Neural correlates of clinical pain processing in neuropathic and inflammatory pain patients and comparison with experimental pain

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Manuscripts arising from this work

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Schweinhardt, P.; Kalk, N.; Wordsworth, P. and Tracey, I. Differential influences of emotional and cognitive determinants of the pain experience on cerebral processing of clinical and experimental pain in patients with rheumatoid arthritis. (Submitted 2006)

Schweinhardt, P.; Glynn, C.; McQuay, H.; Chessell, I.; Bountra, C. and Tracey, I. Assessment of functional magnetic resonance imaging as a new tool for drug development in humans. (In review 2006)

Schweinhardt, P.; Tracey, I. and Bountra, C. MR in drug development – Pain. MR in Drug Development; special issue NMR Biomedicine. (In press 2006)

Tracey, I.; Schweinhardt, P. and Bountra, C. Brain FMRI in Clinical Pharmacological Studies. Chapter in: In Vivo MR Techniques in Drug Discovery and Development (CRC Press, LLC). (In press 2006)

Declaration

I declare that the work presented in this thesis is my own and has not been submitted for any other degree in this or in any other University or Institute of learning.

Anatomical abbreviations

ACC	anterior cingulate cortex
BG	basal ganglia
cAI	caudal anterior insula
CNS	central nervous system
LPFC	lateral prefrontal cortex
M1	primary motor cortex
MFC	medial frontal cortex
MTL	medial temporal lobe
NCF	cuneiform nucleus
Ncl	nucleus
OFC	orbitofrontal cortex
PAG	periaqueductal grey
PBN	parabrachial nucleus
PFC	prefrontal cortex
PCC	posterior cingulate cortex
rAI	rostral anterior insula
RVM	rostroventral medulla
S1	primary somatosensory cortex
S2	secondary somatosensory cortex
SMA	supplementary motor area
STT	spino-thalamic tract
VMpo	ventral medial nucleus (of the thalamus)

Other abbreviations and acronyms

5-HT	5-hydroxytryptamine, serotonin
AFP	atypical facial pain
ARC	Arthritis Research Campaign
BET	Brain Extraction Tool
BDI	Beck Depression Inventory
BOLD	blood oxygenation level dependent
CBF	cerebral blood flow
CBV	cerebral blood volume
CLBP	chronic low back pain
CRP	C-reactive protein
CS	central sensitization
CSF	cerebrospinal fluid
DAS28	Disease Activity Score 28
DMA	dynamic mechanical allodynia
DTI	diffusion tensor imaging
EFIC	European Federation of IASP Chapters
EPI	echo planar imaging
ESR	erythrocyte sedimentation rate
FILM	FMRIB's Improved Linear Model
FLAME	FMRIB's Local Analysis of Mixed Effects
FLASH	Fast Low Angle Shot (sequence)
FMRI	functional magnetic resonance imaging
FoV	field of view
FWHM	full width half maximum
GABA	gamma-aminobutyric-acid
GLM	general linear model
Hb	haemoglobin
HPA	hypothalamic-pituitary-adrenal
HRF	haemodynamic response function
IASP	International Association for the Study of Pain
IBS	irritable bowel syndrome
IL	interleukin
IBS	irritable bowl syndrome

ISI	inter-stimulus interval
MD	major depression
MEG	magnetencephalography
MMSE	Mini Mental State Examination
MNI	Montreal Neurological Institute
MRI	magnetic resonance imaging
NIRS	near infrared spectroscopy
NMR	nuclear magnetic resonance
NPSI	Neuropathic Pain Symptom Inventory
NRS	numerical rating scale
OA	osteoarthritis
PCS	Pain Catastrophizing Scale
PE	parameter estimate
PET	positron emission tomography
PiA	placebo-induced analgesia
PGIC	patient global impression of change
RA	rheumatoid arthritis
RF	radio frequency
RoI	region of interest
SAM	Synthetic Aperture Magnetometry
STAI	State Trait Anxiety Inventory
SD	standard deviation
T_1	longitudinal relaxation
T_2	transverse relaxation
TE	echo time
TI	inversion time
TMS	transcranial magnetic stimulation
TNF	tumour necrosis factor
TR	repetition time
TTL	transistor transistor logic

Abstract

Neural correlates of clinical pain processing in neuropathic and inflammatory pain patients and comparison with experimental pain

Petra Schweinhardt, Christ Church, DPhil, Hilary Term, 2006

The goal of this thesis was to examine the processing of clinical pain in two patient groups with well defined primary pathologies, i.e. neuropathic pain patients and patients with rheumatoid arthritis (RA). It was hypothesized that chronic pain is associated with plastic changes in pain processing brain structures that can be detected using functional magnetic resonance imaging (FMRI). The first study, presented in Chapter 3, demonstrates that the neural representation of experimental heat pain is different in neuropathic pain patients than in age- and gender-matched healthy control subjects, although the pain stimulus was applied outside clinically affected areas. Increased activation was found in amygdala and anterior insula in the patient group and was accompanied by increased state anxiety and depression scores. Anterior insula is the focus of Chapter 4 in which it is demonstrated that clinical pain processing is located significantly more anteriorly in the insula than experimental pain processing, in close proximity to neural correlates of highly negative emotions and the conscious perception of bodily sensations. This offers a potential explanation for the shift of clinical pain processing. In Chapter 5, clinical pain is contrasted with experimental pain in the same patient population, i.e. patients with RA. In addition to comparing clinical and experimental pain processing, it was investigated if emotional and cognitive determinates of the pain experience, specifically depression and catastrophizing, exert different influences on the two types of pain. It is shown that clinical pain, but not experimental pain, is likely to be driven partially by depressive symptoms whereas catastrophizing is associated with the same neural activation pattern in both conditions. The cerebral representation of allodynic pain in neuropathic pain patients is presented Chapter 6. Chapters 6 and 7 demonstrate that the FMRI signal encodes the perceived intensity of clinical allodynic pain across subjects and that it reflects longitudinal variations of the perceived intensity within subjects.

This thesis illustrates that FMRI can reveal subtle differences in the processing of clinical and experimental pain, despite brain activation patterns being similar on the whole. It also indicates that FMRI can be used to elucidate the origin of these differences, for instance by studying the influence of emotional and cognitive variables. This suggests that neuroimaging methods, in particular FMRI, have the potential to dissect clinical pain into its constituent parts, including central sensitization, brainstem facilitation and amplification by psychological factors. Such knowledge could potentially be exploited to target treatment selectively at different components of clinical pain and to monitor longitudinal changes of these components separately.

CHAPTER 1: Introduction

Acute pain serves an important evolutionary purpose, notably as warning system for the organism to prevent tissue damage. Likewise, the persistence of pain beyond the healing time of the injury carries a biological function in that it promotes recovery by temporary inactivity. In contrast, chronic pain remains an enigma because it is difficult to appreciate an evolutionary advantage for an organism suffering from chronic pain. Instead of asking why chronic pain has evolved, it might be more worthwhile to ask why specific mechanisms that avoid the occurrence of chronic pain did *not* evolve (Millan 1999). The answer to this question might lie in evolutionary selection pressure: many chronic pain conditions affect predominantly the elderly who are not part of the reproductive pool. Hence, while acute and sub-chronic pain serve important biological functions and are sometimes crucial for survival, chronic pain is probably not an integral part of life and does not provide any biological advantage for the organism.

1.1 The problem of chronic pain

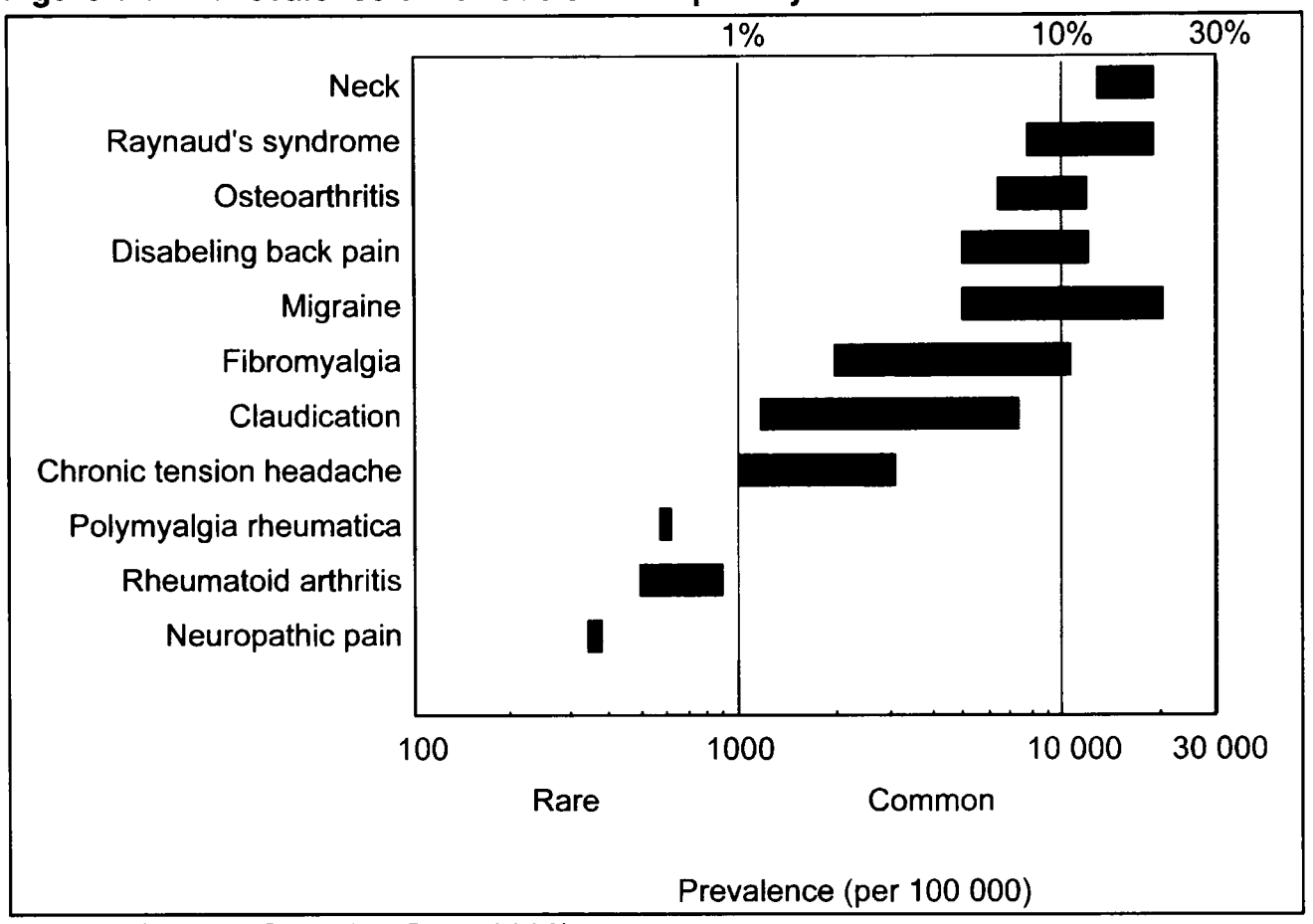
Chronic pain may begin as acute pain, but it continues beyond the normal time expected for resolution of the problem or persists or recurs for other reasons (EFIC 2004). The World Health Organization has identified chronic pain as a common health problem with 22% of primary care patients reporting pain that is present for most of the time during a period of six months (Gureje et al. 1998). Chronic pain is not only a physical problem but can be associated with extensive psychological and social

difficulties that further impact on the quality of life of the patient. Economically, chronic pain generates substantial direct and indirect costs to society caused by high demand on health services, lost working days, reduced productivity, early retirement and the associated sickness and insurance benefits (Faucett and McCarthy 2003; Kvien 2004).

Chronic pain occurs in a variety of conditions, for example nerve injuries (neuropathic pain, see Section 1.2), cancer, angina pectoris, musculoskeletal problems and diseases affecting the joints (Figure 1.1). Pain related to neuropathic pain conditions and to rheumatoid arthritis has been investigated in this thesis and therefore, these two disease entities are described in more detail in Sections 1.2 and 1.3. Risk factors for the development of chronic pain have been investigated most extensively in musculoskeletal conditions, including chronic low back pain (CLBP). Psychosocial and psychological factors have been identified in addition to workplace-related mechanical factors (Harkness et al. 2004; Harkness et al. 2003; Nahit et al. 2003). High levels of psychological distress, anxiety disorders and depression have also been reported to be important risk factor in other forms of chronic pain. The relation between chronic pain and depression is discussed in more detail in Section 1.6. In addition to environmental and psychological factors, it is likely that individual vulnerability for chronic pain is also determined by genetic factors. "Pain genetics" is a novel area of research and has already provided some insight into differing pain sensitivity or opioid response in animals and humans (Fillingim et al. 2005; Mogil et al. 2003; Stamer et al. 2003). In animals, there are additional data for heritable pre-disposition to

developing chronic pain after nerve injury (Devor and Raber 1990; Mogil et al. 1999). Evidence is more difficult to obtain in humans; however, multiple nerve injures that are consistently associated with chronic pain in individual patients strongly suggest genetic susceptibility (Devor 2004).

Figure 1.1 Prevalence of various chronic pain syndromes



Adapted from McQuay (McQuay 2002).

Ideally, treatment is aimed at removal of the dysfunction that is causing pain, for example replacement of an osteoarthritic joint. In many instances, however, this is not possible (e.g. nerve injury) or pain persists after the supposed cause has been treated. In such cases, treatment options include pharmacological, physical and psychological approaches or a combination of these. Still, only incomplete pain relief can be achieved for many patients (Collins and Chessell 2005). The introduction of multidisciplinary pain management programmes has been a milestone in teaching patients to live with their pain where it cannot be eliminated. At

the same time, multidisciplinary pain management programmes are effective in reducing pain and functional disability (Flor et al. 1992; Keefe et al. 2004; Patrick et al. 2004).

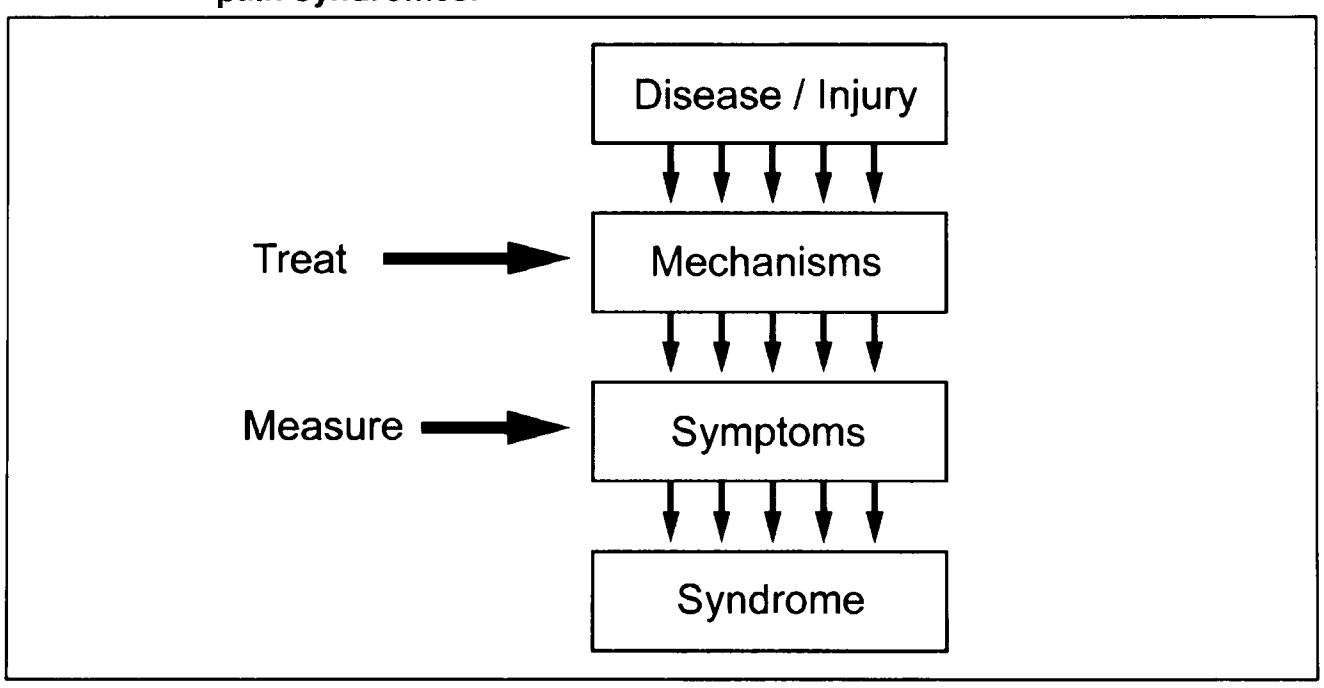
1.2 Neuropathic pain

Neuropathic pain, defined as pain caused by a lesion or dysfunction of the nervous system (Merskey and Bogduk 1994), attracts a lot of attention from basic research. Perhaps because it is often associated with intractable pain or because chronic pain following nerve injury constitutes a paradox: areas of skin with diminished sensitivity to touch or temperature exist alongside and even overlap with hypersensitive areas. Normally non-noxious stimuli may become painful (allodynia) or noxious stimuli may result in more pain than normal (hyperalgesia). Stimulus-evoked pain might be elicited by mechanical, thermal, or chemical stimuli. Mechanical allodynia is further classified as dynamic (brush-evoked) or static (pressure-evoked). In addition to stimulus-evoked pain, spontaneous pain is often present. Spontaneous pain is frequently described as a constant burning sensation, but can also present as pain paroxysms, such as intermittent shooting, lancinating sensations or electric shock-like pain. Paraesthesias (abnormal sensations) and dysaesthesias (abnormal unpleasant sensations) are also common symptoms of neuropathic pain. For a detailed overview of symptoms of neuropathic pain see (Scadding and Koltzenburg 2005).

Classifications of neuropathic pain have been based on the underlying disease (e.g. diabetic neuropathy, post-herpetic neuralgia, and so forth)

(Figure 1.1) or the site of lesion (e.g. peripheral nerve, spinal cord, etc.) (Cruccu et al. 2004). As it has become clear that hyperexcitability and plasticity of the nervous system are key phenomena in neuropathic pain and that treatment efficacy probably depends more on the underlying mechanism than on the aetiology (Sindrup and Jensen 1999), a new, mechanism-based classification has been proposed (Jensen et al. 2001; Woolf et al. 1998). Yet, it proves difficult to relate symptoms to their underlying mechanisms; especially as a symptom can be caused by multiple mechanisms and a mechanism can probably cause more than one symptom (Hansson 2003; Max 2000). Also, allodynia and hyperalgesia are not pathognomonic for neuropathic pain but also occur in other pain conditions, such as inflammatory diseases (Rasmussen et al. 2004).

Figure 1.2 Relation between aetiological factors, mechanisms, symptoms and pain syndromes.



Treatment of underlying mechanisms might be the ideal for pain management. The problem is how to identify these mechanisms. At present, symptoms are the only indication of underlying mechanisms; however, symptoms and mechanism are not equivalent. Adapted from Woolf and Max (Woolf and Max 2001).

1.3 Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is studied here as a representative of chronic inflammatory diseases. Affecting between 0.5 and 1 percent of the population worldwide (Lee and Weinblatt 2001), it is the most common inflammatory arthritis. It affects three times as many women as men and is most common in the fifth to eighth decades, albeit described throughout life. RA is a frequent cause of adult disability (Mili et al. 2002), with concomitant social and economic cost: 9.4 million working days are estimated to be lost in the United Kingdom per year because of RA (ARC 2004).

RA is immune-mediated but of unknown cause. Typically, it presents with morning stiffness, joint tenderness and swelling and some degree of systemic malaise, the latter most likely caused by non-specific inflammatory processes. RA mainly affects peripheral synovial joints, most often in a symmetrical fashion. Between 20% and 40% of patients have extra-articular features, for example rheumatoid nodules, pleural and pericardial effusions and vasculitis. The disease habitually runs a fluctuating course characterized by repeated attacks of inflammatory arthritis superimposed on periods of mild discomfort. Destruction of the joint space occurs progressively. Eventually, the inflammatory component of the disease recedes and the patient is left with the functional problems that are the consequence of ruined joints. For a recent overview of RA as a chronic pain condition see (Scott 2005).

Pain is the predominant symptom of RA and the primary reason for

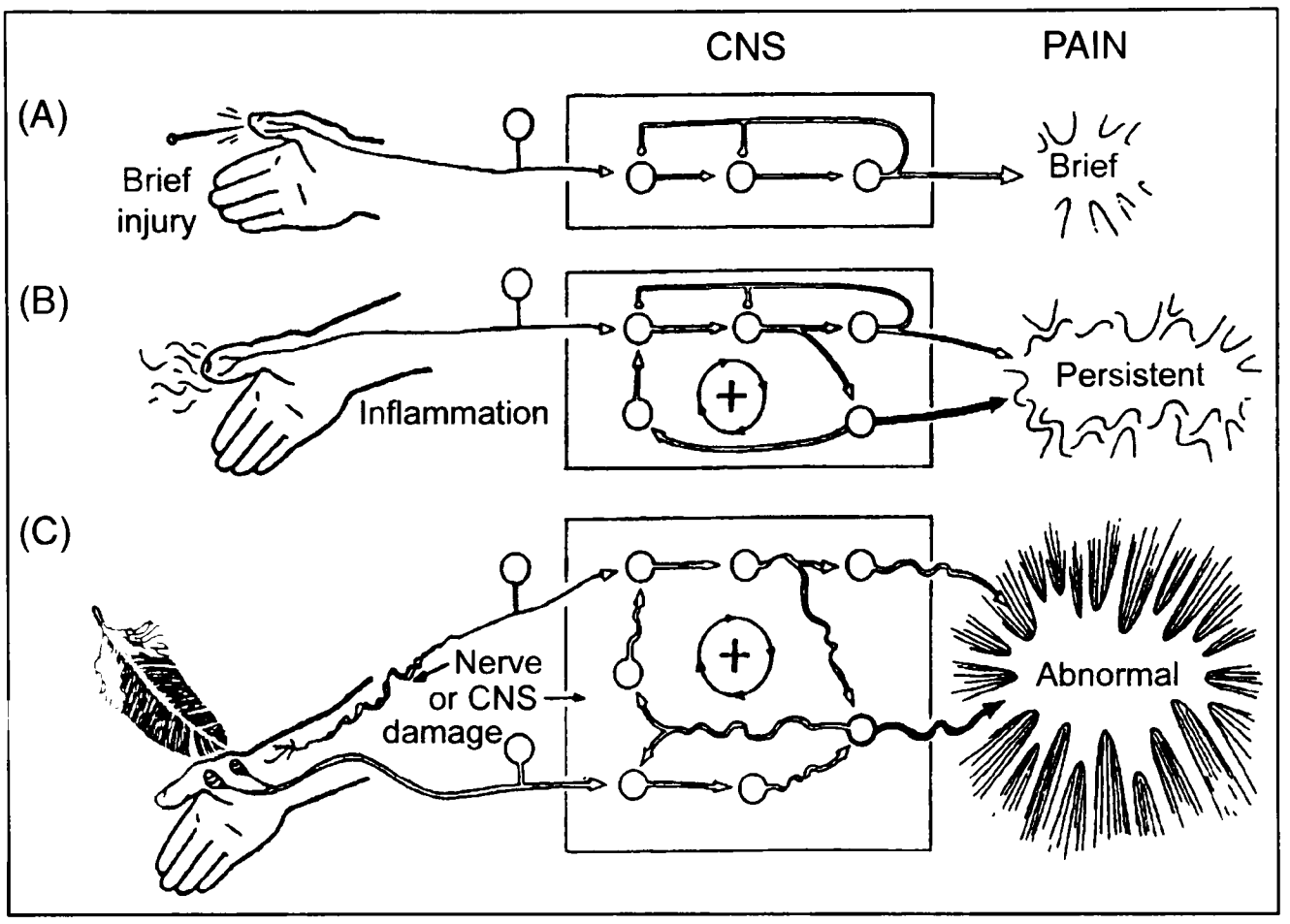
seeking medical attention (Hendiani et al. 2003; Kidd et al. 2000). Far from being a homogeneous entity, pain in RA is multifaceted. Patients may experience painful morning stiffness, pain on joint movement, joint tenderness on palpation, spontaneous joint pain and muscle aches associated with chronic systemic inflammation. In addition, neurological complications of RA may cause pain or discomfort: entrapment neuropathies, such as carpal tunnel syndrome, cervical myelopathy and peripheral neuropathy caused by rheumatoid vasculitis (Scott 2005).

1.4 Nociceptive processing

Nociceptive pain refers to the normal, acute pain sensation evoked by noxious stimuli in intact tissue. Transmission of nociceptive impulses from the periphery to the central nervous system (CNS) signals potential or pending tissue damage and allows initiation of nocifensive behaviour, for example to withdraw from the source of danger, to assume a protective posture or to initiate other measures to deflect harm to the organism. Rather than simply being transmitted, nociceptive information is modulated by the nervous system. Both amplification and inhibition occur under normal physiological circumstances. Temporary hyper-responsiveness of the pain system after injury provides protection and guarding and thereby promotes recovery. Hence, sensitization processes in the peripheral, and to some extent in the central, nervous system can occur as normal responses to pathology but are not necessarily pathological in themselves (Coderre and Katz 1997) (Figure 1.3). Yet, when peripheral nociceptive input continues, progressive structural changes occur in pain transmission pathways. Excessive hyperexcitability, by promoting excitotoxicity, cell

dysfunction and loss of inhibitory mechanisms, can lead to a pathological state (Moore et al. 2002). The damaged pain system has now become "the equivalent of a defective alarm system that produces false alarms" (Devor 2005) (Figure 1.3).

Figure 1.3 Schematic representation of pain with different degrees of CNS involvement



(A) Nociceptive pain following brief tissue injury with equally brief CNS responses; (B) prolonged nociceptive input, for example caused by inflammation, leads to increased responsiveness of dorsal horn neurons; (C) ongoing input, for instance from damaged nerve fibres, induces dorsal horn alterations so that information of non-nociceptive fibres (e.g. brush-sensitive Aβ-fibres) is transmitted to second order nociceptive neurons. CNS, central nervous system. Adapted from Cervero and Laird (1991).

The transmission and modulation of nociception and pain are integrated systems. However, a classification into "nociceptive processing", "alteration of pain processing" and "pain modulation" is used in the current thesis for the sake of clarity. Nociceptive processing is described in this section, touching on pain modulatory structures. The next section (1.5) covers alteration of pain processing, i.e. peripheral and central

sensitization and descending modulatory networks. Sections 1.6 and 1.7 address pain modulation by psychological factors.

From periphery to cerebral cortex

Nociceptive information is transmitted form peripheral tissues to the CNS by specialized fibres that are characterized by a high threshold to the adequate stimulus. Many nociceptors, a term coined by Sherrington in 1906 (Sherrington 1906), respond to a variety of different stimulus modalities such as heat, cold, chemical and mechanical ("polymodal"), in contrast to other cutaneous receptor types. Thinly myelinated (Aδ-) or unmyelinated (C-) fibres transmit nociceptive information to the dorsal horn at different conduction velocities (> 10 m/s and < 2 m/s, respectively) and are associated with the sensations of first and second pain.

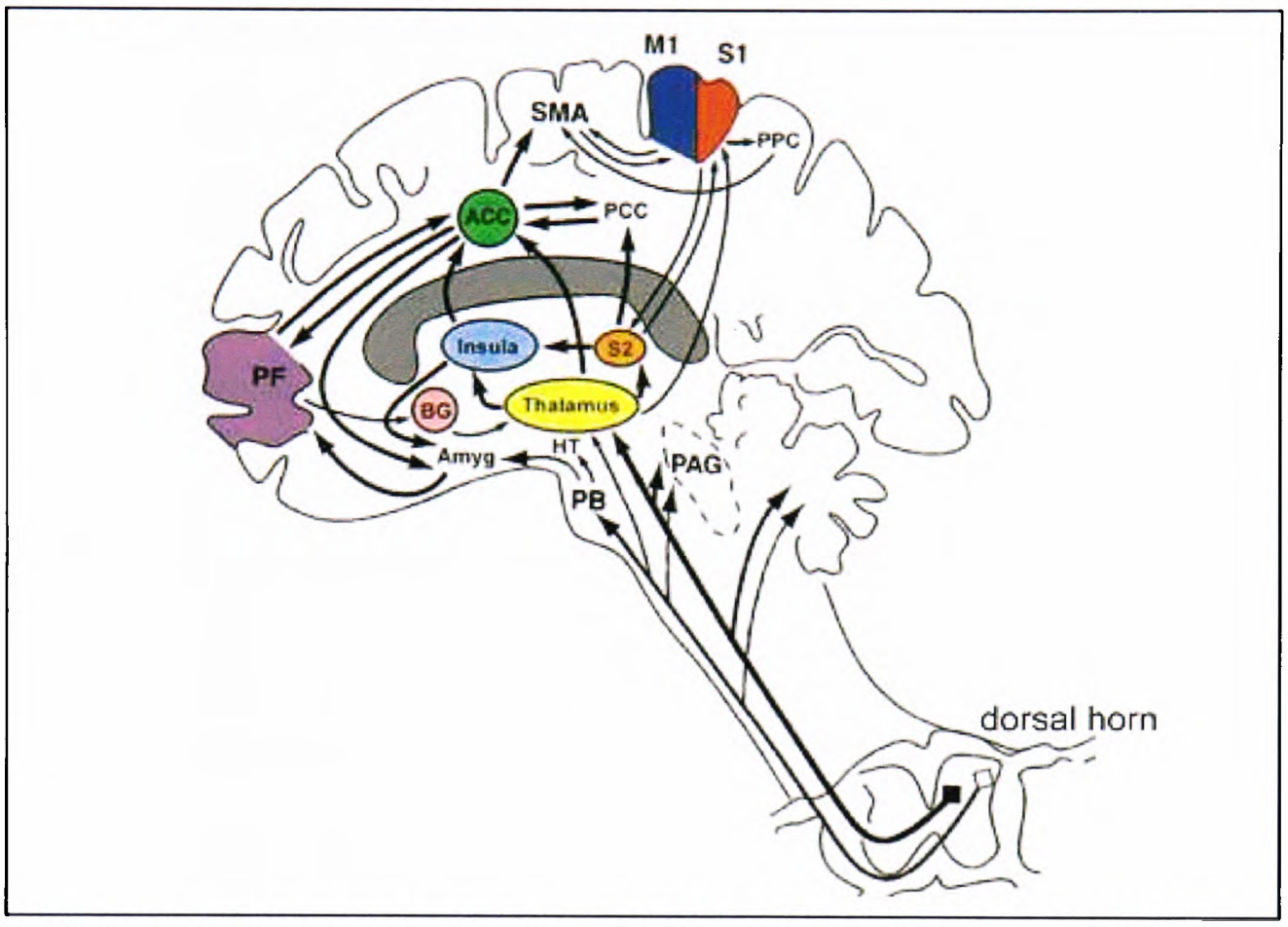
In the dorsal horn, Aδ- and C-fibres synapse with second order neurons, which give rise to ascending nociceptive pathways (Figure 1.4). These pathways project primarily to the thalamus (spino-thalamic tract), to the medulla and brainstem (spino-medullary and spino-bulbar projections) and to the hypothalamus (spino-hypothalamic tract), but there are other ascending nociceptive pathways. For example, a tract has been recently demonstrated to project from the dorsal horn to amygdala, hypothalamus, bed nucleus of the stria terminalis and to the globus pallidus (Braz et al. 2005). In addition to these direct projections, there are indirect projections to the forebrain, for example the postsynaptic dorsal column pathway which is now recognized to be important particularly in visceral pain (reviewed by Joshi and Gebhart (2000)).

The spino-thalamic tract (STT), which is an important ascending tract carrying nociceptive information, originates in the superficial layers of the spinal cord, laminae I and II, and in the deep dorsal horn, laminae IV and V, and projects to different, mainly contralateral, thalamic sites. In primates, primary and secondary somatosensory cortices (S1 and S2) receive noxious and innocuous input from lateral thalamus (Friedman et al. 1986; Shi and Apkarian 1995). Both regions contain neurons that code spatial, temporal and intensity information of noxious (and innocuous) stimuli (Chudler et al. 1990; Kenshalo et al. 1988; Kenshalo and Isensee 1983) and could be involved in sensory-discriminative aspects of pain processing. Medial thalamic nuclei, including the ventrocaudal portion of the medial dorsal nucleus and the parafasicular nucleus, project to anterior cingulate cortex (ACC) (Krettek and Price 1977; Wang and Shyu 2004).

