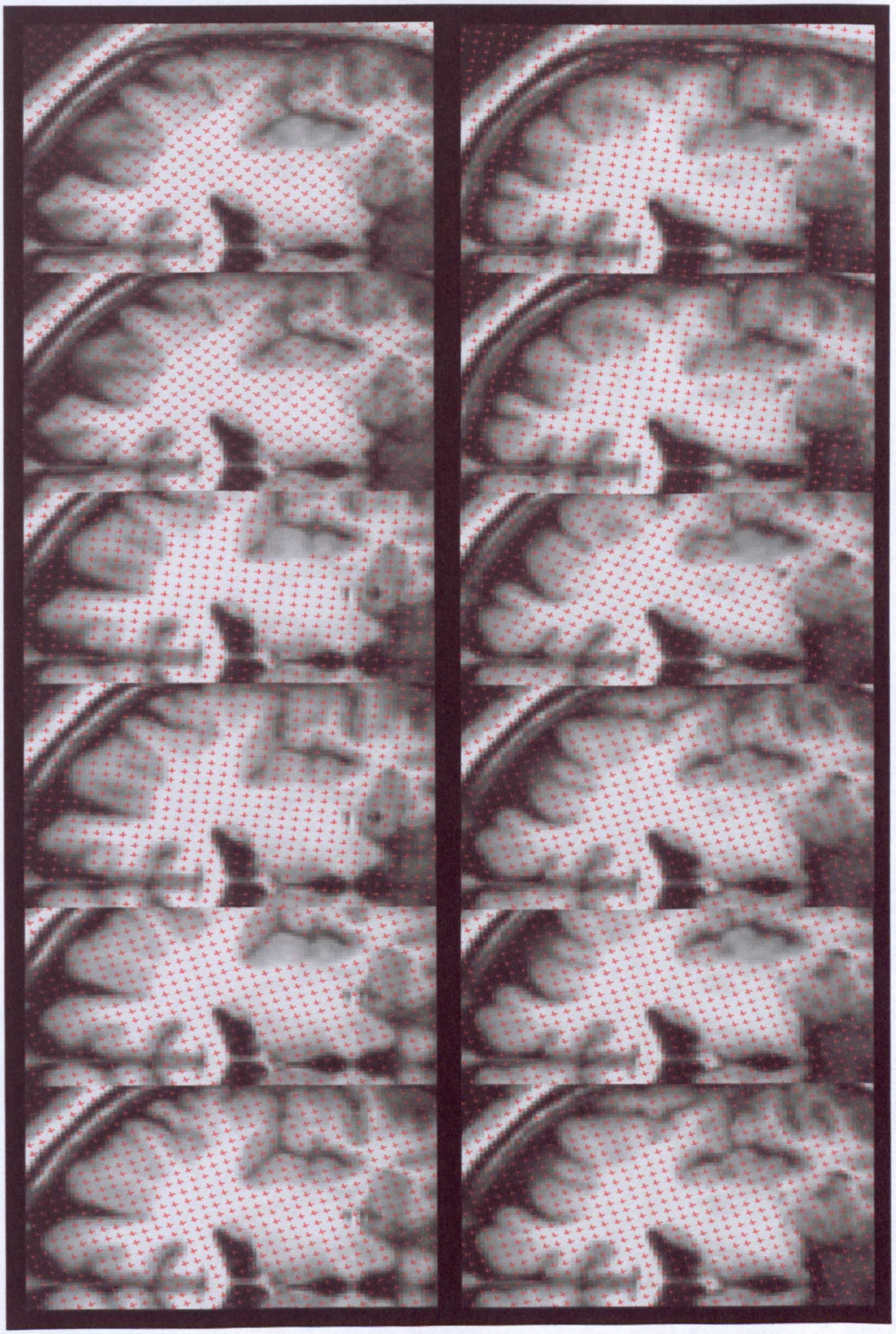


Figure 3.8 Paired Images Illustrating Point Counting For Posterior Insular Cortex (Before; left and after; right).

Figure 3.8 Paired Images Illustrating Point Counting For Posterior Insular Cortex (Before; left and after; right).

3.2.5 Stereology Parameters

The stereological point counting technique provides a heuristic guide to using the technique. For a CE of less than 5% to be predicted, approximately 150-200 points should be counted from the entire structure using between 12-15 slices. To optimise the sampling efficiency for volume estimation, parameters are adjusted for each structure. Suitable parameters have previously been developed for the PFC subfields (Howard et al, 2003) with new parameters being created for volume estimation of the insular cortex. These parameters are listed in Table 3.2.

Table 3.2 Parameters for Volume Estimation

Brain Region	Grid Size (pixels)	Slice Increment (mm)	$\overline{B}/\sqrt{\overline{A}}$
Dorsal Lateral PFC	6x6	2	5.65
Dorsal Medial PFC	6x6	2	5.99
Orbito Lateral PFC	4x4	2	5.48
Orbito Medial PFC	4x4	2	5.19
Insula Cortex	3x3	3	6.95
Anterior Insula	3x3	2	5.44
Posterior Insula	3x3	2	5.34

3.2.6 Biological Variation

When investigating the volume estimates of any biological structure, the variance that is created due to individual biological variability should also be considered. The collection of volume estimates from several participants, when combined together result in a distribution being formed. The spread of the distribution is called the variance. The coefficient of variance (CV) is measured by the square root of the variance, divided by the mean. If the volumes are estimated, the CV will contain contribution from two sources, firstly, the natural or biological variance and secondly, the methodological error due to the imprecision of the volume estimation technique (CE). If the individual predicted CE's on the estimates are known, then the contribution the biological variance makes to the overall variance can be calculated using Equation 3.12 (Gundersen & Osterby, 1981)

$$CV_B^2 = CV_T^2 - CE^2 \qquad \textbf{Equation 3.12}$$

where CE is the predicted mean coefficient of error (based on the mean coefficient of error of the measures volumes), CV_B is the coefficient of variation due to biological variability and CV_T is the overall coefficient of variation based on the sample mean.

3.2.7 Intra-Rater Reliability

Robust methodologies need to be both repeatable and reliable ways of volume estimation. Intra-rater reliability is when the variability between repeat methods by the same observer is small. Reproducibility can be assessed when other observers demonstrate small variability within volume estimates, or when the repetition of the method results in small variability.

For the current study intra-rater reliability was assessed by SK, who measured all structures of interest for 10 randomly chosen participants. Measurements were taken several weeks apart and were applied using a fixed procedure, whereby both the starting slice and the test array remained constant.

Volume estimation of the insular cortex has not been previously demonstrated using stereology in conjunction with point counting. Therefore, a measure of reproducibility needed to be assessed. Reproducibility measures were gained by measuring the structures of interest of 10 participants, using a random procedure. For the random procedure, starting slice and the orientation of the test array are not controlled for. These measurements were taken several weeks apart by SK.

To examine reliability and reproducibility, two statistical measures are used. Firstly, Pearson's product moment correlation coefficient (r) measures the relatedness of two sets of variables. Secondly, an intra-class correlation coefficient (ICC) (Bartko, 1966) is a measure of relatedness between measurements obtained at independent occasions and closeness of the scores to each other and is shown in Equation 3.13:

$$\frac{(2\sum xy)-((\sum x+\sum y)_2/2n)}{(\sum x_2+\sum y_2)-((\sum x+\sum y)_2/2n)} \quad \textbf{Equation 3.13}$$

3.3 Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging (fMRI) is a non-invasive method of examining the localisation and measurement of brain activity. The most commonly used contrast in fMRI is blood oxygenation level-dependent (BOLD) signal. BOLD was first described by Ogawa in 1990, who used strong magnetic fields (7T & 8.4T) to collect very high resolution images of rat brains (Ogawa, Lee, Kay, & Tank, 1990). Images acquired with a gradient-echo pulse sequence were found to demonstrate an artefact, dark lines of varying thickness. Ogawa et al, (1990) discovered this to be signal dropout from blood vessels thus demonstrating a contrast mechanism reflecting blood oxygen levels. Shortly after, the effect was demonstrated in the cat brain during anoxia (Logothetis, 2003; Turner, Le Bihan, Moonen, Despres, & Frank, 1991).

The BOLD contrast is based on the assumption that neuronal activity and haemodynamics (the local control of blood flow and oxygenation) are linked. Signals arise from changes in local haemodynamics which result from alterations in neuronal activity, but the exact nature of this relationship remains unclear. Empirical studies have been conducted to investigate what type of neuronal activity is coupled with the BOLD response. The majority of the evidence has provided support for the association between synaptic processing and the BOLD response.

3.3.1 Neural Origins of BOLD

In 2001 Logothetis and colleagues combined intracortical extracellular recording techniques and BOLD fMRI measurements (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). The insertion of an electrode intracortically in the extracellular space results in a mean extracellular field potential (mEFP) that can be measured. By using highpass (~300 – 400HZ) and lowpass (~300HZ) filters the mEFP can be separated into a low frequency component called a local field potential (LFP) that reflects synaptic activity and a high frequency component called a multiple-unit spiking activity (MUA) that reflects regional neuronal spiking. Logothetis et al, (2001) found LFP's to be more accurate at predicting BOLD responses than MUA's. These findings suggest that the BOLD response directly reflects a local increase in neuronal activity assessed by mEFP signal.

Functional magnetic resonance imaging and electrophysiological recordings suggest that the BOLD contrast mechanism reflects the neuronal responses elicited by a stimulus. BOLD responses and neural responses are shown to have a linear

relationship for stimulus presentation of short duration (Jezzard et al, 2001). The haemodynamic response appears to be correlated with local field potentials, implying activation in an area is most likely to reflect the incoming input and local processing rather than spiking activity.

The underlying characteristic of the fMRI BOLD contrast lies in the inhomogeneity of the magnetic field that is triggered by metabolic demands of increased neuronal activity, which changes the levels of oxygen within the blood. BOLD employs haemoglobin as a convenient endogenous contrast agent relying on the magnetisation difference between oxy-deoxyhaemoglobin to create the fMRI signal.

The model of haemodynamic response posits that an increase in neuronal activity results in an initial increase in oxygen consumption due to increasing metabolic demands. This in turn changes the concentration of oxy-deoxyhaemoglobin in the nearby vasculature increasing the concentration of deoxyhaemoglobin (Heeger & Ress, 2002). Deoxy and oxyhaemoglobin have different magnetic properties. Deoxyhaemoglobin is paramagnetic and therefore introduces an inhomogeneity into the local magnetic field. Oxyhaemoglobin in contrast, is weakly diamagnetic and produces little effect upon the magnetic field. An increase in deoxyhaemoglobin results in a greater field inhomogeneity and a decrease in image intensity, whereas a decrease in deoxyhaemoglobin results in an increase in image intensity.

3.3.2 Haemodynamic Response Function (HRF)

The BOLD response to an increase in neuronal activity measured as an fMRI signal is called the haemodynamic response function (HRF). Two parameters characterise the fMRI signal, the amplitude of the signal intensity change and the time course of this change. Although BOLD has a spatial resolution of 2-5mm, its temporal resolution is only in the magnitude of 5-8s. This is due to the HRF being remarkably slow compared to the underlying changes in neuronal activity (Heeger & Ress, 2002). The HRF undergoes three phases to complete its response to transient neuronal activity.

Firstly, there is an initial small decrease in image intensity below baseline caused by the initial period of oxygen consumption. However this initial dip is inconsistently found and as such remains somewhat controversial. Secondly, approximately 2 seconds after the increased neuronal activity a large increase in

cerebral blood flow occurs. This is over compensatory for the amount of oxygen being extracted which results in an oversupply of oxygenated blood. The result of the oversupply of oxygen is a large decrease in the relative level of deoxyhaemoglobin which in turn increases the BOLD fMRI signal. The oversupply of oxygen is the reason there is a BOLD signal, but the reason for this oversupply of blood is not known. After approximately 6 seconds the BOLD signal has reached its maximum and the signal begins to decline.

Finally, after the oversupply of oxygenated blood has diminished the level of deoxyhaemoglobin slowly returns to normal. The resulting BOLD fMRI signal decreases until it reaches its original baseline level, although a slight 'undershoot' is detected just prior to its full return. The whole haemodynamic response to an initial neuronal activity takes approximately 20 seconds.

3.3.3 Echo Planar Imaging (EPI)

FMRI became robust and practical with the availability of fast gradients integrated with imaging techniques that allow several brain images to be collected over the course of the haemodynamic response (Jezzard et al, 2001). Echo planar imaging (EPI) was conceived by Mansfield in 1977 and is the fastest and most commonly used scanning technique for fMRI. Whole brain images can be collected in 5 seconds or less as the EPI method can collect data after a single radiofrequency excitation pulse or a single "shot" (Jezzard et al, 2001). EPI sequences are characterised by a series of gradient reversals in the readout direction. Each reversal creates a gradient echo, with the second half of one readout sample being rephased by the first half of the subsequent readout period. The reversals are rapidly performed, allowing images to be acquired in 100-200ms. The imaging speed is gained by the use of very high amplitude field gradients, which allow rapid sampling. The trade-off however, is a lower signal to noise ratio than more conventional methods which build up data for an image from a series of signal samples. When using EPI, the signal is decaying with the time constant T2* during *k*-space acquisition, thus limiting the number of lines of *k*-space which can be acquired. For example, a resolution of 64X64 pixel images tends to be gained using single-shot EPI pulse sequence, although 128X128 are possible. Other multi-shot gradient echo sequences have much greater image resolution allowing for as much as 512x512 resolution, but require much longer data acquisition times (Jezzard et al, 2001).

3.3.4 Experimental Design in fMRI

As previously discussed, the fMRI technique allows whole brain images to be collected in a very short period of time, typically < 2seconds. The most commonly used contrast mechanism for fMRI is the blood oxygen-level dependent (BOLD) contrast, which relies upon the haemodynamic response function (HRF). Neural activity can occur in milliseconds, but the haemodynamic response can take between 1-2 seconds to begin, a further 6-8seconds to reach its maximum peak, with an overall time lag of approximately 20 seconds to return to baseline. Therefore the temporal resolution of the fMRI signal is limited by these underlying physiological processes. A further limitation of BOLD imaging is that the signal changes experienced are quite small. A 1.5T machine produces a signal change in the order of 1-5%. A signal change of only 2-10% can be expected when using a 3T machine (Amaro & Barker, 2006). This relationship between physiology and fMRI data collection must inform effective paradigm design (Miezin, Maccotta, Ollinger, Petersen, & Buckner, 2000).

Functional magnetic resonance imaging (fMRI) can incorporate several different design methods. Two methods are more consistently utilised, specifically being 'block design' and 'event-related design'. Both are created based upon the assumptions of the haemodynamic response function. The choice of design will depend upon the most effective design for the nature of the experimental task.

The fMRI experimental studies contained within Chapter Five and Chapter Six used different designs, the basis of which will be presented below.

Block Design

Due to the historical influence of positron emission tomography (PET) studies, the first fMRI studies used block designs, also known as a boxcar design. Essentially, a block design is when events of one type are presented sequentially for a specified period of time. A typical block will last between 15 seconds to a full minute. All blocks are interspersed with durations of 'rest' to allow for the haemodynamic response to return to a 'baseline'. After the rest period, another block containing stimuli associated with another condition will be shown, with multiple task blocks being presented within an fMRI run. The signal acquired during one block is then compared to that of another block.

Block designs are believed to be the most efficient design type (Friston, Zarahn, Josephs, Henson, & Dale, 1999). This assumption relates to the nature of the underlying HRF. Block designs assume that the haemodynamic response is linear in

nature. As such by presenting events of one type within a block ensures the linearity of HRF is maintained and optimised. The ability to detect activation is greater as responses to events of the same type are summed resulting in a greater signal to noise response (Jezzard et al, 2001).

However, block designs are not suitable for all fMRI studies. The presentation of similar events within a block does pose some experimental disadvantages. Participants may develop strategies when being tested, or their attentional levels may drop as they can predict what will be presented next. Studies examining unexpected events, such as oddball paradigms, cannot use block design methods as they are counterintuitive to the phenomena they are investigating.

Event-related Design

An alternative to block designs are event-related designs. In an event-related design signal changes in relation to the onsets of individual trial events are analysed, as opposed to a specific period of time. Studies using event-related designs have the advantage of displaying stimuli in a more naturalistic method, often employing similar presentation techniques that are utilised outside of the scanning environment. This results in the ability to eliminate potential confounds such as habituation, anticipation, set or other strategy effects (Dale, 1999), as well as decreasing the opportunity the participant has for developing alternative strategies or incidental processing (Friston et al, 1999). The strength of the approach is that transient signal changes can be detected and trials can be randomly intermixed for comparison or can be analysed on a post-hoc manner, such as only analysing correct responses.

The original event-related designs used presentation timings similar to previously used block designs. An event was presented and then a sufficient inter-stimulus interval (ISI) was introduced which would allow for the HRF to return to baseline. This meant that an ISI of approximately 10seconds occurred between subsequent stimulus presentations, thus increasing the length of the scan run. However, subsequent developments of rapid event-related designs have overcome this problem.

In recent years technological developments have allowed for event-related designs to be modified. The previously utilised stimulus presentation methods have been replaced by adopting an ISI of shorter duration than the HRF generated by the previous stimuli. Therefore, subsequent events can have an ISI of < 4 seconds. Although the nature of the HRF would appear to not permit this type of design to be

effective, technological advancements allowing for faster data acquisition mean even short stimuli can give rise to measurable changes. In fact a stimulus as short as 34ms has been demonstrated to produce a measurable BOLD response (Donaldson & Buckner, 2001).

The discovery that the shape of the BOLD haemodynamic response is predictable and relatively stable across events has increased the availability of rapid event-related fMRI. The finding that the haemodynamic response summates in a roughly linear fashion means that the HRF for an individual stimulus can be estimated despite any overlap if the initial response to a previous event has not decayed. Therefore estimates of responses to rapidly presented trial events should be similar to, but not identical to estimates obtained when events are widely spaced apart (Miezin et al, 2000).

Distributed Sampling

In fMRI whole brain images are constructed by combining data from slices of the brain that have been collected sequentially, rather than simultaneously. If the presentation of the stimulus always occurs at the same timepoint during the data collection, this results in a slice/timepoint interaction that will impact the resulting data. With an average TR of 3seconds, if stimulus presentation is an integer multiple of the stimulus onset asynchronicity (SOA) and is coordinated with the start of data collection, the brain regions last collected in the image volume, may have as much as a 3 second delay. This effect is most pronounced within event-related designs, which do not have the underlying linear assumptions relating to the BOLD response in the same way block designs do. However, a way to overcome this issue is through using a distributed sampling technique.

Distributed sampling or 'jittering' has been used in a number of studies including auditory perception of the temporal lobes (Specht & Reul, 2003), the neural correlates of processing affective words (Lewis, Critchley, Rotshtein, & Dolan, 2007) and selective movement prevention (Coxon, Stinear, & Byblow, 2008). Jittering can be achieved in one of two ways. Firstly, by fixing the SOA but ensuring that the TR is not an integer multiple of the SOA (Veltman, Mechelli, Friston, & Price, 2002). This overcomes the problem of always collecting the same timepoint in relation to stimulus presentation. A further method often employed when the SOA remains fixed lies in the assumptions of stochastic designs. Stochastic designs are designs where there is a probability of an event occurring at a series of timepoints.

The inclusion of trial free periods during which baseline levels can be attained increases the design efficacy. In the context of stochastic rapid presentation designs this is the equivalent of null events and this provides a more powerful approach (Friston et al, 1999).

Secondly, the presentation of the stimuli can be jittered through a variable ISI. With rapid event-related fMRI there is considerable overlap between successive haemodynamic responses to closely spaced trials. This means that the ability to estimate the BOLD response is highly dependent upon the space between the trials. Fixed spacing between successive trials means that there is not enough information in the time-course data, that there are more unknowns than equations and as such the model is unsolvable. However, the introduction of 'jitter' increases the variance within the data set, providing more information from which to derive estimates from the BOLD response (Jezzard et al, 2001).

Miezin et al, (2000) examined the ISI of trials and the signal percentage change in the visual cortex. The ISI range examined ranged from 5s to 20s. Although there was a modest reduction in amplitude for the trials with the smallest ISI (17% when compared to the slowest rate), the increase in the number of events far outweighed any other advantage. When examining the subjects Z scores obtained for the runs, the fastest rate had the highest score demonstrating a clear power advantage. Response summation is sufficiently linear to use rapid presentation paradigms. Timing of the haemodynamic response remained largely stable across presentation rates in terms of time to onset and time to peak. Therefore, the efficiency of variable ISI designs increases with decreasing mean ISI (Dale, 1999).

It may appear that the use of distributed sampling is only of benefit to rapid event-related fMRI. This method has also been investigated within block designs. Most block designs are analysed with a boxcar regression usually convolved with a haemodynamic response function. The implicit assumption is that steady-state dynamics are attained within each block. However, in fMRI the BOLD response may be short lived and as such the assumption may not be valid. The estimated activation will depend critically on when in the ISI the responses are sampled. If responses are sampled at the same time point, bias may be introduced. Sampling of peaks will lead to an overestimation of steady-state activation, whereas sampling the troughs will lead to an underestimation and a potential loss of sensitivity for small and transient signals. Any biases due to selective (fixed) sampling during the ISI will only be expressed when the underlying responses are transient. In two studies examining

distributed sampling using a jittered TR with a static SOA, Price, Veltman, Ashburner, Josephs, & Friston, (1999) and Veltman et al, (2002), both demonstrated a reduction in sensitivity to responses in language areas. The assumptions were that these activations tended to me more transient, and when analysed within a time locked design, bias was introduced. These results demonstrate that phasic responses occur even in blocked designs (Price et al, 1999).

Distributed sampling techniques were used in both fMRI studies presented within this thesis. See individual study Chapters Five and Six for a discussion of the techniques used.

3.3.5 MRI Scanning Procedure

Scanning sessions for studies contained with Chapters Four and Five were conducted at the Walton Centre for Neurology and Neurosurgery (WCNN). The study contained with Chapter Six was conducted at the University of Liverpool's Magnetic Resonance Imaging and Analysis Centre (MARIARC). All sessions were run by trained radiographers. Participants were required to wear gowns during scanning. They were placed in a supine position head first into the scanner. Earplugs were given and once the receiver coil was placed over their heads, additional head restraints were used to ensure as little motion as possible. A panic buzzer was given for participants to indicate if they were experiencing any problems during the scanning session or wished to terminate their session.

The study presented in Chapter Four did not require participants to complete a task. As such participants were instructed to remain as still as possible during the scanning session. All other safety procedures remained as discussed previously.

The studies discussed in Chapters Five and Six utilised the dot probe task. Once participants were placed upon the scanner bed, task instructions were repeated and participants were given the opportunity to ask any questions for clarification. A mirror placed upon the receiver coil allows the participant to see out of the scanner and this was adjusted accordingly. Once placed within the scanner bore, it was ensured that they could see the screen adequately, were comfortable and that they understood the task requirements. The response box was placed in a comfortable position for the participant with their fingers being positioned over the individual response buttons. Once the researchers returned to the MRI control room, participants were required to press both the panic buzzer and the response buttons, to ensure that both were working adequately.

3.3.6 Data Pre-processing

The aim of fMRI analysis is to identify which areas of the brain demonstrate signal changes that accompany the experimental task. However, the signal changes within the fMRI dataset are relatively small in nature and the data is inherently noisy. Pre-processing is used to reduce the unwanted noise and precondition the data, to enable statistical analyses to be performed. All fMRI analyses described in this thesis were preprocessed and analysed using tools from FSL Version 4.1.

Brain Extraction Technique

The Brain Extraction Technique (Smith, 2002) removes all non-brain tissue from the high resolution structural image. This pre-processing occurs prior to the registration of fMRI dataset to the structural image. All fMRI analyses conducted within this thesis used BET.

Motion Correction

Motion correction is applied to the fMRI dataset to correct for any movement the participant made throughout the scanning session. Although participants were all restricted in movement by the use of head restraints, some motion may still occur. This results in a voxel's time series not referring to the same point in the brain throughout the scanning session. Motion correction was applied to the data using FMRIB'S linear image Registration Tool for motion correction (McFLIRT) (Jenkinson, Bannister, Brady, & Smith, 2002). This aligns all the volumes from a single EPI acquisition with a single volume. FLIRT uses a rigid body transformation which assumed that a single voxel in the brain may change position and size but not its shape.

Spatial Filtering

Spatial filtering, also known as spatial smoothing, is applied for two reasons. Firstly, smoothing can enhance the signal-to-noise ratio in the data, by reducing the noise level whilst retaining the underlying BOLD signal. To avoid reducing the signal along with the noise, the extent of the smoothing must not be larger than the size of the activated region. Secondly, later statistical processing requires the data to be smoothed. To meet the assumptions of Gaussian Random Field (GRF) theory all data must be smoothed (Friston, Worsley, Frackowiak, Mazziotta, & Evans, 1992; Worsley, Evans, Marrett, & Neelin, 1992). All experiments reported in this thesis used a smoothing kernel of 5mm at full width half maximum.

Temporal Filtering

Temporal filtering is applied to each voxel's time series to remove unwanted components, whilst retaining the signal of interest. Temporal filtering uses both 'high' and 'low' pass filtering. High-pass filtering removes any low frequency components of the noise in the data, such as cardio-respiratory drifts or non-physiological scanner drift. In block designs it is important to ensure that the high-pass filter cut off period is not too stringent otherwise the signal of interest may be lost. Event-related designs are more difficult to define a filter for, although it is less important for these designs as they are less likely to be affected by drift.

Low-pass filtering removes any high frequency components and is similar to Gaussian spatial filtering or 'smoothing'. However, FSL does not use a low-pass filter, instead the data is 'pre-whitened' within statistical analysis (Woolrich, Ripley, Brady, & Smith, 2001).

Statistical Analysis of the Data

Statistical analysis is performed upon the pre-processed data. It is used to quantify the degree to which the signal from different voxels is activated by the experimental task. Statistical analysis were conducted using FEAT (FSL's Expert Analysis Tool) which implements General Linear Modelling (GLM) in a univariate way. Thus, each voxel's time series is analysed independently. In GLM analysis a model derived from the timing of the experimental paradigm (explanatory variables) is compared to the time course of each voxel. If the model and data fit well together, it can be assumed that the demonstrated activation was related to the experimental paradigm.

For each explanatory variable this response is modelled using the GLM. The model is convolved with the HRF to produce the expected haemodynamic response of the voxel, essentially delaying and blurring the response. The GLM can be represented as Equation 3.14;

$$\gamma = \beta x + e \qquad \textbf{Equation 3.14}$$

where γ is the data, x is the model, e is the error in the data and β is the parameter estimate (PE) which can be thought of as a scaling factor. To convert a parameter estimate into a statistical parameter, the PE must be divided by the standard error of the PE. This results in a t statistic which can be transformed into a P (probability) or Z statistic using statistical transformations. Furthermore, PE's can be compared to

test whether one PE is better related to the data by subtracting one PE from another, and then divided by the standard error of this subtraction.

Statistical inference or thresholding is then applied to determine which parameters represent significant activation. The experiments contained within this thesis have had statistical inference applied using GRF theory to threshold the image, whereby statistical data is thresholded at a certain Z statistic level with cluster detection then being applied. Gaussian random theory considers spatial extent of clusters prior to estimating significance. Clusters of voxels are created using an initial thresholding level, with each cluster then having a P-value applied which may or may not reach significance.

Cluster based approaches have a greater sensitivity than voxel-based detection. The Bonferroni correction requires a correction based on all multiple comparisons, without a prior thresholding level being established. Secondly, it is more physiologically sound to use cluster based approaches, as activated regions extend over many voxels and do not act in a completely independent manner.

Multi-subject Statistics

The previously discussed analysis techniques are implemented on single subject analyses. Multi-session, multi-subject or group analysis can then be performed by ensuring that all brain images are aligned to a common space. Using FLIRT (FSL's Linear Image Registration Tool) an individual's low-resolution fMRI data is registered to their own high resolution structural scan using a 7 degrees-of-freedom (7dof) linear fit. The high resolution structural scan is then registered to the standard MNI152 brain template using a 12dof linear fit. The two transformations are then convolved to give a single transform taking the fMRI data into standard space (Jenkinson et al, 2002; Jenkinson & Smith, 2001).

The two methods for identifying significant group activation are fixed effects (FE) analysis and mixed (random) effects (ME) analysis. Fixed effects analysis assumes that the experimental effect is constant and focuses only on the within-session error. Results gained using FE analysis are sensitive to extreme results, such as outliers. A further disadvantage is that statistical inference is restricted only to the study population. Mixed effects analysis uses both the FE variance, gained from the within session errors, and random effects variance, which considers between session errors. Results gained using ME analysis can be applied to the whole population the study sample is drawn from. However, ME analyses do tend to give conservative

results. Furthermore, if the study population is small in number, but is very variable in nature, the effectiveness of ME analysis may be reduced.

3.4 Dot Probe Task

The dot probe task entails the presentation of a word pair, one word above the other, which after a predetermined stimulus presentation time is removed from the screen, with one of the words being replaced by a visual probe, often a small dot. The aim of the task is for participants to indicate, via a button press, the location of the dot probe. The paradigm is very flexible, and is open to considerable methodological manipulation.

