Nuffield Department of Anaesthetics

The Role of the Prefrontal Cortex in Pain Modulation

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Christ Church, Michaelmas Term, 2011

Thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Oxford

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Existing knowledge identifies the prefrontal cortex (PFC) as the modulatory area for pain. Previous neuroimaging studies suggest the existence of the cortico-cortical pathway, an alternative pain modulatory pathway distinct from the descending modulatory pathway of pain. However, little is known of the extent, mechanism and underlying substrate of the modulation. The objective of this study is therefore to explore the role of the PFC in pain modulation.

To examine the extent of PFC involvement in pain, meta-analyses of imaging studies in healthy volunteers and patients with chronic pain were performed. Using Gaussian-process regression (GPR) analysis, brain maps were produced from foci of activation as reported in the studies. Since structure dictates function, our next study was to perform probabilistic tractography on diffusion-weighted brain images to ascertain the connection probability of lateral PFC subdivisions and pain-related brain regions as well as intrinsic PFC connections. Two behavioural studies were conducted to investigate cognitive modulation of pain. The first was a study to assess the subjective and physiological correlates of cognitive stress, as previously used in stress-induced analgesia studies. The second was to investigate the involvement of the endogenous opioid system in the cognitive modulation of pain through effortful reappraisal and contextual modulation.

Meta-analyses in healthy volunteers and chronic pain patients revealed activation mainly in the lateral aspect of the PFC due to pain. Distinct pattern of activation was demonstrated in patients with significant ventrolateral PFC (VLPFC) activation across subtypes of chronic pain. Probabilistic tractography further illustrate the functional significance of lateral PFC subdivisions by demonstrating differential connection probability to pain-related brain regions; dorsolateral PFC (DLPFC) regions displayed higher connection probability with brain regions serving more sensory-discriminative function while VLPFC showed high connection probability with both sensory-discriminative and affective regions. Behavioural study of stress showed that cognitive stress failed to induce significant increases in biomarkers of stress, and was not affected by increased level of difficulty. Lastly, behavioural study on contextual modulation and reappraisal confirmed opioid mediation for contextual modulation while negating its involvement in effortful reappraisal.

Findings from this study illustrate the extent of PFC involvement in pain modulation especially in chronic pain patients and provide further evidence of an alternative pathway distinct from the opioid-mediated descending inhibitory pathway.

Acknowledgements

I wish to express my special gratitude to Prof Irene Tracey, who kindly gave me the opportunity to be part of her research group and for her support, guidance and encouragement throughout the course. I am especially indebted to Dr Katja Wiech, from whose teaching, supervision and continued interest I gain invaluable knowledge and experience.

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My humble bow to my parents, who I can always depend on, and who will always be there for me. Finally, I thank my husband and children for their love, understanding, encouragement and acceptance of a number of deprivations throughout the course and period of thesis preparation.

The good is from the Almighty and the shortcomings are from my own weaknesses.

Table of Contents

I. PUBLICATIONS ARISING FROM THIS THESIS

Abstracts:

Ahmad A, Wiech K, Tracey I. Cognitive load of the modified colour-word Stroop task. Autumn School in Cognitive Neurosciences 29 September – 2 October 2008. Department of Experimental Psychology, University of Oxford.

AH Ahmad, C Berna, S Leknes, RN Mhuircheartaigh, K Wiech, I Tracey. Electrodermal correlates of reappraisal and contextual modulation of pain. 13th World Congress on Pain 2010, 29 August – 2 September 2010, Montreal, Canada.

Ahmad A, Wiech K, Jbabdi S, Tracey I. Probabilistic connectivity between the prefrontal cortex and pain-relevant brain regions. Wiring the Brain: Making Connections April 12-15 2011. Powerscourt, Co Wicklow, Ireland.

II. DECLARATION

I declare that the work presented in this thesis, except the derivation of brain maps in Chapters 4 and 5 using Gaussian-process regression analysis, is my own and has not been submitted for any other degree in this or in any other university or institute of learning.
This thesis contains less than 50 000 words.

III. ABBREVIATIONS

ACC	anterior cingulate cortex
AFNI	Analysis of Functional NeuroImages
ALE	activation likelihood estimation
AVS	Advanced Visual Systems
BAS	behavioural activation system
BET	Brain Extraction Tool
BIS	behavioural inhibition system
BOLD	blood oxygen level dependent
CBMA	coordinate-based meta-analysis
CRPS	complex regional pain syndrome
dACC	dorsal ACC
DLPFC	dorsolateral prefrontal
DTI	diffusion tensor imaging
DWI	diffusion-weighted imaging
EDA	electrodermal activity
ER	emotion regulation
FDT	FMRIB's Diffusion Toolbox,
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	functional magnetic resonance imaging
FSL	FMRIB Software Library
GP	Gaussian process
GPR	Gaussian-process regression
GSR	galvanic skin response
HPA	hypothalamic-pituitary-adrenal
IASP	International Association for the Study of Pain
IBMA	image-based meta-analysis
IFG	inferior frontal gyrus
KDA	kernel density approximation
MCC	midcingulate cortex
McFLIRT	Motion Correction in FMRIB's Linear Image Registration Tool
MCMC	Markov Chain Monte Carlo
MD	mediodorsal
MKDA	multi-level kernel density approximation
MNI	Montreal Neurological Institute
MRI	magnetic resonance imaging
NIH-FIDAP	NIH-Functional Imaging Data Analysis Platform
OFC	orbitofrontal cortex
P	probability
PAG	periaqueductal grey
PCC	posterior cingulate cortex
PDD	principal diffusion direction
pdf	probability density function
PET	positron emission tomography
PFC	prefrontal cortex
PO	pars opercularis

rACC	rostral anterior cingulate cortex
rTMS	repetitive transcranial magnetic stimulation
SI	primary somatosensory cortex
SII	secondary somatosensory cortex
SCR	skin conductance response
SF	Sylvian fissure
SHAPS	Snaith-Hamilton Pleasure Scale
SIA	stress-induced analgesia
SIH	stress-induced hyperalgesia
SPECT	spectroscopy
SPM	Statistical Parametric Mapping
VAS	Visual Analogue Scale
VBM	voxel-based morphometry
VLPFC	ventrolateral prefrontal cortex
VPL	ventral posterior lateral

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1. INTRODUCTION

1.1 Pain

Pain, like other conscious sensations, is the result of complex sensory processing at the highest level of the central nervous system (Woolf 1991). The experience of pain is not only subjective but also multidimensional. The International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IASP, 1994).

1.2 Pain pathway

The ‘classic’ ascending pain pathway consists of a three-neuron chain that transmits pain information from the periphery to the cerebral cortex (Cross 1994). A strong and potentially tissue-damaging stimulus is detected by pain receptors and the signal is then transmitted via pain fibers, the A-delta and C fibers, to the dorsal horn of the spinal cord. These are the first order neurons of the ascending pain pathway. The second order neuron synapses with the first order neuron in the spinal cord and ascends in the spinothalamic tract to end in the ventral posterior lateral (VPL) nucleus of the thalamus (Barrett et al 2010). The third order neuron relays the information to the postcentral gyrus in the cerebral cortex. This pathway is organized such that, within tracts and nuclei up to the cortex, topological relations are maintained and different parts of the body are represented in an ordered arrangement, called somatotopy, in the postcentral gyrus (Windhorst 1996). Another

ascending pain pathway that also carries pain signals is the spinoreticular pathway (Barrett et al 2010). In this pathway, tracts from the dorsal horn of the spinal cord synapse in the reticular formation of the brain stem, project to the centrolateral nucleus of the thalamus, and transmit to the cerebral cortex, making up a four-neuron chain pathway.

The pain pathway is now understood to consist of the sensory-discriminative and cognitive-affective aspects of pain (Auvray et al 2010). Fibers from VPL nuclei, also called the neospinothalamic tract, project to SI and SII and subserve the sensory-discriminative aspect of pain. Those from the centrolateral thalamus, also called the paleospinothalamic tract, project to frontal lobe, limbic system and insula, and are responsible for the cognitive-affective aspect of pain (Barrett et al, 2010). In addition to the classic pain pathway, there are also afferents from the spinal cord to local modulating circuits in the spinal cord, pain-mediating areas of the brainstem, and descending pain pathways from the cortex, hypothalamus and brain stem to the spinal cord (Milan 2002). Multiple potential target nuclei as well as several efferent pathways exert modulatory control on pain transmission (Fields 2000).

1.3 Pain-related brain regions

Unlike other sensations that have specific areas in the brain such as vision, touch and auditory, there is not one specific cortical area for pain. Functional magnetic resonance imaging (fMRI) studies of pain have revealed a distributed network of brain regions that are activated during pain. Melzack (1990) coined the term

neuromatrix to denote several brain areas that receive nociceptive and non-nociceptive sensory input and function in an integrated manner following observations on phantom limb patients. The phantom limb phenomena, where the absent or amputated limb was still felt, could not be explained by nerve endings in the stump or the spinal cord level since transection of the spinal cord did not abolish the phantom feeling. Neither is it mediated by the somatosensory cortex as the phantom feeling returns after excision of the postcentral gyrus. It was therefore concluded that the phantom pain perception is subserved by not just one region but a network of brain regions in the brain (Melzack 1990).

The term neurosignature implies that the pattern of activation of brain areas due to pain is peculiar to each person and is congenitally programmed. The neuromatrix theory was gradually replaced by the term pain matrix. However, this term has recently become a subject of debate since the areas that are activated subserve other non-nociceptive functions and are therefore not specific to pain (Iannetti & Mouraux 2010; Tracey & Mantyh 2007).

1.4 Pain modulation: behavioural evidence

The differentiation into sensory-discriminative and cognitive-affective aspects of pain means that the perception of pain is not only determined by nociceptive input, but also depends on affective and cognitive factors (Auvray et al 2010; Wiech et al 2008b; Wiech & Tracey 2009). While the sensory-discriminative aspect of pain involves the intensity, quality and location of pain, the cognitive-affective factors

constitute more subjective psychological variables comprising attention, anxiety, fear, expectation, anticipation and stress (Wiech & Tracey 2009). In short, pain modulation is influenced by the context in which it is experienced as well as longer-lasting factor such as personality. The main factors in the modulation of pain are discussed below.

1.4.1 Anxiety

In general, negative emotions such as anxiety enhance pain. This has been shown experimentally in healthy volunteers (Ploghaus et al 2001) as well as in the clinical setting; presurgical anxiety predicts postsurgical pain intensity (Pinto et al 2011), and in chronic pain patients, anxiety predicts persistence of neuropathic pain (Boogaard et al 2011) while reduction in anxiety increases pain acceptance in individuals with HIV and chronic pain (Huggins et al 2011). Anxiety reduction techniques and anxiolytic drugs reduces pain brought about by medical procedures (Dellemijn & Fields 1994). However, if the stress induced by negative emotions reaches a certain level, the effect is reduction in pain perception, also termed stress-induced analgesia.

The Motivation-Decision model by Fields (2007) stated that analgesia might be the result of averting a bigger threat than pain or the anticipation of obtaining a reward. In the face of menace such as threat of a predator, attending to the dangerous situation takes precedence over attending to the pain, hence analgesia. Likewise, in situations where reward is to be gained, the motivation for reward obliviates the sensation of pain, resulting in analgesia. These concepts summarize the behavioural

reactions to stress resulting in analgesia, i.e. in stressful situations where survival depends on confronting (or fleeing away from) the stressor, attending to the pain is no longer the priority.

Early human studies on stress-induced analgesia by Willer and colleagues (Willer & Albe-Fessard 1980; Willer et al 1981) utilized expectation of strong noxious stimulation as the stressor that was found to inhibit the nociceptive flexion reflex. Several other studies have shown evidence of stress-induced analgesia in humans by stressful stimuli such as cognitive stress (Bandura et al 1988), noxious heat (Willer et al 1989), physical stress (Janal et al 1984), and psychological stress (Flor & Grusser 1999).

1.4.2 Attention

Pain commands attention and disrupts from concurrent tasks and stimuli (Eccleston & Crombez 1999). Attentional studies in pain have been demonstrated by using dual-task paradigms of a distracting task and concurrent pain stimulation (Seminowicz & Davis 2007; Valet et al 2004; Villemure & Bushnell 2002). The attentional bias towards pain over other stimuli is modulated by various factors (Eccleston & Crombez 2005). First, the bias depends on the characteristics of the pain stimulus itself. For instance, novelty, and uncertainty about the intensity, onset or duration of the pain stimulus lead to a stronger bias, but controllability reduces it. Second, it depends on the characteristics of the person experiencing the pain such as the tendency to catastrophize (Vancleef & Peters 2006b) or a predisposition for depression, anxiety (Vancleef et al 2006), hypervigilance (Crombez et al 2005) and

pain-related fear (Vlaeyen & Linton 2000). Third, the wider context in which pain occurs, such as the expectancies of potential benefit from pain, or the emotional valence of concurrent attentional demands (Eccleston & Crombez 2005).

1.4.3 Expectations

Expectations regarding the intensity, duration and onset of pain have been shown to influence pain perception. A person who expects more pain will experience more pain and expectation of a lower pain will result in lower pain perception.

The influence of expectations has been extensively explored in placebo and nocebo studies where the effect of expectation can go both ways. Studies on placebo and nocebo are examples of how expectation can modulate pain to be perceived as less (placebo) or more (nocebo) than the nociceptive input (Benedetti & Amanzio 1997; Benedetti et al 1997). These effects are induced by administration of an inert substance together with the suggestion that it is a potent analgesic for placebo (Benedetti & Amanzio 2011) or a painful stimulation for nocebo (Colloca & Benedetti 2007; Colloca et al 2008). A recent study modulated the expectation of receiving a higher or lower dose of analgesic through prior suggestion of a drug's supposed potency (Bingel et al 2011). Depending on the person's expectancy of pain or analgesia, pain perception is modulated accordingly despite similar dose of analgesic administered.

The effects therefore seem to be based on learning (classical conditioning) and expectations (Benedetti et al 2011). The distinction between expectation and anxiety

is unclear. Expectation of an aversive outcome creates anxiety resulting in increased pain perception as in nocebo expectation (Benedetti et al 1997).

1.4.4 Threat value of pain

Although the above pain modulators are different in nature, their common denominator is the modulation of the perceived threat. The perception of threat induces a heightened sense of awareness and increases interoception towards the threatening stimulus (Paulus & Stein 2006).

The perception of threat thereby depends on the assumed meaning of pain (Arntz & Claassens 2004; Moseley & Arntz 2007). For example heat pain is deemed more threatening than cold pain (Arntz & Claassens 2004) or delayed-onset muscle pain (Dannecker et al 2008) and pain due to cancer is perceived as more intense than pain that is not cancer-related (Smith et al 1998). These examples show that the meaning of pain that can potentially cause harm or tissue injury increases the threat value of pain (Arntz & Claassens 2004).

The threat value of pain determines how much attention will be assigned to the pain resulting in modulation of pain perception. Conflict arises when there is a need to disengage from pain in favour of the more salient need for survival, the 'fight or flight' response (Fields 2007). Similarly, the threat value of pain determines the attentional bias towards pain or concurrent stimuli resulting in changes in task performance causing either deterioration (Vancleef & Peters 2006a) or no significant worsening (Veldhuijzen et al 2006).

The threat value of pain may be modulated cognitively by giving prior information about the pain. Boston & Sharpe (2005) modulated pain-related threat by giving fear-inducing information about a noxious stimuli (cold pressor task). To subjects in the threat condition, the task was introduced as a 'vasodilatation task' that could potentially lead to frostbite, whereas in control subjects the stimulation was introduced as 'the cold pressor task' that would be safe. A study by Van Damme et al (2004) also used information to manipulate the threat value of an electrocutaneous stimulus, informing the subjects in the pain group that the stimulus 'stimulates the pain fibers and that most people find this kind of stimulation unpleasant' while the control subjects were told that the vibrotactile stimulus 'stimulates the touch fibers and that most people find this kind of stimulation not unpleasant'. A similar set of instructions was also used by Vancleef & Peters (2006b) to increase the threat value of electrical stimulation on their subjects. In addition, subjects were also told that the reaction to the stimulus varied across people, inducing a state of uncertainty about the expected sensation.

The threat value also depends on the pain characteristics. Pain of higher intensity has a higher threat value than that of a lower intensity (Crombez et al 1998). Likewise, Dannecker et al (2008) showed that heat and ischaemic pain are deemed more threatening than delayed onset muscle pain and intermittent pain (Vancleef & Peters 2006a) engages more attention than continuous pain (Veldhuijzen et al 2006). Another factor that increases the threat value of pain is novelty. In a study on cancer pain, experiencing pain in a new location has been shown to positively correlate with worrying about the pain and focusing on emotions while in pain (Buck & Morley

2006). Experimentally however, novelty as a threat value of pain is not a factor that has been widely studied.

1.5 Pain modulation: the neural basis

Findings from animal studies have outlined the most completely described pain modulatory circuit, the descending pain modulatory pathway that comprises the rostral anterior cingulate cortex (rACC) and periaqueductal grey (PAG) in the brainstem (Jones & Gebhart 1988; Vaccarino & Chorney 1994; Vaccarino & Melzack 1992; Yaksh & Rudy 1978). This circuit controls pain transmission via the actions of neurotransmitters released by two distinct types of neurons in the brainstem: OFF neurons that inhibit responses to noxious stimuli, and ON neurons that are activated by noxious stimuli and facilitate responses to noxious stimulation (Fields 2004).

In humans, the involvement of this descending pain inhibitory pathway has been shown for each type of pain modulation including distraction (Bantick et al 2002; Tracey et al 2002; Valet et al 2004) and placebo analgesia (Bingel et al 2011; Eippert et al 2009). These studies show increased activation as well as functional connectivity of the brain regions involved in the descending pain inhibitory pathway. This descending pain inhibitory pathway seems to be governed by the prefrontal cortex (Petrovic et al 2010). In most of these studies showing an involvement of the descending inhibitory system, the activation in pain-related brain structures is decreased.

Some other studies on pain modulation only find prefrontal mechanisms such as studies on perceived control over pain (Salomons et al 2004; Wiech et al 2006), religious belief (Wiech et al 2008a), and individual differences in placebo analgesia (Wager et al 2011). Further evidence for cortico-cortical modulation is shown by functional connectivity between anterior insula and midcingulate cortex (MCC). Giving prior information to create bias towards pain has been shown to activate the anterior insula during prestimulation, and the MCC during stimulation (Wiech et al 2010). Functional connectivity between anterior insula and MCC is increased by anticipation of pain (Seeley et al 2007). Unlike in the descending modulation of pain, these studies with prefrontal mechanism do not show decrease in activation in pain-related brain regions, raising the question as to whether this is a second down-modulatory mechanism for pain, the cortico-cortical modulation.

1.5.1 Descending pain inhibitory pathway and the endogenous opioid system

Animal studies show that analgesia is mediated via the endogenous opioid system (Bederson et al 1990; Millan et al 1988; Noda et al 1998). Opioid effects, however, extend beyond analgesia. Studies have shown that opioids are involved in fear acquisition (Eippert et al 2008), reward (Pecina 2008), learning and memory (Carey et al 2009), and mood (Kennedy et al 2006). Apart from its direct effect, opioids also have interactions with other neurotransmitters in the brain such as dopamine (Scott et al 2007) and cannabinoid (Manzanares et al 1999). Dopamine has been implicated in reward and addiction while cannabinoids are also involved in analgesia.

Contextual, expectancy, and attentional aspects of pain such as placebo analgesia (Eippert et al 2009) and stress-induced analgesia (Willer et al 1981) has been shown to be opioid-mediated. This contextual modulation is regulated, at least in part, by the descending modulatory pathway (Colloca & Benedetti 2005; Petrovic & Ingvar 2002). There is also evidence of direct effects of opioids upon cortical nociception-related areas (e.g. anterior cingulate cortex, ACC) (Petrovic et al 2002). Involvement of these areas in analgesia explains the high density of opioid receptors in the ACC, PAG and amygdala. While it is well established that opioid has a role in the descending modulation of pain, its extent of involvement in the cognitive and emotional modulation of pain is still under investigation.

Naloxone is an opioid receptor blocker used clinically as well as in human and animal experimental studies. In human, the traditional clinical role of naloxone is mainly to reverse the depressive effect of exogenous opiates on the respiratory system. Using naloxone to block the opioid receptor also allows the investigation of the underlying mechanisms and sites in the brain and/or spinal cord that are involved in the production of pain to determine whether it is opioid−mediated or non−opioid mediated.

1.5.2 Descending pain modulatory pathway and the prefrontal cortex (PFC)

An interference study provides causal evidence that the descending pain modulatory pathway is governed by the PFC, particularly the DLPFC subdivision, through the cortico-subcortical pathway (Krummenacher et al 2010; Wager et al 2004b). By disrupting the left and right DLPFC using repetitive transcranial magnetic stimulation

(rTMS), expectation-induced placebo analgesia was blocked while the pain experience was not affected (Krummenacher et al 2010).

1.5.3 The cortico-cortical pathway

More recently, another pain modulatory pathway has been suggested to mediate pain modulation, the cortico-cortical modulatory pathway (Figure 1.1; Wiech et al 2008b). Studies have demonstrated that higher areas of the brain are implicated in the cognitive and emotional modulation of pain. Modulation of these higher areas, while driving changes in pain perception, does not cause a change in the lower pain-related brain regions. This modulation is postulated to be achieved through cortico-cortical connectivity of prefrontal areas such as the DLPFC and ventrolateral prefrontal cortex (VLPFC) bypassing areas already established to be activated during pain namely the ACC, SI, SII, insula and thalamus (Wiech et al 2008b).

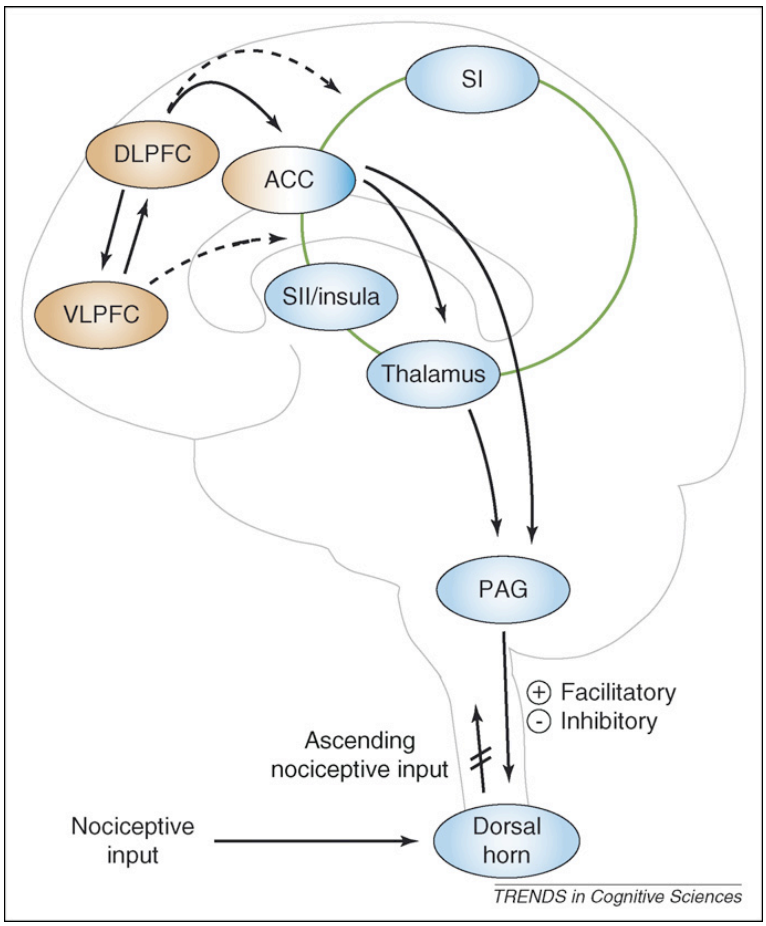


Figure 1-1 Possible pathways of pain modulation. Descending modulation of pain pathway is shown projecting from the ACC to the thalamus and the PAG to modulate pain impulses at the spinal cord. Cortico-cortical pathway involves connections between the DLPFC, VLPFC and ACC. Interconnections between pain-related brain regions are shown by the green circle. The broken lines are hypothesised connections between the PFC and pain-related brain regions. DLPFC: dorsolateral prefrontal cortex, VLPFC: ventrolateral prefrontal cortex, ACC: anterior cingulate cortex, SI: primary somatosensory cortex, SII: secondary somatosensory cortex, PAG: periaqueductal grey. From Wiech et al. (2008).

Studies manipulating cognitive aspects of pain such as reappraisal, control and coping produced changes in the PFC areas that were not accompanied by alteration in pain-related brain regions (Wiech et al 2006) suggesting that modulation takes place in the higher prefrontal regions. Lesion studies have also shown that functional disruption of one pain-related brain region is accompanied by augmentation in the pain-induced activation of one or more pain-related brain region as well as the PFC, suggesting interconnection between the pain-related regions with each other and with PFC (Starr et al 2009). However, the mechanism underlying this modulation is

still not well understood.

There is also first evidence that the PFC plays a key role in belief-related modulation of pain. Examples of this include neuroimaging studies on perceived control of pain (Salomons et al 2004; Salomons et al 2007; Wiech et al 2008b; Wiech et al 2006). In the study by Wiech et al (2006), manipulation of perceived control was used to determine effects on pain perception. Self-controlled stimulation was accompanied by less pain and anxiety and higher activation in the dorsal anterior cingulate, right DLPFC, and bilateral VLPFC. The results suggest that analgesic effect of perceived control relies on activation of right VLPFC.

The neurochemical basis for this modulation is, however, still unclear. PFC function has also been described in chronic pain and experimental models of chronic pain where this brain region is implicated in 'keeping pain out of mind' (Lorenz et al 2003). All these point towards a key role for the PFC in the modulation of pain.

1.6 Prefrontal cortex (PFC)

The PFC (Figure 1.2) comprises 29% of the cerebral cortex (Walker 1940). It occupies half of the frontal lobe anterior to the motor and premotor areas and is part of the neocortex. It is commonly divided into six regions namely, dorsolateral, ventrolateral, frontopolar, orbitofrontal, ventromedial and dorsomedial PFC (Ray & Zald 2011). The PFC is present in mammals but most developed in primates.

Cytoarchitectonically, the PFC is defined based on the presence of cortical granular layer IV. Further details on its subdivisions can be found in Chapter 5.

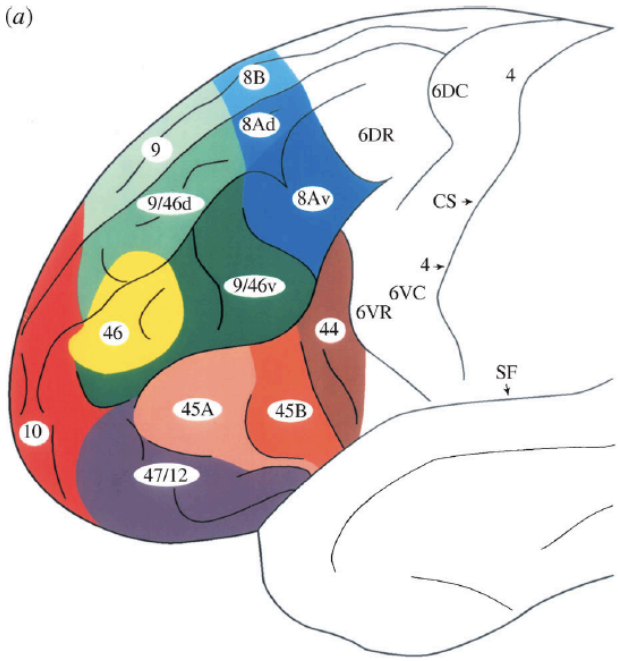


Figure 1-2 Cytoarchitectonic map of the lateral surface of the prefrontal cortex. Numbers indicate Brodmann areas. SF: Sylvian fissure. From Petrides & Pandya (2002)

1.6.1 PFC functions related to pain

The PFC is the executive area of the brain (Gazzaniga 2008). It can synthesise information from other brain regions and exerts control over behaviour (Miller & Cohen 2001; Miller et al 2002). It is also involved in higher level cognitive function such as in resolution of conflict (Egner & Hirsch 2005), decision making (Manes et al 2002) and emotion regulation (Wager et al 2008). These higher level cognitive functions are also factors that affect pain processing resulting in changes in pain perception.

It has been previously held that the DLPFC serve differential functions from the VLPFC. DLPFC is more involved in cognitive conflict, reinforcement and semantic information (Carter et al 1998; Egner 2008), while the VLPFC has predilection for more affective and emotional information processing (Beauregard et al 1998). However, a review of imaging studies on decision making and emotion regulation revealed considerable overlap of neural substrates serving similar function whether the objective is to modulate an operant response or an emotional one (Mitchell & Greening 2011).

In the context of pain, PFC activation has been found in studies on clinical pain (Baliki et al 2006), experimental models of chronic pain (Lee et al 2008), and modulation of pain, for example by attention (Valet et al 2004) and expectation (Atlas et al 2010). Furthermore, interaction between PFC and pain-related brain regions has also been demonstrated in placebo studies (Eippert et al 2009; Krummenacher et al 2010; Zubieta et al 2005). However, some other studies found activation of PFC without pain-related brain regions, such as studies on perceived control over pain (Wiech et al 2006) and religious belief modulating pain (Wiech et al 2008b). A recent study seems to support the idea that prefrontal mechanisms are predictive of placebo analgesia, specifically regions involved in emotional appraisal (Wager et al 2011).

1.6.2 Meta-analysis of PFC functions related to pain

A meta-analysis is a systematic review of literature that combines the results of many studies addressing a single research question (Egger et al 1997). The main outcome of a meta-analysis is the overall magnitude of the effect. The strength of a

meta-analysis is that it is better able to infer findings of a particular research question to the general population because of the bigger sample size. Compared to a single study, meta-analysis has higher statistical power.

Previous meta-analysis on pain imaging studies includes the study by Peyron et al (2000). The focus then was more on defining the brain network activated by painful stimuli, i.e. the somatosensory aspect of pain. Since then many other pain imaging studies have been performed and knowledge on activations due to pain has grown to include other aspects of pain such as pain modulation, placebo and nocebo, and also studies on patients with diverse kinds of chronic pain.

Meta-analysis on patients includes studies on patients with chronic pain (Derbyshire 1999) and patients with fibromyalgia (Dadabhoy et al 2008). The majority of pain imaging meta-analyses (Dadabhoy et al 2008; Derbyshire 1999; Peyron et al 2000) and reviews (Apkarian et al 2005; Kupers & Kehlet 2006; Moisset & Bouhassira 2007; Peyron et al 2000; Seifert & Maihofner 2009; Tracey 2008; Treede et al 1999), used qualitative methods to describe patterns of activations across studies and only a few performed quantitative analysis using coordinates of activation (Farrell et al 2005; Lanz et al 2011).

So far, no known meta-analysis or reviews have specifically looked at the extent of PFC activation during different types of pain modalities in healthy individuals and patients. Wager (2005) in his meta-analysis of placebo studies concluded that activations of the DLPFC due to pain occur over a wide area and not confined to a

localized site.

1.6.3 Connections between PFC and pain-related brain regions

In order to elucidate the mechanisms by which the PFC exerts control over pain, researchers have begun to investigate the anatomical connections between the PFC and pain-related brain regions. Structural connectivity between two brain regions strongly suggests functional connectivity (Behrens et al 2006; Johansen-Berg et al 2005). Knowing the structural connectivity between PFC subdivisions to the various pain-related brain regions can shed light on the means by which modulation of somatosensory and cognitive-affective aspects of pain is achieved.

While the gold standard for connectivity studies are tracer studies in animals and histology studies in humans, diffusion-weighted imaging provides the advantage of studying the white matter connectivity of the human brain in vivo. Probabilistic tractography provides a means to ascertain the probability of connection between areas in the brain. This has been shown by various tractography studies showing connection probabilities between brain regions based on findings from functional connectivity (Beckmann et al 2009; Behrens et al 2003a; Clatworthy et al 2010). These studies are able to define the probability of white matter connections between brain areas as well as parcellate the brain regions according to their connection probabilities.

However, a study by Honey et al (2009) showed that strong functional connectivity also exists as a result of indirect connections and may not be accompanied by

structural connectivity. Thus, direct connections between the PFC and pain-related brain regions may not be the only way that control is exerted. Interconnections between the different PFC areas may provide an indirect connectivity to pain-related brain regions. Nevertheless, defining the structural connections will provide a clue on the differential roles of the PFC subdivisions in pain modulation.

1.6.4 Role of PFC in emotion regulation

Emotion regulation (ER); Gross 1998) refers to the 'processes by which individuals influence which emotions they have, when they have them, and how they experience and express these emotions', i.e. the alteration of emotional processes that serve as a coping mechanism. Emotions result from person-situation transaction that attracts attention, has a valenced meaning and eventually give rise to a multisystem response (Gross et al 2011). This emotion generation may be bottom-up, i.e. elicited by presentation of a stimulus, or top-down, i.e. elicitation of emotion by activation of appraisals that a situation is relevant (McRae et al 2011). ER begins with an emotionally-relevant *situation* that commands *attention* and *appraisal*. These three processes (situation, attention and appraisal) are known as 'antecedents' and subsequently give rise to a multisystem response (Gross 2007; Figure 1.3). ER processes may be antecedent-focused or response-focused. Antecedent-focused ER occurs before emotion is generated and involves four processes, namely, situation selection, situation modification, attentional deployment and cognitive change (Gross 1998), response-focused ER occurs after emotion generation and involves response modulation.

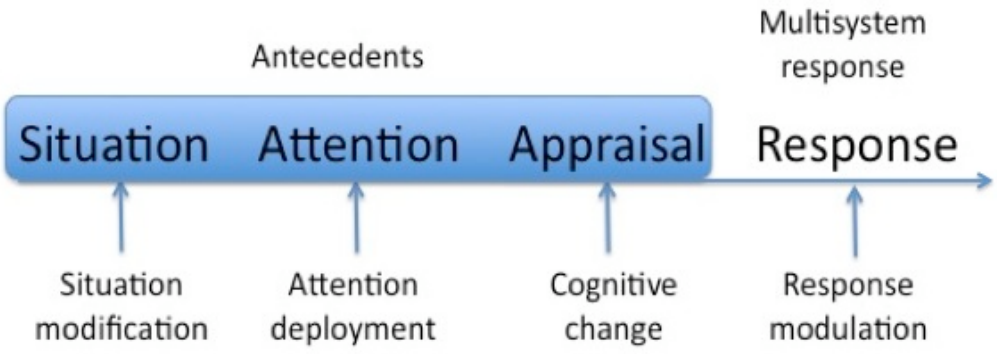


Figure 1-3 The process of emotion regulation. Antecedents (shaded region) precede and influence the multisystem response. Arrows indicate the feedback from the response to the antecedent processes. Adapted from "Emotion Regulation: Conceptual Foundations" (pp. 3–24), by J. J. Gross and R. A. Thompson, in *Handbook of Emotion Regulation*, 2007, New York: Guilford Press. Copyright 2007 by Guilford Press.

Reappraisal is a type of antecedent-focused ER that involves cognitive change that targets the appraisal stage (Urry 2009). Reappraisal involves reinterpreting the meaning of an aversive stimuli or situation in a way that reduces its emotional impact or even changing it into a more positive one (Eippert et al 2007; Pitskel et al 2011; Wager et al 2008). Effortful reappraisal to down-regulate negative emotions requires cognitive control that overrides reflexive or automatic action (Pessoa 2008).

Figure 1.4 illustrates the neural substrates involved in ER. PFC areas most commonly implicated in ER include the orbitofrontal cortex (OFC), DLPFC, VLPFC, dorsomedial PFC and ACC (Phillips et al 2008). Ventromedial aspect of PFC (including OFC, dorsomedial PFC and ACC are generally associated with control of emotional behaviours while the lateral aspect, i.e. DLPFC and VLPFC are more involved in higher executive functions (Phillips et al 2008). However, overlap in function has been demonstrated for the different PFC subdivisions (Mitchell 2011). Wager et al (2008) conducted an fMRI reappraisal study using aversive pictures to test the 'Mediation Hypothesis' which stated that reappraisal success involves a pathway

from the control system in the executive area of the brain (PFC) through an affective reappraisal system mediator rather than a direct pathway. The study showed correlation between right VLPFC and reappraisal success, and confirmed the presence of two pathways, a path through nucleus accumbens that predicted greater reappraisal success, and through ventral amygdala that predicted reduced reappraisal success.

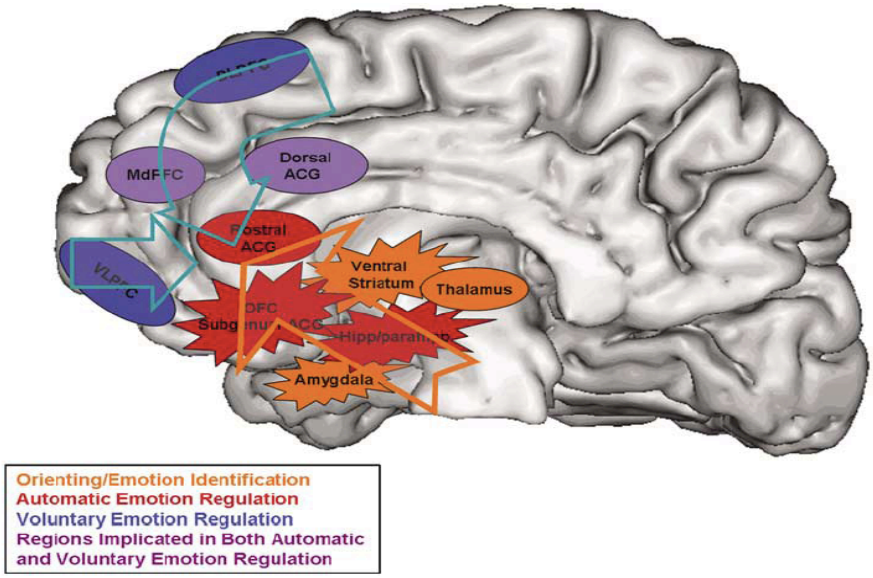


Figure 1-4 Neural substrates for emotion regulation. Blue arrow: feedback pathway for voluntary emotion regulation, red arrow: feed forward pathway for automatic emotion regulation. From Phillips et al 2008.

1.6.5 Role of PFC in cognitive control

The executive function of PFC relies on cognitive control processes mediated by its various subdivisions. Animal models of prefrontal executive function allow the investigation of the contribution of PFC subdivision to specific cognitive processes (Chudasama 2011). Among the animal models used that are comparable to human cognitive elements are models that test planning, working memory, attention, response sequencing, and conditional learning (Robbins 1998). In humans, the study

of cognitive control is often done using conflict (Botvinick et al 2001). The brain has the capacity to rapidly adjust processing strategies to overcome conflict (Botvinick et al 2004). In the face of high conflict the brain exerts cognitive control by amplification of the task-relevant stimuli rather than inhibition of the task-irrelevant one (Egner & Hirsch 2005).

Investigation of conflict has been shown using the Stroop task adaptation of congruent and incongruent paradigm of non-emotional and emotional stimuli. The incongruent information signifies conflict that needs to be resolved. It was found that there are distinct circuits that mediate the processing of emotional and non-emotional stimuli (Egner et al 2008). While a similar area dorsal anterior cingulate cortex (dACC) is implicated in task monitoring, the execution of conflict resolution differs between emotionally salient and non-emotionally salient distracters. The lateral PFC is found to be involved in conflict resolution of non-emotional stimuli and predicts enhancement in the somatosensory cortices, while the rostral ACC mediates the conflict resolution of emotional distracters which is associated with inhibition of emotion-induced amygdala activation (Egner et al 2008).

In the context of pain, cognitive control has mainly been studied with respect to attentional modulation of pain. In these studies, both the task and the pain stimuli are applied at the same time hence the effects are mainly due to attention away from pain and towards the stressor. Petrovic et al (2000) used cold pressor pain during an attention-demanding Maze task to demonstrate decreased activity in the somatosensory association areas and the periaqueductal grey accompanying lower

ratings of pain, with increased activation in the OFC. Using the counting Stroop test as the distractor and applying noxious thermal heat, Bantick et al (2002) showed lowered activations in the thalamus, insula and dorsal ACC, and increased activations in rostral ACC and the OFC. Valet et al (2004) used colour-word Stroop and heat pain to exhibit reduction in pain-related areas and increased activations in the cingulofrontal cortex, the periaqueductal grey, and the posterior thalamus. Wiech et al (2005) studied the effects of a concurrent attention demanding task on capsaicin-induced hyperalgesia and revealed that the pain-related activation in medial PFC and cerebellum was higher during performance of the easy task compared to the hard task. In a similar vein, a study using different combinations of cognitive load and pain intensity (Seminowicz & Davis 2007) showed that pain did not affect activity in cognitive-related areas except when cognitive load was minimal.

These findings from pain studies provide evidence of a supervisory role of the PFC in the modulation of pain during concurrent cognitive task, by assigning resources to the more salient stimuli. This is in keeping with cognitive control outside the pain context showing enhanced cortical responses to task-relevant stimuli and inhibition of neural responses to task-irrelevant ones (Egner & Hirsch 2005).

1.7 Research questions and hypothesis.

Although the PFC has been implicated in pain modulation in a number of studies, there are still many open questions. First, what is the current overall picture and extent of PFC involvement in healthy volunteers and chronic pain patients? Second,

what is the structural connectivity between PFC and pain-related brain regions and does this connectivity reflect the differential roles of the DLPFC and VLPFC in the modulation of pain? Third, what is the subjective and physiological response to cognitive stress? Fourth, what is the role of the opioid system in the modulation of pain during reappraisal and contextual modulation?

It is hypothesized that activations of PFC as reported in the studies are present across a wide range of pain stimulation and conditions. Secondly, structural connectivity of the PFC to pain-related brain regions is hypothesized to reflect their functional connectivity whereby brain regions that are more associated with somatosensation have a higher probability to connect to the DLPFC while regions that are more concerned with cognition and affect are more likely to connect to the subdivisions in the inferior frontal gyrus, the pars opercularis and VLPFC. Thirdly, cognitive stress will show increase in both subjective and physiological indicators of stress, and fourthly, effortful reappraisal modulation of pain will be mostly independent of opioid involvement as opposed to contextual modulation.

1.8 Overview of the thesis

The overall objective of this thesis is to explore the role of the PFC in the modulation of pain. It seeks to complement existing knowledge in pain, firstly through a meta-analysis of pain imaging studies in healthy volunteers as well as pain patients that report PFC activation, secondly by performing a probabilistic tractography study to define the structural relationship between PFC and pain-related brain regions, and

thirdly, through a functional imaging study employing reappraisal and contextual modulation strategies to identify patterns of activation in the brain in the presence of endogenous opioid receptor blockade.

Chapter 2 outlines the methods that were used and their underlying principles.

Chapter 3 describes a pilot study in which we investigated the psychophysiological effects of stress using a Stroop colour-word task that has previously been shown to activate the PFC and interfere with pain activation through distraction. The intention of this study was to explore whether this cognitive task will induce a level of stress that could lead to stress-induced analgesia.

Chapters 4 and 5 describe two meta-analyses of pain imaging studies using positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) to investigate the extent of PFC activation during pain in healthy volunteers and patients. This study employed coordinate-based meta-analysis (CBMA) with Gaussian-process regression (GPR), a newly developed technique in our lab, to statistically define PFC and pain-related areas that are activated during a pain experience across studies.

Chapter 6 describes a study in which probabilistic tractography was used to delineate the relative structural connectivity between the divisions of the lateral PFC with pain-related brain regions in healthy volunteers. The lateral PFC are subdivided into DLPFC, pars opercularis and VLPFC and used as seeds to determine connection

probability to targets comprising pain-related brain regions i.e. brain regions that are consistently activated during an experience of pain as shown by previous pain imaging studies. The purpose is to see whether there are differences in connection probability between the pain-related brain regions and the subdivisions of the PFC as hypothesized.

Chapter 7 describes a behavioural study we performed to further characterize the role of the PFC in the cognitive modulation of pain. In this study, we investigated the involvement of the endogenous opioid system in the cognitive modulation of pain through reappraisal and contextual modulation.

2. METHODS

2.1 Introduction

In this chapter, the relevant methods used in the experiments are discussed. First, the principles underlying diffusion-weighted imaging as well as probabilistic tractography (Chapter 6). Next, the method used in the meta-analysis of pain imaging studies is outlined (Chapters 4 and 5). Finally, we discuss methods to induce pain as well as the subjective and physiological outcome measures used in the experimental chapters (Chapters 3 and 7).

2.2 Diffusion-weighted imaging

Diffusion is the random movement of molecules due to the thermodynamic effect (Huettel et al., 2004). When the probabilities to move in all directions are identical, the microenvironment where the molecule exists is called *isotropic*. In the brain tissue, the microenvironment of a water molecule can be *anisotropic*, i.e. the orientation of movement can be limited by some physical conditions.

Barriers in the body such as cell membrane restrict the Brownian motion of water molecules (Beaulieu 2009). Differences in the diffusion property of different structures form the basis for diffusion-weighted imaging (DWI). In white matter, the numerous longitudinally oriented neurofilaments and microtubules pose a barrier to diffusion perpendicular to the axon while diffusion along the axon is relatively more free (Beaulieu 2009). Therefore, by mapping the degree of anisotropy, the white

matter area (of high anisotropy) and the grey matter area (of low anisotropy) can be distinguished.

The diffusion tensor model assumes that the overall orientation of molecule diffusion in a voxel can be modelled as a tensor, which can be treated as an ellipsoid and therefore characterised by three axes (Basser et al 1994). A tensor with one axis longer than the other indicates greater anisotropy, i.e. diffusion is more orientation dependent. In contrast, a tensor with three axes of equal length indicates isotropy. Diffusion tensor imaging (DTI) shows the spatial features of anisotropy by mapping the dominant orientation of the tensor at each voxel. It therefore becomes a useful tool in investigating some pathological conditions related to white matter disruption, such as stroke, traumatic brain injury, and potentially affective disorders (Voss & Schiff 2009; Sexton et al 2009).

The investigation of anatomical connections between brain regions has previously only been possible through invasive means, by injection of tracers in primates or by the dissection of post-mortem brains (Kobbert et al 2000). DWI offers a non-invasive way of imaging white matter fiber directions and indirectly infering connectivity between brain regions.

DWI utilizes the diffusion property of water molecules in the tissue. By application of large magnetic field gradients during MR image acquisition, the diffusion of water molecules within the voxel can be captured by MR image (Ramnani et al 2004; Ramnani & Owen 2004). Additional diffusion gradients are added into the imaging

sequence to make pulse sequence sensitive to diffusion. These additional gradients cause signal dephasing and result in signal attenuation. Greater signal loss means higher diffusivity and vice versa. From these images, the local direction of greatest diffusion can be computed. These are called the principal diffusion direction (PDD). Images formed by diffusion weighting are a combination of echo-planar imaging (EPI) or fast gradient echo sequences and two large gradient pulses that are applied post-excitation. The gradient pulses are designed to cancel each other out if spins are stationary while phase shift is produced during spin movement. Therefore, in structures where diffusion is restricted by membrane or ligaments, the signal is attenuated while in tissues that have a high degree of diffusivity the signal is increased. The degree of attenuation is dependent on the amplitude (bvals) and direction/vector (bvec) of the gradients.

2.2.1 Probabilistic tractography

Tractography is the technique of tracing brain pathways by integrating voxel-wise fiber orientation into a pathway that connects remote brain regions (Johansen-Berg & Behrens 2006). A simple way to find the tract is to design a streamline composed of the dominant fiber orientation, i.e. the PDD, estimated in each voxel. However, the estimation of PDD is associated with uncertainty due to thermal, physiological and other intrinsic measurement noise during acquisition, as well as uncertainty because of the inadequacy of the model (Behrens et al 2003b). In reality, multiple tracts may exist in a voxel and the model is simpler than the actual diffusion in the data. This uncertainty is low in deep white matter fibers but can be high in areas with complex structures, for example at the intersection of the superior longitudinal

fasciculus, corona radiata and corpus callosum, where there is a high density of crossing fibers.

In areas with complex structures, therefore, the streamline tractography can be error prone because of the accumulating uncertainty (Jones 2003). Also, a streamline may stop prematurely because of low anisotropy in single voxels (Jbabdi & Johansen-Berg 2011). To overcome this limitation, Behrens et al (2003b) incorporates the uncertainty in the form of a posterior probability density function (pdf) at each voxel and globally estimating the chance of finding a continuous streamline between two regions in the brain. This probabilistic tractography model has the advantage of allowing to track regions of low anisotropy and to quantify our confidence of finding a connection between regions (Jbabdi & Johansen-Berg 2011).

As the only non-invasive method to map the structural connectivity in the human brain, DTI tractography has become increasingly popular. Changes in white matter integrity have been shown to be associated with motor and sensory training (Johansen-Berg et al 2007; Johansen-Berg et al 2010) as well as cognitive capacity (Catani et al 2007; Cohen et al 2009). Combining tractography results with the corresponding functional data will provide more insight into the role of brain connectivity in human behaviour (Rudebeck et al 2009). Since structure reflects function (Johansen-Berg et al 2004; Johansen-Berg et al 2005), understanding the anatomical connections of an area can infer its functional connectivity (Passingham et al 2002). While functional imaging detects transient changes in the brain,

structural imaging is more appropriate to detect functionally relevant brain plasticity over time (Johansen-Berg 2011).

To this end, diffusion-weighted imaging offers the opportunity to delineate possible changes in brain connectivity due to pain. Researchers have started investigating the relevance of anatomical connections in the context of pain. Moisset et al (2010) combined fMRI with DWI to elucidate the anatomical relationship underlying the functional connectivity in painful rectal distension. Therefore, it is essential to first know the normal connections between brain regions related to pain.

To determine connectivity between areas in the brain, tracking is done from the origin (seed region) to the target region of interest. Masks for the seed and target regions are first defined. Sampling of the fiber direction at each voxel in the seed region is done repeatedly using Markov Chain Monte Carlo (MCMC) to develop a representation of the pdf at each voxel and identify possible tract directions based on the pdf (Behrens et al 2003b). MCMC is an algorithm for sampling from a probability distribution by constructing a Markov chain that makes trajectories in random directions. This way, diverse paths are repeatedly sampled, subsequently producing a map displaying the number of trajectories passing through each voxel (Bodammer et al 2009). The global connectivity can then be estimated from any point in the brain by taking samples from the local pdfs. Sampling here refers to sampling of the fiber direction from the pdf in each voxel. By default, in the FMRIB's Diffusion Toolbox (FDT); Smith et al 2004) within FMRIB software library (FSL) it is set at 5000, meaning that sampling is performed 5000 times from each voxel using

MCMC. The probability of projection between the seed to the target(s) can then be computed.

2.2.2 Connectivity-based parcellation

The cerebral cortex can be subdivided according to structurally and functionally distinct areas, also termed parcellation or connectional fingerprinting. One application of DWI is the parcellation of the brain into areas with distinct probabilistic connectivity patterns (Johansen-Berg et al 2004).

From the probabilistic tractography, the seed region can be classified according to the target areas with which they have the greatest probability of connection. This can be done a priori without defining target areas that interconnect with a particular seed region (Beckmann et al 2009), or by a posteriori approach using predefined target areas known to have connection with the seed area from primate connectivity studies (Beckmann et al 2009; Behrens et al 2003a). This parcellation enables the defining of borders between brain regions according to their probability of connections in a living brain.

The study in Chapter 5 utilises diffusion-weighted imaging to determine relative connection probability between prefrontal subdivisions and pain-relevant brain regions.

2.3 Meta-analysis of pain imaging studies

Neuroimaging studies usually use relatively small sample sizes to answer a specific hypothesis or research question. Depending on the robustness of the effect under investigation, this can result in small statistical power and a high rate of false positives (Kober & Wager 2010). To increase statistical power and enable a more reliable inference to the general population, meta-analysis is increasingly used in the field of neuroimaging (Farrell et al 2005; Lanz et al 2011). Meta-analyses of neuroimaging data allow the identification of regions that are consistently activated during a particular task or state by pooling data across studies with related research hypotheses.

Contemporary neuroimaging meta-analyses are either coordinate-based meta-analysis (CBMA) or image-based meta-analysis (IBMA) (Salimi-Khorshidi et al 2009). CBMA is the standard method in imaging meta-analysis and is based only on activation foci in a standard space that are commonly reported in a table as a minimal summary of the findings while IBMA methods combine whole-brain statistic volumes. IBMA is superior to CBMA because its use of full statistic images takes into account differing intra-study variance and allows modeling of random inter-study variation (Salimi-Khorshidi et al 2009). However, authors of neuroimaging meta-analyses rarely have access to complete original datasets as most publications only provide information about the magnitude of effects and coordinates of their activation peaks rather than full image data.

Meta-analysis using CBMA may utilize one of several methods, namely activation

likelihood estimation (ALE), kernel density approximation (KDA) and multi-level kernel density approximation (MKDA) (Salimi-Khorshidi et al 2009). In all three methods, a map comprising the coordinates of activation is produced. Significance is assessed using Monte Carlo resampling approach to test the null hypothesis of no coherent activation that the foci are randomly distributed across space (Salimi-Khorshidi et al 2009). An uncorrected p value is obtained at each voxel using the Monte Carlo realizations that equal or exceed the original value while corrected p value is where the maximal image-wise value exceeds the original value.