ACC belongs to the brain areas described by Papez to constitute the limbic system (Papez 1937). Patients who have undergone cingulotomy show attenuated emotional responses to pain (Corkin and Hebben 1981; Foltz and White 1968; Foltz and White 1962) and functional imaging studies have substantiated the view that ACC is involved in processing the unpleasantness of painful stimulation (Rainville et al. 1997). Considering its important role in motor control, response selection and its relationship to the arousal and drive state of the organism, the ACC is in a “unique position to translate intentions to actions” (Paus 2001). With respect to pain, this is often termed the “motivational-affective” component (Melzack and Casey 1968). Yet, the affective element might have been over-

emphasized in some imaging studies; perhaps because experimental restraints make it more difficult to investigate response selection and goal-directed behaviour than to assess the unpleasantness of a painful stimulus.

Figure 1.4 Schematic of pain processing



Cortical and subcortical regions involved in pain processing, their inter-connectivity and ascending pathways. It should be noted that this diagram represents a simplified view of pain pathways in healthy volunteers because it only depicts "bottom-up" processing and does not show any descending modulation, neither facilitation nor inhibition. PB, parabrachial (nucleus); PAG, periaqueductal grey; HT, hypothalamus; Amyg; Amygdala; BG, basal ganglia; S2; secondary somatosensory cortex; PF, prefrontal cortex; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; SMA, supplementary motor cortex; M1, primary motor cortex; S1, primary somatosensory cortex; PPC, posterior parietal cortex. Adapted from Apkarian and colleagues (Apkarian et al. 2005) and originally based on Price (Price 2000).

The insular cortex, which is the most frequently activated cortical structure in human pain imaging studies (Apkarian et al. 2005), has emerged to be key in pain processing (Apkarian et al. 2005; Craig 2003b; Peyron et al. 2002). Inasmuch the ACC is sometimes referred to as the "limbic motor cortex", the insula could be considered as the "limbic sensory cortex". It is in an ideal position to relate sensory information from the external world to

the body's internal milieu: while the posterior insula is engaged in sensory aspects of nociceptive processing, the anterior insula is densely connected to limbic and autonomic areas (Chikama et al. 1997; Mesulam and Mufson 1982a; Mesulam and Mufson 1982b). The posterior insula receives nociceptive-specific input from dorsal horn lamina I via the posterior part of the ventral medial nucleus (VMpo) of the thalamus (Craig 2003b), is somatotopically organised (Brooks et al. 2005; Craig 1995) and encodes stimulus intensity (Craig et al. 2000). The anterior insula has been implicated in the subjective experience of pain and is discussed in detail in Chapter 4.

The role of the prefrontal cortex (PFC) is most likely a modulatory one, given its involvement in high order cognitive functions (Ridderinkhof et al. 2004). In accordance with this, not all pain imaging studies report PFC activation and PFC seems preferably engaged when nociceptive processing is associated with increased cognitive or emotional load (Apkarian et al. 2005). Specific functions of prefrontal subregions are discussed in Sections 1.6 and 1.7.

Aside from thalamus, many other subcortical structures play a role in pain processing. In addition to thalamus, basal ganglia, cerebellum, brainstem and amygdala are frequently activated in human pain imaging studies (Peyron et al. 2000a; Tracey 2005). The functions of these areas in pain processing are gradually being decoded and may prove to be more sophisticated than previously appreciated. For instance, the notion that the role of the cerebellum and basal ganglia is limited to the control of

movement has been challenged in recent years (Middleton and Strick 2000). Both regions contain nociceptive neurons and a direct nociceptive pathway projecting from spinal cord to globus pallidus has been discovered (Braz et al. 2005). It is now thought that basal ganglia may play a crucial role in anti-nociception in the presence of motivational drives that compete with nociceptive stimuli for attentional resources, such as food or sex (Fields 2005).

The amygdala, which receives direct and indirect spinal projections of ascending pain pathways (Barnett et al. 1995; Bernard et al. 1989; Braz et al. 2005) and contains nociceptive neurons (Bernard et al. 1992), may be important for the analgesic effect of systemic morphine (Nandigama and Borszcz 2003; Rodgers 1977) and is engaged in fear-induced anti-nociception (Helmstetter and Bellgowan 1993). In chronic pain conditions, however, the amygdala may enhance nociception via negative emotions (Neugebauer et al. 2004). This possibility is further discussed in Section 5.4.

Spino-bulbar projections target several areas in the brainstem, including periaqueductal grey (PAG) and parabrachial nucleus (PBN). Brainstem regions are important in integrating nociceptive information with autonomic, neuroendocrine and emotional aspects of pain processing. PBN contacts areas such as the amygdala and hypothalamus which in turn project to PAG and indirectly to the rostroventral medulla. By driving a spino-bulbo-spinal loop, brainstem structures are essential in descending modulation, which is discussed in more detail in Section 1.5.3.

Thus, there is evidence that multiple ascending pathways are engaged in signalling nociceptive information that is ultimately integrated at a cortical level. Individual pathways could change their relative contributions to signalling in altered states of pain processing; for instance, information could travel to a larger extent in spinal-parabrachial-amygdala or spinal-parabrachial-hypothalamus connections in chronic pain conditions. This is discussed in more detail in Section 1.5.3.

1.5 Alterations of pain processing

In clinical pain, sensitization of the pain system occurs at several levels of the neuraxis. In this section, peripheral sensitization, sensitization manifest at the level of the spinal cord (central sensitization) and facilitation and inhibition at the level of the brainstem are described.

1.5.1 Peripheral sensitization

In the periphery, tissue injury triggers a cascade of inflammatory events leading to hypersensitivity (*peripheral sensitization*). The stereotypical inflammatory response includes the release of inflammatory mediators and the infiltration of affected tissues by activated immunocompetent cells. Some of these mediators activate peripheral nociceptors directly (e.g. bradykinin) (Dray and Perkins 1993); others, such as tumour necrosis factor (TNF) and Interleukin-1 (IL1), sensitise peripheral nociceptor terminals to subsequent stimuli, e.g. prostaglandins and pro-inflammatory cytokines (Opree and Kress 2000). These processes are involved in the inflammation-induced increase in nociceptor excitability that develops following tissue injury, such as sunburn. They are also thought to

contribute to the maintenance of sensitization in the presence of ongoing or chronic inflammation, for example in RA (Levine et al. 1993; Martin et al. 1987).

1.5.2 Central sensitization

Traditionally, central sensitization (CS) has been tightly linked to neuropathic pain, but it is becoming more and more accepted that this phenomenon can also be observed in other chronic pain conditions, such as inflammatory diseases (Kidd et al. 1995; Leffler et al. 2002; Morris et al. 1997). CS is characterized by increased responsiveness of second order nociceptive neurons in the dorsal horn, which in turn can be caused either by hyperexcitability of the pain transmission neurons themselves or by decreased activity of inhibitory inter-neurons. Prolonged C-fibre input, for example caused by spontaneous activity of injured nerves, is thought to induce CS (Seltzer et al. 1991; Wall and Woolf 1986). CS can manifest in three ways: enlargement of receptive fields, increased response to supra-threshold stimuli and reduction of the activation threshold whereby previously sub-threshold stimuli evoke an action potential. These phenomena correspond to the clinical characteristics of neuropathic pain, such as referred pain, hyperalgesia and allodynia, respectively. Because primary afferent fibres converge to second order nociceptive neurons, hyperalgesia and allodynia spread beyond the territory of the primarily affected nerve. Conventionally, CS was seen as a purely neuronal process. However, there is a growing body of evidence that suggests CS also involves activation of glial cells in the spinal cord, which release pro-inflammatory cytokines and other substances thought to increase neuronal

excitability (Watkins and Maier 2002). Indeed, the pro-inflammatory cytokines TNF, IL1 and IL6 are implicated in the generation of allodynia in animal models of neuropathic pain (DeLeo et al. 1996; Tadano et al. 1999).

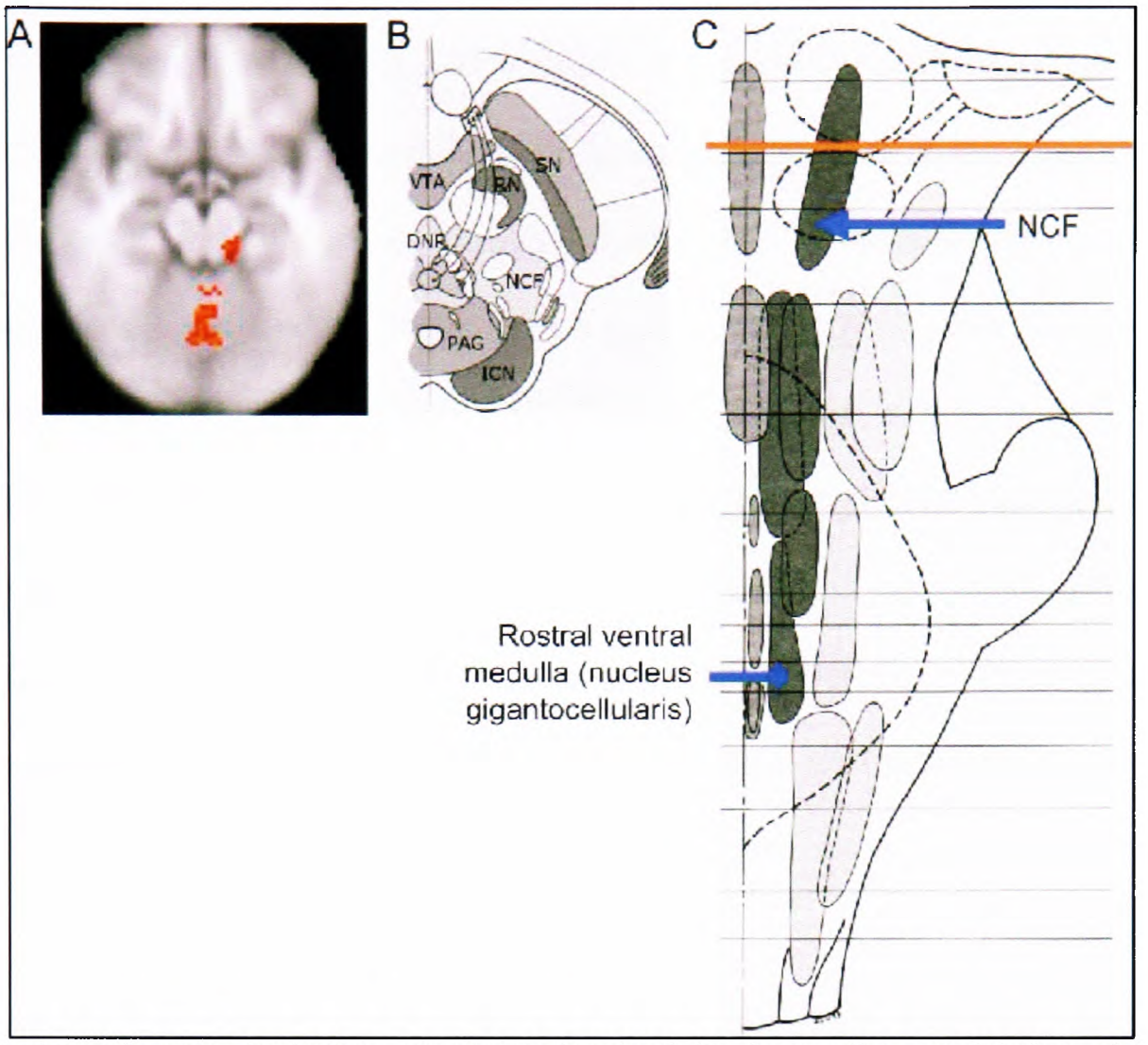
1.5.3 Descending modulatory networks

Transection of the spinal cord in a normal animal leads to a general increase in spinal excitability, resulting in facilitation of nociceptive reflexes (Sherrington 1906). In contrast, abnormally increased responses to mechanical and thermal stimuli in animal models of neuropathy and inflammation are abolished by thoracic spinal cord transection (Bian et al. 1998; Kauppila 1997; Sun et al. 2001; Sung et al. 1998). This illustrates that supraspinal sites contribute to facilitation and inhibition of nociceptive information processing. Bidirectional influences can be exerted by several brainstem structures such as the rostroventral medulla (RVM), cuneiform nucleus and PAG (Fields and Heinricher 1985) (Figure 1.5). All these structures contain ON and OFF cells, which have the ability to facilitate or inhibit spinal transmission, respectively (Heinricher et al. 1989). The RVM is an important relay in the descending projection of facilitatory influences and is perhaps the final common output. ON cells in the RVM have large somatic receptive fields which means they can influence nociceptive processing outside the primary affected area. Activation of facilitatory systems is thought to result from prolonged noxious input (Porreca et al. 2002) – much in the manner of the postulated ongoing C-fibre input in central sensitization. In acute nociception, it is likely that facilitatory and inhibitory systems can be activated simultaneously (Porreca et al. 2002).

In contrast, the balance might tip to the facilitatory side in chronic pain states. The idea that supraspinal influences are crucial in the maintenance of chronic pain is supported by experiments that show *complete* reversal of hyperalgesia in an animal model of neuropathic pain by destruction of the RVM (Bian et al. 1998; Kauppila 1997; Sun et al. 2001; Sung et al. 1998). Serotonin (5-HT) has been described to be an important transmitter in descending facilitatory pathways in animals (Suzuki et al. 2004). In humans, the 5-HT antagonist ondansetron alleviates mechanical allodynia in neuropathic pain (McCleane et al. 2003) and is also effective against diffuse widespread pain in fibromyalgia (Papadopoulos et al. 2000), suggesting that descending facilitation mediated via 5-HT containing pathways might contribute to these pain states.

Although brainstem structures are crucial, they are not necessarily the origin of descending modulation. For instance, midcollicular decerebration, i.e. transection at the upper level of the brainstem, prevents facilitatory effects of vagal stimulation (Randich and Gebhart 1992), and high frequency stimulation of ACC in the rat induces facilitation of the tail flick reflex via the RVM (Calejesan et al. 2000). Equally, descending inhibition induced by cognitive processes such as distraction has been shown in humans to involve brainstem structures (Tracey et al. 2002).

Figure 1.5 Brainstem structures involved in pain



Overview of some brainstem structures involved in descending modulation of pain processing, namely cuneiform nucleus (NCF), periaqueductal grey (PAG) and rostral ventral medulla. (A) and (B) show an axial section at the level of the red line in (C). (A) depicts activation in the NCF during experimental secondary hyperalgesia, in accordance with a role of the NCF in pain facilitation in humans (Zambreanu et al. 2005). (C) shows a coronal section through the brainstem. VTA; ventral tegmental area; SN; substantia nigra; RN, red nucleus; DNR, dorsal raphe nucleus; ICN, intercolliculus nucleus. Schematics in (A) and (C) are reproduced from Duvernoy (Duvernoy 1995) and the figure is adapted from Zambreanu and colleagues (Zambreanu et al. 2005).

1.6 Modulation of pain processing by psychological factors and potential neuroanatomical correlates

Section 1.5 essentially described alterations in nociceptive transmission, leading to changes in pain perception. In addition, pain can be altered by attentional, cognitive and emotional factors. Evidence suggests that psychological factors influence nociceptive transmission as low as at the level of second order nociceptive neurons. Direct electrical recordings in monkeys for example showed that distraction leads to decreased firing of

medullary second order nociceptive neurons (Duncan et al. 1987). As discussed in Section 1.5.3, alterations in descending modulatory systems are likely to contribute to chronic pain states. On the one hand, impairment of descending inhibitory pathways might occur in chronic pain; on the other hand, descending facilitation might be enhanced. But the neural representation of pain in the brain also provides an interface by which bodily sensations, such as nociception, and psychological factors could interact. To date, the relative contributions of descending systems modulating ascending nociceptive transmission and pure cerebro-cerebral interactions that alter the perception of a noxious stimulus are not known for most psychological factors.

Psychological factors influencing pain perception are manifold. Just as distraction decreases pain perception (Miron et al. 1989), attention to pain increases it (Bushnell et al. 1985). Similarly, induction of positive emotions decreases pain and negative emotions increase pain under experimental conditions (de Wied and Verbaten 2001; Lewkowski et al. 2003; Rainville et al. 2005; Weisenberg et al. 1998; Whipple and Glynn 1992; Zelman et al. 1991). A recent study showed that also pain-related sadness and anger, induced by hypnosis, increase the perceived intensity of a constant nociceptive stimulus in healthy volunteers (Rainville et al. 2005).

Chronic pain differs from acute pain with respect to psychological states. Epidemiologically, anxiety-related and depressive disorders show a substantial overlap with chronic pain (Kremer and Atkinson 1983; Romano and Turner 1985; Sternbach 1968), and negative affect, such as

depressed mood and anxiety, is related to the magnitude of daily pain in chronic pain patients (Linton and Gotestam 1985). The neuronal circuitry underlying the, probably reciprocal, relationship between chronic pain and negative affect is largely unknown. That said, limbic structures, such as anterior insula, ACC, amygdala, entorhinal cortex and medial prefrontal cortex, are candidate regions to mediate such a relationship. These areas are not only densely inter-connected (Carmichael and Price 1995; Ongur and Price 2000; Swanson 1981) but have also multiple projections to brainstem structures (Da Costa Gomez et al. 1996; Jasmin et al. 2004; Krettek and Price 1978). This means that limbic structures could interact with descending systems that influence nociceptive processing. In humans, functional brain imaging has been used to demonstrate that anxiety-induced increases of pain perception are linked to activation of limbic structures in the medial temporal lobe (MTL) (Ploghaus et al. 2001). Interestingly, prolonged pain in animal models of chronic arthritic pain leads to enhanced responsiveness in a subset of amygdala neurons (Neugebauer and Li 2003). At the same time, the amygdala is connected via PAG and RVM to the 5-HT descending facilitatory pathway which increases transmission at spinal level (Suzuki et al. 2004). This could be an important circuitry by which nociceptive transmission and emotional aspects reinforce each other.

Chronic stress might provide an additional explanatory framework for the link between pain and depression, particularly in chronic inflammatory conditions. Chronic pain can be regarded as an inescapable and therefore chronic stressor and chronic stress in turn induces dysfunction of the

hypothalamus-pituary-adrenal (HPA) axis (Blackburn-Munro and Blackburn-Munro 2001). At the same time, chronic stress in known to be an important risk factor for depression (Pancner and Jylland 1996) and HPA axis dysfunctions are a common finding in major depression (Barden et al. 1995; Linkowski et al. 1987). Interestingly, patients with RA (Bomholt et al. 2004; Huyser and Parker 1998) as well as rats with adjuvant-induced arthritis (Shanks et al. 1998) have changes of the HPA-axis that are similar to those induced by chronic stress. This theory might be of particular relevance for autoimmune conditions, such as RA, in which susceptibility to stress-induced dysregulation of homeostatic functioning has been described (Zautra et al. 1999). RA patients are more reactive to interpersonal stressors than matched patients with osteoarthritis (OA) and the negative affective response to interpersonal stressors predict subjective and objective disease activity in RA (Zautra et al. 1999).

Supraspinal structures can not only enhance the sensation of pain. It has been reported anecdotally for a long time that the perception of pain can be blocked under certain circumstances, a famous example being the soldier wounded in battle. It is now well established that disinhibition of opioidergic transmission in the brainstem contributes substantially to anti-nociception in fight or flight situations (Fields et al. 1983; Watkins and Mayer 1982). But this endogenous opioid network is not restricted to the brainstem. Opioid injections in frontal cortex and amygdala produce powerful analgesic effects in animals (Fields et al. 1991) and human PET studies have confirmed high opioid receptor density in several forebrain structures, including the ACC (Sadzot et al. 1991). Recent brain imaging

studies suggest that the endogenous opioid circuitry plays an extraordinary role in endogenous pain control in humans. Placebo-induced analgesia (PiA) is an extensively studied example of endogenous pain suppression in humans. Attenuated pain perception manifests itself in decreased activity in pain-related brain areas, accompanied by increased activation of ACC and brainstem structures, which are thought to mediate PiA (Bingel et al. 2006; Petrovic et al. 2002; Wager et al. 2004).

Another cortical structure that has recently been proposed to be involved in pain control in humans is the lateral prefrontal cortex (PFC) (Lorenz et al. 2002; Mayer et al. 2005). Indirect evidence from correlation and functional connectivity analyses suggests that lateral PFC exerts control over brainstem structures. In cognitive neuroscience, the lateral PFC is associated with higher cognitive functions and in particular with the implementation of control processes (Ridderinkhof et al. 2004). Hence, the lateral PFC could provide a neural interface by which cognitive processes can modulate pain perception. This is discussed in more detail in Chapter 5.

1.7 Coping with chronic pain

Coping refers to the "cognitive, emotional and behavioural strategies patients employ in their day-to-day attempt to manage the consequences of their disease" (Covic et al. 2000). Psychological studies of chronic pain patients have indicated that coping alters prognosis with respect to pain (Jensen et al. 1991). Three main strategies have been identified that are predictive of behavioural and emotional adjustment to chronic pain: (1) cognitive coping and suppression, (2) helplessness and (3) diverting attention and praying or hoping (Rosenstiel and Keefe 1983).

Pain catastrophizing is considered a "maladaptive" coping strategy because it is associated with increased pain and decreased functionality (Geisser et al. 1994b; Keefe et al. 1989). Catastrophizing refers to the tendency to focus excessively on pain sensations, to exaggerate their threat value, and to feel helpless in the effort to reduce or manage pain (Sullivan and Neish 2000). Catastrophizing during painful stimulation contributes to more intense pain and increased emotional distress (Sullivan et al. 2001b) and catastrophizing is the most powerful predictor of pain chronicity for some chronic pain conditions (e.g. back pain) (Burton et al. 1995). Consequently, many cognitive-behavioural interventions include modules that are designed to reduce the tendency of pain patients to catastrophize (Sullivan et al. 2005b; Vlaeyen et al. 2002). Flor et al. observed that patients, mainly with rheumatic disorders and chronic back pain, who improved after treatment showed a reduction in catastrophizing, whereas those who did not improve failed to reduce their levels of

catastrophizing (Flor et al. 1993). The mechanisms by which catastrophizing influences the pain perception are not fully understood. Heightened attention, perception of threat, expectation of pain and perceived lack of control are all factors that might provide a link between catastrophizing and increased pain experience (Crombez et al. 1998; Crombez et al. 2004; Jackson et al. 2005; Rosenstiel and Keefe 1983; Sullivan et al. 2001a).

Cognitive modulators of pain could exert their influences, at least partly, via pain modulatory circuits described above and in Section 1.5.3, including the opioidergic network. For example, opioid-mediated hypoalgesia is compromised when the stressor is perceived as uncontrollable (Bandura et al. 1988) and the analgesic effect of self-efficacy (the conviction that one can successfully execute a course of action to produce a desired outcome) is blocked by the opioid-antagonist naloxone (Bandura et al. 1987). Cortical representations of such processes are only starting to be investigated and it is not well established how cognitive processes could modulate pain processing. At this stage, it seems likely that cognitive factors might involve cortico-cortical loops or decreasing systems acting on spinal cord neurons, or both.

1.8 Study aims and hypotheses

This thesis had two major goals. The first aim was to investigate if pain processing is fundamentally altered in patients with chronic pain conditions. This question was sub-divided into three components. Firstly, it was examined if the processing of experimental pain differs in patients and healthy control subjects. Secondly, the neural correlates of clinical pain in patients were compared with those of experimental pain in healthy subjects. Lastly, it was investigated if experimental pain and clinical pain are processed differently in the same patient population. The second major goal of this work was to establish if the FMRI (functional magnetic resonance imaging) signal reflects the perceived pain intensity in patients and if it can be used to identify longitudinal variations of perceived pain intensity.

These goals were pursued via the following experiments. In Chapter 3, comparison of experimental pain processing in neuropathic pain patients and control subjects was performed. On the one hand, it was hypothesized that brain activation in neuropathic pain patients and control subjects overlap to a large extent as experimental nociceptive stimuli were applied to a body site that was not clinically affected in any of the patients. On the other hand, it was tested if subtle differences exist in the processing of experimental pain between patients and controls. Animal models of neuropathic pain have indicated that brainstem mechanisms can facilitate processing of stimuli that are applied outside classical areas of secondary hyperalgesia (Porreca et al. 2002). It was therefore

examined if activation in brainstem structures and in brain areas that are involved in the early stages of nociceptive processing suggests facilitated processing in patients. Also, chronic pain is associated with an increased incidence of affective disorders, such as anxiety and depression (Linton and Gotestam 1985; Romano and Turner 1985), which in turn have been shown to influence pain perception (Affleck et al. 1992; Rainville et al. 2005; Zelman et al. 1991). Consequently, it was investigated if patients display higher activation in areas that process affective aspects of pain.

A meta-analysis of the neural correlates of clinical and experimental pain in the anterior insula is presented in Chapter 4. The insular cortex is not only implicated in pain processing but in the processing of stimuli that signal the physiological state of the body, including hunger, thirst, warmth, cold and air hunger. The anterior portion is thought to be involved in representing these bodily sensations as subjective feelings (Craig 2002; Critchley et al. 2004; Damasio et al. 2000). As clinical pain is likely to carry more meaning for the individual and to be associated with emotions different to those of experimental pain, the anterior insula is a candidate region where processing of experimental and clinical pain could differ.

Chapter 5 addresses the possibility that chronic pain patients process clinical and experimental pain differently. Specifically, the influence of an emotional variable, i.e. depression, and of a cognitive variable, i.e. catastrophizing, on pain processing was compared between administration of experimental heat pain and provocation of clinical pain in patients with rheumatoid arthritis. It was hypothesized that depression and

catastrophizing influence pain processing and perception to a larger extent in clinical pain than in experimental pain.

The neural correlates of clinical allodynia in neuropathic pain patients are presented in Chapter 6. Previous studies investigating the processing of brush-evoked allodynia (Petrovic et al. 1999; Peyron et al. 2004) were extended by two queries. First, it was examined if ongoing pain influences the processing of allodynic pain. Second, it was investigated if the magnitude of the FMRI signal reflects the perceived intensity of allodynic pain and might thus be useful as a surrogate marker of pain perception.

Whereas Chapter 6 focussed on the question of whether the FMRI signal encodes the perceived pain intensity across patients, Chapter 7 is devoted to whether the FMRI signal can detect longitudinal changes in pain intensity within subjects. A surrogate marker of clinical pain intensity would be especially useful if it reflected pharmacological modulation of pain processing. As a first step in the development of FMRI as marker of analgesic drug effect in patients, the regular analgesic medication of neuropathic pain patients was altered and participants underwent FMRI investigation before and after drug modulation.

CHAPTER 2: Methods

2.1 Background

In this section, a short introduction to magnetic resonance imaging (MRI), the principles of functional MRI (FMRI) and FMRI image analysis is given to provide the methodological background for this thesis.

2.1.1 Magnetic resonance imaging

Nuclear spin is a property of atomic nuclei with an odd number of protons or neutrons, such as Hydrogen (^{1}H), Carbon (^{13}C) or Phosphorus (^{31}P). Because nuclei are charged, the spin creates a magnetic moment and this is exploited for MRI. MRI is preferentially based on ^{1}H nuclei because of their high concentration in the human body. Nuclear spin can be thought of as rotation of the magnetic moment around its own axis (Figure 2.1A). When an external magnetic field is applied, the spins align with respect to the principal axis of the magnetic field (z-axis) and precess around this axis (Figure 2.1). The frequency with which the nuclei precess is given by the Lamour equation:

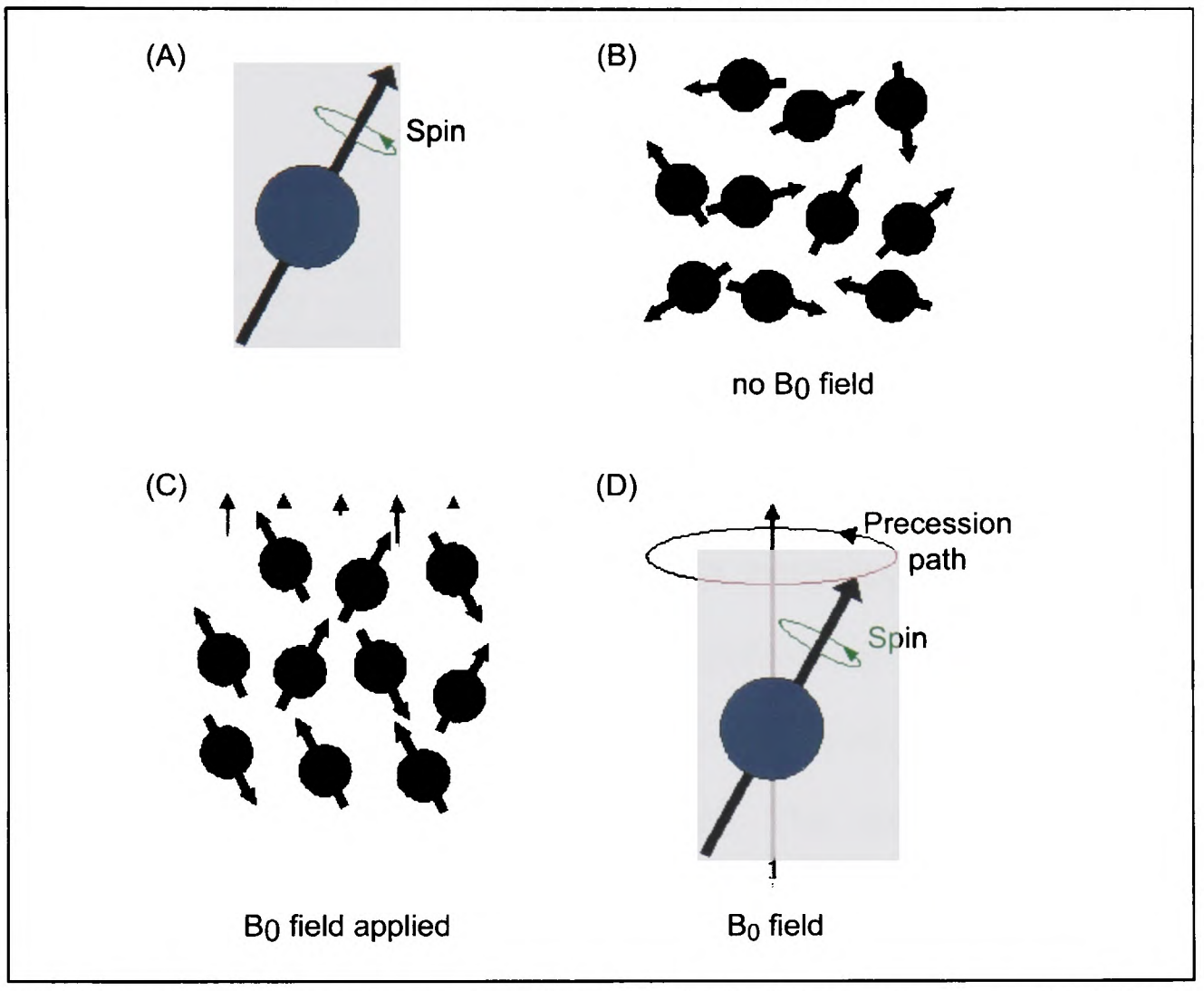
$$f = \gamma * B_0$$

where γ is the gyromagnetic constant, characteristic for the nucleus, and B_0 is the strength of the external magnetic field.