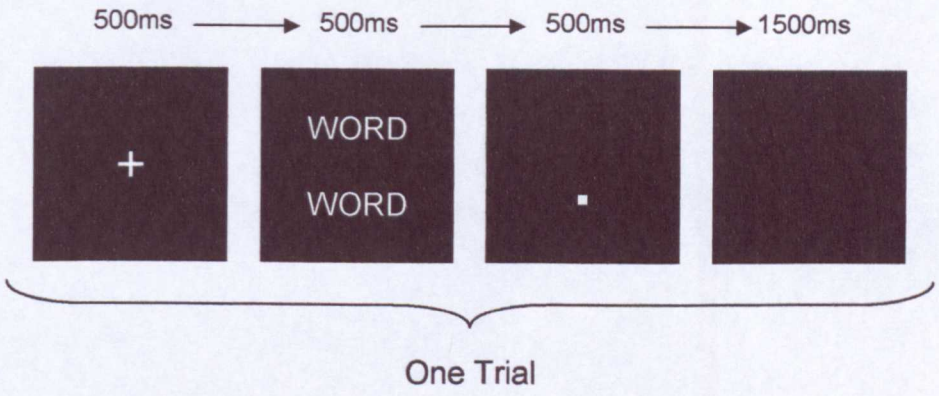
For the studies conducted for this thesis, several considerations relating to the scanning environment had to be included within the task. Firstly, filler trials were excluded from the paradigm. Some studies include probes only for a percentage of the trials. However, for the current studies all trials presented a probe to be detected. This was to ensure participants remained vigilant. Furthermore, the inclusion of probes for the non-semantic condition (Chapter Five) and for the neutral-neutral condition (Chapter Six) provided a behavioural baseline for use in fMRI analysis.

The second consideration for the scanning environment related to the visual probe presentation time. The dot probe task can be self-paced, with the probe remaining on the screen until detection. However, within the fMRI environment this may pose problems for the analysis as participants may recruit different cerebral processes over significantly different periods of response time. Furthermore, chronic pain patients may display longer response latencies, resulting in a disproportionate percentage of responses being recorded beyond the 1000ms response cut off allowed for the task. It was therefore decided that the probe itself would only remain on the screen for a brief period of time, 500ms resulting in participants having to remain attentive to the task.

The dot probe paradigms used within this thesis contained the same presentation and timing of trials. All visual presentations were white on a black background. Trials consisted of the following; a central fixation cross presented for 500ms, the word pair presented for 500ms, a 500ms presentation of the visual probe and 1500ms blank screen to allow for responses to be collected as illustrated in Figure 3.9.

Each trial has four possible combinations of pain-related word and dot probe location, for example pain-related word up + dot up; pain-related word up + dot down; pain-related word down + dot up; pain-related word down + dot down. In both dot probe studies contained within this thesis, each word pair was presented four times to ensure word and dot probe locations were counterbalanced.

Figure 3.9 Schematic for Dot Probe Trials



Prior to statistical analysis, reaction times below 300ms or those exceeding 1000ms are discarded. Incorrect responses and responses greater than three standard deviations from an individual's mean were also removed. Response latencies were analysed to establish if there was any statistical difference in the responses between groups (responses depending on group allocation) or within groups (responses depending on wordtype). To investigate the nature of this interaction the data was analysed using a bias index. A positive score on the index indicates vigilance, a selective attention bias towards the location of the pain-related word. A negative score on the index indicates avoidance, an orientation away from the location of the pain-related word.

A bias index is calculated using the following Equation 3.15;

$$\text{Bias Index} = [(eudl - eldl) + (eldu - eudu)]/2 \qquad \textbf{Equation 3.15}$$

where e = emotional word, d = dot, u = upper location, l = lower location. Thus the term eudl for a pain/neutral word pair would indicate a trial where the pain word appeared in the upper location of the screen, with the dot appearing in the lower location of the screen.

In addition to the total bias index the inclusion of a neutral-neutral condition in the empirical study contained within Chapter 6, allows for the calculation of congruency and incongruency effects. Congruency effects are calculated for trials when the dot replaces a pain-related word, with incongruency effects being calculated for trials when the dot replaces the neutral word.

It should be noted that a higher value on the bias index indicates a speeded response. However, for congruency effects, the neutral condition is used as a baseline, therefore a higher value on the indexes indicates slowed responding to that wordtype.

Congruency and incongruency effects are calculated using Equations 3.16 and 3.17 respectively;

Congruency Index= [(pudu+pddd)/2]-Neutral **Equation 3.16**

Incongruency Index = [(pudl+pldu)/2]-Neutral **Equation 3.17**

The neutral trials are calculated according Equation 3.18:

Neutral Trials = [(nudu+nudl+nldl+nldu)/4] **Equation 3.18**

Additional statistical tests were then applied to the mean reaction time data using SPSS v.16. At minimum a significant interaction between group, target position and probe position is needed to demonstrate an attentional bias to specific stimuli.

3.4.1 Response Methods

All response latencies recorded during the scanning sessions were done so by a MRI compatible response button box. The button box is placed under the participant's right hand. Buttons are positioned in an arc manner allowing for the natural placement under the participant's fingers. Responses were collected using the index and middle fingers and responses were counterbalanced across participants. The use of a single response box allows for the panic buzzer to be accessible to the participant in case they need to alert the researcher to a problem during the scanning session.

3.4.2 Task Instructions

In both fMRI studies contained in Chapters 5 & 6, prior to scanning participants were presented with an information sheet detailing the instructions for the study. They were informed of the nature of the task, and what responses were required of them. Instructions were reinforced once the participant was comfortably placed within the scanner. They were explicitly informed that they would "*see two words appear on the screen, one above the other. One word will be replaced by a small dot. Using the button box in your hand, you must indicate if the dot appears over the word presented at the top or the bottom of the screen. You must answer as quickly as possible, but you must not sacrifice speed for accuracy. A small cross will appear in the middle of the screen for you to focus upon for the next trial.*"

3.4.3 Word Database

An online database was used to assess the suitability of the word stimuli for the study contained in Chapter Six and as a method of post hoc assessment for the word stimuli found in Chapter Five. The English lexicon Project (ELP) provides a standardised behavioural and descriptive dataset for 40,481 words and 40,481 nonwords and online access to the current findings (http://elexicon.wustl.edu). At the time of use the database contained data from 816 participants on a lexical decision task, and data from 444 participants from a speeded naming task. The lexical decision task involved participants being presented with a string of letters, either a word or a nonword. The participant is required to indicate as quickly as possible, via a button press, what the letter string is. The speeded naming task requires participants to name as quickly and accurately as possible, a word which is presented to them visually. The ELP database provides both reaction time and accuracy data relating to both tasks.

The ELP database was accessed to gain frequency and behavioural data for potential word stimuli. For the experimental study in Chapter Six, the ELP was used to help create suitable wordlists. However, the study contained within Chapter Five used the ELP for post hoc analyses of the experimental wordlists. These processes are discussed further in the individual study chapters.

3.5 Pain Behaviour Assessment

Chronic pain patients who participated within experimental studies contained within this thesis were all assessed regarding their levels of pain behaviour (PB). The assessment measure used were the Waddell Signs (WS), a series of clinically validated signs used to assess excessive or inadequate responses to pain, specifically in chronic low back pain patients (CLBP).

To overcome some of the concerns relating to WS (See Chapter 1.6) the CLBP patients recruited for the studies presented within this thesis had to have the most extreme scores; 0/1 for non-PB and 4/5 for PB. This method of recruitment had been used successfully in a previous fMRI study and had produced different results between the patients groups, as well as strong relationships to the self-report measures used (Lloyd et al, 2008).

The WS can be separated into five measures consisting of the following:

(i) Tenderness: superficial skin tender to light touch or non-anatomic deep tenderness not localised to one area,

(ii) Simulation: axial loading pressure on the skull of a standing patient induces low back pain or rotation: shoulders and pelvis rotated in the same plane induces pain,

(iii) Distraction: difference in straight leg raising in sitting and supine positions,

(iv) Regional weakness: many muscles groups, "give-away weakness" (patient does not give full effort on minor muscle testing) or sensory loss in a stocking or glove distribution, i.e. Non-dermatomal,

(v) Overreaction: disproportionate facial or verbal expression (i.e. pain behaviour).

Patient assessment of the WS was performed by one of three pain specialists (GF/TJN/AS). Study inclusion or exclusion criteria based on WS scores are discussed in Chapters Four and Six.

3.6 Self-Report Measures

3.6.1 Beck Depression Inventory II

The BDI-II (Beck, Steer, & Brown, 1996) is a 21 item self-report measure which assesses levels of depression. Each item contains four statements relating to a symptom of depression. Items are arranged to increase in severity. Respondents are required to consider the item in relation to how they have felt for the preceding two weeks. Scores of 13 or below are considered a normal range, 14-19 indicates mild depression, 20-28 indicates a moderate level of depression and finally severe depression is indicated by a score of 29 and greater.

The BDI-II is considered a reliable and valid measure of depression for both clinical and non-clinical samples, including chronic pain patients (Poole, Bramwell, & Murphy, 2006).

3.6.2 Edinburgh Handedness Inventory

The Edinburgh Handedness Inventory (Oldfield, 1971) is a ten item assessment measure to investigate the handedness of the respondent. Responses are recorded by indicating the dominant hand used for a variety of tasks, such as writing and striking a match. Five responses can be given indicating the preferred hand and the strength on this preference (usually right, always right). Each response is given a score of one, all scores for each hand are added (Total), left scores are subtracted from the right scores, with the result being divided by the Total and multiplied by 100. Scores of below -40 indicate left handed dominance, between -40 - +40 the respondent is ambidextrous and scores greater than +40 indicate right hand dominance.

The Edinburgh Handedness Inventory is a popular and widely used instrument for assessing hand dominance. It was administered in all three experimental studies.

3.6.3 Fear of Pain Questionnaire (FPQ)

The fear of pain questionnaire (McNeil & Rainwater, 1998) is a 30 item self-report measure used to assess fear related pain. Responses are indicated on a numerical scale of 1-5, 1 being anchored with 'not at all' and 5 representing 'extreme'. Scores range from 30-150 with high scores on the FPQ indicating a high level of fear of pain. However, there are no numerical indicators of representing cut off levels.

The FPQ is designed to be administered quickly and includes an assessment of different types of pain and painful situations, thus allowing for administration within both clinical and non-pain populations. The FPQ has been shown to have good internal reliability and validity when administered to pain-free populations (Osman, Breitenstein, Barrios, Gutierrez, & Kopper, 2002; Roelofs, Peters, Deutz, Spijker, & Vlaeyen, 2005). The questionnaire provides both an overall global fear of pain score, as well as scores relating to three separate subscales; severe pain, minor pain and medical pain. For the experimental study discussed in Chapter Five, only the global fear of pain score was used for screening purposes, although examination as to whether scores were different across the subscales was also performed.

3.6.4 Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) was developed to identify anxiety and depression in patients from nonpsychiatric clinics (Zigmond & Snaith, 1983). The HADS is a 14 item self-report measure, with each item consisting of a four-point Likert scale ranging from zero to three. The HADS contains two subscales, an anxiety subscale (HADS-A) and a depression subscale (HAD-D) each containing 7 items. For each subscale a score can range from 0-21. Scores below 8 are regarded as normal; scores of 8-10 are regarded as borderline, and scores greater than 11 on either of the two subscales are considered indicative of possible psychological distress.

The HADS has been used extensively, with a systematic review identifying 747 published articles using the scale. The scale has good internal reliability and consistency when being applied to both clinical and healthy populations (Bjelland, Dahl, Haug, & Neckelmann, 2002).

3.6.5 Pain Catastrophising Scale (PCS)

The Pain Catastrophising Scale (Sullivan, Bishop, & Pivik, 1995) was developed to assess other elements of catastrophising which had not been addressed in previous measures. The PCS is a 13 item measure consisting of 5 point Likert scale ranging from 'not at all' to 'all the time'. Respondents rate the extent they experience each item by recalling a previous pain experience. Scores range from 0-52, mean score 28 with a standard deviation of 13. Research suggests that patients obtaining a score above 38 (the 80^{th} percentile) are particularly prone to adjustment difficulties and poor progression through pain rehabilitation (Quartana et al, 2009).

As well as a global score, factor analysis indicates that the PCS yields three

second order factors, rumination, helplessness and magnification. A number of studies have replicated this factor structure using confirmatory factor analytic methods in a variety of populations, differing in ages, healthy status and cultural differences (Meyer, Sprott, & Mannion, 2008; Osman, Barrios, Gutierrez, Kopper, Merrifield, & Grittmann, 2000; Tremblay, Beaulieu, Bernier, Crombez, Laliberte, Thibault & et al, 2008).

3.6.6 Pain Anxiety Symptoms Scale (PASS)

The PASS contains forty items which assess behaviours relating to fear of pain (McCracken, Zayfert, & Gross, 1992). Items are scored on a five point Likert scale ranging from 0 (never) to 5 (always). The PASS has four subscales as well as a Total score, which ranges from 0-200. A high level of anxiety is indicated by a high score.

The measure tends to be used exclusively within chronic pain populations and has demonstrated good internal consistency in a range of .7 to .9. Test-Rest correlations have also demonstrated correlations of .9. Furthermore, the PASS has been found to be consistent with physical assessments (Burns, Mullen, Higdon, Wei, & Lansky, 2000).

3.6.7 Tampa Scale of Kinesiophobia (TSK)

The short version of the TSK is an 11 item self-report measure designed to assess fear of movement (Woby, Roach, Urmston, & Watson, 2005). Responders are asked to state how much they agree or disagree with the given statements. Items are scores on a four point Likert scale, with a range of score being 11-44. Higher scores are indicative of greater fear levels.

The TSK-11 has demonstrated good internal consistency of .7 and reliability of .8 It has been claimed that a reduction of four points is suggestive of a significant reduction in fear levels (Woby et al, 2005).

3.6.8 Spielberger State-Trait Anxiety Inventory (STAI)

The STAI is a 40 item self-report measure of state and trait anxiety (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). The inventory contains two subscales, each comprising a twenty item checklist. Items are scored from 1-4 with greater scores indicating an increase in severity. To avoid response bias, some questions are related to the absence of anxiety and as such are scored in a reverse manner. The range of scores is from 20-80 per scale, with an overall score of 160.

Barnes, Harp & Jung (2002) explored the reliability of the STAI in 816 research publications between 1990 and 2000. Internal consistency of .91 was found for the state scale, with the trait scale demonstrating a .89 reliability coefficient.

3.6.9 Visual Analogue Scale (VAS)

A visual analogue scale is a horizontal line of a fixed length, with endpoint anchors. The VAS is used in a variety of clinical and healthy populations and using different types of medium, such as paper or electronic methods. The VAS examines a linear relationship to a defined variable, for the presented studies, this was pain levels (Price, McGrath, Rafii, & Buckingham, 1983). The respondent indicates upon the line what their pain level is, with a numerical value being gained by the distance of the response from the lowest anchor.

The VAS itself was 10cm long, with the endpoint anchors of 'no pain' to 'worst pain imaginable'. The type of anchors used has been found to have little impact upon the responses given (Hofmans & Theuns, 2008).

CHAPTER FOUR
Reduced Prefrontal and Insular Cortices in Chronic Low Back Pain Patients

4.1 Introduction

There is a growing body of evidence for both structural and functional differences in the brains of people suffering from a variety of chronic pain conditions when compared to pain-free controls (For reviews see Apkarian et al, 2005; May, 2008; Wood, 2010). Localised differences in brain structure between chronic pain patients and controls have been consistently reported, with the majority of findings indicating deficits in gray matter (GM) for the patient population. These structural differences have been reported for patients with fibromyalgia (FM), irritable bowel syndrome (IBS), osteoarthritis (OA) and chronic low back pain (CLBP; For a detailed discussion regarding structural differences for specific pain conditions please refer to Chapter 2.4). Specifically, CLBP patients have been shown to have reduced gray matter in the dorsolateral prefrontal cortex (DLPFC), primary somatosensory cortex (SI), thalamus, brainstem, posterior parietal cortex, anterior cingulate cortex (ACC) and insular cortex (Apkarian et al, 2004a; Buckalew et al, 2008; Schmidt-Wilke et al, 2006; Seminowicz et al, 2010). These areas of GM reduction are consistent with findings from other patient populations, with specific overlaps being reported for DLPFC, ACC and insular cortex. Behavioural deficits in affective (Apkarian, Sosa, Krauss, Thomas, Frerickson, Levy & et al, 2004b; Verdejo-Garcia, Lopez-Torrecillas, Calandre, Delgado-Rodriguez, & Bechara, 2009) and cognitive functioning, most notably attentional and memory impairments demonstrated by CLBP sufferers indicate that the GM changes in these regions may be functionally relevant (Grisart & Plaghki, 1999; Ling, Campbell, Heffernan & Greenough, 2007; Weiner, Rudy, Morrow, Slaboda, & Lieber, 2006).

In contrast differences in global gray matter volume between chronic pain sufferers and controls have been inconsistently reported, with a small number of studies reporting GM deficits for patients (Apkarian et al, 2004a; Kuchinad et al, 2007), but the majority failing to replicate this finding (Buckalew et al, 2008; Burgmer et al, 2009; Hsu et al, 2009; Schmidt-Wilke et al, 2006; 2007). Similarly,

correlations between GM volume and pain duration, used as a method of investigating if GM deficits are a consequence of persistent pain have also produced inconsistent results (Apkarian et al, 2004a; Kuchinad et al, 2007; Schmidt-Wilke et al, 2006; 2007).

As the relationship between pain duration and GM deficits has been tenuous, studies have investigated to what extent these GM deficits may be related to other patient characteristics. Trait anxiety scores have been correlated with GM deficits in two FM studies (Hsu et al, 2009; Lutz et al, 2008). Similarly, Blankstein et al, (2010) reported a negative correlation between cortical thickness of the DLPFC and catastrophising levels in patients with IBS. In CLBP patients, a subgroup has been identified using the WS (For a discussion on WS please see Chapter 1.5), with positive scores believed to be indicative of excessive or inadequate responses to pain. These patients are said to display high levels of pain behaviour (PB). If there are morphological changes associated with PB, it argues against the idea that these patients simply report more pain, or are psychologically less able to cope with their pain. Rather, GM deficits may suggest a biological basis for PB.

In the present study, we attempted to replicate the published findings using a method of analysis previously unused in the chronic pain literature. We applied the Cavalieri method of modern-design stereology in conjunction with point counting to two regions. The prefrontal and insular cortices have both been identified in the literature as showing GM reduction in pain populations. Behavioural deficits in affective and cognitive functioning demonstrated by CLBP sufferers indicate that the changes in these regions may be functionally relevant. As we employed a region of interest analysis, whole brain GM volume was investigated using an automated method, previously used in the literature. The study sample was comprised of 26 CLBP patients and 14 healthy controls. We further extended our analysis to investigate if GM changes are concomitant with patients' pain behaviour status. Of the 26 CLBP patients included within the study, 11 had excessive pain behaviour, as measured by the WS. We hypothesised that relative to controls, CLBP patients would have a reduction in both global and regional GM volume. Additionally, reductions in GM volume would be greatest for the PB patient group.

4.2 Methods

4.2.1 Ethical Approval

The study was approved by the Sefton Research Ethics Committee. All participants gave written informed consent prior to study participation.

4.2.2 Participants

Inclusion and exclusion criteria

Inclusion criteria required that all patients had to be right-handed and suffer from chronic low back pain without sciatica. All patients had to have pain duration of 6 months or greater. Low back pain patients who had undergone any spinal surgery, had three or more degenerate discs, or any evidence of concomitant radicular pain, were excluded. Assessment of pain behaviour was conducted using the Waddell signs. To participate in the study, patients needed a WS score of either 0-1 to be placed in the non-pain behaviour (non-PB) group and a score of 4-5 for placement in the pain behaviour (PB) group.

Inclusion criteria for the controls required that they be right-handed and pain-free, with no history of a chronic pain condition.

Additional exclusion criteria for all participants required them to be free of major neurological or psychiatric disease, head trauma, current or previous drug or alcohol abuse, evidence of cognitive decline or MRI contraindications.

Recruitment

Chronic pain patients were recruited through the Walton Centre Pain Clinic. Participants were approached and assessed by either a Spinal Surgeon (G.F.) or a pain specialist (T.J.N.).

Healthy controls were recruited through an advert placed upon the University of Liverpool's intranet announcement system. The announcement specified the inclusion/exclusion criteria and gave an overview of the study.

To ensure there were no MRI contraindications all participants were given a health screening prior to scanning, performed at WCNN. No participants were excluded due to MRI contraindications.

Participant Demographics

The study sample consisted of fourteen healthy controls and twenty-six CLBP patients. The patient group were further sub-divided based upon their pain behaviour scores, resulting in 11 PB and 15 non-PB patients.

Summary information for age, gender and self-report measures can be found in Table 4.1.

4.2.3 Self-Report Measures

Clinical participants were required to complete several self-report measures. The VAS_{now} was used to examine the level of current pain, and VAS_{5day} recorded the average pain experienced in the 5 days prior to scanning. The Pain Catastrophising Scale (PCS), and the Hospital Anxiety and Depression Scales (HADS) were used to assess catastrophising, anxiety and depression levels respectively.

Healthy controls completed both VAS measures and the HADS to verify that they did not experience significant levels of pain, anxiety or depression. (For a full discussion about each self-report measure please refer to Chapter 3.6).

Table 4.1 Participant Demographics (Please See Overleaf)
PCS-pain catastrophising scale; **HADS**- hospital anxiety and depression scale (**A**) anxiety (**D**) depression; **VAS**- visual analogue scale. Standard deviations in parentheses. Pain Duration mnths- duration in months.

Table 4.1 Participant Demographics

Group & Study No.	Age	Sex	Pain Duration (mnths)	PCS	HADS-A	HADS-D	VAS NOW	VAS 5DAY
CLBP-PB								
1	36	M	168	-	13	8	5	5
2	53	M	96	26	11	10	5	5
3	48	F	216	-	16	10	8	7
4	53	M	72	-	9	8	8	0
5	57	F	31	26	10	11	4	5
6	46	F	76	35	11	14	6	8
7	36	F	24	43	12	8	3	0
8	29	F	38	8	3	4	7	8
9	56	M	252	26	10	12	7	7
10	45	M	60	47	21	15	3	5
11	41	F	72	-	-	-	-	-
Mean (S.D)	45.5 (9.1)	6F	100.4 (77)	30.1 (13)	11.6 (4.6)	10 (3.2)	5 (1.6)	5 (2.9)
CLBP								
1	45	F	85	-	12	9	3	6
2	61	F	18	36	11	5	7	5
3	67	M	-	0	6	9	1	1
4	55	M	-	14	7	8	4	7
5	44	M	-	12	7	4	6	4
6	45	M	360	2	7	5	4	5
7	46	F	-	28	10	7	5	7
8	67	M	240	18	12	4	7	7
9	49	F	60	-	9	14	2	3
10	38	M	54	18	3	9	7	7
11	58	M	180	16	9	6	3	5
12	36	M	66	16	5	2	1	2
13	37	F	6	13	7	4	7	7
14	32	F	-	34	12	4	3	8
15	21	F	49	-	-	-	-	-
Mean (S.D)	46.7 (13.1)	7F	111.8 (113.2)	17.2 (11)	8.4 (2.8)	6.4 (3.1)	4 (2.1)	5 (2.1)
Controls								
1	37	F			0	0	8	3
2	42	F			0	3	6	3
3	52	M			8	7	11	8
4	53	M			0	0	3	1
5	39	M			0	0	2	1
6	32	M			0	0	7	1
7	26	F			0	0	7	1
8	31	F			0	0	2	0
9	30	F			-	-	-	-
10	34	M			0	0	3	1
11	53	M			1	1	2	0
12	57	F			0	0	-	-
13	39	M			-	-	-	-
14	60	F			-	-	-	-
Mean (S.D)	41.8 (11.1)	7F			4.9 (2.8)	1.9 (2.1)	.8 (2.1)	1 (2.1)

4.2.4 MR Image Acquisition

MR imaging of the brain was performed at WCNN. All scanning sessions were run by trained radiographers. Scanning was performed using a 1.5 Tesla GE Signa LX/Nvi neuro-optimised MRI scanner (General Electric, Milwaukee, WI). A high-resolution T_1-weighted 3D inversion recovery prepared gradient echo (IRp-GRASS) sequence was acquired for each participant (TE = 5.4 ms, TR = 12.3 ms, TI = 450 ms, 1.6-mm slice thickness, FOV = 20 cm, 256 x 192 matrix), with 124 axial slices covering the whole brain.

4.2.5 Structural Analysis

Two methods of analysis were performed: an automated whole brain technique to provide a quantitative analysis for GM, WM and CSF and a manual technique for volume estimation of the prefrontal and insular cortices.

Automated Morphometric Technique

Images were normalised. Segmentation tools available within SPM2, (http://www.fil.ion.ucl.ac.uk/spm/software/spm2) partitioned images into GM, WM, CSF and 'other' classes using a modified mixture model cluster analysis technique. Using a priori knowledge about spatial distribution of tissue class, a map was produced which was an estimate of the belonging probability distribution for each tissue class. Output images were then transformed into their native space. Tissue volumes within each individual voxel were calculated on the probability matter in each voxel. Output values represent the amount of GM, WM and CSF contained within the data, when summed provides total intercranial volume (TIV= GM+WM+CSF).

Manual Technique

Preprocessing of the data was performed to ensure that all PFC subfields would transect similar anatomical landmarks for all participants. Images were reformatted and realigned. A parcellation technique was used to create 8 subfields for analysis.

The Cavalieri method was applied in conjunction with point counting for estimates of prefrontal cortex and insular cortex subfield volumes. The technique used for PFC volume estimation has been reported previously (Howard et al, 2003). Stereological estimation of the insular cortex was developed for this thesis. For a full and detailed explanation of the methods refer to Chapter 3.2.

4.3 Results

4.3.1 Participant Characteristics

Table 4.1 shows the participant characteristics for the three groups. A one way ANOVA confirmed that the three groups were matched in terms of age, ($F(2,36) < 1$).

For the two patient groups, independent t-tests were performed on patient duration and the various self-report measures. Differences were found to be unreliable for pain duration ($t(19) = 0.271$, $p=.790$) and for VAS_{5day} ($t(22) = 0.058$, $p=0.955$). Significant differences were found for VAS_{now} ($t(22) = -1.893$, $p=0.03$), pain catastrophising scale ($t(17) = -2.304$, $p=0.01$) and for both the anxiety ($t(22) = -2.122$, $p=0.01$) and the depression ($t(22) = -2.719$, $p=0.001$) subscales of the HADS. As shown in Table 4.1, in each case the PB patient group scored significantly higher on the measures than the non-PB group.

4.3.2 Whole Brain Analysis

Global Tissue Volume

The descriptive statistics for the global measures of gray matter (GM), white matter (WM), cerebrospinal fluid (CSF) and total intra-cranial volume (TIV) for all groups is shown in Table 4.2. Prior to analysis the Shapiro-Wilk Test of Normality was conducted to test if the distribution was normal ($p > 0.05$).

Whole brain analysis investigated if the three groups differed on volumes of GM, WM, CSF or a combined TIV. A second analysis was performed to investigate if any differences in brain volumes existed between all CLBP patients and healthy controls. No significant differences between any of the groups were seen for the whole brain volumes ($p > 0.2$ for each tissue type, one-way analysis of variance).

Age and Pain Duration

To investigate the effects of age and pain duration on TIV and GM, Pearson's Product Moment Correlation Coefficient was calculated. No significant relationships were found ($p > 0.05$).

Table 4.2 Descriptive Statistics for Global Brain Tissue Volumes

Tissue	Group	Min cm^3	Max cm^3	Mean cm^3	S.Dev
GM	Controls	620	860	761.2	61.3
	Patients			763.1	87.4
	Non-PB	600	950	779.9	101.6
	PB	650	880	741.8	61.5
WM	Controls	320	420	372.9	27.2
	Patients			382.6	40
	Non-PB	330	480	384.3	43
	PB	330	450	382	35.9
CSF	Controls	270	510	358.4	64.8
	Patients			361.5	45.7
	Non-PB	280	480	365.9	55.8
	PB	310	400	353.7	24.7
TIV	Controls	1230	1680	1492.5	113.5
	Patients			1503.3	136.5
	Non-PB	1210	1760	1530	158.9
	PB	1300	1620	1477.5	94.5

Patients group are patients combined irrespective of pain behaviour.

4.3.3 Stereology Results

Intra-rater Reliability Study

To ensure reliability of the results, 10 randomly chosen datasets were rated on two separate occasions for the PFC and insular cortex by SK. These occasions were approximately four weeks apart. Intra-class correlations demonstrated a good agreement in measures. Results are presented in Table 4.3.

Table 4.3 Intra-Class Correlations for Intra-Rater Reliability Study

Region	ICC
L DLPFC	0.99
R DLPFC	0.99
L DMPFC	0.98
R DMPFC	0.99
L OLPFC	0.93
R OLPFC	0.94
L OMPFC	0.97
R OMPFC	0.98
L Ant Insula	0.96
R Ant Insula	0.97
L Post Insula	0.98
R Post Insula	0.96

PFC Analyses

Group mean volumes are summarised in Table 4.4. All PFC volumes were normalised by TIV, resulting in a percentage of the total intra-cranial volume.