Existing CBMA techniques such as ALE (Turkeltaub et al 2002), KDA and MKDA (Salimi-Khorshidi et al 2009; Wager et al 2004a; Wager et al 2007), suffer from their strong dependency on their arbitrarily-selected kernel size, solely use “activation coordinates” without incorporating their corresponding effect size (e.g. Z-stats), and cannot assess the extent of heterogeneity present in the study pool (e.g. caused by slight variations in the study design, or different numbers of subjects taking part in each study) (Salimi-Khorshidi et al 2011). In order to avoid these problems, Gaussian-process regression (GPR) has been introduced to CBMA (Salimi-Khorshidi et al 2011).

Gaussian process (GP) assumes that the data (i.e. z-stat) is a 3D Gaussian distribution ~N(0,S), where S is the covariance. The covariance is estimated from the data, i.e. the coordinates and the z-stat and not set arbitrarily or by a rule-of-thumb (Salimi-Khorshidi et al 2011). Using S, the value of a voxel can be estimated given the neighbouring voxels' values (i.e. the more covarying two voxels are, the closer their values will be). Such covariance functions can be modelled mathematically with

certain parameters, which will formulate the similarity of the Z-stat values of a volume based on their spatial distance (e.g. two close-by voxels are expected to have very similar values). Using this GP model, the parameters can be estimated given the samples, i.e. the foci of peak activations obtained from imaging studies (Salimi-Khorshidi et al 2011).

The introduction of GPR to CBMA provides the following advantages: (1) It makes the joint usage of activation and deactivation possible; (2) It enables the meta-analysis to incorporate the effect size and the location information (i.e. coordinates) rather than location alone; (3) It provides a solution for estimating the random effects variance/heterogeneity, which provides the meta-analysis with both fixed effects and random effects options; (4) It enables the analysis to predict the effect size at voxels that have no observation associated with them (i.e. regression); and (5) It allows for the estimation of the scale of the spatial covariance from the data instead of setting it arbitrarily or by a rule-of-thumb.

2.4 Experimental pain in humans

2.4.1 Noxious stimulation

In experimental settings, pain has been induced by various types of stimulation, including heat, cold, electrical stimulation, pressure and chemical irritants. In this thesis, noxious thermal stimulation was used in the study investigating the role of the opioid system in pain (Chapter 6). Noxious thermal stimuli have been reliably used in the investigation of opioid-mediated analgesia (Eippert et al 2008;

Wanigasekera et al 2011) and are also the most frequently used modality to induce pain in neuroimaging studies as found in the meta-analysis study in Chapter 4. Nociceptors for thermal heat have been identified as a component of the vanilloid receptor-system, part of a larger transient receptor protein (TRP) in the receptor membrane (Arendt-Nielsen & Chen 2003). Heat-related nociceptors exist at peripheral nerve-endings, primary sensory neurons and spinal cord. Transmission of thermal heat and pain are mediated by thinly-myelinated Aδ and unmyelinated C fibers.

2.4.2 Behavioural measures

Since pain is a subjective experience and not readily measured by objective means, pain is commonly assessed by subjective report and the analysis of pain-related behavior. In humans, a Visual Analogue Scale (VAS) is widely and reliably used to measure pain characteristics (e.g. pain intensity, unpleasantness), as well as associated psychological states such as stress, mood and anxiety (Wewers & Lowe 1990). It consists of a 10-cm line with anchors at both ends defining the range of perception such as “not painful” at the minimum end and “very painful” at the maximum end. Participants are required to move an indicator along the line using either button press on a computer keyboard or a sliding potentiometer and stop at a point that best describes their perception.

In this thesis, a VAS scale was used in the experimental studies (Chapters 3 and 6). The scale was projected onto a computer screen during behavioural experiments or the projector screen during scanning. Apart from pain perception in the form of pain

intensity and unpleasantness, a VAS scale was also used to assess the level of stress, mood and anhedonia. The specific scales for each study will be described in the relevant chapters.

2.4.2.1 Evaluation of factors that influence pain - questionnaires

Emotional and affective aspects play a major role to modulate pain perception. Psychological questionnaires are an indispensable tool to assess the psychological construct of the individual. Relevant questionnaires given to participants in the experimental study in Chapter 7 were the Snaith-Hamilton Pleasure Scale (SHAPS), the Behavioural Inhibition System (BIS) and Behavioural Activation System (BAS). SHAPS is a validated instrument to measure hedonic tone or the ability to experience pleasure (Franken et al 2007; Snaith et al 1995). SHAPS assesses to what extent a person is capable of experiencing pleasure in four domains, namely interest/pastimes, social interaction, sensory experience, and food and drink (Snaith et al 1995). The psychometric properties of SHAPS have been measured in conditions such as major depressive disorder (Nakonezny et al 2010) and Parkinson disease (Loas & Krystkowiak 2010). In this thesis, SHAPS is used to assess how hedonic tone can influence pain modulation due to contextual influences and reappraisal of pain.

The BIS and BAS measure the personality dimensions that reflect two opposing motivational systems in an individual (Scheres & Sanfey 2006). While BIS is associated with a tendency towards concern and anxious rumination, BAS is more associated with optimism and orientation towards reward and impulsivity. Personality differences in anxiety and impulsivity respectively influence the degree

of activation of BIS and BAS. The BAS is further subdivided into three subscales, namely Fun-seeking, Reward-responsiveness and Drive. In the experimental study in Chapter 7, BIS and BAS were used to assess the relationship between each dimension with the ability to reappraise pain and to contextually modulate pain.

2.4.2.2 Autonomic measures of stress

Like pain, stress is a subjective experience. What is deemed stressful to a person may not be so for another; similar situations will produce entirely different reactions in different individuals. In experiments that induce stress, it is therefore essential to objectively measure the stress response in each individual.

The stress response is mediated by the central and peripheral nervous system (Charmandari et al 2005). The brain interprets what is stressful while the hormones and other systems in the body mediate the effects of stress (McEwen 2000). Stress is detected by an interface consisting of brain regions that include the hippocampus, amygdala and PFC, which are in turn connected to the hypothalamus (Joels et al 2006). This results in activation of the hypothalamic-pituitary-adrenal (HPA) axis (Makino et al 2002) and the autonomic (sympathetic) nervous system (McEwen 2007).

Activation of the HPA axis causes increased secretion of cortisol that can be measured in the plasma, saliva, or urine. The sympathetic system response to stress causes increased secretion of catecholamines leading to increased heart rate and blood pressure. Studies have found significant increases in systolic and diastolic

blood pressure (Wang et al 2007), heart rate (Bandura et al 1988) and salivary cortisol level (Tessner et al 2006) on exposure to psychosocial and cognitive stress.

2.4.2.2a Cortisol level

Cortisol level as the endpoint of the HPA axis reactivity is a good indicator of stress level. In situations where there is high degree of uncontrollability, unpredictability, novelty, and ego involvement, corticotrophin-releasing hormone and ACTH are released resulting in cortisol secretion (Kirschbaum & Hellhammer 1994). Although plasma cortisol is considered the gold standard for stress reactivity, salivary cortisol has been by far the easiest and most reliable measurement of challenge tests such as the psychological stress tests that assess the responsiveness of the HPA axis towards the stressor (Seeman et al 1995). Only the free, biologically active proportion of cortisol is present in the saliva. Salivary cortisol is highly correlated with serum/plasma cortisol and more closely correlated to the free/unbound fraction of serum cortisol than total serum cortisol (Kirschbaum & Hellhammer 1994). Salivary cortisol varies with reported levels of daily stress, affect (Smyth et al 1998), and time of day. Because cortisol secretion follows a circadian rhythm with peak levels in the morning and lowest in late evening, cortisol samples should be obtained at about the same time of the day. Furthermore, the time between the onset of a stressor and the acquisition of the cortical sample should be kept constant.

In our study on the influence of a cognitive task on subjective and physiological markers of stress, we used a Stroop task as the stressor (Chapter 3). Previous studies have shown variable responses of salivary cortisol to the Stroop test, ranging from elevated cortisol level (Brydon et al 2008) to no significant change (Seldenrijk et al 2012). A review of studies on the cortisol response found that factors that may influence the cortisol response include uncontrollable outcome of the event and presence of social evaluative threat (Dickerson & Kemeny 2004).

2.4.2.2b Skin conductance response

Sympathetic activity can be measured using the skin conductance response (SCR). SCR is a change in the electrical properties of the skin due to activation of a person's sweat glands causing damp skin, allowing a small electrical current to pass under the measuring electrodes. When the sweat dries off, the resistance rises and the conductance falls again. This activation of the sweat glands is the result of neurophysiologic arousal with increased activity in the sympathetic nervous system.

A galvanic skin response (GSR) amplifier applies a constant voltage to the skin through electrodes and the current that flows through the skin can be detected and displayed. The output given by the GSR amplifier is the skin's conductance expressed in microSiemens. The term skin conductance refers to how well the skin conducts electricity when an external direct current is applied to the skin (Figner & Murphy in press). The SCR is a sensitive index for pain and is not influenced by circulatory changes, cardioactive or vasoactive drugs and neuromuscular blockade (Storm

2008). It has also been shown to correlate with pain ratings (Chapman et al 2002; Chapman et al 2001). An fMRI study using laser-evoked pain showed that SCR responses reflect actual variations of the brain's response to painful stimuli; a higher amplitude of laser evoked potentials evoke higher electrodermal activity (EDA) response and correspondingly stronger blood oxygen level dependent (BOLD) responses compared to a lower amplitude of laser evoked potential (Mobascher et al 2009b). Habituation of the GSR response to acute pain exposure has also been demonstrated indicating habituation of the autonomic responses to acute pain (Petrovic et al 2004).

Figure 2.1 shows an example of an SCR tracing. Following a stimulus, the SCR rises from baseline to a peak and then decreases relatively more slowly to baseline. The components of an SCR that can be used to quantitatively characterize SCRs include the amplitude (the difference between the conductivity at baseline and the peak) and other temporal parameters of SCR such as the rise time, onset latency and recovery half time (Figner & Murphy in press). A more recent indicator is the area bounded by the curve. For this thesis the amplitude is used to quantify SCR in the behavioural studies in Chapters 3 and 7.

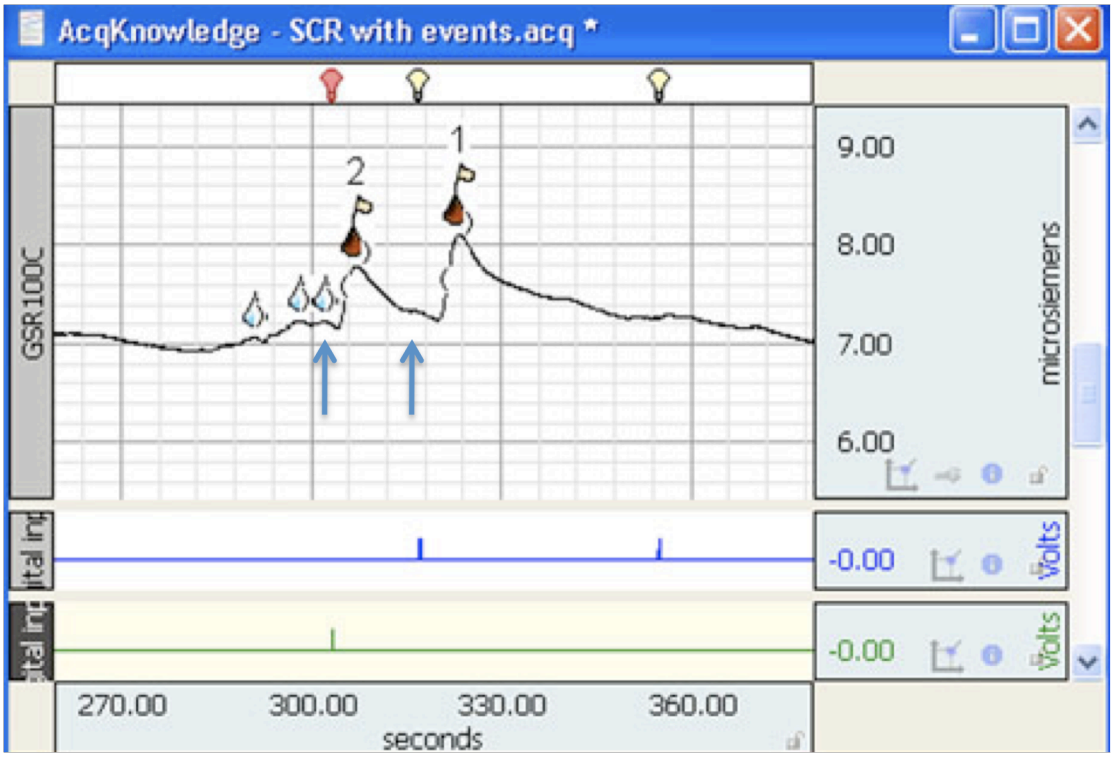


Figure 2-1 Example of EDA time course in response to stimuli (indicated by arrows)

3. BEHAVIOURAL STUDY: EFFECT OF A SELECTIVE ATTENTION TASK ON THE PERCEPTION AND BIOMARKERS OF STRESS

3.1 Introduction

Although stress is commonly understood to increase pain, their relationship is more complex. Stress-induced analgesia (SIA) is the reduction in pain perception as a result of exposure to a distressing event (Amit & Galina 1986). While animal studies of SIA used mainly physical stressor such as swim stress (Connor et al 1997), footshock (Kowalski et al 1988) and restraint (Korzeniewska et al 1995), studies in human mainly utilise psychological stressor such as cognitively demanding task such as mental arithmetic (Slawomira et al 2011) and Stroop task (Fechir et al 2011). SIA may be opioid or non-opioid mediated (Grau 1987) and results from the descending modulation of pain.

In experimental studies, a stressor is implemented before pain testing. Induction of SIA can be achieved either by a physical (Willer et al 1981) or a psychological stressor such as mental arithmetic (Flor & Grusser 1999). To investigate SIA we need a valid and reliable model, i.e. a stressor that induces stress at both behavioural and physiological levels.

In studies on SIA in humans, cognitive tasks such as the Stroop Task (Stroop 1935) are often used to induce stress. The Stroop Task is a colour-word interference task in which congruent and incongruent colour names are presented. In the incongruent

colour-word condition the word names a colour that is incongruent with the colour of the word, whereas in the congruent colour-word condition the name of the colour and the colour of the word are the same. The task is to indicate the colour of the word and not its semantic meaning.

It has been shown that the incongruent colour-word condition is more difficult to perform resulting in lower percentage of correct responses and slower reaction time compared to naming the colour of the congruent word. This is known as the Stroop effect or interference. The task was initially designed to study interference in serial verbal reactions, but has since been modified to be a cognitive test (MacLeod 1991). The Stroop task has also been shown to induce stress, as indicated by an increase in stress parameters such as heart rate, blood pressure and plasma cortisol (Brydon et al 2008; Salahuddin et al 2007).

The aim of this study was to pilot the effect of a Stroop task with different levels of difficulty on subjective ratings as well as physiological indicators for stress including electrodermal activity and salivary cortisol. For this study, two designs were used. The purpose of this approach was to compare stress reactivity induced by a more difficult design, Design 2, compared to a less difficult Design 1 (Gianaros et al 2005). The purpose is to assess whether task difficulty has any effect on subjective and objective stress reactivity.

3.2 Method

3.2.1 Participants recruitment and selection

Fourteen healthy volunteers aged between 18 and 48 years (9 females), mean age 33.6 ± SD 8.9 years, participated in this study. All participants were right-handed, had normal colour vision and had no history of hypertension, heart disease, HPA axis disorder, or psychiatric illness. Participants who were on steroid treatment were also excluded. Informed consent was obtained and all procedures were in accordance with the Helsinki Declaration and approved by the Institutional Ethics Committee. Participants were not given any reimbursement for their participation.

3.2.2 Study design

The study was based on a within-subjects design with the factors DESIGN (Design 1 and Design 2), DIFFICULTY (congruent and incongruent) and BLOCKS (six blocks signifying advancing time). It consisted of two sessions separated by at least one day. Participants were presented with Design 1 or Design 2 (Figure 3.1) of the modified colour-word Stroop task during either session in a pseudorandomized order. During each session, seven blocks of congruent and six blocks of incongruent trials with 30 trials in each block were presented, resulting in 13 blocks overall per session. For both designs, the first congruent block was excluded from the analysis to allow for adaptation to the Stroop task, leaving six blocks each for congruent and incongruent trials. Participants were instructed to indicate by pressing a button, which of four words displayed at the bottom of the computer screen corresponded to the colour of the word in the center of the screen. For Design 1, the choice words were in black

for both the congruent and incongruent task. For Design 2, the choice words for the congruent task were in colours congruent to the colour that they had to name while the incongruent task was made more difficult by using choice words in colours that were incongruent to the colours that they name (Figure 3.1). The order of the choice words for incongruent task in both designs was pseudorandomized. A diamond shaped green cue was shown for six seconds before the congruent task block while the incongruent task was cued using a red triangle, also for six seconds.

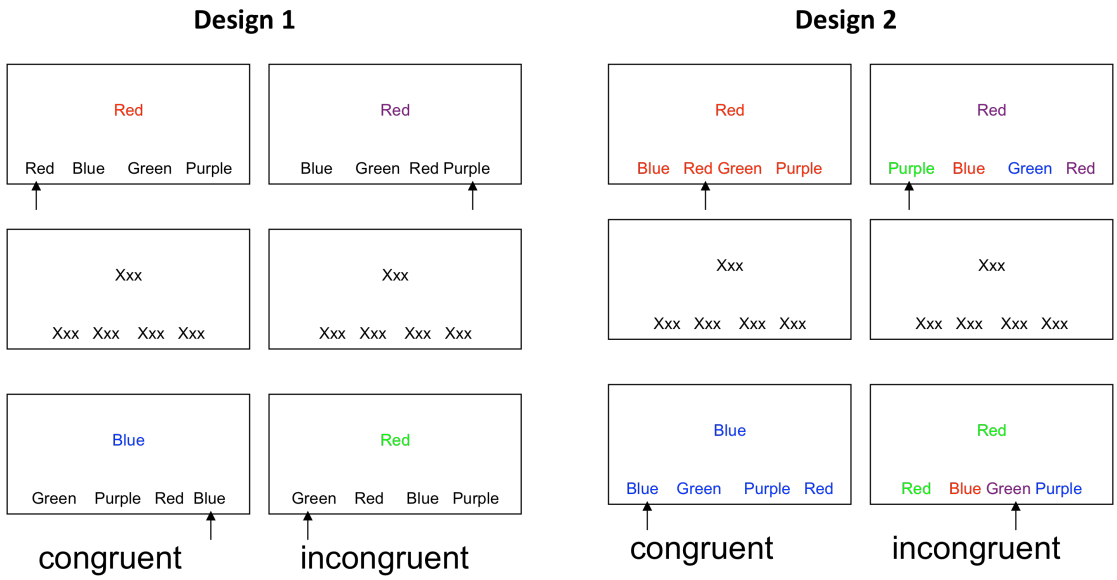


Figure 3-1 Design 1 and 2 of the colour-word Stroop task. Each design comprised seven blocks of congruent and six blocks of incongruent tasks with 30 trials in each block. The trials (examples shown in the first and third rows), lasted 1.2 seconds each followed by 0.8 seconds of fixation cross (second row). Arrows indicate the correct responses. In between trials, participants were asked to fixate on the cross that was displayed at the center of the screen. Trials in Design 1 had choice words in black while Design 2 (the more difficult task) had colour choice word with congruent colours for the congruent trials and incongruent colours for the incongruent trials.

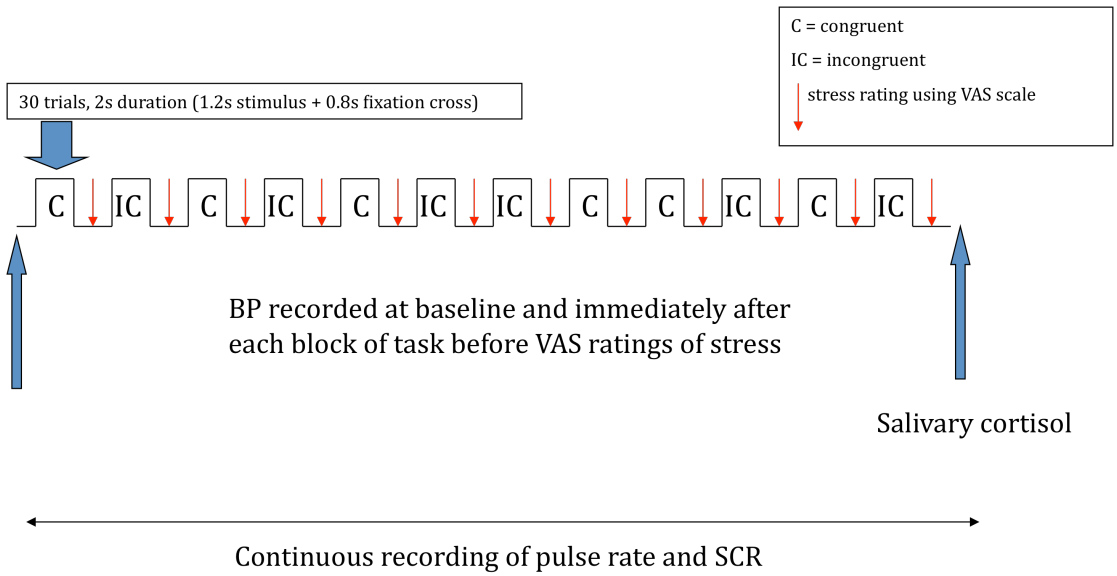


Figure 3-2 Study paradigm showing the congruent and incongruent blocks and measurement of physiological biomarkers of stress.

3.2.3 Experimental procedure

The experiments were conducted in the Electrophysiology lab at the FMRIB Centre, University of Oxford between 1 pm and 5.30 pm when the cortisol level is most stable (Dickerson & Kemeny 2004). Participants were first instructed on how to perform the task and allowed to do a practice run of the task for ten trials. They were instructed to give the correct answer in as little time as possible but not compromising accuracy for speed. They were informed that performance in the task would be assessed based on their correct responses and reaction time.

Participants were asked to perform the Stroop task seated on a chair facing a laptop placed on a table while behavioural and physiological measures were recorded. The timing for each trial was 1.2 seconds with 0.8 seconds intertrial interval where they had to fixate on a fixation cross at the center of the screen. All responses and reaction times were recorded. Physiological indices of stress, pulse rate, blood

pressure and EDA, were monitored while the participants were performing the cognitive task (Figure 3.2).

3.2.4 Behavioral measures

Recorded behavioural measures included reaction times and all button presses to the colour-word stimuli. Responses were categorized as correct when the participant chose the correct colour of the word within the allocated time before the stimulus changed to the next one. Reaction time was defined as the time from the start of the stimulus until the response button was pressed. If no response was recorded, the reaction time is recorded as maximum response i.e. 2s. The perceived level of stress was assessed at the end of each block using a Visual Analogue Scale anchored at 0= "not stressed" and 10= "extremely stressed".

3.2.5 Physiological measures

3.2.5.1 Salivary cortisol

Salivary cortisol is a reliable measure of stress response (Smyth et al 1998) and is highly correlated with plasma cortisol (Kirschbaum & Hellhammer 1994). Studies employing variants of the Stroop task such as the emotional Stroop (van Honk et al 1998) and the Stroop colour-word task (Hlavacova et al 2008) have shown increased salivary cortisol after performing the tasks.

Saliva samples were collected from participants using Salivettes (Sarstedt, Leicester, UK) before the start and at the end of the experiment. To avoid positive cortisol testing due to factors unrelated to stress, participants were requested to abstain from consuming black liquorice for two weeks, using prescription skin creams (containing steroid) for 1 day, and smoking 2 hours before the start of the experiment. They were also instructed not to eat, drink, brush their teeth or rinse their mouth 30 minutes prior to the study. Participants were asked to chew on the Salivettes cotton swab for about one minute or until they felt they could no longer keep from swallowing their saliva. The swab was then returned into the test tube and centrifuged at 1000 rpm for 3 minutes (Hettich Zentrifugen, Tuttlingen, Germany). The saliva samples were stored at -20°C until they were sent to the Endocrine Unit, Southampton General Hospital for analysis.

3.2.5.2 Blood pressure, pulse rate and electrodermal activity recording

Blood pressure was measured with the participants sitting on a chair at the beginning of each session and after each block of the Stroop task using a digital blood pressure monitor (Omron) with the arm cuff applied to the left upper arm of each participant. Systolic blood pressure is the parameter that is known to be more sensitive to stress and will show an increase compared to diastolic blood pressure. Pulse rate and EDA in the form of SCR were recorded using the software Chart v5.5.6 (ADInstruments) on an 8-channel PowerLab Data Acquisition Systems (PowerLab 8/30, ADInstruments). For both measures, data were sampled at 1000 Hz. Pulse rate was measured continuously using a pulse oxymeter (Datex Ohmeda, Hatfield, Hertfordshire, UK) connected to the participant's left index finger. SCR was recorded

continuously using the GSR bioamplifier (ADInstruments) with the electrodes attached to the palmar surface of the left index and ring finger. Electrolyte gel was applied to ensure good contact with the skin. The data were filtered using a low pass filter at 1 Hz. SCR measurement during the task was obtained for the 30 seconds duration of each congruent and incongruent task and analysed separately. The response was defined as the difference between the minimum and maximum value (amplitude) within the time window and averaged over 30 trials.

3.2.6 Statistical analysis

Results were analysed using SPSS v18 (SPSS Inc., Chicago, IL, USA). Stress ratings, correct responses, reaction time, pulse rate, blood pressure and SCR were analyzed using repeated-measures ANOVA with within-subject factors DESIGN (two levels: Design 1 and Design 2), DIFFICULTY (two levels: congruent and incongruent) and BLOCKS (six levels). Subsequently, the two designs were analysed to investigate the effects of each design on each variable. This is to ascertain the design with the least training effect and more capable of inducing a stress response. Greenhouse-Geisser corrections were applied when assumption of sphericity was not met in Mauchly's test. Post-hoc tests were performed using paired t-tests corrected for multiple comparisons.

In order to test whether the Stroop task had significantly increased the salivary cortisol level, a one-tailed paired t-test comparing the cortisol level prior to and after task performance was used.

To study the relationship between variables, simple correlation was performed to assess the relationship between stress with performance measures. For all tests significance level of 0.05 was used.

3.3 Results

3.3.1 Behavioural measures

3.3.1.1 Correct responses

The repeated measures ANOVA for correct responses showed significant main effects of the factors DESIGN ($F(1,13) = 14.1$, $p = 0.002$), DIFFICULTY ($F(1,13) = 35.9$, $p<0.001$)) and BLOCKS ($F(5,65) = 29.5$, $p<0.001$). There was significant interactions between DESIGN and DIFFICULTY ($F(1,13) = 33$, $p<0.001$), DIFFICULTY and BLOCKS ($F(3.2,41.7) = 17.7$, $p<0.001$), and DESIGN, DIFFICULTY and BLOCKS ($F(2.2,28.6) = 3.7$, $p<0.03$). Multiple comparisons with Bonferroni correction showed that correct responses were significantly lower for Design 2 relative to Design 1 ($p=0.002$) and for the more difficult incongruent compared to the congruent task ($p=0.001$). There was also an improvement in performance with advancing blocks as shown in Figure 3.3, indicating a training effect. The two designs were then analysed separately to explore the effect of each of the designs on the correct responses over time.

For Design 1, the 2-way repeated measures ANOVA with the factors DIFFICULTY and BLOCKS showed significant main effects for DIFFICULTY ($F(1,13) = 18.38$, $p=0.001$) and BLOCKS ($F(5,65) = 6.31$, $p = 0.008$). There was also a significant interaction

between DIFFICULTY and BLOCKS ($F(5,65) = 2.48$, $p = 0.041$). Post hoc paired t-tests corrected for multiple comparisons were performed at significance level of $p<0.003$. Results revealed that incongruent trials resulted in less correct responses compared to congruent and that this difference occurred at the third and fourth block (first block: $t(13)=3.11$, $p=0.008$; second block: $t(13)=3.56$, $p=0.003$; third block: $t(13)=4.32$, $p=0.001$; fourth block: $(13)=4.62$, $p<0.0005$; fifth block: $t(13)=3.68$, $p=0.003$; sixth block: $t(13)=3.18$, $p=0.007$.

For Design 2, we also found a significant main effect for DIFFICULTY ($F(1,13) = 40.07$, $p<0.0005$) and BLOCKS ($F(2.44,65) = 20.26$, $p<0.0005$), and a significant interaction between DIFFICULTY and BLOCKS ($F(2.48,65) = 11.94$, $p<0.0005$) on the correct responses. Incongruent trials resulted in less correct responses compared to congruent. Post-hoc paired t-tests corrected for multiple comparisons ($p<0.003$) showed that this difference occurred at every block except the last block (first block: $t(13)=10.21$, $p<0.0005$; second block: $t(13)=6.03$, $p<0.0005$; third block: $t(13)=5.05$, $p<0.0005$; fourth block: $t(13)=3.95$, $p=0.002$; fifth block: $t(13)=4.55$, $p=0.001$; sixth block: $t(13)=3.70$, $p=0.003$.

When comparing congruent and incongruent trials in both designs, participants performed worst during the first two blocks of the incongruent condition of Design 2 (Fig. 3.3) but subsequently was not significantly different from the performance in the incongruent condition of Design 1 as shown by post-hoc paired t-test corrected for multiple comparisons with significance level $p<0.003$ (first block ($t(1,13)=4.59$, $p=0.001$); second block ($t(1,13)=4.18$, $p=0.001$); third block ($t(1,13)=3.43$, $p=0.004$);

fourth block (t(1,13)=2.86, p=0.013), fifth block (t(1,13)=2.73, p=0.017), sixth block (t(1,13)=1.9, p=0.08).

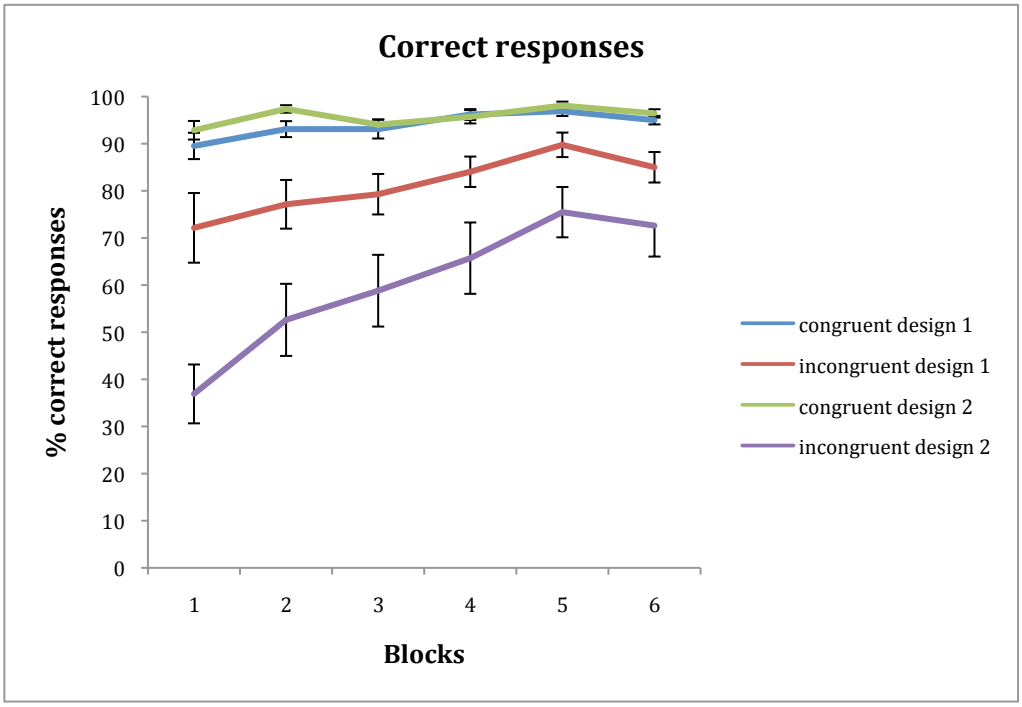


Figure 3-3 Mean ± SEM percentage correct responses for congruent and incongruent tasks.

3.3.1.2 Reaction time

The repeated measures ANOVA for reaction time revealed no significant main effect for DESIGN ($F(1, 13) = 3.96$, $p=0.068$) but for DIFFICULTY ($F(1,13) = 340.85$, $p<0.001$) and BLOCKS ($F(2.28, 29.7) = 16.8$, $p<0.001$). There was also significant interaction between DESIGN and DIFFICULTY ($F(1,13)=65.48$, $p<0.0005$, DIFFICULTY and BLOCKS ($F(2.6,33.82)=6.08$, $p=0.003$) and DESIGN, DIFFICULTY and BLOCKS ($F(5,65)=2.6$, $p=0.033$). Pairwise comparison with Bonferroni correction revealed that it took participants longer to perform the incongruent task compared to the congruent task ($p<0.0005$) (Figure 3.4), but at both difficulty levels they responded faster over time,

indicating a training effect. Further analysis was then done separately for Design 1 and Design 2.

For Design 1, the repeated measures ANOVA with the factors DIFFICULTY and BLOCKS showed a significant main effect for DIFFICULTY ($F(1, 13) = 87.85$, $p<0.0005$) and BLOCKS ($F(1.72, 22.32) = 5.18$, $p = 0.018$) as well as a significant interaction between both factors ($F(5, 65) = 2.97$, $p = 0.018$). As revealed by post-hoc paired t-tests at $p<0.003$, reaction times were faster for congruent compared to incongruent trials at every block (first block: $t(13)=5.52$, $p<0.0005$; second block: $t(13)=6.75$, $p<0.0005$; third block: $t(13)=5.75$, $p<0.0005$; fourth block: $t(13)=5.87$, $p<0.0005$; fifth block: $t(13)=4.97$, $p<0.0005$; sixth block: $t(13)=4.55$, $p=0.001$. Within-subjects contrast however, showed that the difference in reaction time between congruent and incongruent trials was not significantly different when comparing the first with the second block ($F(1,13)=3.3$, $p=0.92$).

For Design 2, we also found significant main effects for DIFFICULTY ($F(1, 13) = 309.23$, $p<0.0005$) and BLOCKS ($F(2.42, 31.41) = 13.82$, $p<0.0005$), and a significant interaction between DIFFICULTY and BLOCKS ($F(2.88, 65) = 4.96$, $p=0.006$). Post-hoc paired t-tests corrected for multiple comparisons ($p=0.003$) showed that the reaction time was faster for congruent compared to incongruent trials at every block (first block: $t(13)=8.5$, $p<0.0005$; second block: $t(13)=11.35$, $p<0.0005$; third block: $t(13)=12.80$, $p<0.0005$; fourth block: $t(13)=8.72$, $p<0.0005$; fifth block: $t(13)=7.72$, $p<0.0005$; sixth block: $t(13)=9.69$, $p<0.0005$. Within-subjects contrast showed that the difference in reaction time between congruent and incongruent trials was not

significantly different when comparing the first with the fifth block ($F(1,13)=2.82$, $p=0.12$).

As shown in Figure 3.4, reaction times improved over the time course of the experiment in all conditions. Reaction times during incongruent trials in Design 2 were significantly slower at the beginning of the experiment in comparison to the other three conditions. Although participants also improved in this condition, they were significantly slower at the end of the experiment as shown by post-hoc paired t-tests corrected for multiple comparisons ($p<0.003$) between the incongruent conditions of Design 1 and Design 2: first block ($t(1,13)=2.64$, $p=0.021$); second block ($t(1,13)=3.18$, $p=0.007$); third block ($t(1,13)=2.24$, $p=0.043$); fourth block ($t(1,13)=0.015$, $p=0.015$), fifth block ($t(1,13)=5.15$, $p<0.0005$), sixth block ($t(1,13)=5.29$, $p<0.0005$)).

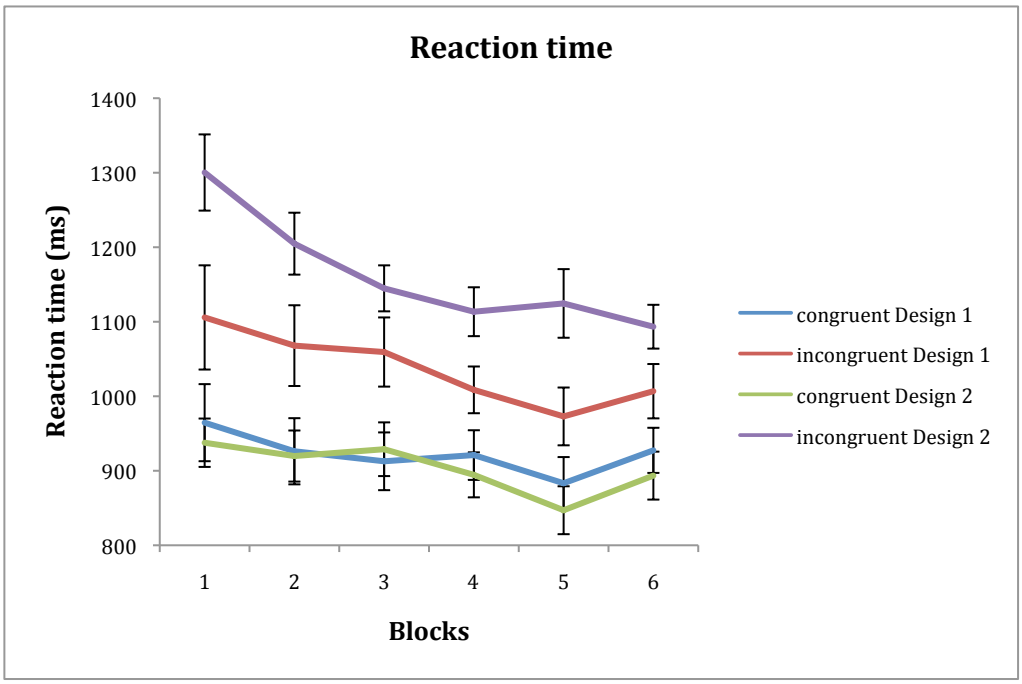


Figure 3-4 Mean ± SEM reaction time for congruent and incongruent trials for Design 1 and 2.

The Stroop interference effect can be seen in Table 3.1, further illustrating the reduction in interference over time.

Table 3.1 Stroop interference effect for Design 1 and Design 2.

		Interference ($RT_{ic} - RT_c$) in ms (SD)		
		mean across all 6 blocks	first 3 blocks (a)	last 3 blocks (b)
Design 1	Congruent	922.5 (133.1)	934.5 (26.9)	910.6 (23.9)
	Incongruent	1036.9 (159.1)	1077.7 (24.7)	996.1 (20.1)
	Interference	114.4 (18.4)	143.2 (1.7)	85.5 (3.1)
	% interference ($Interference/RT_c)/100$	12.4 (1.9)	15.3 (0.4)	9.4 (0.5)
Design 2	Congruent	903.6 (112.5)	928.8 (8.9)	878.4 (27.1)
	Incongruent	1163.5 (116.5)	1216.6 (78.4)	1110.5 (15.8)
	Interference	260.0 (35.3)	287.9 (42.4)	232.1 (23.4)
	% interference	28.8 (3.7)	31.0 (4.5)	26.5 (3.2)

RT_c = reaction time for congruent condition
RT_{ic} = reaction time for incongruent condition

3.3.1.3 Stress ratings

The subjective stress ratings did not show significant main effects for DESIGN ($F(1,13) = 3.61$, $p=0.08$). There was a significant main effect for DIFFICULTY ($F(1,13) = 40.62$, $p<0.0005$) with higher ratings for incongruent compared to congruent trials and for BLOCKS ($F(2.2,65) = 12.98$, $p<0.0005$) indicating a reduction in stress over time (Figure 3.5). There was also a significant interaction between DESIGN and

DIFFICULTY ($F(1,13) = 11.64$, $p=0.005$) with higher stress ratings for incongruent trials in Design 2 compared to Design 1, and between DIFFICULTY and BLOCKS ($F(5,65) = 4.55$, $p=0.001$). Pairwise comparison with Bonferroni correction showed that the stress ratings were significantly higher during the first three blocks compared to the last two blocks (difference between first and fifth block: $p=0.015$, first and sixth block: $p=0.015$, second and fifth block: $p=0.004$, second and sixth block: $p=0.013$, third and fifth block: $p=0.001$, third and sixth block: $p=0.017$). Further analysis was then done separately for Design 1 and Design 2.

For Design 1, there was significant main effects for both DIFFICULTY ($F(1,13) = 22.19$, $p<0.0005$), with higher stress ratings for incongruent compared to congruent trials and for BLOCKS ($F(2.52,65) = 11.47$, $p<0.0005$) with reduction of stress ratings over time. Furthermore, the interaction between DIFFICULTY and BLOCKS reached significance ($F(5,65) = 3.23$, $p=0.012$). Post-hoc paired t-tests corrected for multiple comparisons (significance level $p<0.003$) showed that participants rated the incongruent as significantly more stressful than congruent trials in the first and the fourth block (first block: $t(13)=6.24$, $p<0.0005$; second block: $t(13)=3.36$, $p=0.005$; third block: $t(13)=3.68$, $p=0.003$; fourth block: $t(13)=4.48$, $p=0.001$; fifth block: $t(13)=3.16$, $p=0.008$; sixth block: $t(13)=3.47$, $p=0.004$. The incongruent trials caused significantly higher stress ratings than congruent initially, then decreased over time but were significantly higher again in the middle of the task before decreasing again (Figure 3.5).

Design 2 also displayed significant main effects for DIFFICULTY ($F(1,13) = 37.72$,

p<0.0005), with higher stress ratings for incongruent compared to congruent trials, and for BLOCKS (F(2.3,30) = 5.66, p=0.006) with reduction of stress ratings over time. There was however, no significant interaction between DIFFICULTY and BLOCKS (F(2.68,34.84) = 2.65, p=0.07).

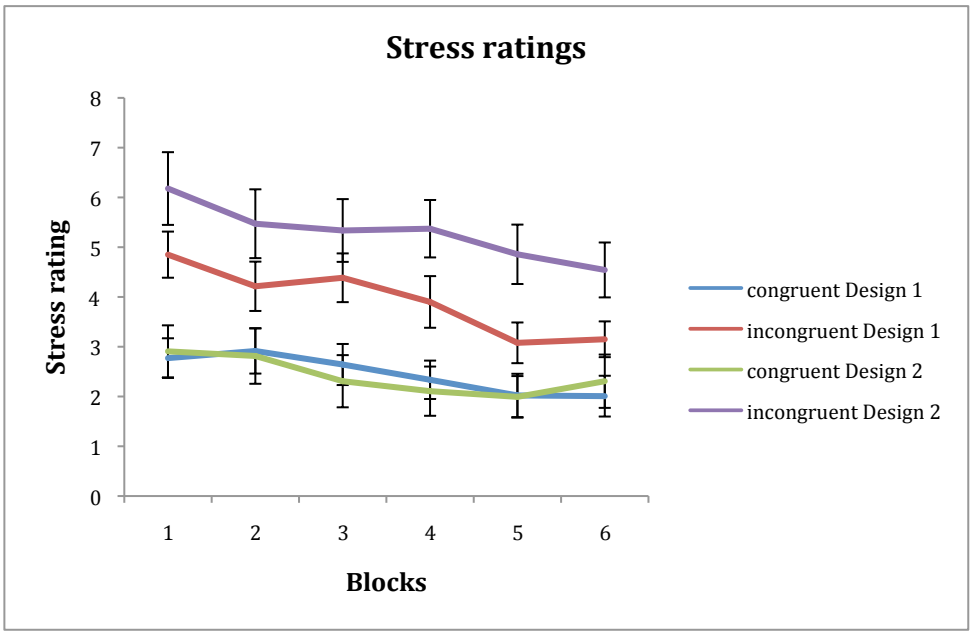


Figure 3-5 Mean stress rating **(± SEM) on a VAS scale for congruent and incongruent trials of Design 1 and 2. 0=not at all stressed, 10=extremely stressed**

Taken together, the behavioural data show that all three measures show an interference effect (i.e. incongruent gave less correct responses and longer reaction times than congruent as well as higher stress ratings). Design 2 was more difficult than Design 1 as indicated by a lower correct response rate and longer reaction time coupled with higher stress ratings. Performance and stress improved over time but remained poorer in incongruent trials of Design 2 in comparison with the other three conditions.

3.3.1.4 Relationship between performance measures and stress ratings

Bivariate correlation between subjective stress ratings and performance measures (correct responses and reaction times) were not significant for all congruent and incongruent conditions in both designs (Table 3.2).

Table 3.2 Correlations between stress and performance measures in the form of correct responses and reaction time

		r	p
Correct responses	**Congruent Design 1**	**-0.29**	**0.31**
	Incongruent Design 1	**-0.26**	**0.36**
	Congruent Design 2	**-0.09**	**0.77**
	Incongruent Design 2	**-0.012**	**0.97**
Reaction time	**Congruent Design 1**	**-0.003**	**0.99**
	Incongruent Design 1	**-0.01**	**0.97**
	Congruent Design 2	**0.019**	**0.95**
	Incongruent Design 2	**0.16**	**0.59**

3.3.2 Physiological measures

3.3.2.1 Pulse rate and blood pressure

The repeated measures ANOVA for the pulse rate with the factors DESIGN, DIFFICULTY and BLOCKS did not produce any significant main effects for any of the factors (DESIGN: $F(1,13) = 0.69$, $p=0.42$; DIFFICULTY: $F(1,13) = 0.007$, $p=0.93$; BLOCKS: $F(2.91,65) = 2.18$, $p=0.11$) nor any interactions between them (Figure 3.6).

Similarly, mean arterial pressure did not show any significant changes when analyzed with repeated measures ANOVA. There was no significant main effect for DESIGN ($F(1,13) = 0.26$, $p=0.62$), DIFFICULTY ($F(1,13) = 1.10$, $p=0.31$), or BLOCKS ($F(1.88,65) = 2.53$, $p=0.10$) nor significant interactions between DESIGN and DIFFICULTY ($F(1,13) = 0.5$, $p=0.49$), DESIGN and BLOCKS ($F(2.04,65) = 1.25$, $p=0.30$), DIFFICULTY and BLOCKS ($F(1.55,65) = 1.52$, $p=0.24$), and between DESIGN, DIFFICULTY and BLOCKS ($F(1.98,65) = 1.50$, $p=0.24$).

Systolic blood pressure also showed no stress-induced alternations. Repeated measures ANOVA for the factors DESIGN, DIFFICULTY and BLOCKS did not produce significant main effects for DESIGN ($F(1, 13) = 2.53$, $p=0.14$), DIFFICULTY ($F(1, 13) = 0.45$, $p=0.51$), or BLOCKS ($F(5, 65) = 1.33$, $p=0.27$). The only result that reached statistical significance was for the interaction between DESIGN, DIFFICULTY and BLOCKS ($F(5, 65) = 2.65$, $p=0.03$). No significant interactions were found between DESIGN and DIFFICULTY ($F(1, 13) = 1.93$, $p=0.19$), DESIGN and BLOCKS ($F(5, 65) = 1.82$, $p=0.15$), and DIFFICULTY and BLOCKS ($F(5, 65) = 1.93$, $p=0.14$). Post-hoc paired t-tests showed significantly higher SBP only at block 4 for incongruent trials in Design 1 ($t(13)=2.83$, $p=0.014$).

These results indicate that the poor performance and higher stress ratings in the incongruent trials was not accompanied by changes in the autonomic indices of stress.

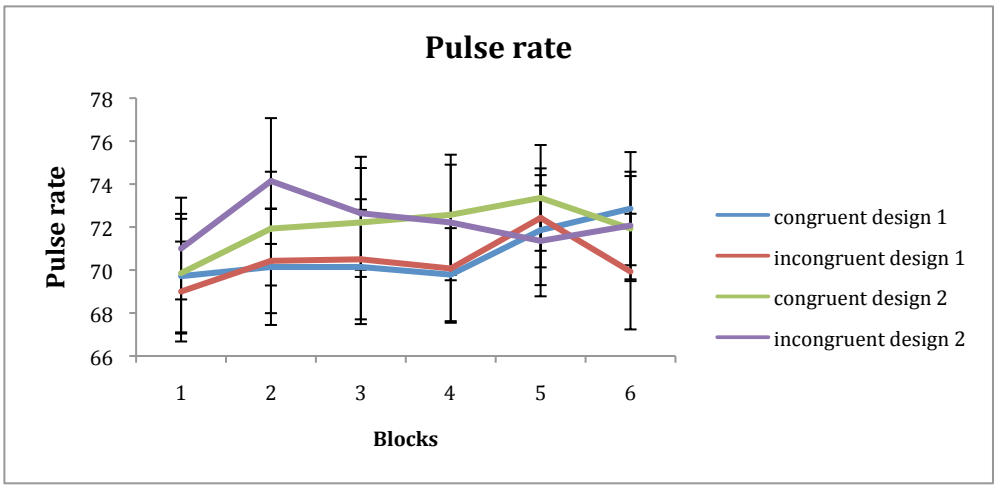


Figure 3-6 Mean ± SEM pulse rate for congruent and incongruent trials for Design 1 and 2.

3.3.2.2 Salivary cortisol

Repeated measures ANOVA with the factors DESIGN (1 and 2) and TIME (pre and post) revealed no significant main effect (DESIGN: F(1, 13) = 3.27, p=0.09; TIME: (F(1, 13) = 0.52, p=0.49), nor interaction (DESIGN and TIME: (F(1, 13) = 0.38, p=0.55) (Figure 3.7).

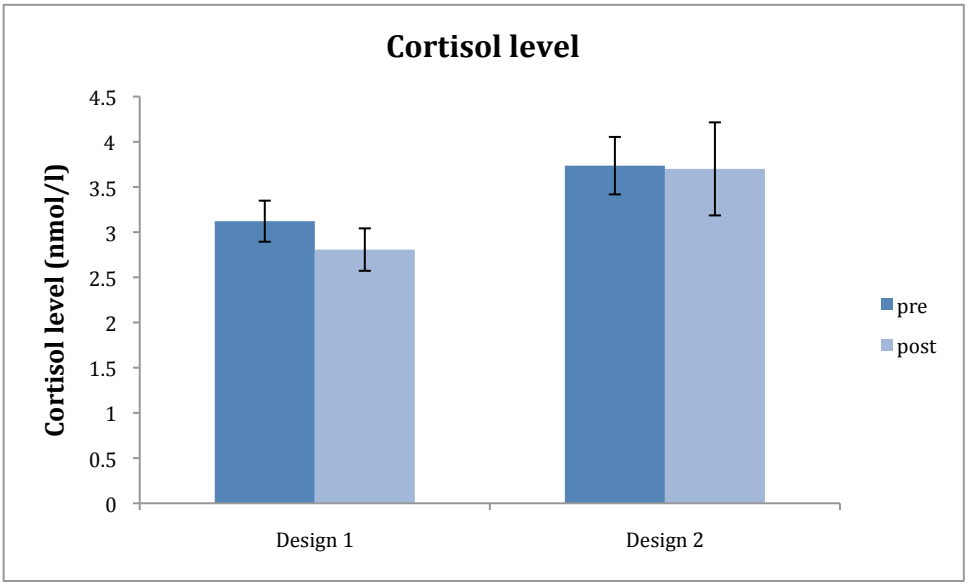


Figure 3-7 Mean ± SEM salivary cortisol level pre and post Stroop task for design 1 and 2

3.3.2.3 Skin conductance response (SCR)

Repeated measures ANOVA for the SCR with the factors DESIGN and DIFFICULTY showed no significant main effects of DESIGN ($F(1,10) = 0.22$, $p=0.65$) or DIFFICULTY ($F(1,10)=0.00$, $p=0.98$) and no significant interaction between DESIGN and DIFFICULTY ($F(1,10) = 2.54$, $p=0.14$) (Figure 3.8).

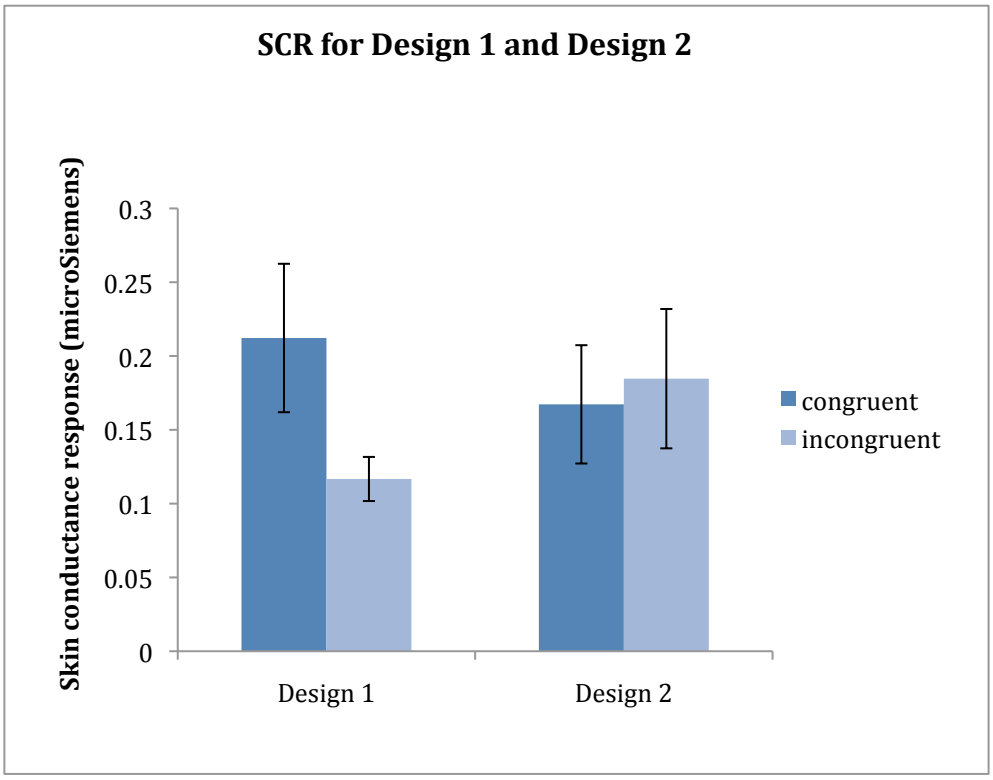


Figure 3-8 SCR during congruent and incongruent trials of Design 1 and Design 2

3.4 Discussion

The aim of this study was to investigate whether the well-known Stroop test induces stress indexed not only by subjective report but also by physiological measures. The purpose initially was to find a design suitable to be implemented in an imaging study investigating the role of the PFC in SIA but this plan was aborted due to reasons stated below.