Only quantum mechanics allow a full description of nuclear magnetic resonance (NMR), but depiction of the nuclei as vectors helps to visualize important principles. ^{1}H nuclei can align either with the magnetic field ("parallel") or against it ("anti-parallel"). As the parallel state is the lower

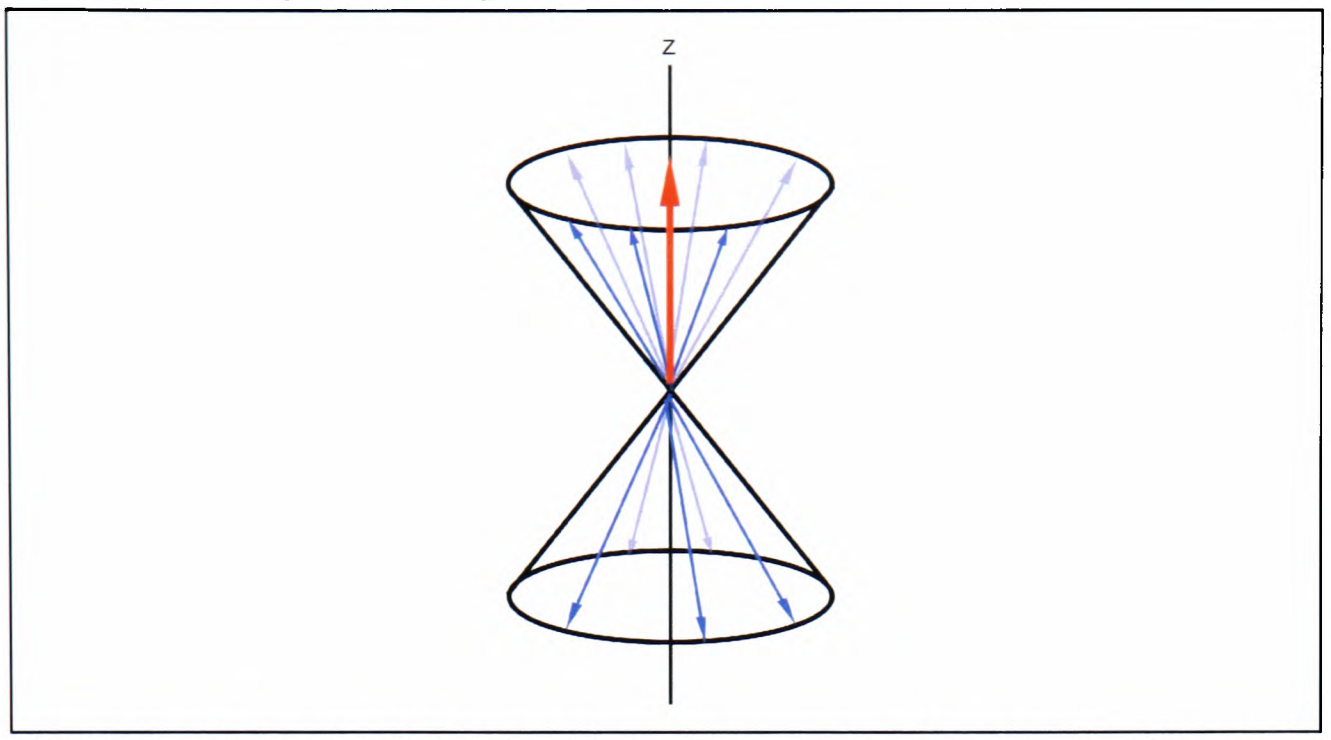
energy state, slightly more nuclei align with the field than anti-parallel to it and the resulting magnetization vector, i.e. the net magnetic moment, is parallel to the z-axis of the magnetic field B_0. Net magnetization in z-direction is called longitudinal magnetization or M_z (Figure 2.2).

Figure 2.1 Nuclear spins and their behaviour in a magnetic field



(A) The nuclear spin creates a magnetic moment. (B) When no magnetic field (B_0) is applied, the nuclei are randomly oriented. (C) When a magnetic field is applied, the nuclei align with the field ("parallel") or against it ("anti-parallel"). (D): The nuclei precess around the axis of the magnetic field. Figure adapted from Jezzard and Clare (2001).

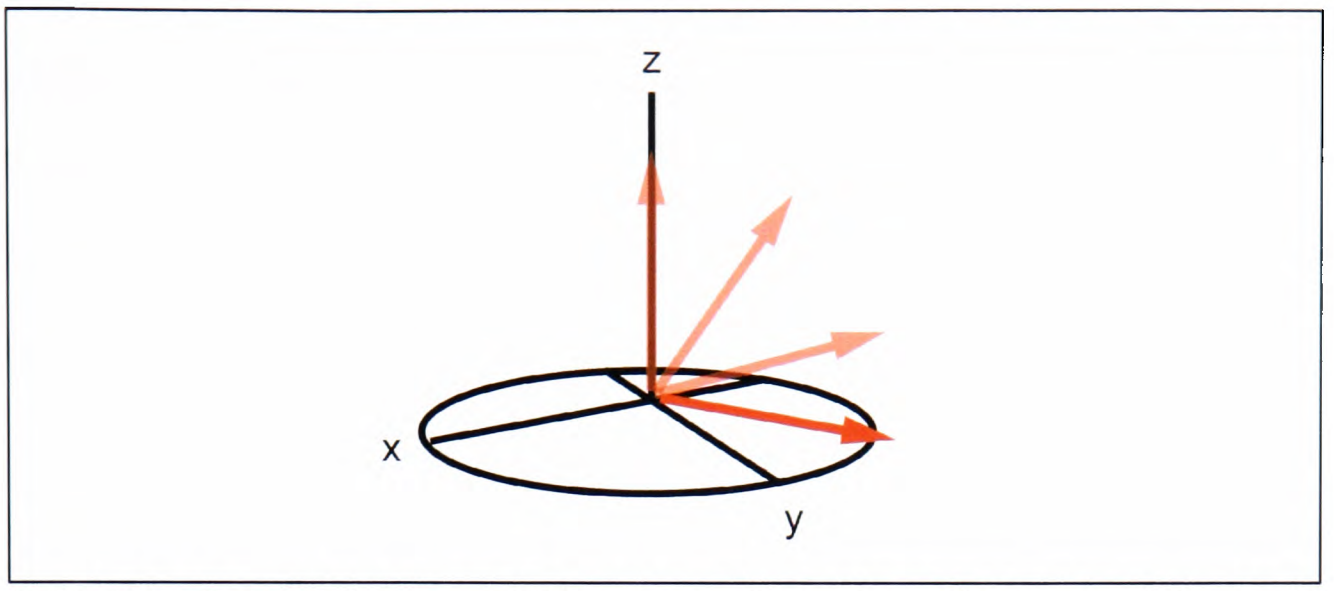
Figure 2.2 Longitudinal magnetization



At equilibrium, the nuclei (depicted by blue arrows) precess randomly around the z-axis of the magnetic field. As more spins are aligned with than against the field, the resulting sum vector of the magnetic moment (net magnetic moment, depicted by the red arrow) is oriented parallel to the z-axis (longitudinal magnetization, M_z).

Radiofrequency (RF) pulses are transmitted to the sample under investigation from a transmitter coil. If the frequency of the RF pulse matches the Lamour frequency of the precessing nuclei, energy is transmitted to the nuclei and some of the nuclei are elevated to the higher energy level, i.e. to the anti-parallel state. This means that the net magnetization in z-direction becomes smaller. In addition, the RF pulse causes the nuclei to precess *in phase*, thereby creating a transverse component to the net magnetization. In the vector model, this can be depicted as the net magnetic moment being "flipped" away from the z-axis to the xy- plane (Figure 2.3) and accordingly, net magnetization in the xy-plane is called M_{xy}. The angle by which the net magnetic moment is flipped depends on the strength and duration of the RF pulse ("flip angle"). By definition, a 90° pulse flips the net magnetic m oment completely into the xy- plane (Figure 2.3). The precessing transverse net magnetization generates an oscillating electrical signal in a surrounding receiver coil.

Figure 2.3 Behaviour of the net magnetic moment when an RF pulse is applied



Application of a radiofrequency (RF) pulse at the resonant frequency of the nuclei elevates some nuclei to a higher energy level, i.e. the anti-parallel state. When as many nuclei are in the anti-parallel as in the parallel state, the resulting net magnetization is fully in the xy- plane (i.e. M_{xy} is maximal) and no magnetization is left in z-direction (i.e. $M_z = 0$).

Image contrast

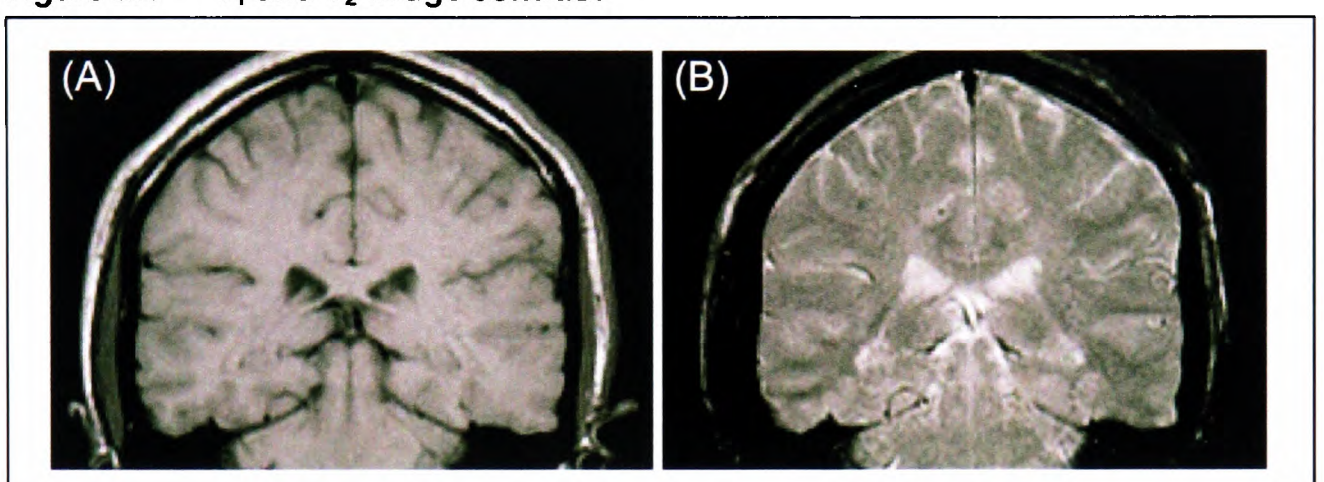
There are different ways in which contrast can be generated in MR images. The recorded signal is maximal immediately after an RF pulse because the magnetization in the xy- plane (where signal can be detected) is maximal. If the signal is recorded at this point, the strength of the signal depends only on the density of ^1H nuclei, resulting in proton-density weighted images. As soon as energy transmission into the system stops, the MR signal decays due to two separate processes, which can both be exploited to create image contrast. On the one hand, there is a recovery of M_z and on the other hand, M_{xy} decreases. Recovery of M_z after an RF pulse occurs because individual nuclei revert back to the lower energy state (parallel with the external field). The rate of recovery is described by the longitudinal relaxation time, T_1. T_1 is different for different types of tissue (Damadian and Cope 1974). There are several ways by which T_1-weighting can be manipulated. Firstly, if the time between two RF pulses (repetition time, TR) is short, tissues with a long T_1 time have not

recovered their full longitudinal magnetization when the next RF pulse is applied and have low signal intensity on T_1-weighted images (Figure 2.4A). Secondly, T_1 contrast can be manipulated by changing the flip angle of the RF pulse because nuclei need a longer time to recover their longitudinal magnetization from a larger flip angle.

As described above, application of an RF pulse causes all nuclei to precess *in phase*, resulting in a transverse component of the net magnetic moment. In an ideal sample and a perfect magnet all nuclei would experience the same magnetic field and transverse magnetization would remain coherent and strong until T_1 processes occur. Yet, random fluctuations of the Lamour frequencies at a molecular level cause the transverse magnetization to decay much more quickly (on a scale of milliseconds whereas longitudinal magnetization is recovered within seconds). Nuclei precessing with slightly different Lamour frequencies lose their phase coherence and as a result, transverse magnetization and the detected signal decrease (Figure 2.5). The rate of the decay is described by the transverse relaxation time, T_2. Different degrees of T_2-weighting are typically achieved by varying the echo time TE, which describes the time between an RF pulse and the read-out of the signal. If TE is long, nuclei in most tissues have dephased and the signal that is left to give contrast stems mainly from water, which has a long T_2 time (Figure 2.4B). A related decay parameter, T_2^*, is exploited for functional imaging (see Section 2.1.2). T_2^* is determined by local field inhomogeneities, which cause further dephasing of the nuclei (due to a larger spread of Lamour frequencies). Therefore, T_2^* is even shorter than T_2. Local field

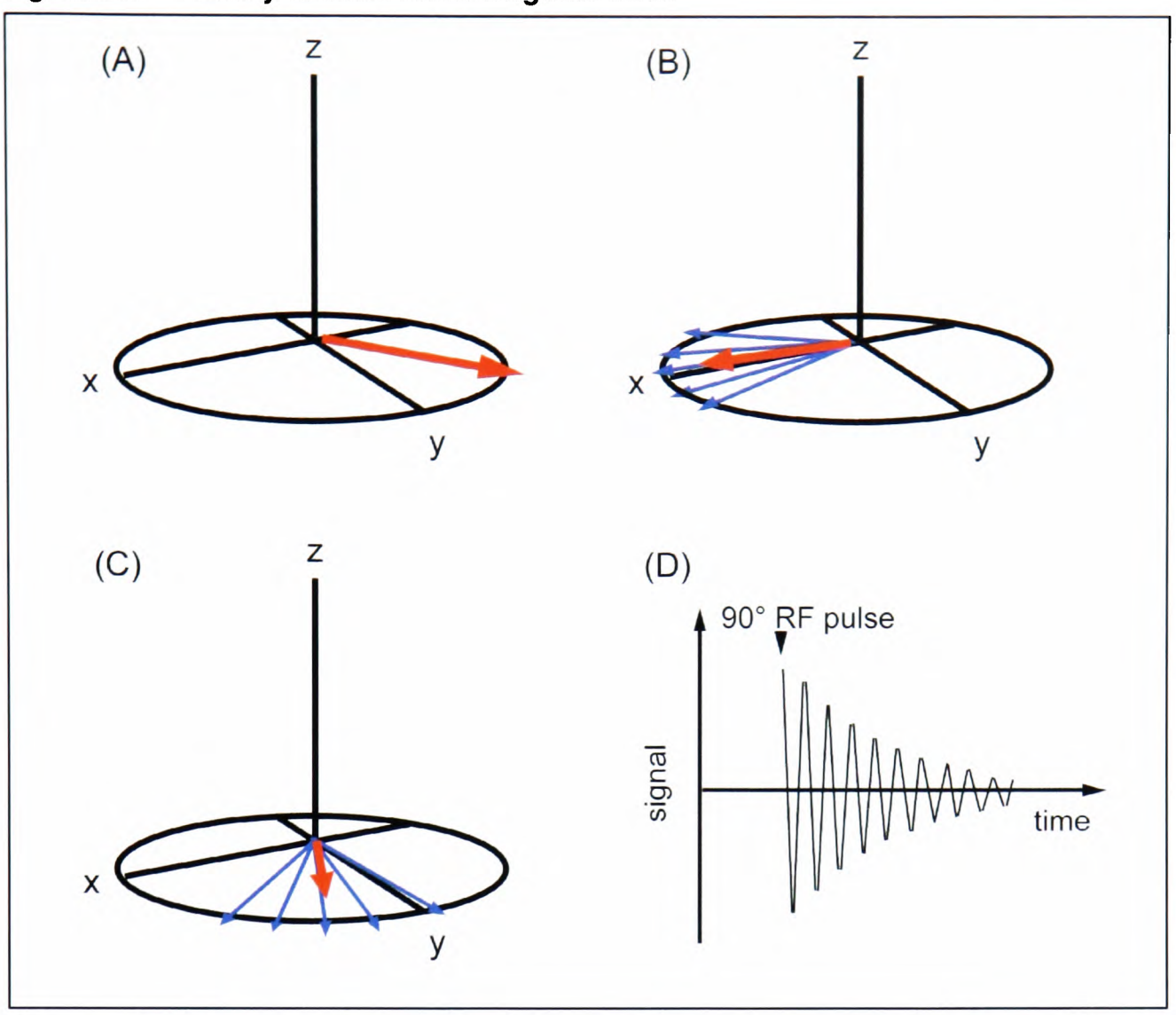
inhomogeneities can be induced deliberately for example by application of the paramagnetic contrast agent gadolinium-DPTA. Gadolinium-DPTA shortens T_2^* and causes a concentration-dependent signal loss on T_2^*-weighted images. Similarly, the endogenous blood oxygen level-dependent (BOLD) contrast, which is described in more detail in 2.1.2, causes signal loss on T_2^* -weighted images.

Figure 2.4 **T_1 and T_2 image contrast**



(A) Cerebrospinal fluid (CSF) appears dark on T_1-weighted images because of its long T_1 time. (B) Tissues that dephase quickly, i.e. that have a short T_2 time, appear dark on T_2-weighted images and tissue with a long T_2 time appear bright (e.g. CSF).

Figure 2.5 Decay of transverse magnetization



(A): Directly after a 90°radiofrequency pulse, all nuclei precess in phase and the resulting transverse magnetization (M_{xy}) is maximal (net magnetization depicted by red arrows). (B) and (C): Inhomogeneities in the magnetic field cause some nuclei to lag behind and some to precess at higher frequencies. M_{xy} becomes increasingly smaller as the phase coherence decreases. (D) The net magnetization precessing in the xy- plane results in an oscillating signal that decays away in an exponential envelope as the nuclei dephase.

Spatial encoding of information

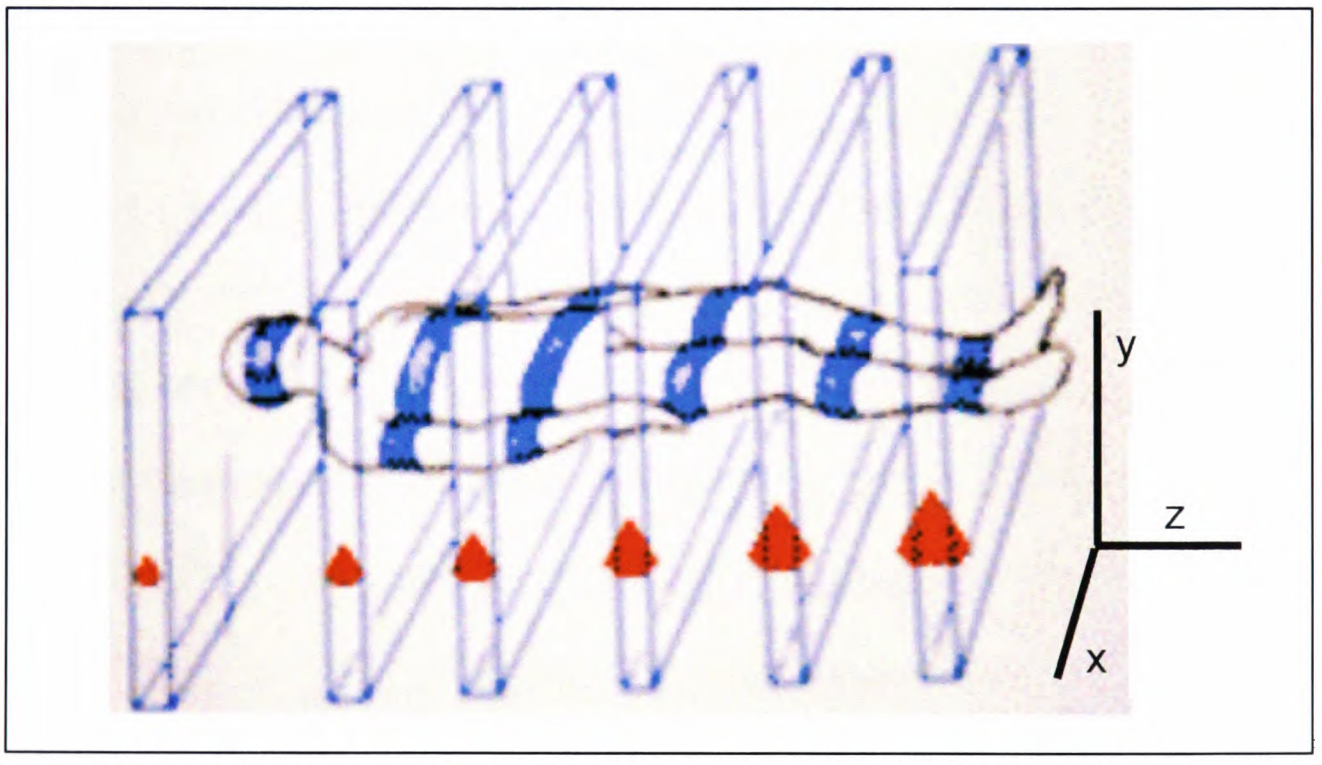
Spatial information about the origin of the signal is obtained by application of linearly increasing small magnetic fields (B_1) superimposed on the static magnetic field, B_0. These B_1 fields are generated by three gradient coils (Gx, Gy, Gz), which generate their magnetic fields in the orthogonal x, y and z directions. By definition, z refers to the principal axis of the B_0 field, which corresponds to the long axis of the magnet. With respect to the brain, z corresponds to superior – inferior, x to left – right and y to anterior – posterior when the subject lies supine in the scanner (Figure 2.6). Through application of the z magnetic field gradient, slice selection is

achieved by altering the resonant (Lamour) frequency of the nuclei along the z-axis (in the case of axial slice acquisition). It is then possible to excite only nuclei in a certain position along the z-axis ("slice") by adjusting the centre of frequency and bandwidth of the applied RF pulse. Once the signal from a slice has been isolated, the remaining in-plane dimensions need to be encoded. One of the directions is encoded by frequency changes cause by the respective magnetic field gradient. However, if a gradient was also applied in the third dimension to alter the frequency of the nuclei, frequencies could not be ascribed unequivocally to the x and y coordinates of the nuclei. Therefore, the signal is encoded in terms of *phase* for the last dimension. After the phase-encoding gradient is removed, the resonant frequency of all nuclei is the same but they will be "out of phase" with each other, which is used for spatial encoding in this direction.

The raw data that is generated by signal detection does not resemble a real image but is a spatial frequency transformation of "real-space". In order to reconstruct the image in real-space, a Fourier transform is applied to the data in frequency space (called "k-space") (Bracewell 1986). Pulse sequences or imaging sequences describe the way RF pulses and gradient fields are applied. Most pulse sequences sample one line of k-space after each RF pulse. To increase acquisition speed and therefore, temporal resolution, functional imaging sequences typically sample all of k-space after one RF pulse. Echo planar imaging (EPI) is the most commonly used pulse sequence for functional experiments and allows acquisition of a whole brain image in three seconds or less. For structural

image acquisition, a FLASH (Fast Low Angle Shot) sequence can be combined with the inversion recovery technique to achieve heavy T_1-weighting. In an inversion recovery sequence, a 180° RF pulse is first applied which rotates the longitudinal magnetization down the z-axis. The magnetization undergoes longitudinal relaxation and returns toward its equilibrium along the z-axis. Before it reaches equilibrium, a further 90° RF pulse flips the magnetization into the xy-plane and the signal is recorded after TE. During the time between the 180° and the 90° pulse (called inversion time, TI), various tissues relax with their intrinsic T_1 time. Tissues in which the longitudinal magnetization was small when the 90° RF pulse was applied have low signal intensities on the images.

Figure 2.6 The three main axes relative to the magnet



X, y and z indicate the three perpendicular axes relative to the magnet. When the subject lies in supine position in the scanner, z corresponds to the superior – inferior axis of the brain, x to left – right and y to anterior – posterior.

2.1.2 Functional MRI (FMRI)

Activity of the human brain was first recorded with electroencephalographic methods in the 1920s by the German psychiatrist Hans Berger. Since then, other methods that assess brain activity have been developed, including FMRI, positron emission tomography (PET), magnetencephalography (MEG) and near infrared spectroscopy (NIRS). Each of these methods has distinct advantages and disadvantages and they vary hugely with respect to the spatial and temporal resolution they provide. In general, electrophysiological methods based on direct mapping of transient electrical dipoles generated by neuronal depolarization (EEG) or the associated magnetic dipoles (MEG) reflect the underlying neuronal activity almost in real time (on the scale of 10 – 100 milliseconds), but provide poor spatial resolution. A more recent analysis tool for MEG has improved spatial localization (Synthetic Aperture Magnetometry, SAM, (Robinson and Vrba 1999)), but MEG provides only limited information on subcortical structures. In contrast, FMRI and PET are indirect measures of neuronal activity with relatively high spatial resolution (approximately 1 – 10 millimetres), but have a temporal resolution that is limited by the much slower haemodynamic changes that accompany increases in neuronal activity. In addition, physical details of the image acquisition technique influence temporal and spatial resolution and as a tool to quantify brain activation, FMRI surpasses the older technique of PET on both measures. A further important advantage of FMRI over PET is that it does not require radioactive tracers. Consequently, FMRI has been widely adopted in research aimed at understanding how the human brain functions since its

emergence in the beginning of the 1990. More recently, FMRI methods are beginning to be used as tools in clinical settings, for example for pre-surgical planning. Different components of the haemodynamic response can be used as contrast mechanisms in FMRI, such as cerebral blood flow (CBF), cerebral blood volume (CBV) and the BOLD (blood oxygenation level-dependent) contrast. The BOLD contrast is the most widely used contrast mechanism for FMRI and was used for all imaging studies in this thesis.

Physiological basis of the BOLD signal

BOLD FMRI is a "noisy" technique that does not, in contrast to PET, allow measurement of an absolute baseline and hence, ongoing neuronal activity cannot easily be measured using FMRI. However, any *change* in neuronal activity over time can be investigated with FMRI. Most commonly, neuronal activity is increased by external stimuli or tasks that the subject has to perform during the experiment. Increase in neuronal activity leads to an increase in energy utilization and to an increase of oxygen extraction from capillary blood. As a consequence, the ratio of oxygenated haemoglobin (Hb) to deoxygenated Hb decreases and blood flow to the respective area increases. This blood flow increase over-compensates the increased oxygen extraction and as a net result, the oxy-Hb / deoxy-Hb ratio increases. In contrast to oxy-Hb, deoxy-Hb is paramagnetic, which means it has a disrupting effect on the local magnetic environment, such that a reduction in deoxy-Hb increases the homogeneity of the local magnetic field. T_2^* imaging sequences are particularly sensitive to changes in the local magnetic field and the

improvement in the magnetic field translates to a signal increase on T_2^*-weighted images.

Neurovascular coupling and interpretation of the BOLD response

If the FMRI signal is only an indirect measure of neuronal depolarization, how well does it depict increases in neural activity? This is an important question as most FMRI experiments are interpreted under the assumption that the signal reflects excitatory neuronal activity. The exact mechanisms of the coupling between increased oxidative metabolism and increased local blood flow are still under investigation (Gjedde 2002; Pellerin and Magistretti 2004). Nevertheless, empirical work that combined intracerebral recording of electrical activity with simultaneous FMRI in awake monkeys has provided evidence for spatial and temporal agreement of neuronal activity and the BOLD response (Logothetis et al. 2001). These experiments showed that local field potentials (LFPs) are the most important single predictor of the positive BOLD response (Logothetis and Pfeuffer 2004), which suggests that increases of the BOLD response mainly reflect input to and processing within an assembly of neurons, rather than their spiking activity, indicative of the neuronal output. This is in accordance with findings that spiking activity itself is not very energy consuming (Arthurs and Boniface 2002) and that metabolic demand reflects primarily the steady-state level of post-synaptic membrane depolarization (Gjedde 2002). However, in the experiments by Logothetis and colleagues, spiking activity was also significantly correlated with the BOLD signal, which is important for the interpretation of the spatial localization of the signal: areas with BOLD signal increases may be areas

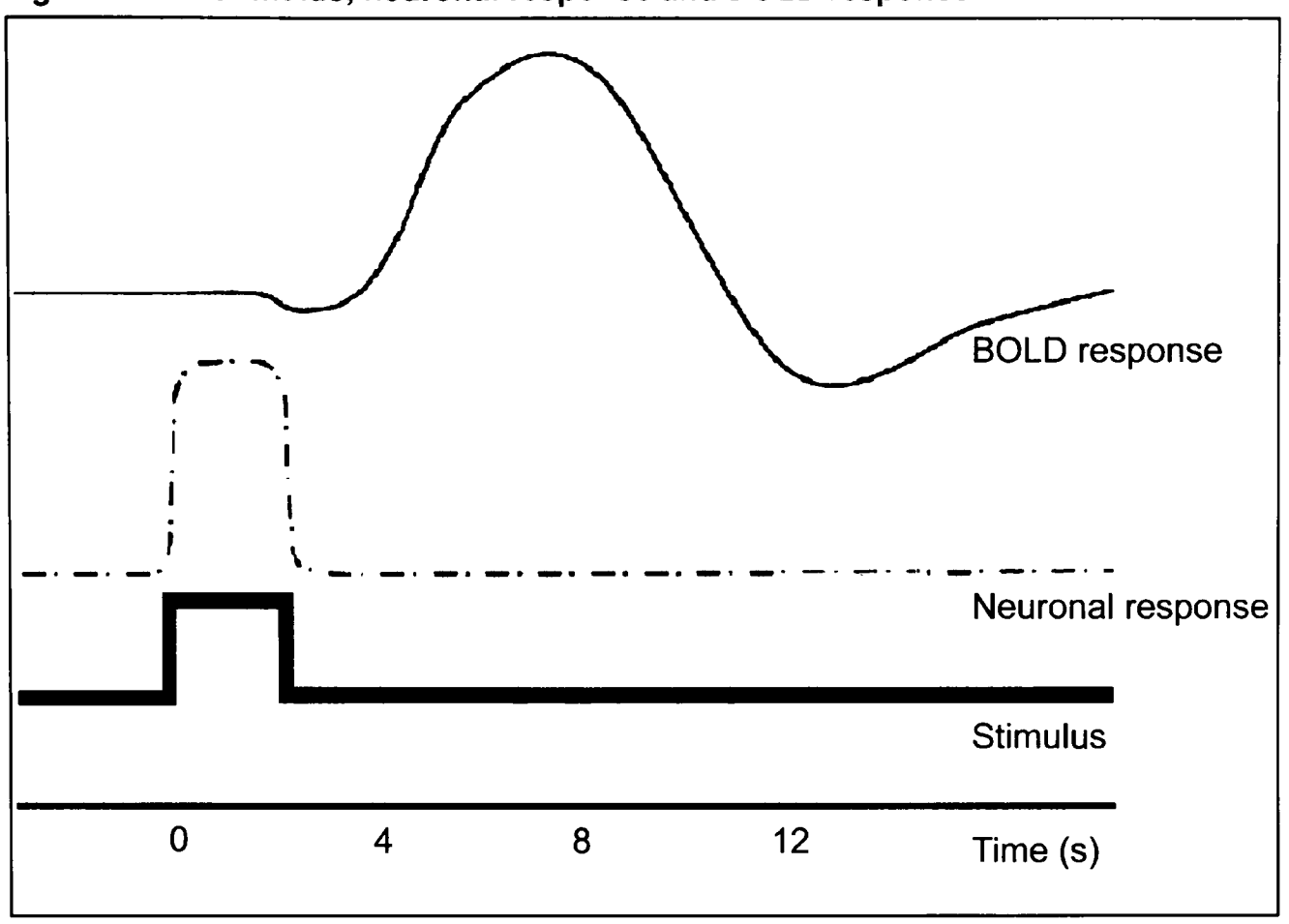
that have an increased output activity themselves but may also be those that receive excitatory projections from other areas. Inhibitory projections might have the opposite effect and cause a local *decrease* of the BOLD response in their target area. Recent monkey studies have shown that decreases in neuronal activity are indeed associated with negative BOLD responses (Shmuel et al. 2003). Although neuronal inhibition itself is an energy demanding process (Nudo and Masterton 1986), a contribution of the activation of inhibitory neurons to the positive BOLD response at their site of origin has not been demonstrated, which might be explained by several observations. First, the number of inhibitory neurons in the brain is relatively small: only 15 to 30% of neurons are reported to be inhibitory (Waldvogel et al. 2000). Second, inhibitory neurons have fewer synapses and these operate more efficiently than excitatory ones (Waldvogel et al. 2000). Taken together, BOLD signal increases in a given brain area are likely to reflect increased excitatory input into the area and increased excitatory output from this area. Local contributions of inhibitory neurons to BOLD signal increases are likely to be limited but inhibitory neurons probably contribute to negative BOLD responses in projection areas.

Importantly for the studies presented in this thesis, it has been shown empirically that a linear relationship between the neural and the BOLD response exists under certain circumstances (Brinker et al. 1999; Rees et al. 2000). In Chapters 6 and 7, alterations in the BOLD response that might be useful as surrogate markers of the pain experience are explored. The extent to which the magnitude of the BOLD signal can be explained by perceived pain intensity is investigated. A (partially) linear association

between neural activity and BOLD response facilitates this analysis as it is performed within a general linear model framework (see Section 2.1.4).