Table 4.4 Descriptive Statistics for PFC Subfields by Group

Region	Controls		Patients		Non-PB		PB	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
Dorsolateral L	1.70	.46	1.64	.30	1.59	.34	1.72	.24
R	1.83	.48	1.59	.34	1.59	.37	1.57	.32
Dorsomedial L	1.95	.37	1.79	.30	1.83	.34	1.73	.22
R	1.90	.37	1.68	.23	1.65	.27	1.74	.15
Orbitolateral L	.60	.29	.61	.18	.64	.20	.57	.16
R	.57	.27	.57	.19	.62	.19	.50	.18
Orbitomedial L	.86	.31	.81	.21	.84	.22	.77	.20
R	.78	.27	.80	.25	.83	.26	.77	.24

Main Effects and Interactions

A mixed factorial 3 x 2 x 2 x 2 ANOVA was performed with normalised volume as the dependent variable, group as the between-subjects factor, and hemisphere (left/right), subfields (dorsal/orbital) and (medial/lateral) as the three within-subject factors.

Overall main effects were found to be significant for the three within-subjects factors: hemisphere (left, 1.28; right, 1.20; $F(1,36) = 6.691$, $p=0.014$), dorsal/orbital subfields (dorsal, 1.74; orbital, 0.70; $F(1,36) = 250.589$, $p=0.001$) and medial/lateral subfields (medial, 1.31; lateral, 1.13; $F(1,36) = 22.369$, $p= 0.001$). There was no significant effect of group, indicating that normalised volumes for PFC regions were not reliably different between the three groups (control, 1.28; PB, 1.20; non-PB, 1.18; $F(2,36) = 1.925$ $p = 0.16$). These main effects indicate that when all participant groups were combined, grand mean volumes were greater for left hemisphere compared to right; for dorsal regions compared to orbital; and for medial regions compared to lateral.

A significant three-way interaction was found between group-by-hemisphere-by-dorsal/orbital subfield ($F(2,36) = 5.75$ $p < 0.01$). As can been seen in Table 4.5 there was consistency with respect to left/right volumes across groups for the orbital regions, whereby the left subfields were equal to or larger than right subfields. In the case of the dorsal subfields, this relationship was only evident for the patient groups, with controls displaying larger GM volumes for the right subfields.

As the medial/lateral subfields did not feature in any interaction, for all subsequent analyses these volumes were combined. To investigate the nature of this interaction further, simple interaction analyses were conducted separately for each group. The hemisphere-by-dorsal/orbital interaction was found to be significant for the control group ($F(1,13) = 7.012$, $p = 0.020$), but not for the two patient groups: PB, $F(1,14) = 3.038$ $p = 0.103$; non-PB, $F(1,9) = 1.596$ $p = 0.238$.

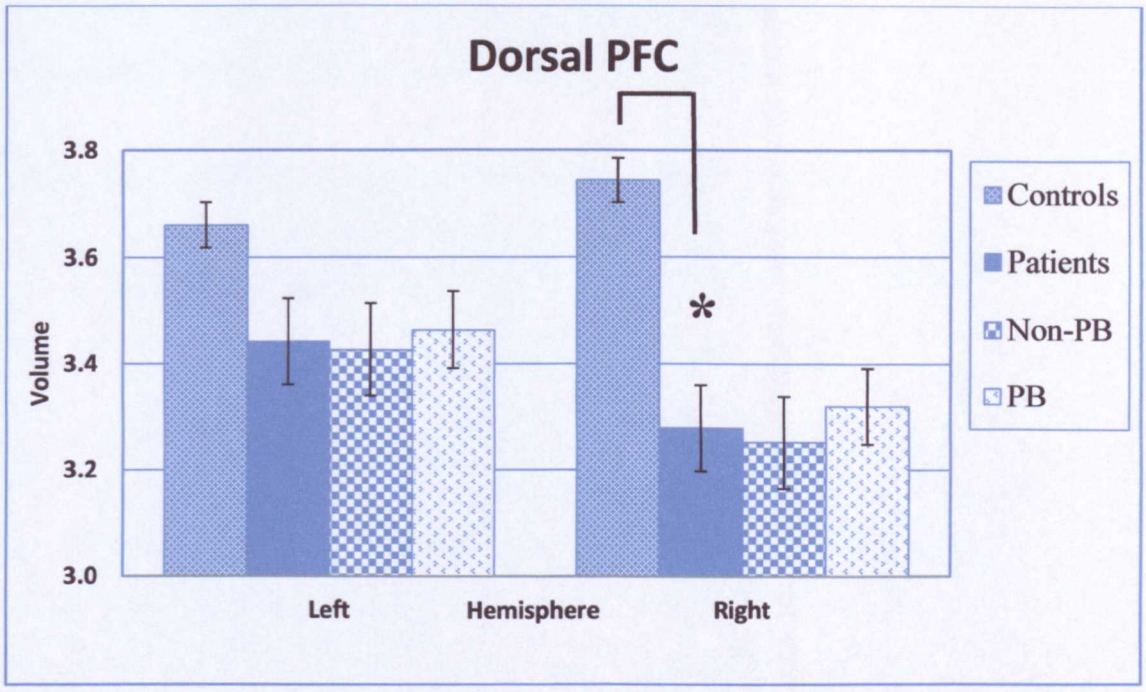
Table 4.5 Descriptive Statistics for Dorsal / Orbital Regions

Region	Controls		Patients		Non-PB		PB	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
Dorsal L	3.6	.58	3.4	.50	3.4	.60	3.4	.34
R	3.7	.66	3.2	.41	3.2	.49	3.3	.26
Orbital L	1.4	.58	1.4	.37	1.4	.39	1.3	.34
R	1.3	.53	1.3	.42	1.4	.43	1.2	.41

Closer inspection of the descriptive statistics indicates that for the patient groups, both dorsal and orbital regions are larger for the left hemisphere. In comparison, the control group demonstrates this hemispheric effect for the orbital region only, with the dorsal region indicating a larger right sided volume. This result suggests the clinical population are demonstrating a reduction in GM volume within the dorsal PFC region.

To investigate this, simple main effects analyses of sub-field across the three groups were conducted. One-way ANOVAs revealed a significant group effect for the normalised right dorsal volume, $F(2,38) = 3.62$ $p = 0.037$, but not for the other three volumes: left dorsal, left orbital, right orbital (all F-values < 1). Further, contrast analyses revealed that for the two patient groups, right dorsal volumes were not reliably different, $t(25) < 1$, but when combined, were significantly different from the control group, $t(36) = 2.613$ $p = 0.013$. Therefore, compared to controls, chronic low back pain patients have a significant reduction in GM volume of right dorsal PFC. This result is illustrated in Figure 4.1.

Figure 4.1 Mean (±SEM indicated by error bars) Dorsal PFC Volume By Group



$p<0.05$ indicates a significant difference in right dorsal PFC volume between controls and patients (Non-PB and PB groups combined).

Asymmetry Analyses

To investigate differences in hemispheric volume, we calculated asymmetry values for the combined dorsal and orbital regions, with the formula (r-l)/((r+l)/2). Group means and results are reported in Table 4.6. Typically, a right-sided effect results in a positive index, with negative values indicating the effect is left-sided.

A right greater than left asymmetry effect is evident only for the dorsal region of the control group. In contrast, left-sided asymmetry was demonstrated for the clinical groups for both orbital and dorsal regions, a pattern consistent with the earlier interaction. Hemispheric results are illustrated in Figure 4.2.

A one-way ANOVA revealed a significant main effect of group for dorsal asymmetry, $F(1,38) = 5.253$ $p = 0.028$, but not for orbital asymmetry ($F < 1$). Contrast analyses showed that asymmetry indexes did not differ between the patient groups, $t(22.630) = -0.226$ $p = 0.82$. When the patient groups were combined they were significantly different from the control group $t(31.700) = 2.533$ $p = 0.08$. As shown in Table 4.6 and illustrated in Figure 4.2, CLBP patients demonstrated a larger left-sided effect for dorsal PFC. In comparison the control group demonstrated a significantly different effect, with larger volumes for the right hemisphere.

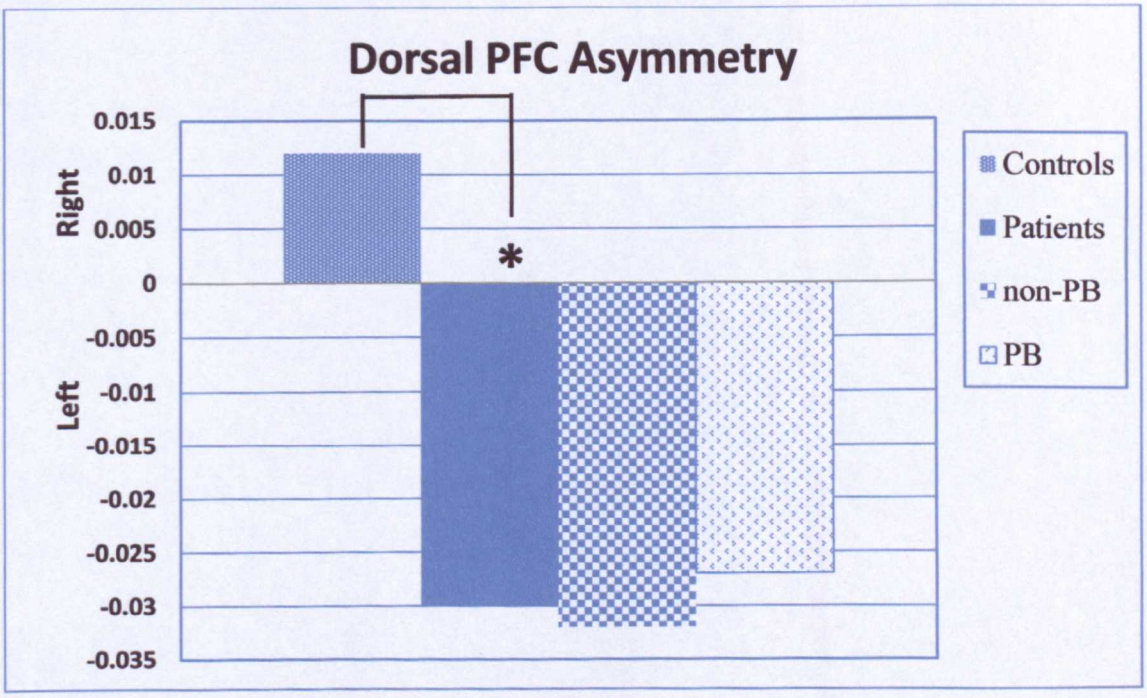
In conclusion, CLBP patients demonstrate a significant GM deficit in right dorsal PFC, compared to left dorsal PFC and furthermore, this brain region is significantly smaller than the right dorsal PFC of pain-free controls.

Table 4.6 Asymmetry Analysis of PFC Dorsal and Orbital Regions

Region	Group	Mean	S.D	R>L	L>R	R=L
Dorsal	Controls	.012	.04	8	5	1
	Patients	-.030	.06	6	20	0
	Non-PB	-.032	.07	3	12	0
	PB	-.027	.04	3	8	0
Orbital	Controls	-.047	.07	3	11	0
	Patients	-.030	.06	10	16	0
	Non-PB	-.020	.06	6	9	0
	PB	-.045	.06	4	7	0

R>L = right larger than left, L>R = left larger than right, R=L = no difference in laterality.

Figure 4.2 Asymmetry Results of Dorsal PFC by Group



*$p<0.05$ indicates a significant difference in right dorsal PFC volume between controls and CLBP patients, with patients displaying reduced volume.

Insula Analyses

Group mean volumes are summarised in Table 4.7. All insula volumes were normalised by TIV, resulting in a percentage of the total intra-cranial volume.

Table 4.7 Descriptive Statistics for Insular Cortex

Region	Controls		Non-PB		PB	
	Mean	S.D	Mean	S.D	Mean	S.D
Insula L	.41	.03	.40	.04	.38	.02
R	.38	.04	.39	.04	.39	.04
Anterior Ins L	.25	.04	.25	.04	.25	.02
R	.24	.04	.25	.03	.25	.03
Posterior Ins L	.15	.03	.15	.04	.14	.02
R	.14	.01	.14	.02	.15	.03

Main effects and Interactions

Normalised volumes were used to perform a mixed factorial 3 x 2 x 2 ANOVA, with laterality (left/right) and region (anterior/posterior) as within-subject factors, and group as the between-subjects factor. Main effects were found for laterality (left, 0.199 right, 0.194; $F(1,36) = 5.516$ $p = 0.024$), and for region (anterior, 0.249 posterior, 0.124; $F(1,36) = 341.852$ $p < 0.001$). The group main effect was non-significant, $F(2,36) < 1$. These results indicate that the left hemisphere and the anterior insular both had larger GM volume across all participants.

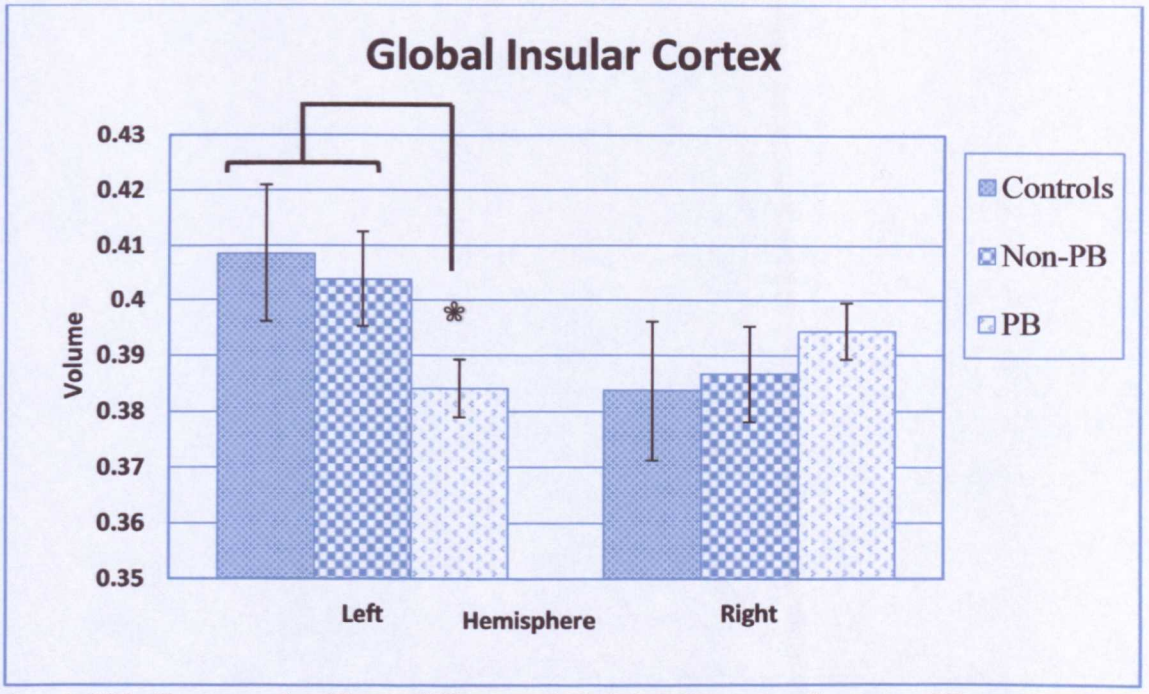
Of most interest was the significant two way interaction between group and laterality, $F(2,36) = 4.994$ $p = 0.012$, demonstrating that the volume asymmetries were different across the three groups. No interaction was found for region (anterior/posterior). Therefore, subsequent analyses were conducted on global insular cortex volumes. GM volumes were larger on the left than the right for the controls (0.41, 0.38) and non-PB (0.40, 0.39) groups, whereas the opposite pattern was evident for the PB group (0.38, 0.39; refer to Table 4.7).

To investigate laterality further, paired sample t-tests were conducted. They revealed that the normalised global insular cortex volume was significantly larger on the left for both the control ($t(13) = 3.823$ $p = 0.002$) and non-PB ($t(14) = 2.197$ $p = 0.045$) groups. However, this effect was not found for the PB group ($t(9) = -1.165$ $p = 0.274$).

Further, contrast analyses of the simple main effects of group revealed that the PB group's left insula volume was significantly smaller than the control and non-PB groups combined ($t(26,301) = 2.255$ $p = 0.033$; the latter two not being

significantly different from each other). The results indicate that CLBP patients, who also have concomitant PB, have significantly smaller GM volume estimation of the left insular cortex, when compared to a combined group of CLBP patients without PB and pain-free controls. This result is illustrated in Figure 4.3.

Figure 4.3 Mean (±SEM indicated by error bars) Global Insular Cortex Volume By Group



$p<0.05$ indicates a significant difference in left insular cortex between PB group and controls and Non-PB combined.

Asymmetry Analyses

Given there was no evidence to suggest a differential anterior-posterior effect across groups, asymmetry indexes were computed for the global insular cortex (see Table 4.8).

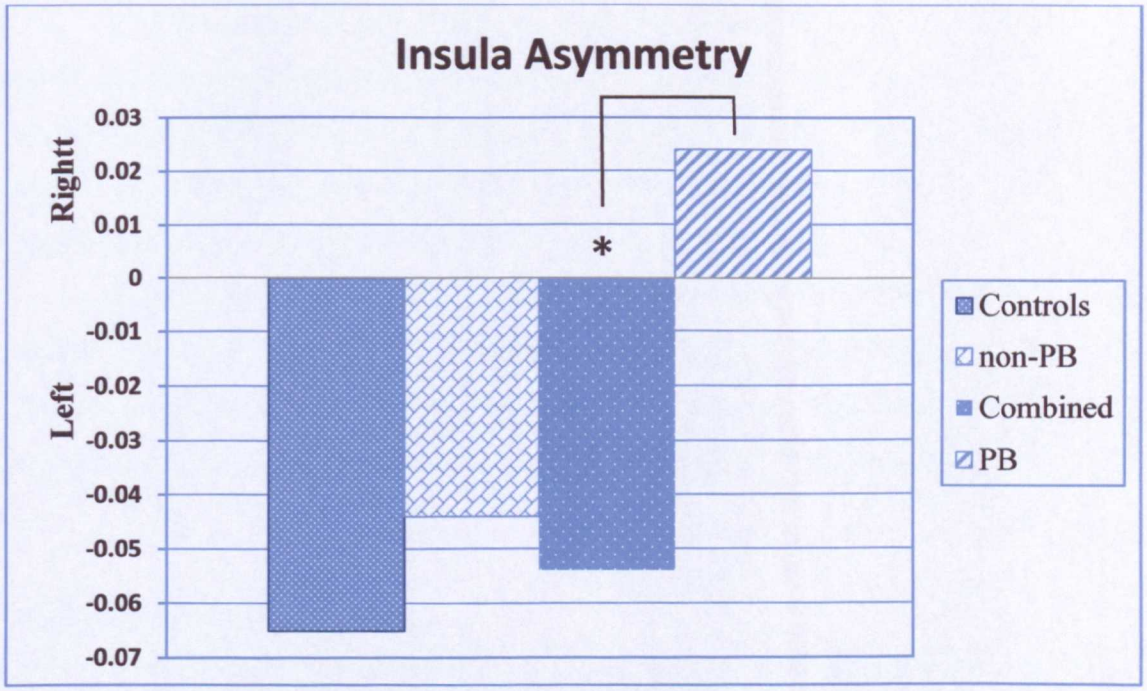
Table 4.8 Asymmetry Analysis for Insular Cortex

Region	Group	Mean	S.D	R>L	L>R	R=L
Insula	Controls	-.065	.066	3	11	0
	Non-PB	-.044	.078	4	11	0
	Combined GP	-.054	.090	7	22	0
	PB	+.024	.074	7	3	0

R>L = right larger than left, L>R = left larger than right, R=L = no difference in laterality.

In contrast to the previous PFC analysis, both the control group and non-PB groups displayed a greater left than right asymmetry for the insular cortex. However, the PB group displayed the reverse, with larger volume estimation for the right insular cortex. Hemispheric results are illustrated in Figure 4.4.

Figure 4.4 Asymmetry Results of Insular Cortex by Group



*$p<0.01$ Indicates a significant difference in left whole insular cortex between the PB group and the combined group of controls and non-PB participants.

A one-way ANOVA revealed a significant main effect of group, $F(2,38) = 4.579$ $p = 0.017$, and contrast analyses showed that whilst the asymmetry indexes were not reliably different for the control and non-PB groups, $t(26.721) < 1$, these two groups combined were significantly different from the PB group, $t(15.350) = 2.921$ $p = 0.010$. Finally, one sample t-tests revealed that the insula volume asymmetry indexes were significantly different from zero (which would indicate no asymmetry effect) for the control, $t(13) = -3.687$ $p = 0.003$, and non-PB groups, $t(14) = -2.184$ $p = 0.047$, but not for the PB group, $t(9) = 1.034$ $p = 0.328$.

In conclusion the results of the asymmetry analyses indicate that CLBP patients with PB demonstrate a left sided asymmetry effect, which is significantly different when compared to non-PB patients and pain-free controls.

Age and Pain Duration

To investigate the effects of age and pain duration on right dorsal PFC and left insula volume estimates, Pearson's Product Moment Correlation Coefficient was calculated. No significant relationships were demonstrated.

4.4 Discussion

This is the first study to utilise the Cavalieri method of modern-design stereology in conjunction with point counting to investigate differences in brain structure between CLBP patients and pain-free controls. Furthermore, in comparison to previous studies, we have used a clinical method of assessment (WS) to investigate if GM volume differs between subgroups of pain patients.

Region of interest analyses were performed for prefrontal and insular cortices. Analyses revealed that compared to pain-free controls, CLBP patients demonstrate GM deficit of right dorsal PFC. Furthermore reduced GM volume was evident for left insular cortex, but this was confined to CLBP patients with PB.

4.4.1 Whole Brain Analysis

A whole brain automated technique was used to investigate global GM and TIV between controls and CLBP patients. No significant differences were found. Furthermore, there was no evidence of significant relationships between both age and pain duration to either global GM or TIV.

In CLBP cohorts, only one study has reported a significant 5-11% global GM deficit (Apkarian et al, 2004a). However, three further studies investigating whole brain differences in CLBP, have failed to replicate this finding (Buckalew et al, 2008; Schmidt-Wilcke et al, 2006; Seminowicz et al, 2011). Similarly, empirical studies investigating global tissue deficits in FM have reported mixed findings, with one study demonstrating an effect (Kuchinad et al, 2007) which, to date, no other VBM study investigating FM patients has replicated (Please refer to Chapter Two, Figure 2.1 for more details).

Although the literature has failed to provide a consensus of global GM or TIV differences in chronic pain patients, localised morphological differences between patient populations and controls are consistently reported. This indicates that whilst some morphological differences exist, they are not sufficient enough to produce a

statistically significant whole brain variation. It is perhaps of more empirical interest that current evidence suggests localised differences relating to the pain experience, which will allow for more focused empirical investigations allowing more directed compensatory methods of improvement than could be achieved when faced with an undefined global effect.

The current study did not find a significant relationship between GM, TIV and age. The literature investigating chronic pain states has produced inconclusive findings relating to age and GM/TIV volume. Significant age and volume relationships have been demonstrated in FM patients (Kuchinad et al, 2007) and CLBP (Apkarian et al, 2004a). However, Valet et al, (2009) found no such relationship in patients with pain disorder and more surprisingly a study investigating whole brain volume in elderly CLBP patients (aged >65) found no significant relationship (Buckalew et al, 2008). One explanation is that although GM volume does start to decline in individuals from as early as 20 years of age, significant decline does not occur until mid to late fifties. In the current study, the average age was early to mid-forties. Therefore, the participants groups may have not been old enough to demonstrate a significant age-related effect.

4.4.2 Pain Duration

There was no significant relationship between pain duration and any of the brain tissue measures (GM, TIV, PFC and Insular cortex). When investigating morphological differences in pain cohorts, questions of causality are raised; do the observable differences precede the pain condition, or are they a consequence of a persistent pain condition? A significant relationship between structural differences and pain duration infers that the differences are a consequence of persistent pain. This relationship has been demonstrated in several pain conditions (Apkarian et al, 2004a; Blankstein et al, 2010; Kuchinad et al, 2007; Seminowicz et al, 2010; Valet et al, 2009; Younger et al, 2010), but is no means unequivocal as other studies investigating the same pain conditions have failed to find any such relationship (Burgmer et al, 2009; Hsu et al, 2009; Lutz et al, 2008; Schmidt-Wilke et al, 2006; 2007; Wood et al, 2009). The differences in findings may be further compounded by the ability to accurately define when a condition started. Pain duration may be ill-defined by the sufferer if the condition was not preceded by a traumatic event.

Finally, the cross sectional nature of these studies means that any assumption of causality should be treated with caution. However, three recent studies which have

investigated morphological changes in pain patients preceding and after successful treatment (Gwilym et al, 2010; Rodriguez-Roecke et al, 2009; Seminowicz et al, 2011), provide partial support that GM differences are the consequence of chronic persistent nociceptive activity and highlight the need for longitudinal studies to better investigate the issue of causality.

4.4.3 Region of Interest Analysis

The ROI analyses conducted on the PFC and insular cortices revealed a deficit in volume estimation for both regions in CLBP patients. Specifically, compared to controls, patients demonstrated a 14% deficit of right dorsal PFC. In comparison, a 5% GM deficit of left insular cortex was found for the PB group, when compared to the control and non-PB patient groups combined. Therefore, the PFC deficit appears to be a function of pain condition (pain vs. control), whereas the deficit of left insula cortex appears to be a function of pain state, specifically PB.

Our findings provide partial support for previous studies which have reported GM differences for CLBP patients in prefrontal regions. Specifically, Apkarian et al, (2004a) and Seminowicz et al, (2011), reported bilateral and left lateralised DLPFC reductions respectively. However, Buckalew et al (2008) failed to find any frontal deficits, with Schmidt-Wilcke et al, (2006) reporting GM increases in right DLPFC, although at an uncorrected threshold level. To date, no study investigating morphological changes in CLBP patients have reported insular cortex differences. Nonetheless, the ROI findings of the current study are comparable to the findings of studies investigating FM (Kuchinad et al, 2007; Hsu et al, 2009; Robinson et al, 2010), pain disorder (Valet et al, 2009), OA (Rodriguez-Roecke et al, 2009) and persistent idiopathic facial pain (Schmidt-Wlike et al, 2010).

Both the insular cortex and prefrontal regions have been identified as part of the pain matrix, with over 50% of fMRI studies using experimentally induced pain, reporting activation of the insular cortex (Apkarian et al, 2005). However, the region is not believed to be pain specific with activity being demonstrated as part of other interoceptive processing (Craig, 2009; Small & Apkarian, 2006). The anterior and posterior subdivisions of the insular cortex represent functionally different processing, with anterior insula being critically involved in affective and motivational aspects of the pain experience (Neugebauer et al, 2009) in contrast to the sensory-discriminative and cognitive functions of the posterior subdivision (Brooks et al, 2005).

A global GM deficit may impact all aspects of pain processing; sensory, affective and cognitive. That smaller GM volume of this region was confined to CLBP who experience PB is of great interest. These patients often report greater levels of pain intensity and display greater affective responses to pain, with a greater reliance on pain medication (Apeldoorn et al, 2011). This behaviour may in part be attributable to an underlying GM deficit of insular cortex, resulting in the development of maladaptive behaviours (Draganski et al, 2004). For example the study by Hsu et al, (2009) reported reduced GMV in anterior insular cortex for FM patients with affective disorder, whereby increased levels of trait anxiety were inversely correlated with GMV. Furthermore, Veldhuijzen, Greenspan Kim, & Lenz, (2010) found altered pain sensation in patients with insular cortical lesions. An inability to accurately determine pain levels may lead to misjudgements about pain-eliciting activities, leading to fear and avoidance of disconfirming behaviours.

Finally, a case study investigating spontaneous pain attacks demonstrated that stimulation of the posterior insula could reproduce a specific pain sensation previously elicited by epileptic seizure (Isnard, Magnin, Jung, Mauguiere & Garcia-Larrea, 2011). Intracortical stimulation of other pain matrix regions failed to replicate this effect, or showed sufficient time delay to suggest that the insular discharge was the first neural event related to the seizures. If the conclusions of Schmidt-Wilcke et al, (2010) and Valet et al, (2004) are correct, whereby GM deficits of the insular cortex represent 'burnout' due to an overutilization of both sensory and subsequent emotional processing in response to pain, this could also raise the possibility that aspects of the pain experience are generated in the cortex.

The frontal cortex is important for many different aspects of attentional processing such as working memory functions, continuous monitoring of external stimuli, cognitively demanding tasks and other activities which are included in the umbrella term of executive functions (Fuster, 2000; 2001). During experimental pain paradigms, the frontal regions have been observed to represent the cognitive and attentional processing of the painful stimuli (Casey, 1999; Coghill, Sang, Maisog, & Iadarola, 1999; Ploghaus, Tracey, Gati, Clare, Menon, Matthews & et al, 1999). This overlap between areas associated with pain and attentional processing has been investigated by studies which have increased cognitive load to demonstrate diminished pain intensity and unpleasantness ratings (Bantick, Wise, Ploghaus, Clare, Smith, & Tracey, 2002; Valet et al, 2004). Seminowicz, Mikulis & Davis (2004) asked participants to complete a classic stroop task whilst receiving a strong

painful sensation. Increased ability on the task resulted in a greater reduction in pain-related activity.