Our results showed that overall, the incongruent condition led to poorer performance (as shown by lower correct responses and longer response times), indicating interference. However, the picture for stress was more ambiguous; while the ratings suggest that the incongruent condition induced stress, the physiological indicators were not significantly different between congruent and incongruent conditions.

Two designs were used in this study, Design 1 and Design 2. Design 2 was the more difficult task because the choice words were in colours incongruent to the colours that they name. The purpose of using two designs was to compare the level of stress that was induced by the incongruent trials in both designs and to assess the training effect over time that would have reduced the stress response. As expected, Design 2 generated more errors than Design 1 in the incongruent task as evidenced by lower correct responses.

The Stroop test, while effective in producing a level of difficulty that compromised task performance, failed to elicit a sufficient level of stress. Although the subjective stress ratings were significantly higher in the more difficult incongruent condition, the physiological parameters only showed a marginally significant increase in systolic blood pressure for the more difficult task. The pulse rate, deemed the most sensitive cardiovascular parameter to respond to stress, also did not manifest any significant differences between congruent and incongruent trials.

Failure to produce significant changes in the autonomic biomarkers in this study may be due to several factors. Firstly, the stressor used might have been too weak to drive a physiological response. Previous studies using colour-word Stroop test have demonstrated increases in mean arterial blood pressure (Gianaros et al 2005), heart rate and salivary cortisol for the incongruent compared to the congruent condition (Brydon et al 2008). However, both studies were performed under more challenging conditions than our study: The former study was conducted in an MRI scanner while the latter combined colour-word Stroop test with recorded public speaking.

The Stroop task alone is not effective in inducing a stress response and other studies have used social threat to induce stress, either alone or in combination with mental arithmetic. It has been shown that social context has a significant influence on emotion, physiology, and health (Seeman & McEwen 1996) and social evaluative threat is associated with an increase in cortisol level (Dickerson et al 2008; Gruenewald et al 2004). Negative feedback in combination with a more challenging cognitive task such as mental arithmetic or public speaking used in the Trier Social Stress Test (Kirschbaum et al 1993) might have been more efficient in eliciting stronger stress responses. It has previously been shown that this combined cognitive challenge induces a strong stress response on both the subjective and the physiological level. Grillon and colleagues (2007) used a similar speech presentation and mental arithmetic paradigm as a social stressor and found increases in subjective ratings as well as physiological biomarkers i.e. blood pressure, heart rate and salivary cortisol. Functional imaging also utilised social threat, for example the Montreal Imaging Stress Task, an adaptation of the Trier Social Stress Test, to

investigate the processing of psychological stress in the human brain (Dedovic et al 2005). A negative social context, such as failure feedback, can potentially result in deleterious physiological responses (Dickerson et al 2004; Dickerson & Kemeny 2004) while a positive social context may confer protective effects (Lepore et al 1993).

Secondly, the failure of the task to manifest significant physiological changes may be due to compensatory mechanisms such as the barorereceptor reflex driving the changes back to baseline. Increases in heart rate such as that induced by stress will cause increased cardiac output resulting in increased arterial pressure. This increase is sensed by the baroreceptors, stretch receptors located in the large arteries and the walls of the heart. The baroreceptors increase their firing rate that is signalled to the nucleus of the tractus solitarius in the medulla oblongata. This will cause inhibition of the sympathetic discharge and excitation of the vagal innervation of the heart and thereby lead to a decrease in heart rate back to baseline (Barrett et al 2010). In line with this notion, a study in which all congruent trials were presented prior to the incongruent trials showed significantly higher heart rate during the conflict task compared to the congruent task (Renaud & Blondin 1997). When the order was reversed (i.e. congruent followed by incongruent), the heart rate did not significantly differ. The design of this study that presented the congruent block first may have allowed for early compensatory mechanism to take place.

Thirdly, the participants were not promised any reward in terms of remuneration for participating in this study. Reward has been shown to induce motivation and

increase arousal (Fujiwara et al 2009). A previous study using similar Stroop colour-word task without rewards or punishment also failed to produce significant changes in SCR (Renaud & Blondin 1997).

Since this study, another research group (Yilmaz et al 2010) had performed an fMRI study to investigate the neural correlates SIA in humans. This study used mental arithmetic as the cognitive stressor and white noise as additional distressing element with mechanical pressure as the pain stimulus. The participants were recruited through public announcement and monetary remuneration was rewarded for their participation. With this setup, an adequate stress response was elicited as evidenced by physiological changes. The results show that the analgesia (as indexed by increased pain tolerance from pre to post-stress) correlates with the BOLD response for post versus pre-stress contrast in the rACC and right SI. Correlation between the differences in pain unpleasantness from pre to post with the BOLD contrast was significant in the dACC. The authors, however, did not report activation in the PFC and given that the predefined regions of interest did not include the lateral PFC, no conclusion can be made regarding the PFC involvement in stress-induced analgesia.

Due to the above factors, it was decided to not further pursue a project examining stress-induced effects on pain but explore how to cognitively manipulate the pain experience using other approaches.

4. META-ANALYSIS OF PAIN IMAGING STUDIES WITH PREFRONTAL ACTIVATION IN HEALTHY VOLUNTEERS

4.1 Introduction

Prefrontal activation has been found in numerous pain studies but its activation has been associated with various functions. Involvement of the PFC in the processing of pain is evidenced by animal (Hardy & Haigler 1985) as well as human studies (Lorenz et al 2003). While activation of PFC in pain was found during the early years of pain imaging, its significance in the modulation of pain was first highlighted by Lorenz et al (2003), recognizing the role of the DLPFC in 'keeping pain out of mind'.

Of the many subdivisions of PFC, the DLPFC is the governing structure and is pivotal for executive function. In addition to the DLPFC there is growing evidence for an involvement of the VLPFC that has a different functional profile. Despite the segregation of roles ascribed to the two areas, there appear to be parallel and often synergistic actions of both to modulate a common function (Mitchell 2011). This commonality is evident from the involvement of both DLPFC and VLPFC in decision making and ER. In decision making processes, the DLPFC is involved in conflict resolution through attention while the VLPFC reconfigures stimulus-response functions and commands attention to salient stimuli (Mitchell 2011). In ER, the role of DLPFC is resolution of emotional conflict through attention and generation of reappraisal strategies, while the VLPFC inhibits and modulates emotional response as well as commands attention to emotionally significant stimuli.

Most of the meta-analysis of brain activations in pain were qualitative in nature and did not take into consideration statistical significance of the brain activations. Two known quantitative meta-analyses on pain imaging studies in healthy volunteers revealed PFC activation alongside pain-related brain regions in thermal heat pain (Farrell et al 2005) as well as pain in sensitised and non-sensitised states (Lanz et al 2011). So far, no known meta-analysis has specifically looked at the extent of PFC activation during an experience of pain in healthy volunteers.

This meta-analysis focuses on PFC activation in pain imaging studies using PET and and fMRI to investigate (i) whether there is a distinct pattern of activation in phasic compared to tonic pain, (ii) whether the activation pattern differs between different types of noxious stimulation used to induce pain, and (iii) whether there is differential involvement of the PFC subregions in acute and chronic pain. Note that the meta-analysis presented in this chapter only includes studies performed on healthy volunteers. A parallel analysis focusing on chronic pain will be presented in Chapter 5.

4.2 Method

4.2.1 Study selection

The Pubmed Medline database (http://www.ncbi.nlm.nih.gov/pubmed/) was searched for articles containing the keywords “pain”, “prefrontal OR frontal” and “fMRI OR PET”. No time limits were set. Then, pain imaging laboratories were searched using names of investigators and the papers produced by these labs were

reviewed. See Appendix 5 for the list of pain imaging investigators. Each paper was scanned for inclusion and exclusion criteria through careful review of the title, abstract and full text.

Original studies reporting pain-related PFC activations in healthy volunteers were selected. These include studies performed exclusively on healthy volunteers or studies on patients that used healthy volunteers as controls. Studies that fulfilled the inclusion criteria (see below) were entered into an Excel table containing the following categories: first author, year of publication, title of publication, title of paper, volume and issue of publication, sample size, type of noxious stimulation, duration of noxious stimulation, site of stimulation, type of neuroimaging (i.e. fMRI or PET), and type of analysis software (e.g. FSL or Statistical Parametric Mapping (SPM; www.fil.ion.ucl.uk/spm)).

Subsequently, information regarding the relevant results were extracted including the coordinates of significant activations and deactivations, their z-score, cluster size, the cluster-forming threshold, smoothing kernel, whether a fixed or random effects analysis was used, and whether the coordinates of peak activation were reported in Montreal Neurological Institute (MNI) or Talairach space. In case only t-scores were reported, the p-value, degree of freedom, t-scores and F-scores were recorded and converted to z-scores. Talairach coordinates were converted to MNI coordinates using a linear transformation (Lancaster et al 2007).

4.2.2 Inclusion and exclusion criteria

Studies were included in the meta-analysis if they were published original articles of whole brain PET or fMRI studies using noxious stimulation and reported activation in prefrontal areas in healthy volunteers. The papers had to be published in peer-reviewed journals beginning from the first imaging study on pain that report prefrontal activation until June 2010. Accepted terminologies for prefrontal as reported in the papers include DLPFC, middle frontal gyrus and areas 8, 9, and 9/46 for DLPFC; and VLPFC, pars opercularis, area 44, area 45 and area 47/12 for VLPFC.

The following types of publications were excluded from the analysis: case reports, review articles, meta-analyses, papers in languages other than English, voxel-based morphometry (VBM), SPECT or spectroscopy studies, studies that did not report coordinates of activation and studies on other meanings for the word pain such as 'social pain'. Also excluded were contrasts not due to pain, coordinates that were quoted from a different study and functional connectivity analyses. Additional exclusion criteria comprised the investigation of modulatory aspects of pain (i.e. studies on attention, anxiety, expectation, predictability, placebo and nocebo), the use of pain-related stimuli other than noxious stimuli (i.e. visual stimuli depicting painful situations, aversive painful sounds), hypnosis with suggestion of pain, imaginary pain, pain-related words, facial expression of pain, empathy towards another person in pain and drug studies. Two studies used hypnosis as intervention (Raij et al 2005; Vanhaudenhuyse et al 2009) but only the session in the absence of hypnosis was considered here. Finally, studies using experimental models of chronic pain (e.g. studies on experimentally induced allodynia or hyperalgesia) were also

excluded from this analysis. However, the control arm of these studies, if present, that utilised pain stimuli without the modulations stated above, is included in the meta-analysis.

We performed four sets of analysis on this data of healthy volunteers. The first analysis was on all pain stimulation, using all the data with any types of painful stimulation. The second analysis was done according to the duration of pain stimulation i.e. tonic and phasic. Tonic pain is categorized as pain lasting more than a minute while phasic pain is pain lasting less than a minute. The third analysis categorized pain as surface and visceral, surface including pain inflicted on the skin while visceral includes pain arising from or inflicted on visceral organs. The fourth analysis distinguished between different types of pain stimulation such as thermal heat, cold, electrical and laser. Therefore, the same coordinates were considered more than once, i.e. once in the analysis on phasic vs. tonic, once in the analysis differentiating between the different types of stimulation etc.

4.2.3 Brain mapping

All compiled coordinates for each category (e.g. tonic or phasic pain) were mapped onto a z-score map using GPR analysis (see Chapter 2).

The resulting brain maps were thresholded at z= 2.3 and the cluster size thresholded at 30 voxels. These are the optimal options for this analysis. The resulting voxel coordinates were transformed into mm coordinates in MNI space.

4.3 Results

4.3.1 Descriptive analysis

A total of 102 studies reporting 2258 activation or deactivation coordinates were included. 12 of these studies focused on chronic pain, but had included healthy volunteers as a control group (Albuquerque et al 2006; Baliki et al 2006; Baliki et al 2010; Brefel-Courbon et al 2005; Derbyshire et al 2002; Derbyshire et al 1994; Elsenbruch et al 2010; Gracely et al 2002; Hall et al 2010; Jones & Derbyshire 1997; Kwan et al 2005b; Verne et al 2003)

Out of the 102 studies, 76 used fMRI and 26 used PET. A majority of the studies used Statistical Parametric Mapping (SPM); www.fil.ion.ucl.ac.uk/spm) for data analysis, followed by FSL (Smith et al 2004; Woolrich et al 2009) and Brain Voyager (Brain Innovation, Maastricht, The Netherlands). Other types of software used were Analysis of Functional NeuroImages (AFNI); Cox 1996; six studies), MedX (Sensor Systems Inc., 1996; two studies), Advanced Visual Systems (AVS) software (AVS, Waltham, MA; 1 study), fMRIstat-multistat (http://www.math.mcgill.ca/keith/BICstat/; three studies, NIH-FIDAP (NIH-Functional Imaging Data Analysis Platform, developed by JM Maisog; two studies), and XBAM (http://brainmap.co.uk/; one study). One study utilised MedX for analysis and SPM echo planar imaging template for registration into standard space. In 68 studies, results were reported in Talairach space, 34 studies reported MNI space coordinates. The sample size for the studies ranged from 4 to 30.

Pain duration varied across studies but was classified as phasic and tonic with the cut-off duration for phasic pain arbitrarily set at 60 seconds. Following this definition, 94 studies used phasic pain with 2159 coordinates while 11 studies used tonic pain with 99 coordinates. For the second set of analysis, studies were categorized according to the noxious stimulation used (see Figure 4.1).

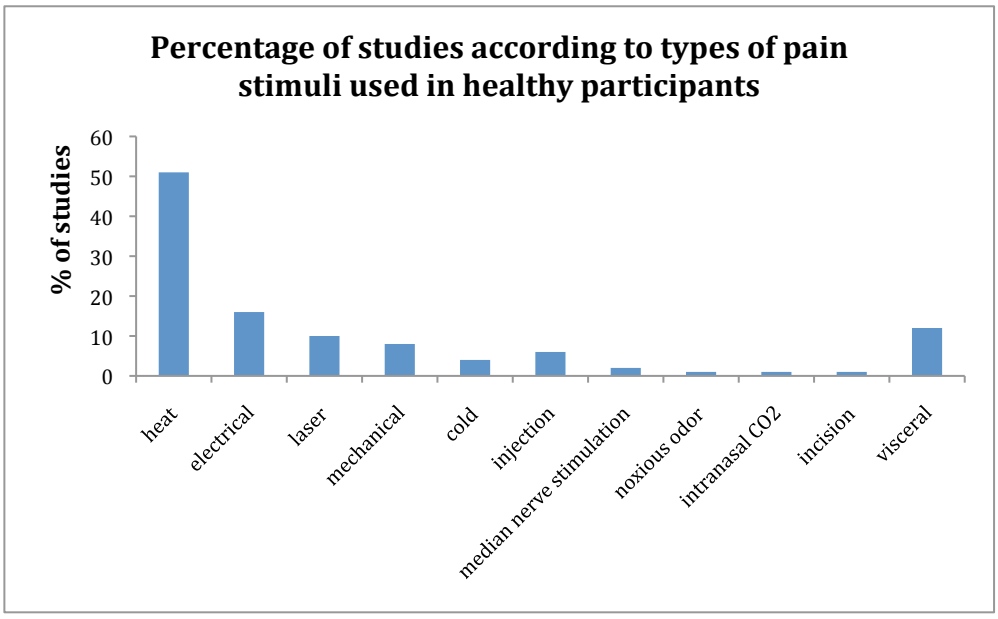


Figure 4-1 Types of pain stimuli used

Heat, electrical, laser, mechanical and cold stimuli were summarized as surface stimulation while visceral stimulation comprised esophageal, gastric and rectal stimuli (Figure 4.2). Other types of pain stimuli, namely injection, median nerve stimulation, noxious odour, intranasal CO_2 and incision were excluded from the reclassification. Following these criteria, 11 studies were excluded, leaving 91 studies for further analysis.

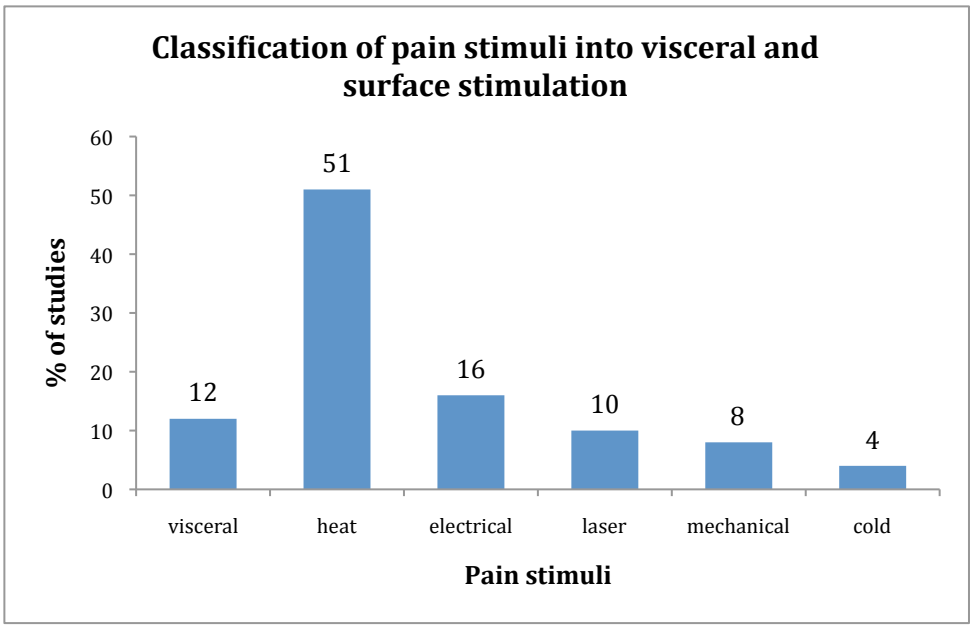


Figure 4-2 Percentages of studies according to visceral and surface stimulation. Visceral stimulation comprises oesophageal, gastric and rectal stimulation while surface stimulation includes heat, electrical, laser, mechanical and cold.

4.3.1.1 Noxious stimulation

Heat

Contact heat was the most common type of noxious stimulation, constituting more than 50% of 91 studies. Common sites of stimulation were the dorsal side of the hand (18 studies; Becerra et al 2001; Becerra et al 2004; Becerra et al 1999; Brooks et al 2005; Derbyshire et al 2002; Derbyshire et al 1994; Derbyshire et al 1998; Geuze et al 2007; Jones & Derbyshire 1997; Peyron et al 1999; Ploghaus et al 2001; Ploghaus et al 1999; Schmahl et al 2006; Smith et al 2002; Tracey et al 2000; Vartiainen et al 2009; Vogt et al 1996; Wise et al 2007) and forearm (14 studies; Adler et al 1997; Casey et al 1996; Casey et al 2001; Coghill et al 2003; Eippert et al 2008; Gundel et al 2008; Kong et al 2007; Kong et al 2006; Kurata et al 2005; Maihofner et al 2006; Ochsner et al 2006; Paulson et al 1998; Sprenger et al 2006; Valet et al 2004). Other sites include the foot (five studies; Becerra et al 2004; Brooks

et al 2005; Dunckley et al 2005; Tseng et al 2010; Verne et al 2003), lower back (four studies; Baliki et al 2006; Baliki et al 2009; Baliki et al 2010; Dunckley et al 2005), leg (three studies; Dube et al 2009; Koyama et al 2005; Villemure & Bushnell 2009), chest (two studies; Strigo et al 2005; Strigo et al 2003), face (three studies; Albuquerque et al 2006; Brooks et al 2005; de Leeuw et al 2006), finger (two studies; Freund et al 2009; Gelnar et al 1999), wrist and upper arm (one study each; Bar et al 2007; Coghill et al 1999). While the majority of the studies used a contact heat pain device, two studies used immersion in hot water (Derbyshire & Jones 1998; Hofbauer et al 2001). The duration of heat stimulus applied varied from a few seconds to three minutes.

Cold stimulation

Out of four studies using cold stimuli, two studies used a cold peltier thermode on the forearm or dorsum of the hand (Seifert & Maihofner 2007; Tracey et al 2000) while the other two studies used cold pressor test (i.e. immersion of the hand in cold water) (Brefel-Courbon et al 2005; Casey et al 1996). Stimulus duration was 20 seconds to 105 seconds.

Laser stimulation

Laser stimuli were delivered mainly to the hand (seven studies; Bingel et al 2007; Bornhovd et al 2002; Derbyshire et al 1997; Mobascher et al 2009a; Raij et al 2005; Vanhaudenhuyse et al 2009; Xu et al 1997). One study delivered the stimuli to the forearm (Svensson et al 1997) and three studies to the foot (Ploner et al 2010;

Veldhuijzen et al 2009; Xu et al 1997). Stimulus durations ranged from 1 millisecond to 100 milliseconds.

Electrical stimulation

Of the 16 studies on electrical stimulation, 12 applied the stimuli to the hand and wrist (Carlsson et al 2006; Christmann et al 2007; Christmann et al 2002; Laureys et al 2002; Petrovic et al 2008a; Rottmann et al 2010; Singer et al 2004; Straube et al 2009; Symonds et al 2006; Talmi et al 2009; Wiech et al 2008b; Wiech et al 2006). One study each stimulated the forehead (Iannilli et al 2009) and leg (Piche et al 2010) while two studies stimulated the tooth area (Jantsch et al 2005; Weigelt et al 2010). Stimulus duration was between 200 milliseconds to 20 seconds.

Mechanical stimulation

Mechanical stimuli included pressure pain or impact pain. Mechanical pressure pain is applied using sustained pressure usually by a hydraulic or pneumatic device with calibrated weights (Cole et al 2006; Gracely et al 2002; Lopez-Sola et al 2010; Lorenz et al 2008; Lui et al 2008). Impact pain is delivered to the subject's skin using a ballistic apparatus and the pain intensities can be adjusted using different velocities of the projectile (Jantsch et al 2005; Maihofner et al 2006; Maihofner et al 2007). Sites of stimulation were thumbnail (Cole et al 2006; Gracely et al 2002; Lopez-Sola et al 2010), forearm (Maihofner et al 2006; Maihofner et al 2007), finger (Jantsch et al 2005), dorsum of hand (Lui et al 2008) and anterior tibia (Lorenz et al 2008). Stimulus duration ranged from 1.5 seconds to 20 seconds.

Visceral stimulation

Visceral stimulation included oesophageal distension (five studies: Aziz et al 1997; Coen et al 2009; Lu et al 2004; Strigo et al 2005; Strigo et al 2003), gastric fundus distension (1 study: Lu et al 2004), and rectal distension (five studies: Dunckley et al 2005; Elsenbruch et al 2010; Hall et al 2010; Kwan et al 2005a; Moisset et al 2010). The duration of visceral distension ranged from 3 seconds to 3 minutes.

4.3.1.2 Reported PFC activation

In general, the lateral PFC was more frequently activated than the medial PFC (Figure 4.3). The PFC division that was most frequently activated across all studies was the DLPFC, either alone or in combination with the VLPFC or medial PFC. Co-activation with VLPFC was the highest followed by co-activation with medial PFC.

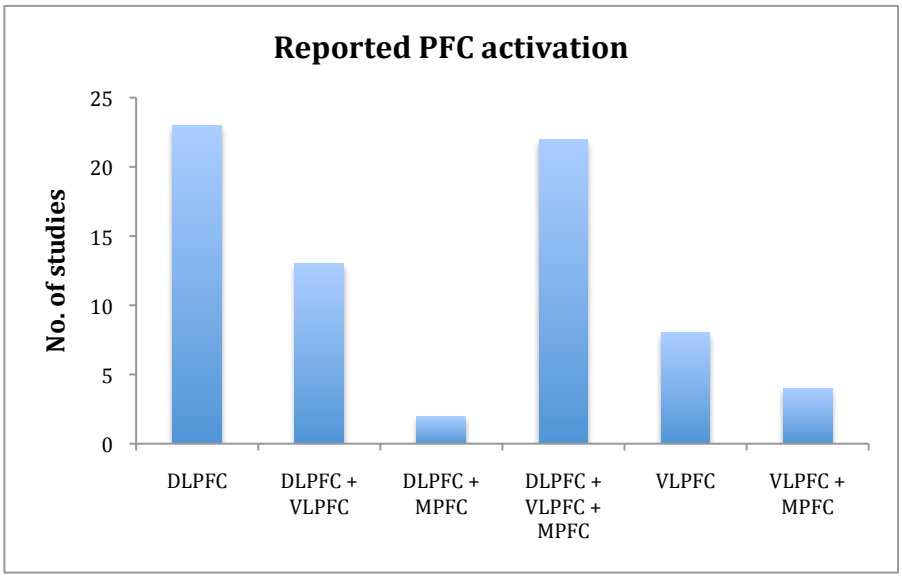


Figure 4-3 PFC activation as reported by studies in healthy volunteers.

4.3.2 Brain maps

4.3.2.1 Pain-related activation (across all types of stimulation and stimulus durations)

At a threshold of z= 2.3 an extensive area of PFC activation was found (Figure 4.4 and 4.5) where most of the VLPFC and DLPFC were covered on the right hemisphere. At a more conservative threshold of z= 2.8, significant PFC activation was only found in the VLPFC. The left PFC did not display much increase at z=2.3 and activations were centered on the lower part of pars opercularis and upper part of pars triangularis. Only the lower part of the left DLPFC survived the z=2.3 threshold.

Apart from the PFC, significant activation following noxious stimulation was found in various pain-related brain regions (Figure 4.4). The biggest cluster of activation was in the cerebellum, followed by the posterior cingulate cortex (Table 4.1). Additional foci of activation in the cingulate cortex were found in the posterior aspect of the dACC. Other clusters were identified along the precentral and postcentral gyri, lateral occipital cortex, and bilateral thalamus. Foci of pain-related activations in the lateral sulcus extend from the frontal and central opercular cortex to the anterior, mid and posterior insula. Extensive activation was found along the lateral sulcus comprising the frontal, central and parietal operculum.

4.3.2.2 Phasic pain

For phasic pain, the pattern of brain activations was very much similar to that of all pain (Figure 4.4). Prefrontal activation was extensive, especially in the right PFC and included the right middle frontal gyrus and inferior frontal gyrus (Figure 4.5). This cluster extended medially to include insula bilaterally.

Other than PFC, the right lateral occipital cortex was the area most activated, followed by the posterior cingulate cortex and the anterior lobe of the cerebellum (Table 4.2). Other pain-related brain regions activated were the thalamus, SI and SII.

4.3.2.3 Tonic pain

For tonic pain, PFC activation was small and limited to the right inferior frontal gyrus in the region of the pars opercularis (Figure 4.5). The rest of the PFC did not show any activation at this threshold. Lateralization to the right was also seen for activation in the frontal pole.

Pain-related brain regions that were most significantly activated were the thalamus, SII and supplementary motor cortex (Table 4.3). The subcallosal cortex, which is part of the rACC, was also activated in tonic pain.

4.3.2.4 Surface and visceral stimulation

Surface stimulation (i.e. heat, mechanical, cold, laser and electrical stimulation) produced patterns of activations almost similar to phasic pain. Foci were mainly in

the frontal operculum extending posteriorly into the central and parietal operculum, VLPFC and SII, and medially to include the insula. The spread of activation was more extensive on the right hemisphere covering up to the inferior part of the DLPFC (Figure 4.6). Also significantly activated was the lateral occipital cortex extending to angular gyrus, preuneus, and thalamus (Table 4.4).

Visceral stimulation comprising oesophageal, gastric and rectal painful stimulation was less extensive and mainly confined to scattered brain regions such as the posterior cingulate cortex, thalamus, cerebellum, precentral gyrus and right anterior insula (Table 4.5).

4.3.2.6 Contrasts

The contrast surface minus visceral produced significant activation in the DLPFC and lateral occipital cortex (Table 4.6). Visceral minus surface produced activation in the posterior cingulate gyrus (Table 4.7).

4.3.2.7 Deactivations

For all the brain maps, deactivations were below threshold and are not shown here.

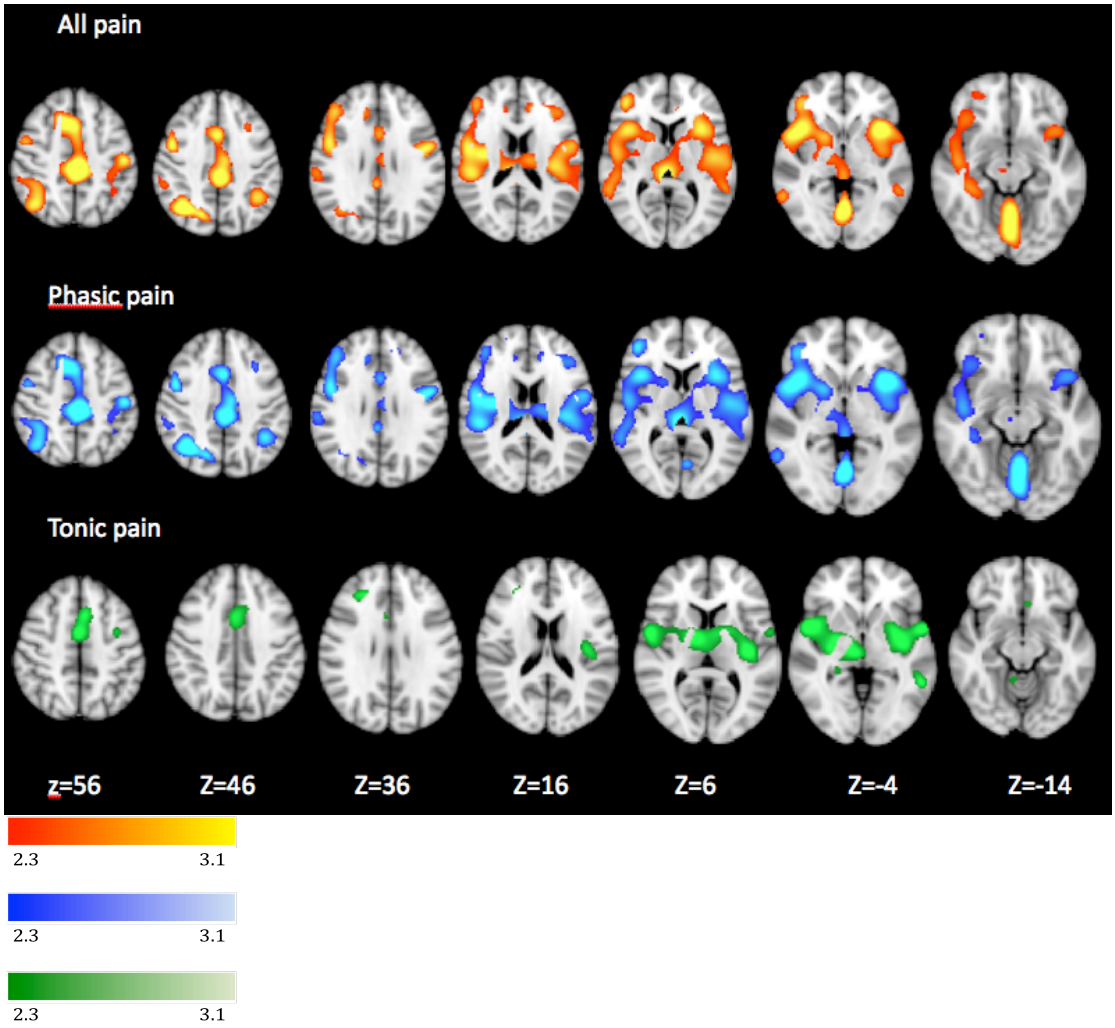


Figure 4-4 Brain activation following noxious stimulation in healthy controls. The upper row shows activation due to all pain stimulation, the middle row shows activation due to phasic pain and the lower row depicts activation due to tonic pain. Activations were thresholded at z=2.3 and cluster thresholded at 30 voxels. Images are in radiological convention.

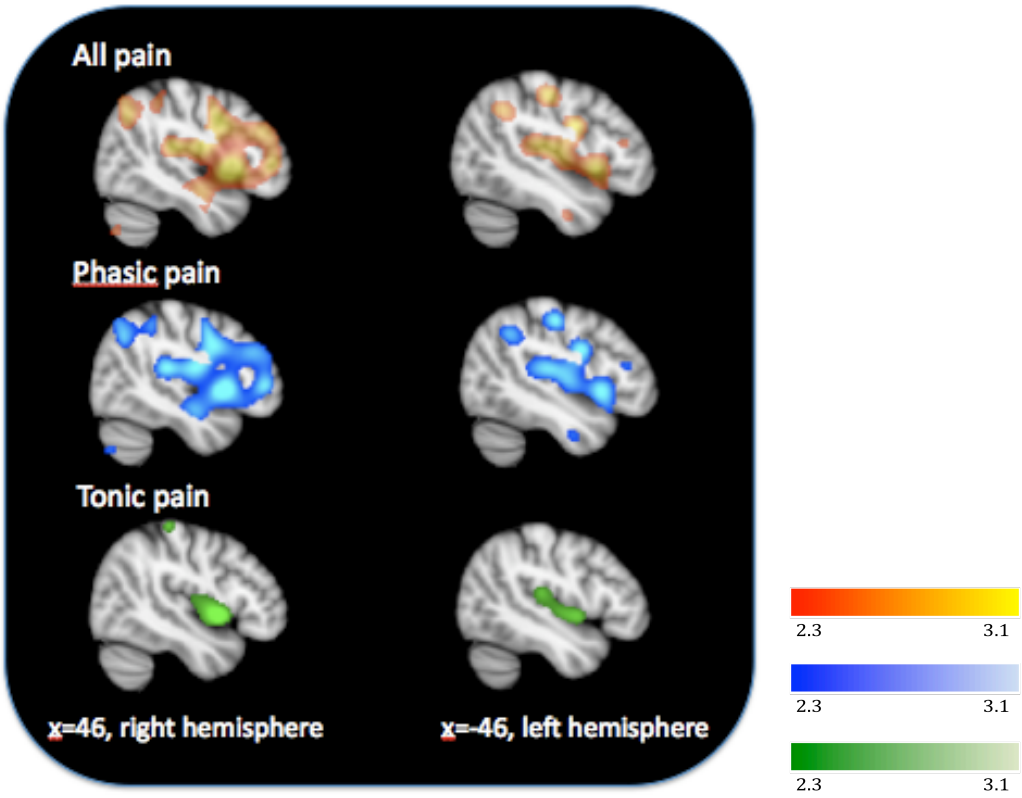


Figure 4-5 Sites of prefrontal activation. The upper row shows activation due to all pain stimulation, the middle row shows activation due to phasic pain and the lower row depicts activation due to tonic pain. Activations were thresholded at z=2.3 and cluster thresholded at 30 voxels.

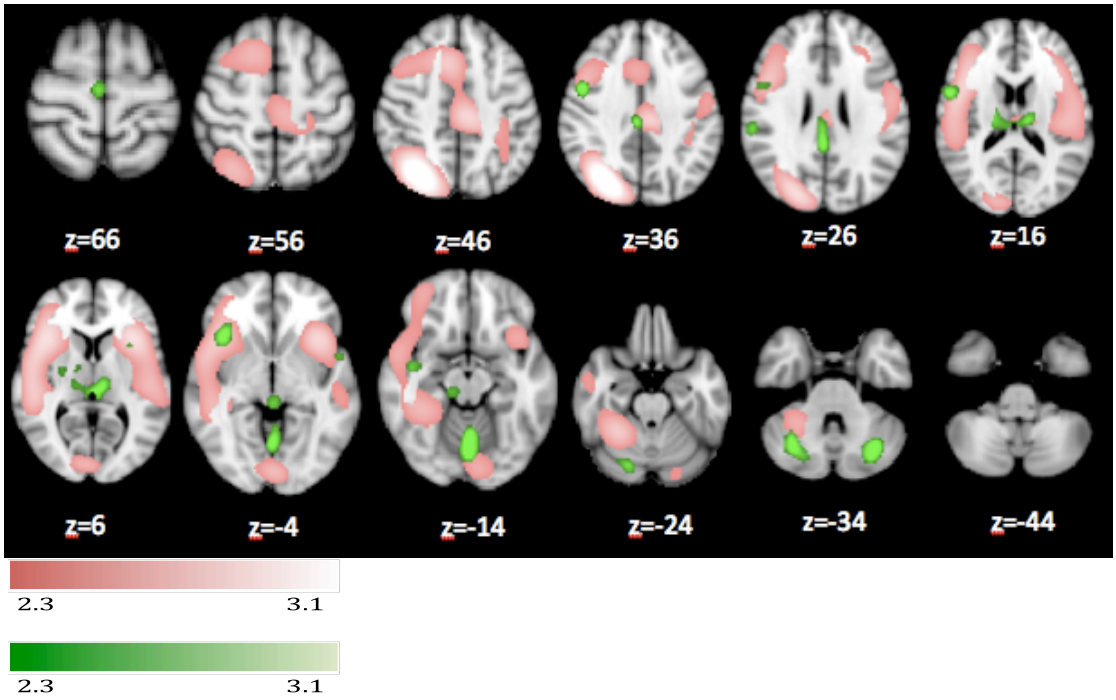


Figure 4-6 Activations due to surface (pink) and visceral (green) stimulation. Activations were thresholded at z=2.3 and cluster thresholded at 30 voxels. Images are in radiological convention.

Table 4.1 Brain responses in healthy volunteers: all pain.

Brain region	Laterality	Brodmann area	MNI coordinates			Cluster size (voxel)	Z score
			x	y	z		
anterior lobe cerebellum	left		0	-64	-6	23573	4.04
posterior cingulate cortex	left	31	-4	-48	26	4695	3.85
SI	right	2	-48	-20	48	1249	2.98
frontal pole	right	10	32	52	-18	141	2.73
paracingulate gyrus	right	32	14	36	24	108	2.69
SI	left	7	-14	-46	70	88	2.51
anterior cingulate gyrus	left	33	-12	36	14	87	2.55
middle frontal gyrus	left	9	-30	22	34	73	2.52
inferior temporal gyrus	left	21	-44	-6	-34	57	2.49

Table 4.2 Brain responses in healthy volunteers: phasic pain

Brain region	Laterality	Brodmann area	MNI coordinates			Cluster size (voxel)	Z score
			x	y	z		
lateral occipital cortex	right	39	36	-62	38	17247	3.63
posterior cingulate gyrus	left	31	-4	-28	46	4560	3.9
anterior lobe cerebellum	left	18	0	-64	-6	3197	3.98
cerebellum	right		22	-48	-28	1881	3.86
SI	left	2	-48	-20	48	831	3.06
angular gyrus	left	7	-42	-52	38	421	2.93
paracingulate gyrus	right	32	14	36	24	99	2.63
frontal pole	right	10	34	52	-18	87	2.72
middle frontal gyrus	left	8	-30	22	34	80	2.56
SI	left	31	-14	-46	40	68	2.5
paracingulate gyrus	left	32	-14	40	18	65	2.48
inferior temporal gyrus	left	20	-44	-6	-34	62	2.51

Table 4.3 Brain responses in healthy volunteers: tonic pain

Brain region	Laterality	Brodmann area	MNI coordinates			Cluster size (voxel)	Z score
			x	y	z		
thalamus	right		14	-6	4	3270	3.58
SII	right	43	54	4	2	1835	3.72
supplementary motor cortex	right	6	2	-2	48	1008	3.27
cerebellum anterior lobe	right		8	-50	-18	301	3.05
middle temporal gyrus	left	21	-56	-48	0	195	3.18
frontal pole	right	10	28	36	24	155	3.13
precentral gyrus	left	4	-36	-2	52	108	2.94
SI	right	1	42	-28	62	79	3.04
hippocampus	right		24	-36	-4	44	2.61
subcallosal cortex	left	24	-4	24	-10	32	2.49

Table 4.4 Brain responses in healthy volunteers: surface pain

Brain region	Laterality	Brodmann area	MNI coordinates			Cluster size (voxel)	Z score
			x	y	z		
frontal operculum cortex	left	44	-36	20	4	24600	2.82
lateral occipital cortex	right	19	30	-70	42	6226	3.42
thalamus	left		-8	-20	20	172	2.42

Table 4.5 Brain responses in healthy volunteers: visceral pain

Brain region	Laterality	Brodmann area	MNI coordinates			Cluster size (voxel)	Z score
			x	y	z		
paracingulate gyrus	left	33	-14	40	18	958	3.42
cerebellum anterior lobe	left		0	-64	-10	679	4.34
precentral gyrus	right	6	48	4	38	510	3.52
cerebellum posterior lobe	right		34	-66	-32	510	3.69
cerebellum posterior lobe	left		-30	-70	-32	434	3.96
posterior cingulate	right	29	2	-42	26	384	3.32
anterior insular cortex	right	13	40	20	-4	256	3.35
planum polare	right	21	44	-8	-14	105	2.75
supramarginal gyrus	right	40	60	-28	26	76	2.8
precentral gyrus	right	6	4	-16	66	67	2.87
frontal operculum cortex	left	44	-32	14	10	53	2.69
parahippocampal gyrus	right		14	-28	-14	44	2.59
putamen	right		26	-8	10	40	2.52
planum polare	left	22	-54	4	-2	31	2.63

Table 4.6 Brain responses in healthy subjects: surface minus visceral pain

Brain region	Laterality	Brodmann area	MNI coordinates			Cluster size (voxel)	Z score
			x	y	z		
middle frontal gyrus	right	46	30	16	50	195	2.47
lateral occipital cortex	right	39	36	-66	34	55	2.35

Table 4.7 Brain responses in healthy subjects: visceral minus surface pain

Brain region	Laterality	Brodmann area	MNI coordinates			Cluster size (voxel)	Z score
			x	y	z		
posterior cingulate gyrus	left	30	-2	-52	18	212	3.17

4.4 Discussion

This study quantitatively analysed the results of pain imaging studies using PET or fMRI that reported PFC activation during painful stimulation in non-sensitised healthy volunteers. The purpose is to investigate the extent of PFC activation in the context of pain. The main findings were that the PFC were activated across a wide range of pain stimulation but were more prominent in surface type of pain stimulation. On the other hand, visceral pain activated mainly the posterior cingulate.

4.4.1 All pain stimuli

In this meta-analysis, although PFC activation as reported in the analysed papers mainly involved the DLPFC, the analysis here showed that the PFC division that was most significantly activated due to all pain stimulation was the VLPFC in the inferior frontal gyrus (IFG), particularly the right side. Existing knowledge shows that the role of the VLPFC seems to be associated with emotion and expectation (Mitchell 2011), two factors that pain are commonly associated with. Another reason for this may be that the clusters in the DLPFC were not grouped together tightly enough to produce significant foci of activation across studies. This could be due to anatomical heterogeneity between individuals and limitations in linear transformation during registration onto standard space. Also, given the subjective nature of pain and the heterogeneity of DLPFC function, obtaining a uniform distribution of pain activation across studies is challenging. Previous meta-analysis by Wager (2005) did not find

any cluster in the PFC in studies on pain. Farrell et al (2005) only found 2 small clusters in the VLPFC and superior frontal gyrus in their meta-analysis of thermal heat pain.

A previous meta-analysis on neuroimaging studies with IFG activation found 4 distinct clusters in the IFG (Liakakis et al 2011). Three clusters in the left IFG for empathy processing, semantic and phonological processing, and working memory, and one cluster in the right IFG for fine movement control. Studies on empathy to others in pain have also been found to activate the IFG (Beeney et al 2011). However, the meta-analysis by Liakakis et al (2011) did not specifically analyse activation due to pain stimulation.

Negative affect induced by sad music (Berna et al 2010; Coen et al 2009) causes increased activation in IFG due to noxious stimuli. VLPFC activation is also seen in placebo treatment (Petrovic et al 2010) although other studies also showed DLPFC activation during placebo-related decreases in pain-related brain regions (Wager et al 2004b). Brain activity in the VLPFC, together with anterior insula and ACC, correlates with activity during noxious stimulation (Maihofner et al 2011).

Consistent with existing knowledge of activation of a network of brain regions due to pain as well as quantitative meta-analyses on pain imaging data (Farrell et al 2005; Lanz et al 2011), this meta-analysis of healthy volunteers showed activation of pain-related brain regions such as cingulate cortex, thalamus, SI and insula during pain stimulation. What sets apart this study from other meta-analysis in pain is that the

present meta-analysis considered only studies that report PFC activation. Therefore, the pattern of activation of pain-related brain regions reported here might be the result of concurrent PFC activation.

Areas with highly significant clusters of activation were cerebellum and posterior cingulate. This finding is in contrast with previous meta-analyses that showed anterior cingulate activation due to thermal pain (Farrell et al 2005) and pain in non-sensitized state (Lanz et al 2011). This study localized significant activations extending from Brodmann area 31 to 33 while the study by Farrell et al (2005) located the activation more anteriorly in area 24 extending to area 32. However, the study by Farrell only analysed thermal heat pain. The meta-analysis by Lanz et al (2011) also produced a more anterior activation of cingulate cortex for non-sensitised pain compared to the present meta-analysis.

A previous meta-analysis on posterior cingulate cortex (PCC) activation segregated pain and memory activation to rostral and caudal parts of the PCC respectively (Nielsen et al 2005). Ironically, however, PCC is one component of the neural network correlates of consciousness and is usually deactivated by aversive stimulation such as pain (Vogt & Laureys 2005). PCC activation during pain was first reported by Tracey et al (2000) in a study using noxious hot and cold stimuli. Involvement of PCC was also demonstrated in visceral pain studies (Baciu et al 1999; Smith et al 2011; Tolle et al 1999).

Cerebellum is another area that featured prominently in this meta-analysis. While the classical role for cerebellum is in the processing of motor function, several non-motor functions have been identified such as tasks associated with attention, executive control, working memory and pain (Strick et al 2009). A closed cerebro-cerebellar loop has been identified that may indicate a modulatory role of the cerebellum in pain processing (Kelly & Strick 2003).

4.4.2 Phasic and tonic

Activations of PFC in phasic pain follow a similar pattern to all pain stimuli. Phasic pain activates the PFC more extensively especially anterior part of PFC.

Distinct patterns of activation for phasic and tonic pain stimulations could be seen in pain-related brain regions. Phasic stimulation induced activations almost as extensive as all pain stimulation and included sensory as well as affect-driven brain areas. The biggest cluster of activation in phasic stimulation was the right lateral occipital cortex followed by posterior cingulate. A meta-analysis by Lanz et al (2011) analysing two types of phasic surface pain showed statistically stronger activation of PFC during thermal pain compared to mechanical pain. This may be explained by the fact that thermal pain exhibits relatively greater consistency of response across subjects compared with other types of pain stimuli (Farrell et al 2005) thus causing less variability of PFC activation.

Significant clusters of activation for tonic pain was much less extensive compared to phasic pain and were confined mainly to sensory and affect-driven areas such as

thalamus, SII, anterior insula and rACC. The reason for this may be the fact that over 90% of the studies were phasic thus producing a confound. Another reason may be that the types of pain stimulation classified under phasic pain were more diversified whereas studies with tonic pain were more homogeneous thereby producing a more confined activation.

It should be noted here that the categorization of duration for tonic and phasic with one minute as the cut-off point is arbitrary and may not accurately reflect underlying differences in the duration of pain stimulation. Duration of pain stimulation may also act differently for different types of pain stimuli depending on the nature and threat value of the pain, and not just its duration.

4.4.3 Surface and visceral

Activations due to surface and visceral stimulation almost mirror that of phasic and tonic pain respectively. This may be explained by the fact that surface stimulation comprises pain stimuli that were mostly phasic in nature and vice versa for visceral.

4.4.4 Limitations

Since the aim of this meta-analysis was to analyze prefrontal activation in pain imaging studies, the strategy used was to only include studies that are tagged with keywords ‘prefrontal’ or ‘frontal’ activations. This may cause limitations in that papers that report prefrontal activation that are not tagged with the keywords may have been missed. To reduce the incidence of missing these papers, we sift through

papers by known authors in pain imaging using their names as keywords and going through each of their papers in Pubmed.

Some studies do not report z-score as well as other parameters that enable calculations of z-score such as t-score, p value and degree of freedom and had to be excluded from the brain mapping analysis. Another limitation is the discrepancy in the number of studies using different types of pain stimulation – heat pain was disproportionately more than any other types of stimuli.

While subcategorization has been done in the present meta-analysis to provide uniformity in the analysis, certain aspects could not be controlled for. For instance, diverse study conditions, diverse study populations, different randomization process across studies, and different experimenters place a limit to the analysis. Different concurrent tasks (or lack thereof) also can potentially cause PFC activation at variable sites and/or intensities. The fact that pain is subjective and does not have a linear relationship with pain stimuli but is influenced by cognitive-affective variable poses another limitation to this analysis that assumed generalization of this variable across studies.

4.4.5 Conclusion

This study on healthy subjects showed evidence of PFC involvement in a wide range of pain studies. However, the pattern of activations differs according to the duration and type of stimulation; phasic surface pain activates more of the DLPFC compared to visceral pain.

5. META-ANALYSIS OF PAIN IMAGING STUDIES WITH PREFRONTAL ACTIVATION IN CHRONIC PAIN PATIENTS

5.1 Introduction

The PFC activity is more characteristic for chronic than for acute pain (Apkarian et al 2005). Findings from experimental models of chronic pain suggest that the PFC has a role in 'keeping pain out of mind' (Lorenz 2003). In non-pain fields, the DLPFC has been shown to have a role in expectations while the VLPFC is more involved in ER (Mitchell 2011). These functions are part of a chronic pain experience.

Chronic pain is defined as persistent pain that lasts longer than three months after the initial insult, despite resolution of the disease or healing of the injury (Merskey & Bogduk 1994). The duration for the healing phase is, however, arbitrary and conditions like back pain may take as long as six months to clinically recover (Apkarian et al 2009).

The role of PFC among the different types of chronic pain may differ. Functional pain syndromes, for instance, have a stronger psychological component. It could therefore be argued that its prefrontal involvement should be more pronounced than in other types of pain.

The underlying mechanism of chronic pain is still an enigma, and some researchers even attribute chronic pain as a disease in its own right (Tracey & Bushnell 2009). It may however be broadly classified into four big groups namely neuropathic, inflammatory, mechanical and functional pain. Neuropathic pain is caused by a

lesion or dysfunction in the central nervous system and persists long after the damage has healed (Smith & Meek 2011). Inflammatory pain results from insult to the integrity of tissues at a cellular level precipitating an inflammatory reaction. The inflammatory reaction consists of aggregation of inflammatory mediators made up of vascular, fibroblastic and tissue cell components that can sensitize nociceptors and in turn activate Aβ touch fibers to be chronically inflammed and transmit pain. Common chronic inflammatory conditions include conditions affecting the joints such as osteoarthritis and rheumatoid arthritis (Aletaha & Smolen 2006). Mechanical pain consists of pain resulting from the wear and tear of prolonged usage and is confined to chronic back pain, with the exclusion of pain resulting from neoplasia, fracture or inflammatory arthropathy (Endean et al 2011). Functional pain syndromes are chronic symptom-based syndromes of unknown aetiology and characterized by chronic pain referred to various regions of the body, and frequent comorbidity with affective disorders (Mayer & Bushnell 2009).

So far, no known studies have compared prefrontal activations among the different types of chronic pain. We performed a meta-analysis of imaging studies on chronic pain patients using GPR analysis as used in the healthy volunteers meta-analysis in Chapter 4. The aims of this study are to investigate the extent of PFC activation in chronic pain patients and to identify any pattern of activation in subtypes of chronic pain conditions.

5.2 Methods

5.2.1 Study selection

The Pubmed Medline database (http://www.ncbi.nlm.nih.gov/pubmed/) was searched for articles containing the keywords "pain", "prefrontal OR frontal", "patient" and "fMRI OR PET". For this meta-analysis, the items recorded were first author, year of publication, title of publication, title of paper, volume and issue of publication, sample size, type of noxious stimulation, duration of noxious stimulation, site of stimulation, type of neuroimaging (i.e. fMRI or PET), type of analysis software (e.g. FSL or SPM; www.fil.ion.ucl.uk/spm)), and the type of chronic pain suffered by the patients. This was then classified under one of four categories namely neuropathic, inflammatory, mechanical, and functional pain.

5.2.2 Inclusion and exclusion criteria

Included in the meta-analysis of chronic pain patients were original articles of fMRI or PET studies on patients with chronic pain with or without noxious stimulation and reported PFC activation. Articles published up to June 2010 were included.

Exclusion criteria specific to the chronic pain meta-analysis were studies on patients with acute pain such as angina pectoris, cluster headache or acute postoperative pain, and studies with mixed sample comprising patients with unrelated pain conditions such as migraine, work-related injury, chronic shoulder pain and pain due to motor vehicle accident (Mailis-Gagnon et al 2003).

5.2.3 Brain mapping

The coordinates for each category were mapped onto a z-score map using GPR analysis as in the meta-analysis of healthy participants in Chapter 4. The resulting brain maps were thresholded at t= 2.3 and the cluster size thresholded at 30 voxels. The resulting voxel coordinates were transformed into mm coordinates in MNI space. A separate map was created for the four categories of chronic pain. To compare the four types of chronic pain activations, differential maps for each type of chronic pain minus all the other types (e.g. functional pain – (neuropathic + mechnical + inflammatory pain) were created.