Although FMRI combines relatively high spatial and temporal resolution, it is far from being an ideal reflection of the neuronal response. Spatially, the signal is not restricted to areas of increased neuronal activity as changes in oxygen extraction and, to an even greater extent, changes in blood flow take place over a larger region (Menon and Goodyear 2002). In addition, draining veins are responsible for an extension and even a spatial shift of the BOLD signal (Menon et al. 1993). One factor that limits the temporal resolution of the FMRI signal is the haemodynamic response. The BOLD response typically peaks after four to six seconds after onset of a stimulus (Figure 2.7); however, the variability of the BOLD response across brain regions, subjects and tasks (Aguirre et al. 1998; Rajapakse et al. 1998) limits the temporal resolution more than the lag itself (Bandettini 1999). If the shape of the BOLD response was accurately known for a given subject under given experimental circumstances, the underlying neuronal response could be theoretically inferred. In addition to the limits imposed by the BOLD response, temporal resolution in a real FMRI experiment is further governed by T_1 relaxation processes and hardware characteristics. As outlined in Section 2.1.1, the time between two RF pulses, TR, has to be long enough to allow substantial recovery of the longitudinal magnetization M_z and should therefore be longer than the longitudinal recovery time T_1. This means in practical terms that TR should at least be 1 – 1.5 seconds. In an FMRI experiment in which whole brain coverage is achieved, TR is typically 3 seconds.

Figure 2.7 Stimulus, neuronal response and BOLD response



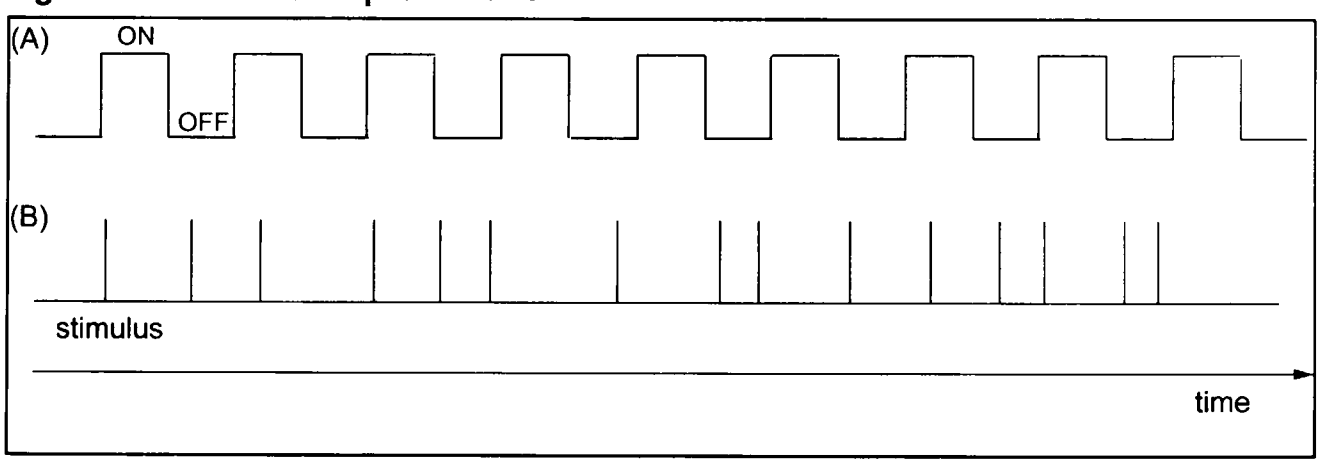
Schematic representation of the temporal relationship between stimulus, neuronal response and BOLD response. Application of a stimulus leads to a neuronal response within milliseconds. In contrast, the BOLD response happens on a time scale of seconds. It takes about 4 to 6 seconds for the BOLD response to reach its peak and about 12 to 15 seconds to return to baseline. s, seconds.

Design of BOLD FMRI experiments

As signal changes of the BOLD response are relatively small (in the order of 0.5 to 3% at 1.5 Tesla), it is necessary to repeat stimulation several times in an FMRI experiment to increase the signal-to-noise ratio by signal averaging and noise cancellation. In a typical experiment, a low resolution image of the brain is acquired every few seconds and over the course of the experiment, 100 images or more are usually recorded. The stimulus can either be presented in a block design, which alternates relatively long periods of rest and stimulation, or "event-related", which typically presents short events at varying intervals (Figure 2.8). Whereas a given number of stimuli can be presented most time-efficiently in block designs, this design

assumes a sustained haemodynamic response over the length of a block, which might not necessarily apply to the stimulus type chosen (rapid habituation is for example observed in studies of olfaction (Sobel et al. 1998)). FMRI data on provoked neuropathic pain are scarce and a published FMRI study of mechanical allodynia suffered problems with signal detection, despite a large number of participating patients (Peyron et al. 2004). Therefore, block designs were used in this thesis in studies of provoked neuropathic pain to provide enough statistical power to detect significant BOLD changes while keeping experimental time for the patients to a minimum. In contrast to provoked neuropathic pain, experimental heat pain is known to provide robust activation in the human brain and event-related designs were employed in studies that used this type of stimulation to minimize sensitization to noxious thermal stimulation.

Figure 2.8 Stimulus presentation



During an FMRI experiment, stimuli can be presented either in a block design (A) or in an event-related design (B). Block designs provide the most time efficient way to administer long periods of stimulation whereas event-related designs are more versatile. In the specific case of thermal pain experiments, event-related designs suffer less from habituation or sensitization than block designs.

2.1.3 Pharmacological FMRI (PhFMRI)

In simple terms, phFMRI is the combination of administering drugs with the collection of FMRI data. PhFMRI performed with analgesic drugs aims to determine either a direct modulation of regional brain activation through centrally acting drugs or an indirect modulation of regional brain activation by altered afferent input after treatment with peripherally acting drugs. In addition to the desired specific effect, drugs penetrating the blood-brain barrier can have direct and indirect non-specific effects influencing the FMRI signal, including non-specific neuronal effects, altered neurovascular coupling and effects on the haemodynamic response. It is therefore important to include control tasks in the experiment to test for non-specific effects of the drug on the FMRI signal if the aim is to investigate FMRI signal changes between ON and OFF drug sessions.

2.1.4 Principles of FMRI data analysis

The time series of data acquired in an FMRI experiment is analysed for stimulus-related changes in the signal. Before statistical analysis of signal intensity changes is carried out, several pre-processing steps are commonly applied to FMRI data to minimize artefacts and to increase the sensitivity of statistical analysis.

Brain extraction

Non-brain structures are removed based on signal intensity differences between brain tissue and layers surrounding the brain surface.

Motion correction

Subject head movement during the experiment is a major source of artefact in FMRI data. Motion correction transforms each image of a time series ("volume") using rotations and translations so that the image of the brain within each volume is aligned with that in every other volume.

Spatial smoothing

Spatial smoothing is mainly applied to increase the signal-to-noise ratio. If noise varies randomly from voxel to voxel, then smoothing should cancel out noise. In contrast, signal intensity changes that are larger than the chosen smoothing kernel should not be compromised by the procedure. A second reason for spatial smoothing is that later statistical inference techniques use Gaussian random field theory, which is based on the assumption that the data is spatially smooth.

Intensity normalization

Changes in the global blood flow to the brain as well as instability of the scanner hardware can cause the mean intensity of the images to vary with time, regardless of functional activity. This is much less of a problem in FMRI than in PET where radioactive decay causes global variations of the signal intensity. In addition, a recognized problem associated with normalising every FMRI volume to the same mean intensity is that non-activated regions are falsely detected as being negatively correlated with the stimulation in volumes where strong activations increase the mean intensity of the volume (Smith 2001). At the same time, carrying out no normalization at all introduces problems at second-level analyses (i.e.

multi-subject or multi-session) because a random effects analysis could falsely see a greater mean intensity as greater activation when in fact activation was the same. A way around this is to scale each 4D (single session) data set (as opposed to each *volume* of a 4D data set) to have a preset mean intensity ("grand mean" normalization).

Temporal filtering

Temporal filtering is applied to remove noise at frequencies that do not correspond to the frequency of the stimulation and do therefore not contain any signal of interest. High pass temporal filtering removes low frequency noise such as scanner-related drifts. Low pass filtering can be used to remove high frequency noise such as cardio-respiratory effects; however, this procedure potentially removes signal of interest, especially in event-related designs. Another danger of low pass filtering arises because it increases the temporal smoothness of the time series, i.e. the intensity at any given time point is likely to be closer to its neighbours' values than values elsewhere in the time series. Smoothed data contains fewer "effective" (i.e. truly independent) time points and later analysis needs to correct for this smoothness. An alternative approach to low pass filtering involves estimating the temporal smoothness of the data and removing this temporal autocorrelation during the statistical analysis stage ("pre-whitening").

Once pre-processing has been performed, the next step is to asses the degree to which the signal from each voxel corresponds to the stimulation (or task). To detect signal intensity changes associated with the

stimulation, the stimulus input function is used as regressor in a general linear model (GLM) in which the time series of pre-processed functional images is the dependent variable (Figure 2.9) (Friston et al. 1995; Worsley and Friston 1995). To account for the time lag of the haemodynamic response, the stimulus input function is convolved with a canonical haemodynamic response function (HRF) before being used as regressor. Other explanatory variables can be included into the model, such as reaction times, age or indeed pain ratings. If the aim is to investigate whether the effect of one stimulus on brain activation is influenced by another stimulus, a non-linear interaction analysis can be performed. Most frequently, a multiplicative interaction term is built, which identifies voxels where the signal during simultaneous application of both stimuli was greater than the sum of the signals when the two stimuli were applied separately. In cases in which two regressors are not completely independent from each other, they partly explain the same variance in the data. To ensure that variance is uniquely associated with a certain regressor (say regressor 1), regressor 1 can be orthogonalised with respect to the other regressor (regressor 2). In this case all variance that is shared by the two regressors in attributed to regressor 2 and activation attributed to regressor 1 is unequivocally explained by regressor 1.

Once the linear model is built it is fit separately to the data at each voxel. An estimate of the goodness of fit (called parameter estimate, PE) is calculated for each voxel. To convert the PEs into useful statistics, their values are divided by their standard errors, which express the uncertainty in the estimation, and converted into T- or Z-statistical parameters for each

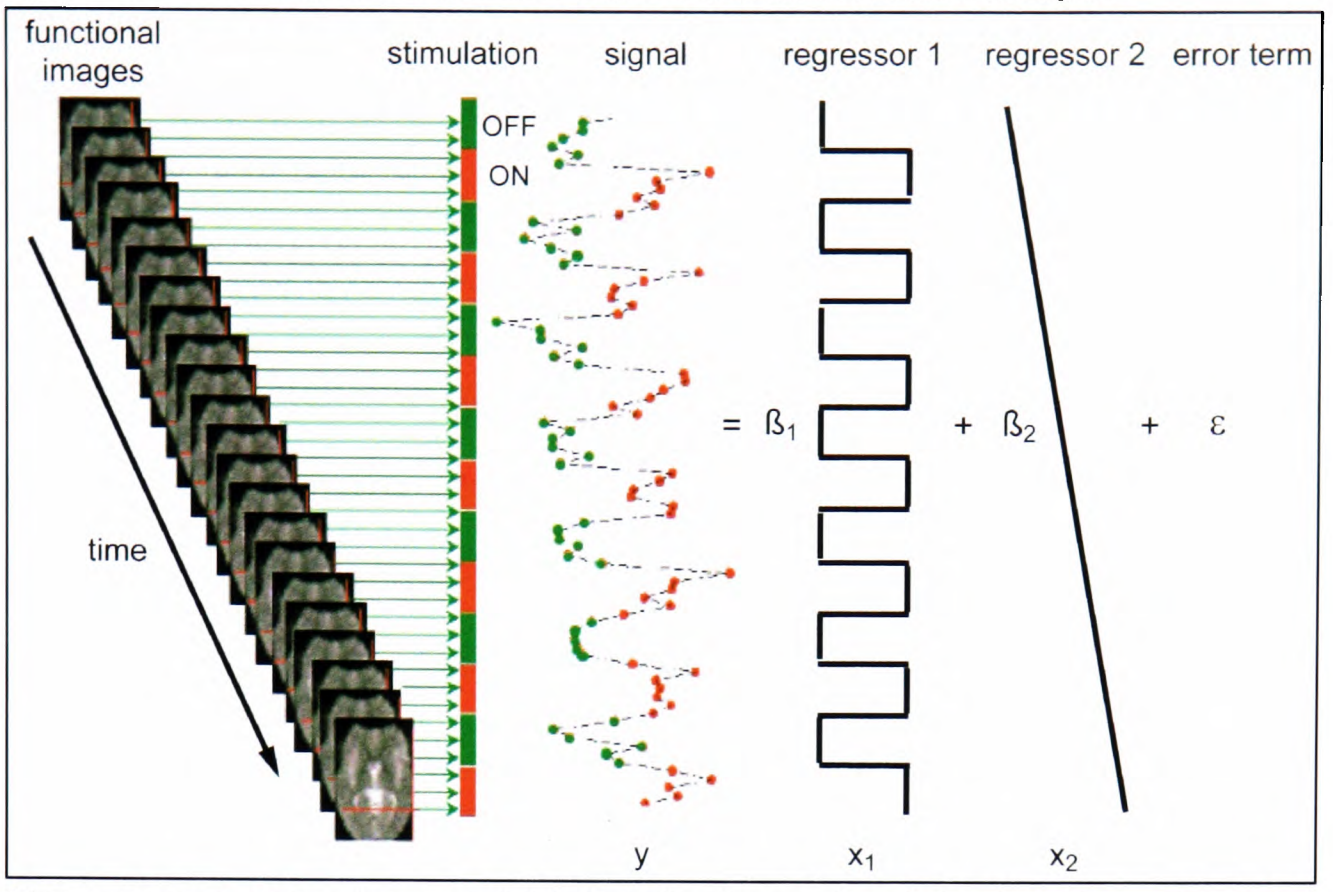
voxel, resulting in a statistical map of the brain. Activation for different conditions (i.e. different regressors) can also be compared directly to each other by performing contrasts of parameter estimates. For example, to detect whether activation in response to regressor 1 is greater than in response to regressor 2, the PE for regressor 2 is subtracted from the PE of regressor 1 and this new estimate is divided by its standard error to create a T- or a Z-value.

Once a statistical parameter has been calculated for each voxel, it is necessary to decide which voxels were activated. T-or Z-values are associated with a probability (p-value) which can be directly used for statistical thresholding. However, there are typically tens of thousands of voxels in the brain and therefore, conventional thresholding leads to many false positives. Bonferroni correction is overly conservative as voxels are not independent from each other (due to regional neuroanatomical characteristics and smoothness imposed by spatial filtering). An alternative voxel-based thresholding approach uses Gaussian random field theory. This takes the spatial smoothness of the statistical map into account and hence, the number of independent volume elements (resolution elements - resels) is smaller than in the original map. This means that correction for multiple comparisons is less conservative than traditional Bonferroni correction. A third approach takes the size of activation into account. A voxel-based threshold is first applied to the statistical map (e.g. $Z > 2.3$). It is then possible to simply define clusters as significantly activated that contain more than a certain number of contiguous voxels exceeding the voxel-based threshold. Alternatively,

Gaussian random field theory can be used to assign a probability value to each cluster of voxels above voxel-based threshold, dependent on its size and shape (Cao and Worsley 2001). Cluster-based corrections are typically more sensitive than voxel-based corrections (Friston et al. 1996) and at the same time, they are arguably physiologically more meaningful as activated regions are expected to extend over several voxels. It should be noted that no firm consensus exists on how high the threshold should be for the Gaussian random field theory to work (Hayasaka and Nichols 2004).

To perform group analysis, no statistical inference is performed at a single-subject level. Rather, the statistical maps of all subjects are combined before the resulting group map is thresholded. To allow subject-based statistical maps to be combined, they have to be transformed into a defined anatomical space ("standard space"). This is done by first co-registering the functional images of an individual subject to a high-resolution T_1-weighted image of the same subject and then to a template brain in standard space. These transforms are then applied to the subject-based statistical maps. Several statistical methods can be used to combine results across subjects, including fixed-effects and random-effects analyses. In all studies of this work, mixed-effect models were employed, in which the within-session variance at single subject level is estimated assuming fixed effects and the inter-session and inter-subject variance of the data is estimated assuming random effects. Thresholding for group statistical maps is done according to the same principles described above for single subject analysis.

Figure 2.9 Overview of the linear model used for FMRI data analysis



After pre-processing, a linear model is fit to the time series of the signal in each voxel of the functional images. In this example, the model consists of two regressors: regressor 1 corresponds to the times when the stimulation was applied and the contrast for regressor 2 tests if the signal increases linearly over the course of the experiment. In addition, an error term is included in the model. β_1 is the parameter estimate for first regressor, i.e. β_1 indicates by which number regressor 1 has to be multiplied to achieve the best model fit. The same applies to β_2.

2.2 Experimental protocols

Methods that were used in at least three of the studies of this thesis are outlined in this section. Details of the specifics for each protocol are described in the respective chapters.

2.2.1 Participants

Participants were only included if they fulfilled the safety criteria for MRI, in addition to specific inclusion and exclusion criteria detailed in the respective study sections. Written informed consent was obtained from all participants prior to the start of the experiment. All studies were approved by the local ethics committee and were in accordance with the declaration

of Helsinki.

2.2.2 State and Trait Anxiety Inventory (STAI)

The STAI differentiates between the temporary condition of "state anxiety" (S-anxiety) and the more general and long-standing quality of "trait anxiety" (T-anxiety) (Spielberger 1970). The essential qualities evaluated by the state anxiety scale are feelings of apprehension, tension, nervousness and worry. Scores on this scale increase in response to physical danger and psychological stress and decrease as a result of relaxation training. On the trait anxiety scale, psychoneurotic and depressed patients generally have high scores, consistent with the trait anxiety construct (Mindgarden 2005). Each item is scored on a four-point Likert scale with higher scores representing increasing levels of anxiety. The STAI has consistently demonstrated adequate psychometric properties and is among the most commonly used measures of anxiety (Nelson and Novy 1997; Nelson et al. 1998). Adult normative data are found in Table 2.1.

Table 2.1 Adult normative data of the STAI

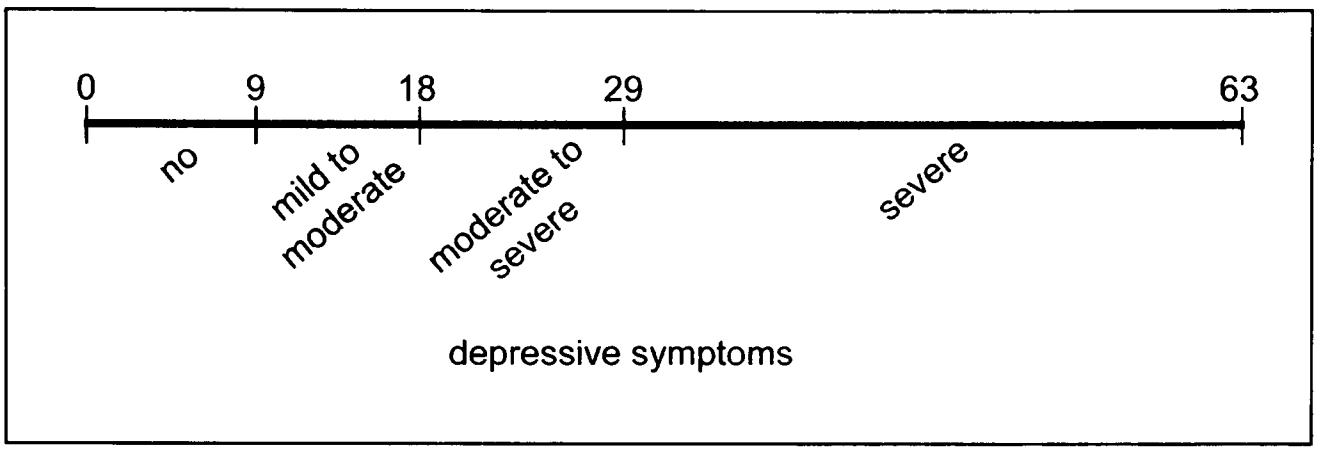
	Female	Male
S-anxiety	35.2 (10.6)	35.7 (10.4)
T-anxiety	34.8 (9.2)	34.9 (9.2)

Listed are the mean and the standard deviation (in brackets). STAI, State and Trait Anxiety Inventory; S-anxiety; state anxiety; T-anxiety, trait anxiety.

2.2.3 Beck Depression Inventory (BDI)

The BDI is a 21-item self-report rating inventory measuring characteristic attitudes and symptoms of depression (Beck et al. 1961). The BDI demonstrates high internal consistency for psychiatric and non-psychiatric populations (Beck et al. 1961). Figure 2.10 displays the score evaluation of the inventory.

Figure 2.10 Score evaluation of the BDI



Scores between 0 and 9 are considered normal; scores between 10 and 18 indicative of mild to moderate depression; scores between 19 and 29 indicative of moderate to severe depression and scores over 30 point at severe depression. BDI, Beck Depression Inventory.

2.2.4 Neuropathic Pain Symptom Inventory (NPSI)

The NPSI is a self-questionnaire designed to evaluate the different symptoms of neuropathic pain (Bouhassira et al. 2004). Descriptors assess the quality and intensity of four different dimensions of neuropathic pain syndromes, i.e. spontaneous pain, paroxysmal pain, evoked pain and paraesthesia / dysaesthesia. As discussed in Section 1.2, symptoms of neuropathic pain overlap with symptoms of other chronic pain conditions and it has been difficult to devise questionnaires that unequivocally determine patients with neuropathic pain conditions. The NPSI is a

recently developed assessment tool and it remains to be seen whether it can distinguish neuropathic from non-neuropathic conditions. The psychometric properties presented so far suggest that the NPSI can be used to characterize subgroups of neuropathic pain patients and test whether they respond differentially to various pharmacological agents or other therapeutic interventions (Bouhassira et al. 2004).

2.2.5 Numerical rating scale (NRS)

The NRS is an 11-point rating scale from 0 to 10 with verbal anchors (0 – "no pain at all", 10 – "worst pain imaginable"). 11-point scales of pain intensity have been demonstrated to be valid measures of pain intensity, also for clinical pain (Jensen et al. 1989). All participants gave their ratings verbally in this work.

2.2.6 Thermal stimulation

Noxious thermal stimulation was carried out in the studies described in Chapters 3, 5 and 7. The in-house built, MR-compatible thermal resistor used to deliver heat stimuli has a fast rise time (30 to 60 ℃ in 0.8 seconds) and the contact probe measures 2 cm x 1.5 cm. Delivery of thermal stimuli is automated using in-house written software ("pain gain") that drives the electronics of the thermal resistor via a National Instruments card (National Instruments, Austin, U.S.A.). The subject can stop the stimulus at any time by using a button that is connected to the thermal resistor. Temperatures exceeding 60 ℃ were not used for safety reasons and two subsequent stimuli were spaced by at least 50 seconds to minimize skin sensitization. For all experiments, the probe was attached

to the right medial forearm using Micropore tape and an elastic bandage. Temperatures to be used during the respective experiment were individually determined according to the following protocol. The first stimulus was delivered at a temperature of 48 °C and repeated twice to account for habituation. Subsequently, the stimulus temperature was increased in steps of 2 °C until a pain rating of 4/10 on the NRS was reached and subsequently in steps of 1 °C until the desired pain rating was obtained. The final temperature was repeated twice to confirm the rating. During functional experiments, the thermal resistor was triggered by TTL (transistor transistor logic) pulses produced by the MR scanner to ensure exact timings between image acquisition and stimulus delivery.

2.2.7 MRI data acquisition

Imaging experiments presented in Chapters 3, 6 and 7 were performed on a 1.5 Tesla Siemens Sonata MRI scanner using a single channel head coil. A multi-slice, gradient echo, echo planar imaging (EPI) sequence covering the whole brain was used for functional scans (TR = 3 s, TE = 40 ms, flip angle = 90°, 26 contiguous, 5 mm thick axial slices, field of view (FoV) 192 mm x 192 mm, matrix 64 x 64, resulting in an in-plane resolution of 3 x 3 mm^2). The first two images were discarded to allow establishment of steady state magnetization. High-resolution T_1-weighted images of the whole brain (3D-FLASH-sequence, TR = 12 ms, TE = 4.76 ms, flip angle = 19°, FoV 160 mm x 208 mm x 256 mm, matrix 160 x 208 x 256, hence voxel size: 1 mm^3) were acquired for all subjects for the purpose of co-registration of functional images to a template in standard space (see Section 2.1.9). In addition, individual high-resolution images

were used in Chapter 7 for precise definition of regions of interest.

2.2.8 Analysis of behavioural data

All behavioural data were analysed using SPSS 11.5 (SPSS Inc., Chicago, U.S.A.).

2.2.9 Analysis of FMRI data

Analysis was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.4, part of the FSL Suite Version 3.2 (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl) in all studies. Before statistical analysis, pre-processing of the images was performed. Non-brain structures were removed using an automated procedure (Brain Extraction Tool, BET) (Smith 2002). Motion correction was carried out using rigid body transformation - this means that only translations and rotations are performed and hence, the shape of the brain does not change. This procedure is implemented within FSL (MCFLIRT) (Jenkinson and Smith 2001). Spatial smoothing used a Gaussian kernel of full width at half maximum (FWHM) of 5 mm in all analyses. No global intensity normalization was carried out for the reasons given in Section 2.1.4; instead, each single session data set was scaled to have a preset mean intensity ("grand mean" normalization). The frequency cut-offs of high pass filtering are given in the respective chapters. Low pass filtering was not performed as pre-processing step; instead, pre-whitening was performed during statistical analysis. Statistical analysis was carried out using FILM (FMRIB's improved linear model) (Woolrich et al. 2001) and group analyses were performed using FLAME (FMRIB's Local Analysis of Mixed

Effects) (Beckmann et al. 2003) which implements a mixed effects model in a Bayesian framework. FLAME models and estimates the inter-subject and inter-session random effects component of the mixed-effect variance. This estimation is considered accurate with group sizes of more than six subjects (Woolrich et al. 2004). EPI images were first co-registered to the subjects' high resolution structural images and then to the MNI (Montreal Neurological Institute) 152 template in standard space (Evans et al. 1992) using affine linear transformations with 12 degrees of freedom, which means that translation, rotation, scaling and skews are performed in the three main directions x, y and z (FLIRT) (Jenkinson and Smith 2001). All coordinates are reported in millimetres, relative to the anterior commissure, according to the MNI 152 template. For visual display, the group activation maps are overlaid onto the mean structural image of the respective subjects. Details of construction of explanatory variables and statistical thresholds are given in the respective chapters.

CHAPTER 3: Comparison of experimental pain processing in chronic pain patients and healthy control subjects

3.1 Introduction

Does a patient with chronic pain perceive an experimental nociceptive stimulus differently when compared to a healthy person? Increased sensitivity to such stimuli has indeed been described in patients with a range of chronic pain conditions, including fibromyalgia (Gibson et al. 1994), temporomandibular disorder (Sarlani and Greenspan 2003), irritable bowel syndrome (IBS) (Verne et al. 2001) and vulvodynia (Giesecke et al. 2004a). Several mechanisms could contribute to an altered perception of experimental nociceptive stimuli resulting in enhanced pain sensations. First, negative affect, which often accompanies chronic pain conditions, could enhance pain perception, as described in Section 1.6. State anxiety and depression are important predictors of pain intensity in clinical conditions (Gaskin et al. 1992) and the induction of anxiety or sadness leads to increased pain perception even in healthy subjects (Ploghaus et al. 2001; Rainville et al. 2005). Second, chronic pain could induce altered functioning of CNS structures that are directly involved in the perception of pain. Third, nociceptive input into supraspinal structures could be increased, either by generalized peripheral sensitization as is suggested in fibromyalgia (Gibson et al. 1994) or by amplification of transmission along ascending pathways. Brain imaging could help to identify the neural networks that reflect these putative mechanisms and their relative contributions to altered pain processing in chronic pain patients. To date, several imaging studies have provided

evidence for augmented cerebral responses to painful stimuli induced under experimental conditions in fibromyalgia (Cook et al. 2004; Giesecke et al. 2004b; Gracely et al. 2002), chronic low back pain (Giesecke et al. 2004b), IBS (Verne et al. 2003) and atypical facial pain (AFP) (Derbyshire et al. 1994). With respect to the different putative mechanisms, the following activation patterns could be expected. Increased activation in structures involved in the early stages of processing, such as thalamus, S1, S2 and posterior insula, could reflect increased nociceptive input from peripheral or spinal afferents (Verne et al. 2003). Such increased input could be caused by facilitation at brainstem level, which can be detected with brain imaging (Zambreanu et al. 2005), or by spinal sensitization, which can potentially be visualized by spinal FMRI (Giove et al. 2004). Augmented affective processing could be reflected by increased activation in limbic areas, such as medial prefrontal, anterior cingulate and anterior insular cortices. Imaging studies have reported increased limbic activation in patients with fibromyalgia, IBS and AFP (Cook et al. 2004; Derbyshire et al. 1994; Verne et al. 2003).

All studies discussed above investigated chronic pain conditions in which the primary pathology is poorly localized or unknown. Both psychophysical and imaging studies investigating experimental pain in chronic pain conditions with well defined pathologies are scarce. One such imaging study has been performed in patients with rheumatoid arthritis (Jones and Derbyshire 1997). However, no significant signal increase in response to heat pain was found in the patient group, which made interpretation of the results difficult. There is no current literature on brain activation in

response to an experimental nociceptive stimulus applied to an unaffected area in neuropathic pain patients.

In the present study, it was hypothesized that altered cerebral pain processing in neuropathic pain patients is not restricted to the pain that is characteristic for the condition but occurs in a generalized fashion. To test this, neural correlates of experimental heat pain in patients with neuropathic pain were compared to those in age- and gender-matched healthy control subjects. To minimize contributions of altered peripheral processing or of spinal cord sensitization, the stimulus was applied outside body areas affected by primary or secondary hyperalgesia. Depressive and anxiety-related symptoms were also evaluated to aid interpretation of the imaging data.

3.2 Methods

3.2.1 Participants

Patients with neuropathic pain caused by peripheral nerve injury or by plexus avulsion were recruited from the Oxford Pain Relief Unit. To reduce the confound of fluctuating levels of spontaneous pain during the FMRI experiment, patients who experienced frequent pain paroxysms (more than once every hour) or who perceived that the intensity of their ongoing pain changed over a one hour period were excluded from participation. Patients with neuropathic pain in the volar aspect of the right forearm were excluded because this was the chosen stimulation site for the imaging study. Finally, patients were excluded if they suffered from any major psychiatric disorder or systemic disease. If willing, subjects discontinued

analgesic medication, including antidepressant and antiepileptic drugs.

Healthy control subjects were recruited at the Department of Physiology, Anatomy and Genetics at Oxford University. These subjects were matched to the patients in terms of age (difference of less than three years) and gender. They were excluded if they suffered from any chronic pain condition, any major psychiatric disorder or systemic disease.

3.2.2 Clinical and psychological assessment

A full medical history was obtained from all patients at a separate session prior to the imaging session. At the first appointment, patients underwent a physical examination with emphasis on signs of neuropathic pain. Each patient completed the Neuropathic Pain Symptom Inventory (NPSI) (Bouhassira et al. 2004), which provided further characteristics of their pain state. A short medical history was obtained from the control subjects at the day of FMRI investigation. All participants completed the Beck Depression Inventory (BDI, Beck et al., 1961) and State Trait Anxiety Inventory (STAI, Spielberger, 1970) on the day of FMRI investigation prior to the imaging experiment.

3.2.3 Thermal stimulation

Each thermal stimulus was three seconds long and applied as outlined in 2.2.6. For the patients, the temperature required to produce a pain rating of 6/10 on the numerical rating scale (NRS) was determined after they had been positioned in the scanner. The temperatures determined in this step were subsequently used during stimulation of the corresponding matched

control subject. Control subjects were unaware that they would receive a preset temperature and they were subjected to the same "test" stimuli as used in the patient group before the FMRI experiment. This was done to make the experimental sessions of patients and control subjects as similar as possible.