Therefore, there is evidence that attention is effective in modulating the sensory and affective aspects of the pain experience. The network of structures that enable this alteration of the pain experience is known as the descending pain modulation network. Specifically, this network includes the anterior cingulate cortex (ACC), amygdala, hypothalamus, periaqueductal gray (PAG), nucleus cuneiformis (NCF), rostral ventromedial medulla (RVM), insular cortex and frontal lobe (Tracey & Mantyh, 2007). Although these areas constitute a descending modulation network, it has been suggested that it is the activity of the DLPFC which initiates any analgesic effects.

Several studies have investigated activity levels of the frontal cortex and its association with pain modulation. Results have indicated that activity levels of the bilateral DLPFC were inversely related to ratings of pain intensity and unpleasantness of experimentally induced allodynia, which was interpreted as 'top down' modulatory effects (Lorenz, Minoshima & Casey, 2003). The suggestion that prefrontal regions initiate pain modulation was investigated by Freund, Klug, Weber, Stuber & Wunderlich, (2009). Participants were told to disengage from thermally induced pain to delineate regions involved in the initial suppression of pain and in the maintenance of pain suppression. Study participants successfully disengaged from the pain sensation, resulting in increased activity in ACC, caudate nucleus, insular cortex and bilateral DLPFC. Whilst increased activity in caudate nucleus was associated with initial suppression, DLPFC activity was associated with the maintenance of pain suppression. These studies suggest that the DLPFC can both initiate and maintain pain modulation to physically painful stimulus.

A recent study conducted by Raij, Numminen, Narvanen, Hiltunen & Hari (2009) extended these findings to investigate pain and pain relief created through hypnosis. Similarly to previous findings, the strength of BOLD activity in the right DLPFC, left insula and secondary somatosensory cortex, was inversely related to the intensity of the pain experienced. Another mechanism of pain relief, placebo analgesia, has suggested the involvement of prefrontal regions in the placebo effect. Placebo analgesia is a neurobiological and behavioural modification that occurs after the simulation of a treatment therapy and is influenced by the patients' cognitive and affective state (Carlino, Pollo & Benedetti, 2011). A study by Krummenacher, Candia, Folkers, Schedlowski & Schönbächler (2009) used repetitive transcranial

magnetic stimulation (rTMS) to demonstrate that the disruption of the prefrontal cortex during thermal pain, effectively disrupted the placebo analgesia effect, which had previously been learnt and applied in the study population.

An alternative explanation for the GM deficits is that this difference does not reflect the clinical pain experienced by the patients, but may be related to other aspects of the pain experience. Patients with persistent pain of a long duration and high levels of pain often have increased levels of disability, resulting in decreased levels of exercise, social isolation and reduced cognitive load. For example, a recent CTA study conducted by Seminowicz et al, (2011) in CLBP patients receiving surgical intervention, found that the left DLPFC was reduced prior to surgery, but increased postoperatively in patients who responded to treatment. Overall pain levels were reduced by 44%, but pain-related disability had a greater reduction of 46%, suggesting that a combination of both activity and pain levels contributed to an increase in DLPFC thickening. Similarly, the impact that certain life events can have upon brain structure has been investigated. In an extensive 20 year prospective study adult stress exposure was associated with decreased GMV in hippocampus and orbitofrontal cortex in otherwise healthy individuals (Gianaros, Jennings, Sheu, Greer, Kuller & Matthews, 2007). As chronic pain conditions can be preceded or exacerbated by stressful experiences, some morphological changes may occur, which are not directly related to the clinical pain. Finally, GM deficits in frontal regions have been consistently cited in the literature examining structural changes related to major depressive disorder (Konarski, McIntyre, Kennedy, Rafi-Tari, Soczynska & Ketter, 2008). It is unlikely that the results of the current study are related solely to mood disturbance, as a group effect would be expected due to HADS-D scores (Please refer to Table 4.1). However, persistent pain is often accompanied by disturbances in general affect, which may be contributing to the results.

4.5 Strengths and Limitations

We used a clinical method of assessment, namely the Waddell Signs for patient recruitment. This served two purposes. Firstly, it ensured that the behaviour of the CLBP patients differed in an observable way. There are many issues relating to the completion of self-report measures, such as the reliance on memory, reinterpretation of events and presentational effects. Whilst the WS may also be subject to external influence, the aims of the tests are not easily inferred and as such are less open to manipulation. Secondly, it ensured that any significant findings

between the patient groups were clinically relevant. Rather than assuming that all CLBP patients demonstrate similar behavioural responses to their condition, using a clinical method of assessment means that treatment can be created or better directed to those patients.

The number of participants used within the presented study may be limiting findings and may be responsible for the small, albeit significant, GM deficits being observed. However, unlike VBM, which requires 20 or more participants per group, stereological analysis may be best suited to studies with smaller participant numbers. Manual methods allow individual volume measurements, something automated techniques cannot currently reproduce. Furthermore, of the nineteen VBM studies cited in Chapter Two (Table 2.1) only seven studies have participant numbers greater than the current study. Although this study was based on a relatively small sample, reliability in the obtained results was increased by using a manual method, best suited to small group numbers.

The magnitude of GM reduction in both prefrontal and insular regions is comparable to previous pain studies using VBM. Comparisons between the current study and the VBM literature are difficult. This is in part due to differences in the unit VBM is measuring (GMD or GMV) and threshold levels of individual studies (corrected or uncorrected). However, this is compounded by a failure to cite either the extent of brain changes, or global tissue volume, both of which would enable a calculation of the findings as a percentage of whole brain volume. However, a handful of studies report enough information to allow these calculations to be performed (Gwilym et al, 2010; Kuchinad et al, 2007; Robinson et al, 2010; Rodriguez-Roecke et al, 2009; Schmidt-Wilke et al, 2010; Valet et al, 2009). For these studies GM deficits ranged between 0.002-0.1% for insular cortex and 0.05-0.07 for PFC regions. In comparison, the findings of the current study show a 0.02% reduction of left insular cortex and a reduction of 0.8% for the right dorsal PFC as a percentage of GM volume, making the current findings comparable to the previous literature. Furthermore, as the possible mechanism of change is currently unknown, it would be ill-advised to set a prior threshold level for clinical relevance.

The current study used a region of interest analysis to assess brain changes in controls and CLBP patients. Although the ROI were empirically driven from the literature, other GM deficits may have been overlooked.

The study is cross sectional in design, meaning that whilst differences between controls and patients can be demonstrated there is no method of

investigating if these volumetric differences represent changes to the brains of CLBP patients, or have always been present.

Participants maintained their medication intake. This issue has been raised in relation to the previously presented VBM studies (Please refer to Chapter 2.5). The necessary 'wash out' period that would be needed to control for these effects is unknown, which would raise ethical issues in relation to the reduction of treatment for research purposes. However, the findings of Younger et al (2011) indicate that opioids can affect brain structure, although the changes observed in the study are not reflected in our findings. As such the issue of medication use whilst studying morphological changes remains inconclusive.

4.6 Conclusion

In conclusion the findings of the current study indicate that two areas known to be involved in pain modulation through attentional mechanisms have a reduction of GM volume in patients with chronic low back pain. Furthermore, an area that is known to be associated with the affective-motivational aspects of pain processing, namely the insular cortex, shows a GM deficit, but only in patients who display pain behaviour. It might be inferred that these patients do not have the same system for cortical modulation available to them as other CLBP patients. Therefore, they may possibly experience greater levels of distress and an altered affective state which may either lead to or maintain pain behaviours.

CHAPTER FIVE
Attentional Bias and Pain-related Fear in a Pain-free Population

5.1 Introduction

The fear avoidance model of pain places an emphasis upon psychological factors in the development and maintenance of pain conditions, with specific focus being placed upon catastrophising and pain-related fear (Vlaeyen & Linton, 2000). The model asserts that pain-related fear can lead to a misinterpretation of the pain experience, and that in turn this can result in hypervigilance to pain-related stimuli, demonstrated by the preferential processing of a pain stimulus over other competing demands on attention.

Prospective pain studies have reported that pain-related fear is a significant predictor for both the recovery from acute pain and the transition from acute pain to chronic pain (Chou et al, 2010; Ramond et al, 2011). However, these studies are investigating pain-related fear within the context of an existing pain condition. Pain-related fear may precede a painful episode, whereby it may be a risk factor for developing chronic pain. Alternatively, pain-related fear could be the resulting consequence of a painful event, whereby it may be a maintaining or exacerbating factor. Pain-related fear has been demonstrated within the pain-free general population and has been associated with greater negative responses to acute experimental pain (Hirsch et al, 2008; Houben et al, 2005). If healthy pain-free individuals, who demonstrate high levels of pain fearfulness, also demonstrate a selective attentional bias for pain-related stimuli, this may indicate an important preceding vulnerability for responding to new pain experiences in a negative manner.

There have been a small number of studies investigating pain-related fear and attentional biases in pain-free populations. Keogh et al, (2001a) used FPQ scores to allocate participants to one of three pain-fearful groups; low, medium and high. A dot probe task was then completed, containing sensory pain, social threat, positive and neutral word lists. Results indicated that participants with high FOP levels responded faster to probes replacing the pain words, compared to the other participant groups. A replication of the study conducted by Roelofs et al, (2003) failed to find any significant attentional bias effects.

A follow-up study conducted by Keogh et al, (2003) investigating pain-related fear and the automaticity of attentional biases found no significant attentional bias for the high FOP group. In contrast an effect was present for the low FOP group, who in the unmasked trials oriented their attention away from pain-related material. It would appear that participants with low FOP have the ability to override the tendency to orient towards pain-related material, which is lacking in individuals with high levels of pain-related fear.

Three further studies conducted by Keogh and colleagues (Hunt, Keogh, & French, 2006; Keogh & Cochrane, 2002; Keogh, Dillon, Georgiou, & Hunt, 2001b) used a different measure of fear to investigate attentional biases in pain-free participants. Anxiety sensitivity (AS) is a fear of anxiety-related sensations. Using the dot probe task, it was reported that participants with high AS levels selectively attended to physical threat-related words, whereas low AS participants avoided these words (Keogh et al, 2001b). Hunt et al, (2006) found evidence of hypervigilance for words representing anxiety symptomology in both masked and unmasked conditions for participants with high AS. In contrast, Keogh & Cochrane, (2002) found no evidence for any selective attentional bias, although an interpretational bias was evident for the high AS group. The results of these studies suggest that high levels of fear can be associated with both attentional and interpretational biases for condition specific word stimuli.

The aim of the current study was to investigate pain-related fear and selective attentional biases in a pain-free population. To extend previous findings, a multi-method approach was adopted through the inclusion of fMRI. The reasoning behind this was twofold. Firstly, several studies have reported differential cerebral activation patterns between study groups when performing a variety of cognitive tasks, even when the between group behavioural measures were not statistically different (Britton, Gold, Deckersbach & Rauch, 2009; Canli, Sivers, Thomason, Whitfield-Gabrieli, Gabrieli, & Gotlib, 2004; Seminowicz et al, 2011). In light of the mixed results from previous dot probe studies investigating pain-related fear, it was hoped that the inclusion of fMRI may provide further insights into the attentional processing of pain-related material. Secondly, this study acted as a proof of principle that a pain-related semantic dot probe task could be successfully administered and completed within the fMRI environment. If the task was completed successfully, it was intended that a dot probe study could then be administered to a clinical population (Please see Chapter 6).

In summary, the current study used a visual dot probe task and block design fMRI to test three hypotheses. Firstly, it was hypothesised that participants with high levels of pain-related fear would be vigilant towards pain-related words. Specifically, the high FOP group would respond faster to probes when they replaced a pain-related word. Secondly, it was hypothesised that participants with low FOP would be avoidant to pain-related words. Therefore, the low FOP group will take longer to respond to probes replacing pain-related words. Finally, we predicted that patterns of cerebral activation would differ between the participant groups for the pain-related stimuli. Specifically, if an individual is hypervigilant to pain-related words, when the probe replaces the accompanying neutral word (pain-incongruent trials) this requires attention to be redirected away. In contrast, if the probe replaces the pain-word (pain-congruent trials) attention does not need to be diverted. If there are no differences in the attention bias to threat, then there should be no difference in the attentional demands of the task.

A previous study investigating trait anxiety using a dot probe task with facial stimuli, has demonstrated increased activation in DLPFC (Telzar, Mogg, Bradley, Mai, Ernst, Pine & et al, 2008). The ACC has been demonstrated to be associated with attention to emotional stimuli and has been shown to be activated by both attentional tasks (Derbyshire, Vogt & Jones, 1998; Whalen, Bush, McNally, Wilhelm, McInerney, Jenike & et al, 1998), and when recalling autobiographical memories triggered by pain-related words (Kelly, Lloyd, Nurmikko, & Roberts, 2007). Additionally, selective attentional tasks, tasks of executive function, and tasks using pain words as cues, have demonstrated increases in activity in both frontal and posterior regions, including precuneus, inferior and superior parietal cortex, and occipital regions, (Collette, Hogge, Salmon & Van der Linden, 2006; Eck Richter, Straube, Miltner & Weiss, 2011; Fan, McCandliss, Fossella, Flombaum & Posner, 2005; Richter, Eck, Straube, Miltner & Weiss, 2009). It was predicted that differences in the selective attention to pain-related words would be demonstrated within these regions.

5.2 Methods

5.2.1 Ethical Approval

The study was approved by the Sefton Research Ethics Committee. All participants signed an informed consent form before study participation.

5.2.2 Participants

Inclusion and exclusion criteria

The aim of this study was to investigate fear of pain, rather than response to actual physical pain, thus inclusion criteria required all participants to be pain-free, with no history of a chronic pain experience. Furthermore, it has been suggested that handedness and childhood bilingualism can impact cerebral activation patterns (Hatta, 2007; Hernandez, 2009). To avoid any possible confounds relating to these influences, the inclusion criteria required participants to be right-handed, with English as a first language.

Exclusion criteria required all participants to be free from a history of co-existing neurological disease, stroke, brain injury, meningitis, substance misuse, dementia or severe psychiatric disease, such as psychosis or bipolar disorder. Due to the nature of the experimental task, participants were excluded if they had any reading impairment, for example dyslexia. Finally, participants had to be suitable for MRI scanning. Any contraindications such as any internal metal, possible pregnancy or claustrophobia meant they were excluded from the study.

Recruitment

Participants were recruited through an advert placed upon the University of Liverpool's intranet announcement system. The announcement specified the inclusion/exclusion criteria and gave an overview of the study. Participants were informed that they would be taking part in a study examining attention. Respondents were sent self-report measures (SRM) which included the fear of pain questionnaire, the score of which determined study inclusion and group assignment (see below). Once SRM were returned, participants were approached via the telephone, to ensure that they met the study inclusion and exclusion criteria.

Sixty-six participants responded. The fear of pain questionnaire (FPQ) score determined if the respondent would be included in the study and which group they would be assigned to, either the high or low fear of pain groups. Assignment to the

groups was determined by the 75th and 25th percentiles of scores from the sixty-two responding participants.

Once selected, participants were then given a full screening, performed by a nurse at the University of Liverpool's magnetic resonance image analysis research centre (MARIARC). This was to ensure no MRI contraindications. Scanning was performed at the Walton Centre for Neurology and Neurosurgery (WCNN), where a second screening occurred prior to scanning and was performed by a radiographer. No participants were excluded.

These procedures resulted in twenty-eight participants being selected for study participation, fourteen in each group. fMRI data was collected for twenty-eight participants. Technical problems resulted in the loss of behavioural data for five participants.

Participant Demographics

Each study group consisted of 5 males and 9 females. Participant ages across groups ranged from 18 to 61 years old. Fear of pain scores ranged from 38-68 for the low FOP group, with a range of 84-108 for the high FOP group. All participants had near normal or corrected-to-normal (with contact lenses) visual acuity, thus ensuring that all words could be seen clearly.

Summary information for age, gender and results from the questionnaires used for participant screening can be found in Table 5.1.

Table 5.1 Participant Demographics

Group & subject No.	FPQ	Age	Sex	PCS	HADS-A	HADS-D	VAS NOW cm	VAS 5DAY cm
Low FOP								
1	38	34	M	0	7	8	1	0
2	46	22	F	16	1	0	0	0
3	55	23	M	0	1	3	0	1
4	55	25	M	7	2	6	1	2
5	57	21	F	16	10	2	0	0
6	60	21	F	17	15	10	2	1
7	61	18	F	-	10	6	1	1
8	63	31	M	8	4	1	0	1
9	63	61	F	4	3	8	2	1
10	65	39	M	12	5	5	2	0
11	65	18	F	12	9	8	0	1
12	65	22	F	10	11	7	2	0
13	67	25	F	16	2	1	1	0
14	68	20	F	12	7	1	1	1
Mean	59	27	9F	10	6.2	4.3	.9	.6
(S.D)	(8.5)	(11.5)		(5.9)	(4.3)	(3.5)	(.8)	(.6)
High FOP								
1	84	29	M	6	6	1	1	1
2	84	25	F	11	11	1	1	1
3	89	18	F	27	8	1	3	1
4	89	53	M	5	4	6	1	0
5	89	22	F	14	5	4	1	1
6	93	57	F	8	4	4	0	0
7	94	27	F	28	10	1	1	1
8	96	27	M	17	4	1	1	2
9	96	20	F	32	12	7	1	1
10	100	29	M	12	9	2	1	0
11	101	21	F	-	5	3	0	0
12	106	20	F	-	8	5	0	0
13	108	25	M	7	6	6	1	1
14	108	21	F	-	7	2	1	1
Mean	95	28	9F	15	7.7	3.1	.9	.7
(S.D)	(8.2)	(11.9)		(9.6)	(2.6)	(2.2)	(.7)	(.6)

PCS-pain catastrophising scale; **HADS**- hospital anxiety and depression scale (**A**) anxiety (**D**) depression; **VAS**- visual analogue scale; **R.T**-reaction time data. Standard deviations in parentheses. Missing data is indicated by a '-'.

5.2.3 Self-Report Measures

All participants were required to complete several SRM. Firstly, the FPQ was used to create two experimental groups at the either end of the fear of pain spectrum. The HADS was used as a screening method to ensure that participants only had pain-related fear and that was not symptomatic of other anxiety or mood disorders. Two types of VAS were administered. The VAS-NOW was used for screening purposes and relates to current pain levels. This was used to verify that the participant did not experience significant levels of pain. The second VAS was given upon completion of the scanning session and required respondents to indicate their pain levels over the previous five days (VAS-5day). This was to ensure that the participant had not been experiencing acute pain, such as dental or menstrual pain, prior to the scanning session.

Finally, the PCS was completed by participants after the scanning session to determine if there existed a relationship between pain-related fear and pain catastrophising, or between pain catastrophising and selective attention bias.

A more detailed discussion of the self-report measures used can be found at Chapter 3.6.

5.3 Dot Probe Task

The study used the visual dot probe task to assess attentional bias within a healthy pain-free population. For a more complete discussion of the dot probe task refer to Chapter 3.4.

5.3.1 Word Selection

The wordlists generated for the dot-probe task consisted of pain-related, emotional and neutral words. An initial wordlist was created by pooling wordlists from previous articles; (Asmundson et al, 1997; Kelly et al, 2007; Keogh et al, 2001a) and from the McGill Pain Questionnaire (Melzack, 1975).

Once this wordlist had been established values were taken for word length, number of syllables and frequency levels (Kucera & Francis, 1967). Due to the limited number of pain-related words, the values for this semantic group were used as a baseline for which the other task stimuli were measured against. An overview of these values per word category is presented below in Table 5.2.

Table 5.2 Length, Syllables and Frequency per Wordlist

Wordlist	Length	No. of Syllables	Frequency
Pain	164	47	103
Neutral-Pain	164	50	99
Emotional	162	54	102
Neutral-Emotional	162	48	105

Neutral-Pain and Neutral-Emotional indicate the neutral words paired with the experimental wordlists. Frequency calculations based on Kucera and Francis (1962).

The emotional word category was included to assess if a general emotionality bias could account for any effects found. The wordlist contained equal numbers of positive and negative emotional words, such as adored and revolted.

The final semantic category was neutral words which were used to accompany the experimental stimuli as a word pair. All neutral words were household-related words, ensuring that they were part of one semantic category, not deemed to have any specific pain-related or emotional-related meanings. Each neutral word matched its experimental word for length. However, frequency and number of syllables were controlled for on a groupwise basis.

To ensure that the words were representative of the list they had been placed into the completed wordlists were given to three colleagues from MARIARC, who were asked individually to 'sort' the words into three unnamed categories. All three 'sorters' successfully separated the words into their respective categories.

A non-semantic condition was included to examine if there existed any performance differences between the groups, both in terms of response latencies and activation patterns. For this, a series of digits, specifically a row of zeros ('00000') were displayed instead of words.

An fMRI block design was used (see section 5.4), with three blocks containing eight trials for each semantic category. To ensure no effect of block, individual blocks were also matched on length, syllables and frequency.

A 3 x 3 analysis of variance (ANOVA) was performed with word valance (pain/emotional/neutral) as the independent variable, and word length, word frequency and number of syllables as the within-category factor. No significant main effects or interactions were found (all F-values < 1). The wordlists contained twenty four word pairs, resulting in forty eight experimental word trials. For complete wordlists see Table 5.3.

Table 5.3 Word Stimuli for Dot Probe Task

Pain-Neutral Words		Emotional-Neutral Words	
aching	beaker	admire	aerial
agony	bowls	adored	armchair
blister	candles	aloof	attic
bruise	candlesticks	annoy	banister
choking	cooker	approving	bedspread
cramp	drawers	coward	blinds
crushing	hallway	delicious	boiler
discomfort	kettle	despise	bookcase
excruciating	lamp	disgust	chimney
gash	laundry	enjoys	corkscrew
gasping	lavatory	harass	cutlery
hurting	lightbulbs	hateful	doorbell
inflamed	lino	helpfully	fridge
irritation	ornament	idiot	furnishing
itch	oven	jolly	heater
nipping	pegs	laughs	microwave
numb	pillow	lonesome	photo
pinching	radiator	loser	plugs
scalding	roofing	loveliness	shampoo
sore	stool	pleasing	socket
sprain	teaspoon	relaxing	spoon
stinging	telephones	revolted	tapes
swollen	toaster	super	teapot
twinge	wardrobe	unkind	tiles

5.3.2 Visual Display

The stimuli were presented using the E-Prime Program (Psychology Software Tools, Inc. http://www.pstnet.com) on a Sony Vaio laptop using a LCD projector (Epson LMP73000). Stimuli were projected onto a screen placed at the foot of the scanner bed. Participants were able to see the screen using a mirrored periscope attached to the head coil.

5.4 fMRI Task Design

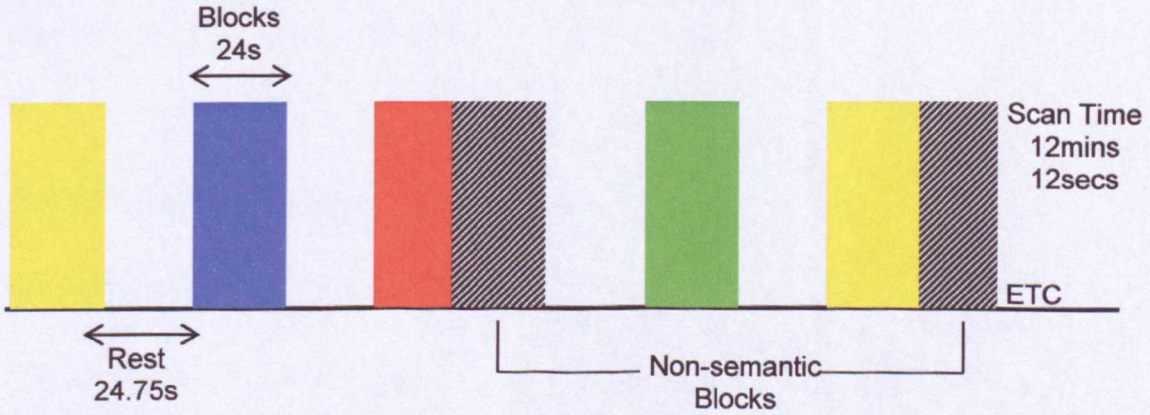
The dot probe task was presented in a block design, which consists of experimental blocks interspersed with Rest. Other fMRI studies have successfully utilised block design in word tasks (Redgrave, Bakker, Belloa, Caffo, Coughlin, Guardaa & et al, 2008; Wigenfeld, Rullkoetter, Mensebach, Beblo, Mertens, Kreisel & et al, 2009). A schematic of the fMRI design is presented in Figure 5.1. This was chosen as the most appropriate design for the current study for two reasons. Firstly, the participants of the current study were all healthy pain-free volunteers separated only by their pain-related fear. Therefore, any effect may be smaller in nature than

that achieved examining a patient population. Secondly, taking this factor into consideration block designed experiments have a greater amount of 'power' to detect a change in the fMRI signal.

Each block consisted of 8 word pairs, either pain or emotional words paired with a matched neutral word, with 3 blocks per condition. Furthermore, under the block design, the same type of experimental stimuli must be presented. For a dot probe task, this meant that the dot always replaced the same semantic category, such as the pain word, regardless of its upper or lower position. This required 6 blocks for each experimental condition; three blocks each of pain congruent, pain incongruent, emotional congruent, emotional incongruent. Words were presented randomly within each block, but it was ensured that all four combinations of experimental word and probe location were presented (pain word up-probe up, pain word up-probe down, pain word down-probe down and pain word down-probe up).

There were 4 blocks which contained eight trials of non-semantic stimuli, essentially a row of zeros. These blocks were pseudo-randomised to immediately follow one block from each of the experimental conditions, preceding the expected REST. Therefore, as well as providing a response baseline, the non-semantic condition was used to break up the certainty of the next rest block.

Figure 5.1 A schematic Representation of the Dot Probe Task



Each colour block represents a different condition; yellow- pain congruent; blue- emotion incongruent; red- emotion congruent; green- pain incongruent; striped- non-semantic.

For the current study the REST blocks comprised of the word 'rest' placed within the centre of the screen. Due to distributed sampling, the duration of the REST block was jittered in relation to the TR (3s). The REST blocks were

manipulated to 8.25TR, resulting in a REST block duration of 24.75s. (Further discussion on distributed sampling can be found in Chapter 3.3.4)

The presentation order of the blocks was counterbalanced across participants. Once in the scan room participants were given a brief practice session. The practice session consisted of 8 trials, using word pairs of animals. The practice session gave feedback for correct and incorrect responses.

5.4.1 Scanning Protocol

Scanning took place at the Walton Centre for Neurology and Neurosurgery. All scanning sessions were run by trained radiographers. Scanning was performed on a 1.5 Tesla GE Signa LX/Nvi neuro-optimised MRI scanner (General Electric, Milwaukee, WI). Functional magnetic resonance imaging was performed with a blood oxygenation level-dependent (BOLD) sensitive T_2^*.weighted multislice gradient echo EPI sequence (TE = 40 ms, TR = 3 s, flip angle = 90°, FOV = 192 cm, 64 x 64 matrix). Twenty-four contiguous 5mm thick axial slices were prescribed parallel to the AC-PC line and covered the entire brain. The overall scan time was 12 minutes 12 seconds, with 244 volumes being acquired.

For the purpose of anatomical referencing and visualisation of brain activation, a high-resolution T_1.weighted 3D inversion recovery prepared gradient echo (IRp-GRASS) sequence was also acquired for each participant (TE = 5.4 ms, TR = 12.3 ms, TI = 450 ms, 1.6-mm slice thickness, FOV = 20 cm, 256 x 192 matrix), with 124 axial slices covering the whole brain.

5.5 Behavioural Analysis and Results

5.5.1 Participant Characteristics

To examine if there was any significant differences for between group characteristics, a between groups ANOVA was conducted with fear of pain group (low vs. high) as the independent variable and self-report measures as the dependant variables.

As was to be expected due to group allocation procedures, a main effect for fear of pain groups was demonstrated for FPQ scores ($F(1,26) = 132.1$, $P < 0.0001$). A second between groups effect was found for PCS scores ($F(1,26) = 2.98$, $P < 0.05$). However, independent t-tests indicated that this was only approaching significance. No other significant differences were found. This suggests that any

significant findings are not related to measures of anxiety and depression. These results indicate that both groups primarily differed upon their levels of pain-related fear, including thoughts when experiencing pain sensations.

To examine further if any relationships existed between the questionnaire measures, correlational analyses were performed as shown in Table 5.4. A significant relationship was demonstrated between HADS-A and HADS-D scores, $r = .381$, p (two-tailed) < 0.05 indicating that greater anxiety levels also meant greater levels of depression. Similarly, a positive correlation was found between the HADS-A and PCS scores, $r = .514$, p (two-tailed) <0.05, indicating that greater anxiety levels were related to greater catastrophising levels.