5.3 Results

5.3.1 Descriptive analysis

A total of 37 fMRI or PET studies on chronic pain reporting activations in the PFC were included and categorized as neuropathic, inflammatory, mechanical or functional pain (Figure 5.1). Table 5.1 shows the types of conditions that were included under each category.

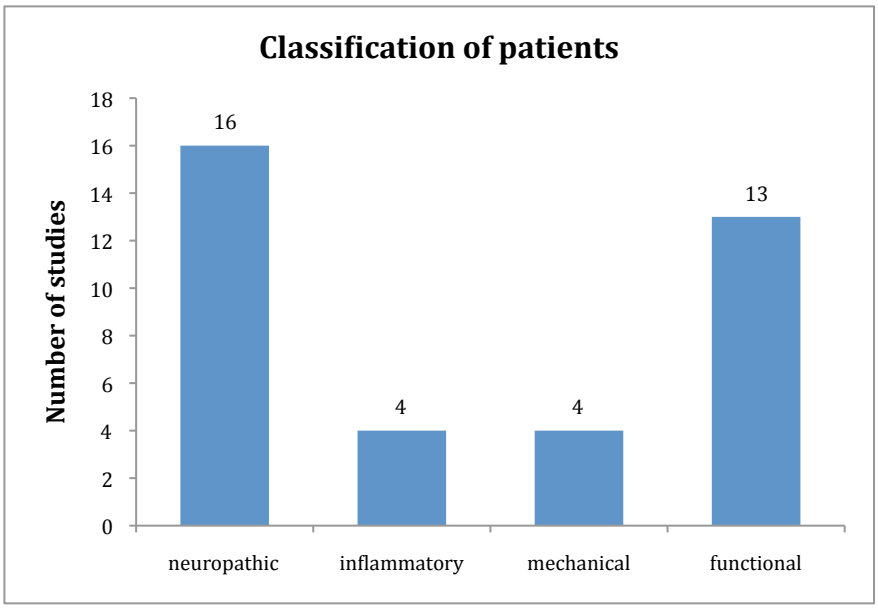


Figure 5-1 Classification of studies according to chronic pain subtypes.

Table 5.1 Classification of types of condition in patients according to specific categories

Neuropathic	Inflammatory	Mechanical	Functional
Burning mouth syndrome Facial pain Syringomyelia Postherpetic neuralgia Painful mononeuropathy Complex regional pain syndrome (CRPS) Spinal cord injury Wallenberg's syndrome (lateral medullary infarct)	Osteoarthritis Rheumatoid arthritis	Chronic back pain	Fibromyalgia IBS Functional dyspepsia

5.3.2 Noxious stimulation

Apart from the background pain suffered by the patients, most studies used one of the following stimulations to evoke pain: contact heat (nine studies), noxious cold (three studies), electrical stimulation (two studies), mechanical stimulation (thirteen studies), incision (one study), and rectal distension (six studies). Studies that investigated brain activation due to background pain without evoked pain included chronic back pain (two studies), complex regional pain syndrome (CRPS; two

studies), fibromyalgia (one study), functional dyspepsia (one study), lateral medullary infarct (one study), neuropathic pain due to central nervous system lesion (one study), peripheral mononeuropathy (one study), and postherpetic neuralgia (one study).

5.3.3 Reported PFC activation

PFC activation as reported by the studies was located mostly in the DLPFC. Medial PFC activation without other PFC activation was found in six studies.

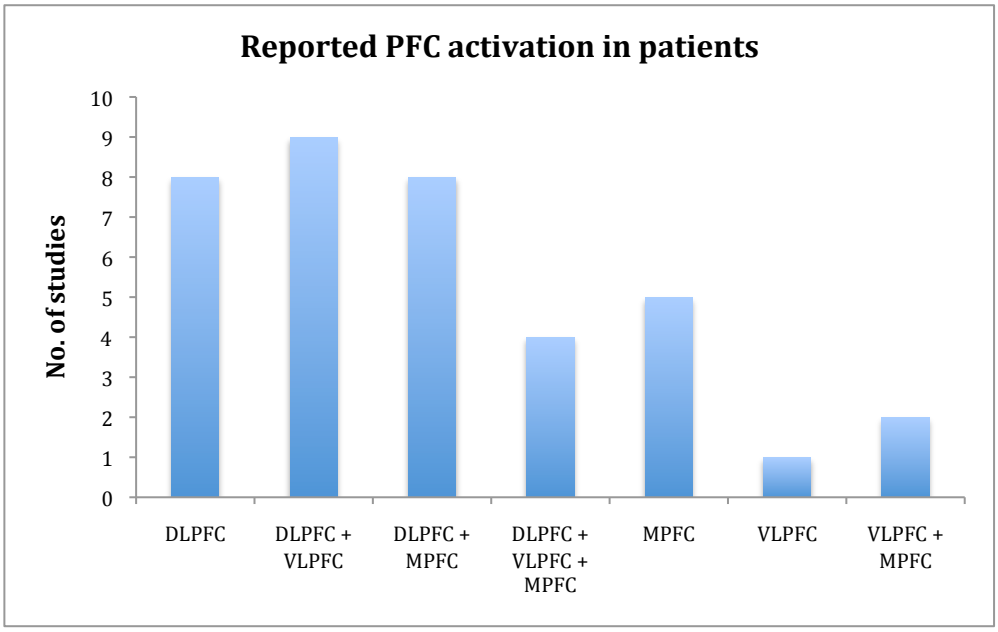


Figure 5-2 PFC activation as reported by studies in patients.

5.3.4 Brain maps

Figure 5.3 shows significant activations for all four categories of chronic pain across studies with and without additional noxious stimulation. Deactivations were below threshold and are not shown. Contrasts are shown in Figure 5.4.

Neuropathic pain

Neuropathic pain did not produce any significant clusters of activation in the PFC. Most of the activations were in the supramarginal gyrus and postcentral gyrus. At a lower threshold (t=1.8), activations of the insular cortex and right midbrain were seen bilaterally. Comparison with the other types of chronic pain did not produce any PFC activation.

Inflammatory pain

Significant PFC activation was seen only in the right inferior frontal gyrus, the VLPFC. Compared with neuropathic, mechanical and functional pain syndromes, inflammatory pain induced the most significant VLPFC activation. Apart from VLPFC, pain-related brain regions such as anterior and posterior insula, thalamus, SI and anterior cingulate cortex were also activated.

Mechanical pain

Significant PFC activation was seen only in the right inferior frontal gyrus. In pain-related brain regions, significant cluster of activation for mechanical pain was found in the anterior cingulate cortex, the left cerebellum and the midbrain region including the PAG area. The right anterior insula also showed significant activation although hardly any cluster was present on the left hemisphere.

Functional pain

The PFC area activated was the right IFG and the frontal pole extending to the anterior aspect of the middle frontal gyrus. Compared to the other types of chronic

pain, functional pain syndromes caused significant activation of the DLPFC. Additional activations were mainly in the right anterior insula and to a lesser extent, the left anterior insula. Significant clusters of activation were also found in the midbrain and pons including PAG.

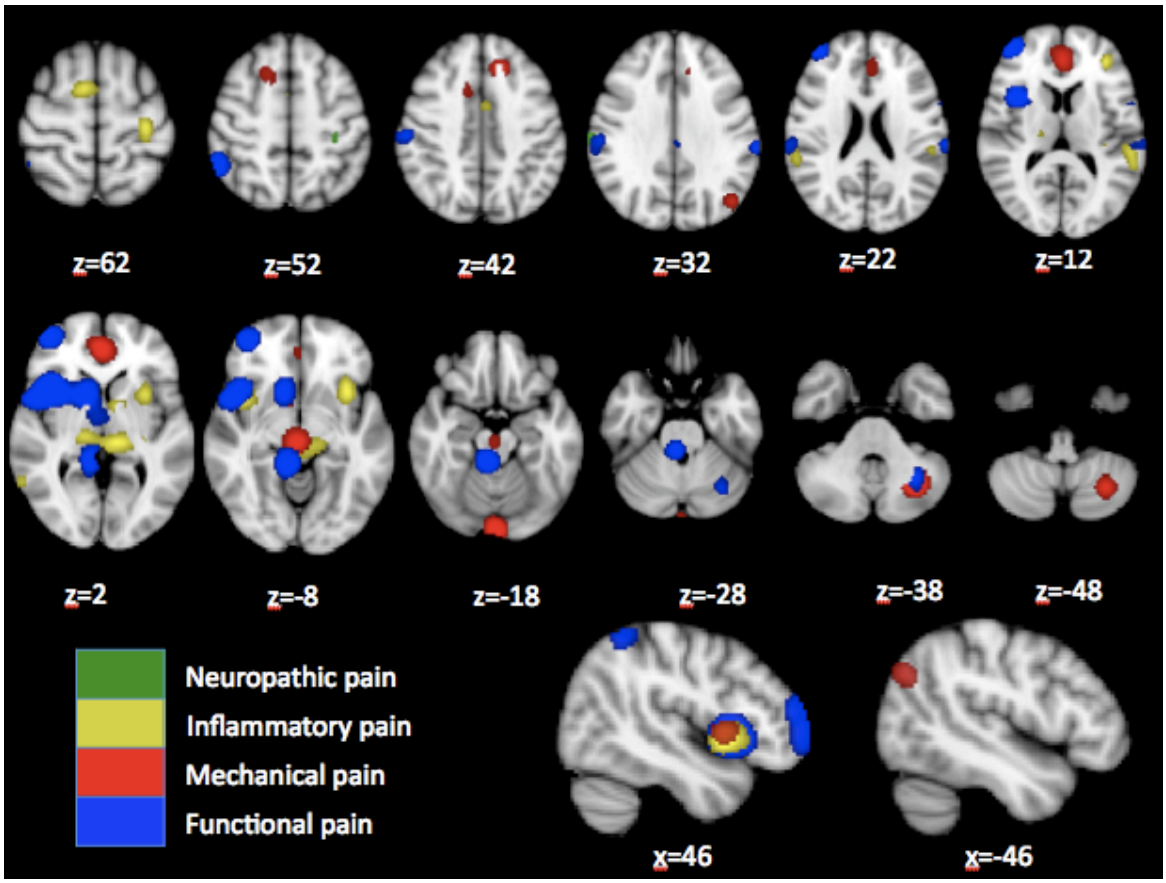


Figure 5-3 Brain activation in patients according to types of pain. Sagittal sections (below) showed activations in PFC. Signals were thresholded at z=2.3 and cluster thresholded at 30 voxels. Images are in radiological convention.

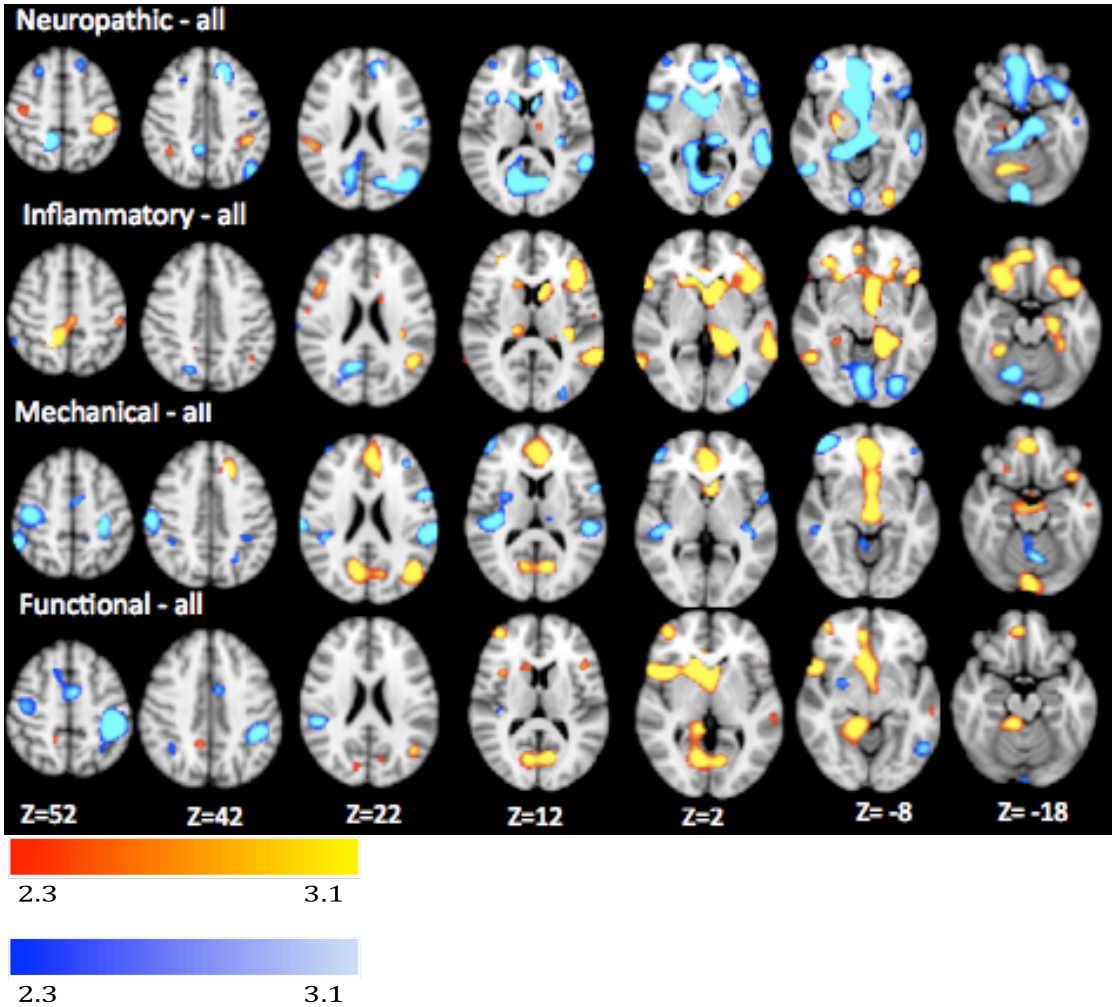


Figure 5-4 Activation (red) and deactivation (blue) for the comparison of each type of chronic pain minus combination of all the other types. Top row: neuropathic minus (inflammatory + mechanical + functional); second row: inflammatory minus (neuropathic + mechanical + functional); third row: mechanical minus (neuropathic + inflammatory + functional); bottom row: functional minus (neuropathic + inflammatory + mechanical). Signals were thresholded at z=2.3 and cluster thresholded at 30 voxels. Images are in radiological convention.

Table 5.2 Peak coordinates of activation for pain condition in patients. All signals were thresholded at z=2.3 and cluster thresholded at 30 voxels.

Type of pain	Brain region	Laterality	Brodmann area	x	y	z	Cluster size (voxel)	Z score
neuropathic	supramarginal gyrus	right	40	66	-22	30	144	2.61
	SI	left	3,1,2	-34	-28	56	104	2.54
inflammatory	ant insula	right	13	42	10	-2	1006	3.51
	thalamus	left		-8	-26	-2	854	3.05
	ant insula	left	13	-34	12	-4	597	3.29
	parietal operculum	left	42	-56	-32	16	304	2.99
	supplementary motor co	right	6	10	2	62	201	2.84
	posterior supramarginal gyrus	right	40	62	-36	18	162	2.71
	SI	left	3,1,2	-32	-26	64	156	2.97
	frontal pole	left	10	-36	42	10	147	2.87
	ant cingulate corex	right	32	2	18	28	65	2.59
	post insula	left	13	-32	-26	6	59	2.73
	ant cingulate	left	32	-4	0	42	47	2.52
	middle temporal gyrus	right	21	62	-56	0	40	2.61
mechanical	ant cingulate		32	2	46	42	1178	3.22
	cerebellum	left		-30	-62	-40	735	4.08
	brainstem			2	-22	-10	640	3.24
	lingual gyrus			0	-92	-18	517	3.65
	ant insula	right	13	42	10	2	381	2.81
	superior frontal gyrus	left	10	-16	30	42	235	2.91
	superior frontal gyrus	right	10	18	18	48	199	2.72
	lat occipital	left	19	-46	-72	34	178	2.8
	subcallosal cortex	right	34	8	8	-12	155	2.67
functional	ant insula	right	13	38	12	4	3251	5.68
	brainstem (pons)			6	-36	-22	1485	4.77
	frontal pole	right		40	54	16	1142	4.6
	supramarginal gyrus	right	1	60	-22	40	487	3.12
	cerebellum	left		-30	-58	-40	366	3.34
	supramarginal gyrus	left	40	-66	-28	32	252	3.26
	precentral gyrus	left	4	-62	8	18	68	2.66
	post cingulate	left	23	-2	-26	28	59	2.6

Table 5.3 Peak coordinates of activation for comparison between chronic pain conditions in patients. All signals were thresholded at z=2.3 and cluster thresholded at 30 voxels.

Contrast	Brain region	Laterality	Brodmann area	x	y	z	Cluster size (voxel)	Z score
neuropathic minus all	SI	left	3b	-42	-32	54	637	4.28
	cerebellum	right		18	-62	-22	284	4.29
	lateral occipital	left	18	-30	-88	-2	250	3.74
	midbrain	right		18	-14	-10	172	3.66
inflammatory minus all	caudate	left		-6	12	0	4114	7.09
	VLPFC	left	45	-44	22	8	1716	4.48
	superior parietal	right	2	18	-48	56	988	5.46
	middle temporal gyrus	left	22	-62	-48	6	952	5.55
	planum temporale	left	22	-34	-30	16	175	4.84
	inferior temporal gyrus	right	20	52	-56	-10	169	3.59
	fusiform cortex	right	37	30	-42	-18	119	3.71
	pars opercularis	right	44	58	20	2	92	3.72
	thalamus	right		16	-24	10	30	3.2
mechanical minus all	paracingulate gyrus	right	11	2	46	-10	3431	6.34
	precuneus	right	18	16	-64	22	710	4.13
	occipital cortex	left	19	-44	-66	26	679	5.61
	occipital fusiform gyrus	right	37	4	-82	-24	408	3.98
	cerebellum	left		-30	-62	-38	212	4.23
	superior frontal gyrus	left	8	-18	28	38	142	4.01
	middle temporal gyrus	left	20	-54	-8	-24	129	3.55
	temporal pole	left	20	-40	16	-24	116	3.26
	temporal fusiform cortex	left	37	-36	-6	-44	52	3.36
functional minus all	subcallosal cortex	right	25	0	12	0	2039	5.01
	intracalcarine cortex	left	17	-10	-70	8	1434	4.26
	DLPFC	right	10	40	52	8	243	3.52

Table 5.4 Peak coordinates of deactivation for comparison between chronic pain conditions in patients. All signals were thresholded at z=2.3 and cluster thresholded at 30 voxels.

Contrast	Brain region	Laterality	Brodmann area	x	y	z	Cluster size (voxel)	Z score
Neuropathic minus all	subcallosal cortex	left		-4	10	0	9709	7.25
	fusiform gyrus	right		4	-82	-24	921	5.09
	middle temporal gyrus	left	21	-60	-48	8	792	4.82
	middle temporal gyrus	left	21	-54	-8	-28	634	3.99
	precuneus	right	2	16	-50	54	413	4.49
	OFC	left	47	-38	18	-16	407	3.41
	pars opercularis	right	44	46	10	2	338	3.72
	parahippocampal gyrus	right	28	22	-6	-30	304	4.51
	SI	left	3b	-48	-14	34	301	4.4
	superior frontal gyrus	left		-16	30	40	281	4.26
	inferior temporal gyrus	right		52	-58	-8	215	3.87
	precentral gyrus	left	4	-22	-16	68	47	3.09
	frontal pole	right	10	40	52	-6	45	3.17
	posterior insula	left	13	-32	-28	14	39	3.16
Inflammatory minus all	cerebellum	right		18	-62	-24	962	4.57
	precuneus	right	18	14	-62	28	692	4.48
	lateral occipital	left	18	-30	-88	2	469	4.76
	supramarginal gyrus	right	4	64	-20	32	66	3.47
Mechanical minus all	SI	right	4	16	-34	68	765	4.55
	cerebellum	right		22	-60	-33	732	4.26
	SII	right	4	64	-20	32	710	4.24
	SII	left	1	-56	-30	18	636	4.67
	Heschl's gyrus	right	2	40	-24	10	390	3.67
	MI	left	4	-32	-24	58	387	3.88
	frontal pole	right	10	42	52	-8	310	4.03
	MI	left	4	-56	8	18	159	3.52
	angular gyrus	right	40	54	-46	54	97	3.99
	posterior cingulate	right		0	-24	30	75	3.18
	posterior cerebellum	left		-4	-64	-16	74	3.29
Functional minus all	SI	left	3b	-38	-30	58	1296	4.85
	juxtapositional lobule	right	6	2	-6	58	400	3.51
	cerebellum	left		-32	-66	-50	201	4.03
	supramarginal gyrus	right	4	52	-36	22	96	3.29
	ant cingulate	left		-6	2	34	53	3.18
	lat occipital	left	18	-50	-64	-6	40	3.09

5.4 Discussion

We performed a meta-analysis of chronic pain imaging studies to investigate PFC activations in four subgroups of chronic pain i.e. neuropathic, inflammatory, mechanical and functional pain syndromes. Results showed that PFC activations were mainly confined to the inferior part of PFC, the VLPFC. Additional activations were also found in pain-related areas such as thalamus, anterior insula, anterior cingulate and SI.

Despite having the largest number of studies, brain activations in neuropathic pain patients did not produce many significant clusters of activation in the brain. No significant cluster was induced in the PFC. This may be due to the high variability of conditions grouped under neuropathic pain ranging from burning mouth syndrome to CRPS and postherpetic neuralgia. This may have caused activations in scattered regions in the PFC that are below threshold. Furthermore, pain activation in neuropathic pain are more somatosensory thus may be more influenced by site and type of pain stimulation. This may explain the involvement of brain regions with somatotopic organization such as SI as shown in this meta-analysis. Previous reviews and meta-analyses (Apkarian et al 2005; Peyron et al 2000; Seifert & Maihofner 2009) suggested that there is no specific network for neuropathic pain.

With the exception of neuropathic pain, all the other types of chronic pain significantly induced activation in the VLPFC. Compared with neuropathic pain, both inflammatory and mechanical pain represent more homogeneous grouping. In this meta-analysis, the four studies grouped under inflammatory pain consisted of

chronic inflammatory conditions i.e. rheumatoid arthritis and osteoarthritis while mechanical pain is confined to low back pain. Inflammatory pain seems to cause the most significant activation of VLPFC compared to the other three types of chronic pain. For chronic back pain, involvement of PFC has been shown previously from fMRI changes in medial PFC (Baliki et al 2006), reduction of N-acetyl aspartate (Grachev et al 2000) and atrophy of DLPFC (Apkarian 2004; Apkarian et al 2004b).

Functional pain includes chronic symptom-based syndromes characterized by chronic pain and discomfort with no obvious underlying pathology such as irritable bowel syndrome and fibromyalgia (Schweinhardt et al 2008; Wilder-Smith 2011). Compared with patients suffering from the other three types of chronic pain, patients with functional pain displayed the most prominent cluster of activation in the DLPFC across studies. Given what is known of the function of DLPFC, this finding may explain the underlying pathophysiology of functional pain that involves a higher cognitive dimension compared to other types of chronic pain.

5.4.1 Lateralization

A feature of pain activation evident in this study is the lateralization of activations to the right PFC. Right lateralization of pain processing has been found previously in DLPFC, thalamus (Coghill et al 2001) and ACC (Hsieh et al 1995; Hsieh et al 1996) in human studies and in amygdala as shown by animal studies (Carrasquillo & Gereau 2008; Ji & Neugebauer 2009). In this meta-analysis, strong activation of right VLPFC is seen in inflammatory, mechanical and functional pain syndromes. This finding is in keeping with the proposed role of the right VLPFC in ER (Mitchell 2011; Wager et al

2008), a crucial factor involved in the coping responses to pain especially chronic pain (Agar-Wilson & Jackson 2011; Apkarian et al 2004a; Connelly et al 2007)

Another finding of this meta-analysis of chronic pain patients is the activation of medial PFC activations. This is consistent with a hypothesis put forward by Apkarian et al (2009) that the interaction between basal ganglia, amygdala, and medial PFC constitutes the emotional, motivational and hedonic components that influence the quality of perceived pain as well as modulate spinal cord nociceptive processing via descending pathways.

5.4.2 Limitation

Apart from the chronic pain suffered by the patients, many of the studies in this meta-analysis used external pain stimuli to induce pain. This may produce a confound that can mask the activations due to the background pain and obliterate the differences between the different types of chronic pain. Chronic pain sufferers often exhibit psychological manifestations such as catastrophizing, pain-related fear and avoidance (Eccleston 2001). Pain associated with a preexisting clinical condition is deemed more salient and evokes more vigilant behaviour. Therefore, it is expected that there are differences between the pain evoked by an experimental stimulus in a site not associated with the clinical condition with a stimulus that aggravates a preexisting clinical pain. However, these differentiations were not performed in this study.

5.4.3 Conclusion

Meta-analysis of chronic pain patients revealed significant foci of activation in the PFC especially the VLPFC with lateralization to the right side. Functional pain syndromes, in addition to inducing activation in the VLPFC, also significantly activated the DLPFC, underlining the cognitive component implicated in the aetiology of this type of chronic pain.

6. STRUCTURAL CONNECTIVITY BETWEEN LATERAL PREFRONTAL CORTICES WITH PAIN-RELEVANT BRAIN REGIONS

6.1 Introduction

Functional imaging studies suggest that the lateral PFC is the key area for cognitive modulation of pain (Lorenz et al 2003; Kalisch et al 2006; Kalisch et al 2005; Wiech et al 2008b; Wiech et al 2006). Subdivisions of the lateral PFC, the DLPFC and VLPFC, seem to have differential yet often overlapping roles.

In cognitive conflict, DLPFC mediates non-emotional cognitive conflict resolution through enhancement of task-relevant stimuli representation in the sensory cortices (Egner 2008). It is also the key controller of top-down attentional processing, biasing attention towards relevant information (Badre & Wagner 2004) or threat-related stimulation (Bishop et al 2004). It is implicated in anticipatory actions such as anticipation of placebo analgesia (Wager et al 2004b; Watson et al 2009). Prior expectation about an upcoming stimulus increases connectivity between the DLPFC with sensory-related medial temporal area (Rahnev et al 2011). High DLPFC activity is correlated with reduced connectivity between subcortical pain-related brain regions resulting in reduced pain perception (Lorenz et al 2003). During attentional modulation of pain, increased DLPFC activity accompanies increased pain intensity (Dunckley et al 2007). Therefore, the role of DLPFC in pain seems to be in the anticipation and attentional modulation of pain.

The role of VLPFC is more evident in the affective aspect of pain from its involvement in perceived control over pain (Salomons et al 2007; Wiech et al 2006), contextual modulation of pain by religious belief (Wiech et al 2008a) and reappraisal (Kalisch et al 2006; Kalisch et al 2005). These studies show that VLPFC mediates the analgesic effect of perceived control and belief, and during high level reappraisal of pain. This is further supported by reappraisal studies in non-pain fields (Wager et al 2008). Wager et al (2008) showed evidence for an interaction between the VLPFC and amygdala, an emotion-related region (Phelps 2004), in the reappraisal of aversive materials. In contrast with VLPFC, DLPFC is involved with exteroceptive rather than interoceptive processing (Faw 2003). Based on the nature of their respective modulations, it is hypothesized that DLPFC is more involved in the non-emotional attentional aspect of pain while the VLPFC mediates modulation of more affective aspects.

In the living brain, tractography, a non-invasive tool based on the diffusion properties of water in tissues, is increasingly used to test specific anatomical hypothesis based on findings derived from functional pain imaging studies (Behrens et al 2003a). There is evidence that function of a brain region is critically determined by its structural connectivity (Johansen-Berg et al 2005; Passingham et al 2002; Toosy et al 2004). The PFC is known to have extensive anatomical connection with other brain areas. It is therefore imperative that the determination of connection strength rather than existence of connection alone is sought for connection between brain areas (Stephan et al 2001) to determine functional connectivity.

It has been suggested that the cognitive modulation of pain by the lateral PFC is based on its functional cortico-cortical and cortico-subcortical connections (Wiech et al 2008b). While the cortico-subcortical pathway connects to pain-related regions, the cortico-cortical pathway does not seem to involve the subcortical pain-related regions such as thalamus, amygdala and PAG. The pain-related brain regions may be classified as those serving more somatosensory function, such as thalamus and SI, and regions more related to the affective-motivational aspects of pain, such as amygdala, anterior insula, OFC and PAG (Treede et al 1999). The somatosensory aspect concerns discriminative and perceptual information such as stimulus location, intensity and duration; while affective-motivational aspects deals more with the quality of pain such as its unpleasantness and is largely affected by psychological variables such as fear and anxiety (Auvray et al 2010).

Tracer studies in primates have established the cortical and subcortical (Gerbella et al 2010; Petrides et al 2011; Schmahmann et al 2008; Yeterian et al 2011) connections of the PFC. However, the relative strength of connections between the PFC and pain-related brain regions as well as intrinsic connectivity between PFC subdivisions still have to be explored.

In order to investigate the structural connectivity of the lateral PFC areas with pain-related regions, we performed probabilistic tractography (Behrens et al 2003b). Seed regions in the lateral PFC comprised the right and left DLPFC, pars opercularis (PO) and VLPFC. Pain-related brain areas included the amygdala, anterior insula, mid insula, posterior insula, dorsal ACC (dACC), rostral ACC (rACC), orbitofrontal cortex

(OFC), periaqueductal grey (PAG), SI, SII, and thalamus.

It is hypothesised that the DLPFC has a higher connection probability with pain-related regions that are related to sensory processing (i.e. SI and thalamus), while VLPFC are more likely to be connected with areas involved in emotional processing (i.e. amygdala, anterior insula, OFC and PAG). Previous tracer studies (Petrides 2005; Petrides & Pandya 1988) and a tractography study (Frey et al 2008) has parcellated VLPFC into pars opercularis and pars triangularis according to their cortico-cortical connections. For the purpose of our tractography, we therefore divided the VLPFC into pars opercularis and VLPFC.

6.2 Method

To investigate whether the lateral PFC is divided into connectivity-based regions that follow our hypothesis, we firstly performed a parcellation of the whole lateral PFC according to its highest probability of connection to pain-related brain regions. Modulation of pain by the prefrontal areas may be influenced by indirect connectivity of these regions to the prefrontal areas by way of cortico-cortical connections between the prefrontal areas. To address this issue, the second part of the study determined intrinsic connection probability between prefrontal subdivisions. The third part of the study focused on determining the connection probability of each PFC subdivision to each pain-related brain region.

Lastly, since the pain-related regions vary in size and distance from the PFC, we determined the connection probability of each pain-related region to PFC subdivisions to determine any preferences in the connection probability of each pain-related region to the PFC subdivisions.

Initial tracking to both hemispheres showed that certain paths (e.g. from the PFC to the cingulate gyrus), suffer from the strong pull of crossing fibers, resulting in an erroneously high connection probability for contralateral compared to ipsilateral targets. In accordance with the majority of tracer studies (Kotter 2004), we therefore performed tractography to the ipsilateral side only.

6.2.1 Definition of masks in structural space

Regions of interest were defined on each subject's T1-weighted image (Figure 6.1) following anatomical landmarks as defined in the Duvernoy's atlases of the human brain (1999) and brainstem (2009). The masks were hand-drawn using fslview (in FSL, FMRIB Software Library, www.fmrib.ox.ac.uk/fsl) starting in one view, e.g. coronal view and double-checked using multiplanar views. Regions of interest comprised three lateral prefrontal regions (i.e. DLPFC, pars opercularis (PO) and VLPFC) and eleven pain-related brain areas including the amygdala, anterior, mid and posterior insula, dorsal and rostral anterior cingulate cortex (dACC, rACC), the orbitofrontal cortex (OFC), periaqueductal grey (PAG), SII, SI and thalamus.

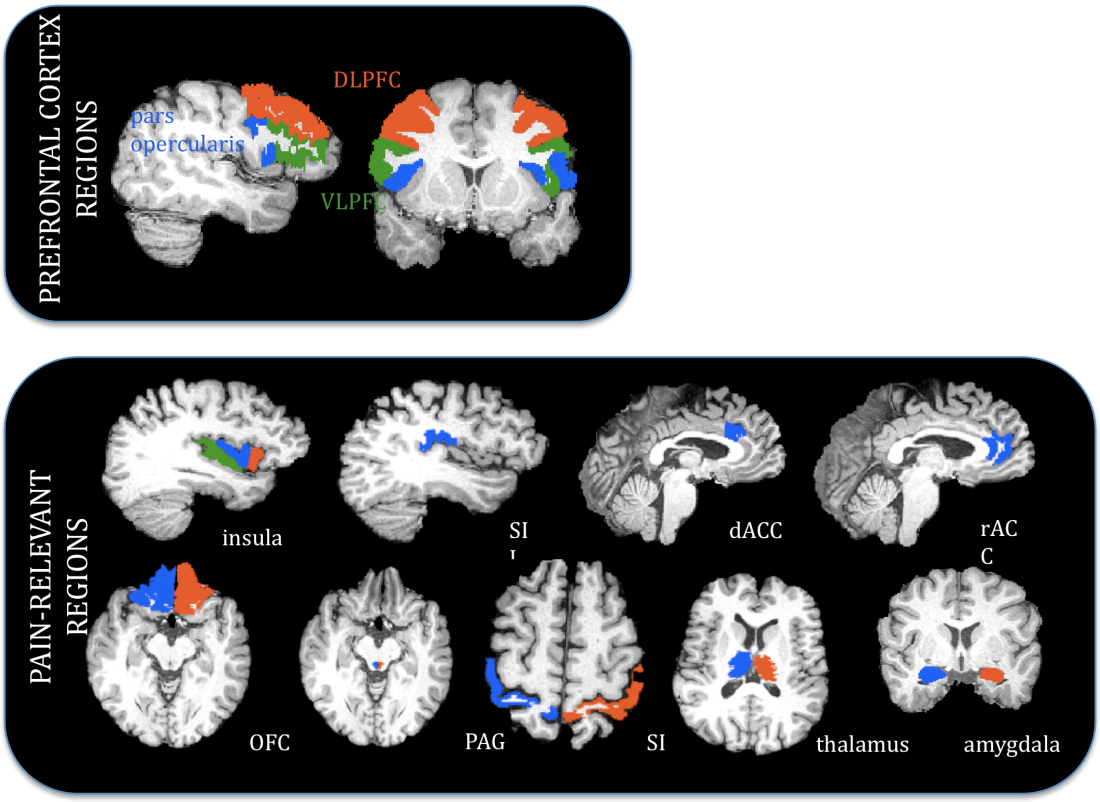


Figure 6-1 Masks of lateral prefrontal subdivisions and pain-related brain regions drawn in the native space of one subject. DLPFC: dorsolateral prefrontal cortex, VLPFC: ventrolateral prefrontal cortex, dACC: dorsal anterior cingulate cortex, rACC: rostral anterior cingulate cortex, OFC: orbitofrontal cortex, PAG: periaqueductal grey, SI: primary somatosensory cortex, SII: secondary somatosensory cortex.

Dorsolateral prefrontal cortex (DLPFC)

The DLPFC includes Brodmann areas 8, 9, 46 and 9/46 located in the superior and middle frontal gyri (Petrides 2005). Cytoarchitectonically, areas 9 and 8B, which lie on the dorsalmost and medial aspect of the PFC are characterized by their sparse granular layer (Petrides & Pandya 1999) while areas 8A, 46 and 9/46 have a well-developed layer IV. As area 9 also comprises the medial surface above the cingulate or paracingulate sulcus, it is often included in the dorsomedial PFC (Croxson et al 2005). For this study, the DLPFC is defined as covering areas 8A, 46 and 9/46 following the similarity in their cytoarchitectonic features. Area 9/46 is further

divided into a dorsal and a ventral subdivision. The dorsal 9/46 occupies the inferior portion of the superior frontal gyrus while the ventral part is situated on the middle frontal gyrus. Area 46 occupies the middle part of the middle frontal gyrus adjacent to area 10 anteriorly (Petrides & Pandya 1999).

Ventrolateral prefrontal cortex (VLPFC) and pars operularis (PO)

The VLPFC occupies Brodman areas 44 (pars opercularis), 45 (pars triangularis) and the lateral part of area 47/12 of the inferior frontal gyrus (Petrides 2005; Petrides & Pandya 2002). The VLPFC extends from the horizontal ramus of the lateral fissure inferiorly to the inferior frontal sulcus superiorly (Croxson et al 2005; Tomassini et al 2007). Rostroventrally, it extends as far as the lateral orbital sulcus (Petrides 2005). Cytoarchitectonically, area 44 is dysgranular whereas the other areas have a well-developed layer IV (Petrides 2005).

Previous studies in humans suggest that the pars opercularis and pars triangularis are differentially connected to other regions (Croxson et al 2005; Frey et al 2008). Based on this observation, we divided the VLPFC into two subregions: i) pars opercularis (area 44) and ii) pars triangularis (area 45) and lateral part of area 47/12 (extending from area 45 to the lateral orbital sulcus). For the remainder of this chapter, the first division of the VLPFC will be referred to as pars opercularis (PO) and the second division will be referred to as VLPFC.

Dorsal and rostral anterior cingulate cortex (dACC and rACC)

Vogt (2005) defined four regions of the cingulate cortex based on cytoarchitecture, connections and functions, namely

- the anterior cingulate cortex (ACC - subgenual and perigenual)
- the midcingulate cortex (MCC - anterior and posterior)
- the posterior cingulate cortex (PCC - dorsal and ventral)
- the retrosplenial cortex (RSC).

The anterior cingulate cortex (ACC) is differentiated from the posterior cingulate cortex (PCC) on the basis of cytoarchitecture and projection patterns found in animals, as well as function observed in animals and humans (www.cingulumneurosciences.org). For subjects with a double parallel cingulate sulcus, the ACC was defined as the region between the corpus callosum and the paracingulate sulcus (Bush et al 2000). The ACC can be further subdivided into a dorsal cognitive division (dACC) and a rostral emotional division (rACC). The dorsal ACC corresponds to the anterior midcingulate cortex (Brodman area 24a', b', c' and 32'), while the rACC corresponds to the perigenual ACC (area 24a, b, c and 32) in Vogt's model. Following the findings of Bush et al (2000) and Vogt (2005), we performed separate tractography analyses on the dorsal (dACC) and rostral division (rACC) of the ACC.

Primary and secondary somatosensory cortex (SI and SII)

SI occupies the posterior bank of the central sulcus and the postcentral gyrus, and extends from the dorsal surface of the brain to the lateral fissure (Behrens et al 2003a) corresponding to Brodmann areas 1, 2, 3a and 3b (Geyer et al 1999).

SII occupies the superior bank of lateral fissure superior to posterior insula (Behrens et al 2003a). Eickhoff et al (2006b) described four cytoarchitectonic regions of SII, OP1-4 which correspond to distinct functional activation in imaging studies, confirming previous pain-related studies that described two separate areas of activation by pain and non-painful stimuli (Coghill et al 1994; Ferretti et al 2003). Area OP1 is the area modulated by pain intensity (Eickhoff et al 2006a).

Amygdala

The amygdala lies in the superomedial aspect of the mesial temporal lobe (Amunts et al 2005). It gradually enlarges posteriorly and decreases in size when in contact with the hippocampus.

Orbitofrontal cortex (OFC)

The OFC is a subdivision of the PFC (Lacerda et al 2003) and occupies Brodmann areas 10, 11, 12, 13, 14 and the orbital part of area 47/12 (Kringelbach 2005; Petrides & Pandya 2002). It extends from the horizontal ramus of the lateral fissure on the lateral surface to the orbital surface and onto the medial surface to include the gyrus ventral to cingulate sulcus and the subcallosal cingulate areas. The

boundary on the medial surface is from the rostral sulcus to the horizontal ramus of the lateral fissure (Croxson et al 2005).

Insula

The insula is a roughly triangular area located deep to the frontal, parietal and temporal opercula in the floor of the Sylvian fissure. The left insula is larger than the right (Varnavas & Grand 1999). Cytoarchitectonically, the insula is divided into rostroventral agranular, intermediate dysgranular and caudodorsal granular areas arranged radially around the piriform olfactory cortex (Chikama et al 1997). It is divided from the frontoparietal operculum by the superior limiting sulcus and the temporal operculum by the inferior limiting sulcus (Chikama et al 1997).

The insula comprises an anterior and a posterior lobule that are separated by the central sulcus of the insula (Naidich et al 2004). The anterior insula consists of the anterior, middle and short insular gyri while the posterior lobule is made up of the anterior and posterior long insular gyri. Schweinhardt et al (2006) divided the anterior lobule into the rostral anterior insula consisting of the anterior short insular gyrus, and the caudal anterior insula consisting of the middle and posterior short insular gyri. For the purpose of this analysis, the insula is subdivided into anterior, mid and posterior regions according to Brooks et al (2002).

Thalamus

The thalamus lies between the foramen of Monro and the posterior commissure and extends from the third ventricle medially to the medial border of the posterior limb of the internal capsule laterally (Ooteman & Cretsinger, Thalamus tracing guidelines: www.psychiatry.uiowa.edu/mhcrc/pdf/papers/thalamus.pdf). Throughout its course it maintains relationship with the internal capsule on the superolateral aspect. Visualization ends just beyond the level of the corpora quadrigemina.

PAG

The periaqueductal grey, as its name implies, surrounds the aqueduct of sylvius in the midbrain (Naidich et al 2008). The neural networks of the PAG are organized in dorsocaudal columns that function in escapable and inescapable stress (Keay & Bandler 2001). It is also a key region for the descending modulation of pain (Fields 2004).

The method for drawing the masks is specified in Appendix 2.

6.2.2 Data acquisition

Diffusion-weighted scans of 15 healthy volunteers (12 males) aged between 22 and 40 years old were obtained from the FMRIB diffusion-weighted imaging database. The study was approved by the Oxfordshire Clinical Research Ethics committee and written informed consent had been obtained from each participant prior to

scanning. Diffusion-weighted data had been acquired on a Siemens Trio 3T scanner using 3 acquisitions in 60 directions and 5 non-diffusion-weighted images. Parameters include b-value of 1000 s mm^{-2}, voxel size 1.5 x 1.5 x 1.5 mm and 100 slices. Probabilistic modelling of diffusion parameters was performed according to previously described methods (Behrens et al 2007; Behrens et al 2003b) using the FMRIB's Diffusion Toolbox, FDT, a tool in FSL (FMRIB Software Library, http://www.fmrib.ox.ac.uk/fsl). Each participant's brain images comprised the diffusion-weighted and T1-weighted structural images. These images and MNI standard space brain template images were skull-stripped using the brain extraction tool (BET; Smith 2002) and affine registration (Jenkinson et al 2002; Jenkinson & Smith 2001) was performed to derive transformation matrices between the three spaces, i.e. matrices for diffusion to structural, structural to standard, and standard to diffusion.

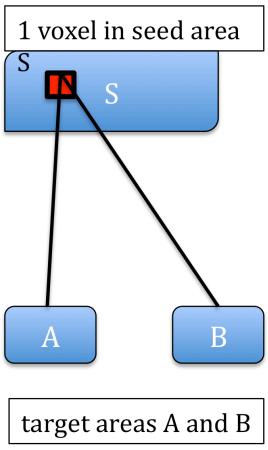
6.2.3 Probabilistic tractography

Probabilistic tractography was performed on high-resolution (1.5mm) diffusion-weighted bedpostX datasets using probtrackx in FDT (Behrens et al 2003b; Smith et al 2004) according to previously described methods (Behrens et al 2003b). Prior to tractography, all masks in structural space were registered onto the subject's diffusion-weighted image using transformation matrix 'structural-to-diffusion' derived from the linear registration function (FLIRT) in FDT. Using Bayesian principle, a probability diffusion function was estimated for every voxel to determine the principal fiber direction. Streamline samples were generated from each seed voxel to build up a connectivity distribution in structural space. Five thousand samples were

used for parcellation of PFC while for tractography, a total of 10000 samples was used. We decided to use this relatively high number of samples because given the high variability in size and distance of the targets and a lower sample would be likely to produce inconsistent results especially for the smaller target regions. Previous studies have shown that the output converges after 10000 samples (Behrens et al 2003a; Hadjipavlou et al 2006). The number of these samples passing through each brain voxel is proportional to the connection probability of the seed voxel. To ascertain that tracking was only through white matter and not indirectly via grey matter, a limit was set so that any tract that was shorter than 2mm (2 voxel length) was discarded. Furthermore, masks of target areas were combined and used as a stop mask so that tracking terminated once it has entered the target mask.

For each subject, probabilistic connectivity distribution from all voxels in each seed region and their connection probability to each target area was quantified. This gave the mean number of samples per voxel in the seed area with positive connection probability to each target area. The mean per voxel multiplied by the volume with positive connection probability gives the connectivity index (Figure 6.2). From the connectivity index of each target, the proportion of samples reaching a particular target out of all probable connections to all target areas is calculated to give the relative connection probability for each target. Calculating the proportion of fiber connectivity allows comparison to be made with the connectivity of other seed areas with the respective target (Rushworth et al 2006). For visualization, the structural brain image from each subject was then transformed into standard space using linear registration (FLIRT) and combined to produce an average brain image across

all subjects in standard space.



Consider tracking from seed area S to target areas A and B.

Connection probability from seed area S to target areas A and B is given by **connectivity index.**

If 5000 samples are initiated from each voxel in seed S, connectivity index for seed S to target A is given by

CI_A = mean (M) x volume (V)

where
M = mean number of samples per voxel in seed area with connection probability to target A
V = number of voxels in seed area with connection probability to target A

Relative connection probability for target A is the connectivity index of target A, CI_A divided by the sum of connectivity index of targets A and B multiplied by 100.

Relative connection probability = $[CI_A / (CI_A + CI_B)] \times 100$

Figure 6-2 Quantification of connection probability.

For the connectivity path images, the output from each subject was transformed to standard space, binarized and overlaid on the averaged structural brain image of all subjects in standard space. Therefore, for each coloured voxel, the intensity indicates the number of subjects with overlapping connectivity. These spatial maps were then thresholded to include the overlap of 14 or more subjects out of the 15 subjects. Tracts from each subject are binarized so that an above zero connection probability is given the value of 1. Therefore each voxel number indicates the number of subjects with an above zero probability of the tract being present. This non-stringent criteria is to ensure the inclusion of smaller tracts to distant targets.

Four types of tractography were performed on the bedpostx datasets. For each analysis, tracking was done from each seed to each ipsilateral target. Figure 6.3 gives an overview on the analysis.

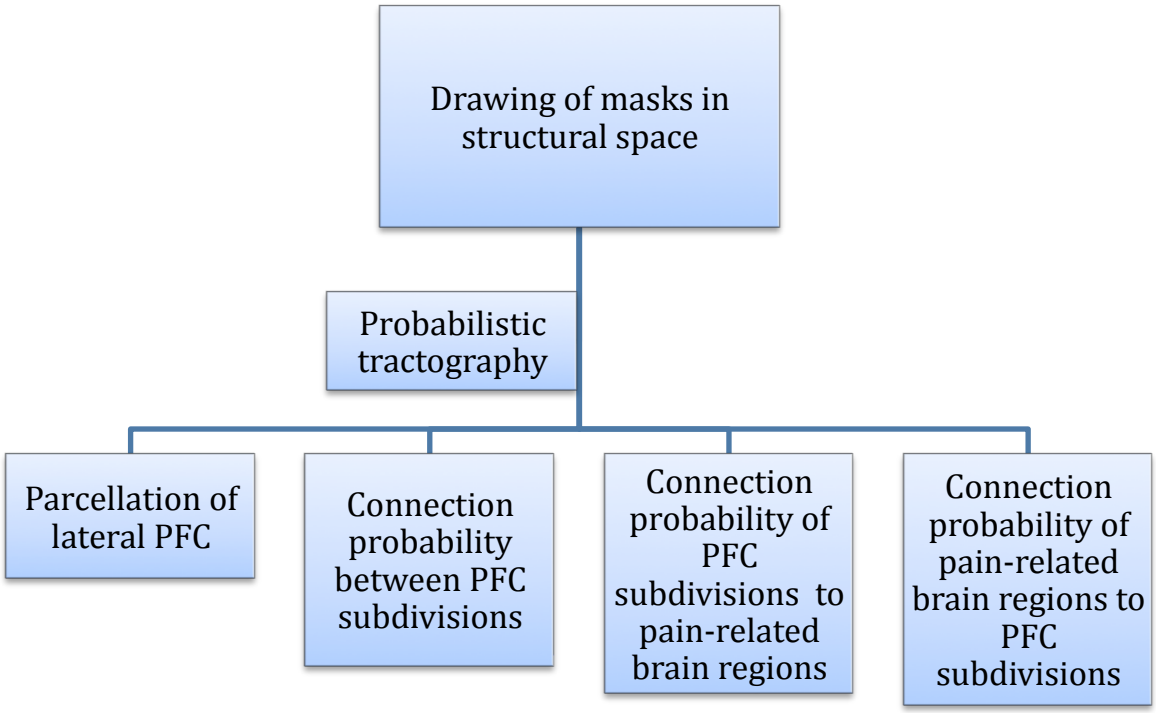


Figure 6-3 Overview of the probabilistic tractography

a) Probabilistic parcellation of the whole lateral PFC according to the strongest connection probability to pain-related brain regions.

In this first analysis, all three subdivisions of the lateral PFC, i.e. DLPFC, PO and VLPFC were combined into a mask and using this combined mask as a seed area, parcellation was performed of the whole lateral PFC according to its highest connection probability to each pain-related brain region. The seed voxels in the lateral PFC are classified according to their highest connection probability to the 11 pain-related brain regions. This produced a connectivity-based map of the lateral

PFC according to the area that has the highest connection probability to the pain-related brain regions. Due to the high spatial variability of the connection across subjects, the results are shown first in the individual structural space of each subject. Second, the maps were registered to standard space individually, and third, a seed-to-target image for each subject was registered to standard space and summed to produce a mean image across all subjects. The third map depicts the spatial location in the PFC for every connection to the pain-related region.

b) Probabilistic tractography between subdivisions of PFC

To investigate the structural connectivity between the subdivisions of lateral prefrontal areas, we performed tractography from each prefrontal area as the seed to the remaining two ipsilateral prefrontal areas as targets.

c) Probabilistic tractography between each lateral PFC subdivision and all pain-related brain regions

To define the relative connection probability between each division of the PFC to pain-related regions, we performed tractography from each prefrontal subdivision (i.e. DLPFC, PO and VLPFC) to all ipsilateral pain-related regions of interest.

d) Probabilistic tractography from each pain-related target region to the prefrontal areas

While the first tractography tracked from the PFC as the seed region and gives the relative connection strength for the different pain related areas, it provides little information as to how the pain-related regions are differentially connected to each

prefrontal area. We therefore performed additional analyses in which each pain-related brain region served as a seed region and the three prefrontal brain regions were defined as targets.

A previous study has performed parcellation of the thalamus according to its connections with cortical regions (Behrens et al 2003a). Our results of the second analysis showed the highest probability of connection between DLPFC and thalamus. We performed an additional analysis to determine the location of highest connection probability with the prefrontal subdivisions. The coordinates of the center of gravity of each subject's thalamic mask was weighted by the connectivity to the prefrontal region and plotted on a standard space brain image to show the location of peak voxels for all subjects.

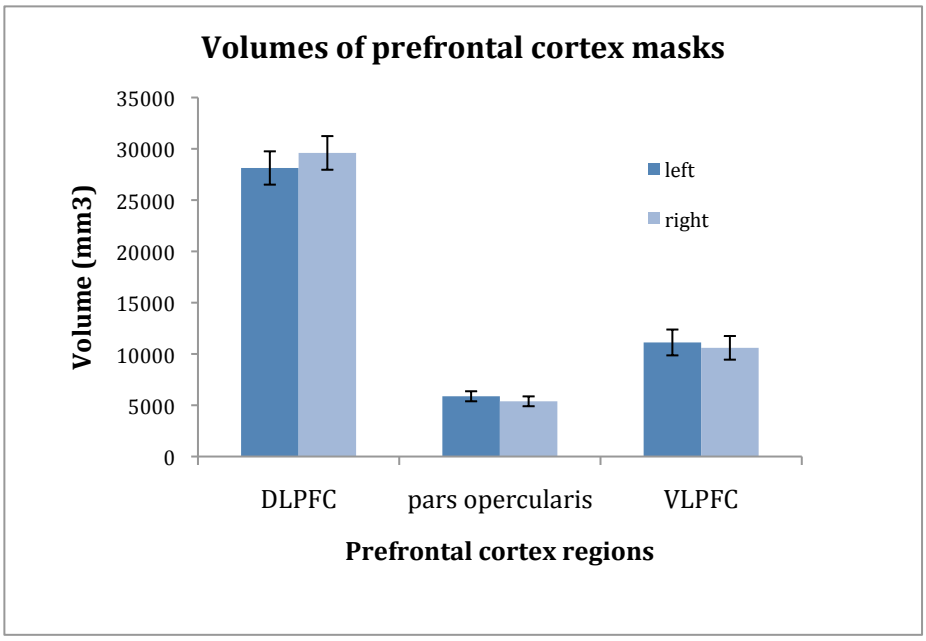
6.2.4 Statistical analysis

The tractography results were explored. A repeated measures ANOVA with factors HEMISPHERE (2 levels: right and left), PFC SUBDIVISIONS (3 levels: DLPFC, PO and VLPFC), and PAIN REGION S (11 levels: anterior insula, midinsula, posterior insula, amygdala, PAG, thalamus, SI, SII, OFC, dACC and rACC) was used to investigate the differences in mask size and connectivity index values for analyses ii, iii and iv. Greenhouse-Geiser correction was applied when Mauchly's assumption of sphericity was not met. Connection probability is the result of connection index of each target divided by the sum of connection probability of all targets and expressed in percentage. The analysis did not take into account the size of the mask and distance into account.