3.2.4 Experimental design

During the functional imaging experiment, 15 thermal stimuli of three seconds duration were delivered to the right volar forearm with various inter-stimulus intervals, which ranged from 52 to 68 seconds, using the temperature determined beforehand. Immediately after the scan, subjects were asked to rate the average pain elicited during the experiment on the NRS.

3.2.5 MRI data acquisition

Data acquisition was performed as described in 2.2.7.

For the functional scan, 326 image volumes were acquired with a TR of three seconds, resulting in an experimental time of 16 minutes and 18 seconds.

3.2.6 Analysis subject data and behavioural data

Age, BDI, state and trait anxiety scores, pain ratings and temperatures were tested for differences between patient and control group using unpaired t-tests. Pain ratings were controlled for temperature and Pearson's product moment correlation coefficient was calculated between residual pain ratings, BDI, state anxiety and trait anxiety scores across all subjects. Correlations between age and psychological scores were

calculated for each group separately and correlations between ongoing pain intensity (rated on the NPSI), disease duration, age and psychological scores were calculated only for the patient group. Significance levels for t-tests and Pearson's product moment correlation coefficient were set to $p < 0.05$ (two-sided tests).

3.2.7 Analysis imaging data

During pre-processing, high pass filtering was performed using a cut-off of 50 seconds. All other pre-processing steps were performed as described in 2.1.4. For statistical analysis on a single-subject level, the timing of the thermal stimulation, convolved with a canonical haemodynamic response function, was entered as the regressor of interest in the linear model. To allow for imperfect timing, the first temporal derivative of the regressor was included in the model as covariate of no interest. On a group level, one statistical map "thermal pain", which represents thermal stimulation compared to baseline, was calculated for the patient group and one for the control group, using a constant regressor for each group. The voxel-based threshold for these main effect statistical maps was set to $Z > 2.3$ and significant clusters defined according to spatial extent at $p < 0.05$ (corrected for multiple spatial comparisons according to Gaussian random field theory (Worsley et al., 1992)). To compare activation in response to thermal stimulation between the groups, an unpaired t-test was performed between patient and control group. Pain ratings were included as regressor of no interest. The search volume for statistical inference was restricted to voxels that showed activation in the thermal pain map of either group. The voxel-based threshold was set to $Z > 2.3$, uncorrected

for multiple comparisons. Only activation clusters that exceeded a minimal contiguity threshold of five voxels were considered significant.

In a second analysis step, state anxiety and BDI scores were incorporated as regressors into two separate group analyses. The first regressor in both analyses was constant across subjects. A second regressor (the regressor of interest) contained either the state anxiety or BDI scores and was orthogonalised with respect to the constant regressor. Contrasts were set to determine voxels in which activation co-varies with state anxiety and BDI scores, respectively. The search volume for statistical inference was restricted to voxels that were activated either in the thermal pain map of the patient or of the control group. The voxel-based threshold was set to $Z > 2.3$, uncorrected. Activation clusters containing five or more contiguous voxels above voxel-threshold were considered significant.

The GLM analysis was complemented by a region of interest (RoI) analysis. RoI analysis combines the statistical information of all voxels in a selected region by calculating a single average statistical parameter. Thereby, RoI analyses on a group level are less affected by false negatives results that are caused by small neuroanatomical differences between subjects. For example, all subjects might activate somatosensory cortex but at slightly different voxel locations, which could lead to false negative results in the GLM. At the same time, RoI analysis can "dilute" results when the RoI chosen exceeds largely the activated area because the calculated statistical parameter comprises also values from non-activated voxels. In this study, the purpose of RoI analysis was to test the

results of the GLM analysis because this analysis was performed with a relatively low statistical threshold. Therefore, RoI analysis was carried out only for brain areas that were activated in the unpaired t-test in the GLM analysis or in the GLM correlation analyses. RoIs were first anatomically defined in standard space and then reduced to voxels that were activated in the thermal pain map of either group to reduce the risk of "dilution". The mean percent signal change in each RoI was calculated for each subject and compared between groups using unpaired t-tests. Correlations between mean signal intensity change and psychological parameters were calculated in the RoIs that arose from the correlation analysis and from the t-test within the GLM framework. Correlations were determined using Pearson's product moment correlation coefficient. Significance levels were set to $p < 0.05$ for t-tests and correlation analysis (two-sided tests).

3.3 Results

3.3.1 Subject data

Twelve patients with neuropathic pain and twelve healthy control subjects took part in the study. Both groups contained five males and a close age match was achieved between the two groups (Table 3.2). All patients were right-handed (although patient 1 in Table 3.1 was left-handed before the accident) and all but one control subject were right-handed. Duration of neuropathic pain ranged from 2 to 32 years in the patient group (mean: 9 years; SD: 9.1 years). One patient described a gradual onset of pain after several operations on a congenital short leg. The data of this patient were not included in the calculation of the average disease duration or in the correlation analyses with disease duration as a variable. The average

intensity of ongoing pain, measured by the NPSI, was 3.5/10 (SD: 2.9). Patient baseline characteristics, including diagnosis and medication, are presented in Table 3.1. Five patients were willing to discontinue their analgesic medication for the imaging session. The exact timing of the withdrawal depended on the drug. Patients taking antidepressant or antiepileptic drugs stopped their medication two weeks and those on opioids one week before scanning. Two patients were not on any medication. In all, seven patients were not taking analgesic medication at the time of investigation.

Table 3.1 Patient characteristics

Pat	Diagnosis	Affected body area	DD (yrs)	Ongoing pain	Current medication
1	Brachial plexus avulsion	left arm	9	4.5	none
2	Post-operative NPP	left foot	5	4	stopped
3	Post-inflammatory NPP	lower back left > right	4	0	stopped
4	Post-injury NPP	left hand	3	7.5	none
5	Post-operative NPP	right knee	2	0	morphine, amitriptyline, paracetamol
6	Post-operative NPP	left thigh, groin	5	3	stopped
7	Post-operative NPP	right knee	15	0	stopped
8	Post-operative NPP	left thorax wall	1.5	5	dihydrocodeine, dothepin, paracetamol
9	Sacral plexus avulsion	right ankle	6	7	clonidine
10	Diabetic neuropathy	feet bilateral	5	6.5	stopped
11	Post-operative NPP	left elbow	32	0	amitriptyline, citalopram, diclofenac
12	Congenital short leg	right leg	gradual onset	4	codeine, paracetamol

DD, disease duration; yrs, years; NPP, neuropathic pain.

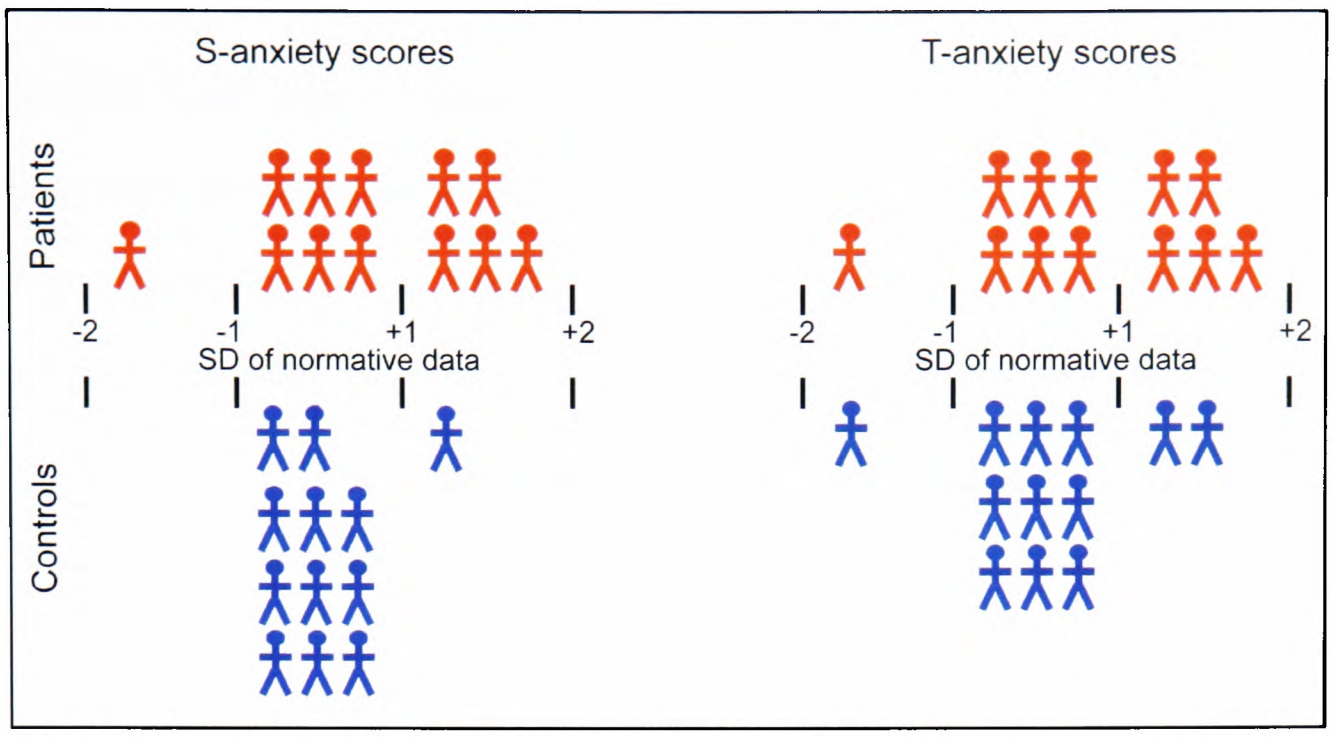
3.3.2 Psychological data

One patient did not complete the BDI and this was only detected after the study. The data from this patient and that from the matched control subject were excluded from all analyses in which BDI scores were used. Five patients and nine control subjects presented with BDI scores in the normal range. The scores of three patients were indicative of mild to moderate depression (two control subjects) and the scores of three patients fell in the category of moderate to severe depression. The average BDI score in the patient group was significantly higher than in the control group (Table 3.2).

The STAI was completed by all subjects. Results of the state and trait anxiety scales are found in Figure 3.1. Whereas trait anxiety scores did not differ between the groups, patients had significantly higher state anxiety scores than control subjects (Table 3.2).

State anxiety scores were significantly correlated with trait anxiety scores ($r = 0.93$, $p < 0.001$) and with BDI scores ($r = 0.75$, $p < 0.001$). Residual pain ratings of heat stimuli were not correlated with any of the psychological scores and correlation coefficients were as follows: $r = -0.18$, $p = 0.4$ (BDI scores); $r = -0.16$, $p = 0.46$ (state anxiety scores); $r = -0.13$, $p = 0.48$ (trait anxiety scores). In the patient group, state anxiety scores were positively correlated with disease duration ($r = 0.66$, $p = 0.02$).

Figure 3.1 STAI scores relative to adult normative data



S-anxiety scores: The scores of six patients lay in the range of one SD below and one SD above the mean of adult normative data (eleven controls), five patients had scores between one and two SDs above the mean normative data (one control) and one patient presented with a score between one and two SDs below the mean of normative data.
T-anxiety scores: In the patient group, the same distribution as for S-anxiety scores was observed. Nine control subjects lay in the range of one SD below and one SD above the mean adult normative data, two between one and two SDs above and one control subject between one and two SDs below.
STAI, State Trait Anxiety Inventory; SD, standard deviation; S-anxiety scores and T-anxiety scores refer to the state and trait anxiety scales, respectively.

Table 3.2 Group characteristics and results of heat pain provocation

	Patients (n = 12)	Controls (n = 12)	p-value
Age (years)	50.7 (17.2) [21 to 76]	51.4 (17.1) [22 to 76]	0.9
Females / Males	7 / 5	7 / 5	n/a
Depressive symptom scores on BDI	10.7 (7.4) [0 to 21]	4.3 (3.9) [0 to 13]	0.02*
State anxiety scores	39.4 (14.3) [20 to 69]	29.3 (8.6) [22 to 46]	0.048*
Trait anxiety scores	38.5 (11.7) [22 to 59]	31.8 (8) [22 to 49]	0.11
Pain rating experimental stimulus	5.4 (0.36) [4 to 7.5]	4.7 (0.41) [2 to 7]	0.27
Temperature experimental stimulus (℃)	51.9 (1.77) [50 to 54]	51.8 (1.68) [50 to 54]	0.9

Mean (SD) and [range] where applicable. SD, standard deviation; BDI, Beck Depression Inventory; n/a, non applicable; *significant at $p < 0.05$.

3.3.3 Psychophysical data

Applied heat pain temperatures ranged from 50 ℃ to 54 ℃. The temperatures between control subjects and the corresponding patients were exactly matched except for one control subject in whom the temperature had to be lowered from 54 ℃ to 53 ℃. Details of the temperatures can be found in Table 3.2. Thermal stimulation elicited pain ratings between 4 and 7.5 in the patient group (mean: 5.4; SD: 1.3) and between 3 and 7 in the control group (mean: 4.8; SD: 1.4). Pain ratings of the heat pain stimulus did not differ between the two groups (Table 3.2).

3.3.4 Imaging results

Provocation of experimental heat pain in healthy volunteers activated a network of pain processing regions, similar to that described in previous imaging studies (Apkarian et al. 2005; Peyron et al. 2000b). This network comprised sensory (thalamus, S2, posterior insula) and motor areas (supplementary motor area, putamen), and regions involved in motivational and affective processing (ACC, amygdala) (Table 3.3, Figure 3.2). Stimulation using the same temperatures on a pair-wise basis resulted in similar, but not identical, brain activation in the patient group (Table 3.3, Figure 3.2). The cerebellum, prefrontal cortex, periaqueductal grey (PAG) and cuneiform nucleus showed activation exclusively in the patient group while substantia nigra was only activated in the control group.

To test if these qualitative differences were statistically significant, an unpaired t-test between patient and control group was performed within

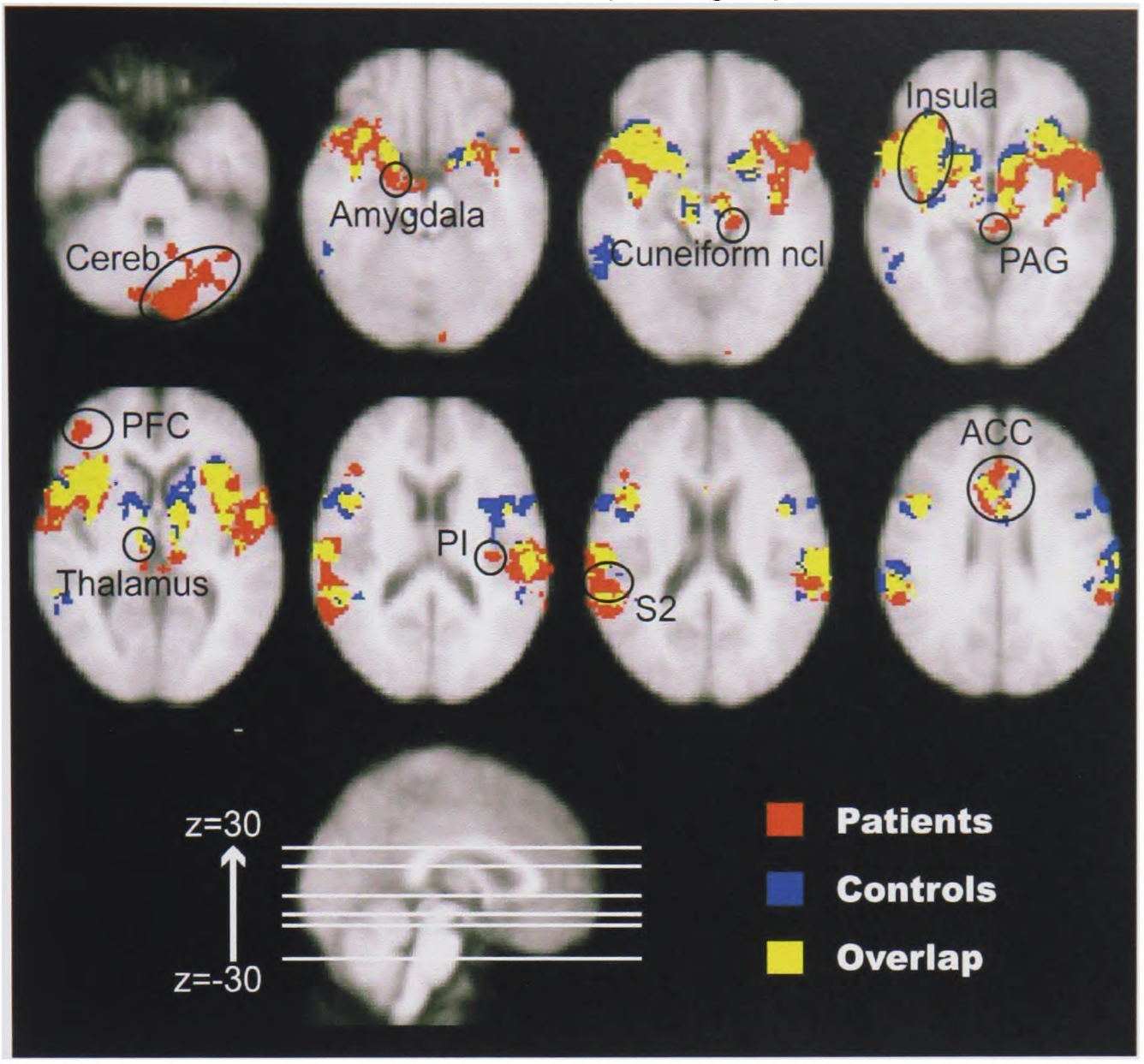
the GLM. Pain ratings were controlled for so that differences in activation could not be caused by minor differences in pain ratings, although ratings were not significantly different (see Table 3.2). The t-test revealed that several regions showed more activation in either group (Tables 3.4 and 3.5). Subsequent RoI analysis was performed for those regions for which clear hypotheses about their differential involvement in pain processing in patients compared to healthy subjects exist, as outlined in Section 3.1. Consequently, RoI analysis was performed for anterior insula and amygdala because of their postulated involvement in emotional processing, for posterior insula, which might reflect altered nociceptive input (Craig 2003b) and for the cuneiform nucleus, which has been implicated in brainstem facilitation in humans (Zambreanu et al. 2005). RoI analysis confirmed that right anterior insula and right amygdala were significantly more activated in the patient than in the control group. For contralateral posterior insula and cuneiform nucleus, RoI analysis failed to confirm the result of the GLM analysis ($p = 0.6$ and $p = 0.9$ respectively). Results of the GLM and RoI analyses for anterior insula and amygdala are shown in Figure 3.3.

Table 3.3 Main effects of thermal stimulation in patients and controls

Brain region		Control group		Patient group	
		[x y z]	Z-score	[x y z]	Z-score
Anterior insula	cl	[-34 12 8]	6.3	[-34 16 2]	8.1
	il	[40 20 -4]	7.8	[40 20 -4]	9.8
Posterior insula	cl	[-38 -14 -4]	4	[-38 -18 -6]	4.2
	il	[38 -16 0]	4	[38 -16 -4]	3.8
ACC	cl	[-6 22 32]	5.3	[-4 6 38]	6.6
Thalamus	cl	[-8 -12 4]	4.4	[-10 -16 6]	4.1
	il	[12 -14 6]	3.6	[14 -16 2]	3.7
S2	cl	[-50 -26 20]	4.4	[-50 -28 16]	3.9
	il	[56 -24 20]	5.2	[62 -26 20]	5
Putamen	cl	[-26 0 -8]	4.7	[-24 4 -6]	5.6
	il	[24 10 -4]	5.9	[20 10 -4]	4.7
SMA	cl	[-4 8 52]	5.2	[-4 -4 52]	4.8
Inferior parietal cortex	cl	[-54 -24 24]	7.1	[-64 -20 18]	7.2
	il	[58 -30 30]	5.2	[60 -32 30]	4.8
Cerebellum	cl			[-28 -62 -32]	5.1
Lateral prefrontal cortex	il			[44 46 8]	4.1
Amygdala	il	[18 -2 -22]	2.8	[18 -2 -18]	4.2
Brainstem					
PAG	il			[2 -30 -4]	3.6
Cuneiform nucleus	cl			[-14 -28 -12]	3
	il			[10 -24 -8]	4.2
Substantia nigra	cl	[-6 -16 -10]	3.3		
	il	[10 -14 -10]	4.1		

Locations of peak Z-scores within an activation cluster are given according to MNI coordinates. Voxel threshold was set to $Z > 2.3$ and cluster-level correction for multiple comparisons across the whole brain to $p < 0.05$. ACC, anterior cingulate cortex; SMA, supplementary motor area; PAG, periaqueductal grey.

Figure 3.2 Brain activation in control and patient groups



Group activation in response to experimental heat pain is depicted in red for the patient group and in blue for the control group. Areas that are activated by both groups are depicted in yellow. Statistical analysis was performed with a voxel–based threshold of $Z = 2.3$ and cluster-level correction at $p < 0.05$. Images are shown in radiological convention. Cereb, cerebellum; ncl, nucleus; PAG, periaqueductal grey; PFC, prefrontal cortex; PI, posterior insula; S2, secondary somatosensory cortex; ACC, anterior cingulate cortex.

Table 3.4 Brain regions showing greater activation in the patient than control group

Brain region		[x y z]	Z-score	cluster size
Anterior insula	il	[32 26 -4]	3.6	28
Posterior insula	cl	[-46 -30 16]	3.3	8
Inferior parietal cortex	cl	[-64 -26 14]	3.9	24
Amygdala	il	[16 -10 -20]	2.7	5
Cuneiform nucleus	cl	[-16 -26 -12]	2.6	8
Cerebellum	cl	[-10 -84 -28]	3	77
Superior temporal gyrus	cl	[-52 -12 2]	3.2	34

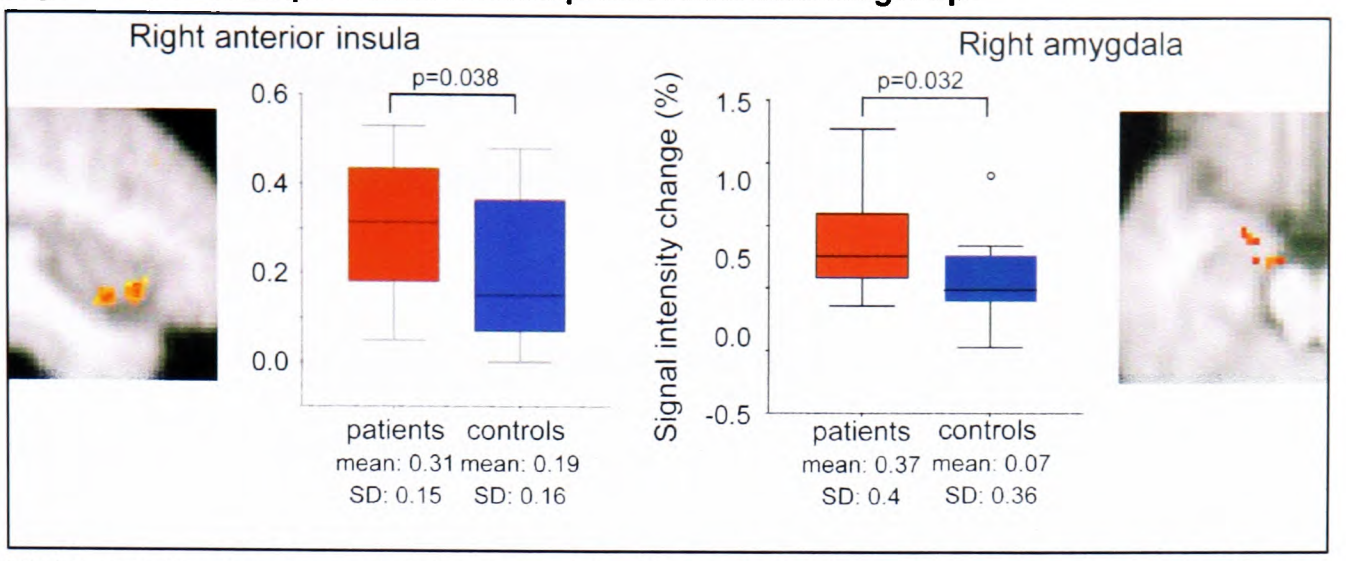
Locations of peak Z-scores within an activation cluster are given according to MNI coordinates. Voxel threshold was set to $Z > 2.3$ in the GLM analysis and clusters with five or more contiguous activated voxels were considered significant. cl, contralateral; il, ipsilateral; GLM, general linear model.

Table 3.5 Brain regions showing greater activation in the control group than patient group

Brain region		[x y z]	Z-score	cluster size
Parietal cortex	cl	[-42 -32 40]	3.2	42
	il	[30 -46 46]	3.1	14
Caudate nucleus	cl	[-18 24 4]	3.3	25

Locations of peak Z-scores within an activation cluster are given according to MNI coordinates. Voxel threshold was set to $Z > 2.3$ in the GLM analysis and activation clusters with five or more contiguous activated voxels were considered significant. cl, contralateral; il, ipsilateral; GLM, general linear model.

Figure 3.3 Comparison between patient and control group



Right anterior insula and right amygdala showed significantly stronger activation in the patient than in the control group, both in the GLM and RoI analyses. The brain images show the results from the comparison within the GLM framework and the graphs show the results from the RoI analysis. GLM, general linear model; RoI, region of interest.

In the final analysis step, the relation between brain activation and BDI and state anxiety scores, respectively, was investigated. No significant correlation between activation and either score was found on a voxel-by-voxel basis (GLM analysis). Also RoI analysis did not reveal any significant correlations between psychological scores and BOLD signal change.

3.4 Discussion

This study demonstrates that provocation of experimental heat pain in neuropathic pain patients and in age- and gender-matched control subjects results in activation of a similar network of brain regions including anterior and posterior insula, ACC, thalamus, S2 and basal ganglia. The extensive overlap of activation patterns might lead to the interpretation that processing of experimental heat pain is not fundamentally altered in neuropathic pain patients; nevertheless, closer examination of these data indicates that subtle differences in pain processing exist between patients and healthy subjects. Because results of studies comparing pain

sensitivity in patients and control subjects vary considerably, some considerations regarding the psychophysical results are presented before the imaging results are discussed in more detail.

Pain ratings in response to experimental heat pain did not differ between patient and control group in this study. In general, stimulus type and duration seem to be important factors whether differences in pain sensitivity between chronic pain patients and control subjects are found. For instance, patients show more frequently increased sensitivity to tonic than to phasic heat pain (Lautenbacher et al. 1990; Sarlani and Greenspan 2003). A study in IBS patients that did observe increased sensitivity to relatively short lasting (20 seconds) heat pain stimuli found that this was more pronounced on the foot than on the hand (Verne et al. 2001). In this case, some degree of convergence at spinal level of input from the rectum and foot could partly explain the increased pain sensitivity of the patients. In neuropathic pain, animal models demonstrate that electrophysiological responses to nociceptive input from homologous body areas contralateral to the affected areas are increased (Carlton et al. 1994; Yasuda et al. 2005). However, this mechanism is unlikely to augment afferent input in the present study, because the left arm, i.e. the homologous body site contralateral to the stimulation side, was affected only in three out of twelve patients. Studies that report increases in perceived pain intensity generally investigate pain syndromes in which the primary pathology is unknown, such as IBS (Verne et al. 2001), vulvodynia (Giesecke et al. 2004b) and fibromyalgia (Gibson et al. 1994). To the best of our knowledge, no published study has investigated sensitivity to

experimental heat pain applied outside the clinically affected area in neuropathic pain patients. More work is needed to establish if heat pain perception is indeed unaltered in neuropathic pain patients or if the present study did not have enough statistical power to detect such difference.

Increased brain activation in response to a nociceptive stimulus has been described in patients with varying chronic pain syndromes (Cook et al. 2004; Giesecke et al. 2004b; Gracely et al. 2002; Verne et al. 2003). In many of these studies patients rated the pain of a given stimulus intensity significantly higher than control subjects (Giesecke et al. 2004b; Gracely et al. 2002; Verne et al. 2003). Although this indicates that amplification of nociceptive input has occurred somewhere along the pain processing pathway, it does not help to elucidate the origin of this amplification because brain activation is largely reflective of the perceived intensity of the stimulus (Coghill et al. 2003; Coghill et al. 1999). Indeed, for stimulation at iso-intense perceived pain levels, rather than iso-intense stimulus intensity (e.g. same temperature), brain activation patterns are largely similar (Cook et al. 2004; Giesecke et al. 2004b; Gracely et al. 2002). But matching the perceived intensity implicates that patients in these studies often receive significantly lower stimulus intensities than control subjects (Cook et al. 2004; Giesecke et al. 2004b; Gracely et al. 2002). Whilst this is an interesting observation in its own right, the differences in peripheral input might confound the interpretation of subtle differences in brain activation and thereby hinder the investigation of the source of signal amplification. A different approach was taken in the

present study. Stimulus intensities (i.e. temperatures) between patients and control subjects were matched, and during analysis variations in pain ratings were controlled for (by including them as regressor of no interest) when the groups were compared. Hence, differences in brain activation revealed by the statistical comparison of activation, such as in anterior insula and amygdala, cannot be caused by (minor) differences in perceived intensity.

Although psychophysical rating did not reveal differences in the perceived *intensity* of the thermal stimulation between patient and control group, imaging results suggest that experimental heat pain is processed differently by neuropathic pain patients. For example, activation in PAG and cuneiform nucleus was only observed in the patient group. As outlined in Section 1.5.3, these structures are involved in the bidirectional descending modulation of pain (Fields and Heinricher 1985). Functional imaging cannot unambiguously distinguish inhibitory and facilitatory influences on pain transmission. Yet, a recent study of experimental secondary hyperalgesia in humans suggested that activation in cuneiform nucleus reflects descending facilitation (Zambreanu et al. 2005). As heat pain stimulation was applied outside the clinically affected area in all patients, facilitation would have to occur in a non-localised fashion. Indeed, brainstem structures involved in descending facilitation have large somatic receptive fields, which means that input from large body areas could be augmented by this mechanism (Fields et al. 1991). Increased nociceptive input in the present study could be indicated by the observation that the patient group showed stronger activation of the

contralateral posterior insula, despite matched temperatures (Craig 2003b). Together with the observed activation in brainstem structures, this might suggest that facilitation of input from thermal stimulation outside clinically affected areas could have occurred in neuropathic pain patients. However, this cannot be fully ascertained. Firstly, increased activation in posterior insula or brainstem structures in the patient compared to the control group was not confirmed by RoI analysis. Secondly, no statistically significant difference in pain ratings was found between the groups and finally, limited evidence from animal studies suggests that brainstem facilitation occurs for mechanical and cold but not for warm stimuli (Sun et al. 2001).

Magnitude changes of sensory aspects of nociception are not the only alterations that could occur in the pain processing of patients. For instance, a network comprising the amygdala, PAG and parabrachial nucleus has been implicated in emotional and autonomic aspects of nociceptive processing (Suzuki et al. 2004) and the amygdala and anterior insular cortex are generally implicated in the processing of emotions and feelings (Craig 2002; Zald 2003). Hence, the finding that these two regions showed significantly more activation in the patient than in the control group is in line with the observed increase in depression and state anxiety scores in the patient group. Concomitant increase of negative affect and activation in limbic areas during pain processing has been described previously: fibromyalgia patients diagnosed with major depression show increased right anterior insula and amygdala activation compared to fibromyalgia patients without depression (Giesecke et al. 2005). In another

study, fibromyalgia patients demonstrated increased anterior insula activation compared to controls, accompanied by higher depression and post-scan state anxiety scores (Cook et al. 2004). In that study, stimulus intensities were matched and pain ratings did not differ between patient and control group, as is the case with the current study. Anterior insular cortex, in particular in the right hemisphere, has been shown to mediate attention to and awareness of bodily sensations such as one's own heart beat (Critchley et al. 2004). In the same study, interoceptive awareness and right anterior insula activation were both correlated with negative affect, i.e. anxiety and depression (Critchley et al. 2004). Attending to a painful stimulus does indeed lead to increased anterior insula activation (Brooks et al. 2002). Hence, attention to and awareness of information arising from the body could explain the association between negative affect and increased anterior insula activation in pain processing. Further studies are needed to investigate this potential relationship.

In conclusion, differences in brain activation between patients and healthy controls might be caused by several factors, which include differences in negative affect and facilitation of nociceptive input. As the neural networks that process these different mechanisms are likely to be inter-linked, it is more difficult to attribute a specific brain activation pattern to a particular mechanism. Investigation of a larger patient sample is likely to help unravel the contribution of different factors, in particular because subtle differences were detected using a relatively small patient sample in the present study.