However, neither HADS scales nor PCS scores correlated with the FPQ scores indicating that these scores were not related to group.

Table 5.4 Correlational Analyses between Self-report Measures

	FPQ	PCS	HADS-A
FPQ	-		
PCS	.387	-	
HADS-A	.132	.514*	-
HADS-D	-.154	-.154	.381*

* $P < 0.05$.

5.5.2 Data Reduction

SPSS version 18 was used for all non-fMRI data analysis.

The responses of five participants were not recorded due to technical difficulties resulting in data for twenty-three participants being collected. This did not influence the validity of their fMRI data.

All responses that fell below 300msec or above 1000msec were removed as outliers. Further, responses which fell beyond 3 standard deviations from an individual's mean were also removed. Analysis of the number of correct responses revealed that errors and outliers accounted for less than 1% of the responses. They were excluded from further analysis. The mean response times for the dot probe task can be found in Table 5.5.

To remove excessive variability in the reaction time of individual participants, median (MD) response scores were used instead of mean reaction times. Although this creates a more conservative approach to data reduction, differences in

attentional bias are often found within tens of milliseconds. The use of median values ensures that any significant findings would be more robust.

5.5.3 Reaction Time Data

A mixed factorial 3 x 2 x 2 x 2 ANOVA was performed with response time (RT) as the dependent variable, group as the between-subjects factor (low FOP/high FOP), and word type (pain/neutral vs. emotional/neutral), target word location (upper vs. lower) and dot location (upper vs. lower) as the three within-subject factors. There were no significant main effects or interactions demonstrated (all F-values < 1).

To investigate mean reaction time data by group to pain congruent trials (when the dot replaced the pain word irrespective of location), an independent t-test was performed which approached significance (t (21) = 1.723, $P < 0.052$). The group descriptive statistics indicates that participants with high pain fearfulness responded faster to dots when they replaced the pain-related words, compared to participants with low pain-related fear. This effect was found to be significant when both the pain word and dot appeared in the lower location, as evidenced by a significant independent t-test (t (21) = 1.981, $P < 0.05$).

One sample t-tests were conducted to investigate within group differences. No significant findings were uncovered in relation to the word stimuli. Therefore, neither group displayed significant differences in mean reaction time to pain-related or emotional words.

Table 5.5 Mean Dot Probe Response Times of Word Valence (pain vs. emotional), Target Word Location (upper vs. lower), Probe Location (upper vs. lower) by FOP Group (high vs. lower)

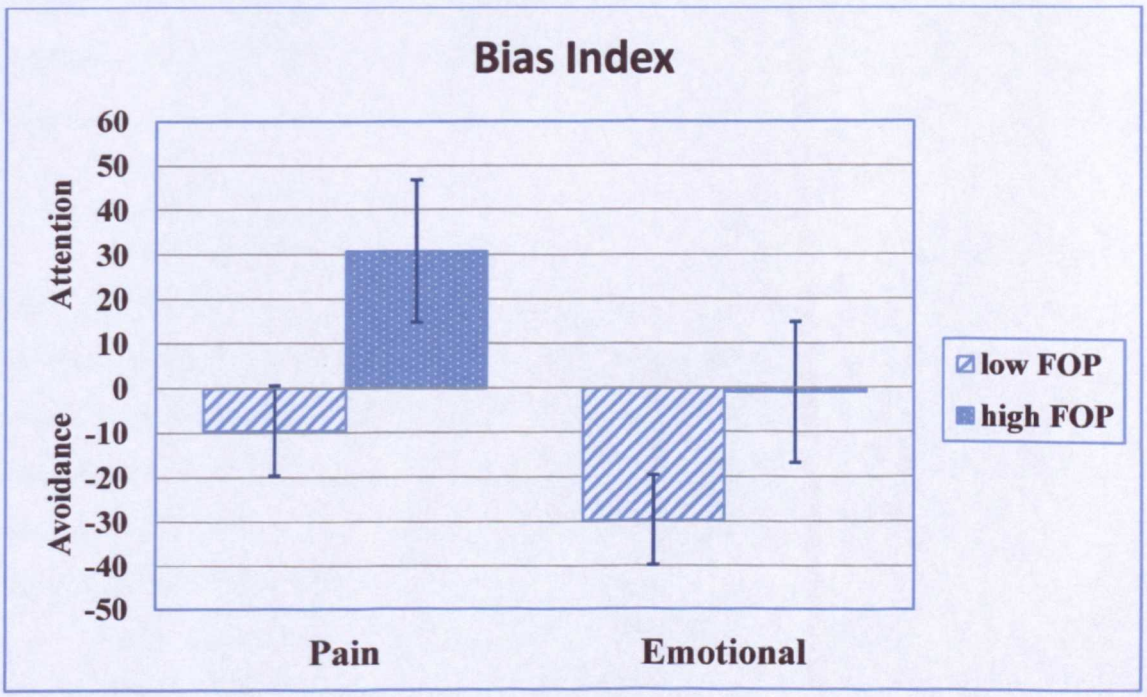
	Low FOP (ms)	High FOP (ms)
Pain upper/dot upper	553.00 (122.50)	499.36 (44.53)
Pain upper/dot lower	515.54 (73.93)	488.36 (55.41)
Pain lower/dot upper	529.21 (85.06)	504.77 (55.55)
Pain lower/dot lower	513.13 (85.91)	460.18 (40.15)
Emotional upper/dot upper	538.92 (87.00)	503.91 (48.08)
Emotional upper/dot lower	505.38 (61.24)	470.36 (37.48)
Emotional lower/dot upper	514.67 (60.33)	496.77 (47.71)
Emotional lower/dot lower	522.92 (103.55)	467.73 (52.56)
Pain Bias Index	-9.48 (80.81)	30.89 (73.85)
Emotional Bias Index	-29.67 (76.42)	-0.93 (42.70)

Standard Deviation contained within parentheses, ms-milliseconds.

Bias Indexes

A further examination of the data was undertaken using bias indexes for the reaction time data. (For further discussion relating to bias index calculations please refer to Chapter 3.4). The results of the bias indexes can be found in Table 5.5 and Figure 5.2. Independent t-tests were conducted between the FOP groups for the two indexes. There were no significant differences between the bias indexes for group (Pain Bias; $t(21) = -1.593$, $P > 0.05$; Emotional Bias; $t(21) = -1.095$, $P > 0.05$). Therefore, although there are differences in the direction of the bias indexes, with the high FOP group attending to pain-related stimuli and the low FOP avoiding both pain-related and emotional stimuli, these differences did not reach statistical significance.

Figure 5.2 Bias Index (±SEM indicated by error bars) for Wordtype



To determine if the bias indexes were related to pain fearfulness, indexes for both conditions were correlated with FPQ scores. No significant relationship was demonstrated indicating that the attention/avoidance demonstrated by the participants was not related to their level of pain-related fear.

In order to determine whether the bias indexes could be attributed to catastrophising, anxiety or depression rather than pain fearfulness, a series of correlations were conducted between the self-report measures and the bias indexes. A significant negative correlation was found between HADS-D score and the pain-

related attentional bias (r = -.453, p = < 0.05), indicating that participants who had lower pain bias index scores, had higher levels of self-reported depression. No significant relationships were found for the emotional word bias index and any of the self-report measures.

The design of the dot-probe task had to be modified for use within a fMRI environment. This resulted in the probe replacing the same word type per block. To rule out the possibility that participants had become aware of the design and could therefore predict the location of the probe, a one sample t-test was conducted to examine if responses differed between the blocks. Response times were significantly faster for the third block than the first block, but only when the dot replaced the pain word (t (22) = 2.276, p = 0.02) or the emotional word (t (22) = 2.642, p = 0.001). This effect was not found when the dots replaced the accompanying neutral word. These results would suggest that the participants did not predict the probe location as a speeded effect would have occurred for all word types. Therefore, the speeding of responses across blocks is interpreted as being related to the emotional nature of the stimuli.

5.5.4 Post-Hoc Analysis of Word Stimuli

During the creation of the studies wordlists, the stimuli were explicitly controlled for on word length, word frequency and number of syllables. However, in recent years an online database has been created which contains additional behavioural data relating to word stimuli. The English Lexicon Projects (ELP) database was used to examine if the wordlists may contain differences in behavioural testing, which had not been controlled for prior to the study. A more detailed discussion of the ELP can be found in Chapter 3.4.3.

To examine if the wordlists differed on either the speed with which the words can be identified, the study wordlists were entered into the ELP database. An ANOVA was conducted with wordtype (pain, emotional, pain paired neutral and emotional paired neutral) as the independent variable and behavioural results for the lexical decision making and speeded naming tasks, from the ELP database, as dependent variables. Interactions were found for wordtype and the reaction time data for the lexicon decision making task (F (3,95) = 2.77, P < 0.05) and wordtype and reaction time data for the speeded naming task (F (3,95) = 2.14, P < 0.05). Examination of the descriptive statistics indicated that the neutral words paired with the pain words, had faster reaction time data for both tasks. Although these findings indicate a previously uncontrolled for factor relating to the wordlists, it is assumed

that this finding had little impact upon the results. The participants did not respond significantly faster to probes replacing the pain paired neutral words which may have otherwise been predicted. The researchers are confident that the linguistic stimuli were suitably controlled for within the study.

5.6 FMRI Data Analysis and Results

Data analysis was performed using FMRI Expert Analysis Tool (FEAT) Version 5.98, part of FMRIB's Software Library (FSL, www.fmrib.ox.ac.uk/fsl). For steady state, two dummy scans are collected at the beginning of scanning and are deleted prior to analysis. The following pre-processing statistics were applied; motion correction using MCFLIRT (Jenkinson, et al, 2002); non-brain removal using BET (Brain Extraction Tool, Smith 2002); spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor; highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=75s). A more detailed discussion of preprocessing steps can be found in Chapter 3.3.6.

Statistical analysis of the time-series data was conducted using a general linear model on a voxel by voxel basis using FILM (FMRIB's Improved Linear Model; Woolrich, 2001). Once statistical analyses were conducted, registration to high resolution structural and/or standard space images was carried out using FLIRT (FMRIB's Linear Image Registration Tool; Jenkinson, et al, 2001; 2002).

Although stimuli were presented in a block design, data acquisition did not occur in a typical ABAC design as this would have been too predictable for the participants. Instead blocks were randomised. As such a three column format was used to define when the events occurred, with five explanatory variables being modelled. These were pain-congruent (dot replaced a pain word), pain-incongruent (dot replaced the neutral word paired with a pain word), emotional-congruent (dot replaced an emotional word), emotional-incongruent (dot replaces the neutral word paired with the emotional word) and the non-semantic condition (00000).

The resulting Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z > 1.8$ and a cluster significance threshold of $P=0.05$ corrected (Worsley, 2001). Random effects analysis were conducted using FLAME (FMRIB's Local Analysis of Mixed Effects) stage 1 (Beckmann, Jenkinson & Smith, 2003; Woolrich, 2004).

FSL View was used as a manual inspection tool to investigate both excessive head motion and the results of BET. The Nifti 4d images were 'played' in a movie mode, allowing for excessive movement to be observed. The BET output images were reviewed within FSLView to ensure that no non-brain matter remained on the images.

5.6.1 Neural Activation for Task Performance

To investigate if there were significant differences in cerebral activation relating to task performance, a between group (low FOP>high FOP) and (high FOP> low FOP) whole brain uncorrected analysis was conducted for the non-semantic condition (00000). No significant peak activations were found. It was therefore deemed unnecessary to subtract task performance (semantic condition>non-semantic condition) prior to between group statistical analysis.

5.6.2 Neural Activation for Main Effect of Task

Participants from both the high FOP and low FOP groups activated similar brain regions in response to the pain-congruent blocks (when the probe replaced the pain word) compared to REST. Specifically, both groups activated bilateral cerebellum, left postcentral gyrus, right precentral gyrus and right inferior frontal gyrus. The insular cortex was activated bilaterally for the high FOP group, but for the low FOP group this activity was left lateralised. The low FOP group also demonstrated bilateral activation of both inferior parietal cortex and ACC, with a further activation in the right superior frontal gyrus. The high FOP group showed additional activation for right sided dominance, with activations in supramarginal gyrus, lateral occipital, superior parietal lobe and precuneus. Furthermore, this group showed left lateralised activation in both the caudate and the putamen.

For the pain-incongruent blocks, (when the probe replaced the neutral word) only the right cerebellum was activated by both groups. The high FOP participants displayed significant bilateral activation within anterior insula, putamen, occipital lobe and inferior frontal gyrus. Additionally, right sided peak activation were present in thalamus, caudate and middle frontal gyrus, with left sided activation found in superior parietal and postcentral gyrus. The low FOP group had significant peak activation in bilateral superior parietal lobe and ACC, with further activations of the left superior frontal gyrus and right precentral gyrus.

5.6.3 Within Group Effects

To test the hypothesis that levels of pain-related fear would be associated with a hypervigilant bias for pain words the contrast pain-congruent >pain-incongruent was conducted. No significant clusters of activation were found for either group, indicating that there were no neural responses associated with hypervigilance for pain-related words.

To investigate the difference in the participants neural responses to probes that replaced the neutral word compared to pain words, the contrast pain-incongruent>pain-congruent was performed. It was hypothesised that participants with low levels of pain-related fear would avoid pain-related words. No significant clusters of activation were found. This indicates that irrespective of pain fearfulness, there was no differential processing of probes replacing neutral words compared to probes replacing pain words.

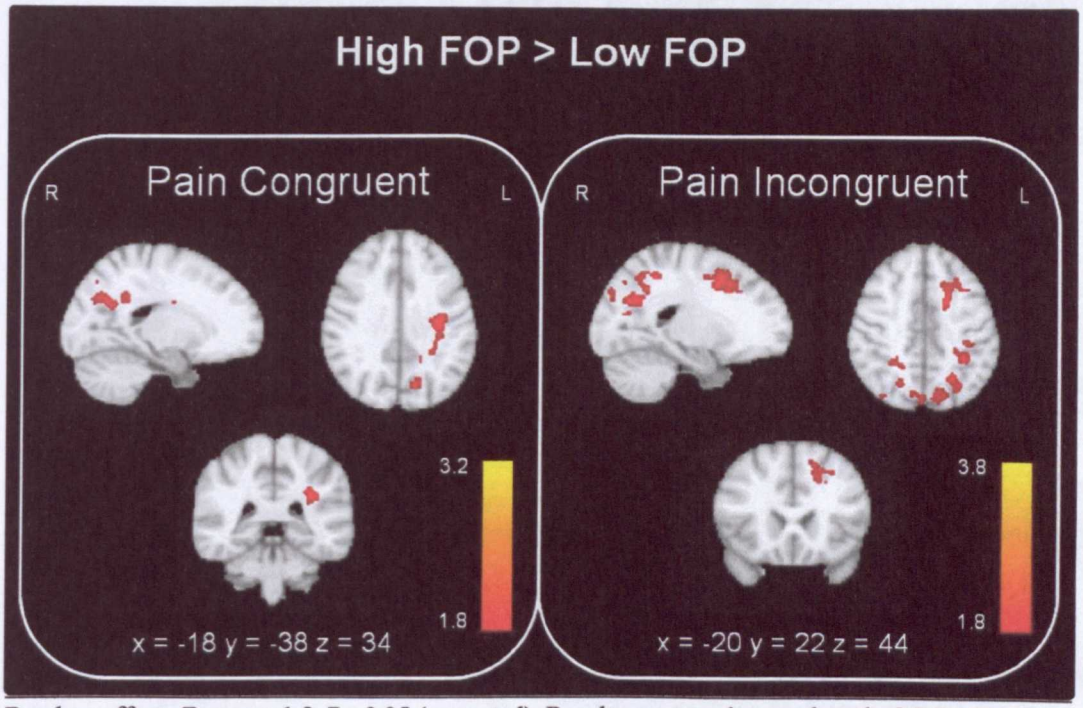
5.6.4 Effect of Group and Congruency

To investigate if the two participant groups differed significantly on their neural response to probes replacing pain-related words, the between group contrast of pain-congruent $_{\text{high FOP}}$>pain-congruent $_{\text{low FOP}}$ was conducted. The high FOP group demonstrated significantly greater left sided activation within precentral gyrus, parietal operculum, supramarginal gyrus and precuneus compared to participants with low FOP. Peak activations are reported in Table 5.6 and illustrated in Figure 5.3. The reverse contrast (pain-congruent $_{\text{low FOP}}$ > pain-congruent $_{\text{high FOP}}$) found no significant activations.

A between group contrast of pain-incongruent $_{\text{high FOP}}$>pain-incongruent $_{\text{low FOP}}$ was performed to investigate if there were any differences in cerebral activity for the avoidance of pain-related words. Significant peak activations were found in bilateral superior parietal lobe and left middle frontal gyrus, superior frontal gyrus and precuneus for the high FOP group. No significant differences in cerebral activity were found for the reverse contrast pain-incongruent $_{\text{low FOP}}$ > pain-incongruent $_{\text{high FOP}}$.

To ensure that these effects were not related to a general emotionality bias, between group contrasts were performed for both congruent and incongruent blocks containing emotional words (emotion -congruent $_{\text{high FOP}}$ > emotion-congruent $_{\text{low FOP}}$ and emotion-incongruent $_{\text{high FOP}}$ > emotion-incongruent $_{\text{low FOP}}$). No significant activations were found indicating that group effects were specific to pain-related words.

Figure 5.3 Activation Map for Between Group Contrasts



Random effects Z score >1.8, P =0.05 (corrected). Results are superimposed on the MNI 152 standard brain template.

Table 5.6 Differential Activation Between Groups

Region of Activation	Talairach Coordinate of Max Z Score			Z Value
	x	y	z	
Pain-Congruent>REST				
High > Low				
L Precentral Gyrus (BA4)	-32	-18	34	3.23
L Parietal Operculum Cortex	-38	-34	22	2.76
L Supramarginal Gyrus	-28	-42	38	2.84
L Precuneus (BA18)	-16	-76	28	2.61
Low > High				
No significant activation				
Pain-Incongruent>REST				
High>Low				
L Middle Frontal Gyrus (BA6)	-20	16	42	2.71
L Superior Frontal Gyrus (BA6)	-26	0	56	3.83
L Superior Parietal Lobe (BA7)	-34	-44	46	3.42
R Superior Parietal Lobe (BA9)	28	-44	36	3.30
L Precuneus (BA7)	-18	-62	30	3.53
Low>High				
No significant activation				

Random Effects, Z >1.8, P=0.05 (Corrected)

5.7 Discussion

5.7.1 Behavioural Findings

The present study investigated if healthy pain-free participants demonstrate selective attentional processing for pain-related words when separated into groups based on levels of pain-related fear. It was hypothesised that attentional biases would be observed towards the pain-related words for participants with high FOP, demonstrated by smaller response latencies for probes replacing pain-related words. In contrast, it was predicted that the low FOP group would demonstrate an avoidance of pain-related words, with smaller response latencies for the probe replacing the paired neutral word.

Bias indexes were calculated for the pain condition. Inspection of the index indicated that high FOP participants did respond faster to probes replacing pain-related words (pain index = 30.89ms). This effect was not demonstrated for the emotional blocks (emotional index = -0.93), indicating that it was a pain specific response. In comparison the low FOP group demonstrated avoidance effects, with the greatest effect found for the emotional blocks (pain index = -9.87ms, emotional index = -19.34ms). However, statistical analyses failed to demonstrate a significantly different effect between the groups, either related to the bias indexes, or when using mean reaction time data.

Correlational analyses found a significant negative relationship between the pain-bias index and the HADS-D score. This suggests that participants with higher levels of depression avoided the pain-related words irrespective of pain-related fear. Patients with mood disorders have been shown to demonstrate preferential processing of negative stimuli (Pincus & Morley, 2001). However, in the current study participants depression scores fell within the normal range, suggesting that avoiding negative stimuli might be a mechanism used to avoid greater levels of depression. That this effect was not found for the emotional bias index could be because the emotional condition contained both positive and negative words.

The current study was in contrast to previous findings suggesting selective attentional processing to pain stimuli by participants separated on either pain-fearfulness or other pain-related assessments such as AS (Asmundson et al, 1997; Keogh et al, 2001, 2003; Khatibi et al, 2009). Specifically, Keogh et al (2001a) used a similar recruitment method and task and demonstrated selective attentional biases for high FOP participants. However, it is of note that the bias index of the current study displayed an effect to pain-related words of a greater magnitude to that found

in the Keogh et al study. Specifically, we found an effect of 30.89ms for pain-related words in the high FOP group, compared to a 20ms effect demonstrated in the Keogh et al. study. Avoidance of pain-related words which was predicted for the low FOP group had an effect of -9.87ms, comparable to that of 8ms for the previous study. Therefore, although results of the current study failed to reach statistical significance, they did demonstrate a trend similar to that of the previous literature.

5.7.2 Neuroimaging Findings

The current study sought to act as a proof of principle that a semantic pain-related dot probe study could be administered and completed within an fMRI environment, with the intention of then administering the paradigm within a clinical pain population. Although non-significant trends were found for the behavioural results, differential fMRI activation patterns were demonstrated between the experimental participant groups. Furthermore, differences in cerebral activation patterns were also found when attention was directed towards or away from the pain-related stimuli. These results indicated that the pain-related semantic dot probe paradigm was suitable for the fMRI environment.

Specifically, increased cerebral activation was found for the high FOP group compared to the low FOP group, for blocks containing pain words, irrespective of probe location. For the pain-incongruent condition, significant differences in peak activation were localised to middle and superior frontal gyri, precuneus and bilateral superior parietal lobe. Both tasks resulted in greater activity in the left hemisphere, which is probably related to the language component of the dot probe task.

For the pain-congruent condition, the between group contrast demonstrated significant activity in precentral gyrus, parietal operculum cortex, supramarginal gyrus and precuneus. The activity within the precentral gyrus was found in Brodmann Area 4, which is the premotor cortex. Previous studies have demonstrated that during experimental pain paradigms, the premotor cortex may become activated in preparation for movement away from the pain stimulus. That the premotor cortex was activated when attention was directed towards the pain-related words, suggests that higher levels of pain-related fear may result in behaviour that urges escape. In persistent pain conditions, escape behaviours may lead to avoidance behaviours which negatively impact the pain experience. The continuous use of avoidance behaviours may lead to dissociations between pain and circumstances that can elicit pain, partly through a lack of opportunities to disconfirm these beliefs (Crombez et al, 1999).

Additional activation found for the high FOP group during the pain-congruent blocks, was found within the supramarginal gyrus, parietal operculum cortex and precuneus. These regions have been found to increase activity during noxious pain stimulation (Lloyd et al, 2008; Peyron et al, 2000). A recent study investigating medication overuse headache (MOH) found increased activity of the supramarginal gyrus to painful mechanical stimulation in both healthy controls and MOH patients after medication withdrawal (Ferraro, Grazzi, Mandelli, Aquino, Di Fiore, Usai & et al, 2011). Similarly, the parietal operculum has been associated with the cortical representation of pain (Treede, Apkarian, Bromm, Greenspan & Lenz, 2000; Kurth, Zilles, Fox, Laird & Eickhoff, 2010; Schlereth, Baumgärtner, Magerl, Stoeter, & Treede, 2003) and with deficits in pain sensation in patients with opercular lesions (Greenspan & Winfield, 1992). These findings suggest that when participants with high levels of pain-related fear selectively attend to pain-related words, they activate areas associated with pain processing.

Previous studies using pain words as cues have demonstrated increased activity in pain-related areas. Gu & Han (2007) found increased activity in PFC and somatosensory cortex when participants rated the intensity of pain words. Osaka, Morishita, Kondo & Fukuyama (2004) found increased ACC activation when participants listened to expressions of suggestive pain compared to nonsense words. The retrieval of autobiographical pain-related memories increased activity in both ACC and IFG compared to recollections for non-pain-related words (Kelly et al, 2007). Finally, two studies investigating imagining pain situations using pain words as cues, demonstrated increased activity in several pain processing areas including, parietal operculum, supramarginal gyrus, ACC, insula and frontal regions in both migraine patients and pain-free controls (Eck et al, 2011; Richter et al, 2009). In the current study, the participants with high FOP had increased activity in areas associated with the sensory-discriminative aspect of the pain experience, to pain-congruent blocks. The lack of activation in areas associated with the cognitive aspects of the pain experience could be because the participants were not explicitly instructed on how to process the words. The dot probe task only requires a response to a probe, therefore elaboration of the meaning of the word was not required.

During the pain-incongruent blocks, participants with high FOP had increased activity of precuneus and bilateral superior parietal lobe. Parietal activation has been demonstrated for the attentional processing of noxious stimulation (Peyron et al, 2000). Peyron, Garcia-Larrea, Gregoire, Costes, Convera, Lavenne & et al

(1999) applied thermal pain of varying intensity to the hand under three attentional conditions; attend towards the stimulus; divert attention away and no attentional task. Increased activation of the parietal cortex was found for the attentional tasks only, irrespective of stimulus intensity. Therefore if areas of pain processing are activated by the pain-related words, it might appear that when attention needs to be diverted away areas associated with the attentional aspects of pain processing also increase activity.

However, in recent years, the concept of the pain matrix as a pain specific network has been investigated (Apkarian et al, 2001; Neugebauer et al, 2009). A recent study by Mouraux et al (2011) questioned the specificity of areas associated with the pain matrix for pain processing with two experiments. In the first study, experimental stimuli were presented in different modalities (nociceptive, non-nociceptive, visual and auditory). The second study consisted of an auditory oddball paradigm. The results indicated increased BOLD activity in several areas of the pain matrix including the somatosensory cortex, insular cortex and ACC indicating that these areas are multimodal and not pain specific. Therefore, an equally plausible explanation for increased activity within areas associated with pain processing, is that these areas are activated as part of a general attentional network or as part of sematic and visual word processing.

Increased activity of the supramarginal gyrus, precuneus and superior parietal regions, have previously been found in studies using tasks of visual word processing and tasks involving 'top down' processing, whereby the goals of the task are dictated by the will of the individual. Stoeckel, Gough, Watkins & Devlin (2009) applied TMS to the supramarginal gyrus when participants were completing semantic and phonological word tasks. Although the results indicated task facilitation rather than the expected disruption, both tasks were equally affected by the TMS, indicating that this area is associated with both aspects of word processing. In relation to the pain-congruent blocks, the bias index would suggest that participants with high levels of pain-related fear preferentially attend to the pain-related words. Therefore activity with the supramarginal gyrus may be associated with the speeded recognition of the pain-related words. As such, when attention has to be reoriented, as is the case in the pain-incongruent blocks, regions associated with top down attentional processing (goal-directed behaviour) increased activity, namely parietal and frontal regions (Legrain, Van Damme, Eccleston, Davis, Seminowicz & Crombez, 2009). Superior parietal lobe has been associated with shifts in attention between different visual

fields. Yantis, Schwartzbach, Serences, Carlson, Steinmetz, Pekar & et al, (2002) used event-related fMRI to investigate neural activity associated with shifts in attention between two peripheral spatial locations. Activation of the right superior parietal cortex was demonstrated only with shifts in attention and not when the attentional state was being maintained. Finally, the pain-incongruent blocks resulted in increased activity in middle and superior frontal regions. An empirical investigation conducted by Fan et al, (2005) used an event-related fMRI attentional network task to investigate three types of attention; alerting, orienting, and executive control networks. Increased activation of the middle frontal gyrus was found for incongruent trials for executive attention, and superior frontal gyrus was found to be activated for orienting attention towards a spatial cue. Although the incongruent trials contained within the current study do not violate a task assumption, the probe replaced a word that was theoretically incongruent to the participants' attention and which therefore required attention to be oriented away from the pain-related word. Activations found for participants with high FOP included structures associated with pain processing, word comprehension and attentional shifts.

5.8 Strengths and Limitations

An explanation for not finding a behavioural selective attentional bias could be due to the small participant numbers. Due to a technical difficulty behavioural data was only collected for twenty three of the twenty eight participants. Selective attentional biases are demonstrated by effects in the tens of milliseconds. The current study did produce small effects, but with large standard deviations. Therefore, due to the small study number the study may have lacked statistical power. The trends exhibited by the groups may have become statistically significant with greater participant numbers.

The between group difference for the low and high FOP groups was smaller than previous studies, with only a between group difference of 16 points (Low FPQ 68/ High FOP 84; Keogh et al, 2001; FPQ = 70/96, 26 point differences and Roelofs et al, 2003a; FPQ = 62/85, 23 point difference). Although the difference was great enough to allow differential cerebral activation to be observed, significant differences in behavioural results may be better demonstrated when the difference in pain fearfulness is more extreme.

Although every care was taken with the word stimuli, some homographs remained within the pain-related condition. However, these words have been utilised within previous literature investigating selective attentional biases to pain words. Furthermore, as this was a block design paradigm, it could be assumed that the other pain-related words contained within the experimental block primed the interpretation of the words.

Although a non-semantic task was included to examine task performance, blocks containing neutral-neutral word pairs were not included within the paradigm. The lack of semantic processing meant that as a low level visual task, it was not a suitable baseline measure for congruency effects calculated, which would have allowed for an investigation of the direction of attention, such as disengagement effects. However, not all studies investigate congruency effects, making the use of the bias index comparable with previous literature.