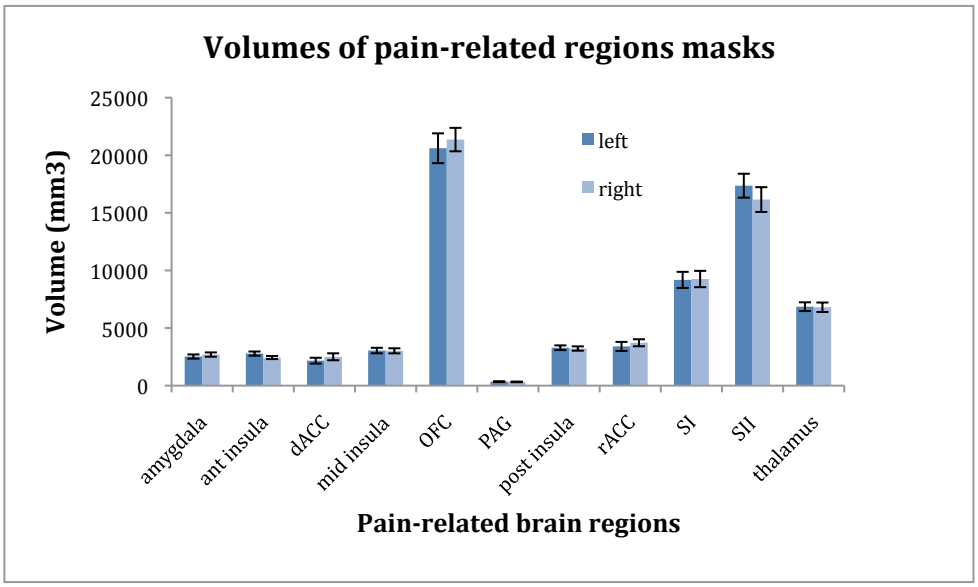
6.3 Results

6.3.1 Mask sizes

The different sizes of the seed and target masks are shown in Figure 6.4. The biggest mask was the one for the DLPFC (right side, mean ± sd = 29600 ± 6493; left side, mean ± sd = 28130 ± 6493) and the smallest was the one for the PAG (right side, mean ± sd = 324.48 ± 121.81; left side, mean ± sd = 349.75 ± 137.48). Repeated measures ANOVA with 2 factors, HEMISPHERE (left and right) and PFC SUBDIVISIONS (DLPFC, PO and VLPFC) showed that the prefrontal masks were not significantly different between hemispheres (main effects of HEMISPHERE: $F(1,14)=0.92$, $p=0.35$) but there was a significant main effect of PFC SUBDIVISIONS ($F(2,28)=137.14$, $p<0.0005$). Similarly, for pain-related brain regions, there was no significant difference between hemispheres ($F(1,14)=0.17$, $p=0.69$) but a significant main effect of PAIN REGIONS (11 levels brain regions) (main effect of PAIN REGIONS: $F(1.8, 25.2)=218.09$, $p<0.0005$).



(a)



(b)

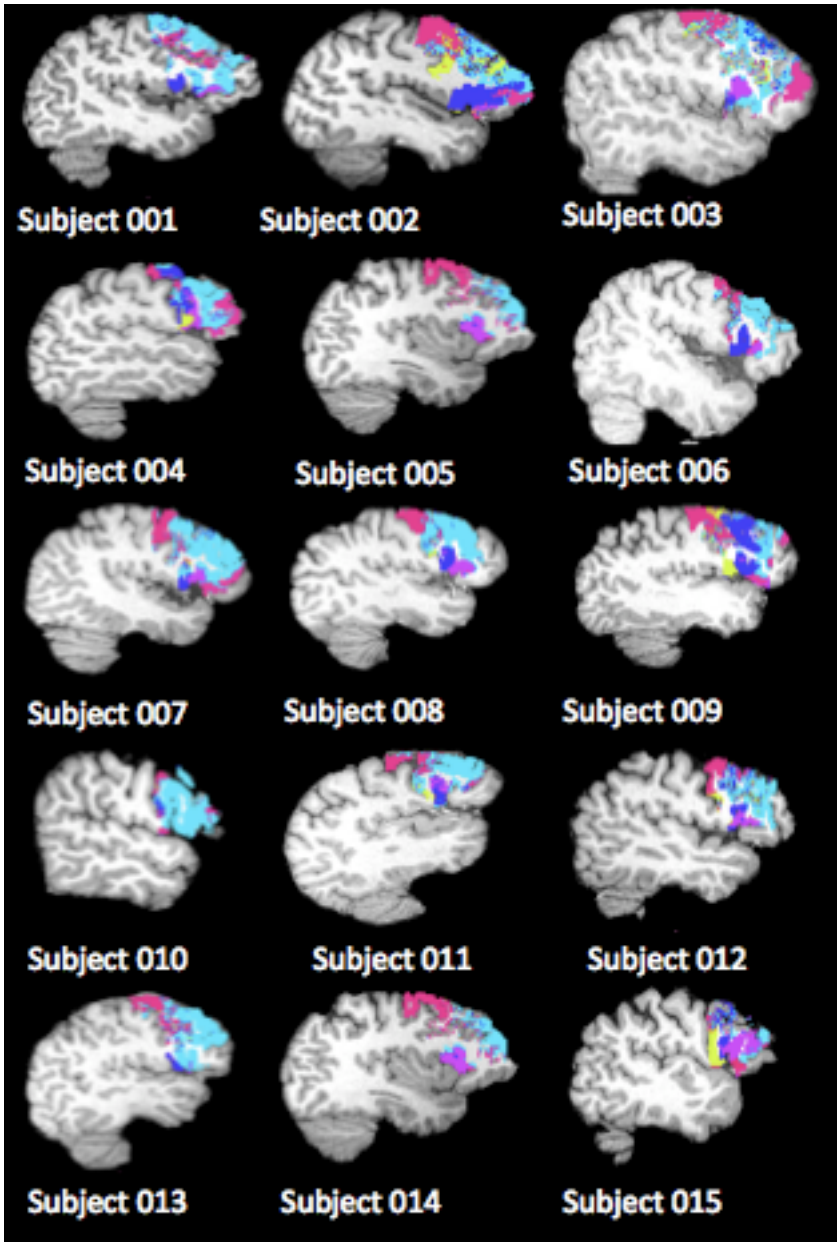
Figure 6-4 Mask sizes of lateral prefrontal subdivisions (a) and pain-related brain regions (b). Values are mean ± S.E.M.

6.3.2 Results of analyses

6.3.2a) Probabilistic parcellation of the lateral PFC according to the strongest connection probability to pain-related brain regions

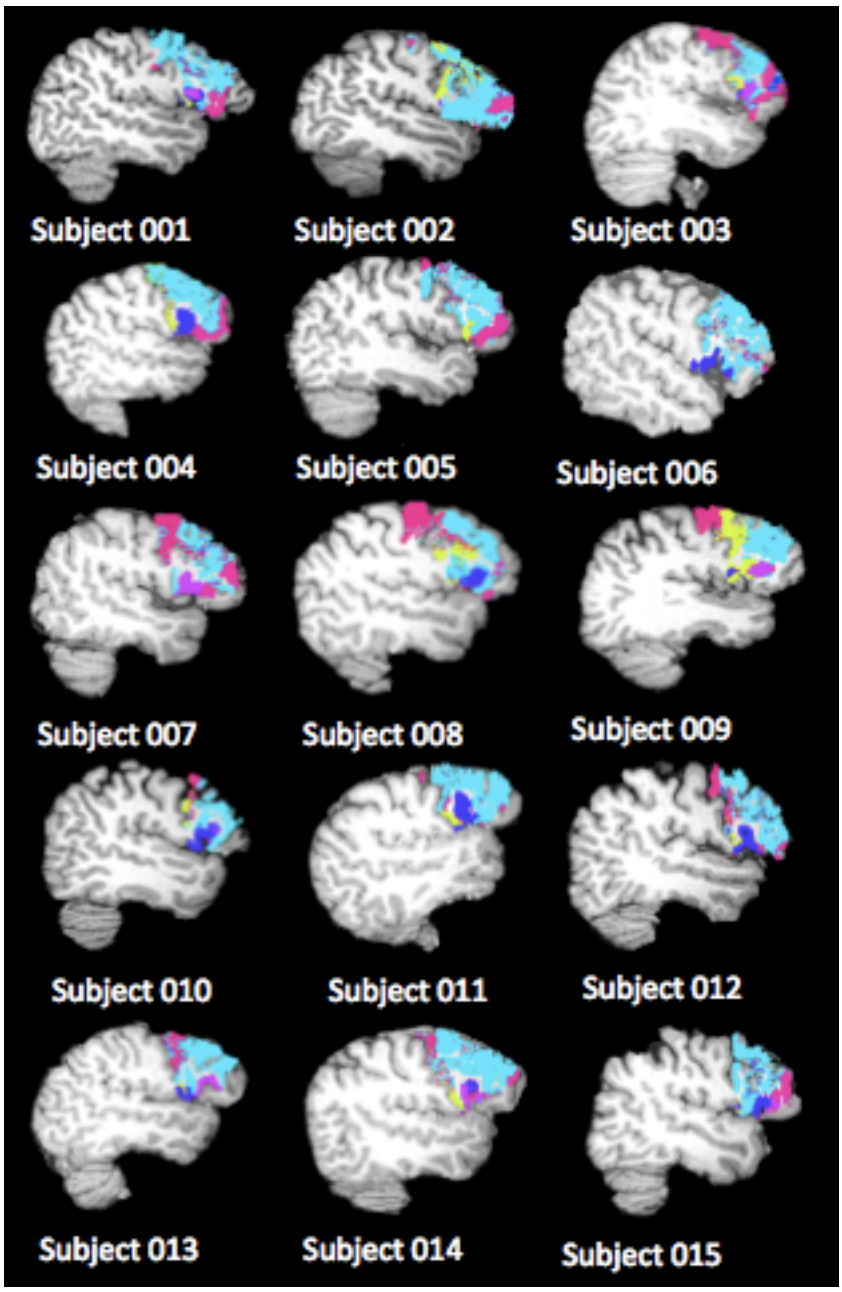
The whole lateral PFC was used as seed and parcellated according to the highest probability of connection with pain-related brain regions. This means that for every

voxel in the lateral PFC, with which of the eleven pain-related brain regions is the voxel has highest probability of connection. This parcellation revealed a high degree of variability across subjects (Figure 6.5 - 6.8). In general, however, the highest connection probability with thalamus, SI, SII, midinsula, anterior insula and OFC occupies the largest spread spatially in the PFC. In most subjects, the postero-superior aspect of the PFC has a high connection probability with SI while more inferiorly, the highest connection probability was shared between anterior and mid insula, SII and thalamus. While the majority of subjects displayed the highest connection probability to thalamus and SI in the superior aspect of PFC, at least 3 subjects also have a high connection probability with midinsula in this region. Other regions with lower probabilities of connection did not appear in the parcellated map.



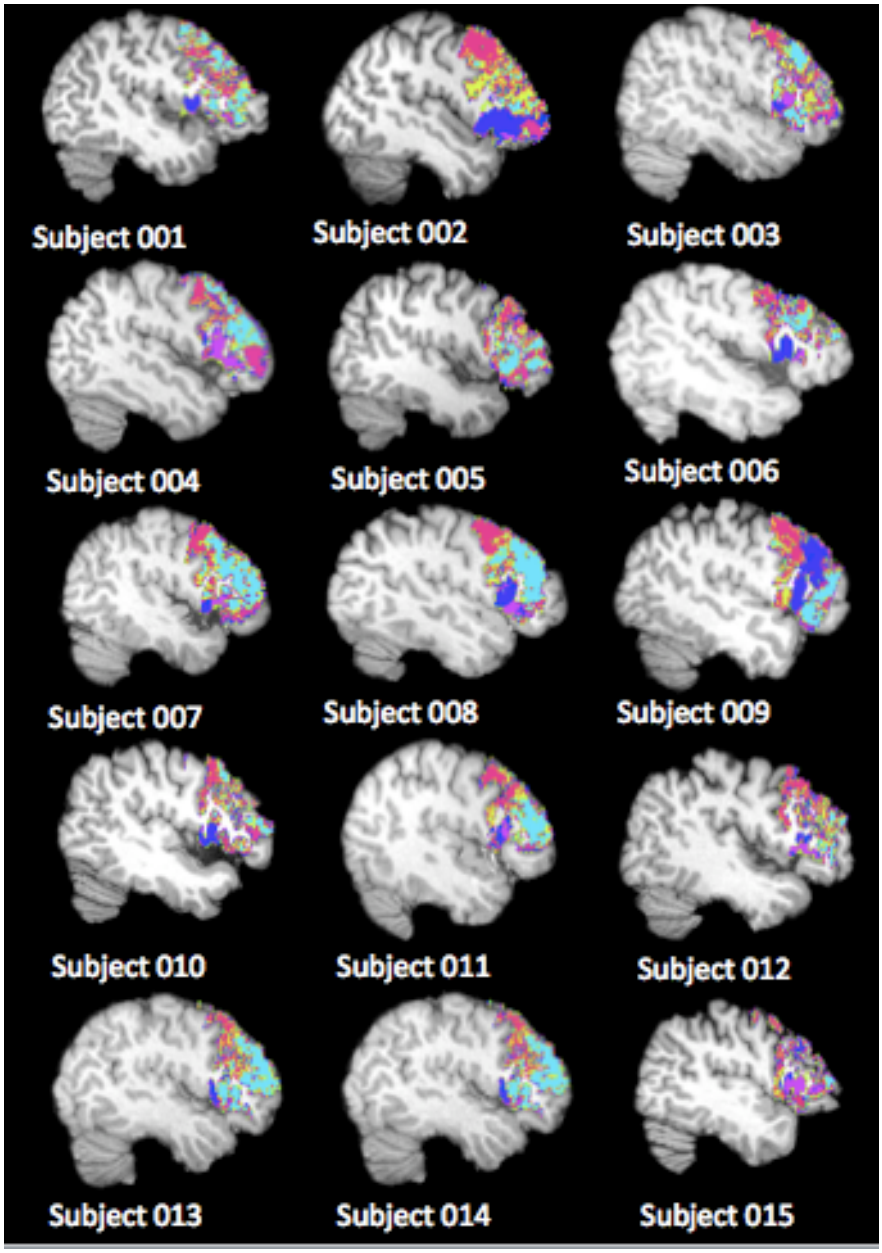
	thalamus
	SI
	midinsula
	ant insula
	SII
	OFC

Figure 6-5 Parcellation of the left PFC in structural space



	thalamus
	SI
	midinsula
	ant insula
	SII
	OFC

Figure 6-6 Parcellation of the right PFC in structural space



	thalamus
	SI
	midinsula
	ant insula
	SII
	OFC

Figure 6-7 Parcellation of left PFC for each subject registered onto standard space: x=-46

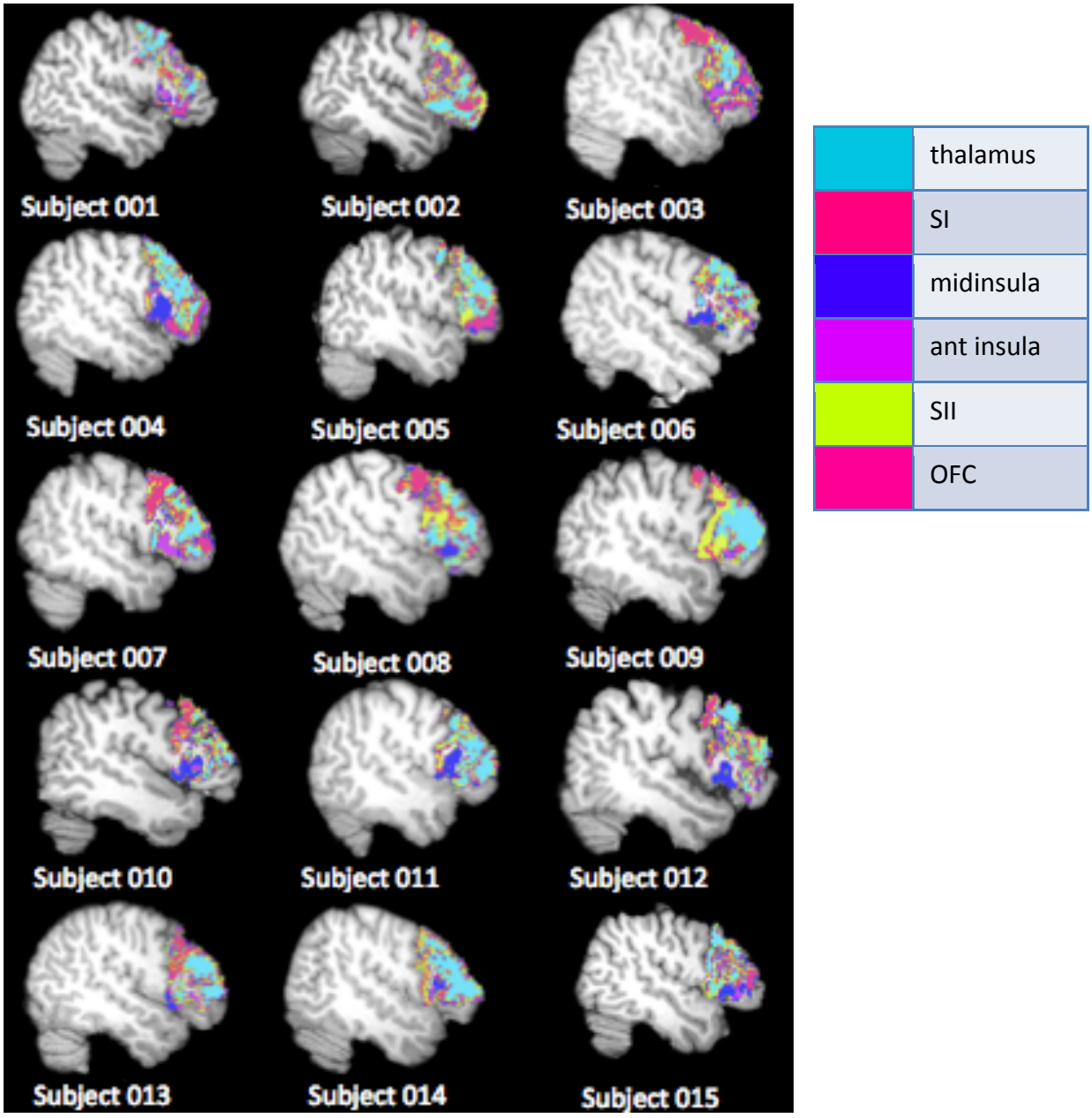


Figure 6-8 Parcellation of right PFC registered onto standard space: x=46

Figures 6.9 and 6.10 show spatial maps depicting where in the PFC is the highest connection probability with each pain-related brain region across subjects. Most of the pain-related brain regions have a high connection probability with the inferior aspect of the lateral PFC except for SI that has a higher likelihood of connection with the postero-superior part of the PFC. While the thalamus displays the highest

connection probability among the pain-related regions and is spatially spread all over the subdivisions of lateral PFC, the highest connection probability seems to be with the inferior aspect of the PFC (Figures 6.9 and 6.10).

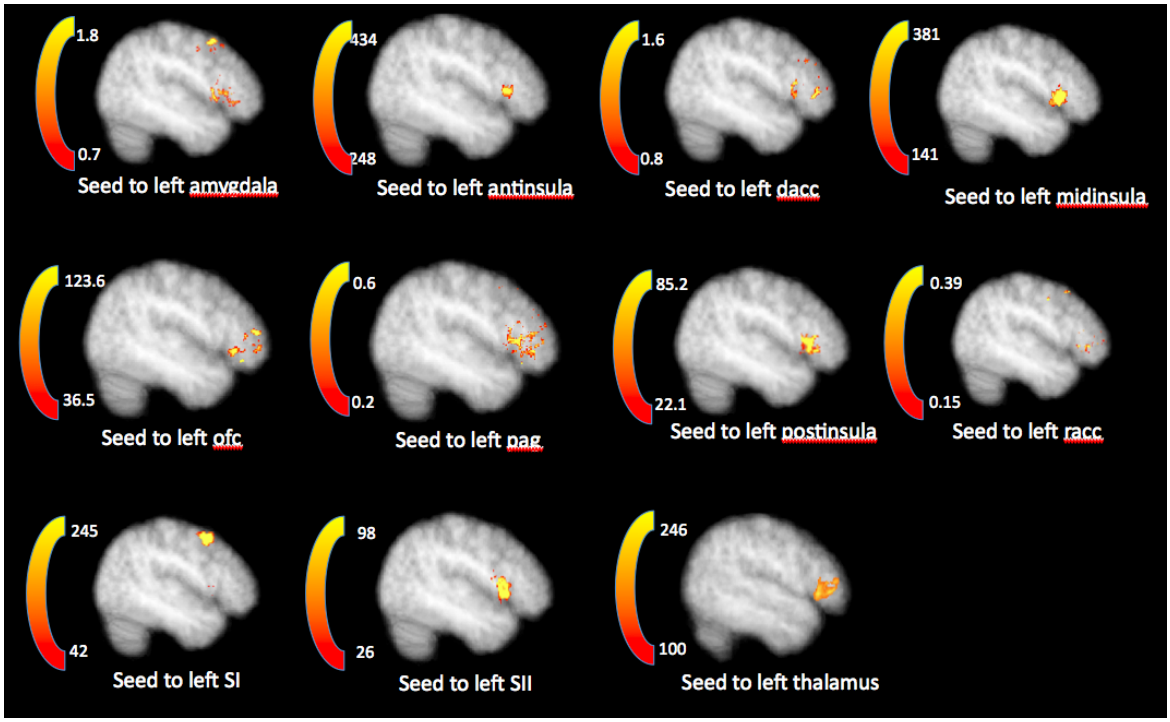


Figure 6-9 Location and intensity of connection probability of left lateral PFC to pain-related targets, summed and normalized across subjects. The numbers indicate the range of the number of samples that reaches the target out of 5000 samples initiated from every voxel in the lateral PFC. Images thresholded at half the maximum intensity out of 5000 samples initiated.

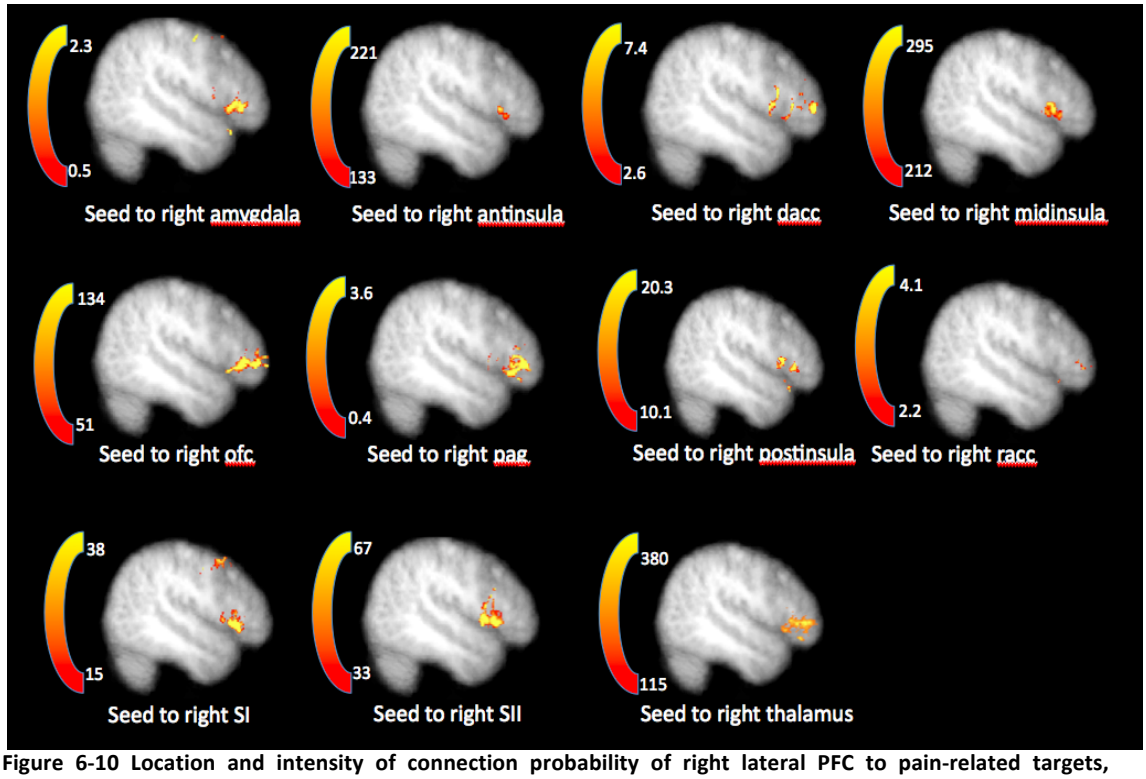


Figure 6-10 Location and intensity of connection probability of right lateral PFC to pain-related targets, summed and normalized across subjects. The numbers indicate the range of the number of samples that reaches the target out of 5000 samples initiated from every voxel in the lateral PFC. Images thresholded at half the maximum intensity out of 5000 samples initiated.

6.3.2b) PFC seeds to other PFC subdvisions (structural connectivity between lateral prefrontal subdivisions)

For intrinsic connectivity between PFC subdivisions, a repeated measures ANOVA was performed with the factors HEMISPHERE (2 levels: left and right) and PFC SUBDIVISIONS (2 levels: for VLPFC, DLPFC and PO,; for PO, DLPFC and VLPFC; and for DLPFC, PO and VLPFC) (Figure 6.11).

i) DLPFC: There was a significant main effect of HEMISPHERE, ($F(1,12)=8.9$, $p=0.011$) but no significant main effect of PFC SUBDIVISION ($F(1,12)=1.04$, $p=0.33$). The left DLPFC had significantly higher connection probability with the other two PFC subdivisions compared with the right DLPFC.

There was no interaction between HEMISPHERE and PFC SUBDIVISION ($F(1,11)=2.34$, $p=0.15$).

ii) PO: The connection probability was not significantly different between the left and right hemisphere (main effect of HEMISPHERE: $F(1,12)=0.29$, $p=0.6$) but there was a significant difference in connectivity with the other two PFC subdivisions (main effect of PFC SUBDIVISION: $F(1,12)=13.44$, $p=0.003$). Connection probability with the DLPFC was higher compared to the VLPFC. No significant interaction was seen between HEMISPHERE and PFC SUBDIVISION.

iii) VLPFC: Similar to the PO, the VLPFC was not differentially connected between the right and left hemispheres (main effect of HEMISPHERE: $F(1,11)=1.34$, $p=0.27$) but had different connection probabilities with the other two PFC subdivisions (main effect of PFC SUBDIVISION: $F(1,11)=11.17$, $p=0.007$). Had higher connection probability was to DLPFC compared to PO ($t(12)=2.87$, $p=0.014$). No significant interaction was seen between HEMISPHERE and PFC SUBDIVISION.

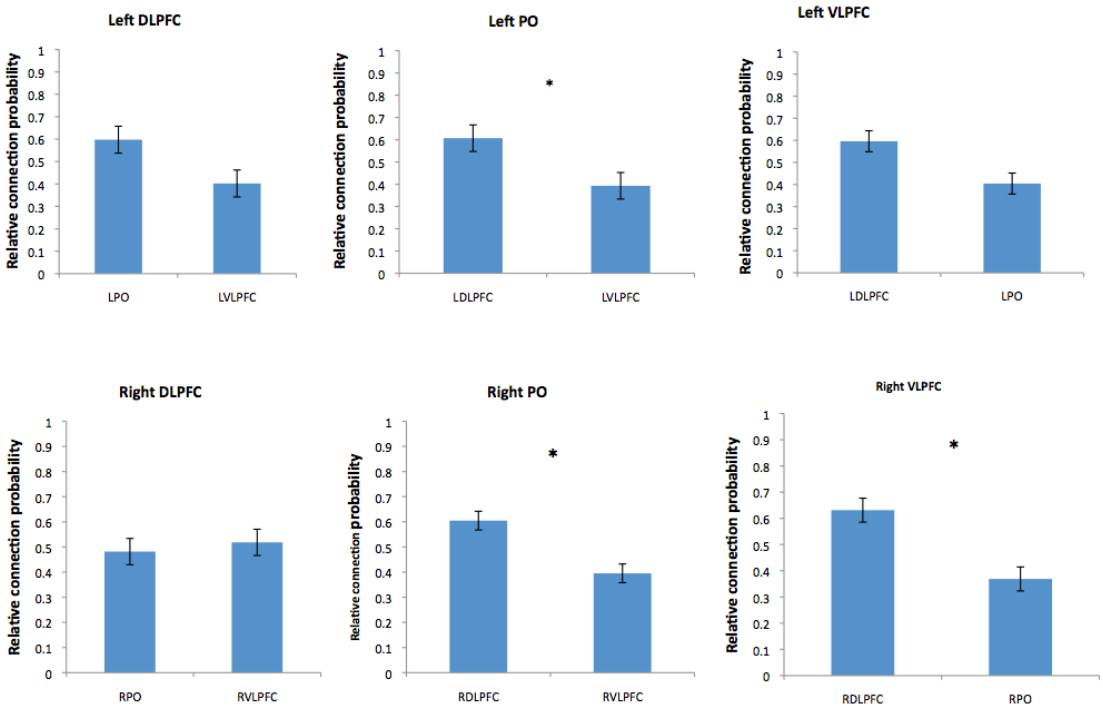


Figure 6-11 Relative connection probability (mean ± SEM) between intrinsic prefrontal areas DLPFC, PO and VLPFC. * p<0.025

6.3.2c) PFC subdivisions to pain-related brain regions

Figure 6.12 shows the paths originating from the DLPFC, PO and VLPFC. Tractography run from these prefrontal seed areas to ipsilateral pain-related brain regions produced connection probability maps as shown in Figure 6.13 while Figures 6.14 – 6.19 provide spatial information (i.e. which part of the PFC is connected to which pain-related brain region).

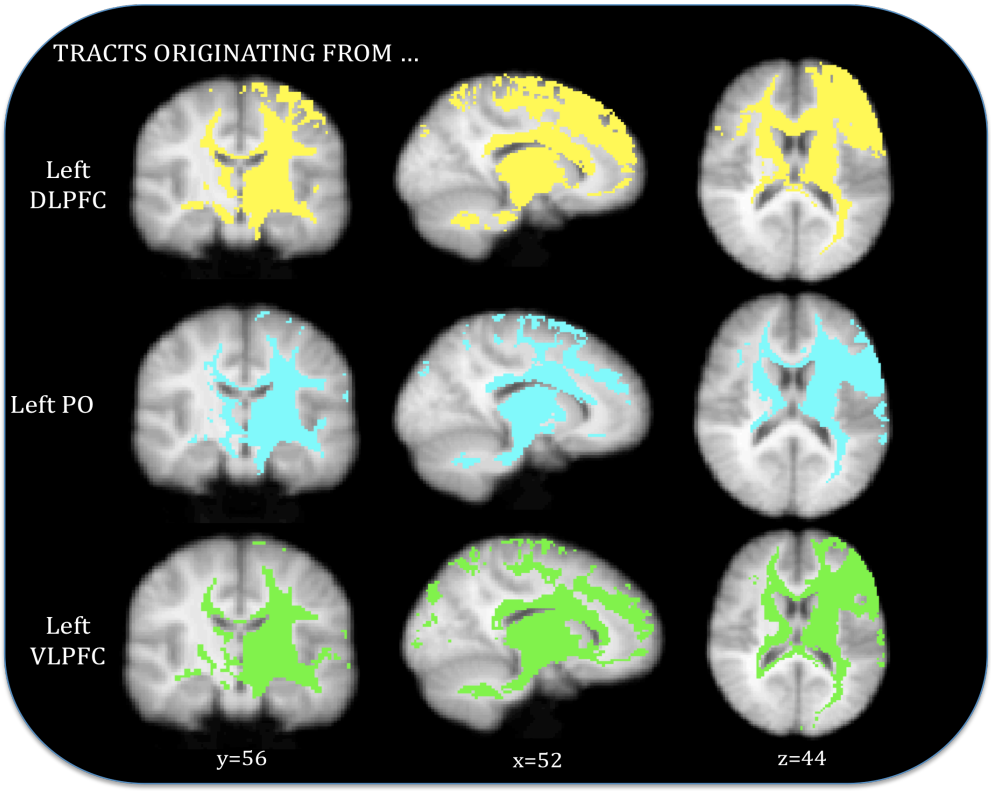
A repeated measures ANOVA with factors HEMISPHERE (2 levels: right and left), PFC SUBDIVISIONS (3 levels: DLPFC, PO and VLPFC) and PAIN REGIONS (11 levels of pain-related brain regions) showed no significant difference between the right and left hemisphere (main effects of HEMISPHERE: $F(1,12)=0.04$, $p=0.84$). There were, however, significant main effects of PFC SUBDIVISIONS ($F(1.13,24)= 18.58$, $p<0.001$)

and PAIN REGIONS ($F(2.07,120)=25.17$, $p<0.0005$). Multiple comparisons with Bonferroni correction revealed that connection probabilities of the DLPFC were significantly lower than those of the PO ($p<0.0005$) and VLPFC ($p=0.001$). The connection probability of the PO was significantly lower than the one for the VLPFC ($p=0.025$). Post-hoc multiple comparisons with Bonferroni correction for PAIN REGIONS are shown in Table 6.1. Furthermore, there was a significant interaction between PFC SUBDIVISIONS and PAIN REGIONS ($F(1.87,240)=15.75$, $p<0.0005$). Within subjects contrasts showed that comparison between DLPFC and PO was not significantly different when comparing connections between amygdala and OFC ($p=0.21$). Comparison between VLPFC and DLPFC was not significantly different when comparing connections between amygdala and dACC ($p=0.18$), PAG ($p=0.13$), rACC (0.47) and thalamus (0.98).

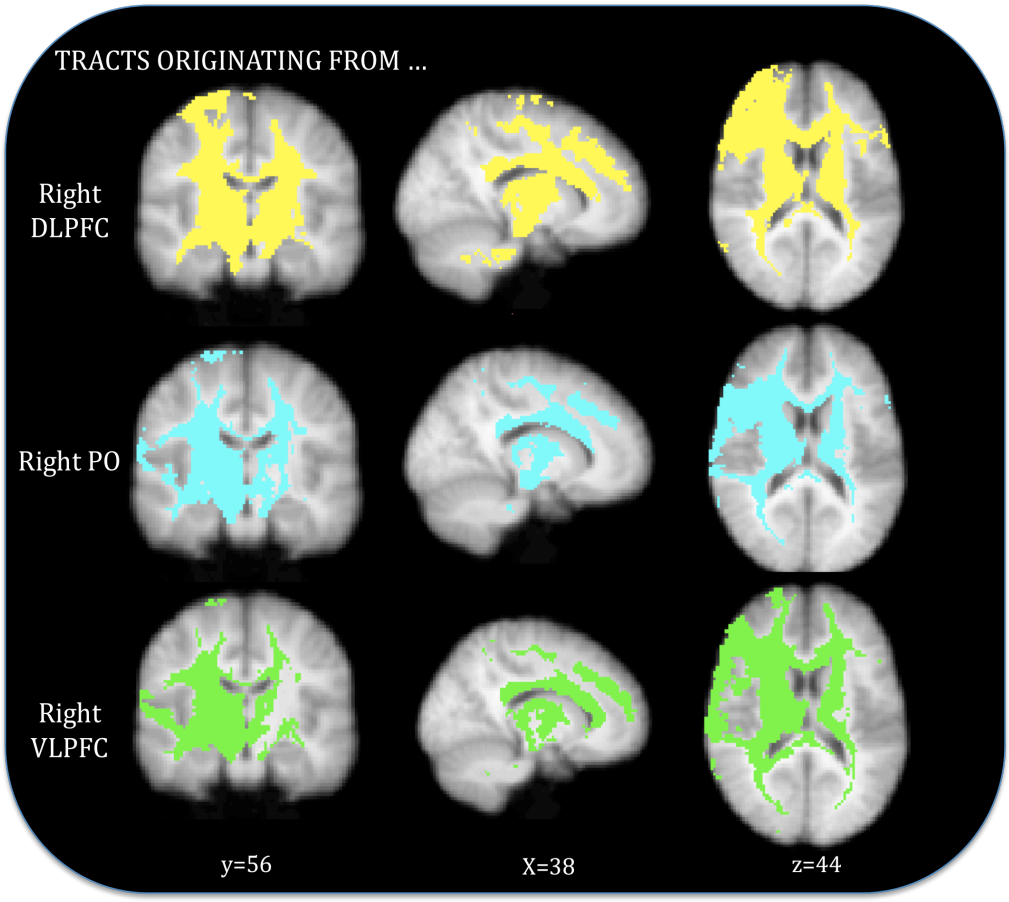
Table 6.1 Post-hoc multiple comparisons with Bonferroni correction for the factor PAIN REGIONS. Significant results are shown in bold. Numbers indicate the mean difference in connectivity index between pain regions in column I and row J

J \ I	amygdala mean diff (I-J)	Sig	antinsula mean diff (I-J)	Sig	dacc mean diff (I-J)	Sig	midinsula mean diff (I-J)	Sig	ofc mean diff (I-J)	Sig	pag mean diff (I-J)	Sig
amygdala			2987849	**0.00**	-26985	0.09	2113794	**0.00**	1528737	0.43	-22881	0.23
antinsula	-2987849	**0.00**			-3014834	**0.00**	-874054	1.00	-1459112	0.10	-3010729	**0.00**
dacc	26985	0.09	3014834	**0.00**			2140780	**0.00**	1555722	0.39	4105	1.00
midinsula	-2113794	**0.00**	874054	1.00	-2140780	**0.00**			-585058	1.00	-2136675	**0.00**
ofc	-1528737	0.43	1459112	0.10	-1555722	0.39	585058	1.00			-1551617	0.41
pag	22881	0.23	3010729	**0.00**	-4105	1.00	2136675	**0.00**	1551617	0.41		
postinsula	-150942	0.76	2836907	**0.00**	-177927	0.27	1962852	**0.00**	1377795	0.88	-173822	0.34
racc	30640	**0.04**	3018489	**0.00**	3654	1.00	2144434	**0.00**	1559377	0.39	7759	1.00
SI	-276956	0.22	2710893	**0.00**	-303941	0.10	1836839	**0.00**	1251781	0.92	-299836	0.10
SII	-219642	0.07	2768207	**0.00**	-246627	**0.03**	1894153	**0.00**	1309095	0.88	-242522	**0.02**
thalamus	-999409	**0.00**	1988440	**0.03**	-1026395	**0.00**	1114385	0.11	529328	1.00	-1022290	**0.00**

J \ I	postinsula mean diff (I-J)	Sig	racc mean diff (I-J)	Sig	SI mean diff (I-J)	Sig	SII mean diff (I-J)	Sig	thalamus mean diff (I-J)	Sig
amygdala	150942	0.76	-30640	**0.04**	276956	0.22	219642	0.07	999409	**0.00**
antinsula	-2836907	**0.00**	-3018489	**0.00**	-2710893	**0.00**	-2768207	**0.00**	-1988440	**0.03**
dacc	177927	0.27	-3654	1.00	303941	0.10	246627	**0.03**	1026395	**0.00**
midinsula	-1962852	**0.00**	-2144434	**0.00**	-1836839	**0.00**	-1894153	**0.00**	-1114385	0.11
ofc	-1377795	0.88	-1559377	0.39	-1251781	0.92	-1309095	0.88	-529328	1.00
pag	173822	0.34	-7759	1.00	299836	0.10	242522	**0.02**	1022290	**0.00**
postinsula			-181582	0.23	126014	1.00	68700	1.00	848467	**0.01**
racc	181582	0.23			307596	0.09	250282	**0.02**	1030049	**0.00**
SI	-126014	1.00	-307596	0.09			-57314	1.00	722453	**0.00**
SII	-68700	1.00	-250282	**0.02**	57314	1.00			779768	**0.00**
thalamus	-848467	**0.01**	-1030049	**0.00**	-722453	**0.00**	-779768	**0.00**		

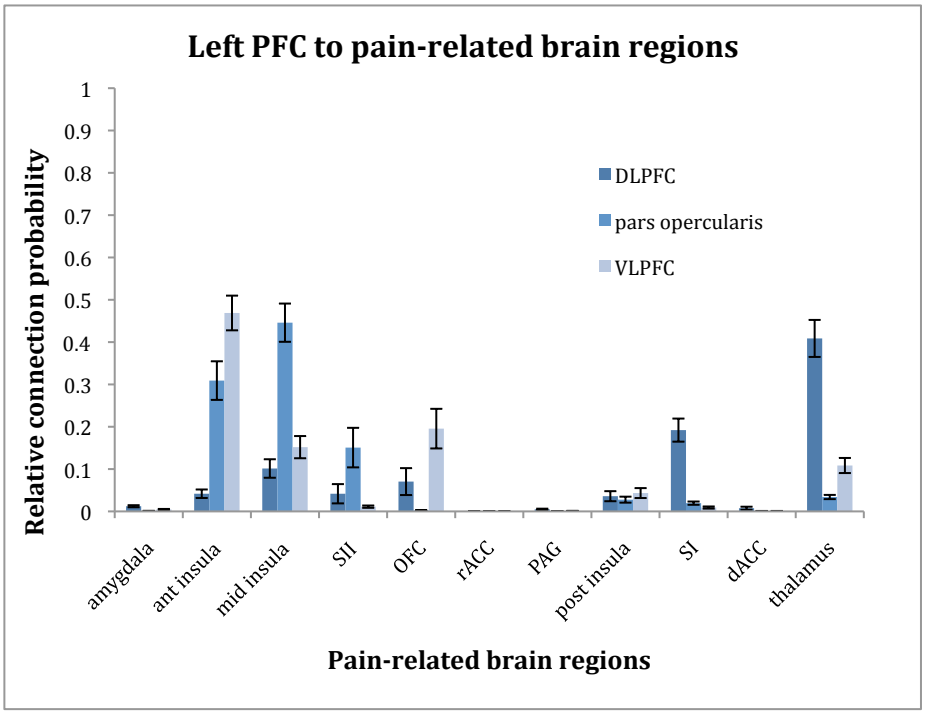


(a)

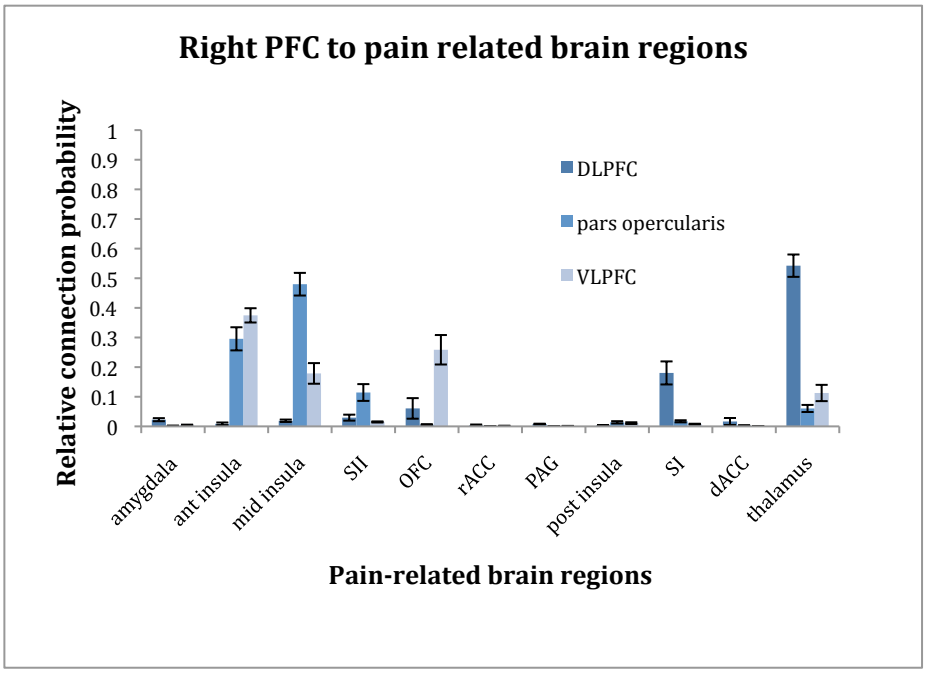


(b)

Figure 6-12 Overlap of tracts originating from (a) left and (b) right lateral prefrontal seed areas shown on the group average structural MRI scan after registration to standard space.



(a)



(b)

Figure 6-13 Relative connection probability between (a) left and (b) right prefrontal areas and pain-related brain regions. Each value is the result of the connection probability of each target, divided by the sum of connection probabilities for all targets.

For each PFC subdivision, repeated measures ANOVA was performed with factors HEMISPHERE (2 levels: right and left) and PAIN REGIONS (11 levels of pain-related brain regions).

i. DLPFC to pain-related regions

Main effects of HEMISPHERE was not significant ($F(1,12)=0.08$, $p=0.79$) but there was significant main effects of PAIN REGIONS ($F(2.09, 25.12)=19.07$, $p<0.0005$). Multiple comparisons with Bonferroni correction for PAIN REGIONS are shown in Table 6.2. The highest connection probability was found for the thalamus, followed by SI. There was no significant interaction between factors HEMISPHERE and BRAIN REGIONS ($F(1.91, 22.96)=0.64$, $p=0.53$).

Table 6.2 Post-hoc multiple comparisons with Bonferroni correction for the factor PAIN REGIONS for DLPFC. Significant results are shown in bold. p significant at <0.05. Numbers indicate the mean difference in connectivity index between pain regions in column I and row J.

J \ I	amygdala mean diff (I-J)	Sig	antinsula mean diff (I-J)	Sig	dacc mean diff (I-J)	Sig	midinsula mean diff (I-J)	Sig	ofc mean diff (I-J)	Sig	pag mean diff (I-J)	Sig
amygdala			17765	1.00	-29716	0.57	84168	0.86	169988	1.00	-22142	1.00
antinsula	-17765	1.00			-47481	0.16	66403	1.00	152222	1.00	-39908	0.12
dacc	29716	0.57	47481	0.16			113884	0.11	199704	1.00	7574	1.00
midinsula	-84168	0.86	-66403	1.00	-113884	0.11			85820	1.00	-106310	0.14
ofc	-169988	1.00	-152222	1.00	-199704	1.00	-85820	1.00				
pag	22142	1.00	39908	0.12	-7574	1.00	106310	0.14	192130	1.00	-192130	1.00
postinsula	-5326	1.00	12439	1.00	-35042	1.00	78842	0.13	164662	1.00	-27468	1.00
racc	35856	0.14	53622	**0.03**	6140	1.00	120024	**0.04**	205844	1.00	13714	0.20
SI	-5809	1.00	11957	1.00	-35525	1.00	78359	0.86	164179	1.00	-27951	1.00
SII	-443242	0.32	-425477	0.36	-472958	0.20	-359074	1.00	-273254	1.00	-465384	0.21
thalamus	-1337792	**0.01**	-1320027	**0.01**	-1367508	**0.01**	-1253624	**0.02**	-1167804	0.06	-1359934	**0.01**

J \ I	postinsula mean diff (I-J)	Sig	racc mean diff (I-J)	Sig	SI mean diff (I-J)	Sig	SII mean diff (I-J)	Sig	thalamus mean diff (I-J)	Sig
amygdala	5326	1.00	-35856	0.14	5809	1.00	443242	0.32	1337792	**0.01**
antinsula	-12439	1.00	-53622	**0.03**	-11957	1.00	425477	0.36	1320027	**0.01**
dacc	35042	1.00	-6140	1.00	35525	1.00	472958	0.20	1367508	**0.01**
midinsula	-78842	0.13	-120024	**0.04**	-78359	0.86	359074	1.00	1253624	**0.02**
ofc	-164662	1.00	-205844	1.00	-164179	1.00	273254	1.00	1167804	0.06
pag	27468	1.00	-13714	0.20	27951	1.00	465384	0.21	1359934	**0.01**
postinsula			-41182	0.51	483	1.00	437916	0.38	1332466	**0.01**
racc	41182.42299	0.51			41665	0.41	479098	0.18	1373648	**0.01**
SI	-483	1.00	-41665	0.41			437433	0.22	1331983	**0.01**
SII	-437916	0.38	-479098	0.18	-437433	0.22			894550	0.34
thalamus	-1332466	**0.01**	-1373648	**0.01**	-1331983	**0.01**	-894550	0.34		

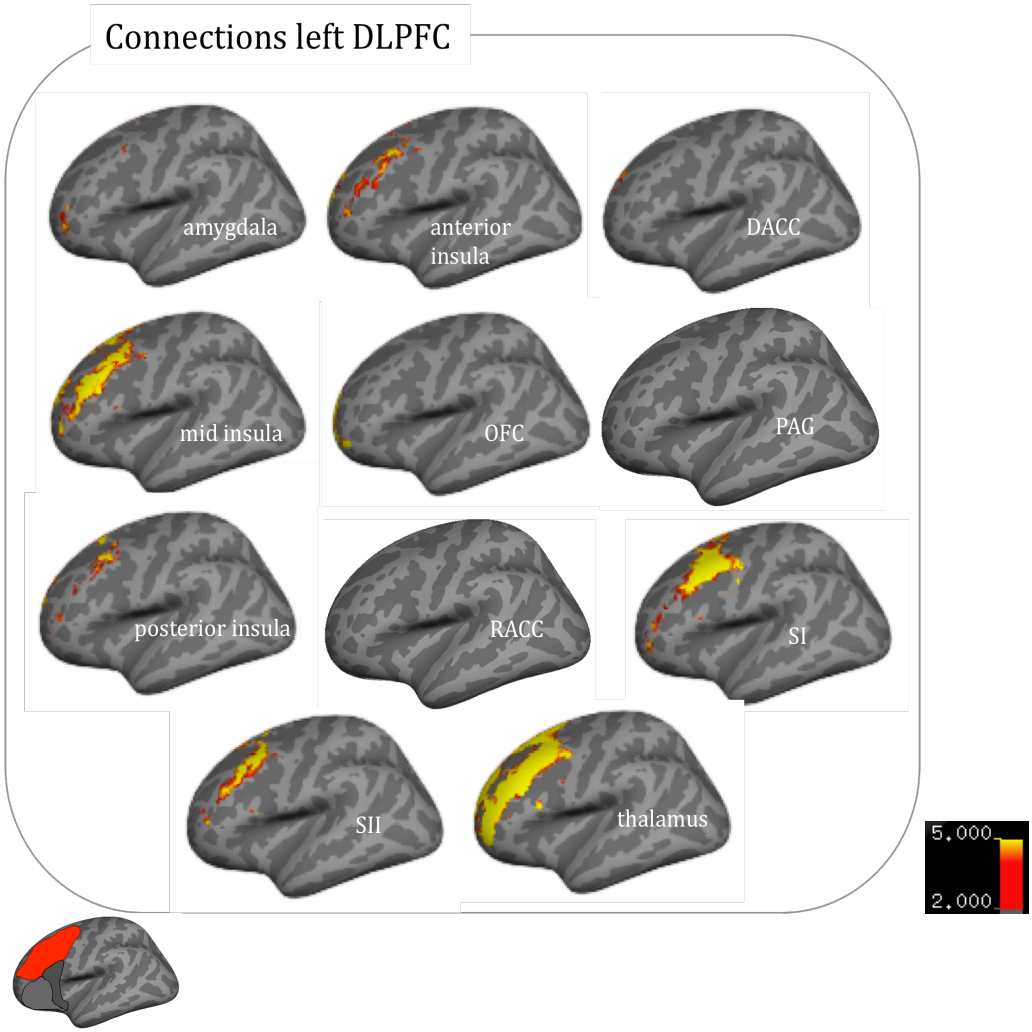


Figure 6-14 Spatial location of connection probability of left DLPFC to pain-related targets, summed and normalized across subjects. Images shown on inflated brain surfaces using Freesurfer software package (Dale et al 1999; Fischl et al 1999). Colour bar indicates minimum number of samples with connection probability with the pain-related target. Inset: left DLPFC mask.