This study identified the anterior insula as a candidate region where pain processing could be altered in patients with chronic pain conditions. In order to investigate this possibility further, a literature review of pain imaging studies was performed. The review contrasts anterior insula processing of clinical pain with that of experimental pain in healthy volunteers and is presented in the next chapter.

CHAPTER 4: The role of anterior insula in clinical pain compared to experimental pain in healthy volunteers

4.1 Introduction

In the 1960s Geschwind suggested that lesions damaging the insula result in *pain asymbolia* by disconnecting the somatosensory cortex from the limbic system (Geschwind 1965). Patients with pain asymbolia recognize painful stimuli but lack appropriate emotional and behavioural responses to nociceptive stimuli applied anywhere on the body surface. Consequently, they have significantly higher pain intolerance thresholds and endure pain for longer whilst pain detection thresholds are not significantly altered (Berthier et al. 1988; Greenspan et al. 1999).

The anatomical connections known from primate studies place the insular cortex in an ideal position to interrelate information from the extra-personal space to the body's internal milieu: the postero-dorsal insula, which is somatotopically organised (Brooks et al. 2005; Craig 1995), is densely connected to sensorimotor areas (Mesulam and Mufson 1982b) and receives direct thalamic input (Craig 2003b). Information is postulated to pass from the postero-dorsal insula to the mid-insula where it is processed to form a sensory re-representation (Craig 2002). Subsequent processing within the anterior insula, which has dense reciprocal connections to the limbic system (Chikama et al. 1997; Mesulam and Mufson 1982a; Mesulam and Mufson 1982b), is thought to add a subjective component to stimulus processing, leading perhaps to interoceptive attention and awareness of one's physical condition (Craig 2003a; Critchley et al. 2004;

Damasio et al. 2000). With respect to pain processing, it has been hypothesized that "the insula integrates somatosensory information of a nociceptive stimulus with contextual information to provide an overall sense of intrusion and threat to the physical body and self" (Price, 2000).

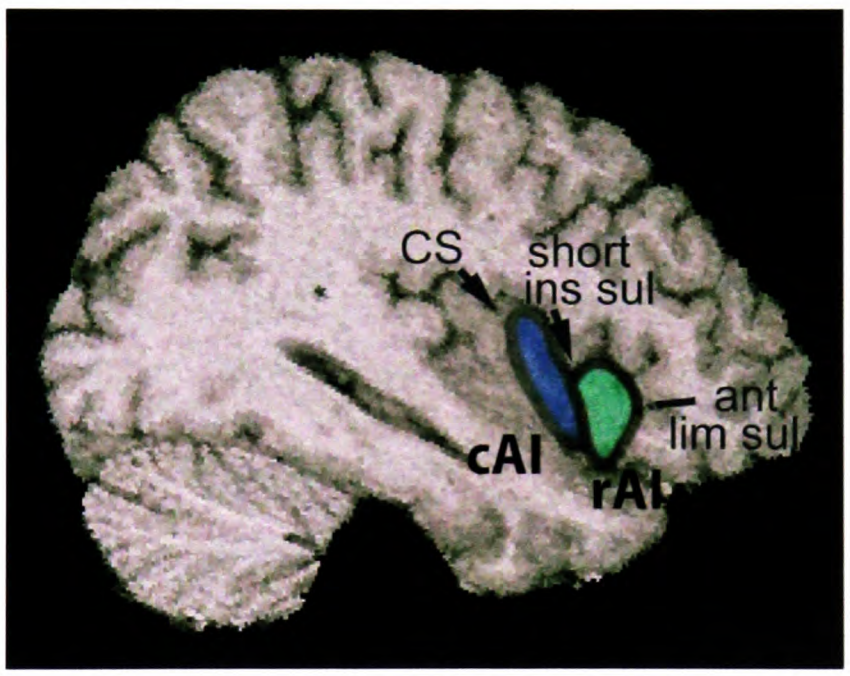
Clinical pain in patients and experimental pain in healthy volunteers have been suggested to differ with respect to emotions directed toward long-term implications of having pain (Price, 2000). This consideration, together with the observations described above, has led to the hypothesis that these two types of pain (clinical and experimental) differ in their processing within the anterior portion of the insula. To investigate this, a literature review of experimental and clinical pain imaging studies reporting anterior insula activation was conducted. Imaging studies commonly divide the insula into an anterior and a posterior portion, and sometimes describe a mid-insular section spanning a region between the arbitrarily defined boundaries of anterior and posterior insula. To facilitate more precise localization of brain activation, two distinct areas within the anterior insula (the portion of the insula anterior to its central sulcus) were defined in this study: rostral anterior insula (rAI), consisting of the anterior short gyrus and caudal anterior insula (cAI), comprising middle and posterior short gyrus. Imaging studies examining cortical processing of highly negative emotions and of interoception were included in the literature review to test if clinical pain processing is located more closely to processing of unpleasant emotions and perception of stimuli originating inside the body when compared to experimental pain in healthy volunteers.

4.2 Methods

4.2.1 Subdivision of anterior insula

In this study, a subdivision of the anterior insula that is based upon its macroscopic anatomy was employed. Middle and posterior short gyri were defined as caudal anterior insula (cAI) and the anterior short gyrus as rostral anterior insula (rAI) (Figure 4.1). This subdivision has been previously used by Brooks and colleagues (Brooks et al. 2002).

Figure 4.1 Insular anatomy



Anatomical details of the delineation of the rostral anterior insula (rAI, green) and caudal anterior insula (cAI, blue). rAI was defined to consist of the anterior short gyrus of the insula and cAI of the middle and posterior short gyrus. The short anterior gyrus is limited anteriorly by the anterior limiting sulcus; the posterior short gyrus is delimited posteriorly by the central sulcus of the insula. Anterior and middle short gyri are separated by the short insular sulcus. ant lim sul, anterior limiting sulcus; CS, central sulcus (of the insula); short ins sul, short insular sulcus.

4.2.2 Literature review

PET and FMRI studies investigating (1) clinical pain, (2) somatic nociceptive pain in healthy subjects, (3) capsaicin-evoked pain in healthy subjects, (4) interoceptive input / interoceptive task, (5) stimuli with highly negative emotional content, or (6) cognitive or emotional aspects / modulation of nociceptive pain processing in healthy subjects were considered for inclusion and were identified by manual search of MEDLINE, covering the period January 1994 to March 2005. Only short-lasting (up to 20 seconds) experimental pain was considered for category (2) as tonic experimental pain is known to have a stronger affective component (Rainville et al., 1992). Single subject studies were excluded from analysis.

For each study, the peak of activation in the anterior insula was determined. All peak activation coordinates given in Talairach space were converted into MNI space using the transformation algorithm TAL2MNI developed by Matthew Brett (http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml) and used in previous meta-analysis research (Murphy et al. 2003). If the peak of insula activation was located outside a predefined bounding box, the study was excluded from the analysis. The bounding box was defined as follows: first, a liberal mask of the insula anterior to the central sulcus was drawn on the MNI 152 template. The mask volume was 11.6 cm^3 (reported literature value for grey matter of this region: 5.34 cm^3 (Kasai et al. 2003). The highest and lowest values in x-, y- and z-direction of this mask were

taken as borders of a cuboid and 4 mm were added in each direction to obtain the bounding box.

To investigate the relative position of clinical pain in the y-direction (i.e. rostral-caudal), Mann-Whitney U-tests ($p < 0.05$, two-sided) were performed between the y-coordinates of clinical pain and nociceptive pain, capsaicin-related pain, negative emotional stimuli and interoception; and between nociceptive pain and capsaicin-related pain.

4.3 Results

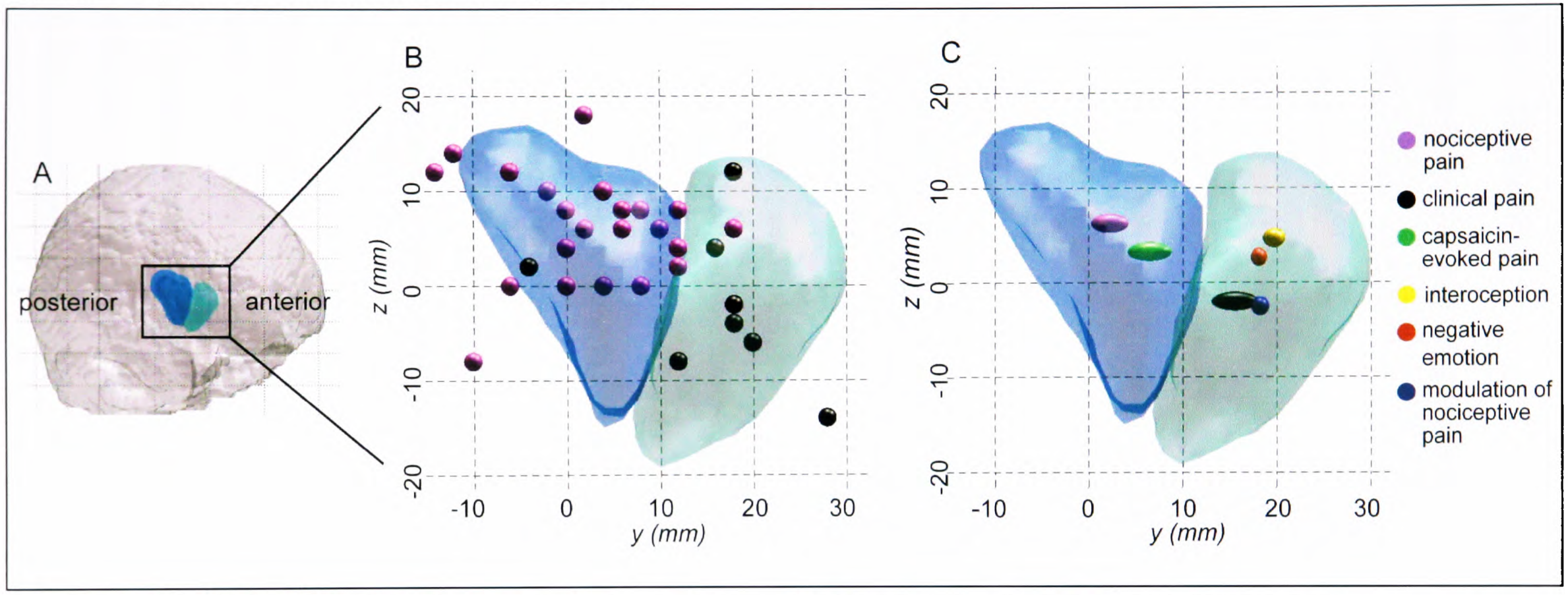
The following number of studies (experiments) were included for categories (1) to (6): clinical pain: 8 (9), somatic nociceptive pain in healthy volunteers: 20 (24), capsaicin-evoked pain: 7 (8), interoceptive input / task: 4 (4), stimuli with highly negative emotional content: 5 (6), cognitive or emotional aspects / modulation of nociceptive pain in healthy subjects: 5 (6). Clinical pain comprised studies investigating allodynia in neuropathic pain patients (Petrovic et al. 1999) or complex regional pain syndrome (Maihofner et al. 2005), ongoing neuropathic pain (Hsieh et al. 1995), cluster headache (Hsieh et al. 1996; May et al. 1998a; May et al. 2000) and angina pectoris (Rosen et al. 1994; Rosen et al. 2002). Studies of nociceptive pain in healthy subjects provoked pain by noxious heat or cold or by laser stimulation (Apkarian et al. 2000; Bingel et al. 2003; Brooks et al. 2002; Casey et al. 1996; Coghill et al. 1999; Coghill et al. 1994; Davis et al. 2002; Derbyshire et al. 2004; Gelnar et al. 1999; Kurata et al. 2002; Lorenz et al. 2002; Paulson et al. 1998; Peyron et al. 1999; Ploghaus et al. 1999; Strigo et al. 2003; Svensson et al. 1998; Svensson

et al. 1997; Tracey et al. 2000; Vogt et al. 1996; Xu et al. 1997). Studies investigating cognitive or emotional aspects / modulation of nociceptive pain in healthy volunteers instructed subjects to attend to their pain (Brooks et al. 2002; Peyron et al. 1999), imaged brain activation during anticipation of pain (Ploghaus et al. 1999) or modulated the unpleasant value of the nociceptive stimulus by hypnotic suggestion (Rainville et al. 1997). The category of capsaicin-related pain comprised studies investigating capsaicin-induced experimental allodynia / hyperalgesia (Iadarola et al. 1998; Lorenz et al. 2002; Maihofner et al. 2004b; Witting et al. 2001) and ongoing pain due to capsaicin application (Andersson et al. 1997; Iadarola et al. 1998; Lorenz et al. 2002; May et al. 1998b). Studies of stimuli with highly negative emotional content included those that investigated viewing pictures of mutilated humans and contamination (Wright et al. 2004), aversive gustatory stimulation (Zald et al. 1998) and neural responses to fearful vocalisations (Morris et al. 1999). In addition, anxiety in normal subjects (Liotti et al. 2000) and worrying statements in subjects with generalized anxiety disorder (Hoehn-Saric et al. 2004) were included in this category. Imaging studies in the category "interoceptive input / task" investigated hunger (Tataranni et al. 1999), food-related visual stimuli in hungry subjects (LaBar et al. 2001), air hunger (Evans et al. 2002) and heartbeat detection (Critchley 2004).

Figure 4.2 depicts the results of the literature review of anterior insula activation. It shows that experimental pain in healthy volunteers is preferentially processed in the cAI, whereas processing of clinical pain is located significantly more anteriorly ($p < 0.001$). Modulation of nociceptive

processing in healthy volunteers or its anticipation significantly shifted the peak of activation from the cAI, where it was located for "pure" nociceptive processing, anteriorly to the rAI ($p < 0.001$). Neuronal correlates of interoception and of stimuli with a highly negative emotional content or anxiety were equally found in the rAI, significantly more anterior than experimental pain ($p = 0.003$ and $p < 0.001$, respectively) and close to where clinical pain is represented. Capsaicin-evoked pain was located in the cAI, although it seems to reside slightly more anteriorly compared to nociceptive pain.

Figure 4.2 Spatial distribution of anterior insula processing



(A) Relative position of rAI (green, anterior short gyrus insulae) and cAI (blue, middle and posterior short gyrus insulae) in the human brain.
(B) Anterior insula activation found in imaging studies investigating somatic experimental nociceptive pain in healthy subjects (provoked by noxious heat or cold or by laser stimulation) (purple spheres) and clinical pain (ongoing and provoked neuropathic pain, angina pectoris, cluster headache) (black spheres). Clinical pain spheres are located significantly more anterior than experimental pain spheres (Mann-Whitney U-test, two-sided: $p < 0.001$).
(C) Studies investigating (1) clinical pain, (2) somatic nociceptive pain in healthy subjects, (3) capsaicin-evoked pain in healthy subjects, (4) interoceptive stimuli / tasks, (5) anxiety or stimuli with highly negative emotional content or (6) cognitive or emotional aspects / modulation of nociceptive pain processing in healthy subjects. Clinical pain is located as anterior as highly negative emotions or interoceptive states (Mann-Whitney U-tests between clinical pain and highly negative emotions / interoceptive states: $p = 0.9$ and $p = 0.4$, respectively). X-, y and z-coordinates of individual studies were averaged for the groups and ellipsoids are relative in size to the standard deviation (SD) in y-direction (size of blue sphere corresponds to SD = 0.4 mm). Coordinates are shown in MNI space. Y-axis, anterior-posterior direction; z-axis; superior-inferior direction.

4.4 Discussion

Although clinical pain and acute nociceptive pain differ with respect to their meaning for the individual and associated psychological states (Bushnell and Apkarian 2005), there is a large overlap between the brain regions processing these two types of pain (Apkarian et al. 2005) and no region is specific for either type of pain. Yet, this literature review shows that clinical and experimental pain processing do differ if sub-regions of pain processing regions are considered, in this case anterior insula.

The meta-analysis performed here demonstrates that clinical pain is mostly processed in the rostral part of the anterior insula (rAI), regardless of whether patients suffered from neuropathic pain, cluster headache or angina pectoris; conversely, activation due to nociceptive pain in healthy subjects was localized predominantly in the caudal part of the anterior insula (cAI). Stimuli with a highly negative emotional content, anxiety and interoception were all found to activate the rAI, close to where clinical pain is represented. From Figure 4.2 it is apparent that nociceptive pain can be shifted into the rAI also in healthy volunteers but only when there is either a cognitive or emotional modulation of the experience: for example, explicitly paying attention to or anticipating a painful stimulus or increasing its unpleasant valence by hypnotic modulation. These are situations in which the subject is required to engage in one way or another with the nociceptive stimulus. Clinical pain is likely to carry more meaning for the individual than experimental pain and it is suggested that clinical pain is located significantly more rostral in the anterior insula because it engages

systems that mediate emotions, subjective feelings and sensitivity to stimuli originating from the body more than experimental pain does.

Models of chronic pain are employed to advance the knowledge of pathological pain processing in the human brain. Capsaicin is frequently used to produce certain characteristics of neuropathic pain in healthy volunteers. However, the literature review showed that processing of capsaicin-evoked pain is located in the cAI, although it seems to reside more anterior compared to nociceptive pain, which could signify that it has the potential to engage negative emotional and interoceptive circuits more than experimental nociceptive pain. It could be expected that ongoing pain induced by capsaicin engages the rostral portion of anterior insula more than capsaicin-induced allodynia does because long-duration pain stimuli are relatively more unpleasant for a given perceived intensity than brief experimental pain stimuli (Price 2000). Post-hoc analysis showed that the mean y-coordinate of ongoing capsaicin-related pain was indeed located more anterior that capsaicin-induced allodynia ($y = 11$ and $y = 4$, respectively), albeit this was not statistically significant (Mann-Whitney U-test, two-sided, $p = 0.19$). These findings emphasize the importance of selecting the appropriate model dependent on the aspect of pain processing that ought to be investigated.

Cytoarchitectonic mapping of human insular cortex is currently being performed (Zilles, 2005), thus the structural subdivision of the human insula in this study according to macroscopic anatomy is somewhat arbitrary. Nevertheless, both the functional imaging data and

cytoarchitectonic data from monkey studies support a division into three sectors. In primate studies, granular cortex is found in the postero-dorsal portion, dysgranular cortex in the antero-ventral part and within this area, an agranular component is found ventrally (Jones and Burton 1976; Roberts and Akert 1963). Different functions can be ascribed to the three areas (Jasmin et al. 2004; Mesulam and Mufson 1982b). The antero-ventral part is not only closely connected to the limbic system via pathways to amygdala, entorhinal cortices, anterior tip of cingulate gyrus (Mesulam and Mufson 1982a; Mesulam and Mufson 1982b) and ventral striatum (Chikama et al. 1997), but also contains the neural specialisation for autonomic responses (Hoffman and Rasmussen 1953; Jasmin et al. 2004; Showers and Lauer 1961). Recent studies in rats have expanded data on the agranular portion and have suggested that the rostral agranular insular cortex (RAIC) plays a particularly important role in pain processing: the RAIC has extensive connections to brainstem structures that are known to be involved in descending anti- and pronociception (PAG, pericerulear region, rostroventral medulla, parabrachial nucleus) (Jasmin et al. 2004) and pharmacological and biochemical interventions in the RAIC alter pain behaviour. For instance, morphine injections, local increases of dopamine as well as of GABA-levels all result in analgesia by enhancing the descending inhibition of spinal nociceptive neurons (Burkey et al. 1999; Burkey et al. 1996; Jasmin et al. 2003). In humans, opioid receptors have been shown to be more densely present in anterior than in posterior parts of the insular cortex (Willoch et al. 2004).

In conclusion, it is suggested that a functional sub-division of the anterior

insula exits, comprising rostral (rAI) and caudal (cAI) functional entities. Further studies are likely to benefit from a clear distinction between rAI and cAI and human cytoarchitectonic data will probably provide refined boundaries for these two regions in the near future. The relative position of activation within anterior insula could help to validate which aspects of chronic pain are represented by different experimental models of pathological pain. Why and how clinical pain engages emotional and interoceptive circuits within the anterior insula needs to be addressed in longitudinal studies aimed at quantifying such potential plastic changes. Such knowledge might be beneficial, as shift of the representation of chronic pain processing back to cAI might be useful as marker of therapeutic efficacy.

The literature review did not contain a category that comprised studies investigating acute nociceptive pain in chronic pain patients. The comparison of such studies with experimental pain in healthy volunteers and with clinical pain would have been interesting but only two such studies met the inclusion criteria of the literature review: one study investigated heat pain in atypical facial pain and found the peak of activation in the cAI (Derbyshire et al. 1994) and the other, which examined heat pain processing in patients with fibromyalgia, reported the peak in the rAI (Cook et al. 2004). To expand the data on experimental pain processing in patients, cerebral heat pain processing in patients with rheumatoid arthritis was investigated and is presented in the next chapter. In addition, clinical pain processing was examined in the same patients, which allowed the direct comparison of experimental and clinical pain

processing in this patient group. It was also examined if clinical pain in a chronic inflammatory condition is processed in the rAI, in analogy to the clinical pain conditions studied in the present chapter.

CHAPTER 5: Contrasting processing of clinical and experimental pain in patients with rheumatoid arthritis

5.1 Introduction

Although joint pain and stiffness are the most distressing symptoms of rheumatoid arthritis (RA) (Hendiani et al. 2003; Kidd et al. 2000), pain in RA is incompletely understood. Every day practice reveals differences in the level of pain suffered by patients that are difficult to explain on the basis of peripheral joint swelling and inflammation alone. For instance, RA patients can report high pain levels in the absence of swollen joints or elevated inflammatory markers, while others report no pain but have evidence of active disease (Thompson and Carr 1997).

Considering RA as a chronic pain condition as much as a rheumatological disease helps to explain the discrepancy between reported pain intensity and clinical disease. Alterations in the CNS caused by persistent nociceptive input are traditionally described for neuropathic pain conditions (Woolf 1993). But the notion that persistent or recurrent firing of nociceptors potentially leads to plastic changes in the CNS also in other disease states, including inflammatory conditions, is becoming increasingly recognized (Ji and Woolf 2001; Yaksh et al. 1999). Moreover, alterations in psychological states are associated with chronic pain conditions (Bushnell and Apkarian 2005) and psychological and behavioural variables can alter the amount of pain that is perceived (Craig 2005). CNS alterations and psychological variables should therefore be taken into account when studying the degree of pain in RA.

Depressive mood and the tendency to catastrophize are two important psychological variables that are related to the pain experience (Sullivan et al. 2001a). Epidemiologically, there is considerable evidence for an overlap between chronic pain and clinical depression (Bair et al. 2003; Currie and Wang 2004; Dworkin and Gitlin 1991; Romano and Turner 1985). Depression is associated with a heightened pain experience (Romano and Turner 1985; Taenzer et al. 1986) and pain intensity and depression scores are significantly correlated also in RA (Affleck et al. 1992; Mangelli et al. 2002). This close relationship between negative affect and pain is not limited to chronic pain patients but can also be observed in healthy volunteers: psychophysical studies have repeatedly shown that induction of negative mood leads to increased pain sensitivity (de Wied and Verbaten 2001; Rainville et al. 2005; Zelman et al. 1991).

While depression has been discussed as a significant emotional determinant of the pain experience (Gaskin et al. 1992), it has been suggested that catastrophizing is an important cognitive determinant (Chaves and Brown 1987; Jensen et al. 1991; Sullivan et al. 2001a). Catastrophizing has been defined as "the tendency to focus on and exaggerate the threat value of painful stimuli and negatively evaluate one's ability to deal with pain" (Rosenstiel and Keefe 1983) and is one of the most important psychological predictors of the pain experience (Geisser et al. 1994b; Sullivan et al. 2005a; Sullivan et al. 1998). Specifically, the degree of catastrophizing predicts some of the pain experienced by RA patients at a later time point (Keefe et al. 1989). Consequently, cognitive-behavioural intervention programmes for chronic

pain have incorporated strategies to reduce catastrophizing (Bennett et al. 1996; Keefe et al. 1990; Vlaeyen et al. 1995) and these strategies have been shown to reduce pain and improve function in RA (Keefe et al. 1989).

Both depression and catastrophizing have been shown to influence the cerebral response to pain in clinical populations (Giesecke et al. 2005; Gracely et al. 2004). Because psychological factors are particularly related to clinical pain, they might influence the processing of clinical pain to a larger extent than that of experimental pain. To address this question, this study investigated processing of clinical and experimental pain in RA patients. Specifically, cerebral responses to a stimulus that mimics RA-related pain experienced on a daily basis were contrasted with cerebral responses to an experimental noxious stimulus. To explore potential reasons for differential processing, influences of depression and catastrophizing on the processing of clinical pain were compared to the influences on experimental pain processing. Two hypotheses were investigated: (1) clinical and experimental pain are processed differently, i.e. clinical pain engages brain regions that are related to emotional and cognitive aspects of the pain experience to a larger extent, and (2) depression and catastrophizing influence clinical and experimental pain processing to a different degree, which should be particularly evident in regions that are involved more in clinical than in experimental pain processing.

5.2 Methods

5.2.1 Patients

16 patients were recruited through the Department of Rheumatology at the Nuffield Orthopaedic Centre in Oxford, England. To be included in the study, patients had to present with active RA (Disease Activity Score 28 (DAS 28) greater than 3.2; see 5.2.2). Exclusion criteria were any condition causing pain other than RA and any neurological or psychiatric disease, apart from depressive disorders. In addition, patients in whom cognitive impairment was suspected according to the Mini Mental State Examination (see below) were excluded from participation to ensure that they were capable of providing adequate information in the psychological self-assessment. Study participants were asked to refrain from analgesic medication 24 hours before the experiment, but disease modifying drugs were not altered.

5.2.2 Assessment tools

DAS 28

The DAS 28 (Prevoo et al. 1995) is a validated outcome measure used by the European League Against Rheumatism (EULAR) to differentiate between high and low disease activity (van Gestel et al. 1996; van Riel and van Gestel 2000). It is a composite of four parameters, incorporating subjective and objective measures: (1) swelling and (2) tenderness on palpation (assessed in twenty eight joints), (3) subjective level of disease activity during the past week (on a Visual Analogue Scale (0 – 100 mm)) and (4) the erythrocyte sedimentation rate (ESR), a blood marker for

inflammation. A DAS 28 score greater than 3.2 is considered as moderately active disease and greater than 5.1 as very active disease (Prevoo et al. 1995).

Clinical pain assessment

Self-report of clinical pain intensity was obtained by asking the subjects to rate the average pain they experience on a typical day using the numerical pain rating scale (NRS), described in 2.5.

Beck Depression Inventory (BDI)

Please refer to Section 2.3 for details of the BDI. Its administration is described in Section 5.2.3.

Pain Catastrophizing Scale (PCS)

The PCS measures catastrophizing as a multi-dimensional concept including the tendency to ruminate (e.g. "I keep thinking about how much it hurts"), magnification (e.g. "It's terrible and I think it's never going to get any better"), and helplessness (e.g. "There's nothing I can do to reduce the intensity of the pain") (Sullivan et al. 1995). Tendency to catastrophize is conceptualized as a continuum with no cut-off for 'normal' levels. The possible scores of the PCS range from 0 to 52.

Mini Mental State Examination (MMSE)

The MMSE is a brief 11-question measure that tests five areas of cognitive function: orientation, registration, attention and calculation, recall and language (Folstein et al. 1975). The maximum score is 30 and a score of

23 or less is indicative of cognitive impairment. Patients obtaining a score of 23 or less were excluded from this study. The MMSE was performed in this study because it was suspected that patients might be elderly and were therefore more likely to present with cognitive impairment than the patients recruited for the other studies of this work.

5.2.3 Clinical assessment

On the same day and before FMRI investigation, a medical history focusing on RA and associated pain was obtained from each patient. Patients were assessed for other joint conditions, other chronic pain syndromes unrelated to RA, conditions affecting peripheral sensation, and antecedent history of depressive illness and other psychiatric diseases. Subsequently, joints were assessed for the DAS 28 and patients rated the subjective disease activity. The MMSE was performed by the investigator. Next, the BDI and PCS were explained to the subjects. After having demonstrated that they understood the instructions, participants completed both questionnaires in privacy.

Blood samples were obtained on the same day after the FMRI investigation in order to avoid painful venepuncture prior to the experiment. ESR and C-reactive protein (CRP), another blood inflammatory marker, were determined in the respective laboratories at the John Radcliffe Hospital in Oxford. CRP is considered to be a better test for the acute phase of inflammation while it has been suggested that the ESR is a better measure of disease severity in RA because it is also sensitive to haematological measures (e.g. decreased haemoglobin and

haematocrit), rheumatoid factor and other immunoglobulins (Wolfe 1997).

5.2.4 Provocation of clinical pain

The objective was to mimic the pain that patients with RA experience in their affected joints on a day-to-day basis. To this end, a purpose-built magnet-safe pressure device was developed. The surface area over which the pressure was to be applied measured 1 cm in diameter. The device was graded so that similar pain ratings could be produced in patients with varying levels of disease activity. The pressure device had been tested in six other RA patients in an earlier pilot study and they had confirmed that it replicated the pain they associated with their condition and experienced on a chronic basis. Patients typically described the pressure pain applied to their hand as very similar or identical to the pain they experience when they try to open a bottle. Importantly for the paradigm used in the FMRI experiment in this study, the pressure-evoked pain decayed very quickly after termination of the stimulus. In addition, the pain intensity provoked by the stimulus was found to be constant in most instances, i.e. there was little variation in pain intensity with repetitive stimulation.

Initial testing of clinical pain provocation took place after the clinical assessment but before the patient was put into the MR scanner. During each stimulus, the pressure device was pressed down for two seconds on a joint on the right hand, selected for its tenderness during the DAS 28 assessment. Stimuli were separated by 50 seconds to prevent sensitization. Patients were asked to rate the pain provoked by the stimulus on a NRS from 0 to 10. Once a location on the joint that elicited a

rating of 6/10 had been identified, this was marked with a cross. After the patient had been positioned in the scanner, two to three further test stimuli were applied before the start of the experiment to confirm the NRS rating of 6/10, thereby taking into account the lower ambient temperature and altered position of the limb.

5.2.5 Provocation of experimental pain

Experimental heat pain was applied using the thermal resistor described in Section 2.6. Heat pain was chosen as experimental nociceptive stimulus for two reasons: first, it activates those areas associated with pain processing in the brain reliably and therefore provides a good standard to compare with clinical pain processing. Second, the type of stimulus (heat) is very different from the mechanical stimulus used to provoke clinical pain and should therefore be very distinct and not reminiscent for the patients of their clinical pain.

Initial testing of heat pain stimuli took place after the testing of clinical pain. The thermode was applied to the right volar forearm, at the midpoint. Stimuli lasted for two seconds and were separated by 50 seconds to minimize sensitization. Once a stimulus yielded a rating of 6/10, testing outside the MR scanner was concluded.

5.2.6 Experimental protocol

Two FMRI experiments were performed during which clinical pain and thermal pain were provoked, respectively. During the clinical pain experiment, ten mechanical stimuli lasting two seconds were applied to

the previously identified joint on the right hand using the same pressure device and the pressure determined previously. Intervals between the stimuli varied in the course of the experiment to reduce anticipation of the stimulus (50 s, 55 s, 60 s, 65 s or 70 s); the same timing protocol was used for each patient. The identical protocol (ten stimuli lasting two seconds each and the same inter-stimulus intervals) was employed in the thermal pain experiment. Immediately after each experiment, patients were asked to rate the stimulus-produced pain on average using the NRS. The order of the clinical and thermal pain experiments was balanced across subjects. As it was crucial for this study to attain the same pain ratings in both experiments, the temperature was adjusted to match the post-scan rating of the clinical pain experiment in patients on whom the clinical pain experiment was conducted first. In patients who underwent the thermal experiment first, the temperature was adjusted to match the clinical pain rating acquired outside the scanner. Hence, the procedures to obtain matched pain ratings differed slightly between patients who underwent the clinical pain experiment first and patients who underwent the thermal pain experiment first. Therefore, it was tested if the obtained match between thermal and clinical pain differed between the two groups. The difference between thermal and clinical pain ratings was calculated for each patient and compared between the two groups. The mean difference for the group which received the thermal stimulation first was 0.9 (SD: 0.3) and for the group which received clinical pain first, the difference was 0.7 (SD: 0.25). The match of clinical and experimental pain did not differ between the two groups ($p = 0.5$, two-sided Mann-Whitney U-test).