5.9 Conclusion

The current study verified that a pain-related dot probe task could be successfully completed within the fMRI environment. Furthermore, that differential cerebral activation can be found for pain-related words according to the attentional focus induced by the tasks. In addition the fMRI findings demonstrate that even when behavioural effects are not found, there may still be differential attentional processing of pain-related stimuli. This suggests that pain-free individuals who have high levels of pain-related fear may have a predisposition to attend to pain-related stimuli. In the event of an acute pain experience, this may place them at a greater risk for the misinterpretation of the pain experience, resulting in cognitions and behaviour which may ultimately lead to a transition from acute pain to persistent pain.

CHAPTER SIX
Attentional Bias in Chronic Low Back Pain Patients Attending a Pain Management Programme

6.1 Introduction

The fear-avoidance model of pain (Vlaeyen & Linton, 2000) regards hypervigilance as a possible mechanism for exacerbating and maintaining chronic pain conditions. The empirical support for hypervigilance to pain-related stimuli, measured through selective attentional biases, has produced inconsistent results. In chronic pain populations dot probe studies have reported attentional biases for sensory pain words (Dehghani et al, 2003; Haggman, Sharpe, Nicholas, & Refshaugez, 2010; Liossi et al, 2009; Sharpe et al, 2009), but other studies have failed to replicate this (Asmundson et al, 2005; Roelofs et al, 2005; Please refer to Chapter 1.7.3 for a discussion on pain-related attentional biases).

If pain-related fear mediates a selective attentional bias to pain-related stimuli, as proposed by the Fear-avoidance model, interventions which reduce pain fearfulness should produce a concomitant change in selective attentional processing. The psychological approach to pain treatment, cognitive-behavioural therapy (CBT) targets the emotional, psychological, and social factors believed to maintain and exacerbate the pain condition. Originating from the work of Albert Ellis and Aaron Beck, CBT challenges misconceptions and distorted beliefs, such as pain-related fear and fear-avoidance behaviours. It relies on patients learning new behaviours, abandoning or revising existing behaviours, and challenging unhelpful and negative ways of constructing their pain experiences (Curran, Williams, & Potts, 2009; Gamsa, 1994). Frequently CBT is combined and applied as part of a multidisciplinary pain management programme (PMP), using a variety of treatments including relaxation, biofeedback, stress management, behavioural pacing and meditation (Molton, Graham, Stoelb, & Jensen, 2007).

The effectiveness of CBT and pain treatment programmes for treating components of the Fear-avoidance model has been investigated. Specifically, Woby, Watson, Roach, & Urmston (2004) found that PMP-associated reductions in fear-avoidance beliefs about work and physical activity were related to reductions in

disability. Similarly, reductions in disability and pain intensity levels have been associated with reduced levels of catastrophising (Burns, Kubilus, Bruehl, Harden, & Lofland, 2003; Sullivan, Stanish, Waite, Sullivan, & Tripp, 1998; Turner, Mancl, & Aaron, 2005). A meta-analysis conducted by Hoffman, Papas, Chatkoff & Kerns (2007) investigating the effectiveness of psychological intervention in CLBP, reported positive effects for psychological interventions on pain intensity, quality of life and depression. Morley (2011) refers to the efficacy of CBT treatments, whereby CBT is superior to no treatment and the relative efficacy of CBT, whereby it may be marginally superior when compared against other treatments.

The primary aim of CBT is changing maladaptive cognitions. Therefore, changing pain-related fear may allow the individual to overcome beliefs which are believed to maintain a pain condition. The success of both PMP and CBT in reducing pain-related fear allows for this prediction to be investigated. Research investigating selective attentional processing within emotional disorders has already demonstrated that anxiety-related processing biases are sensitive to cognitive behavioural change (Legerstee, Kallen, Dieleman, Treffers, Verhulst & Utens, 2009). To date, one study has examined selective attentional biases in chronic pain patients undergoing a three week multidiscipline PMP (Dehghani et al, 2004). Chronic musculoskeletal pain patients were tested on a semantic dot probe task containing sensory, affective, disability and threat words. Patients were tested when starting the programme, immediately upon completion and at a one month follow-up. At pre-treatment, patients demonstrated a hypervigilance to sensory pain words, which was still evident at post-treatment. However, this attentional effect was not present at follow-up. Furthermore, change in fear of movement assessed using the TSK was predictive of concomitant changes in attentional bias. The results of this study support a relationship between pain-related fear and hypervigilance in chronic pain patients and that attendance of a PMP can reduce both pain-related fear and selective attentional processing of pain-related information. The study is not without its limitations. Primarily, these relate to the lack of a control group and the heterogeneity of the patients. Both these factors limit the generalizability of the findings. The attentional effect might be present in pain-free controls, or may be condition specific, but the inclusion of several pathologies is obscuring this effect. However, as all patients were enrolled upon a PMP, it might be inferred that they share some similarities in the ability to cope with their pain conditions.

The aim of the current study was to overcome these concerns by studying selective attentional bias in a homogenous group of patients, namely CLBP patients who display pain behaviour (Please refer to Chapter 1.5 for an in-depth discussion). Pain-free volunteers were included as a control group. Pain participants completed a dot probe task prior to and upon completion of an intensive multidisciplinary PMP. It was predicted that prior to attending a PMP chronic low back pain patients will demonstrate a selective attentional bias for pain-related words. Specifically it was hypothesised that they would respond faster to probes when they replaced a pain-related word. Furthermore, upon completion of the PMP, it was expected that patients would display a significant reduction in their selective attentional processing of pain-related words.

The study presented in the preceding chapter (Chapter Five) found differential activation patterns in pain-free participants separated into groups based upon levels of pain-related fear. This confirmed that a semantic dot probe task could successfully be conducted within the fMRI environment. Therefore to extend the current knowledge regarding selective attentional biases within chronic pain populations, the current study aimed to investigate if chronic low back pain patients demonstrate enhanced cortical activations in response to pain-related words, both as an indication of pain-related fear but also as an indication of pain status. Specifically, cerebral activation patterns were predicted to differ between pre-PMP and post-PMP testing (pain-related fear) and between CLBP patients and pain-free controls (pain state).

While differential activation patterns were demonstrated in the dot probe study presented in Chapter Five, the mechanism underlying attentional biases in CLBP patients may differ to those found in pain-fearful healthy individuals. Specifically, as threat-related processes are believed to underlie selective attentional biases, it was predicted that areas associated with the detection and response to threat, such as the PFC and the amygdala will show enhanced activation to pain-related words (Bishop, 2008). Amygdala activation has been demonstrated in tasks using fearful faces (Carlson, Reinke, & Habib, 2009) and by threat-related semantic stimuli in phobic controls (Britton et al, 2009) and patients with major depressive disorder (Canli et al, 2004).

6.2 Methods

6.2.1 Ethical Approval

The study was approved by the Sefton Local Research Ethics Committee. The study recruited chronic pain patients and as such further ethical approval was sought and granted by the Research Governance Committee at the Walton Centre for Neurology and Neurosurgery and Aintree Hospital Trust (NHS). All participants signed an informed consent form before study participation.

6.2.2 Participants

Inclusion and exclusion criteria

To be recruited and included within the study, clinical participants had to meet the following criteria; (i) their primary pain condition had to be chronic low back, although other concomitant pain conditions were allowed, as long as they were secondary; (ii) they completed a 16-day PMP at WCNN. Failure to complete the full programme would result in exclusion from the post-PMP testing; (iii) they had positive scoring on 4-5 out of 5 categories on the Waddell Signs, indicative of pain behaviour.

To ensure the pain population had similar clinical profiles the exclusion criteria for CLBP patients were; (i) recent (<1 year) spinal surgery; (ii) 3 or more degenerate discs; (iii) evidence of concomitant radicular pain. Finally, patients were excluded if they were taking strong opioids, hypnotics or high doses of central nervous drugs (greater than; amitriptyline-50mg/day; gabapentin-1200mg/day; codeine/dihydrocodeine-240mg/day). Paracetamol and anti-inflammatory drugs were allowed in recommended doses. Any drug intake must have been stable for 4 weeks minimum.

Inclusion criteria for the control group were; (i) that they were currently pain-free and did not have a history of a previous pain condition; (ii) that they were of a similar age to the clinical group, therefore, a minimum age of thirty five was set.

For both study groups the inclusion criteria were that all participants had to be right handed and have English as a first language. They were excluded if they had any evidence of co-existing neurological disease, stroke, brain injury, history of meningitis, history of substance misuse, post-traumatic stress disorder, dementia or severe psychiatric disease, such as psychosis or bipolar disorder.

If participants had any MRI contraindications such as internal metal, pregnancy and claustrophobia, they were excluded from the study. Finally, due to the

nature of the experimental task, participants were excluded if they suffered from any reading impairments, such as dyslexia.

Recruitment

The clinical group were all recruited at the WCNN Pain Management Programme Assessment clinic (PMPAC). Participants are required to complete a battery of questionnaires, and were assessed by a pain specialist, physiotherapist, an occupational therapist and a clinical psychologist. If deemed suitable, a place will be offered to the patient, with a start date for the PMP programme being agreed upon.

Prior to the assessment clinic, the case notes for all patients attending the PMPAC were reviewed for suitability, which was based upon the exclusion and inclusion criteria cited above. Any possible recruits who were offered a place on the 16-day PMP were interviewed immediately. During the interview, the inclusion and exclusion criteria were discussed and the WS were conducted. The WS and all other clinically related decisions were conducted by two clinical specialists (AS/TJN). Those who met the inclusion criteria were then informed about the requirements of the study. If a patient did not meet the study requirements they were thanked for their time and were not detained any further.

Initially a randomisation process was used to assign participants to one of two groups (PMP group/Waitlist group). All randomisation numbers were computer generated. However, three waitlist participants were excluded from the study as they attended the PMP within two weeks of recruitment. Due to a limited number of possible recruits, the randomisation procedure was removed. Instead every consecutive recruit was automatically allocated into the PMP group. Recruitment for the waitlist group would occur after completion of the PMP group recruitment.

Participants were scanned the week prior to PMP attendance and the week following completion of the PMP. Eight participants were recruited but were not scanned due to scanner malfunction, claustrophobia, possible MRI contraindications and significant pain levels preventing test attendance.

Healthy volunteers were recruited through advertising upon the University of Liverpool's intranet announcement system, MARIARC's volunteer files and word of mouth. All healthy volunteers received an information sheet and were questioned about their suitability for the study based upon the inclusion and exclusion criteria cited above. Five participants were recruited but not scanned due to claustrophobia and medication issues. In total fifteen controls were scanned for the study.

All scanning was conducted at the University of Liverpool's MARIARC centre. Prior to scanning all participants underwent a full screening by the centre's radiographer (VA) to ensure MRI suitability. All participants were naive to the aims of the experiment. Healthy volunteers were debriefed upon completion of their scanning session, whereas patients were debriefed after their second scan session.

6.2.3 Pain Management Programme

The Pain Management Programme attended by the chronic pain patients is an intensive multidisciplinary programme. Attendance is for 16 days, four days a week, for a period of four weeks. A typical day runs from 9am until 4:30pm each day. The PMP includes psychological group discussions, relaxation training, physiotherapy treatments, pacing techniques, problem solving, goal setting, graduated exercises and cognitive therapy. The aim of the programme is to improve disability functioning, increase confidence, reduce excessive medication intake and encourage patients to resume avoided activities. A reduction in sensory pain levels is not an aim of the programme.

6.2.4 Participant Demographics

Participant recruitment resulted in fourteen CLBP patients being tested pre-PMP and twelve of these patients returned for post-PMP testing. The clinical group consisted of 10 females and 4 males, with an age range of 34-54 years. The control group had fifteen volunteers, with 11 females and 4 males and an age range of between 37-58 years of age.

All participants had near normal or corrected-to-normal (with contact lenses) visual acuity, thus ensuring that all words could be seen clearly.

Summary information for the participants is presented in Table 6.1.

Table 6.1 Participant Demographics

Clinical Group	Age	Sex	D mths	WS	BDI	STAI (State)	STAI (Trait)	TSK	PCS	PASS
1	48	F	132	5 / -	44 / 31	- / 35	- / 50	- / 24	38 / 22	96 / 112
2	49	M	264	4 / 0	38 / 4	41 / 66	58 / 59	30 / 26	43 / 21	154 / 94
3	43	M	133	5 / 3	28 /-	62 / 30	64 / 54	35 / 24	34 / 21	152 / 126
4	46	F	156	4 / 1	31 / 38	37 / 27	59 / 37	31 / 28	40 / 34	96 / 146
5	52	M	18	5 / 3	9 /-	42 / 44	44 / 43	28 / 24	23 / 22	98 / 76
6	43	F	96	4 / 3	- / -	43 / 56	51 / 55	38 / 31	- /-	- / -
7	50	F	120	5 / -	18 / 28	39 / -	53 / -	20 / -	3 / 4	120 / 56
8	48	M	72	4 / 3	33 / 2	33 / 29	38 / 32	23 / 21	16 / 7	98 / 74
9	54	F	240	4 / 3	47 / 38	52 / 52	52 / 59	20 / 27	33 / 36	150 / 134
10	45	F	60	5 / 3	25 / 12	43 / 31	51 / 42	31 / 17	39 / 9	114 / 52
11	50	F	19	5 / 3	25 / 16	58 / 30	54 / 37	39 / 21	32 / 11	118 / 34
12	44	F	120	5 / -	24 / 6	43 / -	40 / -	32 / -	20 / 1	112 / 68
13	53	F	18	4 / 2	23 / 19	41 / 51	53 / 57	33 / 32	45 / 15	120 / 90
14	34	F	36	4 / 2	27 / 18	46 / 46	59 / 56	27 / 23	34 / 19	76 / 60
Mean	47	11F	106		28.6/21.8	43.5/38.2	52.2/44.6	27.6/21.2	30.8/18.8	110/110
(S.D)	5		78		9.8/14	8.6/16.8	7.8/16.3	9.8/.9.8	11.6/12.2	20/42.5
Control										
1	44	F			0	20	20			
2	43	M			3	26	26			
3	37	M			1	20	41			
4	40	M			0	22	22			
5	51	F			0	20	20			
6	58	F			5	24	30			
7	46	F			0	23	24			
8	56	F			2	24	29			
9	49	F			0	20	25			
10	44	F			12	35	20			
11	50	F			11	20	33			
12	37	F			5	25	34			
13	39	M			4	31	32			
14	38	F			2	41	24			
15	37	F			1	38	29			
Mean	45	10F			3	25.9	27.2			
(S.D)	7				3.8	7	6			

D (mths)-pain duration in months. Clinical self-report scores are indicated by pre-PMP/post-PMP values.

6.2.5 Self-report Measures

A detailed discussion of the self-report measures used can be found in Chapter 3.6. For the clinical cohort, the BDI, PCS, and PASS were administered at the PMPAC (pre-PMP scores) and upon completion of the PMP (post-PMP scores). The STAI was completed on the day of scanning by all participants. Additionally, the control group completed the BDI at this time, with the clinical group completing the TSK at both scanning sessions.

Pain-related fear was measured twice in the clinical cohort, through the completion of the TSK and the PASS. PCS completion ensured a measurement of catastrophising levels. Therefore, the two components believed to precede hypervigilance, as suggested by the Fear-avoidance model, were measured. The BDI and STAI were completed to investigate if any attentional effects were related to depression or anxiety levels.

6.3 The Dot-Probe Task

6.3.1 Word Selection

The wordlists generated for the dot probe task consisted of pain-related, emotional and neutral words. The emotional wordlists contained only negative words and were used to ensure results did not reflect a negative emotional bias. Neutral wordlists were created from household related words. They were used both to accompany the experimental stimuli as a word pair and also as a neutral-neutral word pair to replace the non-semantic condition used in the previous attentional bias study presented in Chapter 5.

An initial wordlist for the pain-related words was created from the wordlist used in Chapter 5 (see Table 5.3). Each individual word was then inputted into the online database of the English Lexicon Project (ELP), where values were gathered for word length, two measures of frequency, and behavioural data from a lexical decision making task and a speeded response task (for additional detail of the ELP see Chapter 3.4.3). Words with frequency values that could not be matched successfully were removed, resulting in a wordlist containing 14 words. Similarly to the previous study, these values were used as a baseline to create the other wordlists.

The wordlists for the emotional and neutral-neutral conditions were matched with the pain-related words on a word by word basis for letter length. Word frequency, number of syllables and behavioural data were controlled for on a

groupwise basis. The wordlists contained fourteen word pairs, for each of the three conditions. For counterbalancing purposes each word pair is presented four times, resulting in a total of 168 trials. The list of word pairs used is presented in Table 6.2 with descriptive statistics being presented in Table 6.3.

The completed wordlists were given to three colleagues who were asked individually to sort the words into three unnamed categories. All sorters successfully separated the words into their respective categories. This provided support that the words were representative of the lists they had been placed into.

Table 6.2 Word Stimuli for Dot Probe Task

Pain-Neutral		Emotional-Neutral		Neutral-Neutral	
gash	sill	glum	wick	turf	pram
itch	rake	mope	vase	loft	pail
sore	tidy	wail	bunk	pane	sofa
agony	stool	snide	tiled	broom	decor
cramp	grill	loner	patio	dryer	foyer
bruise	tenant	sorrow	beaker	gutter	saucer
sprain	pillow	feeble	washer	teflon	duster
twinge	kettle	sombre	boiler	teapot	tripod
blister	spatula	anguish	cushion	chimney	platter
inflamed	ornament	grieving	doorbell	vacuumed	doorstep
pinching	radiator	lonesome	dwelling	wardrobe	driveway
stinging	teaspoon	revolted	emulsion	saucepan	pendulum
discomfort	windowpane	unbearable	renovation	thermostat	tablespoon
excruciating	refurbishing	helplessness	housekeeping	conservatory	condensation

To ensure that wordlists were not significantly different, a 3 x 5 ANOVA was performed. Word valence (pain/emotional/neutral) was the independent variable and word length, word frequency, number of syllables and mean reaction times (lexical decision making/naming tasks) were the dependent variables. No significant main effects or interactions were found (all F-values <1).

Table 6.3 Length, Syllables, Frequency and Behavioural Data per Wordlist

Wordlist	Length	No. of Syllables	Frequency	RT. Lexical Task	RT. Naming Task
Pain	93	26	6.35	707.51	675.35
Neutral-Pain	93	31	6.51	718.83	675.43
Emotional	93	28	6.07	738.24	648.63
Neutral-Emotional	93	29	6.42	701.39	647.33
Neutral-Neutral	93	29	6.47	704.40	675.58
Neutral-Neutral	93	30	6.62	727.73	667.01

Neutral-Pain and Neutral-Emotional indicate the neutral words paired with the experimental words. Frequency calculations based on Hyperspace Analogue to Language (HAL) frequency norms (Lund & Burgess, 1996). Neutral-neutral wordlists were divided as though two separate wordlists.

6.3.2 Visual Display

The stimuli were presented using the Presentation® Program (Neurobehavioral systems http://www.neurobs.com) on an Acer laptop (Travelmate 8100) using a LCD projector (Epson LMP73000). Stimuli were projected onto a screen placed at the foot of the scanner bed. Participants were able to see the screen using a mirrored periscope attached to the head coil.

6.4 fMRI Task Design

This study used an event-related dot probe task. This was chosen as the most appropriate design for the current study as it would mimic the behavioural testing used in previous research and remove possible task habituation. Trials were presented in a random manner. Participants were given the opportunity to have a practice session prior to scanning. (For a detailed discussion regarding the dot probe tasks please refer to Chapter 3.4.)

To allow for distributed sampling in relation to stochastic designs, null events were included. These consisted of the fixation cross remaining on the screen for the duration of a trial period, 3seconds. As event-related designs do not contain a REST period, null events are used to provide a baseline measure. A heuristic guide to calculating the number of null events needed for an adequate baseline, is to use >20% of the total number of trials i.e. experimental trials and null events, (Friston et al, 1999). A total of 50 null events were included. Distributed sampling was also achieved by offsetting the individual trial length (3s) with the TR (2.5s).

Further discussion on fMRI task design and distributed sampling can be found in Chapter 3.3.4.

6.4.1 Scanning Protocol

All scanning sessions were run by a trained radiographer (VA). Scanning was performed on a 3-Tesla Siemens Trio MRI scanner (Erlangen, Germany). FMRI was performed with a blood oxygenation level-dependent (BOLD) sensitive T_2^*.weighted multislice gradient echo EPI sequence (TE = 30ms, TR = 2.5s, flip angle = 90°, FOV = 192 x 192 matrix). Thirty-Two 2.5mm thick axial slices, with 1mm gap, were prescribed 30degrees to the AC-PC line and covered the entire brain. The overall scanning time was 11 minutes 26 s with 274 volumes being collected. To prevent participants from becoming fatigued and to ensure task compliance, the task was divided into two runs of 5 minutes 43s.

For the purpose of anatomical referencing and visualisation of brain activation, a high-resolution T_1.weighted 3D MP-RAGE sequence was also acquired for each participant (TE = 4.52ms, TR = 2300ms, TI = 1100ms, with an in-plane resolution of 0.6 x 0.6mm, FOV = 200 x 200matrix), with 192 axial slices covering the whole brain.

6.5 Behavioural Analysis and Results

6.5.1 Participant Demographics

Participant demographics can be seen in Table 6.1. Independent t-tests were performed on participant age, gender and the various self-report measures between patient and controls. Differences were found to be unreliable for age ($t(27) = 1.081$, $p=0.289$) and for gender ratio ($t(27) = 0.111$, $p=0.913$). Unsurprisingly significant differences were found for BDI ($t(27) = 9.308$, $p=0.001$, STAI-S ($t(26) = 6.508$, $p=0.001$ and STAI-T ($t(26) = 9.580$, $p=0.001$, due to patients scoring higher on all measures.

For the patient group, paired t-tests were conducted to investigate the change from pre to post-PMP on all self-report measures and WS. Significant differences were found for the PCS ($t(11) = 4.873$, $p=.001$; TSK ($t(10) = 2.704$, $p=.02$; BDI ($t(12) = 2.419$, $p=0.03$, and WS ($t(10) = 7.416$, $p=.001$. In each case pre-PMP scores were higher on the measures.

6.5.2 Data Reduction

All responses that fell below 300msec or above 1000msec were removed as outliers. Analysis of the number of correct responses revealed that errors and outliers accounted for less that 5% of the responses. They were excluded from further statistical analysis, (See Table 6.4 for group mean reaction times). Similarly to the dot probe study presented in Chapter Five, median reaction time latencies were used to create individual means prior to analyses.

6.5.3 Reaction Time Data

To investigate the dot probe reaction time data, a mixed factorial ANOVA was conducted. Group (controls/pre-PMP) was the between-groups factor, and wordtype (pain vs. emotional), target word location (upper vs. lower) and probe location (upper vs. lower) were the within-group factors. There were no significant main effects or interactions demonstrated ($P > 0.05$).

It had been hypothesised that post-PMP scores would differ significantly from pre-PMP scores. A repeated measures 2 x 2 x 2 ANOVA was conducted to investigate the effects of the PMP on reaction times. Within-subjects factors were time (pre/post-PMP), wordtype (pain vs. emotional), target word location (upper vs. lower) and probe location (upper vs. lower). A significant three-way interaction of time by wordtype by word location was found $F(1,11)=5.177$, $p=0.044$ reflecting the finding that at pre-PMP testing, pain-related words in the lower position were responded to more slowly than at post-PMP testing. Additionally, a significant two-way interaction was found for wordtype by probe location $F(1,11) =14.924$, $p=0.003$ indicating that during the pain-related word condition, slower reaction times were found for probes in the upper position.

Table 6.4 Mean Dot Probe Response Times of Word Valence (pain vs. emotional), Target Word Location (upper vs. lower), Probe Location (upper vs. lower) by Group

	Pre-PMP	Post-PMP	Controls
Pain upper/dot upper	614 (109)	621 (110)	573 (117)
Pain upper/dot lower	604 (114)	601 (119)	566 (130)
Pain lower/dot upper	631 (90)	627 (85)	558 (110)
Pain lower/dot lower	633 (100)	604 (121)	580 (132)
Emo upper/dot upper	613 (117)	600 (109)	573 (132)
Emo upper/dot lower	621 (116)	601 (115)	573 (134)
Emo lower/dot upper	598 (75)	596 (123)	562 (117)
Emo lower/dot lower	608 (99)	595 (104)	577 (118)
Neutral	615 (88)	613 (81)	583 (134)

Standard Deviation contained within parentheses, ms-milliseconds.

To investigate congruency effects, a mixed factorial 2 x 2 x 2 ANOVA was conducted with within-subjects factors of wordtype (pain/emotional) and congruency (congruent/incongruent) and a between-subjects factors of group (controls/pre-PMP). A significant main effect was found for congruency $F(1) = 5.060$, $p=0.034$. However, neither of the interactions involving group were significant. The significant congruency effect reflected the fact that mean reaction times were slower for probes replacing the experimental words.

A repeated measures 2 x 2 x 2 ANOVA was conducted to investigate the effects of the PMP on congruency reaction times. Within-subjects factors were time (pre/post-PMP), wordtype (pain/emotional) and congruency (congruent/incongruent). There were no significant main effects or interactions demonstrated ($P>0.05$).

6.5.4 Bias Indexes

Three measures were calculated for the reaction time data, a bias index, a congruency index and an incongruency index (Summary information is shown in Table 6.5). Discussion of the equations used for index creation can be found in Chapter 3.4 (Equations 3.16 – 3.18). A positive score on the bias index indicates an attentional effect, with a negative score representing avoidance. In contrast, for both the congruency and incongruency indexes, as they are compared to the neutral condition, a positive score indicates an avoidance effect (slower than baseline) and a negative score indicates an attentional effect (faster than baseline).

Table 6.5 Descriptive Statistics for Index Calculations

Word Type	Group	Indexes (ms)		
		Bias	**Congruency**	**Incongruency**
Pain	Pre-PMP	-60.5(286)	85.5(341)	25.0(266)
	Post-PMP	20.5(317)	-9.2(376)	11.4(303)
	Controls	-144.4(325)	-64.0(312)	-208.2(453)
Emotional	Pre-PMP	-7.9(433)	-45.1(384)	-53.1(324)
	Post-PMP	7.4(298)	-155.7(427)	-148.2(541)
	Controls	-76.6(245)	-75.0(415)	-151.6(388)

Standard Deviation contained within parentheses, ms-milliseconds.

To investigate if bias indexes differed between groups, a mixed factorial 2 x 2 ANOVA was performed with bias indexes (pain/emotional) and group (pre-PMP/controls) as within and between group factors respectively. To extend this enquiry to congruency effects, a further 2 x 2 x 2 ANOVA was conducted with congruency (congruent/incongruent) and wordtype (pain/emotional) as within group factors and a between group factor of group (pre-PMP/controls). No significant main effects or interactions were found for either analysis ($F<1$).

We hypothesised that response latency pre-PMP would significantly differ to post-PMP response. To investigate if a significant change occurred for bias indexes two repeated measures ANOVA's were performed. Firstly, the bias indexes (pain/emotional) were compared against time (pre-PMP/post-PMP). Secondly, congruency effects over time (pre-PMP/post-PMP) were investigated by the within group factors of congruency (congruent/incongruent) and wordtype (pain/emotional). No significant main effects or interactions were reported ($F<1$).

Finally, one sample t-tests were run on all of the indexes by group (controls/pre-PMP/post-PMP). A score of zero on a bias index is indicative of no attentional effect. None of the indexes were significantly different from zero for any group ($P>0.05$) indicating that no attentional effect was recorded.

Correlational Analyses

To determine if the results of the indexes could be attributed to pain-related fear, depression or anxiety, a series of correlations were conducted between the self-report measures and the bias indexes.

Results indicated associations with the emotional bias index and trait anxiety scores of the STAI for the pre-PMP group ($r = 0.56$, $p = 0.045$). Similarly the control group had a significant association between the emotional bias index and state anxiety levels ($r = 0.55$, $p = 0.032$).

The pain congruency index was significantly associated for the pre-PMP group and the PASS ($r = 0.771$, $p = 0.001$), and for the post-PMP group the responses on the pain congruency index were significantly related to BDI scores ($r = 0.62$, $p = 0.041$).