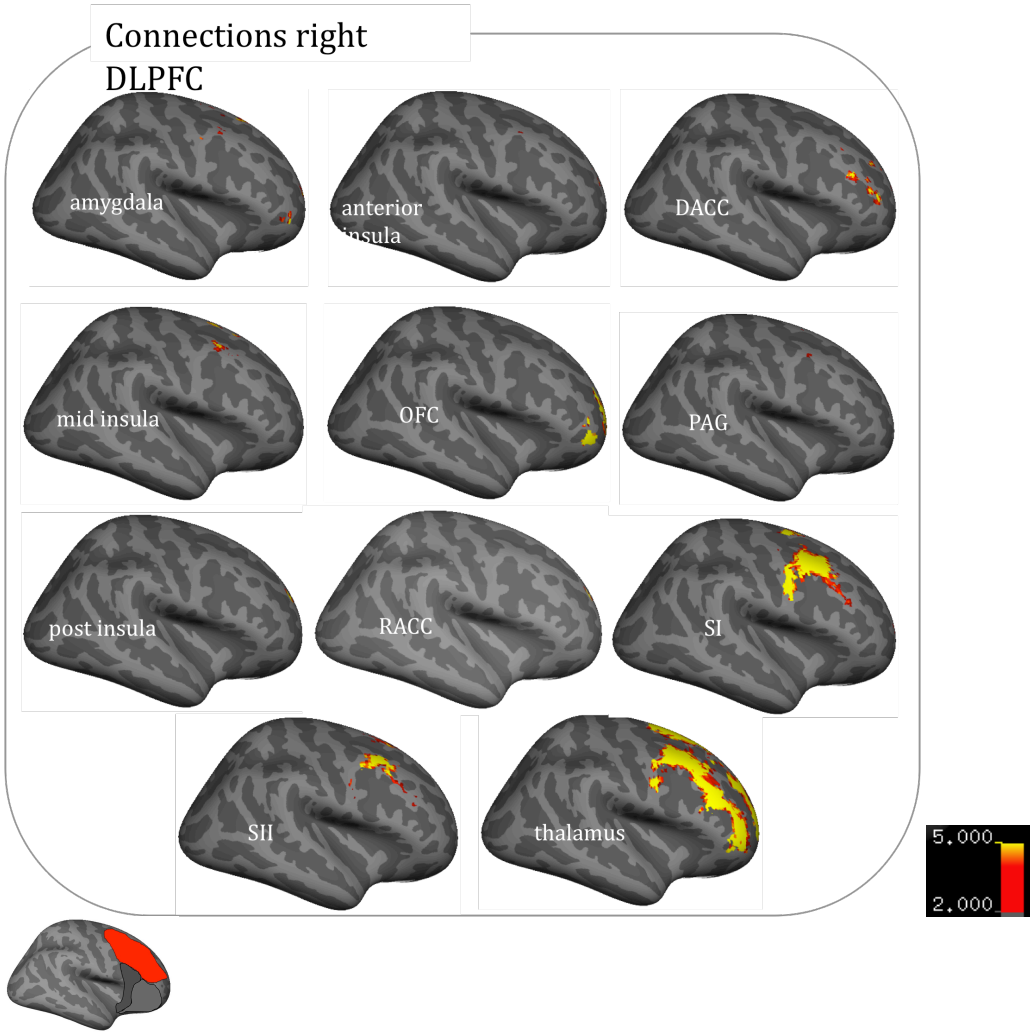


Figure 6-15 Spatial location of connection probability of right DLPFC to pain-related targets, summed and normalized across subjects. Images shown on inflated brain surfaces using Freesurfer software package (Dale et al 1999; Fischl et al 1999). Colour bar indicates minimum number of samples with connection probability with the pain-related target. Inset: right DLPFC mask.

ii. Pars opercularis to pain-related regions

No significant main effects of HEMISPHERE was found ($F(1,14)=0.51$, $p=0.49$) but there was main effects of PAIN REGIONS ($F(1.51, 21.12)=25.94$, $p<0.0005$). Post hoc tests are shown in Table 6.3. Both left and right PO showed the highest connection probability with the mid insula, followed by the anterior insula. There was no significant interaction between HEMISPHERE and BRAIN REGIONS ($F(2.14, 29.93)=0.17$, $p=0.86$).

Table 6.3 Post-hoc multiple comparisons with Bonferroni correction for the factor PAIN REGIONS for PO. Significant results are shown in bold. p significant at <0.05. Numbers indicate the mean difference in connectivity index between pain regions in column I and row J.

J \ I	amygdala mean diff (I-J)	Sig	antinsula mean diff (I-J)	Sig	dacc mean diff (I-J)	Sig	midinsula mean diff (I-J)	Sig	ofc mean diff (I-J)	Sig	pag mean diff (I-J)	Sig
amygdala			2752271	**0.00**	-6873	1.00	4701492	**0.00**	33149	1.00	-8539	0.73
antinsula	-2752271	**0.00**			-2759144	**0.00**	1949221	0.87	-2719122	**0.00**	-2760810	**0.00**
dacc	6873	1.00	2759144	**0.00**			4708365	**0.00**	40021	1.00	-1667	1.00
midinsula	-4701492	**0.00**	-1949221	0.87	-4708365	**0.00**			-4668343	**0.00**	-4710031	**0.00**
ofc	-33149	1.00	2719122	**0.00**	-40021	1.00	4668343	**0.00**			-41688.00	1.00
pag	8539	0.73	2760810	**0.00**	1667	1.00	4710031	**0.00**	41688	1.00		
postinsula	-179611	0.13	2572660	**0.00**	-186484	0.10	4521881	**0.00**	-146462	0.35	-188150	0.10
racc	13439	**0.04**	2765710	**0.00**	6567	1.00	4714931	**0.00**	46588	1.00	4900	0.02
SI	-1582785	1.00	1169486	1.00	-1589657	1.00	3118707	**0.00**	-1549636	1.00	-1591324	1.00
SII	-137350	**0.00**	2614921	**0.00**	-144223	**0.00**	4564142	**0.00**	-104201	**0.00**	-145889	**0.00**
thalamus	-341789	**0.00**	2410482	**0.00**	-348661	**0.00**	4359703	**0.00**	-308640	**0.00**	-350328	**0.00**

J \ I	postinsula mean diff (I-J)	Sig	racc mean diff (I-J)	Sig	SI mean diff (I-J)	Sig	SII mean diff (I-J)	Sig	thalamus mean diff (I-J)	Sig
amygdala	179611	0.13	-13439	0.04	1582785	1.00	137350	**0.00**	341789	**0.00**
antinsula	-2572660	**0.00**	-2765710	**0.00**	-1169486	1.00	-2614921	**0.00**	-2410482	**0.00**
dacc	186484	0.10	-6567	1.00	1589657	1.00	144223	**0.00**	348661	**0.00**
midinsula	-4521881	**0.00**	-4714931	**0.00**	-3118707	**0.00**	-4564142	**0.00**	-4359703	**0.00**
ofc	146462	0.35	-46588	1.00	1549636	1.00	104201	**0.00**	308640	**0.00**
pag	188150	0.10	-4900	**0.02**	1591324	1.00	145889	**0.00**	350328	**0.00**
postinsula			-193050	0.08	1403174	1.00	-42261	1.00	162178	1.00
racc	193050.2332	0.08			1596224	1.00	150789	**0.00**	355228	**0.00**
SI	-1403174	1.00	-1596224	1.00			-1445435	1.00	-1240996	1.00
SII	42261	1.00	-150789	**0.00**	1445435	1.00			204439	**0.01**
thalamus	-162178	1.00	-355228	**0.00**	1240996	1.00	-204439	**0.01**		

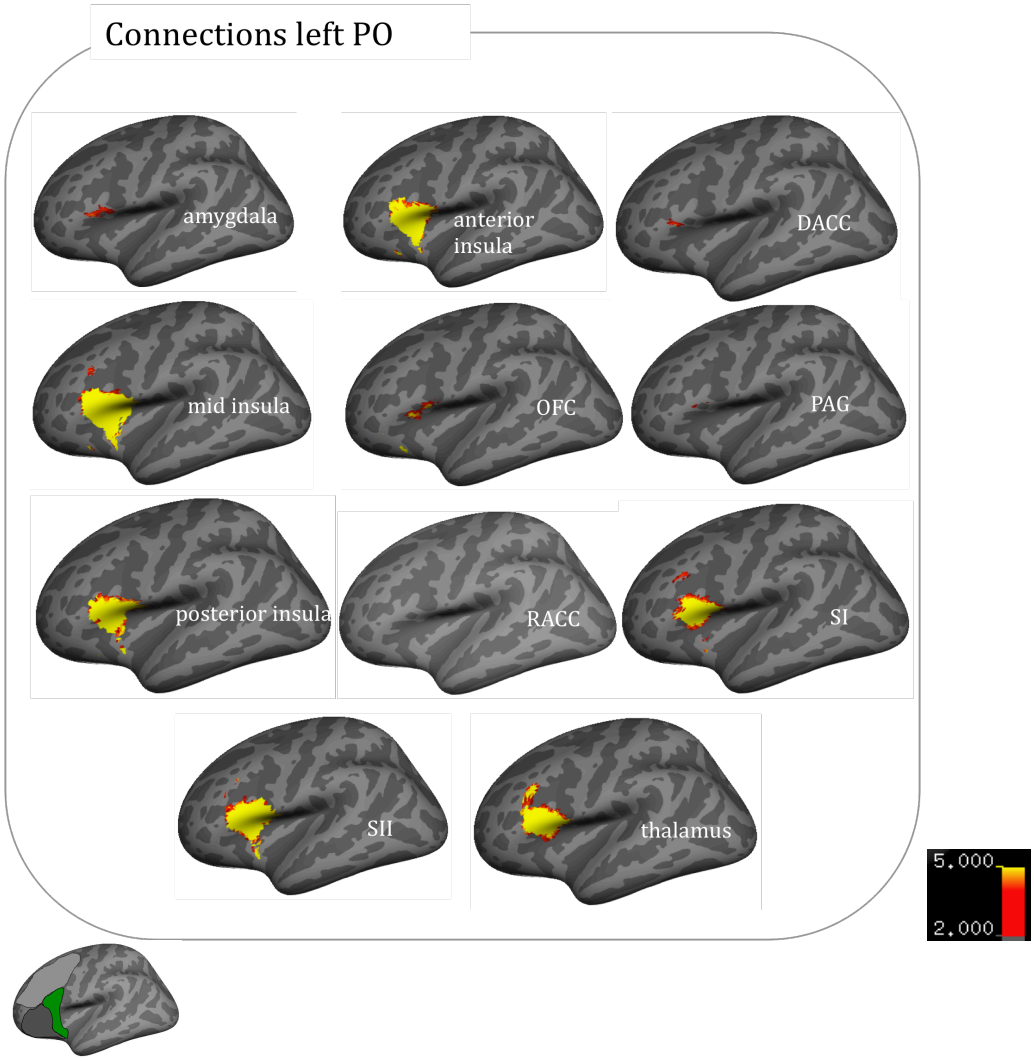


Figure 6-16 Spatial location of connection probability of the left PO to pain-related targets, summed and normalized across subjects. Images shown on inflated brain surfaces using Freesurfer software package (Dale et al 1999; Fischl et al 1999). Colour bar indicates minimum number of samples with connection probability with the pain-related target. Inset: left PO mask.

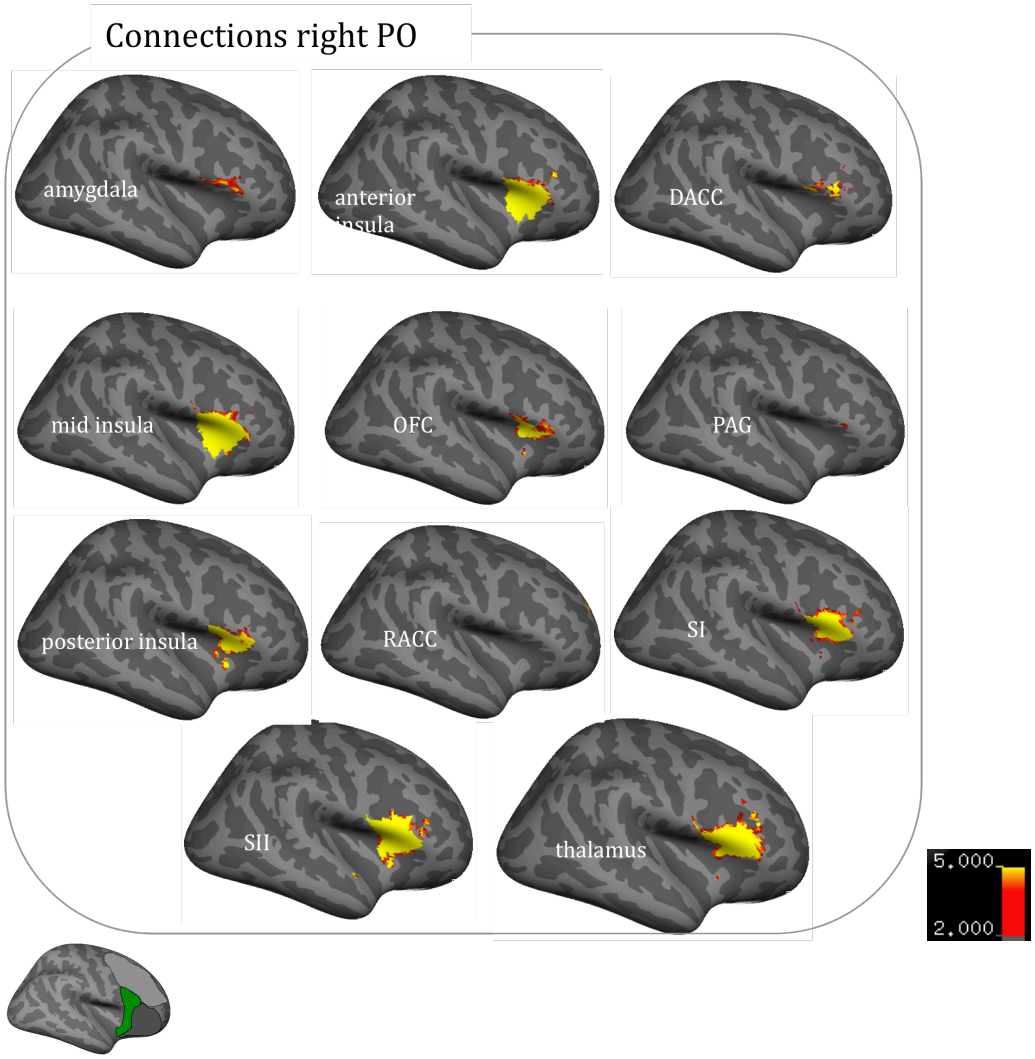


Figure 6-17 Location of connection probability of the right PO to pain-related targets, summed and normalized across subjects. Images shown on inflated brain surfaces using Freesurfer software package (Dale et al 1999; Fischl et al 1999). Colour bar indicates minimum number of samples with connection probability with the pain-related target. Inset: right PO mask.

iii. VLPFC to pain related regions

Similar to the other 2 subdivisions, there was no main effects of HEMISPHERE ($F(1,14)=0.84$, $p=0.37$) but main effects of PAIN REGIONS reached significance ($F(1.44, 20.2)=17.78$, $p<0.0005$). Post hoc tests are shown in Table 6.4. Both left and right VLPFC was most strongly connected to the anterior insula and the OFC (Figure 6.13). Interaction between HEMISPHERE and PAIN REGIONS was not significant ($F(2.14, 29.93)=0.17$, $p=0.86$).

Table 6.4 Post-hoc multiple comparisons with Bonferroni correction for the factor PAIN REGIONS for VLPFC. Significant results are shown in bold. p significant at <0.05. Numbers indicate the mean difference in connectivity index between pain regions in column I and row J.

J \ I	amygdala mean diff (I-J)	Sig	antinsula mean diff (I-J)	Sig	dacc mean diff (I-J)	Sig	midinsula mean diff (I-J)	Sig	ofc mean diff (I-J)	Sig	pag mean diff (I-J)	
amygdala			6952269	**0.00**	-53308	**0.03**	3065559	**0.03**	5739388	0.25	-49760	0.07
antinsula	-6952269	**0.00**			-7005577	**0.00**	-3886710	**0.01**	-1212881	1.00	-7002030	**0.00**
dacc	53308	**0.03**	7005577	**0.00**			3118867	**0.03**	5792696	0.24	3547	1.00
midinsula	-3065559	**0.03**	3886710	**0.01**	-3118867	**0.03031931**			-2673829	1.00	-3115319	**0.03**
ofc	5739388	0.25	1212881	1.00	5792696	0.24	-2673829	1			5789148	0.24
pag	49760	0.07	7002030	**0.00**	-3547	1.00	3115319	**0.03**	5789148	0.24		
postinsula	-345216	0.52	6607053	**0.00**	-398524	0.26	2720343	0.05	5394172	0.32	-394977	0.30
racc	55401	0.09	7007670	**0.00**	2093	1.00	3120959	**0.03**	5794788	0.24	5640	1.00
SI	-201166	0.50	6751103	**0.00**	-254474	0.12	2864393	**0.03**	5538222	0.28	-250927	**0.10**
SII	-95917	0.54	6856352	**0.00**	-149225	**0.03**	2969642	**0.04**	5643471	0.28	-145677	**0.02**
thalamus	-1509636	**0.00**	5442633	**0.00**	-1562944	**0.00**	1555922	1.00	4229751	0.96	-1559397	**0.00**

J \ I	postinsula mean diff (I-J)	Sig	racc mean diff (I-J)	Sig	SI mean diff (I-J)	Sig	SII mean diff (I-J)	Sig	thalamus mean diff (I-J)	Sig
amygdala	345216	0.52	-55401	0.09	201166	0.50	95917	0.54	1509636	**0.00**
antinsula	-6607053	**0.00**	-7007670	**0.00**	-6751103	**0.00**	-6856352	**0.00**	-5442633	**0.00**
dacc	398524	0.26	-2093	1.00	254474	0.12	149225	**0.03**	1562944	**0.00**
midinsula	-2720343	0.05	-3120959	**0.03**	-2864393	**0.03**	-2969642	**0.04**	-1555922	1.00
ofc	-5394172	0.32	-5794788	0.24	-5538222	0.28	-5643471	0.28	-4229751	0.96
pag	394977	0.30	-5640	1.00	250927	0.10	145677	**0.02**	1559397	**0.00**
postinsula			-400617	0.28	-144050	1.00	-249299	1.00	1164420	**0.01**
racc	400617	0.276087638			256567	0.12	151317	**0.04**	1565037	**0.00**
SI	144050	1.00	-256567	0.11850216			-105250	1.00	1308470	**0.00**
SII	249299	1.00	-151317	**0.04**	105250	1			1413720	**0.00**
thalamus	-1164420	**0.01**	-1565037	**0.00**	-1308470	**0.00**	-1413720	**0.00**		

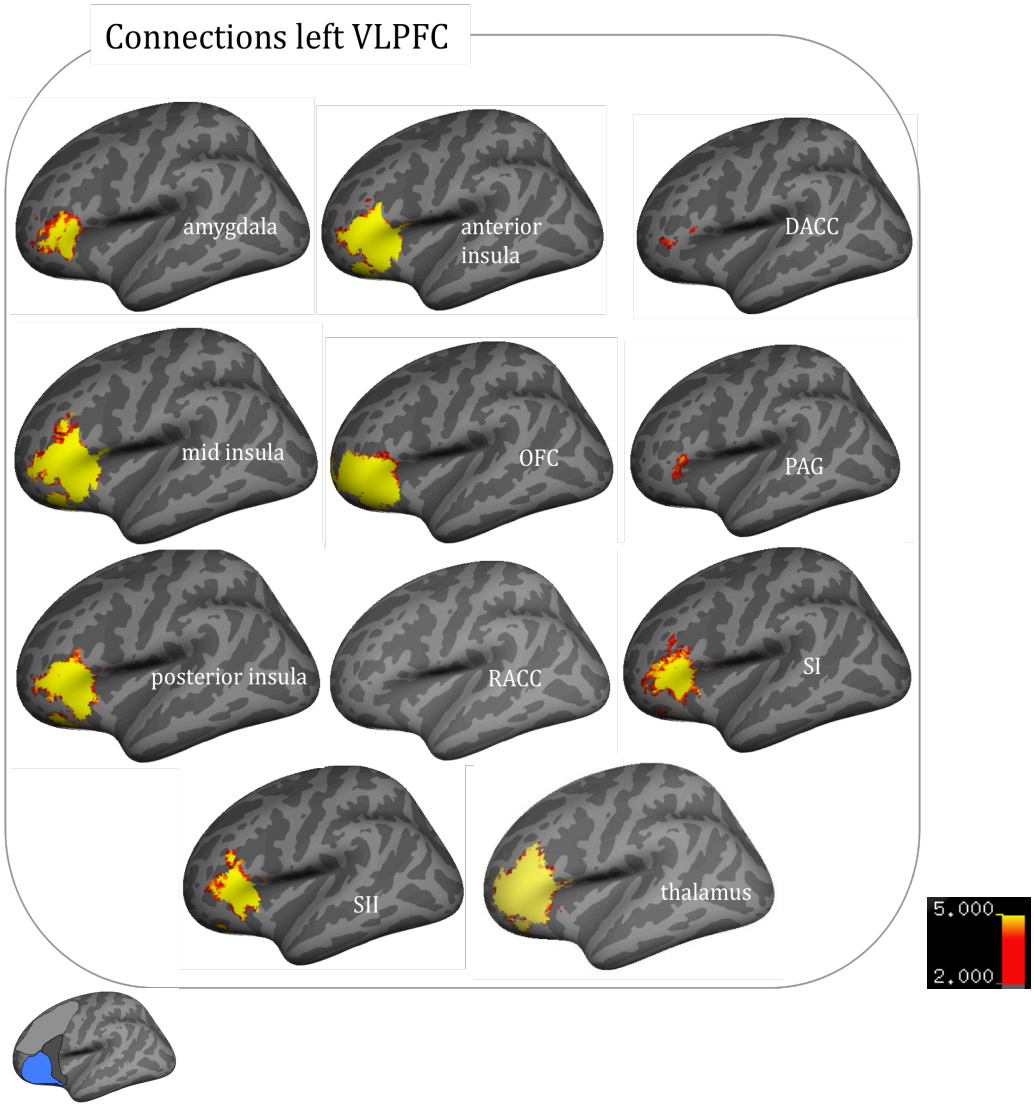


Figure 6-18 Location of connection probability of the left VLPFC to pain-related targets, summed and normalized across subjects. Images shown on inflated brain surfaces using Freesurfer software package (Dale et al 1999; Fischl et al 1999). Colour bar indicates minimum number of samples with connection probability with the pain-related target. Inset: left VLPFC mask

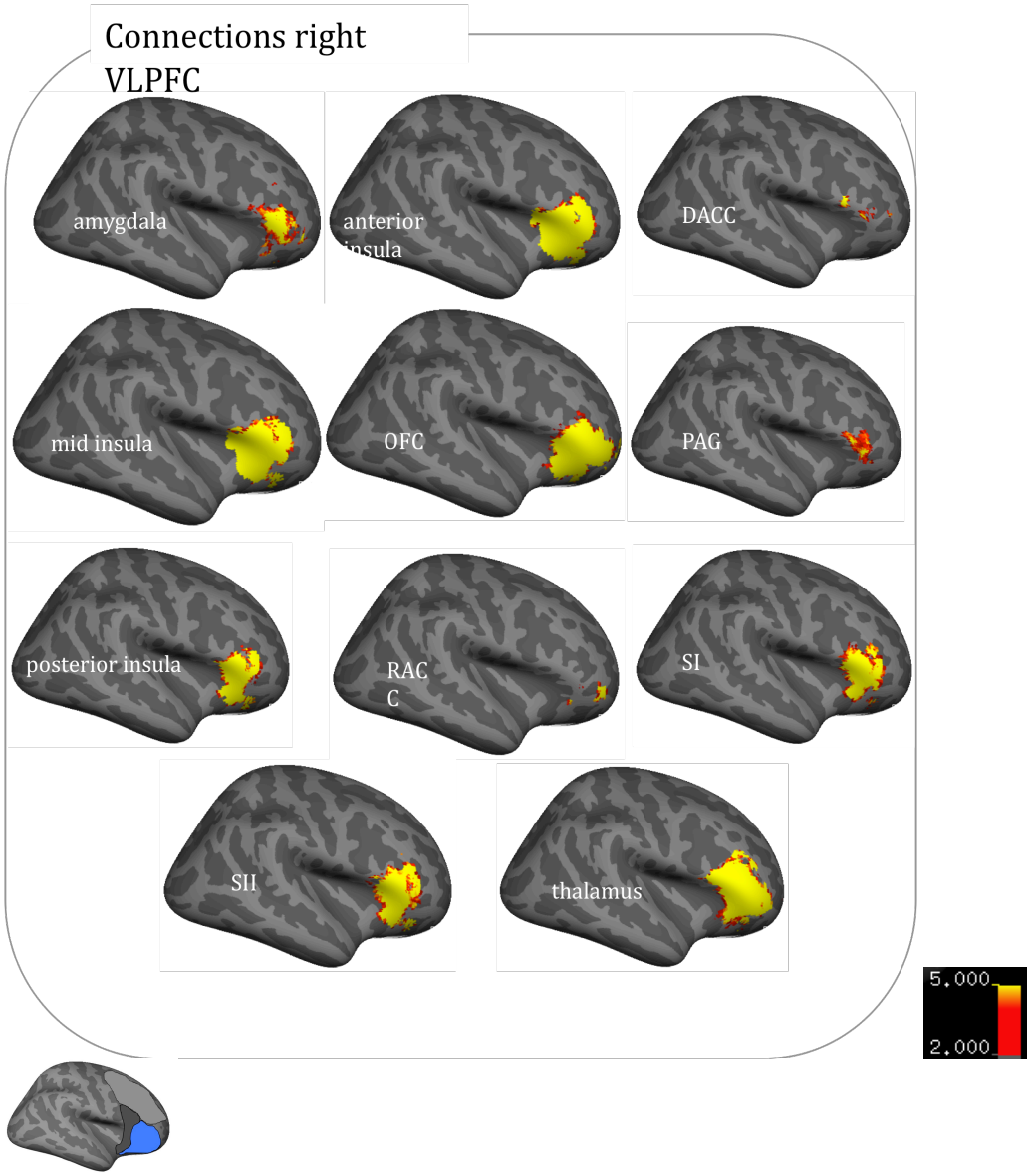


Figure 6-19 Location of connection probability of the right VLPFC to pain-related targets, summed and normalized across subjects. Images shown on inflated brain surfaces using Freesurfer software package (Dale et al 1999; Fischl et al 1999). Colour bar indicates minimum number of samples with connection probability with the pain-related target. Inset: right VLPFC mask.

6.3.2d Pain-related brain regions to PFC

To determine differences in connection probability between each pain-related brain region to PFC subdivisions, we performed repeated measures ANOVA with the factors HEMISPHERE (2 levels: left and right) and PFC SUBDIVISION (3 levels: DLPFC, PO and VLPFC) for each pain-related region (Table 6.5). Main effects of HEMISPHERE was significant only for anterior insula whereby the left anterior insula had higher

connection probability compared to the right. Post-hoc paired t-tests corrected for multiple comparisons were performed for the factor PFC SUBDIVISIONS (Table 6.6).

Interaction between HEMISPHERE and PFC SUBDIVISIONS was significant for SII, posterior insula and thalamus. Post hoc multiple comparisons with Bonferroni correction showed that for SII, connection probability to DLPFC was lower than PO ($p<0.0005$) and VLPFC ($p=0.001$), and not significantly different between PO and VLPFC. Connection to PO was higher on the left side compared to the right while connection to VLPFC was vice versa, higher on the right side compared to the left.

In a similar pattern to SII, posterior insula also had lower connection probability to DLPFC compared to PO ($p<0.0005$) and VLPFC ($p<0.0005$), and not significantly different between PO and VLPFC. Connection to PO was also higher on the left side and VLPFC on the right. Figure 6.20 shows the relative connection probability of pain-related brain regions to PFC subdivisions.

For thalamus, connection probability was lower to PO compared to DLPFC ($p<0.0005$) and VLPFC ($p=0.001$). Connection to PO was significantly lower compared to VLPFC. Connection to PO was higher on the right side while connection to VLPFC was higher on the left side.

Table 6.5 Results of repeated measures ANOVA for connection probability between pain-related regions and PFC subdivisions. Significant results are shown in bold.

Pain region	Main effect/interaction	df	F	p
Amygdala	HEMISPHERE	1, 11	0.97	0.35
	PFC SUBDIVISION	2, 22	4.99	**0.016**
	HEMISPHERE * PFC SUBDIVISION	1.33, 22	1.2	0.31
Ant insula	HEMISPHERE	1, 12	7.42	**0.018**
	PFC SUBDIVISION	1.15, 24	44.16	**0.000**
	HEMISPHERE * PFC SUBDIVISION	1.38, 24	3.61	0.064
Mid insula	HEMISPHERE	1, 12	0.23	0.64
	PFC SUBDIVISION	1.2, 24	36.91	**0.000**
	HEMISPHERE * PFC SUBDIVISION	2, 24	0.51	0.61
SII	HEMISPHERE	1, 10	0.1	0.76
	PFC SUBDIVISION	2, 20	19.62	**0.000**
	HEMISPHERE * PFC SUBDIVISION	2, 20	9.23	**0.001**
OFC	HEMISPHERE	1, 10	0.05	0.82
	PFC SUBDIVISION	1, 20	7.18	**0.023**
	HEMISPHERE * PFC SUBDIVISION	1, 20	0.12	0.74
rACC	HEMISPHERE	1, 10	0.02	0.883
	PFC SUBDIVISION	2, 20	10.15	**0.001**
	HEMISPHERE * PFC SUBDIVISION	2, 20	0.09	0.913
PAG	HEMISPHERE	1, 9	2.51	0.15
	PFC SUBDIVISION	2, 18	13.02	**0.000**
	HEMISPHERE * PFC SUBDIVISION	2, 18	3.43	0.055
Post insula	HEMISPHERE	1, 11	3.67	0.08
	PFC SUBDIVISION	1.29, 22	20.86	**0.000**
	HEMISPHERE * PFC SUBDIVISION	2, 22	4.6	**0.036**
SI	HEMISPHERE	1, 12	2.19	0.164
	PFC SUBDIVISION	1.23, 24	19.5	**0.000**
	HEMISPHERE * PFC SUBDIVISION	1.11, 24	4.24	0.056
dACC	HEMISPHERE	1, 11	3.03	0.109
	PFC SUBDIVISION	1.19, 22	5.85	**0.026**
	HEMISPHERE * PFC SUBDIVISION	1.26, 22	1.36	0.273
Thalamus	HEMISPHERE	1, 12	0.27	0.61
	PFC SUBDIVISION	2, 24	13.36	**0.000**
	HEMISPHERE * PFC SUBDIVISION	2, 24	4.49	**0.022**

Table 6.6 Post hoc paired t-tests

	Left						Right					
	DLPFC vs PO		DLPFC vs VLPFC		PO vs VLPFC		DLPFC vs PO		DLPFC vs VLPFC		PO vs VLPFC	
	t	p	t	p	t	p	t	p	t	p	t	p
Amygdala	3.58	0.003	-1.49	0.16	-2.18	0.049	3.41	0.005	-0.02	0.98	-3.74	0.003
Ant insula	-6.36	<0.0005	-8.28	<0.0005	-6.28	<0.0005	-4.77	<0.0005	-7.03	<0.0005	-3.43	0.005
Mid insula	-5.83	<0.0005	-3.84	0.002	5.4	<0.0005	-6.45	<0.0005	-3.77	0.002	3.99	0.002
SII	-4.44	0.001	-4.2	0.001	3.5	0.005	-5.26	<0.0005	-5.48	<0.0005	-2.01	0.069
OFC	1.91	0.08	-3.05	0.01	-3.51	0.004	0.52	0.61	-3.04	0.011	-3.07	0.01
rACC	2.91	0.012	1.77	0.102	-2.71	0.019	3.25	0.008	1.51	0.16	-2.81	0.017
PAG	2.42	0.033	0.1	0.92	-2.96	0.012	4.28	0.001	0.51	0.62	-4.27	0.002
Post insula	-5.52	<0.0005	-6.2	<0.0005	2.03	0.064	-4.85	<0.0005	-5.59	<0.0005	-1.74	0.108
SI	3.13	0.008	3.53	0.004	2.99	0.01	3.73	0.003	1.52	0.15	-2.83	0.014
dACC	3.15	0.008	1.72	0.11	-2.84	0.014	2.68	0.02	0.69	0.503	-3.19	0.007
Thalamus	5.03	<0.0005	-1.61	0.134	-3.82	0.002	4.04	0.001	1.7	0.114	-6.59	<0.0005

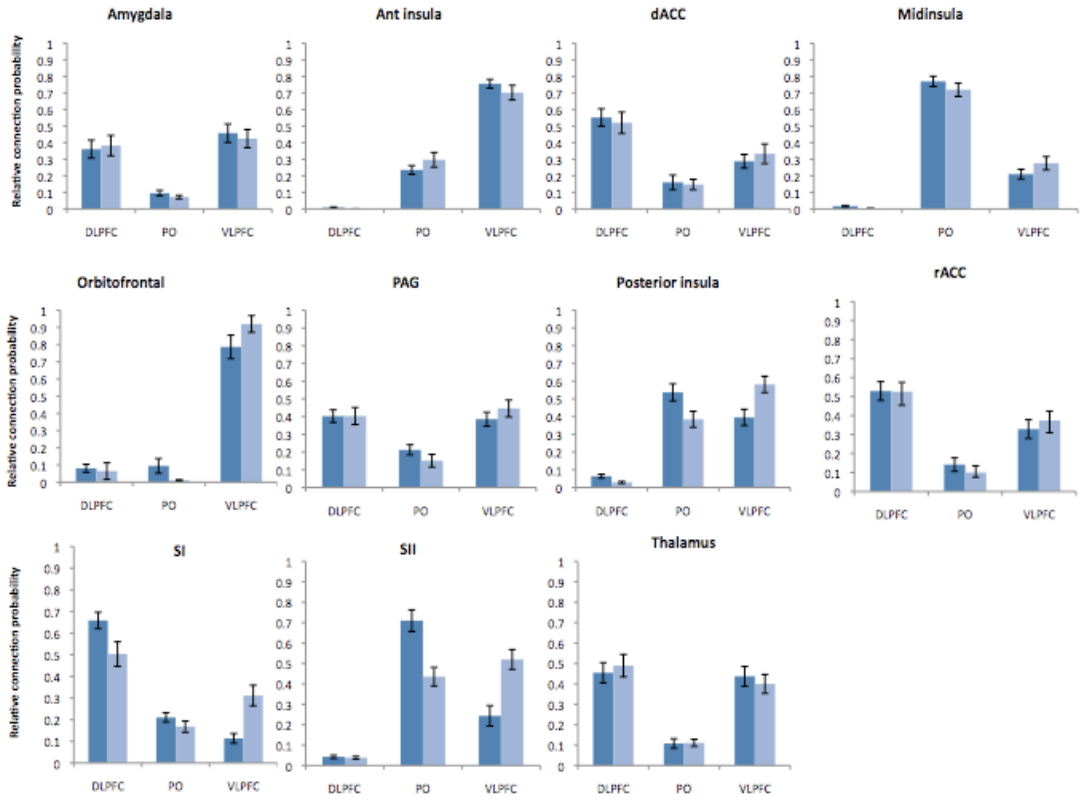


Figure 6-20 Relative connection probability between pain-related brain regions as seeds to prefrontal areas as targets (dark blue: left, light blue: right). Each value is the result of the connection probability of each target, divided by the sum of connection probabilities for all targets (in %). Data are expressed as mean ± SEM.

Amygdala

The amygdala was differentially connected to PFC subdivisions but not between left and right hemispheres. Connection probability was significantly higher to the DLPFC and VLPFC compared to PO.

Anterior insula

For both hemispheres, connection probability was highest to the VLPFC, followed by PO and DLPFC. Differential connection probability was found between hemispheres; connection probabilities were higher on the left compared to the right side for DLPFC ($t(13)=4.19$, $p=0.001$) and VLPFC ($t(13)=3.68$, $p=0.003$).

Mid insula

The mid insula demonstrated the highest connection probability with PO followed by VLPFC and DLPFC. However, no significant difference was found between hemispheres.

Posterior insula

The posterior insula did not exhibit differential connection probability between the left and right hemisphere. Stronger connection probability was found with the PO and VLPFC compared to the DLPFC.

Orbitofrontal cortex (OFC)

The OFC was preferentially connected to the VLPFC with minimal connection probability with the other two prefrontal areas on both the left and the right hemisphere.

Periaqueductal gray (PAG)

The PAG had almost similar connection probability with DLPFC and VLPFC, with significantly lower connection probability with the PO, on both the left and right hemisphere.

Primary somatosensory cortex (SI)

For left SI, the highest connection probability was with DLPFC followed by PO and lowest to VLPFC. For the right SI, both DLPFC and VLPFC had significantly higher connection probability compared to PO.

Secondary somatosensory cortex (SII)

In contrast with SI, connection probability was lowest with DLPFC for both hemispheres. Left SII has highest connection probability with PO while right SII showed no significant difference between PO and VLPFC.

Dorsal anterior cingulate cortex (dACC)

For both hemispheres, the connection probability with the PO was significantly lower than with DLPFC and VLPFC, with no significant difference between DLPFC and VLPFC.

Rostral anterior cingulate cortex (rACC)

The rACC showed the lowest connection probability with the PO for both the left and right hemisphere.

Thalamus

Both the right and left thalamus had shown an almost identical connection pattern with the highest connection probability similarly shared between DLPFC and VLPFC. The connection probability to PO is significantly smaller.

Peak voxels for thalamus' connection probability to PFC subdivisions

The coordinates of the center of gravity of each subject's thalamic mask were extracted and weighted by the connectivity to the prefrontal region. These coordinates were plotted on a standard space brain image to show the location of peak voxels for all subjects.

Figure 6.21 shows the thalamic regions that exhibited the highest connection probability with each prefrontal subdivision overlaid on the thalamic connectivity map (Behrens et al 2003b). The projections of all three PFC subdivisions fell within the cluster previously parcellated and shown to have the highest thalamic-prefrontal connection by Behrens et al (2003b). This cluster corresponds to the mediodorsal, ventral anterior and parts of the anterior complex thalamic nucleus. Figure 6.22 illustrates the centre of gravity of voxels in the thalamus that have positive connection probability to the three subdivisions of the prefrontal cortices weighted by the strength of probabilities in all subjects.

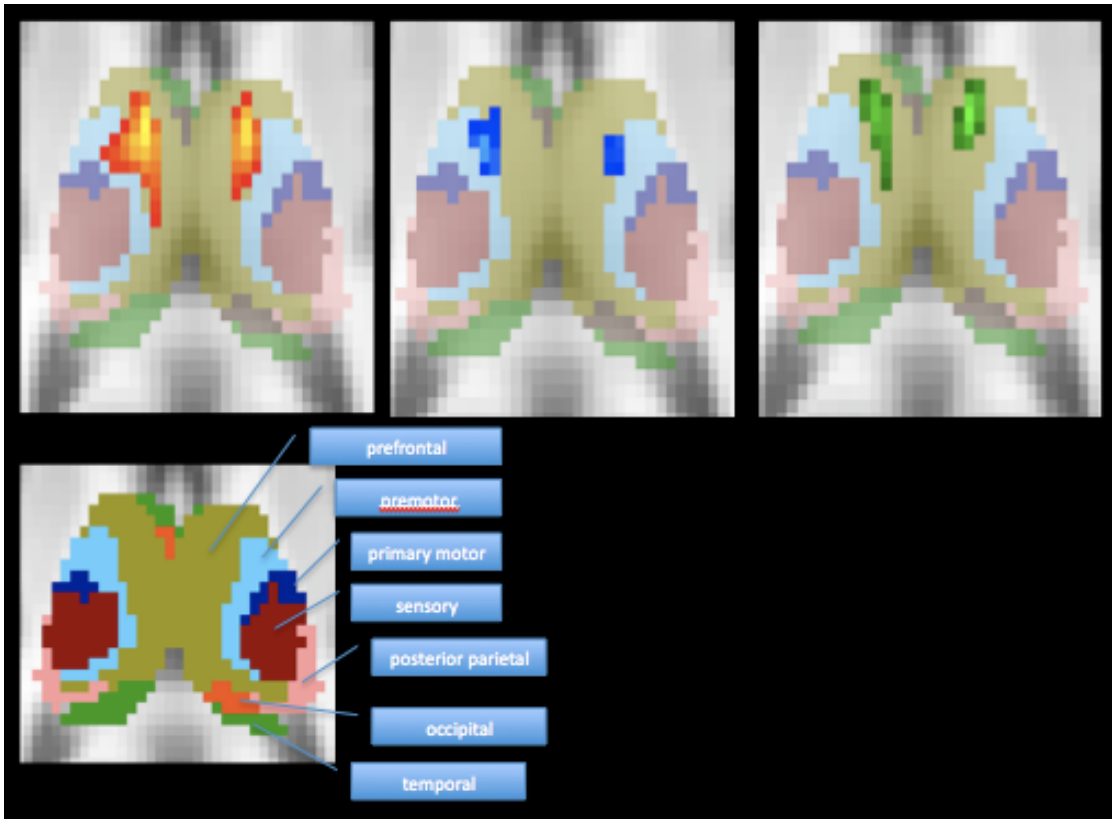


Figure 6-21 Top from left: Highest connectivity of thalamus to DLPFC (red), PO (blue), and VLPFC (green) using the dataset from the current study and overlaid on thalamic connectivity map from the dataset used by Behrens et al (2003b, below).

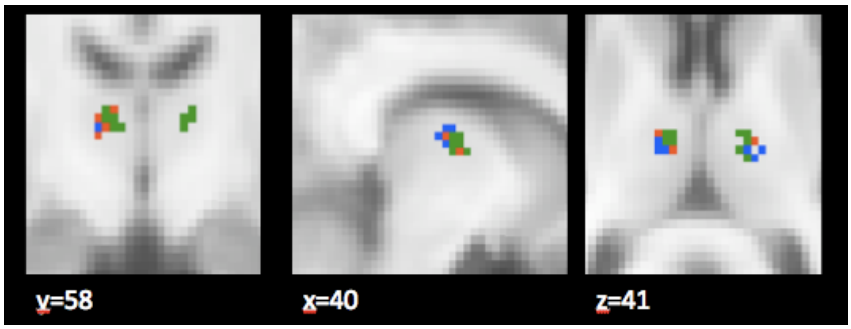


Figure 6-22 Centre of gravity of voxels with positive connection probability to PFC weighted by strength of probabilities in all subjects. Red (DLPFC), blue (PO), green (VLPFC). For each subject, the centre of gravity for the ROI consisting of the 'seed_to_target' output (voxels with positive connection probability to a certain target) was calculated and weighted by the connection probability.

6.4 Discussion

The aims of this study were to firstly, ascertain whether the different subdivisions of the lateral PFC are differentially connected to pain-related brain regions, and secondly, to determine the intrinsic connectivity within the PFC subdivisions. Note

that usage of the term ‘connection’ strictly means the probability of finding a tract connecting the seed to target region(s).

Our hypothesis was that the DLPFC is more preferentially connected to pain-related brain regions that serve a more sensory-discriminative function while PO and VLPFC are more connected to regions more involved in cognitive and affective modulation of pain.

Our data revealed significantly different connection probability patterns between the three prefrontal subdivisions and pain-related brain regions (Table 6.6). As hypothesized, the DLPFC is more connected to sensory-discriminative regions (i.e. thalamus and SI) while the VLPFC and PO (which show a very similar pattern) have strong connections with both sensory-discriminative (thalamus and SI) and cognitive-affective regions (i.e. anterior insula, midinsula, SII and OFC). In the following, our results are compared with findings from primate tracer studies as well as previous human and primates diffusion imaging studies.

6.4.1 Parcellation

To our knowledge, this is the first study that has performed parcellation of the lateral PFC according to its highest connection probability to pain-related brain regions. Parcellation of the PFC revealed large variations in the connectivity pattern between subjects. A few generalizations, however, can be made. Firstly, out of the 11 pain-related target regions, only six targets consistently appeared in the map that shows the highest connection probabilities across subjects. These are SI, thalamus,

anterior insula, mid insula, SII and OFC. This finding suggests strong structural and functional connectivity between the PFC and these pain-related regions.

Secondly, the upper part of the PFC, corresponding to the DLPFC, is mainly connected to SI and thalamus. SI and thalamus are two pain-related regions most associated with somatosensation (Treede et al 1999). However, strongest connection to thalamus can also be seen extending to the lower part of the PFC, i.e. the PO and VLPFC.

Thirdly, the lower portion of PFC, corresponding to PO and VLPFC, displayed high connection probability to thalamus, SII, OFC and insula, especially the anterior and midinsula. It has been previously shown by functional studies that the anterior and mid insula are often associated with affect as well as interoception (Craig 2009) as opposed to posterior insula that is more associated with nociceptive processing (Peltz et al 2011). While posterior insula did not appear in our parcellation map of PFC, the spatial location of highest connection probability with insula was shown to be in the region of PO and VLPFC as shown in Figures 6.9 and 6.10.

The spatial location of highest connection probability to individual pain-related region showed for most pain-related brain regions, highest connection probability was to PO and VLPFC. Only SI and left amygdala also show high connection probability to DLPFC as well.

6.4.2 Probabilistic tractography

6.4.2.1 Intrinsic prefrontal connection probability

As shown in the meta-analysis in Chapters 4 and 5, functional studies have found evidence for a co-activation of prefrontal subdivisions during the experience of pain (Baliki et al 2008, Becerra et al 2006, Freund et al 2009). Our study suggests that the basis for this functional connectivity lies in the extensive structural connectivity between the PFC subdivisions. Recent research in our lab revealed the existence of a hierarchical structure in DLPFC and VLPFC activation during pain modulation; DLPFC activation precedes VLPFC activation that in turn acts to down-regulate pain (Wiech et al, in preparation). The results of our tractography study show strong connections between the PFC subdivisions, with the VLPFC and PO displaying higher connection probability to the DLPFC than to each other. In line with this finding, animal tracer studies confirm the existence of strong intrinsic connectivity between prefrontal subdivisions (Yeterian et al 2011).

6.4.2.2 Prefrontal subdivisions and pain-related brain regions

Using probabilistic tractography, we investigated how each PFC subdivision are connected to pain-related regions in more detail. Consistent with functional studies that ascribe a sensory-discriminative function to thalamus and SI (Kulkarni et al 2005, Bingel et al 2003, Coghill et al 1999) and a modulatory function to DLPFC with concomitant increased (Dunckley et al 2007) or decreased (Lorenz et al 2003) pain

intensity, the results of this study showed a relatively higher connection probability between DLPFC to thalamus and SI compared to the more affective pain regions.

Animal studies show that the lateral PFC is closely associated with sensory input (Miller & Cohen 2001). Connections between SI and DLPFC have been outlined by Petrides and Pandya (1999). Reciprocal corticothalamic connections are present ipsilaterally as well as contralaterally in some thalamic nuclei (Preuss & Goldman-Rakic 1987). The lateral PFC is connected to the lateral subdivision of the mediodorsal nuclei of the thalamus (Tobias 1975).

For PO, highest connection probability was to midinsula followed by SII, while for VLPFC, highest connection probability was to anterior insula followed by OFC. The pattern for relative connection probability for PO and VLPFC to pain related regions was quite similar with relatively lower connection probability to thalamus and SI. Anterior and mid insula, SII and OFC are regions that have been shown to serve a more affect-related function (Kulkarni et al 2005, Singer et al 2004). Cipolloni & Pandya (1999), in a tracer study, has also showed connections between SII and the ventral part of area 46. Connections between the PFC and OFC have been found in previous tracer studies both to the VLPFC (Petrides & Pandya 2002) as well as the DLPFC (Petrides & Pandya 1999).

Another finding from this study was that connection probabilities from the lateral subdivisions of the PFC were very low to PAG relative to other pain-related brain regions. This is consistent with tracer studies that found connections with the PAG

predominantly on the medial and dorsomedial aspect of the PFC and only sparse connections with the other parts of the PFC (An et al 1998; Frankle et al 2006). A previous tractography study (Hadjipavlou et al 2006) reported a path from PAG to the lateral prefrontal although the strength of connection probability was relatively low compared to medial prefrontal. PAG is a major region for descending modulation of pain that serve mainly 'automatic' responses to pain, and functional studies have suggested increased functional connectivity between DLPFC and PAG during placebo analgesia (e.g. Wager et al 2004). This scarcity of direct connection with the lateral PFC may suggest that functional connectivity is achieved mainly through indirect connections.

Another finding of our study is the relatively low connection probability between the lateral PFC and the amygdala. This is consistent with tracer studies in primates showing abundant fiber connections between amygdala with medial and orbitofrontal PFC and only sparse connection with the lateral PFC (Barbas et al 2011). Studies on anxiety modulation found functional and structural connection between amygdala and medial PFC (Bishop 2007; Kim & Whalen 2009). Although DLPFC does modulate anxiety, the functional connection seems to be with ACC and not amygdala (Bishop 2009). This again suggests that functional connection between lateral PFC and amygdala is mainly through indirect pathways.

6.4.2.3 Pain-related brain regions to PFC subdivisions

In this analysis, we isolated each pain-related brain region to see its connection probability to PFC subdivisions. Since the pain-related brain regions also serve other

functions apart from pain, their connections to PFC are influenced by these other functions as well.

Among the pain-related brain regions, only SI displayed significantly higher connection probability to DLPFC compared to PO and VLPFC. The other pain-related regions had higher or similar connection probability to either PO or VLPFC compared to DLPFC.

For dACC and rACC, our results revealed similar connectivity pattern to PFC subdivisions. Tracer studies in animals have established connection between the anterior cingulate cortex (ACC) and the DLPFC (Petrides & Pandya 1999) as well as the VLPFC (Petrides & Pandya 2002). Diffusion spectrum imaging in humans showed that fibers from the cingulate gyrus project to the lateral PFC through the cingulum bundle (Schmahmann et al 2007). Beckmann et al (2009) performed a meta-analysis of functional pain imaging studies that showed peak activations due to pain in the mid supracallosal cingulate cortex that also displays highest connection probability with dorsal PFC (both dorsolateral and dorsomedial PFC). However, the study by Beckmann et al (2009) did not include VLPFC as a target in the parcellation of cingulate cortex so a comparison could not be made with our study.

A tracer study in old world monkeys found connections between the anterior and mid insula and the granular part of the PFC corresponding to area 46 and lateral 12 (Mufson & Mesulam 1982). In contrast, injection of tracer into posterior insula produced fewer labelled neuron in the prefrontal area. In the present study, the

anterior and midinsula, the seat of interoceptive awareness and subjective emotional experience as shown in functional studies (Coen et al 2009; Craig 2009; Critchley et al 2004), showed high connection probability with the VLPFC compared to DLPFC. The mid insula has higher probability of connection with PO while anterior insula with VLPFC. This is consistent with the tractography study by (Cerliani et al 2011) who highlighted the gradual change in connectivity corresponding to the gradual change in cytoarchitectonic features of the insula from agranular anteriorly to granular posteriorly. Posterior insula, known for its more sensory functions, also exhibited higher connection probability with PO and VLPFC compared to DLPFC.

6.4.2.4 Importance of thalamic connections to PFC

The role of thalamus in pain is well documented both in animal and human studies. Being the critical relay site for pain projection from the spinal cord to cortical and subcortical areas, the thalamus is crucial for transmitting pain information from the periphery to modulatory regions in the PFC.

Connections of the thalamus have been extensively studied in animals and humans. Connections with the prefrontal areas have been shown to be densely concentrated in the mediodorsal (MD) region of the thalamus in macaque monkeys (Ray & Price 1993). This site of strong connection probability to the PFC in the mediodorsal thalamus has also been studied using diffusion imaging studies both in macaques and humans (Behrens et al 2003a; Klein et al 2010). Our study also shows that the center of gravity for thalamic connections to the PFC is in the MD, ventral anterior and parts of the anterior complex nuclei. Incidentally, these are also the sites

receiving dense projections from lamina 1 of the dorsal horn of the spinal cord that ascends through the contralateral spinothalamic tract (Craig & Blomqvist 2002; Pralong et al 2004). This shows that the pattern of connection between thalamus and the PFC lends itself well to pain modulation. The strong connection probability between DLPFC and thalamus may also explain the decreased gray matter volume of bilateral DLPFC and right thalamus peculiarly seen in chronic pain patients (Apkarian et al 2004b).

6.4.3 Limitations

A limitation of this study is that the cytoarchitectonic areas do not always correlate with anatomical landmarks such as sulci and gyri. Also individual variation in anatomical region definition limits the accuracy of connectivity analysis. Problems related to tractography include crossing fibers especially when tracking to distant targets (Jbabdi & Johansen-Berg 2011). The high variability in size and distance of seed and target regions in this study also posed another limitation.

While the limitations of *in vivo* tractography techniques do not allow the achievement of results comparable to tracer studies, most of the results shown here are compared and found to be consistent with available connectivity studies in non-human primates and with *in vivo* diffusion studies in humans thus providing the validation required for the tractography findings.

6.5 Conclusion

In this study, the relative connection probabilities between the subdivisions of lateral PFC and pain-related brain regions were examined. Our findings show that the lateral prefrontal subdivisions are differentially connected to pain-related brain regions with the DLPFC displaying a higher connection probability to brain regions that are more associated with sensory-discriminative functions while subdivisions in the lower part of PFC (i.e. PO and VLPFC), are strongly connected to both sensory-discriminative as well as affective regions.

7. BEHAVIOURAL STUDY: ROLE OF THE OPIOID SYSTEM IN COGNITIVE MODULATION OF PAIN

7.1 Introduction

Existing studies on pain modulation have predominantly focused on an external type of modulation induced by changes in expectation and/or context. This contextual modulation includes beliefs, learning and conditioning that influences the experience of pain (Tracey 2010). Examples of contextual modulation include the placebo response (Petrovic et al 2010), perceived coping efficacy (Bandura et al 1987) and learning (Flor et al 2002).

On the other hand, studies on reappraisal have mainly been studied in the context of ER (Wager et al 2008) where it was shown that reappraisal crucially involves activation of (right) lateral PFC areas. ER involves utilization of strategies to increase or decrease emotions (Gross 1998). These strategies can be categorized according to its relative reliance on attentional control or cognitive change. While attentional control, for example distraction from an unpleasant stimulus, is more automatic, cognitive control requires more volitional effort that frequently employs reappraisal of an aversive stimulus. Since pain is a salient and aversive stimulus, using reappraisal as a strategy to cognitively change it into a less aversive or even a pleasant stimulus may potentially reinterpret the meaning of pain, making it less threatening and consequently reducing the perception of pain (Wiech et al 2008b).