5.2.7 Data acquisition

Scans were acquired with a Siemens Trio 3 Tesla MRI/MRS system using a single channel head coil. A standard whole-brain gradient echo EPI sequence was used for functional scans (repetition time TR = 3 s, echo time TE = 30 ms, flip angle 90°, 36 axial slices, field of view (FOV) = 192 mm x 256 mm, matrix 64 x 64, voxel size 3 x 3 x 3.3 mm^3, 195 volumes). The first two volumes were discarded to allow steady-state magnetization. In addition, a structural scan was acquired using a 3D-FLASH-sequence with inversion recovery (TR = 204 ms, TE = 5.56 ms, TI = 1100 ms, flip angle 8°, isotropic volume acquisition, rectangular field of view, voxel size 1 mm^3) for co-registration purposes.

5.2.8 Data analysis clinical data

To examine the relationships between baseline data and clinical variables, Pearson's product moment correlation coefficients were calculated between age, disease duration, average daily pain, inflammatory markers, disease activity, morning stiffness, BDI scores and residual PCS scores. Standardized residuals of the PCS scores were calculated by regressing the BDI scores on the PCS scores to statistically control for self-reported depression in catastrophizing. Catastrophizing is suggested to play an important role in the experience of chronic pain *independent* of depression (Geisser et al. 1994b; Sullivan et al. 2001a), although catastrophizing and depression are known to be related (Haaga et al. 1991; Jensen et al. 1992). The standardized residuals of the PCS scores were also used as a regressor in the analysis examining the relationship between catastrophizing and brain activation.

Based on a previous publication investigating the influence of catastrophizing on brain activation (Gracely et al. 2004), additional median split of the data was performed. The group was divided into patients with high and low catastrophizing scores and patients with high and low depression scores, respectively. As each sub-group consisted of eight subjects, non-parametric Mann-Whitney U-tests were performed for baseline and clinical data between the sub-groups. Correlations and Mann-Whitney U-tests were considered significant at $p < 0.05$ (two-sided tests).

5.2.9 Data analysis imaging data

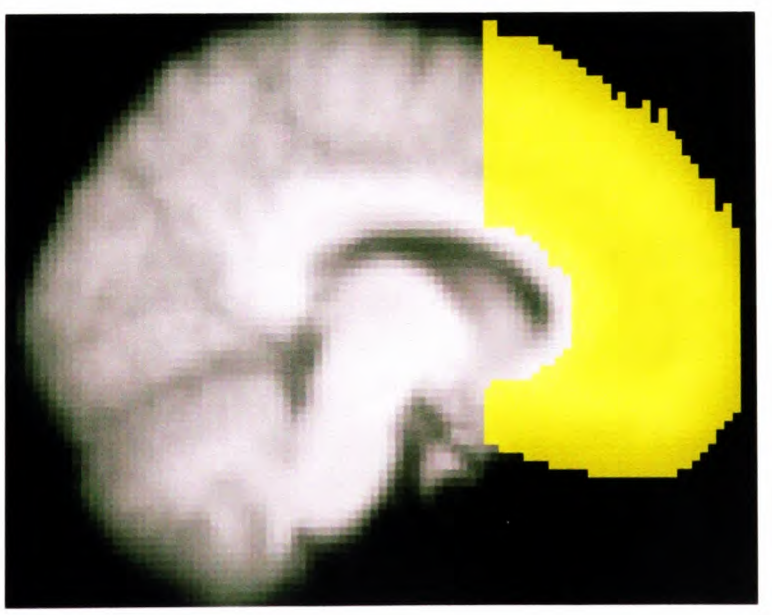
The following pre-processing steps were applied to each individual data set: motion correction using MCFLIRT (Jenkinson and Smith 2001), non-brain removal using BET (Smith 2002), spatial smoothing, mean-based ("grand mean") intensity normalization and high pass temporal filtering using a cut-off of 50 seconds. The same general linear model (GLM) was applied on a voxel-by-voxel basis to the pre-processed data of the thermal and of the clinical pain scans. One explanatory variable (EV) was constructed by convolving the stimulus input function with a haemodynamic response function (the regressor of interest) and the temporal derivative of this EV was included as covariate of no interest. Voxel-wise parameter estimates were derived for the regressor of interest. In addition, the differential contrasts that examine voxels in which activation is stronger in response to either type of pain were calculated for each subject. Before subject-specific contrast images were entered into

group analysis, they were transformed into MNI standard space using the transforms obtained by registration of the pre-processed functional data to the individual anatomical scans and subsequent registration to the MNI 152 template (Collins et al. 1994).

Group analyses were performed using FLAME (FMRIB's Local Analysis of Mixed Effects), which is a mixed effects model (Smith et al. 2004) estimating the random effect component of the inter-subject variance. The main effects of the clinical pain and of the experimental pain condition were calculated using a regressor constant across all subjects. To test for statistically significant differences between clinical and thermal pain, a paired t-test was performed within the GLM. The relationship between depression and catastrophizing, respectively, and brain activation was determined in two ways. Firstly, correlation analysis was performed separately for clinical and for thermal pain. In two separate analyses, BDI scores or residual PCS scores were entered as regressor into the GLM. Importantly, a regressor constant across all subjects was retained and BDI (or residual PCS scores) were orthogonalised with respect to this regressor. These procedures ensure that activation that is found to co-vary with BDI scores (or residual PCS scores) is independent of activation common to all subjects. To test if potential relations between BDI or residual PCS scores and brain activation are significantly different for clinical than for experimental pain, additional correlational group analyses were performed using the same model as before but in which the within subject differential contrast images between clinical and experimental pain served as inputs. Secondly, to determine if results of the correlation

analysis can be replicated using a different methodological approach, median splits of the data were performed into patients with high and low catastrophizing scores and patients with high and low depression scores, respectively, and unpaired t-tests between the groups were performed. Because this study aimed at investigating a differential effect of catastrophizing and depression on clinical and experimental pain processing and the paired comparison showed differences between these two types of pain in the prefrontal cortex (see Section 5.3.5), a prefrontal cortex mask was employed for all analyses investigating the influence of depression and catastrophizing on brain activation. This mask was drawn in standard space and was limited caudally by a vertical plane through the anterior border of the anterior commisure and excluded subcortical structures, the ventricles and the corpus callosum. Consequently, the mask comprised most of BA6 and 24 and all of BA8, 9, 10, 11, 12, 44, 45, 46, 32 (Figure 5.1).

Figure 5.1 Prefrontal cortex mask



A prefrontal cortex mask was employed for statistical analysis of the influence of depression and residual catastrophizing scores on brain activation. The mask comprised Brodmann areas (BA) 8, 9, 10, 11, 12, 44, 45, 46, 32 and most of BA6 and 24.

The voxel-based threshold was set to $Z > 2.3$ for all group statistical maps. For main effects analyses, cluster-level correction was performed for multiple comparisons across the whole brain using Gaussian random field theory (Worsley et al., 1992) and the cluster threshold was set to $p < 0.05$. For paired comparison and correlation analyses, no correction for multiple comparisons was applied and all clusters exceeding 100 contiguous voxels are reported. Coordinates are given in MNI space (Evans et al., 1992).

5.3 Results

5.3.1 Subject characteristics and clinical history

The subject group consisted of 16 RA patients, mean age 57 years (SD: 10.8 years), eleven females and five males. Disease duration ranged from 3 to 37 years (mean: 14 years; SD: 10 years). All subjects had joint involvement, but the presence of extra-articular manifestations or complications of RA was not universal. The most common RA-associated condition reported was a history of carpal tunnel syndrome (three patients). This would have been of concern if symptomatic because it affects peripheral sensation in the hand. However, in all cases, the carpal tunnel syndrome had resolved spontaneously or after curative surgery. Other extra-articular features included keratoconjunctivitis sicca (two patients), Sjogren's syndrome (one patient) and flexor tendonitis (two patients). None of the patients reported a diagnosis of co-morbid joint conditions. However, some degree of secondary osteoarthritis (OA) should be expected in advanced cases of RA and one patient had Heberden's nodes, which are a typical finding in OA. Other co-morbid conditions which

may affect peripheral sensation were not found, although one patient suffered from Diabetes Mellitus type 1. However, this was well-controlled and there was no history of peripheral neuropathy. Similarly, no conditions affecting cerebral blood flow were reported. Although one patient had a history of asthma, this was well-controlled so hypercapnia was unlikely.

Subjects were taking a range of medication, including disease modifying anti-rheumatoid drugs, steroid preparations, anti-inflammatory medications and analgesics. Subjects were encouraged to omit their analgesic medication for 24 hours before the scan; however, it was not a prerequisite for participation in the study. As a result, eight patients refrained from analgesic medication on the day of imaging; the remaining eight had taken their analgesic medication in the morning, including non-steroidal anti-inflammatory drugs and dihydrocodeine.

5.3.2 Present clinical assessment

Subjects' current level of disease severity varied. The majority of patients rated their average daily pain as moderate to severe (NRS ratings: mean: 5.7; SD: 1.8; range: 3 to 9). Morning stiffness, a common clinical indicator of disease activity, was variable and lasted from a couple of minutes up to five hours (Table 5.1). On joint examination, nine patients had more tender than swollen joints. According to DAS 28 scores, 14 patients had very active disease (DAS 28 score > 5.1) at the time of investigation; the remaining two showed moderately active disease (3.2 < DAS 28 score < 5.1) (mean: 5.8; SD: 1.0). Data on clinical patient characteristics can be found in Table 5.1. Analysis of the clinical data revealed that average daily

pain correlated positively with CRP (r = 0.6, p = 0.014), length of morning stiffness (r = 0.6, p = 0.014) and self-assessed disease activity (r = 0.5, p = 0.04). The only other statistically significant correlation was found between CRP and ESR (r = 0.65, p = 0.007).

Table 5.1 Patient characteristics

Patient	Sex	Age (yrs)	Disease Duration (yrs)	Average daily pain	DAS28	CRP (mg/L)	BDI	PCS	Morning stiffness (min)	MMSE
1	F	44	8	7	5.7	62	17	49	180	30
2	M	59	15	3.5	5.1	9	21	27	10	30
3	M	63	4	7	6.8	35	17	35	20	26[1]
4	F	54	3	5	5.8	< 8[2]	2	14	60	29
5	F	45	22	6	5.1	36	29	24	0[3]	29
6	F	65	7	5	6.7	15	7	14	0[3]	29
7	F	72	17	6	5.7	14	13	9	60	29
8	M	57	32	4.5	5.5	11	2	14	5	28
9	F	59	20	8	6.4	20	9	14	45	29
10	M	57	3	5.5	3.9	< 8[2]	13	14	120	29
11	F	72	37	6	6.4	16	16	11	210	28
12	F	53	5	9	5.7	35	7	15	300	30
13	F	70	20	7.5	7	41	12	15	120	29
14	M	45	8	2.5	3.7	< 8[2]	13	29	30	30
15	F	62	14	6	6.8	67	9	21	75	30
16	F	34	12	3	6.9	25	9	5	20	29
mean		56.8	14	5.7	5.8	27.5	12.3	19.4	78	29
SD		10.8	10	1.8	1	19.7	6.9	11.1	87	1

[1] Patient could not hold a pen because of the pain. Hence, the last two points of the examination, which require writing, were omitted.
[2] The laboratory does not give exact values for values below 8 mg/L. In these cases, a value of 8 was entered in the calculation of the mean and standard deviation.
[3] Do not experience stiffness – rather describe localised ache which lasts all day.

F, female; M, male; yrs, years; DAS 28, Disease Activity Score 28; CRP, C-reactive protein; mg, milligram; L, litre; BDI, Beck Depression Inventory; PCS, Pain Catastrophizing Scale; MMSE, Mini Mental State Examination; SD, standard deviation.

5.3.3 Psychological assessment

Three patients had an antecedent history of depression; in two cases, this had been associated with a specific stressor and had long been resolved. One patient had ongoing depression that had run a chronic course for years and was treated by low-dose citalopram. Consistent with this history, this patient presented with the highest BDI score of 29 indicating moderate to severe depression (Beck et al. 1961). One more patient had a score suggestive of moderate to severe depression; all other subjects had either BDI scores in the normal range or scores that are consistent with mild to moderate depressive symptoms (mean of all patients: 12.3; SD: 6.9; range: 2 to 29). PCS scores varied markedly in the group (mean: 19.4; SD: 11.1; range: 5 to 49). A non-significant association was found between BDI and PCS scores ($r = 0.47$, $p = 0.065$).

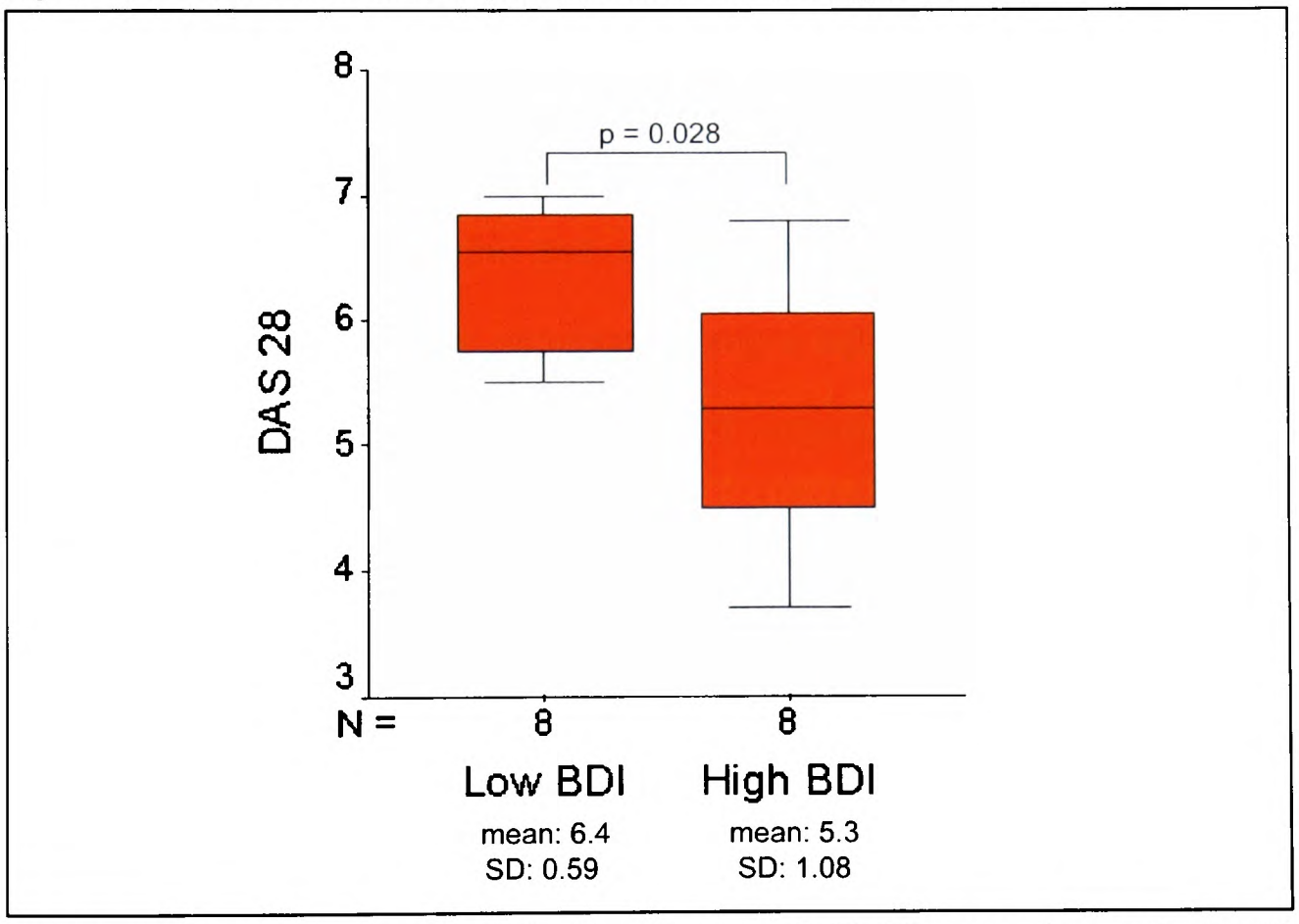
Although no correlation was found between BDI scores and clinical patient characteristics, median split of the data into patients with high and low BDI scores revealed that patients with high BDI scores had a significantly lower disease activity as measured by the DAS 28 than patients with low BDI scores ($p = 0.027$) (Figure 5.2) even though there was no significant difference in average daily pain between the two groups ($p = 0.7$). Similarly, median spit of the data into patients with high and low residual catastrophizing scores showed that patients with high catastrophizing scores displayed a lower ESR (mean: 25 mm/h; SD: 14.8 mm/h) than patients with low catastrophizing scores (mean: 40 mm/h; SD: 21.9 mm/h); however, this did not reach statistical significance ($p = 0.1$).

5.3.4 Psychophysical results

Pain ratings for clinical pain stimuli obtained immediately after the imaging experiment ranged from 4 to 6 (mean: 4.9; SD: 0.7). Matching of the perceived intensity of clinical and thermal pain stimuli was achieved: ratings for the two experiments did not differ more than one unit on the NRS for any patient. Mean pain rating for thermal pain was 4.8 (SD: 0.8).

All subjects confirmed at the end of the imaging session that application of the pressure pain stimulus provoked pain that felt similar to their typical RA-related pain.

Figure 5.2 DAS 28 scores in patients with low and high BDI scores



Patients with low BDI scores had significantly higher DAS 28 scores than patients with high BDI scores (Mann Whitney U-test, two-sided, p = 0.027) although both groups reported no difference in average daily pain (p = 0.7). Mean (SD) average daily pain in group with low BDI scores: 6.0 (2.0); mean (SD) average daily pain in group with high BDI scores: 5.4 (1.6).

5.3.5 Imaging results

Provocation of experimental heat pain resulted in activation of brain regions commonly observed in imaging experiments of pain (Apkarian et al. 2005; Peyron et al. 2000b), including anterior and posterior insula, ACC, pre-motor cortex, thalamus, S2, inferior parietal cortex, prefrontal cortex, basal ganglia and brainstem. The activation map resulting from the thermal pain experiment is shown in Figure 5.3. Details of activation regarding location and peak Z-scores are given in Table 5.2.

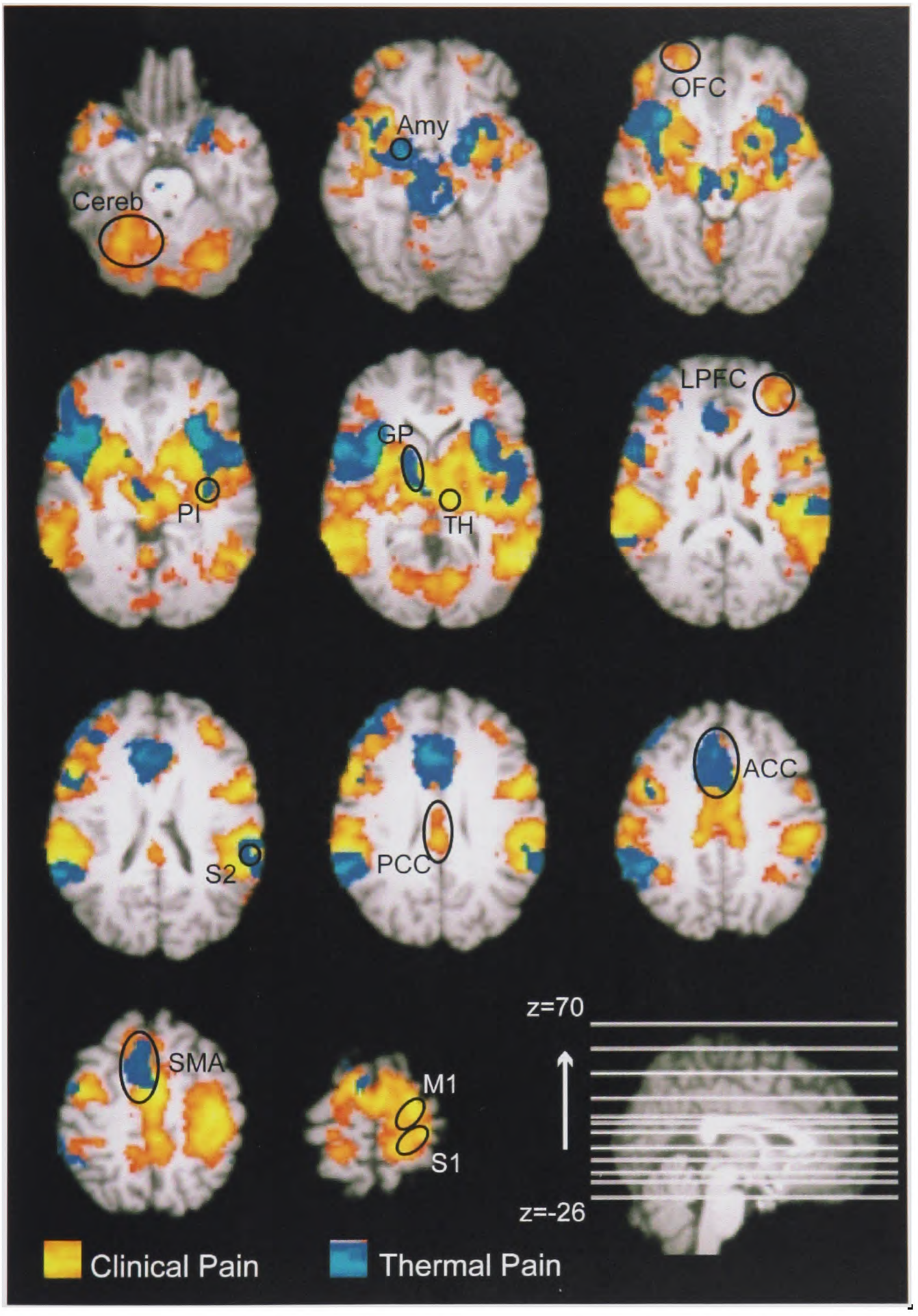
Clinical pain, matched in intensity to the heat pain, equally activated typical pain processing regions but yielded activation in additional areas (Table 5.2, Figure 5.3). To test if statistically significant differences between clinical and experimental pain processing existed, a paired t-test was performed between the two conditions. This analysis showed that several brain regions were significantly more activated during processing of clinical pain than during processing of experimental pain, despite the equality of perceived pain intensity. These regions included orbito- and prefrontal cortices, S1, M1, SMA, thalamus, basal ganglia, cerebellum, temporal cortices and posterior cingulate cortex (Table 5.3). For an exploratory analysis of voxels that were more activated in the thermal than in the clinical pain condition, the criterion of minimal cluster size of 100 contiguous voxels was abandoned. Even in this analysis, no region showed significantly more activation in the experimental pain condition than in the clinical pain condition.

Table 5.2 Brain activation in response to experimental and clinical pain

		Experimental Pain		Clinical Pain	
Brain region		**[x y z]**	**Z-score**	**[x y z]**	**Z-score**
Anterior insula	cl	[-32 16 2]	5.1	[-34 16 -2]	5.6
	il	[38 16 0]	5.3	[42 12 -2]	5.6
Posterior insula	cl	[-36 -22 10]	3.6	[-34 -20 6]	5.3
	il			[34 -16 4]	4.8
ACC		[2 24 26]	4.1	[-4 10 30]	5.1
Thalamus	cl			[-16 -24 6]	5.0
	il	[8 -10 0]	3.0	[8 -16 6]	4.9
S1	cl			[-36 -32 64]	5.4
S2	cl	[-62 -26 18]	3.4	[-58 -26 16]	5.4
	il			[50 -26 16]	5.1
Globus pallidus	cl			[-12 -2 2]	5.0
	il	[12 -2 4]	3.5	[16 6 2]	4.8
Putamen	cl			[-30 2 2]	5.6
	il			[32 8 2]	5.5
SMA	cl			[-54 14 22]	4.7
	il	[6 28 44]	4.3	[58 8 24]	4.8
Primary motor cortex	cl			[-28 -16 68]	5.1
Cerebellum	cl			[-36 -56 -36]	4.6
	il			[32 -50 -32]	4.3
LPFC	cl			[-34 42 18]	4.2
	il	[26 46 28]	4.3	[38 36 18]	3.7
Orbitofrontal cortex	il			[24 58 -14]	3.6
Inferior parietal cortex (BA39/40)	cl	[-62 -48 -10]	3.3	[-58 -42 24]	5.8
	il	[50 -48 36]	4.4	[58 -38 28]	6.0
Temporal gyrus	cl			[-54 -56 6]	5.4
	il			[62 -52 5]	4.8
Amygdala	cl	[-18 -6 -16]	4.1	[-22 0 -16]	4.6
	il	[18 -4 -18]	4.5	[26 0 -14]	4.6
Brainstem					
SN / red nucleus	il			[10 -26 -10]	4.4
Cuneiform nucleus	cl	[-8 -32 -14]	3.5	[-8 -26 -12]	4.2
	il	[10 -30 -14]	4.3	[8 -28 -12]	4.6

Locations of peak Z-scores within an activation cluster are given according to MNI coordinates. Voxel threshold was set to $Z > 2.3$ and cluster-level correction for multiple comparisons across the whole brain to $p < 0.05$. ACC, anterior cingulate cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; LPFC, lateral prefrontal cortex; BA, Brodmann area; SN, substantia nigra; cl, contralateral; il, ipsilateral.

Figure 5.3 Brain activation in response to clinical and experimental pain



Provocation of clinical and experimental thermal pain that were matched for perceived pain intensity result in different brain activation patterns. Thermal pain activates a network of typical pain processing regions; clinical pain results in activation of additional brain areas including prefrontal cortex and sensorimotor areas. Images are in radiological convention. Cereb, Cerebellum; Amy, amygdala; OFC, orbitofrontal cortex; PI, posterior insula; GP, globus pallidus; TH, thalamus; S2, secondary somatosensory cortex; PCC, posterior cingulate cortex; ACC, anterior cingulate cortex; SMA, supplementary motor cortex; M1, primary motor cortex; S1, primary somatosensory cortex.

Table 5.3 Paired t-test: clinical > experimental pain

Region	Side	x	y	z	Z-score	Cluster size
Orbitofrontal cortex	cl	-24	44	-6	3.4	199
	il	38	36	-8	4.3	194
Medial frontal cortex		0	60	-8	3.8	157
Cingulate cortex / SMA	il	12	-12	46	3.2	443
PCC	cl	-2	-30	48	2.7	112
Cerebellum	il	30	-42	-30	4.3	200
Primary motor cortex	cl	-36	-10	62	3.2	532
	il	42	-16	62	4.1	361
Secondary somatosensory cortex	cl	-42	-26	54	3.9	815
	il	36	-36	50	3.0	123
Premotor cortex	cl	-54	0	28	3.3	231
	il	58	0	28	3.3	187
Thalamus	cl	-12	-32	8	3.8	288
	il	18	-24	0	3.0	170
Putamen	cl	-28	-10	2	3.1	107
	il	32	-6	0	3.7	302
Superior / middle temporal gyrus	cl	-54	-2	-28	3.3	231
	il	56	-70	10	4.6	6576
Temporal pole	cl	-52	-4	-16	3.6	289
Parieto-occipital junction	il	2	-70	44	3.0	169

Locations of peak Z-scores within an activation cluster are given according to MNI coordinates. Voxel threshold was set to Z > 2.3, uncorrected, minimal cluster size: 100 voxels. SMA; supplementary motor area; PCC, posterior cingulate cortex; cl, contralateral; il, ipsilateral.

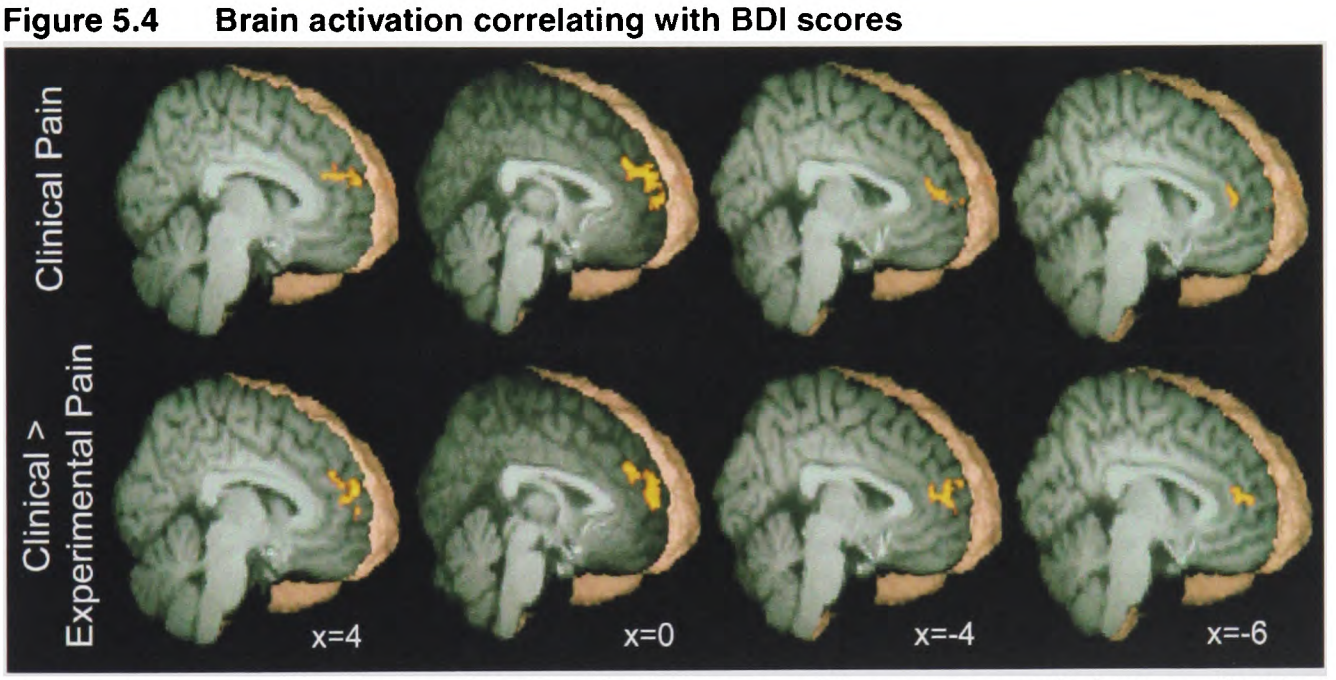
In a next step, brain activation was investigated in relation with BDI and with residual PCS scores respectively. During clinical pain, a cluster in the medial prefrontal cortex (MFC) correlated positively with depression scores (Table 5.4, Figure 5.3). No correlation with BDI scores was observed for experimental pain. The stronger relationship between BDI and brain activation for clinical pain was ascertained by using the differential contrast images on single subject level (clinical > experimental pain) as inputs (Table 5.4, Figure 5.3). No brain area correlated negatively with depression scores. Conversely, no region showed a positive correlation with catastrophizing scores but several lateral prefrontal cortex (PFC) areas correlated negatively with residual catastrophizing scores. This negative correlation between residual PCS scores and PFC activation was observed in the clinical and in the experimental pain condition (Table 5.5, Figure 5.4) and no correlation with residual PCS scores was observed when the individual differential contrast images were used. Median-spilt of the data into patients with low and high BDI scores confirmed the results of the correlation analysis because patients with high BDI scores displayed significantly more MFC activation than patients with low BDI scores. Median spilt of the data into patients with low and high catastrophizing scores failed to show higher LPFC activation in patients with low catastrophizing scores both for experimental and for clinical pain.

Table 5.4 Brain activation correlating with BDI scores

Region	Side	x	y	z	Z-score	Cluster size
Clinical pain						
Medial frontal cortex		0	42	26	3.7	584
Clinical > experimental pain						
Medial frontal cortex	cl	-6	58	18	3.6	622

Locations of peak Z-scores within an activation cluster are given according to MNI coordinates. The search volume was restricted to prefrontal cortex and voxel threshold was set to Z > 2.3, uncorrected, minimal cluster size: 100 voxels. The lower row shows the correlation with BDI scores when the differential contrast images (clinical pain > experimental pain), formed on single subject level, were used as inputs in the group analysis. BDI, Beck Depression Inventory; cl, contralateral; il, ipsilateral.