Figure 6.2 Bias Index (±SEM indicated by error bars) for Pain Words by Group

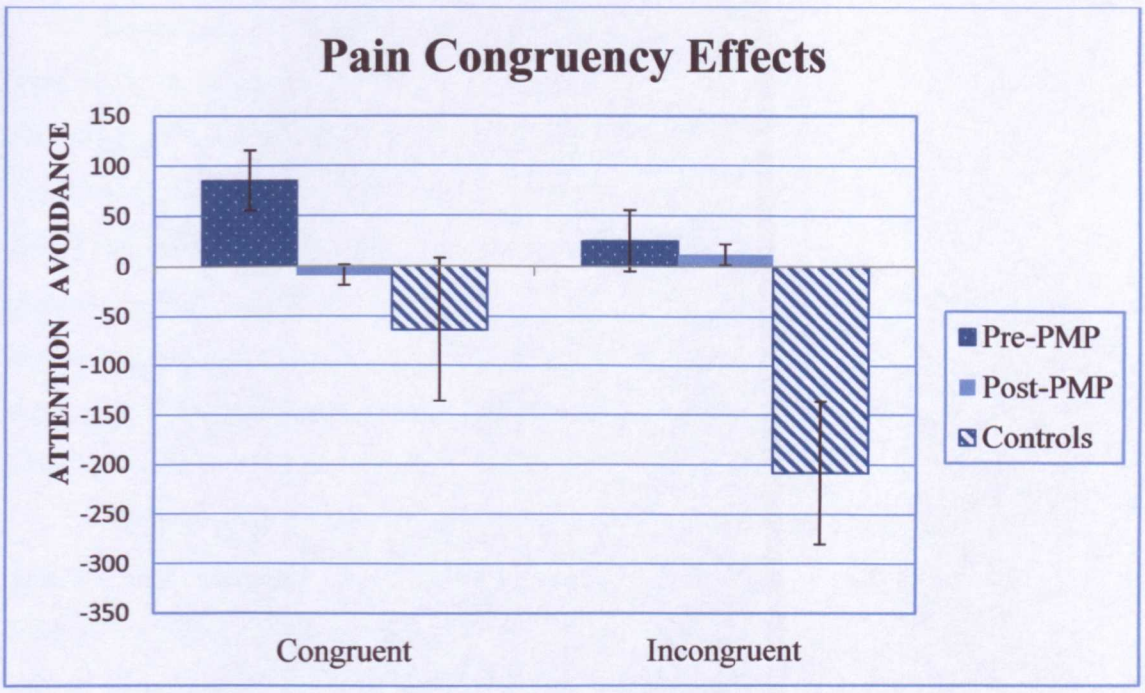
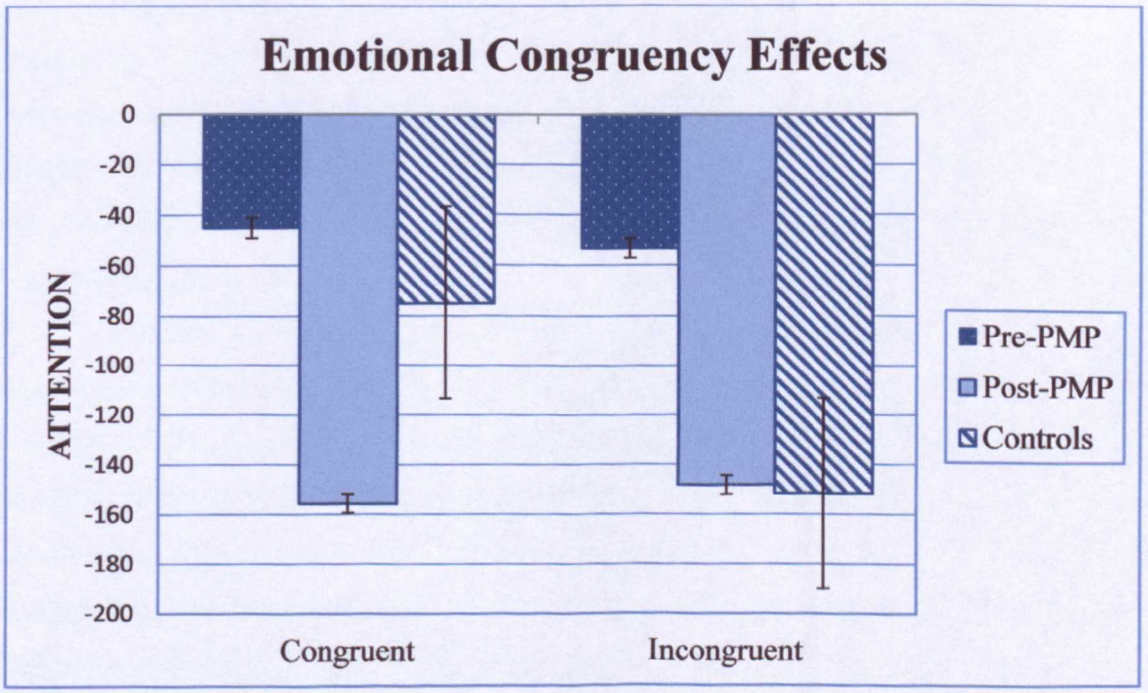


Figure 6.2 Bias Index (±SEM indicated by error bars) for Emotional Words by Group



6.6 FMRI Data Analysis and Results

Data analysis was performed using FMRI Expert Analysis Tool (FEAT) Version 5.98, part of FMRIB's Software Library (FSL, www.fmrib.ox.ac.uk/fsl). The following pre-processing statistics were applied; motion correction using MCFLIRT (Jenkinson, 2002); non-brain removal using BET (Brain Extraction Tool, Smith 2002); spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor; highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=25s). A detailed discussion of preprocessing steps can be found in Chapter 3.3.6.

Statistical analysis of the time-series data was conducted using a general linear model on a voxel by voxel basis using FILM (FMRIB's Improved Linear Model; Woolrich, 2001). Registration to high resolution structural images was carried out using FLIRT (FMRIB's Linear Image Registration Tool; Jenkinson, 2001, 2002). The resulting Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z > 1.8$ and a cluster significance threshold of $P=0.05$ corrected for multiple corrections (Worsley 2001).

FSLView was used as a manual inspection tool to investigate both excessive head motion, whereby Nifti 4d images were 'played' in a movie mode to observe head movement and to review the output images of BET to ensure that no non-brain matter remained. The MCFLIRT motion correction report was also inspected to ensure that any movement was no greater than a voxel. No participants were excluded on this basis.

To allow for a rest period to reduce fatigue especially within the clinical participants, fMRI data was acquired across two runs. First level analyses (lower level FEAT directories of individual subjects) were conducted for each run. A second level analysis used fixed effects to combine the two lower level results files into a single results file containing the mean BOLD response for each of the conditions. To ensure that participants had not moved between the two runs registration output files were checked. All files were satisfactory and no exclusions were necessary. Group analyses were performed as a third level analysis on individual contrast of parameter estimate (cope) files.

A three column format was used to define when the events occurred, with six explanatory variables being modelled. Individual files were created for each participant removing errors and outliers from the analysis. The six EV's were pain-

congruent (probe replaced a pain word), pain-incongruent (probe replaced the neutral word paired with a pain word), emotional-congruent (probe replaced an emotional word), emotional-incongruent (probe replaced the neutral word paired with the emotional word), neutral wordpairs and incorrect responses.

6.6.1 Neural Activation for Main Effect of Task

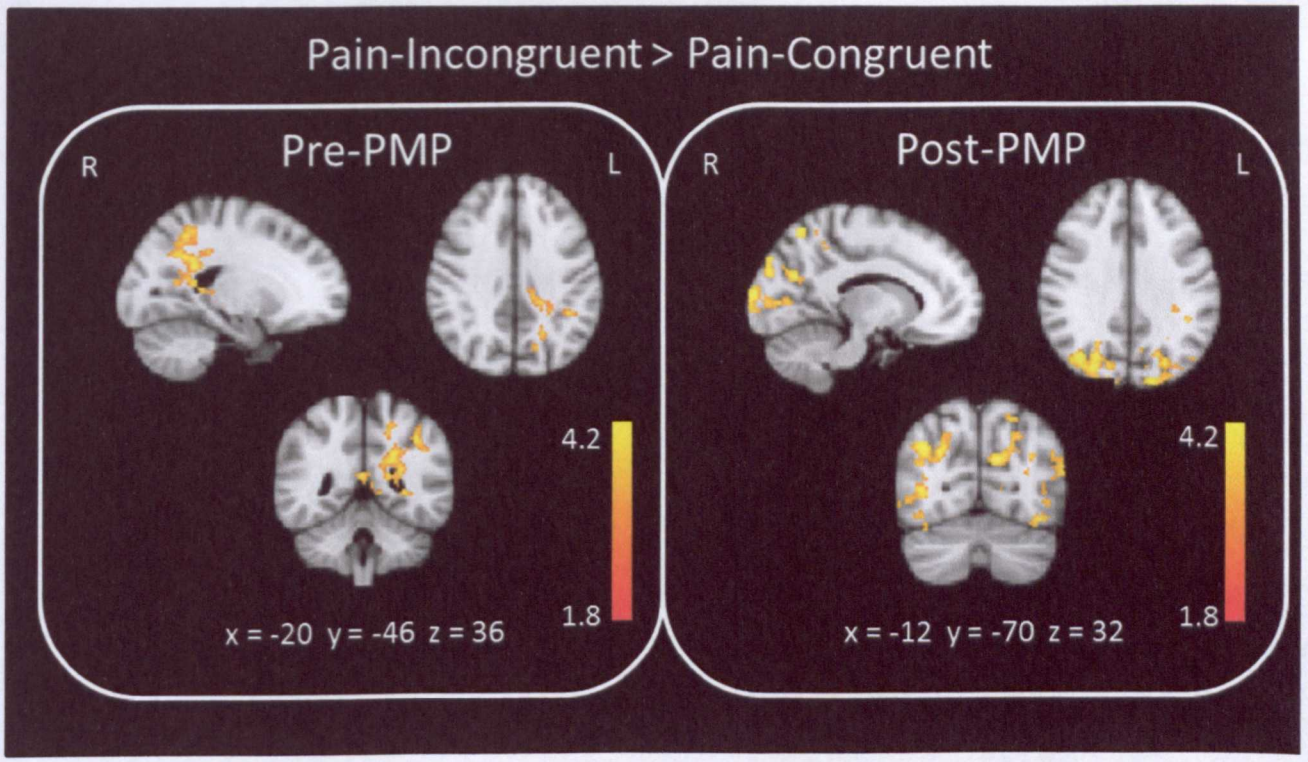
The inclusion of the neutral-neutral word pairs allowed for an investigation of cerebral activation associated with a baseline task performance (neutral > null events). All participant groups demonstrated an extensive network of activation, with a left lateralised dominance. Specifically, for the baseline task increased cerebral activity was evident in precentral and postcentral gyrus, supplementary motor area, superior parietal lobe, occipital cortex and cerebellum. Additionally, CLBP patients had cerebral activation in both fusiform gyrus and central/parietal operculum at both pre/post-PMP testing. Finally, a neural response to neutral words was found in the ACC for post-PMP patients and controls.

An extensive network of activation was demonstrated by all participant groups for both pain-related conditions (pain-congruent > null events and pain-incongruent > null events). Specifically increased activity was found in superior and inferior parietal lobe, fusiform gyrus, cerebellum, supplementary motor area and precentral and postcentral gyrus. In addition, the pre-PMP group demonstrated activity in the putamen for pain-congruent trials and ACC for pain-incongruent trials. Finally, the CLBP patients demonstrated increased cerebral activity within supramarginal gyrus and middle frontal gyrus for the pain-incongruent trials, at both pre/post-PMP testing.

6.6.2 Within Group Effects

The direct comparison of pain-incongruent>pain-congruent conditions revealed activation for the clinical group only. Prior to PMP attendance, this contrast resulted in activation of left supramarginal gyrus, extending to precuneus. Upon PMP completion, the contrast elicited significant activity of two clusters which contained bilateral activation of middle temporal gyrus, precuneus and cuneal cortex. Additional activation was found in left inferior and superior parietal lobe.

Figure 6.3 Activation Maps for Within Group Contrast



Mixed Effects, Z >1.8, P=0.05 (Corrected). Activation superimposed onto MNI Standard brain

Table 6.6 Clusters of Activation for Pain-Incongruent>Pain-Congruent Condition for CLBP Patients

Region of Activation	Talairach Coordinate of Max Z Score			Z Value
	x	y	z	
Pre-PMP				
L Supramarginal Gyrus (BA40)	-40	-44	40	3.45
L Precuneus (BA7)	-20	-54	32	3.43
Post-PMP				
L Middle Temporal Gyrus (BA39)	-46	-64	12	3.49
R Middle Temporal Gyrus (BA39)	38	-70	32	4.29
L Intraparietal Sulcus	-28	-46	38	3.48
L Superior Parietal Lobe (BA7)	-12	-66	60	3.47
L Precuneus (BA18)	-12	-70	24	3.46
R Precuneus (BA7)	24	-78	36	3.41
L Cuneal Cortex (BA18)	-10	-102	10	3.46
R Cuneal Cortex (BA19)	24	-90	26	3.41

Mixed Effects, Z >1.8, P=0.05 (Corrected)

6.6.3 Effect of Group and Condition

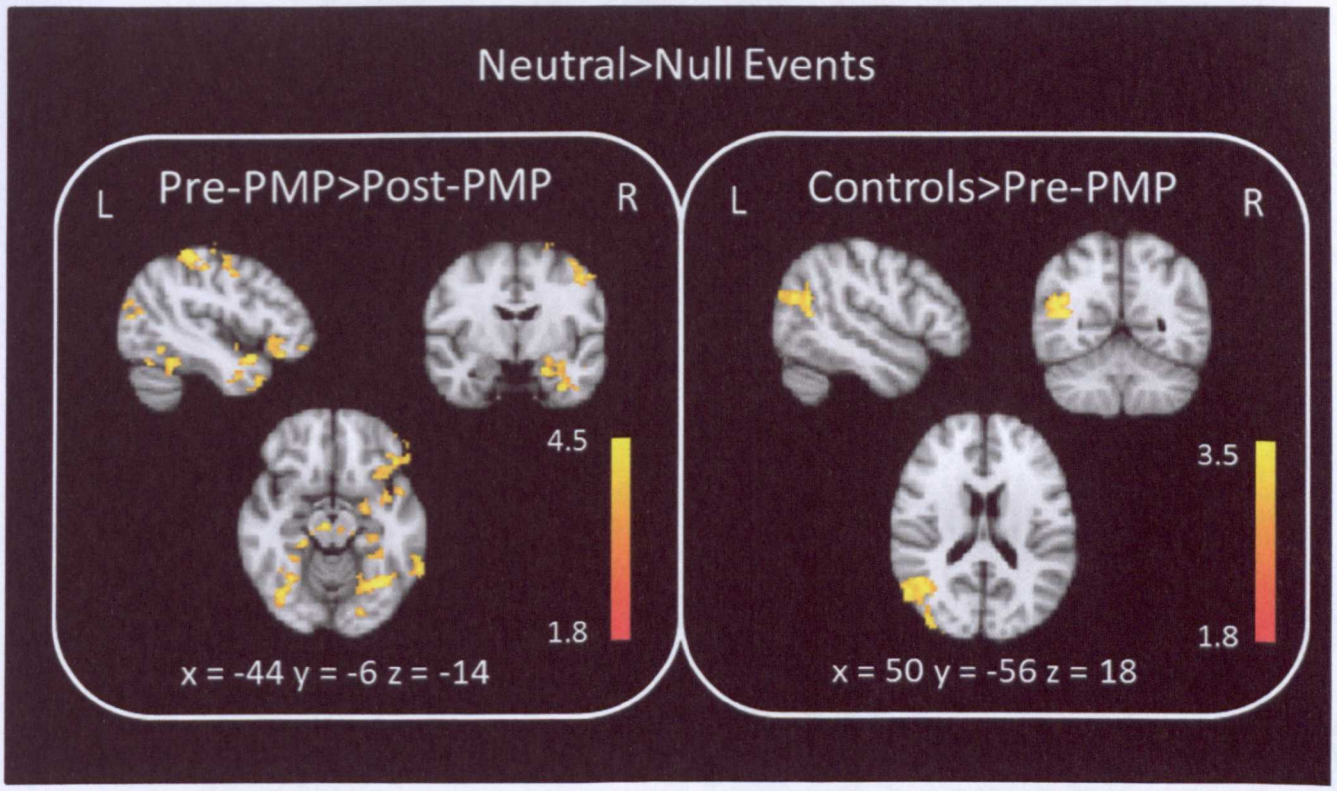
Neutral Condition

Prior to analysis investigating attention to or avoidance from the pain-related words, between group analyses were performed for the neutral condition (when a probe replaced one of the words in a neutral word pair). This was to ensure that any significant difference in neural activity demonstrated in the experimental conditions, was the result of the experimental stimuli and not simply an artefact of baseline performance. Between group contrasts for the neutral condition, indicated significant differences in baseline task performance. Specifically, Neutral $_{controls}$ > Neutral $_{prePMP}$ found significantly greater cerebral activity in right middle temporal gyrus, middle occipital gyrus and right angular gyrus. The reverse contrast Neutral $_{pre\text{-}PMP}$ > Neutral $_{controls}$ revealed no significant peak activation.

However, the Neutral $_{pre\text{-}PMP}$ > Neutral $_{post\text{-}PMP}$ paired t-test indicated extensive left lateralised activation in inferior frontal gyrus, superior frontal gyrus, hippocampus, amygdala, middle/superior temporal gyrus, precentral and postcentral gyrus, inferior parietal cortex and cerebellum. In addition bilateral activations were found in fusiform gyrus and cuneal cortex, which extended into right parahippocampal gyrus. No significant activation was found for the reverse contrast Neutral $_{post\text{-}PMP}$ > Neutral $_{pre\text{-}PMP}$.

Due to the significant differences in cerebral activation to the baseline task it was necessary to subtract task performance (experimental condition > neutral) prior to between group statistical analysis.

Figure 6.4 Activation for the Between Group Contrast for Neutral Trials



Mixed Effects, Z >1.8, P=0.05 (Corrected). Activation superimposed onto MNI Standard brain

Table 6.7 Areas of Activation for Neutral>Null Events

Region of Activation	Talairach Coordinate of Max Z Score			Z Value
	x	y	z	
Pre-PMP > Post-PMP				
L Inferior Frontal Gyrus (BA47)	-46	30	-16	3.39
L Middle Frontal Gyrus (BA6)	-34	-2	64	3.79
L Amygdala	-20	-8	-22	3.07
R Parahippocampal Gyrus	22	-32	-14	3.14
L Primary Somatosensory Cortex	-44	-36	58	4.29
L Precentral Gyrus (BA6)	-40	-18	64	3.92
L Postcentral Gyrus (BA3)	-34	-36	42	3.31
L Superior Temporal Gyrus (BA22)	-66	-24	0	3.53
L Middle Temporal Gyrus (BA21)	-70	-40	-6	3.43
L Fusiform Gyrus (BA37)	-34	-62	-14	4.54
L Cuneal Cortex (BA18)	-12	-82	20	3.12
R Cuneal Cortex (BA19)	12	-84	18	3.54
L Cerebellum (V)	-32	-40	-32	3.42
Controls>PrePMP				
R Middle Temporal Gyrus (BA39)	46	-66	24	3.06
R Middle Occipital Gyrus (BA18)	34	-84	10	3.06
R Angular Gyrus (BA39)	48	-56	20	2.86

Mixed Effects, Z >1.8, P=0.05 (Corrected)

Pain-Congruent Condition

To investigate neural activation for attention to pain-related words, we performed a between group contrast for the pain congruent condition (pain-congruent-neutral > pain-congruent-neutral). In the pre-PMP > Control contrast two clusters of activation were found. The first cluster contained activation in right middle temporal gyrus extending to fusiform gyrus. The second cluster contained bilateral activation of cerebellum including midline brainstem activation. The reverse contrast (Controls > pre-PMP) demonstrated no significant activation.

The pre-PMP > post-PMP contrast produced only one cluster of activation found in right inferior and superior parietal lobe, extending into precuneus and superior/middle temporal gyrus. The reverse contrast of post-PMP > pre-PMP produced no significant activation.

Pain-Incongruent Condition

The between group contrast pre-PMP > Controls for incongruent trials produced two cluster of activation. The first cluster was found in right middle temporal gyrus, extending to precuneus and inferior parietal lobe. The second cluster of activation was found in bilateral posterior cingulate cortex, with additional activation of the right lingual gyrus.

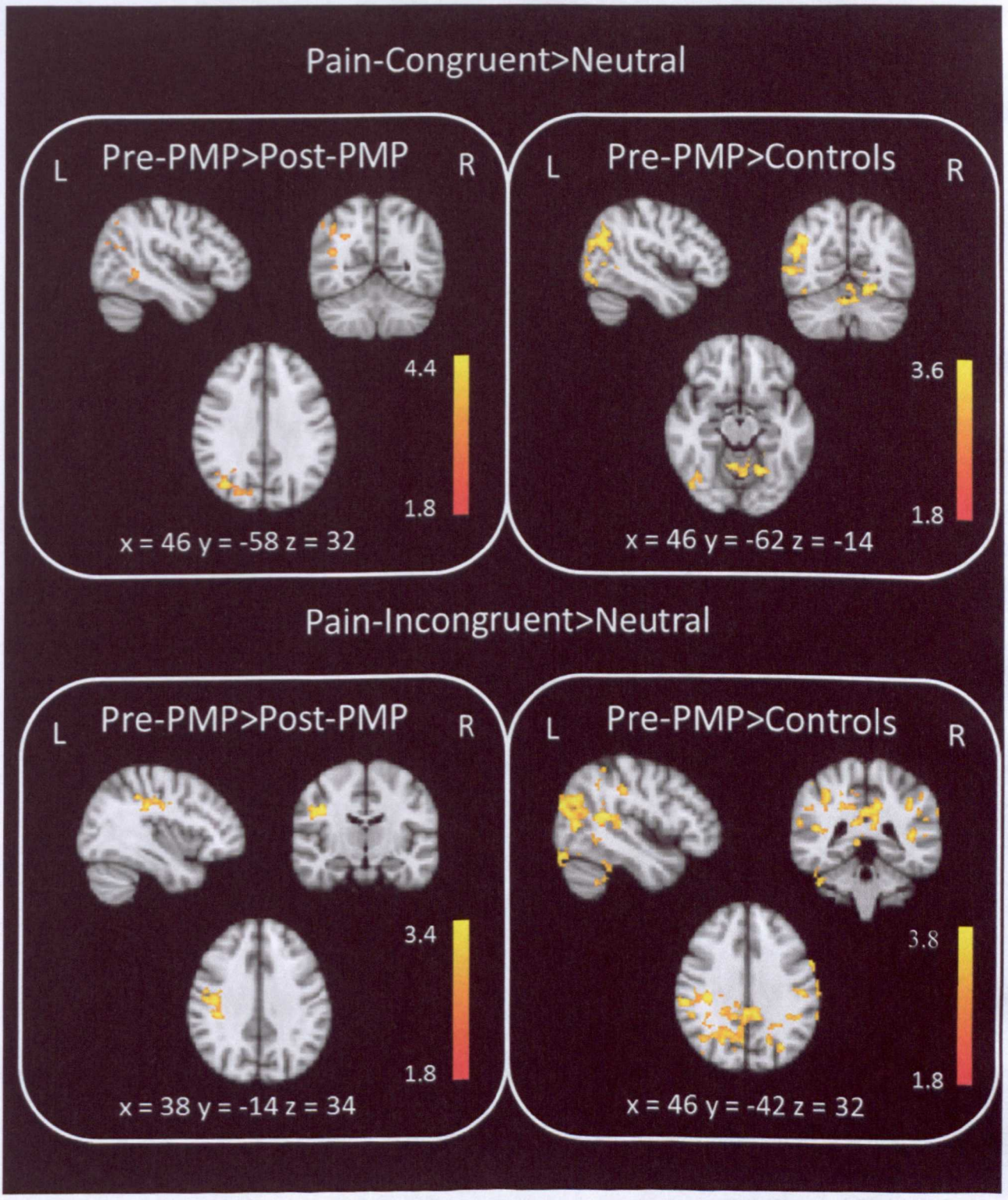
The pre-PMP > post-PMP contrast produced one cluster of activation found in right precentral and postcentral gyrus extending into supramarginal gyrus. The reverse contrast of post-PMP > pre-PMP produced no significant activation.

Table 6.8 Effects of Group and Word Condition

Region of Activation	Talairach Coordinate of Max Z Score			Z Value
	x	y	z	
Pain-Congruent				
Pre-PMP>PostPMP				
R Inferior Parietal Lobe (BA39)	40	-60	42	2.80
R Superior Parietal Lobe (BA7)	38	-70	34	4.44
R Middle Temporal Gyrus (BA37)	42	-56	4	2.98
R Precuneus (BA19)	24	-76	34	2.70
Pre-PMP>Controls				
R Middle Temporal Gyrus (BA39)	46	-66	24	3.62
R Fusiform Gyrus (BA37)	42	-68	-18	3.27
L Cerebellum	-6	-52	-10	3.07
R Cerebellum	2	-60	-26	3.07
Brainstem	0	-44	-20	3.56
Pain-Incongruent				
Pre-PMP>Post-PMP				
R Primary Somatosensory Gyrus (BA3)	42	-14	32	3.43
R Precentral Gyrus (BA6)	50	-6	32	2.62
R Supramarginal Gyrus (BA40)	38	-38	40	2.59
Pre-PMP>Controls				
R Middle Temporal Gyrus (BA39)	60	-60	12	2.81
L Posterior Cingulate Gyrus (BA23)	-12	-42	36	2.58
R Posterior Cingulate Gyrus (BA23)	10	-38	20	2.72
R Inferior Parietal Lobe (BA39)	44	-66	24	2.62
R Precuneus (BA18)	22	-60	28	2.72
R Lingual Gyrus (BA18)	10	-54	4	2.59

Mixed Effects, $Z > 1.8$, $P = 0.05$ (Corrected)

Figure 6.5 Activation for the Between Group Contrast for Pain Trials



Mixed Effects, Z >1.8, P=0.05 (Corrected). Activation superimposed onto MNI Standard brain

6.7 Discussion

6.7.1 Behavioural Findings

The present study sought to investigate to what extent CLBP patients with PB and pain-free controls selectively attend to pain-related words. Additionally, we investigated if the attendance of an intensive multidisciplinary PMP would modify any pre-attendance effects. To extend the discussion of pain-related selective attentional biases, all participants underwent event-related fMRI scanning during task completion. In addition to mean reaction time data, three indexes of selective attention were calculated to further elucidate the directionality of any attentional effects. Statistical analyses performed on both mean RT data and the bias indices, failed to find any of the predicted between-group differences. Typically the data has small effects with large standard deviations, indicating large within-group variability.

Results did show two within group effects. Specifically, pre-PMP patients responded more slowly when pain-related words were in the lower position. Furthermore, all CLBP patients responded more slowly when probes replaced pain words in the upper position. Previous dot probe studies have reported similar findings of spatial preferences, whereby responses are faster for words presented in one screen location (Keogh et al, 2001a). However, the issue of congruency was investigated further and revealed that slowed responding on congruent trials was evident for all participants. This pattern of responding suggests attentional avoidance of the experimental words, with attention being directed towards the accompanying neutral word. Inspection of the congruency indexes (See Table 6.5 and Figures 6.1 & 6.2) confirmed longer response latencies for congruent trials.

In contrast to the predicted performance, pre-PMP CLBP patients did not demonstrate an attentional effect towards the pain-related words; rather an avoidance effect was evident. Previous studies have reported incongruency effects, whereby participants are impeded from disengaging from the pain-related stimuli (words or pictures; Koster et al, 2004b; Roelofs et al, 2005; Sharpe et al, 2009), illustrated by an inverse relationship, whereby speeded responding on congruent trials (vigilance) results in slower responses for incongruent trials (inability to disengage). If CLBP patients were only avoidant of the pain-related stimuli, when probes replaced the neutral words during incongruent trials, these responses should be faster as attention is focused at that location. However, inspection of the incongruency index revealed that pre-PMP patients were faster on incongruent than congruent trials, but in comparison with baseline, they still exhibited a slowing response. This suggests that

patients avoided both the pain-related words, and to a lesser extent, the neutral word accompanying it.

To investigate if attentional avoidance was pain specific or an effect for all negative stimuli, the bias indexes for emotional word pairs were examined. These revealed that in contrast to the pain indexes, all groups displayed a hypervigilant effect for both congruent and incongruent emotional trials. The effect of congruency was evident, with faster responding on incongruent trials, suggesting that attention was directed towards neutral words. The control group demonstrated this pattern of responding for all trials, which has been reported previously (Asmundson et al, 1997; Keogh et al, 2003) and was an effect observed in the study presented in Chapter 5 for pain-free participants with low FOP.

The prediction that the completion of a PMP would modify the pattern of responding was supported, although findings were not statistically significant. Post-PMP patients demonstrated a hypervigilant effect for pain-related words on congruent trials (-9.2ms), indicating a speeded response of 94.7ms compared to pre-PMP performance. Pain-related incongruent trials demonstrated a reduction in response time of 13.6ms, but still reflected an avoidance effect compared to baseline suggesting a failure to disengage from the pain-words. The greatest difference in post-PMP testing was evidenced in the emotional bias indexes whereby there was a threefold increase in the hypervigilant effect. The post-PMP group were slightly faster on congruent trials, suggesting that they were engaged by the emotional word. Analysis of change scores for the self-report measures failed to find any significant relationships between the extent of change and the bias indexes indicating that post-PMP behavioural data was not mediated by either pain fearfulness or pain catastrophising as predicted by the fear avoidance model.