Studies have shown that contextual modulation of pain is mainly opioid-mediated

and engages the descending pain modulatory pathway (Bandura et al 1988; Eippert et al 2009; Petrovic et al 2002). Reappraisal of pain is hypothesised to involve cortico-cortical interactions that bypasses this descending pathway (Wiech 2008). Previous studies have pointed towards a key role for the VLPFC in pain modulation related to reappraisal and there is some evidence that this prefrontal mechanism does not engage the classic descending pain control system (Kalisch et al 2006; Wiech et al 2006). Since the opioid receptors are relatively scarce along the cortico-cortical pathway, it is hypothesised that the opioid system has less role in the analgesic effects generated through this pathway by reappraisal. It has been shown by Petrovic et al (2010) that placebo analgesia, long considered to involve the opioid system through the descending modulatory pathway, also has a non-opioid cognitive component acting through the VLPFC.

The involvement of the opioid system in the two types of emotion regulation in reappraisal is not fully explored. It is also unknown whether success in contextual modulation of pain also predicts success in implementation of reappraisal of pain.

This study investigates the neural mechanism of emotion regulation of pain through effortful reappraisal and compares it with the less effortful contextual pain modulation. For the contextual modulation, moderately painful heat stimuli were presented in two different settings. In the first condition, participants either expected a non-painful warm stimulus or the moderately painful heat stimulus. In the second condition, participants expected to receive a strongly painful stimulus or the moderately painful stimulus. Critically, the moderately painful stimuli in the two

conditions were calibrated to the same pain intensity level but in the first condition, this stimulation level was the worse outcome (because alternatively, non-painful heat stimuli would be applied) whereas in the second condition, this stimulation level would be the better outcome because alternatively, high-intensity pain stimuli would be applied. We hypothesized that, depending on the context in which the moderately painful heat stimuli are presented, the unpleasantness and/or intensity of the pain is perceived differently.

For effortful modulation, similar moderately painful stimuli were presented but now the participants were asked to volitionally change the meaning of the pain into a pleasant stimulus through imagery. This condition was then compared with imagining a neutral stimulus for the pain. The hypothesis is that reappraisal of pain to a more pleasant stimulus would reduce pain perception.

A second aim of the study was to investigate whether effortful regulation of pain through reappraisal is opioid−mediated. Participants underwent two sessions, one with naloxone, an opioid receptor antagonist, one with saline (placebo). It is hypothesized that reappraisal-based pain modulation is not primarily based on the descending inhibitory pain system but is achieved through cortico−cortical connectivity, and is largely opioid-independent.

The research questions for this study were: (1) Is pain modulation that is based on effortful reappraisal opioid-dependent? (2) Is the effect of contextual modulation related to the effect of effortful reappraisal? We hypothesized that the contextual

modulation is opioid-dependent (i.e. sensitive to naloxone) because it is mediated by the descending pain modulatory pathway that is known to operate on endogenous opioids. Pain modulation based on effortful reappraisal was expected to be opioid-independent (i.e. insensitive to naloxone) and to depend on cortico-cortical interactions instead of the descending pain inhibitory pathway.

7.2 Method

The data of this study were collected during the course of an fMRI experiment. Only the behavioural data are presented here.

7.2.1 Participants

Participants were recruited through advertisements placed on public notice boards, word-of-mouth and mailing lists. They underwent a preliminary health screening over the phone for absence of any medical or psychiatric illness, had no contraindications to magnetic resonance imaging and were not on opiates such as morphine or codeine either for medication or recreation. Only participants who passed the phone screening were invited to participate in the study. Informed consent was obtained in writing before the start of each session. Participants were remunerated £35 per session they participated in. Informed consent was obtained and all procedures were in accordance with the Helsinki Declaration and approved by the Milton Keynes Research Ethics Committee (09/H0603/17).

Out of 27 healthy participants recruited, 20 completed the study. Three dropped out after the first session due to side effects from naloxone infusion. Expected side effects from naloxone were headache, nausea, severe vomiting, dry mouth, dry skin, blurred vision, sedation and dizziness. The three participants who dropped out had severe vomiting either during the experiment or after the first experiment. Four participants had to be excluded due to scanner problems while undergoing the first session of the experiment. The remaining 20 participants (11 female) who completed the study were all right-handed and aged between 20 and 38 years of age (mean age 27 years old, s.d. 5.4 years).

7.2.2 Study design

A within-subject crossover design with two sessions separated by at least one day was used (Figure 7.1). Participants received a saline or naloxone infusion during either session in a pseudorandomized order. They were blinded to the order of the drug. The study had two factors: reappraisal-based pain modulation with two levels (pleasant vs neutral) and contextual pain modulation with two levels (moderate pain as best vs worst outcome).

During each session, there were three runs with four conditions in total. In one run, *reappraisal-based* modulation was investigated with the two conditions 'reappraise neutral' and 'reappraise pleasant' presented in a pseudorandomized order. Moderately painful heat was used for both conditions. Another run was the *intense* run with moderately painful heat stimuli presented alternating with strongly painful heat in a pseudorandomized order; here, the moderately painful heat was the

'better outcome' relative to the high-intensity stimulation. In the third run, non-painful warm stimuli were applied alternating with moderately painful heat stimuli; here, the moderately painful heat was now the 'worse outcome'. The 2 latter runs constituted the *contextual* modulation. The order of the 3 runs was pseudorandomized across participants as well as between sessions. However, prior to each run participants were informed about the type of run and the kind of stimuli they would be getting. During the experiment they were also informed about the type of heat stimuli that was going to be delivered by a visual cue. At the end of each trial, participants rated the intensity and unpleasantness of the stimulation.

Participants and two experimenters who had direct communication with the participants were blinded to the treatment received. Only the experimenter responsible for calculating the dose (see Drug administration) and administering the infusion was not blinded to the treatment. Unblinding was done only after the whole experiment had been completed. After the second session of the experiment participants were debriefed on the order of naloxone and placebo they received.

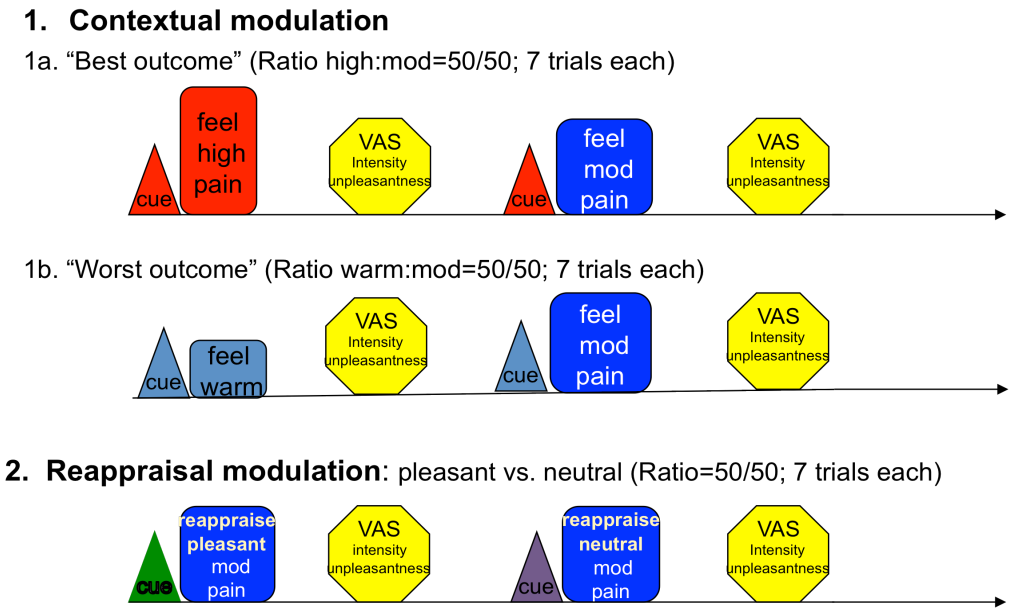


Figure 7-1 Study design.

7.2.3 Experimental procedure

Participants were given instructions on the design of the study using a PowerPoint presentation before they were positioned in the scanner.

Details of the experimental procedure are given below.

7.2.3.1 Reappraisal-based modulation

This condition used moderately painful stimuli presented simultaneously with two different tasks. The ‘reappraise pleasant’ task required the participants to reappraise the pain through the imagination of a positive context. The ‘reappraise neutral’ task required the participants to visualize the device or any neutral heat-dissipating device that was delivering heat to their skin. The ‘reappraise pleasant’ task was

supposed to trigger positive reappraisal in contrast to 'reappraise neutral'. For the 'reappraise pleasant' condition, example strategies were given such as: "Imagine you are freezing cold and you are sitting close to the fire. The fire feels hot but pleasant on your skin". Alternatively, participants could choose their own strategy and use it during the experiment. Participants practised the reappraisal strategy before going into the scanner until they were able to do the imagery. For 'reappraise neutral', participants were instructed to "visualize and feel the device that is delivering heat to your skin". A total of 7 trials of each task were presented in pseudo-randomized order over two runs. The 'reappraise neutral' condition was cued with the word 'DEVICE' while the 'reappraise pleasant' condition was cued with the word 'DIFFERENT' written in a rectangular box with yellow and purple-coloured frame. The colours were pseudorandomized between the two cues.

7.2.3.2 Contextual modulation.

For the contextual modulation, two tasks were presented in two runs. Both required the participant to feel different stimuli and rate each stimulus for pain intensity and pleasantness on a Visual Analogue Scale (VAS) anchored at one end with 'not painful' and 'extremely painful' at the other end. The moderately painful stimulus, which was delivered in both runs was the object of the modulation. The participants were instructed to just feel the stimuli and not try to change the sensation.

-*Best outcome*. The participant was told that they would be getting mostly intense pain stimuli, which would sometimes be followed by lower temperature stimuli (moderate pain). This instruction was used to induce the feeling of relief when the intense stimulation was not applied (therefore the moderate pain was

the “best outcome possible”). There were 7 trials of moderate pain and 7 trials of intense pain. The exact number of stimuli or the ratio between moderate and intense stimuli was not disclosed to the participants. The intense pain was cued with the word ‘FEEL’ in a red rectangular box while the moderate pain was cued with the word ‘FEEL’ in a white arrow pointing downwards.

-*Worst outcome.* In the ‘worst outcome’ condition the participants were told that they would be getting mostly non-painful warm stimuli, which were sometimes followed by higher temperature stimuli (moderate pain). This run therefore created a disappointment when the moderate stimulus was delivered (“worst outcome”). Overall, there were 7 trials of the moderate pain and 7 trials of the warm temperature per run. The warm stimuli were cued with the word ‘FEEL’ in a blue rectangular box while the moderate pain was cued with the word ‘FEEL’ in a white arrow pointing upwards (Figure 7.2).

Unbeknownst to the subject, the same temperature was used for the moderately painful stimuli applied in all the above conditions, which allowed us to compare the perception of physically identical stimuli under different conditions and modulations.

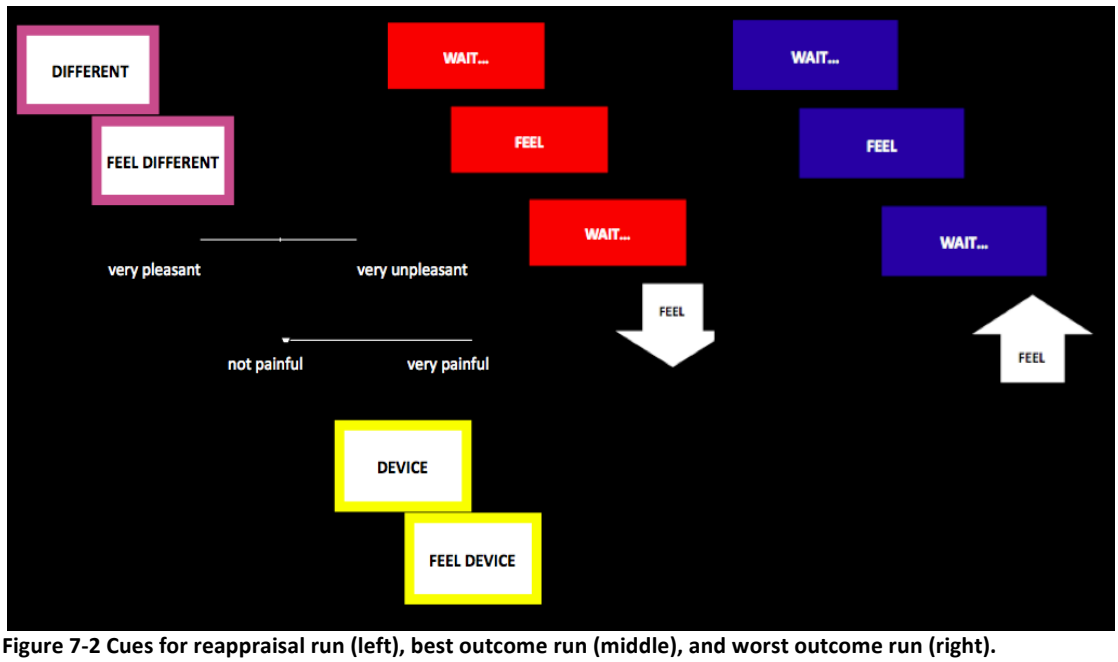


Figure 7-2 Cues for reappraisal run (left), best outcome run (middle), and worst outcome run (right).

7.2.4 Stimuli

7.2.4.1 Delivery method

Two in-house built thermal heat pain devices (thermodes) were used to deliver contact heat stimuli. The thermodes were attached to the ventral aspect of the left forearm of the participants using a Velco strap. The thermodes were positioned along the length of the forearm with a gap of at least 6 cm in between (Figure 7.3). The purpose for using two thermodes was to alternate the delivery of the stimuli between the two sites to allow enough time for the skin to cool off at one site of stimulation before the next stimulus was given.

The duration of the thermal stimuli used in this study was 5 seconds. This duration was chosen after piloting with a shorter duration (4 seconds) and a longer duration (8 seconds) of heat pain stimuli. With the shorter stimulus duration, participants did not have sufficient time to reappraise the stimuli while a longer duration lent itself

more to distraction and loss of concentration. As a result, both the 4 second and the 8 second stimulation failed to produce successful contextual modulation. In view of the findings from the pilot studies, the pain duration was set at 5 seconds and participants were asked to start the reappraisal from the cue onset.

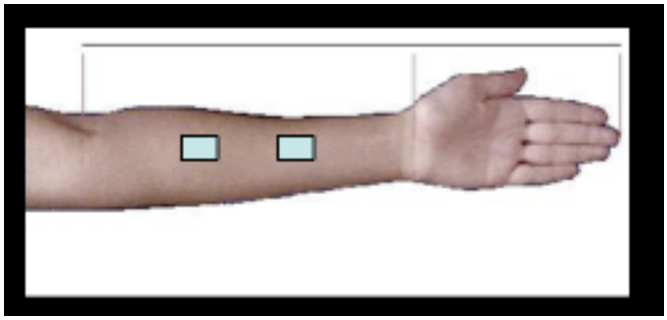


Figure 7-3 Position of thermodes on the ventral surface of the left forearm of the participants

7.2.4.2 Calibration of stimulation intensities

Prior to the experiment, the temperature levels for non-painful warmth, moderate and strong pain were adjusted to the individual sensitivity level to ensure a perception-locked stimulation. First, a non-painful warmth stimulus was delivered, followed by a higher temperature in 0.5 to 1°C increments until a maximum temperature of 50°C or rating of intense pain. Participants were asked to categorize each stimulus as 'warm' (not painful), 'mild pain', 'moderate pain' or 'intense pain'. 'Mild pain' was defined as slightly painful while 'intense pain' was defined as pain that is intense but bearable and that they would be able to withstand when applied repetitively over a duration of about 10 minutes. 'Moderate pain' was defined as considerably more painful than slight pain but less than intense pain. The temperatures were delivered until an intensity slightly more than intense rating was obtained. Then the temperature was lowered to retest the ratings. The calibration

was repeated until a constant temperature for the ratings were obtained for at least three times for the moderate pain and two times for the intense pain. A gap of at least 60 seconds was allowed in between two consecutive stimuli for each thermode site. The calibration procedure was repeated prior to the second session. As a consequence, different stimulation intensities might have been used to induce the same percept in the naloxone and the placebo session. The temperatures used are shown in the Results section 7.3.1. The calibration was done after the start of the placebo or naloxone infusion.

7.2.4.3 Ratings of stimuli

During the actual experiment, participants rated the intensity and unpleasantness of each stimulus using a visual analogue scale (VAS). The rating scales ranged from 'not painful' to 'very painful' for the intensity and from 'very pleasant' to 'very unpleasant' for unpleasantness. Each scale was projected onto a computer screen for 6s with a gap of 1s between the scales. The intensity was then transformed into numerical score ranging from 0-10 while the unpleasantness was rated on a scale from -5 to 5.

7.2.5 Psychological questionnaires

Prior to the experiment, participants filled in psychological questionnaires to assess psychological states and traits related to pain modulation. These included motivation (BIS/BAS, Carver et al 1994), anxiety (STAI, Spielberger et al 1964), and life orientation (LOT-R, (Scheier et al 1994). For the assessment of the participants'

mood, 4 items of the Bond-Lader mood scale (Bond et al 1974) were projected onto the screen at the beginning and the end of each run. The 4 items were 'sad', 'happy', 'calm', and 'anxious' and written as 'At this moment I feel …. sad'. The scale was a continuous scale with anchors 'not at all' and 'very much so'. The Snaith-Hamilton Pleasure Scale (SHAPS; (Snaith et al 1995) for anhedonia was shown after the intense run to assess and compare the hedonic tone between placebo condition and naloxone condition. It consists of 13 items written as 'I would enjoy reading a book, magazine or newspaper' that are rated on a scale from 'not at all' and 'very much'. Ratings for the Bond-Lader mood scale and SHAPS were transformed into scores ranging from 0 (= not at all) to 10 (= very much (so)).

7.2.6 Urine test for opiates

Participants were screened for consumption of opiates using a urine test kit (Instalert, Innovacon, San Diego) prior to each session. None of the participants tested positively for opiates.

7.2.7 Electrodermal activity measurement

During the experiment, SCR data was acquired using the GSR 100C amplifier for Biopac MP150 system equipment. GSR electrodes were attached to the middle phalanges of the left index and ring fingers. Participants were instructed to wash their hands with soap and water and dry them thoroughly before the electrodes were attached. Electrolyte gel was applied to the electrodes to ensure good contact with the skin. Data were processed using Acqknowledge software version 3.9.2-

150M after downsampling from 5000Hz online sampling to 156.25Hz, one of the default settings in the software. The data were filtered using an IIR low pass filter at 0.001 Hz to 1 Hz. Further processing was done using Matlab 2007. SCR was obtained from 10-second epochs segmented corresponding to the 7 repetitions of each condition beginning from the start of the thermal stimulation until 5 seconds after its cessation. The response was defined as the difference between the minimum and maximum amplitude within this time window.

7.2.8 Drug administration

The opioid antagonist naloxone was used to block endogenous opioid receptors at a dose higher than that used to offset exogenous opiates from opioid receptor binding. This is because the affinity of endogenous opioids to bind to opioid receptors is higher than exogenous opiates (Mangold et al 2000).

Prior to the experiment and before signing the consent form, participants were informed about the potential side effects of naloxone which include nausea and vomiting. After the participant had been positioned inside the scanner, intravenous cannulation was performed over the dorsum of right hand for naloxone and placebo infusion. Naloxone or placebo was administered at a bolus dose of 0.15mg/kg intravenously over a duration of about 15 minutes before the start of the experiment. This was followed by an infusion at a rate of 0.2mg/kg/hr until the end of the experiment. It has previously been shown that at this rate the plasma level is kept constant and a continuous complete blockade of opioid receptors is ensured

(Eippert et al 2009; Mangold et al 2000). The plasma level for naloxone was not checked for this experiment.

Participants were informed that naloxone is a drug that interacts with the opioid receptors, but its effects on pain perception were still not well determined, hence the study. They were also informed that they might not be able to notice any differences between naloxone and placebo infusion. After the experiment, the participants were asked for side effects of naloxone using a questionnaire.

7.2.9 Statistical analysis

The analysis of all behavioural data was done using Excel and SPSS v16. To assess the success of both modulations, repeated measures ANOVA and two-tailed paired t-tests were performed comparing the unpleasantness and pain intensity ratings and SCR obtained during moderate pain in the ‘best outcome’ vs. ‘worst outcome’ and during the 'reappraise pleasant’ vs. ‘reappraise neutral’ task. The differences in pain intensity and unpleasantness ratings for both modulations (reappraisal-based modulation: ‘reappraise neutral’ minus ‘reappraise pleasant’; for contextual modulation: ‘worst outcome’ minus ‘best outcome’) signify the success of reappraisal and contextual modulations respectively. Potential differences in intensity and unpleasantness ratings due to the order of runs (i.e. placebo followed by naloxone or vice versa) were investigated using unpaired t-tests.

To test whether individual psychological constructs influence the success of modulations, correlation analyses between the psychological scores and the

differences in ratings of pain intensity and unpleasantness were performed for both types of modulation.

For all analyses, significance was assumed at $p < 0.05$.

7.3 Results

7.3.1 Stimulation temperatures

Figure 7.4 shows the mean temperatures that were used for each stimulation level after calibration. A repeated measures ANOVA with the factors DRUG (2 levels: placebo and naloxone) and TEMPERATURE (4 levels: warm, moderate site 1, moderate site 2 and intense) showed a significant main effect of DRUG ($F(1)=5.16$, $p=0.035$) and TEMPERATURE ($F(1.54)=352.7$, $p<0.0005$). The temperature needed for the naloxone session was significantly higher than the placebo session. There was no significant interaction between DRUG and TEMPERATURE ($F(2.15, 40.83)=2.76$, $p=0.07$).

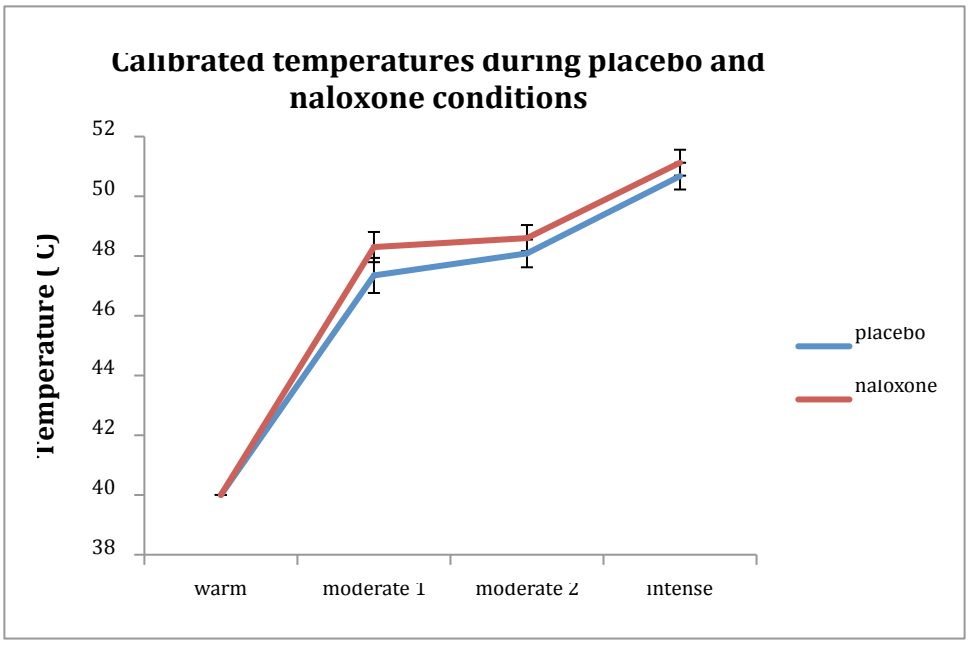


Figure 7-4 Mean temperatures (^{0}C) used for different intensities of heat stimuli during the placebo and the naloxone conditions

7.3.2 Effects of reappraisal-based modulation

During both placebo naloxone conditions, all participants were able to reappraise the pain stimuli to a more pleasant sensation (Figures 7.5 and 7.6).

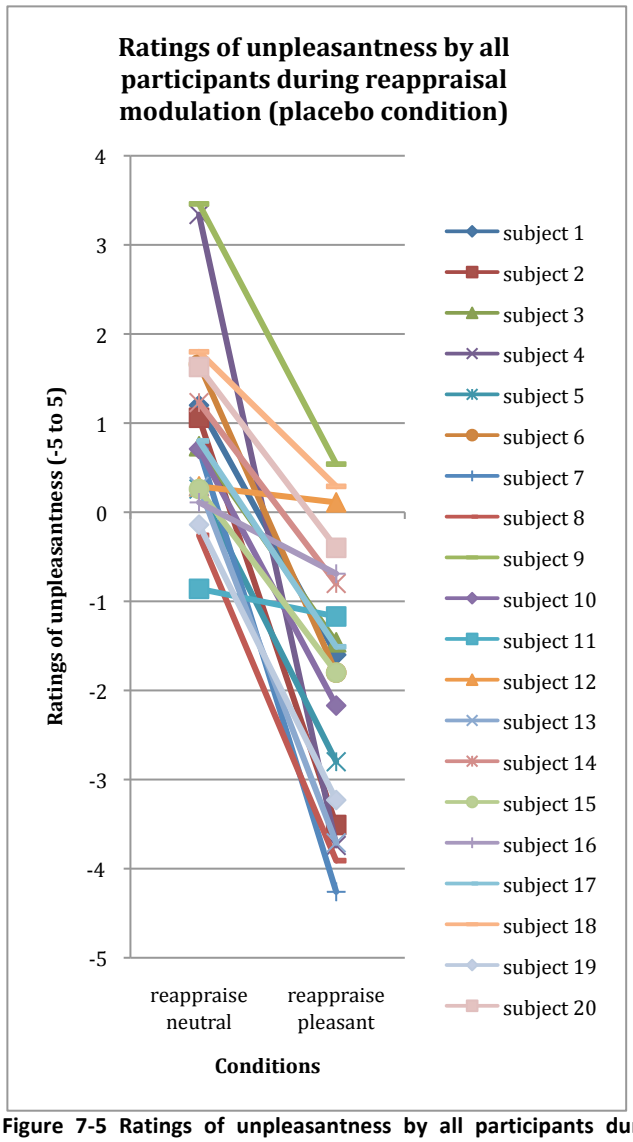


Figure 7-5 Ratings of unpleasantness by all participants during the reappraisal-based modulation in the placebo condition

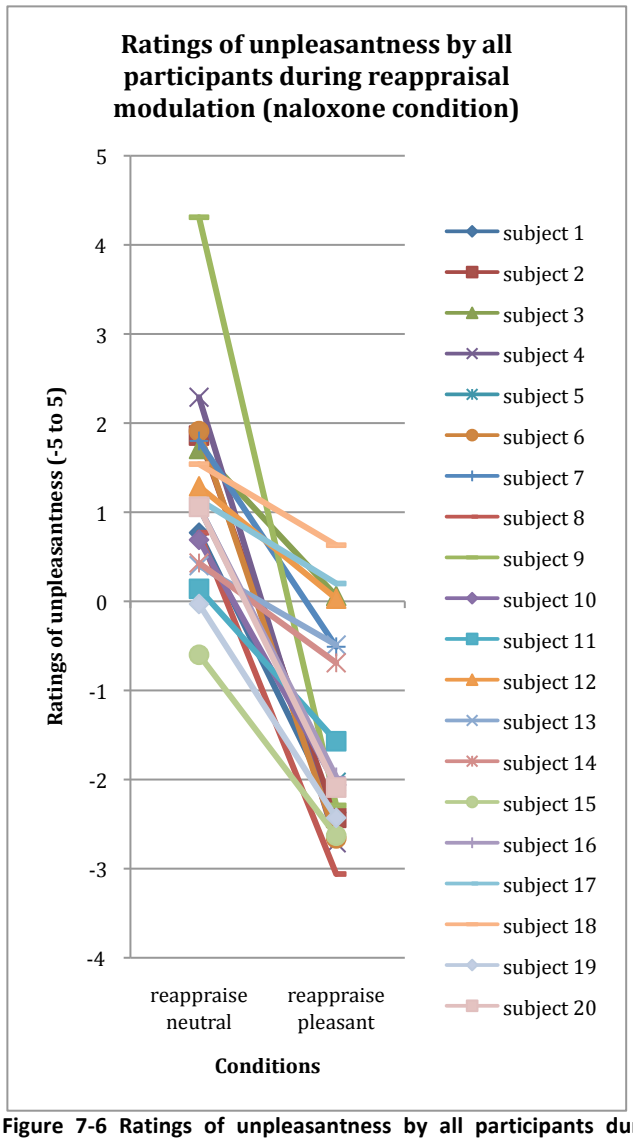


Figure 7-6 Ratings of unpleasantness by all participants during the reappraisal-based modulation in the naloxone condition

Repeated measures ANOVA with factors DRUG (two level: placebo vs naloxone) and REAPPRAISAL (two level: pleasant vs neutral) was performed for both unpleasantness and intensity ratings. For unpleasantness, there was no significant main effect of DRUG ($F(1,19)=1.83$, $p=0.19$) but there was significant main effect of REAPPRAISAL ($F(1,19)=79.25$, $p<0.0005$). The reappraise neutral condition produced higher unpleasantness ratings compared to the reappraise pleasant condition (Figure 7.7). There was no significant interaction between DRUG and REAPPRAISAL ($F(1,19)=0.008$, $p=0.93$).

For pain intensity, there was also no significant main effect of DRUG ($F(1,19)=2.59$, $p=0.12$) but there was significant main effect of REAPPRAISAL ($F(1,19)=40.22$, $p<0.0005$). The reappraise neutral condition produced higher pain intensity ratings compared to the reappraise pleasant condition (Figure 7.8). No significant interaction between DRUG and REAPPRAISAL ($F(1,19)=0.06$, $p=0.80$) was seen.

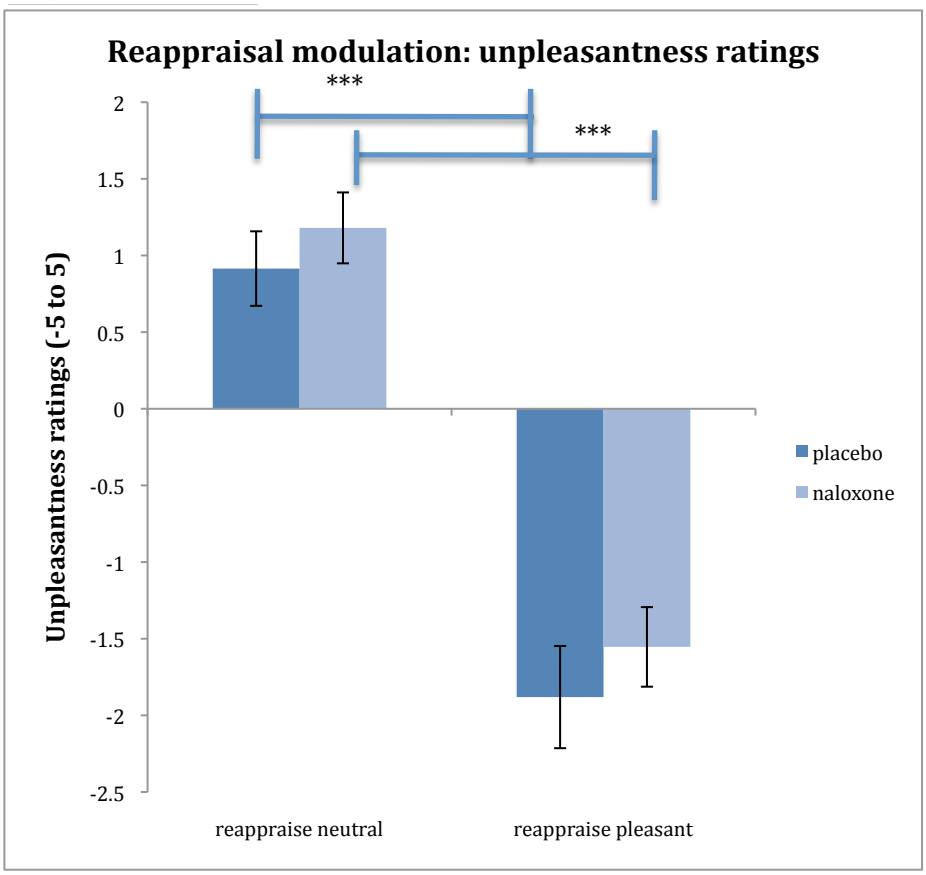


Figure 7-7 Unpleasantness ratings (-5 = very pleasant, 0 = neutral, 5 = very unpleasant) during the reappraisal modulation in the placebo and naloxone condition. ***p≤0.001;p≤0.01;*p<0.05**

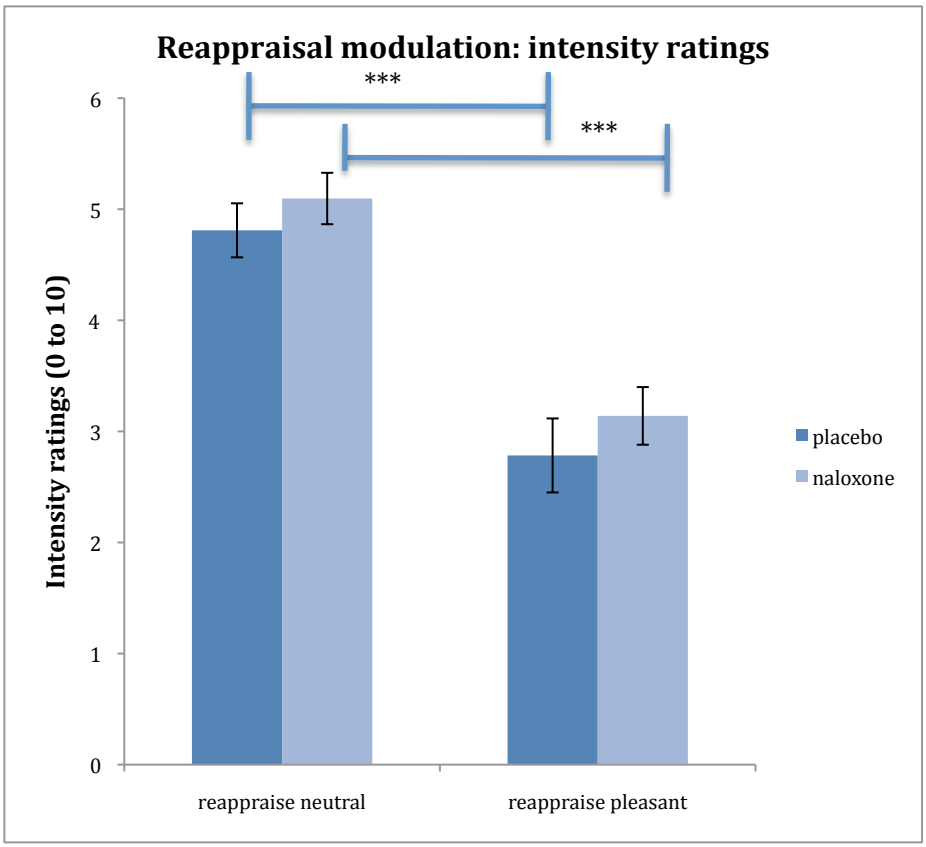


Figure 7-8 Pain intensity ratings (0 = not painful, 10 = very painful) during the reappraisal-based modulation in the placebo and the naloxone condition. ***p≤0.001;p≤0.01;*p<0.05**

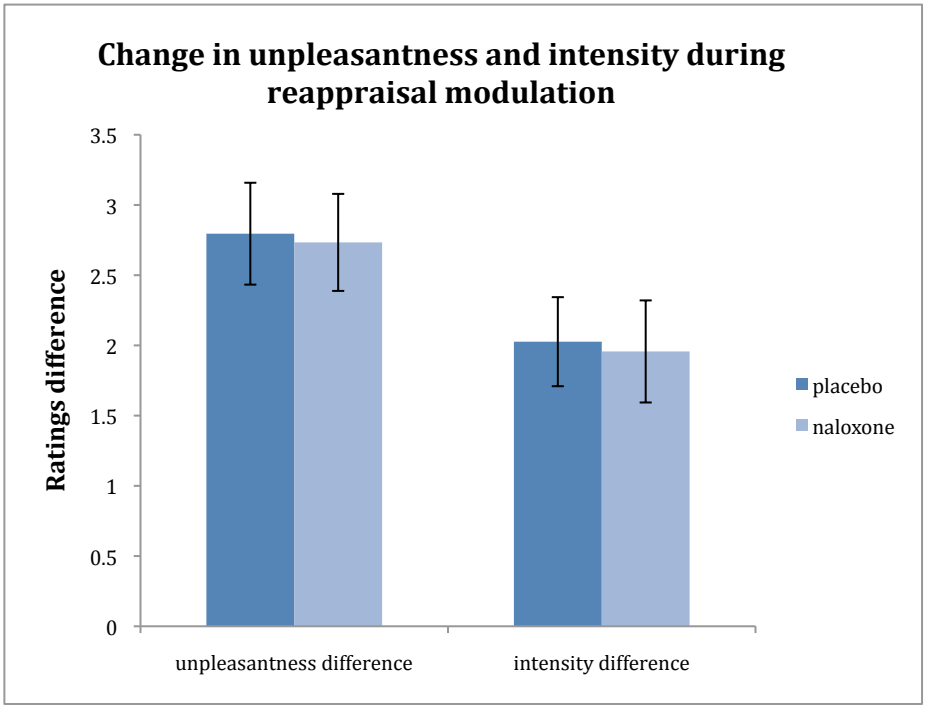


Figure 7-9 Change in unpleasantness and pain intensity ratings ('reappraise neutral' minus 'reappraise pleasant') during the reappraisal-based modulation.

In the placebo condition, the 'reappraise pleasant' condition resulted in significantly lower unpleasantness ($t(19) = 7.51$, $p<0.001$) and intensity ($t(19) = 6.39$, $p<0.001$) ratings compared to the 'reappraise neutral' condition. The blockade of endogenous opioid receptors with naloxone did not affect the effortful reappraisal of pain. 'Reappraise pleasant' during naloxone infusion also resulted in less unpleasantness ($t(19) = 7.91$, $p<0.001$) and less pain intensity ($t(19) = 5.39$, $p<0.001$) compared to 'reappraise neutral' (Figures 7.7 and 7.8).

The difference between 'reappraise pleasant' and 'reappraise neutral' (i.e. 'reappraise neutral' minus 'reappraise pleasant') for unpleasantness and pain intensity (Figure 7.9) illustrates the ability to change the unpleasantness and intensity of the pain to a more pleasant sensation. Interestingly, reappraisal had a stronger effect on unpleasantness than on intensity ratings.

The direct comparison of the placebo and the naloxone condition (Figure 7.9) revealed no significant difference in reappraisal (difference in unpleasantness: $t(19) = -0.18$, $p = 0.86$; difference in intensity, $t(19) = 0.25$, $p = 0.80$), suggesting that the reappraisal-based modulation of pain is not opioid-sensitive.

7.3.3 Effects of contextual modulation

For the contextual modulation, only moderate pain trials applied during the intense and warm runs (i.e. 'best outcome' and 'worst outcome') were compared. Unlike in the reappraisal-based modulation, the effect of the contextual modulation varied considerably between subjects for both placebo and naloxone conditions. While

some gave higher pain intensity and unpleasantness ratings in the 'best outcome' relative to the 'worst outcome' condition, the pattern was reversed in others (Figures 7.10 and 7.11).

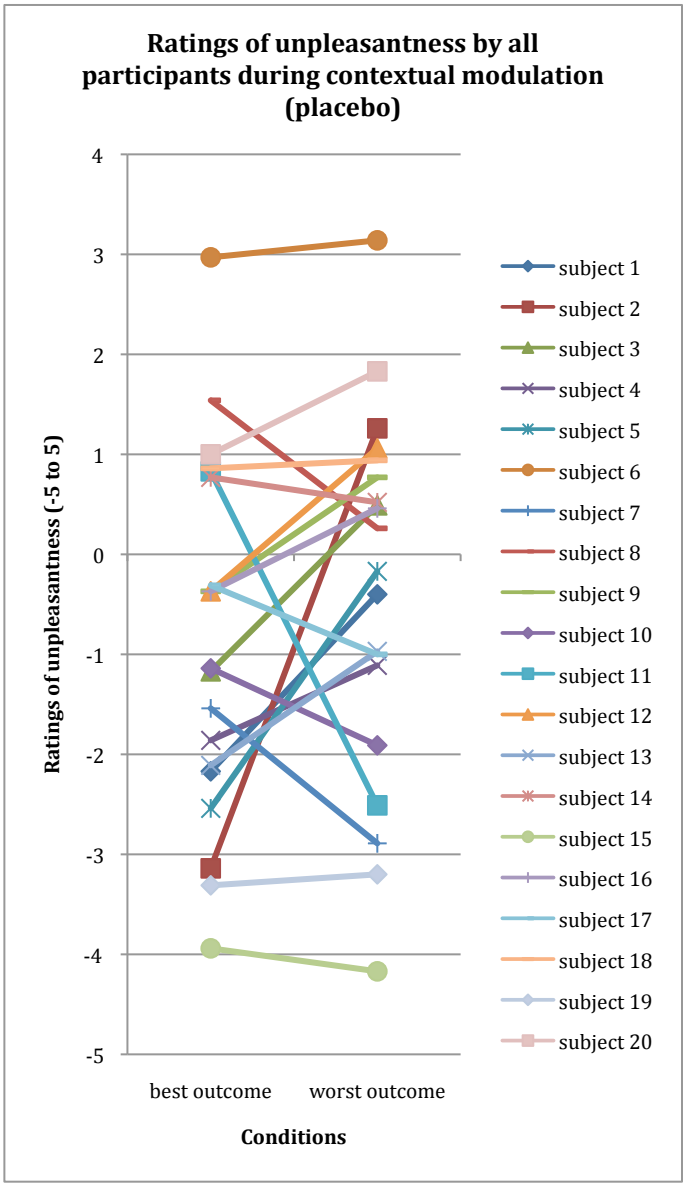


Figure 7-10 Ratings of unpleasantness by all participants during the contextual modulation in the placebo condition

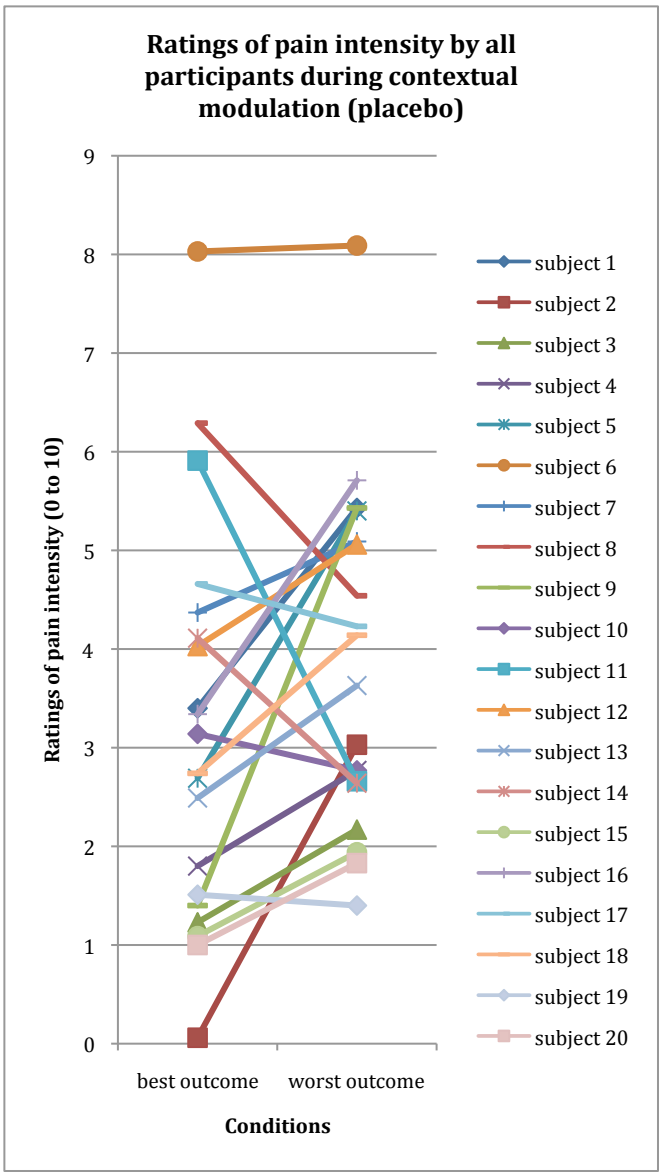


Figure 7-11 Ratings of pain intensity by all participants during the contextual modulation in the placebo condition

Repeated measures ANOVA with factors DRUG (2 level: placebo vs naloxone) and CONTEXT (2 level: best outcome vs worst outcome) for unpleasantness showed that there was trend level effect of DRUG ($F(1,19)=3.18$, $p=0.09$) and CONTEXT ($F1,19)=0.03$, $p=0.87$) and no significant interaction between DRUG and CONTEXT ($F(1,19)=3.52$, $p=0.08$) (Figure 7.12). For pain intensity, there was no significant main effect of DRUG ($F(1,19)=2.63$, $p=0.12$) or CONTEXT ($F1,19)=1.5$, $p=0.24$) and trend level interaction between DRUG and CONTEXT ($F(1,19)=3.08$, $p=0.1$) (Figure 7.13).

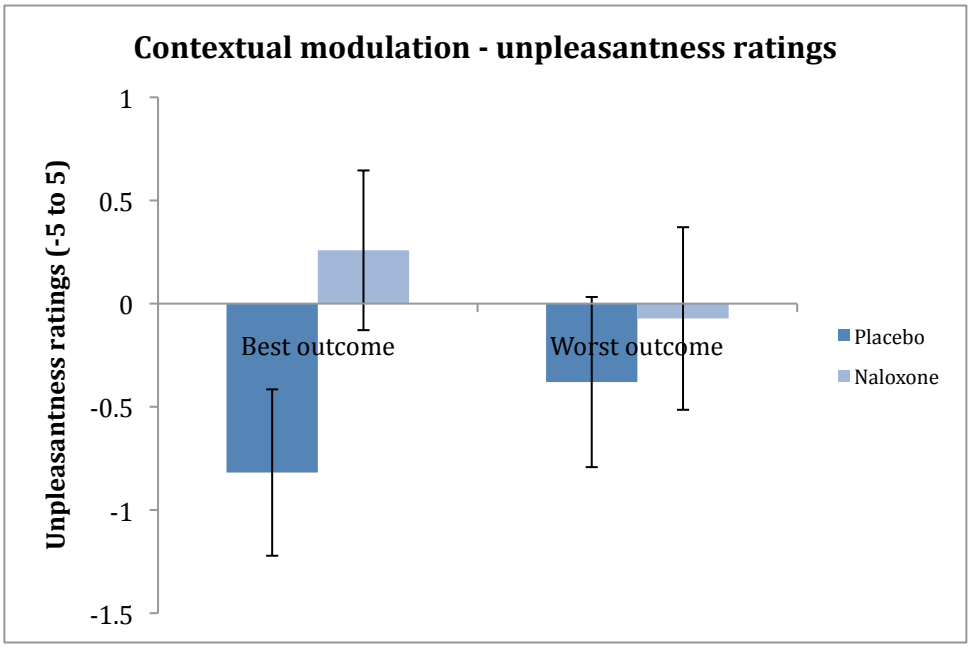


Figure 7-12 Unpleasantness ratings (-5 = very pleasant, 0 = neutral, 5 = very unpleasant) during contextual modulation in the placebo and naloxone condition.

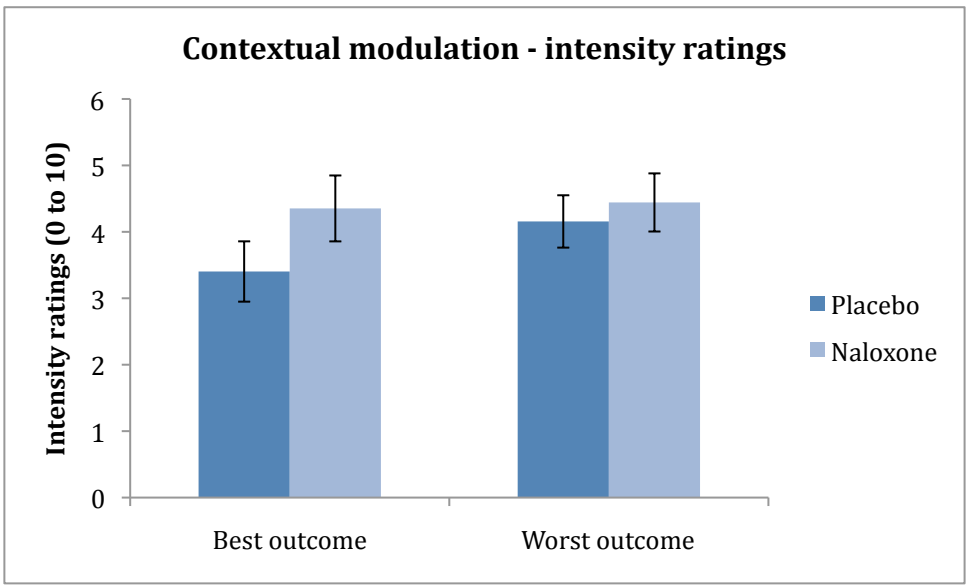


Figure 7-13 Pain intensity ratings (0 = not painful, 10 = very painful) during contextual modulation in the placebo and the naloxone condition.

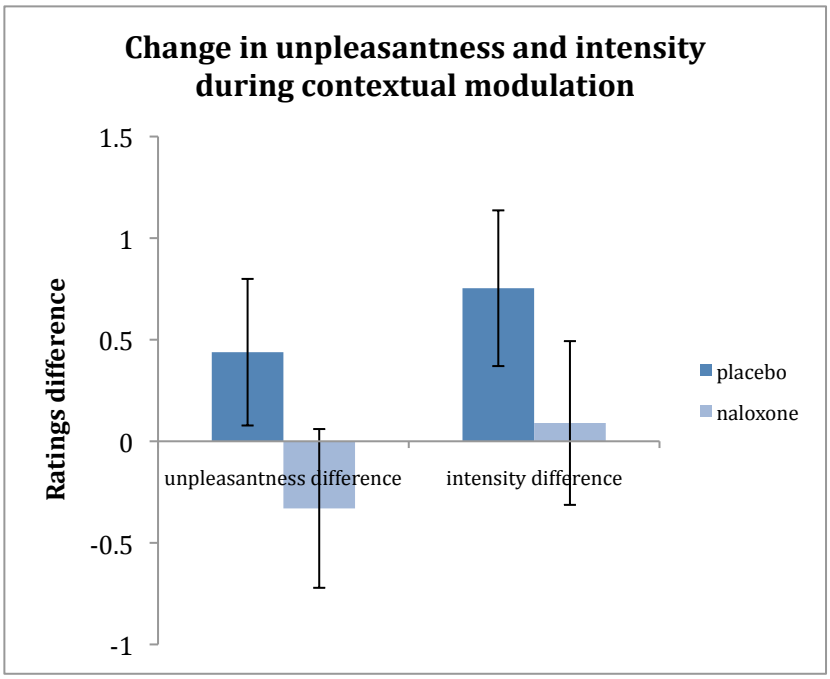


Figure 7-14 Change in unpleasantness and pain intensity ratings (worst outcome minus best outcome) during the contextual modulation.

In the placebo condition, there was no significant difference between the best outcome and the worst outcome condition for unpleasantness ($t(19) = 1.22$, $p=0.24$) but there was trend level effect of intensity ($t(19) = 1.92$, $p=0.07$) ratings. The best outcome condition was perceived as less intense compared to the worst outcome.

In the naloxone condition, there was no significant difference between the best outcome and the worst outcome conditions for both unpleasantness ($t(19) = 0.85$, $p=0.4$) and intensity ($t(19) = 0.23$, $p=0.82$). This suggests that naloxone blocked the perception of less intense pain in the best outcome condition.

The difference between 'best outcome' and 'worst outcome' (i.e. 'worst outcome' minus 'best outcome') for unpleasantness and pain intensity illustrates the ability to change the unpleasantness and intensity of the pain to a more pleasant sensation.

The direct comparison of the placebo and the naloxone condition (Figure 7.14) revealed trend level differences in contextual modulation (difference in unpleasantness: $t(19) = 1.88$, $p = 0.08$; difference in intensity, $t(19) = 1.7$, $p = 0.1$). These results suggests trend level significance of the ability to contextually modulate pain that is blocked by administration of naloxone suggesting opioid mediation.

Out of 20 participants who completed the study, five did not show reduced intensity and unpleasantness ratings during the 'best outcome' situation as expected. Given that the purpose of the study was not to show the effect of contextual modulation on the perception of pain but to compare a successful contextual modulation with a reappraisal-based modulation, these subjects were excluded and further analyses were performed on the preselected subjects with a sample size of N= 15.

Repeated measures ANOVA performed on the preselected sample (N=15) with factors DRUG (2 level: placebo vs naloxone) and CONTEXT (2 level: best outcome vs worst outcome) for unpleasantness (Figure 7.15) showed that there was trend level effect of DRUG ($F(1,14) = 3.5$, $p = 0.08$) and significant main effect of CONTEXT ($F(1,14) = 8.64$, $p = 0.01$). There was no significant interaction between DRUG and CONTEXT ($F(1,14)=2.73$, $p=0.12$). For pain intensity (Figure 7.16), the effect of modulation was stronger with higher trend level for DRUG ($F(1,14) = 4.06$, $p = 0.06$) and significant effect of CONTEXT ($F(1,14) = 23.91$, $p < 0.0005$). There was also trend level interaction between DRUG and CONTEXT ($F(1,14) = 3.32$, $p = 0.09$).

The direct comparison of the placebo and the naloxone condition (Figure 7.17) revealed no significant difference in contextual modulation for unpleasantness ($t(14) = 1.65$, $p = 0.12$); and trend level difference in intensity ($t(19) = 1.82$, $p = 0.90$).

Although there is possibility that findings on a preselected sample might be related to a regression to the mean, the earlier analysis on N=20 showing trend level significance suggests that the difference may not just be due to chance.

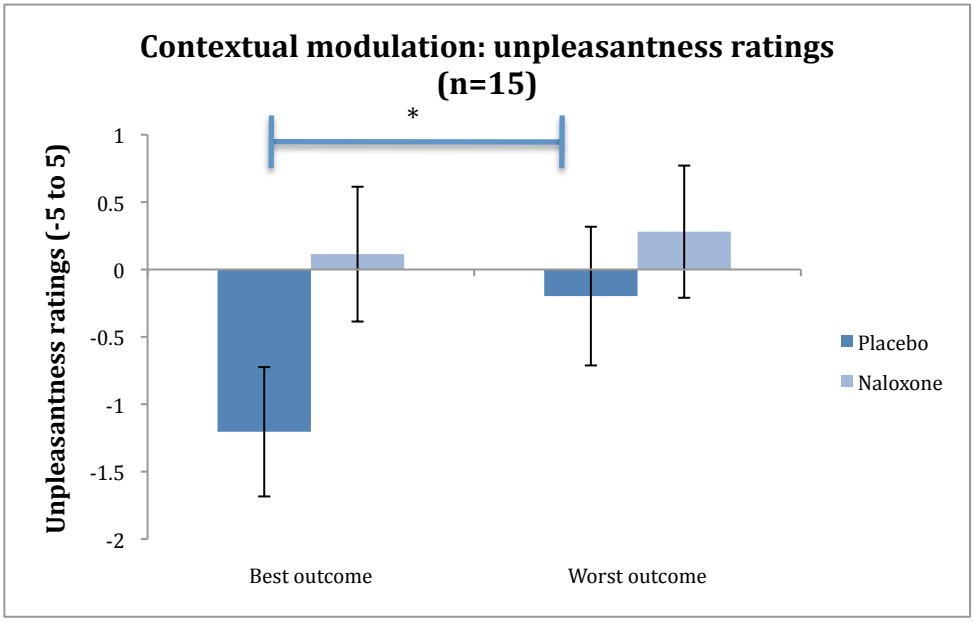


Figure 7-15 Unpleasantness ratings (-5 = very pleasant, 0 = neutral, 5 = very unpleasant) during contextual modulation in placebo and naloxone condition. Note that this analysis was on preselected sample (responder of contextual modulation; n=15).

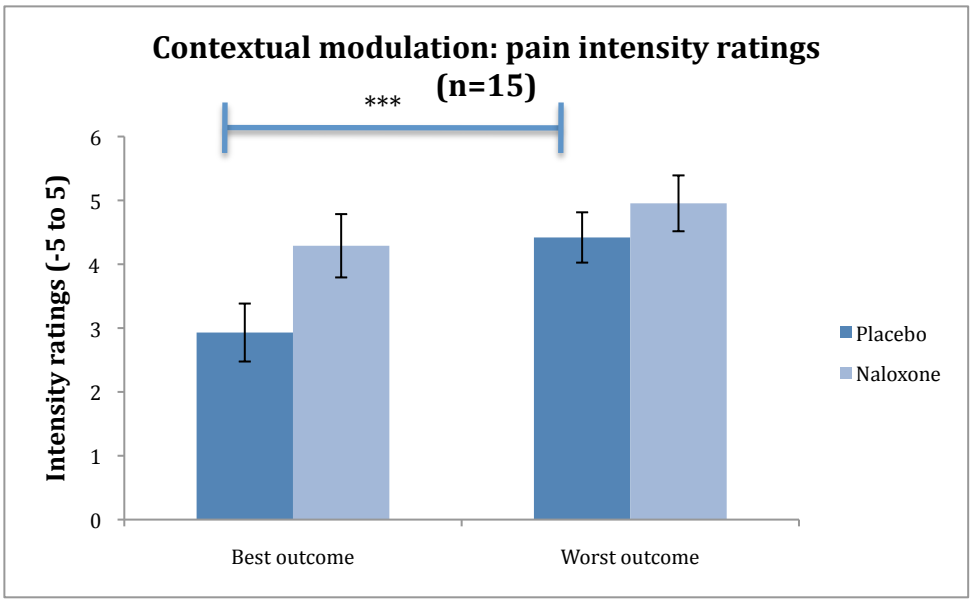


Figure 7-16 Pain intensity ratings (0 = not painful, 10 = very painful) during the contextual modulation in the placebo and naloxone condition (analysis was on preselected sample (responder of contextual modulation; n=15).

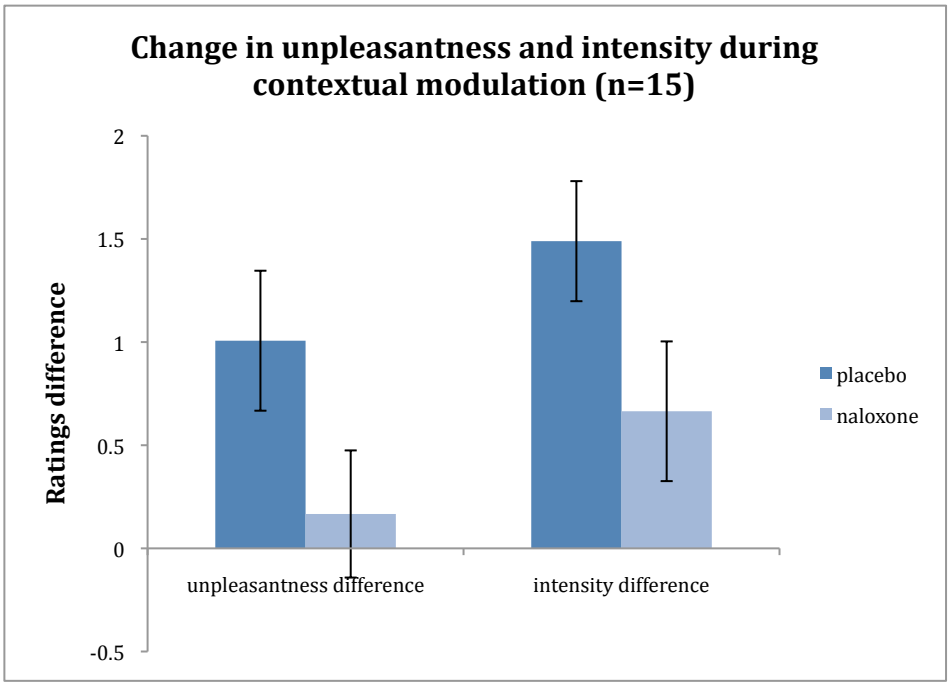


Figure 7-17 Differences in unpleasantness and intensity ratings ('worst outcome' minus 'best outcome') during the contextual modulation in the placebo and naloxone condition (analysis was on preselected sample (responder of contextual modulation; n=15).

7.3.4 Assessment of order effect

Given that half of the participants started with the naloxone session while the other half was administered placebo first, the observed effects could in principle be

explained by an order effect. To test this assumption, pain intensity and unpleasantness ratings were compared between participants who started with placebo and those who started with naloxone.

The data showed that participants who started with placebo had higher unpleasantness ratings during naloxone session compared to those who started with naloxone (unpaired t test: t(18)=2.33, p=0.032) (Figure 7.18).

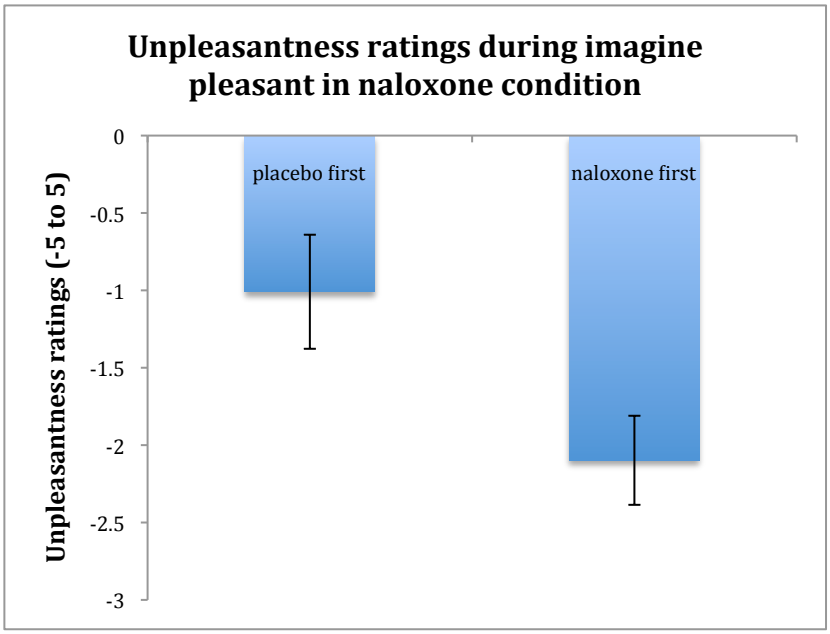


Figure 7-18 Order effect: differences in unpleasantness ratings during imagine pleasant in naloxone condition in participants who started with placebo or naloxone.

7.3.5 Psychological ratings

7.3.5.1 SHAPS score

Using the SHAPS questionnaire, anhedonia could only be assessed in 19 participants as data from one participant was missed out. Across the group, scores were

significantly higher during the placebo compared to the naloxone session (t(18) = 3.08; p=0.006) (Figure 7.19), indicating that naloxone reduced hedonia.

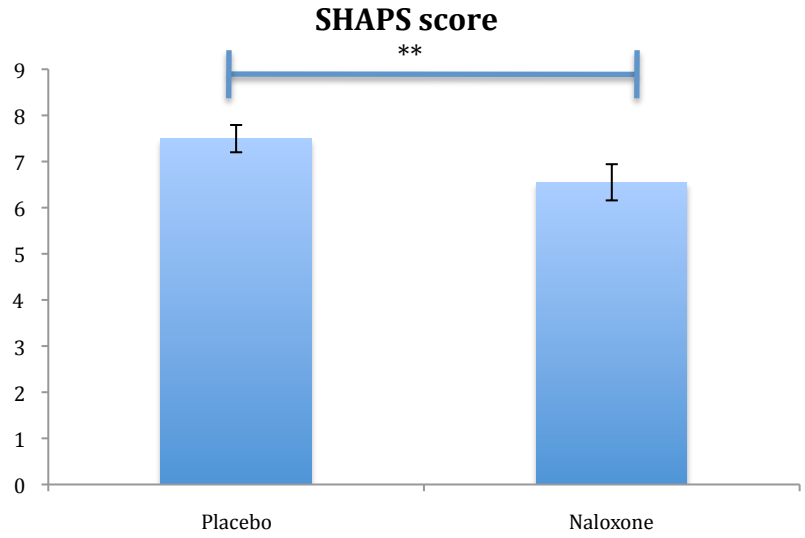


Figure 7-19 SHAPS score for anhedonia during the placebo and naloxone sessions. Values are mean ± SEM. ** p<0.01.

7.3.5.2 Mood ratings

Mood ratings (sad, anxious, happy and calm) rated at the beginning and the end of each run were not significantly different between placebo and naloxone (Table 7.1), indicating that naloxone had no significant effect on these mood dimensions.

Table 7.1 Differences in mood ratings between placebo and naloxone conditions rated at the beginning (pre) and end (post) of each run.

Modulation	Run	Mood	t(df)	
			pre	post
Contextual modulation	warm run	anxious	1.18(19), ns	-0.13(19), ns
		sad	2.09(19), ns	1.22(19), ns
		calm	-1.56(19), ns	0.18(19), ns
		happy	0.65(19), ns	-0.98, ns
	intense run	anxious	0.69(19), ns	0.04(19), ns
		sad	-0.63(19), ns	-1.09(19), ns
		calm	-0.75(19), ns	0.47(19), ns
		happy	-0.96(19), ns	0.19(19), ns
Reappraisal modulation	reappraisal run	anxious	-0.42(19), ns	1.49(19), ns
		sad	-0.24(19), ns	-0.42(19), ns
		calm	-0.93(19), ns	-1.18(19), ns
		happy	0.04(19), ns	0.16(19), ns

7.3.5.3 Correlations of pain intensity and unpleasantness ratings with psychological questionnaire scores

Reappraisal-related differences in unpleasantness (i.e. 'reappraise neutral' minus 'reappraise pleasant') during the placebo condition correlated significantly with the scale that measures the persistent pursuit of desired goals (BAS Drive) (Pearson's correlation: r = 0.47, p = 0.04) (Figure 7.20) and the scale that measures willingness to approach a new event on the spur of the moment (BAS Fun-seeking) (Pearson's correlation: r = 0.62, p = 0.003) (Figure 7.21). These results show that individuals with higher BAS Drive and BAS Fun-seeking scores are more capable of reappraising pain to a more pleasant sensation.

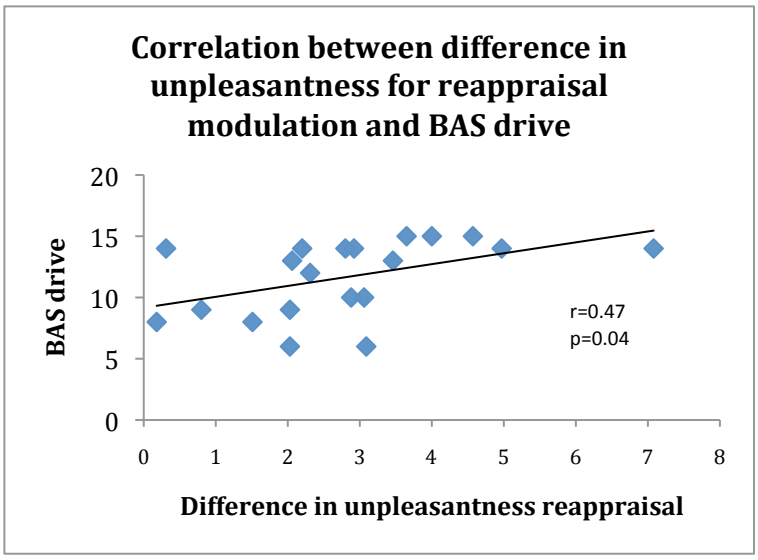


Figure 7-20 Correlation between the difference in unpleasantness for the reappraisal-based modulation and BAS drive

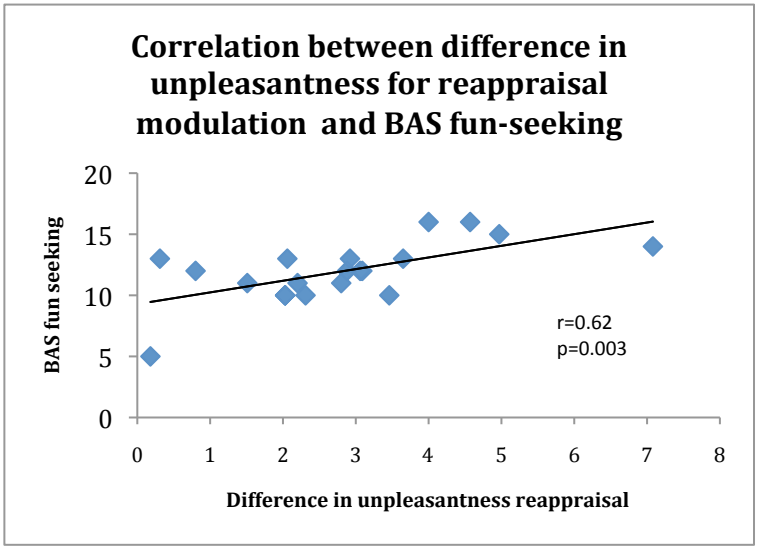


Figure 7-21 Correlation between the difference in unpleasantness for the reappraisal-based modulation and BAS fun-seeking.

The change in unpleasantness ratings related to the contextual modulation (i.e. 'worst outcome' minus 'best outcome') during the placebo condition was negatively correlated with the BIS scale that measures aversive motivation (Figure 7.22). This finding suggests that the more pronounced one's tendency to avoid negative outcome, the less one benefits from the contextual modulation with respect to the unpleasantness of pain.

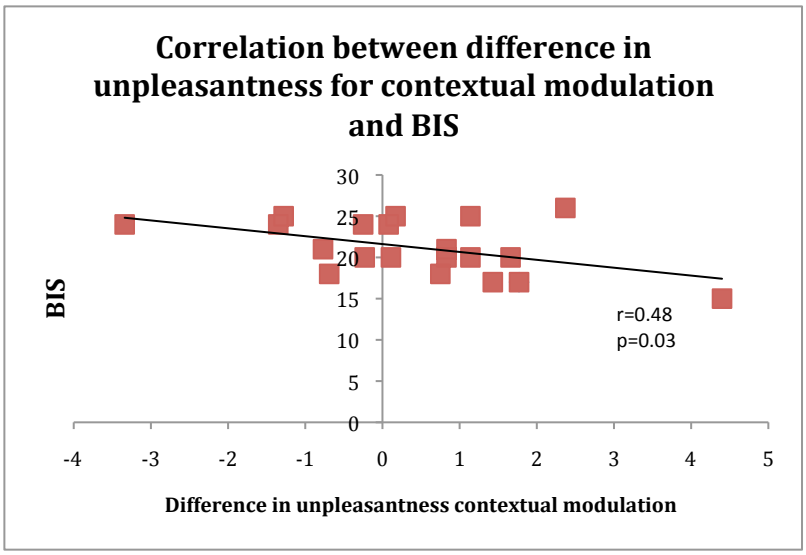


Figure 7-22 Correlation between the difference in unpleasantness for the contextual modulation and the BIS score.

7.3.6 Skin Conductance Response (SCR)

The SCR during moderately painful stimulation was compared between sessions and conditions.

7.3.6.1 Reappraisal modulation

Out of 20 participants, the dataset from one participant was mistakenly deleted and in four additional participants data were contaminated by interferences either from the pain devices or the scanner trigger and were therefore excluded. The remaining 15 datasets were analysed.

Repeated measures ANOVA of SCR with within subjects factors DRUG (placebo and naloxone) and CONDITION ('reappraise pleasant' and 'reappraise neutral') revealed a main effect of CONDITION ($F(1,14)=8.44.$, $p=0.012$) but no main effect of DRUG ($F(1,14)=0.28$, $p=0.61$). There was no interaction between DRUG and CONDITION

(F(1,14)=1.90, p=0.19) (Figure 7.23). Reappraisal of pain to a more pleasant sensation led to a reduction in SCR compared to neutral pain appraisal.

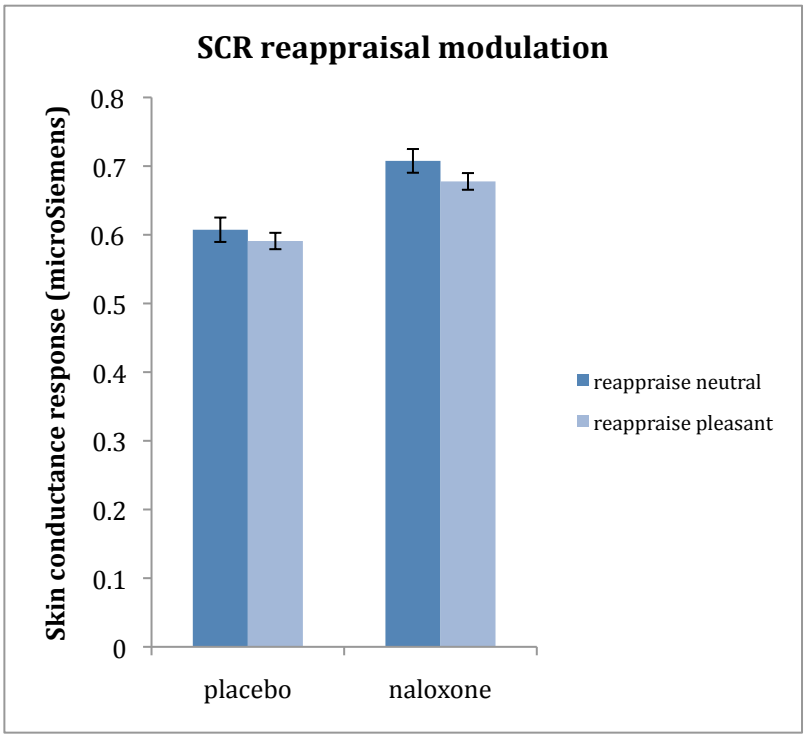


Figure 7-23 SCR during the contextual modulation in the placebo and naloxone condition (data are mean ± SEM). *p<0.05

7.3.6.2 Contextual modulation

Datasets from 14 out of 15 responders of contextual modulation were of good quality for analysis. One dataset was contaminated by noise from the heat pain devices.

Repeated measures ANOVA with the within-subjects factors DRUG (placebo and naloxone) and CONDITION ('best outcome' and 'worst outcome') showed that there was no significant main effect of DRUG or CONDITION nor a significant interaction between the two factors (DRUG: F(1,11)=0.91, p=0.36; CONDITION: F(1,11)=2.37, p=0.15; DRUG * CONDITION: F(1,13)=1.98, p=0.19) (Figure 7.24). This shows that the

behavioural effect of the contextual modulation was not sufficient to drive changes in SCR.

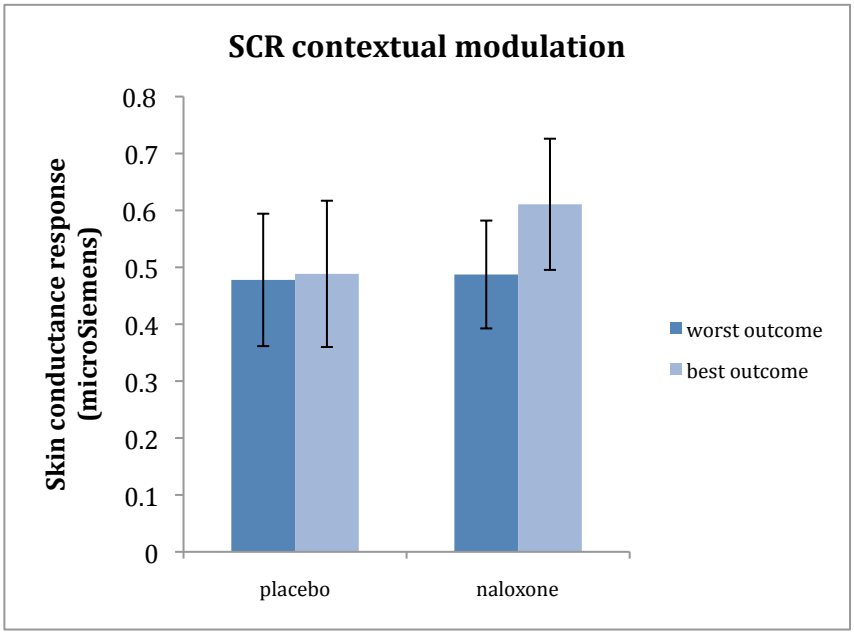


Figure 7-24 SCR during the contextual modulation in the placebo and naloxone condition (data are mean ± SEM).

7.4 Discussion

This study investigated opioid involvement in two types of emotion regulation: reappraisal and contextual modulation of pain. Contextual modulation is a type of emotion regulation that depends mainly on attentional control (Ochsner & Gross 2005). It is less effortful and requires less cognitive change compared to reappraisal, which involves volitional reinterpretation of the aversive stimuli (Tracey 2010).

The aims of this study are firstly, to determine whether effortful reappraisal is opioid-dependent, and secondly, to ascertain whether the effect of contextual modulation is related to the effect of effortful reappraisal. The hypothesis is that

contextual modulation is opioid-dependent due to modulation through the descending modulatory pathway while effortful reappraisal is less opioid-dependent and depends mainly on cognitive cortico-cortical interactions.

The data showed that while all participants were successful in reappraising pain to a more pleasant experience, they differ in the ability to contextually modulate pain. Furthermore, reappraisal modulation was not affected by opioid receptor blockade. There is the possibility that, in contrast to contextual modulation, the explicit instructions for reappraisal to positively change the sensation might have led to social desirability bias and causing decreased pain reporting as found in all the participants. In view of this, measurement of SCR as an objective physiological marker was performed to correct for any possible bias in subjective pain reporting. As seen in the results, the decrease in SCR during positive reappraisal was in keeping with the reappraisal success by pain reporting.

Studies on contextual modulation of pain employing attentional and expectation strategies have established opioid involvement of its modulation (Benedetti et al 2005; Wiech et al 2008b). For effortful reappraisal, however, no known studies have investigated whether the opioid system has a role to play in its mediation. In contrast, placebo analgesia, a type of contextual modulation known to be at least partly mediated by the opioid system (Eippert et al 2009) through the descending modulatory pathway, has been shown to also have a non-opioid component (Petrovic et al 2010). This non-opioid component is the cognitive aspect of the placebo response and is shown to involve the right VLPFC (Petrovic et al 2010).

Studies on effortful reappraisal in non-pain fields have also pointed towards the right VLPFC as the modulatory region for reappraisal (Wager et al 2008). Evidence for little or no opioid involvement in VLPFC functioning lies in the low binding potential of opioid in the prefrontal region (Jones et al 1991; Willoch et al 2004; Willoch et al 1999). Also, studies have also shown no activation of PFC following opioid infusion (Adler et al 1997; Wagner et al 2001).

Contextual modulation includes pain relief modulated by expectation (Charron et al 2006; Montgomery & Kirsch 1997; Price et al 1999; Price & Blake 1999). The subjective interpretation of a nociceptive stimulus is an unconscious decision process depending on not only the sensory input but also knowledge about impending threats and available rewards (Fields 2004). In this study, the contextual modulation paradigm creates an expectation of a higher pain (threat) or a lower pain (reward) resulting in a higher or lower perception of pain accordingly for a similar temperature nociceptive stimulus. The relief felt during the 'best outcome' moderate pain is reward in the form of reduction of an aversive, herein painful, event (Leknes et al 2011; Leknes & Tracey 2008). For this modulation, bottom-up emotion generation (heat pain) is combined with top-down emotion generation due to prior information given about the nature of pain delivery and the cue preceding the pain stimuli creating an expectation of the outcome (McRae et al 2011). Emotion regulation is minimal as the participants were just asked to feel the stimuli and not try to reappraise.

Endogenous opioid has been implicated in the modulation of hedonic responses to

reward and losses (Koob et al 1997). Blockade of opioid receptor by naloxone attenuates the pleasure of reward outcome with corresponding reduction in the activation of brain areas implicated in reward (rACC), and enhances unpleasantness of losses with increases in areas associated with aversive processing (anterior insula and caudal ACC) (Petrovic et al 2008b).

From the results, a majority but not all the participants were able to experience relief during the best outcome moderate pain. The explanation for this may be individual differences in the response to opioid such as sex, age, psychological attributes and genetic profile variation in the response to opioid. Opioid efficacy is influenced by complex interaction of these factors i.e. individual sensitivity to pain stimulation, opioid sensitivity of a particular pain mechanism, and opioid efficacy at particular receptor subtypes (Kindler et al 2011). As a result, certain people are more opioid-responsive than others. Altered mu-opioid receptor availability has been found in brain regions thought to be mainly involved in mediating the affective components of pain (Sprenger et al 2006; Zubieta et al 2001). Zhou et al (2008) measured sustained muscle pain-induced opioid release using PET with selective mu-opioid receptor radiotracer [^{11}C]carfentanil and found that higher levels of mu-opioid system activation in various brain regions is predicted by lower haplotype-driven NPY expression. Studies on mice lacking mu opioid receptor indicate that, among the three subtypes of opioid receptors (mu, delta and kappa), mu opioid receptor is a mandatory component for both endogenous and exogenous opioid actions (Sora et al 1997).

A previous study in our laboratory has revealed the existence of individuals that are opioid responders and non-opioid responders that determines their propensity to develop opioid-induced hyperalgesia (Wanigasekera et al 2009). The psychological ratings of participants in this study showed that the ability to reappraise pain to a more pleasant sensation correlated significantly with the behavioural activation systems in two dimensions, BAS Drive and BAS Fun-seeking. However, the correlation with the psychological scale is indicative of personalities with different opioid resonsiveness and does not infer that the mechanism for reappraisal is driven by opioid.

In contextual modulation, a participant's ability to feel relief correlated negatively with the behavioural inhibition system (BIS). Again, this is indicative of positive correlation with the opioid responsiveness in the participants driving the relief felt.

SHAPS score that requires participants to retrieve pleasurable events, was higher in placebo group indicating better capacity to experience pleasure compared to naloxone group. This suggests that the dose of naloxone was sufficient to block the opioid tone and reduce the pleasurable effect of opioid. Although a dose-response trial was not done in this study, the dose used is based on previous studies (Eippert et al 2009; Mangold et al 2000) that have demonstrated opioid blockade at this dose. A similar dose was also used in the study by Eippert et al (2008) in a fear-conditioning paradigm that used naloxone to block the endogenous opioid receptor. Using the bolus and infusion protocol, the researchers demonstrated that the plasma naloxone level was maintained until the end of the experiment. In this study,

three participants suffered severe side effects and had to abort the experiment. The variability in responses to opioid blockade by naloxone further strengthens the notion of individual variation in responses to opioid by reasons stated above.

Participants did not display significant mood changes before and after each run as indicated by the mood ratings measured before and after each run. Mood also did not differ significantly between placebo and naloxone indicating that the manipulation did not significantly affect either the negative or positive mood. This indicates that there was no mood induction in any of the runs. Mood also was not affected by naloxone infusion. However, change in mood was not assessed specifically for each different manipulation, eg the difference between reappraise neutral and reappraise pleasant, and between best outcome and worst outcome moderate pain.

Consistent with the subjective ratings, the SCR was accordingly reduced as a result of reappraisal. Apart from the reduction in pain perception, the pleasant sensation brought about by the reappraisal may also be the cause for the decrease in SCR. The reduction in SCR was also seen during naloxone condition, further negating opioid involvement in reappraisal modulation.

In contextual modulation, however, there was no decrease in SCR during the moderate pain in the best outcome situation compared to worst outcome situation as expected. Similar results were also obtained during pilot study outside the scanner. One possible explanation for this may be that the intense cue used in the

intense run signified increased threat, thus increasing sympathetic output. It has been shown that pain-predictive cues alone may cause increased reported pain (Keltner et al 2006). Another reason could be that the intense pain felt during the intense run somehow 'overshadowed' the relief effect during the best outcome moderate pain in the same run causing only subjective sense of relief yet not enough to produce reduction in SCR.

Taken together, the results of this study supported the hypothesis that reappraisal modulation and contextual modulation operate via separate and independent pathways. Success in one modulation does not indicate capability to modulate in the other. The data suggest that all individuals can successfully reappraise but not all were susceptible to contextual modulation. A more effortful and less automatic means of analgesia tend to be more robust perhaps due to the explicit instruction to positively reappraise to a more pleasant sensation caused engagement of cognitive resources. The instructions for contextual modulation were more implicit and the variation in the ability to attain analgesia is, more often than not, the result of variability in contextual modulation that is in turn mediated by the opioid system. These findings may give a clue to the underlying mechanism of chronic pain especially functional pain disorder that has little evidence of organic cause. It also opens up a potential avenue for the treatment of chronic pain conditions using reappraisal strategies especially in patients with dysfunctional descending modulatory pathway such as fibromyalgia patients (Goffaux et al 2009).

7.5 Conclusion

This study suggests that the extent of endogenous opioid activity mediation of contextual modulation of pain varies across individuals while effortful reappraisal is largely independent from involvement of the opioid system.

8 Discussion and conclusion

In this thesis, the role of the PFC in pain modulation is explored, using a multimodal approach. Although the PFC as a whole has a common role in solving temporally complex events (Wilson et al 2010), each subdivision of the PFC seems to have a specific function. This thesis attempted to make use of existing data by systematically reviewing previous related research work to provide the foundation for the studies in the subsequent chapters.

8.1 Meta-analysis of pain imaging studies with PFC activation

The meta-analysis was performed to ascertain the extent of PFC activation in pain imaging studies. Another aim was to determine whether there is a specific pattern of activations in the subdivisions of PFC according to various aspects of pain. The results showed that PFC activations occur across various types and duration of pain stimuli, and under different psychological influences that modulate pain in both healthy volunteers and patients. The meta-analysis also showed that the activations were not limited to one defined site but are widely dispersed throughout the vast expanse of the PFC. Also, there was a high degree of co-activation, whereby more than one subdivision of PFC were activated within one study paradigm. This suggests presence of an interaction between the subdivisions of PFC.

An obvious limitation of the meta-analysis is the wide variation across studies with regard to study design, even with studies that have similar research question. Confounding this is the highly subjective affective component of individuals

participating in the studies. This is also the issue with meta-analysis of imaging studies in other non-pain fields. For instance, a meta-analysis of fMRI studies that attempted to localize working memory found it to be located in many different regions by many different researchers (Cabeza & Nyberg 2000). A longitudinal study is therefore warranted to better able narrow the schism between the biology and psychology of pain (Derbyshire 1999).

8.2 The right VLPFC

One significant finding from the meta-analysis is that across studies, there is strong activation of the right VLPFC. Although the DLPFC is the region most frequently reported, this meta-analysis showed that significant foci of activations mainly reside in the inferior aspect of the PFC. This is particularly true in chronic pain patients where the different types of pain seem to activate mainly the VLPFC. The VLPFC is also the main PFC subdivision that co-activated with other subdivisions of the PFC, particularly the DLPFC. This finding suggests interaction between the VLPFC and DLPFC in the modulation of pain and may be the basis for cortico-cortical connectivity.

Top-down modulation involving the VLPFC is also evident in expectation-induced analgesia. While it has long been accepted that the placebo effect is mediated through the descending modulatory pathway, a reanalysis of a study on placebo and opioid treatment revealed prefrontal involvement, particularly VLPFC (Petrovic et al 2010) that is not opioid-mediated for placebo. Furthermore, activity of rACC, an

opioid-rich region, co-varied with the VLPFC in the placebo condition specifically. These results demonstrate prefrontal top-down influence on rACC.

8.3 Structural connectivities between PFC and pain-relevant brain regions

The study in Chapter 5 utilized another approach to further define the underlying mechanism by which PFC modulates pain. To ascertain whether modulation of pain by PFC is exerted through anatomical connections, the probable structural connectivity of the PFC to pain-relevant brain regions was defined. Histological and tracer studies in animals show that the PFC can be divided into anatomical subdivisions and has varying degree of connections to other brain regions. This tractography study found that the various subdivisions of the PFC have distinct probabilities of connection to the different pain-relevant brain regions, consistent with the primate tracer studies and post-mortem studies, as well as functional imaging as in the findings from the meta-analysis of functional imaging studies of pain described in Chapter 4.

The experience of pain encompasses a whole network of brain regions that also subserve various other functions. The findings of the tractography study in Chapter 5 suggest that anatomical segregation of the PFC dictates functional distinctiveness in pain as suggested by its relative probabilities of connection to pain-relevant brain regions. The somatosensory aspect of pain is evidenced by activation of brain regions such as the thalamus and posterior insula in functional imaging studies. This

tractography findings show high probability of connection between these brain regions with DLPFC. On the other hand, functional studies show that the psychological dimension influencing the affective-motivational aspect of pain causes activation of structures such as anterior insula, OFC, PAG and amygdala. This tractography results show that the VLPFC displayed high connection probability with affective as well as somatosensory regions.

One finding from the tractography study is the relatively lower connection probability of the lateral PFC with PAG, a structure prominent for the descending modulation of pain. One possibility to explain this finding is that the lateral PFC is more involved in functions that require cortico-cortical interaction compared to that involving descending modulation of pain. Animal studies have revealed the presence of columns in the PAG that mediate somatic and autonomic components of emotional coping (Keay & Bandler 2001). The dorsolateral and lateral columns seem to integrate active emotional coping while the ventrolateral column mediates passive emotional coping. Tracer experiments in monkeys indicate that projections from distinct cortical areas have three distinct patterns of termination in individual longitudinal PAG columns. These fibers arise from 1) medial PFC terminating predominantly in the dorsolateral columns, 2) orbital areas terminating primarily in the ventrolateral column, and 3) dorsomedial areas terminating mainly in the lateral column (An et al 1998).

The PFC areas that project to the PAG include two distinct areas; the ‘‘medial prefrontal network’’ that represents the visceromotor system, and the ‘‘orbital

network'' that is sensory related (An et al 1998). These findings suggests that specific cortical areas, i.e. the orbital and medial PFC, but not lateral PFC, differentially influence the analgesic, somatic, and autonomic functions integrated within specific PAG columns (Bandler & Shipley 1994).

8.4 Contextual and reappraisal modulation of pain

The study in Chapter 7 compared two modulations of pain with and without opioid blockade by naloxone, modulation by reappraisal of pain, where participants reappraised moderate pain as a pleasant or neutral sensation, and contextual modulation, where the same intensity of pain was perceived differently in different contexts.

By reappraising a pleasant scenario for pain, participants were able to change the perception of pain and reduced activation in pain-relevant brain regions. Reappraisal has previously been shown to down-regulate emotion to reduce the intensity of an adverse situation, such as when viewing aversive pictures (Hayes et al 2010; Kanske et al 2011; Wager et al 2008).

The findings from Chapter 7 provide one more evidence for the existence of a dual pathway for pain processing. The opioid-mediated pain modulation, as shown in the contextual modulation condition, and the cortico-cortical modulation of pain through reappraisal that is less opioid-mediated. Further work should investigate the neural network for these two modulations.

8.5 Conclusion

In summary, this thesis aimed to unravel the role of the PFC in pain modulation. By using a multimodal approach, certain aspects related to the role of the PFC in pain modulation have been identified. First, PFC activation is present across a wide range of pain studies; second, the modulatory function of PFC can be segregated into functional subdivisions that mostly conform to anatomical connectivity; third, there seems to be interaction among the subdivisions of the PFC that is supported by intrinsic cortico-cortical structural connectivity; fourth, cognitive modulation of pain through reappraisal does not seem to be dependent on opioid mediation as much as contextual modulation of pain that operates through the descending pain modulatory pathway, suggesting that reappraisal mainly involves the cortico-cortical pathway.

Further work should build upon these findings to further enhance our understanding of the functional connectivity of the pain network. One avenue that can potentially shed light on the role of the PFC is interference study using tools such as transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS; (O'Shea et al 2008). These techniques involve non-invasive focused stimulation of a localized region in the brain using short magnetic pulses generated by a specialized electromagnet placed on the person's scalp. Stimulation of the underlying cerebral cortex has been shown to induce temporary disruption in brain activities. Using tDCS or TMS to a subdivision of PFC such as DLPFC to temporarily disrupt its function can potentially infer causality of its function in pain modulation.

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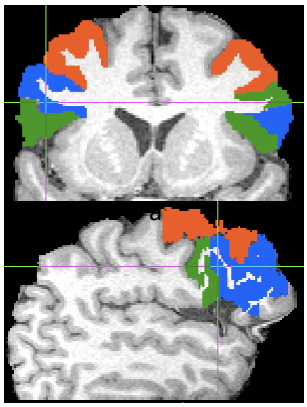
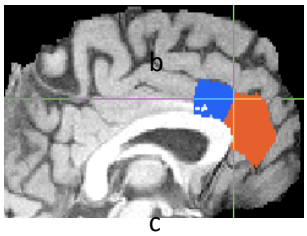
VII. APPENDICES

Appendix 1 – Drawing of masks in structural space

These figures show the masks for each seed and target region drawn on one dataset's T1-weighted image. All figures are in radiological orientation (left is right).



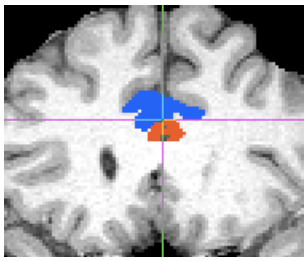
Figures 1a and b. Dlpfc: The dlpfc (red) is limited posteriorly by the superior precentral sulcus (Croxson et al., 2005) on the lateral surface and extends anteriorly to where the middle frontal gyrus can no longer be defined, i.e. where it borders the frontopolar area 10. The pars opercularis (green) is bordered posteriorly by the inferior precentral sulcus and anteriorly by the first sulcus connecting the lateral sulcus and inferior frontal sulcus. The vlpfc (blue) extends from the pars opercularis posteriorly to the frontopolar sulcus anteriorly.
Drawing of the dorsolateral and the ventrolateral masks were done on the coronal view. From the sagittal slice, the rostral and caudal borders were first outlined at the frontopolar sulcus anteriorly, and the superior and inferior precentral sulci posteriorly (Croxson et al., 2005). From the axial view, the superior frontal sulcus was identified as the superior border for dlpfc.



b

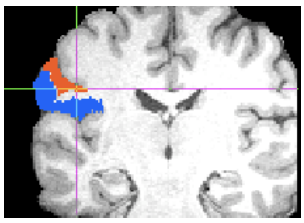
c

Figure 1c and d. ACC: The masks for dorsal ACC (or anterior MCC) and rostral ACC (or perigenual ACC) were outlined according to the borders defined by Vogt et al (1995). The anterior cingulate cortex lies between the cingulate sulcus and the corpus callosum. Variations in surface features of the ACC include the presence of either single cingulate sulcus with or without segmentation or double parallel cingulate sulci (paracingulate sulcus) with or without segmentation (Bush et al., 2002). Rostral ACC corresponds to areas 24a-c and 32 while dorsal ACC corresponds to caudal divisions of areas 24b-c and 32 (written as 24b', 24c' and 32') (Bush et al., 2000).

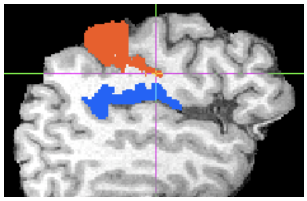


d

The mask for rostral ACC was traced on the coronal view. From the sagittal section, the outline of the rostral ACC was drawn first as a guide to the borders when drawing in coronal view. Starting from the rostralmost cingulate sulcus on the sagittal view, the mask was drawn in the corresponding coronal section.

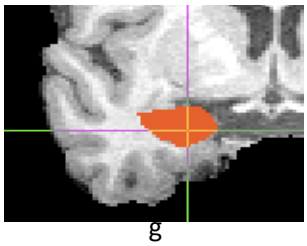


e

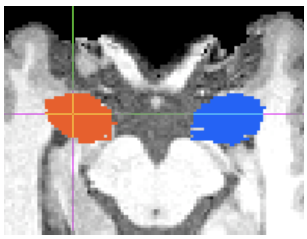


f

Figure 1e and f. SI and SII: Tracing of SI was done in the coronal plane. The outline of the SI area was first delineated over the lateral aspect of the brain in axial and sagittal view. In the coronal view, the central sulcus was located starting from where it lies at the most medial and superior aspect. The postcentral gyrus is the one inferior to the central sulcus on coronal view. At the same time the axial and sagittal views were opened at the upper section to confirm the location of the postcentral gyrus. The mask was drawn over the posterior bank of the central sulcus and the postcentral gyrus following the demarcation by (Geyer et al., 1999).
Tracing of SII was done firstly in the sagittal section and in the coronal section according to (Eickhoff et al., 2006). Mask was drawn over the superior bank of the posterior insula where it can be viewed in the sagittal section and continued in the coronal section over the superior bank of the lateral fissure starting from the rostralmost slice corresponding to the plane of the line passing through anterior commissure.

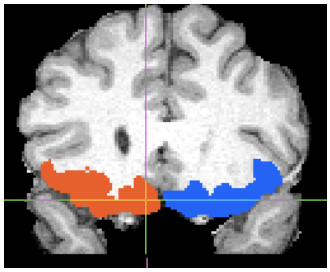


g

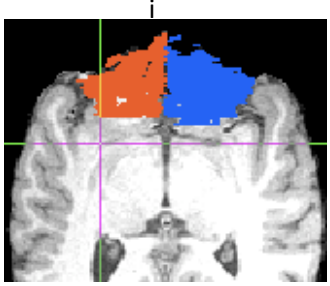


h

Figure 1g and h. Amygdala: The mask for amygdala was drawn in the coronal section starting from where the structure can be visualized maximally (around the plane of the anterior commissure) and moving in the y-axis anteriorly and posteriorly. The margins of the amygdala are limited by either the CSF or white matter tract. The transition slice, where the hippocampus first appears on the more posterior slices and borders the amygdala inferomedially, is identified by white matter tracts on the medial border of the hippocampus, which appears as a line of gray matter curving superomedially. The two structures (amygdala and hippocampus) may also be separated by white matter or CSF. The border between amygdala and hippocampus was also double-checked on sagittal section where the CSF or white matter are clearly visible separating the two structures.

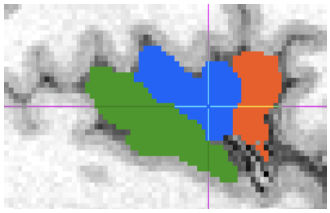


i

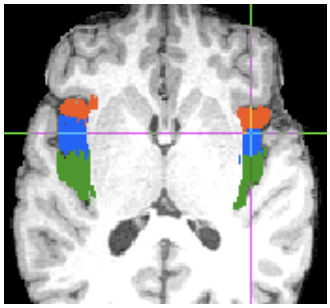


j

Figure 1i and j. Ofc: Tracing of OFC was done in the coronal plane. From the sagittal view the borders of the OFC were outlined starting from the rostralmost where it borders the frontopolar gyrus anteriorly until the anterior perforated substances caudally. The superior border of the OFC is the cingulate sulcus. In the coronal view, areas included in the tracing start from the gyrus rectus medially to lateral orbital gyrus laterally.
Tracing of OFC was done in the coronal plane. From the sagittal view the borders of the OFC were outlined starting from the rostralmost where it borders the frontopolar gyrus anteriorly until the anterior perforated substances caudally. The superior border of the OFC is the cingulate sulcus. In the coronal view, areas included in the tracing start from the gyrus rectus medially to lateral orbital gyrus laterally.

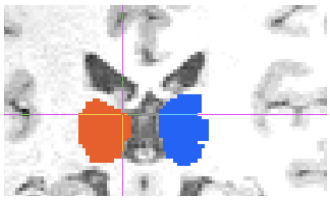


k



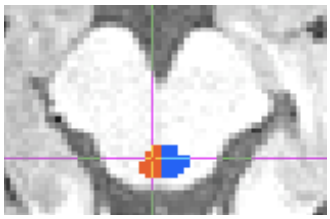
l

Figure 1k and l. Insula: Drawing of the insula was done in the sagittal section. The anterior insula consists of the anterior short insular gyrus which is limited anteriorly by the anterior limiting sulcus (Schweinhardt et al., 2006). The middle insula consists of the middle and posterior short insular gyri (Schweinhardt et al. (2006) named this area the caudal anterior insula), while the posterior insula is made up of the anterior and posterior long insular gyri.
Drawing of the insula was done in the sagittal section. The anterior insula consists of the anterior short insular gyrus which is limited anteriorly by the anterior limiting sulcus (Schweinhardt et al., 2006). The middle insula consists of the middle and posterior short insular gyri (Schweinhardt et al. (2006) named this area the caudal anterior insula), while the posterior insula is made up of the anterior and posterior long insular gyri.



m

Figure 1m. Thalamus: The mask for thalamus was drawn in the coronal view starting from the rostral aspect, at the plane where the anterior commissure (Naidich et al., 1986) can be visualized maximally, and moving caudally. The thalamus first appears in the section following the anterior commissure plane.



n

Figure 1n. PAG: The outline of PAG was first delineated in the sagittal view and the mask drawn in the axial view. The PAG is situated in the pons around the cerebral aqueduct.

Appendix 2 - List of studies included in meta-analysis of healthy subjects

First Author	Year	Journal
Adler	1997	Anesth Analg
Albuquerque	2006	Pain
Aziz	1997	Gastroenterology
Baliki	2010	Neuron
Baliki	2006	J Neurosci
Baliki	2008	J Neurophysiol
Bar	2007	Biol Psychiatry
Becerra	2001	Neuron
Becerra	1999	Magn Reson Med
Becerra	2004	J Neurophysiol
Bingel	2007	Neuron
Bornhovd	2002	Brain
Brefel-Courbon	2005	Mov Disord
Brooks	2005	Neuroimage
Carlsson	2006	Neuroimage
Casey	1996	J Neurophysiol
Casey	1996	J Neurophysiol
Casey	2001	J Neurophysiol
Christman	2002	Neurosci Lett
Christman	2007	Neuroimage
Coen	2009	Gastroenterology
Coghill	2001	J Neurophysiol
Coghill	2003	Proc Natl Acad Sci U S A
Coghill	1999	J Neurophysiol
Cole	2006	Brain
de Leeuw	2006	Oral Surg Oral Med Oral Pathol Oral Radiol Endod
Derbyshire	1994	J Neurol Neurosurg Psychiatry
Derbyshire	1998	Pain
Derbyshire	1998	Exp Brain Res
Derbyshire	2002	Neuroimage
Derbyshire	1997	Pain
Dube	2009	Neuroimage
Dunckley	2005	Neuroscience
Eippert	2008	J Neurosci
Elsenbruch	2010	Gut
Freund	2009	Somatosens Mot Res
Gelnar	1999	Neuroimage
Geuze	2007	Arch Gen Psychiatry
Gracely	2002	Arthritis Rheum
Gundel	2008	Pain
Hall	2010	Neurogastroenterol Motil
Hofbauer	2001	J Neurophysiol
Iannilli	2009	Pain
Jantsch	2005	Pain

First Author	Year	Journal
Jantsch	2005	Pain
Jones	1997	Ann Rheum Dis
Kong	2006	Hum Brain Mapp
Koyama	2005	Proc Natl Acad Sci U S A
Kurata	2005	Anesth analg
Kwan	2005	Neurology
Laureys	2002	Neuroimage
López-Solà	2010	Neuroimage
Lorenz	2008	Br J Anaesth
Lu	2004	Neurogastroenterol Motil
Lu	2009	Pain
Lui	2008	Pain
Maihofner	2006	Eur J Neurosci
Maihofner	2007	Eur J Neurosci
Maihofner	2006	Eur J Neurosci
Mobascher	2009	Neuroimage
Moisset	2010	Eur J Pain
Ochsner	2006	Pain
Paulson	1998	Pain
Petrovic	2008	Soc Cogn Affect Neurosci
Peyron	1999	Brain
Piche	2010	Pain
Ploghaus	1999	Science
Ploghaus	2001	J Neurosci
Ploner	2010	Proc Natl Acad Sci U S A
Raij	2005	Proc Natl Acad Sci U S A
Rottmann	2009	Eur J Pain
Schmahl	2006	Arch Gen Psychiatry
Seifert	2007	Neuroimage
Singer	2004	Science
Smith	2002	Br J Psychiatry
Sprenger	2006	Pain
Straube	2009	Hum Brain Mapp
Strigo	2003	J Neurophysiol
Strigo	2005	Neurosci Lett
Strigo	2003	J Neurophysiol
Strigo	2005	Neurosci Lett
Svensson	1997	J Neurophysiol
Symonds	2006	J Neurophysiol
Talmi	2009	J Neurosci
Tracey	2000	Neurosci Lett
Tracey	2000	Neurosci Lett
Tseng	2009	Hum Brain Mapp
Valet	2004	Pain
Vanhaudenhuyse	2009	Neuroimage
Vartiainen	2009	Pain
Veldhuijzen	2009	Pain
Verne	2003	Pain

First Author	Year	Journal
Verne	2003	Pain
Villemure	2009	J Neurosci
Vogt	1996	Eur J Neurosci
Weigelt	2010	Pain
Wiech	2006	J Neurosci
Wiech	2008	Pain
Wise	2007	Magn Reson Imaging
Xu	1997	Neuroreport

Appendix 3 - List of studies included in meta-analysis of patients

First Author	Year	Journal
Albuquerque	2006	Pain
Baliki	2008	J Neurosci
Baliki	2008	Molecular pain
Baliki	2010	Neuron
Baliki	2006	J Neurosci
Becerra	2006	J Neurosci
Burgmer	2010	J Neural Transm
Derbyshire	1994	J Neurology, Neurosurgery & Psychiatry
Derbyshire	1999	Eur J Pain
Derbyshire	2002	Neuroimage
Ducreux	2006	Brain
Eisenbruch	2009	Gut
Geha	2007	Pain
Geha	2008	Pain
Giesecke	2005	Arthritis & Rheumatism
Gracely	2002	Arthritis & Rheumatism
Gracely	2004	Brain
Hall	2009	Neurogastroenterol Motil
Hsieh	1995	Pain
Hsieh	1996	Pain
Jensen	2009	Pain
Jones	1997	Ann Rheum Ds
Kwan	2005	Neurology
Lebel	2008	Brain
Lieberman	2004	Neuroimage
Maihofner	2007	Brain
May	2000	Neurology
Naliboff	2001	Psychosomatic Medicine
Nicotra	2006	Brain
Peyron	1998	Brain
Peyron	2004	Neurology
Pukall	2005	Pain
Schweinhardt	2006	Neuroimage
Schweinhardt	2008	Neuroimage
Shiraishi	2006	Radiat Med
Verne	2003	Pain
Zeng	2009	Neurosci Lett

Appendix 4- List of investigators doing pain imaging research

Apkarian V
Bingel U
Borsook D
Bouhassira D
Büchel C
Bushnell C
Clauw DJ
Coghill RC
Davis KD
Derbyshire SWG
Dolan RJ
Fields HL
Flor H
Gracely RH
Iadarola MJ
Jones AKP
Mackey SC
Maihofner C
Petrovic P
Peyron R
Ploghaus A
Price DD
Rainville P
Schweinhardt P
Tölle TR
Tracey I
Treede R-D

List courtesy of Professor Irene Tracey