The finding of slowed responding for pain congruent trials amongst CLBP patients with pain behaviour was against prediction and in contrast to a previous study investigating the modification of attentional biases through a PMP (Dehghani et al, 2004). The fear avoidance model of pain suggests that highly fearful patients develop hypervigilance to pain signals which leads to avoidant behaviour (Vlaeyen & Linton, 2000). The findings of the current study support this assumption that CPP may selectively avoid pain-related information, possibly as a way of avoiding further cognitive processing. Slowed responding has been reported in previous studies investigating selective attentional biases in CPP. Chronic musculoskeletal patients were found to respond more slowly to affective, disability and threat words relative

to sensory words, with the slowest responders having the highest FOP levels (Dehghani et al, 2003). Khatibi et al, (2009) used a pictorial dot probe study of facial expressions of pain in CPP and controls and reported that both groups shifted attention away from happy faces, with the control group also demonstrating this for painful facial expressions. More recently, Haggman et al, (2010) investigated attentional biases in acute and CPP. All groups demonstrated attentional avoidance for a variety of words (sensory, affective, disability and threat). Hypervigilance was found for groups with low to moderate FOP levels, which was not evident in the high FOP group. Unfortunately, these studies lacked a neutral baseline condition (neutral-neutral trials) so the directionality of attention, such as facilitation or disengagement effects, cannot be ascertained.

One explanation of the attentional avoidance demonstrated in by the pre-PMP group is from the hypervigilant-avoidance hypothesis (Mogg, Bradley, De Bono & Painter, 1997). Primarily discussed within the anxiety literature, the hypothesis asserts that anxious individuals' initially attend to threat, but in an effort to reduce anxiety direct attention away, leading to maladaptive coping methods as potential threats are identified, but disconfirming information or cognitive coping strategies are avoided (Mogg & Bradley, 1998; Koster, Crombez, Verschuere, Van Damme & Wiersema, 2006). Furthermore, these effects have been found to have a temporal lag, whereby anxious individuals demonstrated a hypervigilant effect for stimuli presented at 200ms, but an avoidance effect for stimuli presented at 500ms or longer (Onnis, Dadds & Bryant, 2011). In the current study, stimulus presentation rates were 500ms, allowing strategic processing of the stimulus (Snider, Asmundson & Wiese, 2000). Avoidance of pain words was found in the pre-PMP group, with a hypervigilance to pain-related words being observed at post-PMP testing. One proposal is that completion of the PMP modified previous maladaptive beliefs and cognitions. When faced with a potential source of threat, newly acquired coping strategies were implemented whereby avoidance of the stimulus was no longer necessary. The slowed responding for the incongruent trials could reflect a failure to disengage from the pain-word whilst new coping strategies were being implemented.

One possible coping strategy employed by the post-PMP patients relates to the issue of control. Control over pain refers to the diminishment or management of the pain experience. Attempting to control pain is an adaptive coping strategy, but when faced with chronic persistent pain, maintaining this strategy may be counterproductive. As such when faced with a pain threat that is beyond their

control, pre-PMP participants may initiate avoidance behaviours. Two studies have investigated the behavioural repercussions of losing control over previously controlled pain in pain-free participants (Crombez, Eccleston, De Vlieger, Van Damme & De Clercq, 2008; Notebaert, Crombez, Vogt, De Houwer, Van Damme & Theeuwes, 2011). Although different tasks were administered (Wisconsin card sorting task/visual search paradigm), both studies found that once control over pain was relinquished, participants increased vigilance for pain signals and persisted in their attempts to avoid pain. This behaviour was maintained even when it was unnecessary for task completion. Therefore, when individuals perceive a loss of control over pain they adopt a vigilant strategy as a means of predicting and ultimately maintaining control. These findings relate to experimental pain in pain free participants and as such generalizability to a clinical population should be treated with caution. The studies investigate a recognised loss of control over pain, which in a chronic pain condition may occur over many years and be less well recognised. The multidisciplinary PMP attended by the patients, includes several approaches to help patients regain a greater sense of control over their pain experience such as pacing techniques, problem solving, goal setting and graduated exercises. Therefore, prior to PMP attendance, the avoidance of pain-related words may arise from a lack of coping strategies, borne out of a diminished sense of control. Upon PMP completion, a hypervigilant effect for previously avoided stimuli, may suggest a greater sense of control over pain and a reduced need to implement previously adopted maladaptive behaviours. Future studies may include measures to investigate these factors. Specifically, the direction of attention predicted by the hypervigilance-avoidance hypothesis could be better elucidated by eye tracking methods which directly assess attentional focus through eye gaze (Onnis et al, 2011), with levels of control being ascertained through self-report measures such as Chronic Pain Acceptance Questionnaire (McCracken, Vowles, & Eccleston, 2004).

6.7.2 Neuroimaging Findings

This fMRI study sought to investigate if CLBP patients demonstrate enhanced cortical responses to pain-related stimuli. It was predicted that cerebral activation patterns would differ between pre-PMP and post-PMP testing (pain-related fear) and between CLBP patients and pain-free controls (pain state).

As CLBP patients demonstrated different behavioural responses for when the probe replaced the pain word (congruent trials) and when they replaced the neutral word (pain-incongruent trials) we investigated the underlying neural correlates for these trial types separately. The areas of predicted cerebral activation were PFC, ACC, amygdala and posterior regions including superior and inferior parietal cortex and precuneus. Although participants did not display any significant differences on the behavioural measures, significant differences in cerebral activation patterns were revealed.

Within group analyses were conducted investigating the differences in cerebral activity for when attention is maintained or directed away from pain-related words. Increased activity was only found for the clinical group at both testing sessions, for when their attention was directed away from the pain-related word. The contrast pain-incongruent > pain congruent at pre-PMP testing, revealed left lateralised activity in areas previously identified as both pain-related and involved in semantic processing. At post-PMP testing, a bilateral network of increased cerebral activity was found in regions associated with a variety of tasks including memory, semantic, spatial and self-referential processing. Furthermore, the extent of cerebral activity was much greater than pre-PMP results. The behavioural results suggest that prior to PMP attendance, attention was directed away from both the pain word and its associated neutral word. However, upon completion of the PMP, CPP were hypervigilant to the pain words and unable to disengage from their spatial location, thus possibly relying more heavily on cognitive processes to reorient their attention, resulting in a greater network of activity.

Prior to between group analysis investigating attentional processing of pain-related words, contrasts of neutral task performances were analysed (Neutral-Null > Neutral-Null) to assess if groups differed significantly in their neural activity when completing the baseline task. All groups demonstrated different patterns of neural activity. Interestingly, the contrast between patients (pre-PMP > post-PMP) found greater activation of the left amygdala. Previous studies have reported increased amygdala activation for fearful facial expressions in healthy controls (Carlson,

Reinke & Habib, 2009), and tasks requiring vigilance (Bishop, 2004). One possibility is that pre-PMP patients remained vigilant for pain and emotional words. Anxious individuals are believed to constantly search the environment for threat, and will maintain the search until a possible source of threat is found (Pincus & Morley, 2001). On trials were no negative stimulus appeared, CLBP patients may have remained in a heightened state of arousal either searching for a possible threat, or waiting for the next trial to ascertain the threat level there. However, the differences in activity relating to task completion may simply be a reflection of practice effects. Post-PMP patients had already completed the task approximately five weeks previously and may have felt more relaxed within the scanning environment the second time.

Between group contrasts for both pain congruent and incongruent trials revealed that pre-PMP patients preferentially activated the right hemisphere. There is an assumption that semantic processing is only mediated through the left hemisphere. However, it has been suggested that the right hemisphere is specifically involved in emotional processing of various stimuli and that this may extend to emotional words (For a detailed review please refer to Abbassi, Kahlaoui, Wilson & Alpers, 2011). Furthermore, the right hemisphere may be preferentially activated in attention demanding tasks, such as the stroop task (Britton et al, 2009, Zoccatelli, Beltramello, Alessandrini, Pizzini & Tassinari, 2010) or in the recollection and suppression of emotional memories (Depue, Curran & Banich, 2007). Therefore, in the present study the right lateralisation displayed by the pre-PMP group when responding to pain-congruent and pain-incongruent trials may be reflective of greater emotional processing compared to post-PMP and controls.

Pain-Congruent Condition

The pre-PMP group displayed significant increases in cortical activity for the pain-congruent trials, compared to both post-PMP and control groups. Increased neural activity was found in the inferior and superior parietal lobe, medial temporal lobe (MTL) and precuneus in comparison to the post-PMP group. Compared to control participants, the pre-PMP group displayed greater activity within the medial temporal lobe, fusiform gyrus, cerebellum and brainstem.

Although hypervigilance to pain-related words had been predicted, the behavioural data suggests an avoidance of pain words for the pre-PMP group. For the pre-PMP > post-PMP contrast increased activity within the right parietal cortex was

found. As activity within this region is associated with shifts in attention, this activity may underlie the attentional avoidance of the pain words, indicated by longer response latencies for congruent trials (Yantis et al, 2002).

The medial temporal lobes are associated with processes of memory and the increased activity may suggest that the pre-PMP used additional memory processes when reading the pain related words compared to both controls and the post-PMP group (Guedj, Bettus, Barbeau, Liegeois-Chauvel, Confort-Gouny, Bartolomei, & et al, 2011; Ranganath, 2010; Schacter & Addis, 2009). The schema enmeshment model of pain suggests that for CPP, schema relating to pain, illness and the self, become enmeshed over time (Pincus & Morley, 2001). As self-relevant information is always prioritised and given preferential processing, due to the high level of enmeshment between the schemas, pain-related information is also prioritised. Therefore, compared to the control group, pain-related words are of greater relevance to the patient group, possibly resulting in a more sensitive memory network which is more easily activated by top-down processing, whereby certain types of information are given priority based upon the goals of the individual (Eck et al, 2011). It is also of note, that the pre-PMP group displayed greater MTL activity compared to the control group for the incongruent condition also, suggesting that the processing of the pain-words continued even when attention was oriented away from the word. If increased activity of MTL structures is representative of greater memories associated with pain-related words, the difference in activity between the two testing sessions for the patient group, might suggest that attendance on a multidisciplinary PMP results in a partial separation of the previously interrelated schemas, whereby pain-related words are less associated with self-relevant information.

Additional support that the pre-PMP group processed the pain-related words as more negative than the control participants is provided by posterior activation of the cerebellum for the contrast pre-PMP > controls. Originally associated with the co-ordination of movement, the cerebellum has been identified as being engaged by both cognitive and affective tasks with evidence of topographic organisation (Stoodley & Schmahmann, 2010). A recent study conducted by Schraa-Tam, Rietdijk, Verbeke, Dietvorst, van den Berg, Bagozzi & et al (2011) investigated cerebellar activity in response to pictures of positive and negative facial expressions. Although positive facial expressions activated the cerebellum, a greater posterior network of activity was demonstrated for facial expressions of negative emotions,

suggesting that the pre-PMP patients in the current task may have processed the pain-words in a more negatively affective manner than the controls.

Pain-Incongruent Condition

The pre-PMP group also displayed increased activity compared to both the post-PMP and control groups for pain-incongruent trials. When compared to controls, the pre-PMP group displayed increased activity within MTL, inferior parietal, precuneus, lingual gyrus and posterior cingulate gyrus (PCG). The behaviour data indicated the CLBP patients demonstrated the least reduction in response latencies for the pain-incongruent trials between testing. Increased activations were found in primary somatosensory cortex, extending to the parietal operculum, the precentral gyrus and the supramarginal gyrus, which are areas associated with the sensory-discriminative aspects of pain (Ferraro et al, 2011; Peyron et al, 2000). That these areas are preferentially activated in the pre-PMP group compared to post-PMP testing for the incongruent trials, does suggest that extensive processing of the pain words occurred even when attention was diverted from that area. Additionally, the areas that were activated for the pre-PMP > controls contrast, are all areas associated with more effortful semantic processing (Binder, Desai, Graves & Conant, 2009). Similar activations have been demonstrated in migraine patients when their attention was distracted from the semantic meaning of pain-related words, by being asked to count the number of vowels the words contained (Eck et al, 2011). In the pre-PMP group, if the pain-related words are activating a memory network during the congruent trials, this processing may continue even when attention is being diverted away from the pain stimulus.

6.8 Strengths and Limitations

The current study had many strengths including the homogeneity of the clinical sample, strict recruitment selection criteria, completion of an intensive multidisciplinary PMP and the inclusion of fMRI. However, limitations of the study remain. Firstly, although the study did have a pain-free control group, a waitlist group has not been tested. Therefore, it must be acknowledged that the results gained post-PMP could be the result of practice effects and not the influence of the PMP. This does seem unlikely as neutral trials were not significantly different between testing sessions, but it should not be overlooked. Secondly, whilst every effort was

made to control the word stimuli, valence and arousal measures were not collected. One explanation for the speeded effects on experimental trials, especially for the control group, could be that the neutral word pairs were less arousing. Thirdly, the pain words were not individualised for the pain patients. One possibility of the avoidance effect is that these words were not representative of their pain condition. However, investigations into the self-relevance of experimental stimuli, used within both dot probe and stroop tasks, have proved inconsistent. To overcome this consideration, the wordlists chosen were controlled on several criteria, including behavioural responses relating to word detection and naming.

6.9 Conclusion

The current study provides additional support for CLBP patients demonstrating a cognitive bias for pain-related words. Whilst this effect has been in contrast to the direction proposed by the fear-avoidance model, this does not make the results incongruent to the model. Demonstrating that any cognitive bias exists within the chronic pain population can lead to greater understanding of psychological factors which may contribute to maladaptive behaviours and cognitions believed to be influential on pain related coping. However, it does highlight that the model may need to be adjusted to consider attentional processes other than hypervigilance. These behaviours may impact a chronic pain experience and result in many of the behaviours proposed by the fear avoidance model as negatively impacting a pain experience.

CHAPTER SEVEN
General Discussion

7.1 Introduction

The aims of the presented work were to use MRI to investigate morphological and functional differences in clinical and pain-free participants. Structural analyses were conducted to investigate GM volume estimation within CLBP patients, which included a subgroup of patients with pain behaviour. Two functional MRI studies were used to investigate selective attentional biases. The first study provided a proof of principle that a semantic dot probe study could be successfully administered within an fMRI environment. Pain-free participants, separated on levels of pain fearfulness, demonstrated different behavioural and functional findings for pain-related words. The second study investigated selective attentional biases in CLBP patients with pain behaviour who attended a multidisciplinary PMP. FMRI testing was conducted pre and post treatment with the clinical group demonstrating behavioural and functional differences compared to pain-free controls and their pre-treatment selves.

7.2 Morphological Differences in PFC and Insular Cortex

Chapter 4 investigated morphological differences between pain-free controls and CLBP patients. To extend the findings from the previous literature investing gray matter differences in CPP, the clinical group was comprised of CLBP patients who differed on their levels of PB as measured by WS. We applied the Cavalieri method of modern design stereology in conjunction with point counting, to two regions of interest, namely the prefrontal cortex and the insular cortex. The findings showed a 14% GM deficit in right dorsal PFC for CLBP, as a cohesive group, when compared to pain-free controls. In contrast a GM deficit of the left insula was only demonstrated in CLBP patients with PB compared to the combined volumes of controls and non-PB groups. The extent of GM differences was not associated with either age or pain duration.

The underlying mechanisms of chronic pain are still being investigated; with no one explanation for the GM differences being found within chronic pain populations (Please refer to Chapter 2.7 for an in-depth discussion). Therefore, discussions surrounding the GM deficits as a cause or a consequence of excessive nociception are rather speculative. The GM differences reported in chronic pain states could be in response to cortical and subcortical reorganisation due to either overuse or disuse of transmission over synaptic pathways (Apkarian et al, 2011). The findings from these ROI investigations, suggest that as a group, CLBP patients have a reduction in a brain region posited as initiating the descending modulation of pain, namely the PFC (Tracey & Mantyh, 2007). Furthermore, CLBP patients with significant pain behaviour were found to have a further deficit of this network, demonstrated as a GM reduction of the left insular cortex.

The descending pain modulatory system enables the regulation of nociceptive processing to produce either facilitation or inhibition which is directly influenced by higher centres of the brain (Basbaum & Fields, 1984; Fields & Basbaum, 2005). It consists of a large network of brain regions which includes the ACC, amygdala, hypothalamus, PAG, NCF, RVM, insular cortex and frontal lobe, with the suggestions that the initiation of pain inhibition is associated with activity of the DLPFC (Lorenz et al, 2003; Raij et al, 2009; Tracey & Mantyh, 2007). The findings of the current study suggest that CLBP patients may be limited in their ability to use the pain modulation system for the inhibition of nociception. Furthermore, and as an extension to the findings of the previous literature, our results have found that a subgroup of CLBP patients with significant levels of pain behaviour have an additional GM deficit of the pain modulatory system. The effects of GM reduction in two pain inhibiting brain regions may underlie the pain behaviour exhibited by these patients, whereby attempts at pain inhibition are less effective. Additionally, as well as being involved in the modulation of pain, the insular cortex has also been linked to the subjective experience of pain, specifically in assessing the magnitude of the pain experienced (Apkarian et al, 2011). A GM deficit in this brain region may result in an inability to accurately assess pain levels over a full spectrum, instead leading patients to simply over report some lesser pains, (Fishbain et al, 2003).

7.3 Selective Attentional Biases

The remaining two studies, presented in Chapters 5 and 6, investigated selective attentional biases in pain-free participants and CLBP patients respectively. Selective attentional biases were investigated using a dot probe task, which was performed in conjunction with fMRI scanning to ascertain the neural correlates underlying pain-related attentional biases.

7.3.1 Selective Attention and Fear of Pain

The behavioural results of Chapter 5 failed to reach statistical significance, but did demonstrate the predicted trend, whereby pain-free participants with high levels of pain-related fear displayed a hypervigilant effect for pain related words. This effect was not evident for emotional words indicating that this result was not an emotionality bias. The behavioural results suggest that higher levels of pain-related fear underlie differences in the processing and assigned threat levels, of pain-related information. The hypervigilance to pain words found in individuals with high FOP supports the possibility that pain-related fear may be a vulnerability factor for persistent pain. Specifically, individuals with high levels of pain-related fear are predicted to misinterpret ambiguous physical sensations, which in turn lead to both hypervigilance and avoidant behaviours (Vlaeyen & Linton, 2000). If pain-related selective attentional biases already exist within the general population based on fear of pain levels, an acute pain experience may be prolonged by the preferential processing of pain-related stimuli. Prospective studies have reported pain-related fear as being a significant predictor in the transition from acute to persistent pain (Chou et al, 2010; Ramond et al, 2011). The results from the presented study suggest that hypervigilance to pain-related stimuli, may be one factor mediating this relationship.

Previous studies investigating pain-related fear and selective attentional biases have failed to produce consistent results, with some studies demonstrating fear related biases (Keogh, et al, 2001a) and other failing to replicate this finding (Roelofs et al, 2003). A further behavioural study, although adding to the existing body of work, would not extend existing knowledge. The inclusion of fMRI scanning allowed for an investigation of the underlying neural correlates for the pain-related dot probe task. As the first pain-related semantic dot probe task to be conducted within the scanning environment, the study was used as proof of principle, that the task was suitable for the scanning environment. The results indicated that the cerebral regions activated by the pain-related words, differed according to the

direction attention was oriented, either pain-congruent or pain-incongruent trials. The fMRI results showed that for the pain-related trials, compared to the low FOP group, participants with higher levels of pain-related fear had greater activity in brain regions associated with pain processing. Previous studies using pain words as cues for a variety of tasks, have also reported increased activity in pain-related areas (Eck et al, 2011; Gu & Han, 2007; Kelly et al, 2007; Osaka et al, 2004; Richter et al, 2009). The current findings showed that the activations elicited by pain-related words are indicative of an enhanced processing of pain-related stimuli which is mediated through pain-related fear. Coupled with the behavioural data, these results suggest preferential processing for pain-related words, which engages areas associated with pain processing. One suggestion is that associative learning creates stronger relationships between pain-descriptors and pain sensation, whereby the perception of pain sensations are altered through the verbal pain descriptor which have been previously processed (Richter et al, 2009). When faced with an acute pain experience, these associations may result in the misinterpretation of pain and avoidance of disconfirming evidence.

Avoidance learning in relation to persistent pain experiences, arises as a product of operant conditioning (Fordyce, 1976). This is a learned pattern of behaviour whereby the individual avoids or postpones the presentation of an aversive event, undesirable situation or experience, through avoiding activities known to produce pain in the past. Whilst beneficial for healing to take place in acute injury, if avoidance behaviour persists after the healing process has been completed, limitations in activity become maladaptive. To be successful, avoidance must take place prior to the painful event, making avoidance behaviour extremely resistant to extinction, as successful avoidance stops the individual from challenging and disconfirming their previously held beliefs (Leeuw et al, 2007). In pain-free participants, a higher level of pain-related fear may result in stronger associations between non-noxious pain-related stimuli and potential pain experiences, resulting in greater accessibility to pain-processing areas when faced with a perceived threat.

7.3.2 Selective Attention and Chronic Low Back Pain

In Chapter 6 we extended the investigation of selective attentional biases to a chronic pain population, specifically CLBP patients with pain behaviour. If the assumption of the Fear-avoidance model is accurate, whereby negative pain conditions are the result of hypervigilance behaviours, CPP regarded as having a negative response to pain, should also demonstrate selective attentional behaviours. In addition, it has been posited that if cognitive biases are sensitive to cognitive interventions, that this may guide the psychological management of pain (Keogh et al, 2001a). We tested CLBP patients before and after attendance of a multidisciplinary PMP to ascertain if cognitive biases would be altered, if any change in bias was related to changes in pain-related fear and the neural correlates underlying behavioural performance.

Whilst the behavioural results failed to reach statistical significance, a pattern of behaviour was observed. Contrary to predictions, the pre-PMP group did not display a hypervigilance effect; whereby they were faster at responding to pain-congruent trials. Instead patients took longer to respond to these trials, suggesting avoidance behaviour. Only after completion of the PMP did the group display facilitated responding (hypervigilance) for pain-congruent trials. Although these results suggest that CLBP patients with PB are avoidant of pain-related words, the hypervigilance-avoidance hypothesis suggests a more complex response (Mogg et al, 1997).

The attentional system is not a unitary system, but is comprised of separate attentional mechanisms, such as hypervigilance (facilitated engagement), impaired disengagement and attentional avoidance (Allport, 1989; Koster et al, 2006; Posner & Peterson, 1990). As such it is unlikely that attentional biases are simply the response of a reactive system driven by the threat value of the stimuli contained within the environment (Onnis et al, 2011). Rather it has been proposed that attentional selection can occur at different stages of information processing. The hypervigilance-avoidance hypothesis posits that there is an initial detection of the stimulus (hypervigilance), which in highly anxious individuals attempting to reduce anxiety, is followed by an avoidance of the stimulus.

The results of the current study suggest that prior to attending a PMP CLBP patients with PB reduce their anxiety to pain-related stimuli by avoiding it. However, upon completion of the PMP these patients demonstrated a hypervigilant effect for the same stimuli. However, whilst significant changes in several self-report measures

were recorded, (TSK, WS, PCS, BDI), no associations were found between the measures and the behavioural data. It is of note, that the self-report measures all displayed significantly reduced scores post-PMP, at a time when the patient group became hypervigilant for pain-related words.

The current literature predicts that it is elevated levels of these characteristics that are responsible for selective attentional processing of pain words (Keogh et al, 2001a; Pincus & Morley, 2001). The factor mediating this change however remains elusive. One possible factor, which was not investigated, is the issue of control. Pain-free participants have been found to demonstrate hypervigilance for pain-related targets, when they believe that they have some control over the pain (Notebaert et al, 2011). If avoidance is used as a coping strategy, when more proactive strategies are adopted, the individual may become hypervigilant to threat in the same manner as that proposed for non-anxious individuals. It is assumed that, as a survival instinct, all individuals scan their environment for evidence of threat which can result in a hypervigilant effect for trials that contain information with greater salience (Mogg & Bradley, 1998). However, once threat is detected, unless it reaches an individually specified threat level, it is ignored or avoided. Indeed the results of the presented study support this, as controls demonstrated a hypervigilance for all trials compared to the neutral baseline and additionally, they demonstrated incongruency effects, whereby they were faster for probes replacing the neutral word, thus supporting the suggestion of a subsequent avoidance of the threat word.

The fMRI results demonstrated different neural correlates for pain-congruent and pain-incongruent trials between the participant groups. Specifically, compared to controls and the post-PMP groups, the pre-PMP group demonstrated increased neural activity for all pain-related trials. The majority of this activity was found in the right hemisphere, which is suggestive that pre-PMP patients processed the stimuli in a more emotional manner than the other groups.

The pre-PMP group had increased cerebral activity in regions associated with memory processes, suggesting that these patients have more readily accessible memories relating to pain and furthermore, that these memory structures can be accessed automatically, even without the explicit recollection of pain experiences. The schema enmeshment model of pain suggests that for CPP, schema relating to pain, illness and the self, become enmeshed over time meaning pain-related representations are more readily available. Studies investigating memory biases for pain have provided some support for this proposition, with biases being reported

when assessed using recall as opposed to recognition methods (Pincus & Morley, 2001). Thaler, Meana & Lanti (2009) found compared to controls, women who reported sexual pain had significantly more false memories for pain words and furthermore, pain words resulted in more false memories than any other word type, suggesting that women with sexual pain had pain networks that were more readily accessible. A study conducted by Edwards & Pearce (1994) used a stem completion task to investigate the schematic representations of pain in three participant groups, CPP, health professionals and controls. The CPP group produced the greatest number of pain-related word completions than either control groups. As the mean number of years since qualifying for the health professionals was greater than the pain duration of the CPP, this suggests that it is the subjective experience of pain that creates more accessible schematic representations of pain. This is further supported by memory biases being diminished once the pain has been successfully treated through surgical intervention (Edwards, Pearce & Beard, 1995). In the current study, although the patients did attend a multidisciplinary PMP, the aim of the programme is not in the reduction of sensory pain levels. However, it does appear that after completion of the PMP, CLBP patients had lower activity in brain areas associated with memory, compared to pre-PMP testing, suggesting that pain-related words did not automatically access memory or semantic structures as previously demonstrated. By elaborating less on the pain-related words, patients may be more able to access other coping strategies.

The clinical implications for the findings of the current study are that a subgroup of CLBP patients uses avoidance of pain-related stimuli as a coping strategy. The attempts to reduce pain-related anxiety through avoidance behaviours, may be useful as a short term strategy, but as pain persists, may lead to maladaptive processes. Completion of an intensive multidisciplinary PMP appeared to be successful in reducing the previous avoidance behaviour.

7.4 Future Directions

The findings of the stereology study in Chapter 4 need to be replicated in another CLBP population who demonstrate PB. This work is currently being undertaken with the patients recruited for the study contained in Chapter 6. To extend findings further participants were tested on three tasks assessing PFC functioning namely; trail making task (attention), verbal fluency (language) and digit span (memory), the performance of which will be correlated with stereological analysis of the PFC.

Additionally, the data collected for Chapter 6 can be used to investigate morphological changes associated with attending a multidisciplinary PMP. de Lange, Koers, Kalkman, Bleijenberg, Hagoort, van der Meer & et al, (2008) investigated morphological changes in patients with chronic fatigue syndrome receiving CBT. The prediction that patients would have GM deficits prior to treatment, with GM increases post-treatment was supported. Although CBT was undertaken over a longer time period than the patients attending the PMP (6-9 months) the amount of treatment time was approx. 16 hours. In comparison the attendees of the PMP receive approximately 120 hours of treatment. In addition GM changes have been demonstrated after periods as short as 8 days, albeit to sensory stimulation (Teutsch et al, 2008).

Future research using the dot probe task might also include additional eye-tracking methods, which can be used both within and out of a scanning environment. The discovery that CLBP patients use avoidance as a strategy when encountering pain-related words, could be better investigated using analysis of eye gaze. This would reveal where the patients attention had been oriented to. Furthermore, as an avoidance effect was unpredicted, future research should try and replicate this finding in both CLBP patients with and without pain behaviour. Using two different patient groups will not only provide support for the current finding in patients with PB, but would also ascertain if avoidance strategies are unique to patients with PB. If differential patterns of attention are found in association with pain behaviour, this may better inform any treatment measures and help tailor PMP's to the individual needs of the attendees.

7.5 Conclusion

The original work presented within this thesis makes a clear contribution to the previous literature investigating morphological differences and selective attentional biases in chronic pain patients. To overcome some of the criticisms of earlier work regarding group heterogeneity, we investigated CLBP patients with pain behaviour. The strict inclusion/exclusion criteria meant that the clinical sample was as homogenous as was feasible. Furthermore, only a limited number of dot probe studies investigating pain-related selective attention have been conducted within a pain cohort, with only one previous study testing patients before and after a treatment programme. The strength of the study presented here is that chronic pain patients receiving treatment were tested on a dot probe paradigm prior to and upon completion of a PMP, whilst undergoing fMRI scanning. The study also included a healthy control group, lacking in previous work.

The results of the work presented found; morphological differences in CLBP patients with and without pain behaviour; provided a proof of principle for the successful use of a semantic dot probe study in the fMRI environment; pain fearfulness mediates attentional processes to pain-related words and concomitant cerebral activity in pain-free individuals; CLBP patients with PB use avoidance to threat as a coping strategy, but attendance of a multidisciplinary PMP attendance was successful in changing this behaviour and concomitant cerebral activity.

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