Figure 5.4 Brain activation correlating with BDI scores



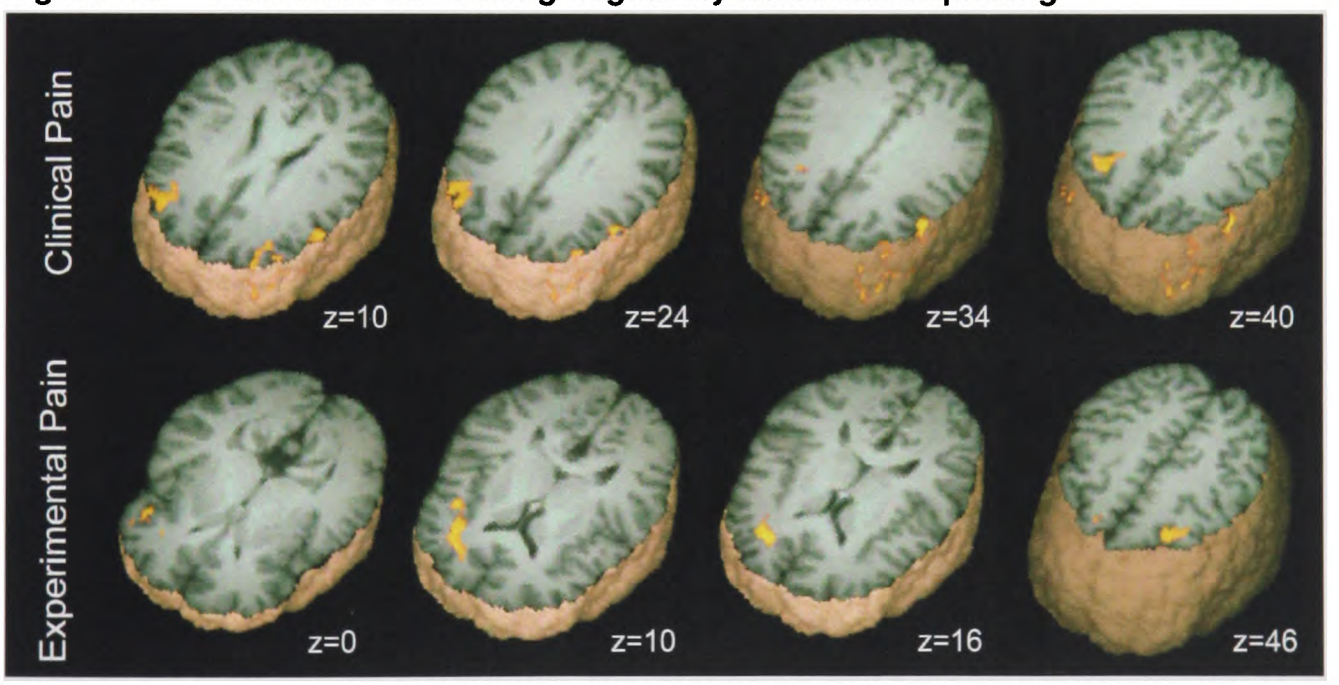
Brain activation correlating positively with BDI scores. The upper panel shows activation during provocation of clinical pain correlating with BDI scores. The lower panel shows the correlation with BDI scores when the differential contrast images (clinical pain > experimental pain), formed on single subject level, were used as inputs in the group analysis. The search volume was restricted to prefrontal cortex and voxel threshold was set to Z > 2.3, uncorrected, minimal cluster size: 100 voxels. BDI, Beck Depression Inventory.

Table 5.5 Activation during clinical and experimental pain correlating negatively with residual PCS scores

Region	Side	x	y	z	Z-score	Cluster size
Clinical pain						
Ventrolateral PFC	cl	-54	32	8	3.5	316
Dorsolateral PFC	il	46	46	28	3.0	294
Dorsolateral PFC	cl	-54	14	34	3.7	168
Dorsolateral PFC	il	30	28	44	3.2	149
Experimental pain						
Ventrolateral PFC	il	26	44	12	3.5	428
Dorsomedial PFC	il	16	44	42	3.2	373
Dorsolateral PFC	cl	-30	24	46	3.6	175

Locations of peak Z-scores within an activation cluster are given according to MNI coordinates. The search volume was restricted to prefrontal cortex and voxel threshold was set to $Z > 2.3$, uncorrected, minimal cluster size: 100 voxels. Residual PCS scores were obtained by correcting PCS scores for BDI scores. PCS, Pain Catastrophizing Scale; BDI, Beck Depression Inventory; PFC, prefrontal cortex; cl, contralateral; il, ipsilateral.

Figure 5.5 Activation correlating negatively with catastrophizing



Brain activation during provocation of clinical (upper panel) and experimental pain (lower panel) correlating negatively with PCS scores, corrected for BDI scores. Search volume was restricted to prefrontal cortex and voxel threshold was set to $Z > 2.3$, uncorrected, minimal cluster size: 100 voxels. BDI, Beck Depression Inventory; PCS, Pain Catastrophizing Scale.

5.4 Discussion

This study compared and contrasted cerebral processing of clinical and experimental pain in RA patients. To this end, processing of clinical pain and experimental pain stimuli that were matched for perceived pain intensity were investigated following an identical experimental protocol. The results of the experiments indicate that areas involved in both types of pain correspond to those activated by painful stimuli in healthy controls (Apkarian et al. 2005; Peyron et al. 2000b). The roles of these common areas in pain processing are briefly reviewed before a more detailed discussion of the differences between experimental and clinical pain processing is presented. Processing of these two types of pain is discussed predominantly as it relates to the influence of depression and catastrophizing.

5.4.1 Areas activated by clinical and experimental pain

Extensive bilateral insular activation was found in both pain conditions and did not show a statistically significant difference in the paired comparison. Insular cortex, particularly caudal anterior insula (cAI), encodes the intensity of both noxious and innocuous sensations (Coghill et al. 1999; Craig et al. 2000) and the observation that the insula was equally engaged in both pain conditions supports the evidence from verbal pain ratings that the stimuli were indeed equally painful. The peak of activation was located in the rostral anterior insula (rAI) for both pain conditions, i.e. clinical and experimental pain. Considering the findings of Chapter 3 that showed stronger rAI activation in response to experimental heat pain in

neuropathic pain patients compared to matched control subjects and the results of Chapter 4 that indicated that experimental pain in healthy volunteers is preferentially processed in cAI, processing of experimental pain seems to be indeed altered in chronic pain patients, including patients with RA. This raises the possibility that experimental pain might be useful as a marker of altered pain processing in patients with chronic pain conditions, which would have the distinct advantage that experimental pain can often be elicited more easily and in a more controlled way than clinical pain. However, the results of this study show that depression influences pain processing mainly in the clinical pain condition, which suggests that experimental pain processing in chronic pain patients does not capture all aspects of clinical pain processing. Further studies are needed to establish which aspects of clinical pain processing can be represented by experimental pain applied to chronic pain patients.

Mid-ACC was also extensively activated in both experiments. Although the ACC has generally been assigned a role in affective processing of pain (Peyron et al. 2000b), a more cognitive / evaluative role has been suggested for the mid-ACC (Vogt et al. 2003). The mid-ACC has also been described as reflecting perceived pain intensity in healthy volunteers (Buchel et al. 2002; Coghill et al. 1999), which might explain why no significant difference in mid-ACC activation between the two conditions was found, similar to the insular cortex findings. In both conditions, ACC activation was extensive and robust. Decreased ACC activation in response to nociceptive input has been reported previously in RA patients (Jones and Derbyshire 1997) and generally, patient studies have tended

to show reduced activation in pain processing areas when compared to studies of healthy volunteers (reviewed in (Apkarian et al. 2005)). Although no comparison with healthy controls was included, the results from the present study do not suggest that chronic pain patients demonstrate greatly reduced activation in response to nociceptive stimuli. Similarly, the data in neuropathic pain patients, presented in Chapter 3, which were directly compared to matched control subjects, do not support the notion that activation is reduced in chronic pain patients. Several reasons could explain why patient studies less frequently report activation in pain processing areas than healthy volunteer studies. Firstly, the patient groups investigated tend to be more heterogeneous than the groups of healthy volunteers, which is likely to increase the variance in the patient data. Secondly, patient groups tend to be older and the haemodynamic response decreases with age (Aizenstein et al. 2004; D'Esposito et al. 1999; Huettel et al. 2001; Schroeter et al. 2003), which emphasizes the importance of using age-matched control subjects. Lastly, ongoing activity in pain processing areas during constant pain (as might happen in chronic pain patients) has been suggested to inhibit increases of the haemodynamic response in response to an additional stimulus by competing for the same neurophysiological resources (Derbyshire et al. 2002). The influence of ongoing pain on the FMRI response to a brief painful stimulus is investigated in more detail in Chapter 6.

5.4.2 Areas activated preferably by clinical pain

In addition to areas activated in both the clinical and thermal pain condition, paired comparison showed that clinical pain activated several areas in addition to experimental pain, or to a greater extent. Since the design of this study was aimed at comparing clinical and experimental pain processing, it was important that patients did not relate the experimental pain to their RA-related pain. Therefore, an experimental stimulus which is qualitatively very different from mechanical provocation of clinical pain was chosen. It is likely that observed differences between clinical and experimental pain processing in somatosensory, proprioceptive and motor areas, such as S1, M1, SMA, thalamus, basal ganglia and cerebellum, are attributable, at least partly, to the nature of stimulation (mechanical versus thermal). An alternative explanation for more extensive activation in "motor areas" in conjunction with orbitofrontal cortex (OFC) activation is a greater behavioural drive generated by the clinical pain. OFC plays a central role in motivational learning and action selection (Gallagher et al. 1999; O'Doherty 2004) and its activation is related to "punishers leading to a change in behaviour" (Kringelbach and Rolls 2004). Not only OFC but also other prefrontal cortex (PFC) areas were significantly more active in the clinical compared with the experimental pain condition. Based on the reasoning that preferential activation of PFC by clinical pain should not be related to the different stimulation modality, a PFC mask was employed in this study to investigate the influence of catastrophizing and depression on clinical and experimental pain processing. Imaging studies that compare processing of

experimental mechanical and thermal pain are needed to ascertain this assumption, but this kind of study has not yet been published. In general, studies investigating experimental mechanical pain are scarce. Two studies were performed using a region of interest (RoI) approach, which did not contain prefrontal cortex as a RoI (Disbrow et al. 1998; Fabri et al. 2002). Two other studies report prefrontal cortex activation in response to noxious mechanical stimulation but these results are inconclusive. One study examined tonic pain (Creac'h et al. 2000) and hence, it is not clear if PFC involvement is related to the nature or the length of the stimulus. The remaining study investigated both phasic and tonic mechanical pain but did not specify which stimulus activated PFC (Ringler et al. 2003). The assumption made here that differential PFC activation reflects differential involvement of the PFC in clinical and experimental pain and not the stimulus modality is supported by a recent meta-analysis showing that PFC is more frequently activated in imaging studies of clinical pain than of experimental pain in healthy volunteers (Apkarian et al. 2005). 81% of clinical pain studies describe PFC activation as opposed to 55% of experimental pain studies. The authors of the review suggest that clinical pain conditions have stronger cognitive, emotional and introspective components than acute pain conditions leading to the observed increase in prefrontal activation. To investigate if the differences in the present study relate to emotional and cognitive variables, depression and catastrophizing scores were incorporated into the analysis. This procedure revealed two distinct patterns of PFC activation: whereas medial PFC (MFC) activation was positively correlated with depression scores in clinical pain, catastrophizing scores were negatively correlated with

activation in lateral PFC (LPFC) both in clinical and experimental pain. These results accord with previously described functions of these two areas: MFC plays a crucial role in emotional processing (Phan et al. 2002) and the LPFC is implicated in the cognitive control of emotions (Levesque et al. 2003; Phan et al. 2005) and in the control of pain (Lorenz et al. 2002). First, the relation between MFC and depression is examined in more detail, followed by a discussion of LPFC, pain control and catastrophizing.

5.4.3 MFC, depression and chronic pain

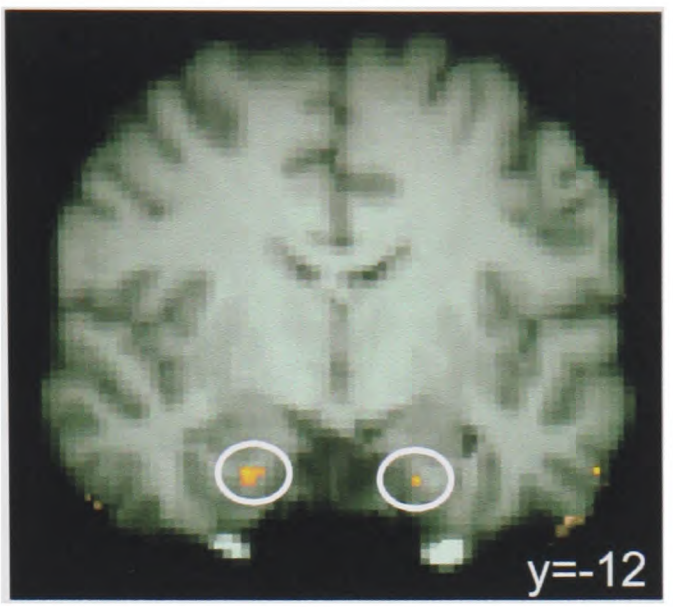
Both human and animal studies suggest a role of MFC in pain facilitation. Stimulation of MFC using transcranial magnetic stimulation (TMS), possibly disrupting MFC activation, inhibits pain in humans (Kanda et al. 2003) and MFC lesions in rats lead to increased latencies in the hot plate test (Pastoriza et al. 1996). However, it should be noted that in both examples MFC encompassed a part of the cingulate cortex and the observed modulation of nociception may have been partially mediated by this brain region. Reviews have implicated the perigenual / rostral portion of ACC in the unpleasantness of pain (Peyron et al. 2000b; Vogt 2005) and this portion of MFC has been shown to be involved in anxiety-induced increase of pain (Ploghaus et al. 2001). In the current study, brain activation correlating with depression scores extended into the perigenual / rostral ACC (Figure 5.3), but the main correlation site was located rostrally in BA9/10. This area has repeatedly been described as site of emotional processing (reviewed in (Phan et al. 2002)). For instance, several studies in healthy volunteers found MFC activation in response to

transient sadness at almost identical co-ordinates as the peak correlation in this study (George et al. 1995; Lane et al. 1997; Pardo et al. 1993). Recently, MFC was demonstrated to be the effect site of emotional regulation: cognitive detachment, which led to reduced anticipatory anxiety, decreased MFC activation in a pain paradigm (Kalisch et al. 2005). In the same study, the source of this emotional regulation was shown to be located in the lateral PFC. This result is interesting in view of the catastrophizing analysis of this study. Lateral PFC processing is discussed in more detail in Section 5.4.4.

Anatomically, the MFC is connected to the amygdala (Amaral and Price 1984). The amygdala forms part of the limbic system, together with other structures of the medial temporal lobe (MTL) such as the hippocampal formation, subiculum and entorhinal cortex (Braak et al. 1996; Insausti et al. 1987; Papez 1937). In 1968, Melzack and Casey proposed that the hippocampus and associated cortices participate in mediating the aversive drive and affect characteristic of pain (Melzack and Casey 1968). Increasing evidence now points to the amygdala as an important interaction site between negative affective states and persistent pain (Neugebauer et al. 2004). In addition to playing a key role in affective disorders, including anxiety and depression (Cardinal et al. 2002; Davidson 2002; Gallagher and Schoenbaum 1999; LeDoux 2000; Zald 2003), the amygdala is a target region of ascending nociceptive pathways (Barnett et al. 1995; Bernard et al. 1989) and contains a nociceptive portion (Burstein and Potrebic 1993; Gauriau and Bernard 2002). Animal studies of chronic arthritic pain show that prolonged pain leads to neural

plasticity and enhanced neuronal responsiveness in the amygdala (Neugebauer and Li 2003). The link in the amygdala between nociceptive processing and emotion was recently substantiated by an imaging study in which amygdala activation differentiated fibromyalgia patients with major depression (MD) from patients without MD (Giesecke et al. 2005). In the present study, both experimental and clinical pain led to amygdala activation. In addition, the magnitude of MFC activation correlated with the magnitude of entorhinal cortex activation in the clinical pain condition (Figure 5.6), revealed by post-hoc analysis. Generally, the pattern of cortical connectivity of the entorhinal cortex resembles that of the amygdala (Van Hoesen 1995) and prenatal lesions of entorhinal cortex in mice have put this region forward as another possible source of pain modulation (Fiore et al. 1999). Indeed, an imaging study in healthy volunteers showed that anxiety-related increases in perceived pain are associated with significant activation in the entorhinal cortex (Ploghaus et al. 2001).

Figure 5.6 Activation co-varying with MFC activation

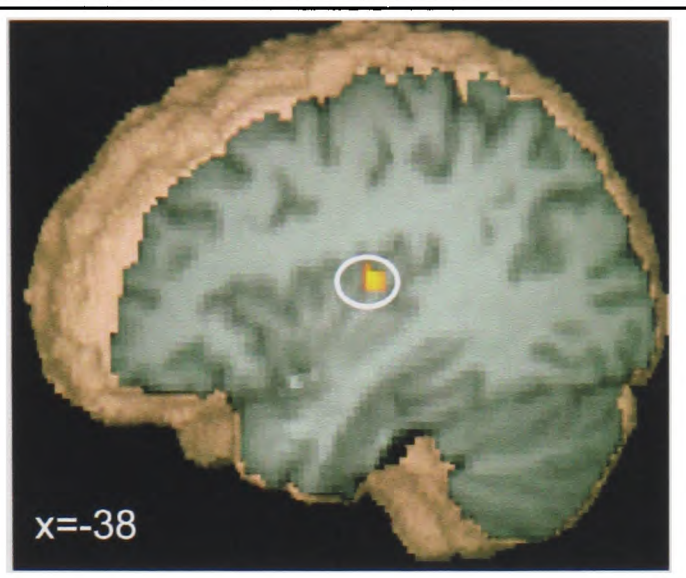


In a post-hoc analysis, activation co-varying with MFC activation was determined. To this end, the highest percent signal change was determined for each subject in the MFC cluster observed in the GLM analysis of clinical pain investigating the correlation with depression scores. These values were entered as regressor in a whole brain GLM analysis of the clinical pain imaging data. Voxel threshold was set to $Z > 3.0$ and no cluster correction was applied. Activation in entorhinal cortex correlated bilaterally with MFC activation and no other correlation was found. The peak of activation was at $x = 22$, $y = -12$, $x = -28$ ($Z = 3.4$) in the right hemisphere and at $x = -20$, $y = -16$, $z = -30$ ($Z = 3.2$) in the left hemisphere. Image is in radiological convention. MFC, medial frontal cortex; GLM, general linear model.

In the present study, the relationship between depression and pain processing is reflected by brain activation patterns in MFC and MTL. Behaviourally, it is well known that depression and pain are closely linked: for instance, depression is associated with a heightened pain experience. Similarly, high levels of pain are related to high depression scores (Affleck et al. 1992; Linton and Gotestam 1985; Mangelli et al. 2002). Also in this study, a behavioural relationship was observed between the amount of pain patients experienced on a daily basis and their depression scores: patients with high and low depression scores experienced the same degree of average daily pain although patients with low depression scores displayed significantly higher disease activity (Figure 5.2). This illustrates that disease activity and degree of inflammation, although weakly related to pain (Rojkovich and Gibson 1998), are not by themselves sufficient to

explain pain in RA. Should this influence of depression scores on pain perception not also be noticeable in the imaging results of this study? To answer this question, a post-hoc analysis was performed that was based on the assumption that activation in contralateral posterior insular cortex reflects the strength of the incoming signal. This assumption is based on the observation that the posterior insula reflects basic sensory aspects of nociceptive input (Apkarian et al. 2005; Brooks et al. 2005; Ostrowsky et al. 2002) and activation in posterior insula is more closely related to the intensity of the nociceptive stimulus than to the perceived pain intensity (Craig et al. 2000). As can be seen in Figure 5.7, posterior insula activation was negatively correlated with depression scores. Considering that pain ratings of the pressure pain stimulus were held constant across subjects, this signifies that patients with low depression scores received a relatively higher stimulus input than patients with high depression scores. This finding supports the view that both stimulus intensity *and* depression contribute to the resulting pain perception. The relationship between depression and pain processing was unique to the clinical pain condition and could not be observed in the processing of experimental pain, pointing at stronger involvement of negative emotional processing in clinical pain and potentially contributing to the pathogenesis and maintenance of RA-related pain.

Figure 5.7 Activation co-varying negatively with BDI scores



It was tested in a post-hoc analysis if contralateral posterior insula activation co-varies negatively with BDI scores. The search volume was restricted to the anatomically defined contralateral posterior insula (posterior to the central sulcus of the insula) and the voxel threshold was set to $Z > 2.3$. It was found that contralateral posterior insula correlated negatively with BDI scores (peak Z-score at $x = -38$, $y = -16$, $z = 14$; $Z = 2.8$).

5.4.4 LPFC, coping and pain control

Apart from amplifying the pain experience, the CNS is also capable of inhibiting and controlling pain. In many circumstances, such as acute stress (keyword: fight or flight), this is evolutionary desirable. Brainstem mechanisms that inhibit nociception have been established in animal models (Basbaum and Fields 1984) and shown to operate also in humans (Tracey et al. 2002). Higher cortical functions that contribute to endogenous pain control have been substantially less studied, but evidence is emerging that the prefrontal cortex plays an important role in generating anti-nociception. Specifically the lateral PFC has been implicated in various ways in the down regulation of pain, including placebo analgesia (Wager et al. 2004), perceived control over pain (Wiech et al.) and endogenous suppression of pain unpleasantness (Lorenz et al. 2002). The regulatory function of lateral PFC is not restricted to pain processing, but rather extents to volitional and controlled processing of

emotions (Levesque et al. 2003; Phan et al. 2005), cognitive anxiety reduction (Kalisch et al. 2005) and placebo-induced anxiolysis (Petrovic et al. 2005).

In this study, LPFC activation was *negatively* correlated with residual catastrophizing scores. Although catastrophizing is most often conceptualized as a cognitive phenomenon (Rosenstiel and Keefe 1983; Sullivan et al. 2001a), encompassing beliefs or cognitive appraisal, it might also contain affective components (Jones et al. 2003). In order to increase the clarity of the results, the variance associated with depression was removed from the PCS scores in the present study. Thus the finding that the less patients catastrophize, the more they engage the LPFC relates predominantly to the cognitive component of catastrophizing. Interestingly, this relationship between brain activation and this presumably cognitive phenomenon was found in the experimental and clinical pain condition, contrasting depression, which only influenced clinical pain processing. What could be the functional significance of decreased LPFC activation in patients with high catastrophizing scores? Patients who catastrophize hold maladaptive beliefs about their ability to cope with pain (Geisser et al. 1994b; Jensen et al. 1991; Keefe et al. 1989) and there is ample evidence that these beliefs relate to the inability to control or decrease one's pain (Harkapaa 1991; Rosenstiel and Keefe 1983). At the same time, it is well established that pain is perceived as less intense when it is deemed controllable by the subject (Geisser et al. 1994a; Jensen et al. 1991; Pellino and Ward 1998). Indeed, higher catastrophizing scores are predictive of worse pain scores and greater disability at a later time point

in RA patients (Keefe et al. 1989) and have been associated with higher pain levels in various other pain populations including patients with post-herpetic neuralgia (Haythornthwaite et al. 2003) and dental pain (Sullivan and Neish 2000). Also in healthy volunteers, the degree of catastrophizing correlates negatively with cold pain tolerance (Edwards et al. 2005). Based on these considerations, it is suggested that catastrophizing interrupts activation of LPFC and may thereby impair endogenous control of pain.

Depressive symptoms and catastrophizing can be seen as important emotional and cognitive determinants of the pain experience, respectively. In general terms, the relationship between emotion and cognition is one of the most pertinent questions in neuroscience (Northoff et al. 2004). The two distinct patterns of prefrontal activation observed in this study – MFC activation related to negative affect and LPFC activation representing a cognitive phenomenon – endorse the notion of a functional dissociation between medial and lateral PFC in which medial PFC is predominantly concerned with processing of affect and lateral PFC with cognitive processes. Moreover, these two regions, and functions, interact intensively. For instance, a cognitive task attenuates emotion-induced MFC activation and an emotional task attenuates cognition-induced LPFC activation (Northoff et al. 2004). This might be the neurophysiological underpinning of the effectiveness of pain management programmes that focus on emotional and cognitive factors (Turk 2003).

The findings of this study suggest that FMRI has the potential to reveal amplification of the pain experience by psychological factors. The results of Chapter 3 indicated that FMRI might also be useful to detect brainstem facilitation and indirectly central sensitization by inferring from activation in brain areas involved in the early stages of nociceptive processing. This could signify that the different constituents of pain can be visualized using FMRI. Ultimately, such knowledge might lead to selective targeting of different components of the pain experience and FMRI might be useful to monitor longitudinal changes of these components. As a first step towards using FMRI as such a tool to objectify the subjective pain perception, it was investigated in the next chapter if FMRI reflects the perceived intensity of clinical pain. In Chapter 7, this was taken a step further and it was examined if the FMRI signal reflects longitudinal changes of perceived intensity, both in clinical and in experimental pain.

CHAPTER 6: Neural correlates of brush-evoked allodynia in neuropathic pain patients

6.1 Introduction

Pain in response to a normally innocuous stimulus, i.e. allodynia, is an important characteristic of neuropathic pain and is one of its diagnostic criteria (Dworkin et al. 2003). Cerebral responses to provoked neuropathic pain have been investigated in several patient studies using different imaging modalities such as FMRI, PET and MEG (Lorenz et al. 1998; Maihofner et al. 2003; Petrovic et al. 1999; Peyron et al. 1998b; Peyron et al. 2000a; Peyron et al. 2004). These studies revealed that a variety of brain regions are involved in the processing of allodynic pain, such as the insular cortex, anterior cingulate cortex, primary and secondary somatosensory cortices, thalamus, cerebellum, prefrontal cortex, and parietal association areas. Instead of being uniquely associated with allodynic pain, these regions are commonly involved in the processing of experimental pain in healthy volunteers (reviews by Apkarian et al. (2005) and Peyron et al. (2000b)).

It has been repeatedly demonstrated in healthy subjects that the magnitude of signal measured by neuroimaging techniques, irrespective of whether they are indirect or direct measures of neuronal activity, reflects the perceived intensity of acute experimental pain, both within and across subjects (for review see Porro (2003); in addition Bromm and Treede (1991); Iannetti et al. (2005a); Timmermann et al. (2001)). Consequently, the signal magnitude can be considered to be a surrogate marker of perceived pain intensity. A surrogate marker of perceived clinical pain

intensity could be useful as it provides a more direct read-out of the pain experience that is independent of pain labelling behaviour and should therefore be less error-prone than commonly used pain rating scales. To date, two PET studies have explored a possible relationship between the perceived intensity of clinical pain and brain activation in patients. Petrovic and colleagues showed that responses in both anterior cingulate gyrus and anterior insular cortex correlated with "total" subjective pain perception, which was composed of ratings of provoked and ongoing pain (Petrovic et al. 1999). A second study reported a correlation between the intensity of ongoing pain and activation of the cingulate gyrus in patients with trigeminal neuropathic pain (Willoch et al. 2003). However, no study has yet explored whether the perceived intensity of provoked clinical pain is encoded in the human brain independently of ongoing pain intensity. As these two constituents of clinical pain do not necessarily follow the same behavioural pattern, it could be important to differentiate between provoked and ongoing pain.

This study investigated the cerebral processing of allodynic pain in neuropathic pain patients. To examine if the FMRI signal has the potential to serve as a marker of perceived clinical pain intensity, it was investigated if the magnitude of the signal encodes the perceived allodynic pain intensity independently of ongoing pain intensity. Furthermore, an interaction analysis was performed to characterize a potential influence of ongoing pain intensity on the neural correlates of allodynic pain intensity.

6.2 Methods

6.2.1 Participants

FMRI data were obtained from eight neuropathic pain patients whose clinical details are outlined in Table 6.1. Patients were recruited from the Oxford Pain Relief Unit and selected according to the following criteria: (1) presence of neuropathic pain of peripheral origin or caused by plexus avulsion, (2) presence of pronounced brush-evoked allodynia (i.e. dynamic mechanical allodynia), (3) constant ongoing pain or no ongoing pain and (4) absence of any major psychiatric disorder or systemic disease. To ensure a constant background of ongoing pain during the FMRI experiments, patients were asked if they experienced frequent pain paroxysms (every hour) or if they perceived fluctuations of the intensity of their ongoing pain over a one hour period. Patient who answered either of these two questions affirmatively were excluded from the study. If possible, subjects discontinued co-analgesic (antidepressant and antiepileptic) and analgesic drugs. As a result all but two patients were not taking (co-) analgesic medication at the time of investigation (see Table 6.1 for details).

6.2.2 Clinical assessment

All subjects underwent a detailed neurological examination prior to the day of the imaging session (for results see Table 6.1). The function of lemniscal pathways was assessed by vibratory and moving tactile stimuli as well as by determination of static sensory detection thresholds using von Frey filaments (Stoelting, Illinois, U.S.A.). The function of spinothalamic pathways was assessed through pinprick and temperature

sensations (warm and cold detection thresholds as well as cold pain and heat pain thresholds tested by quantitative sensory testing using a TSA-II NeuroSensory Analyser (Medoc, Haifa, Israel)). Dynamic mechanical allodynia and punctate hyperalgesia (i.e. an exaggerated pain response to von Frey filaments) were tested using a soft brush (Somedic, Hörby, Sweden) and von Frey filaments respectively. Punctate hyperalgesia was considered to be present if the filament two forces above the detection threshold elicited painful sensations. The most painful body site upon brushing was determined and if the pain rating elicited was greater than 3/10 on the numerical rating scale (NRS), the degree of dynamic mechanical allodynia was considered suitable for the study. This body area was marked and photographed to allow easy recognition on the day of FMRI scanning. Ongoing pain intensity was rated using the NRS and the quality of ongoing pain was inferred from the Neuropathic Pain Symptom Inventory (Bouhassira et al. 2004), which each patient was asked to complete on both visits. The presence of symptoms of depression and anxiety was tested using the Beck Depression Inventory (BDI, Beck et al. 1961) and the Spielberger State-Trait Anxiety Inventory (STAI, Spielberger 1970). During the first appointment, patients were also familiarized with the experimental procedure of the imaging session.

6.2.3 Experimental design

Prior to imaging, the location and intensity of dynamic mechanical allodynia was verified and the extent of the site to be stimulated during the experiment was marked on the skin. Dynamic mechanical allodynia was provoked by brushing as determined at the first visit. During the functional

imaging experiment, three consecutive brush strokes were delivered during a 6 second long stimulation block. The strokes were applied with a force of approximately 100 to 150 mN, stroke length was between 6 and 7 cm and velocity was approximately 3 to 4 cm/s. Each stimulation block was preceded by a 24 second rest period and repeated 15 times. The last stimulation block was followed by an 18 second rest-period, resulting in a total acquisition time of 7 minutes and 48 seconds. The short block length, together with the relatively long rest period, was chosen based on the examination conducted during the first visit, which showed that (1) all patients tolerated 15 stimulation blocks of 6 seconds duration well and (2) that this design caused only minimal after-sensations. An average NRS rating of the pain provoked by brushing was obtained immediately after the scan finished. In the following sections, the term "pain rating" is used to refer to this rating. In addition, patients were asked to rate the ongoing pain before and after the functional scan and were specifically asked if they experienced any pain paroxysms during the scan. Stimulation of the homologous contralateral, unaffected body side (or the region less affected in patient 2; see Table 6.1) was performed using the same design (control condition). In half of the patients, the allodynic scan was performed before the control scan and for the remaining patients the order was reversed. Subjects were instructed to keep their eyes closed and to keep as still as possible during the experiments. At the end of the imaging session, a structural scan was performed.

6.2.4 Data acquisition

FMRI data were acquired on a 1.5 Tesla Siemens Sonata MRI scanner using the protocol described in 2.7.4.

6.2.5 Statistical analysis of clinical and behavioural data

Pearson's product moment correlation coefficient (two-sided test, $p < 0.05$) was calculated pair-wise for the following variables: age, disease duration, allodynic pain intensity and intensity of ongoing pain.

6.2.6 Statistical analysis of imaging data

All image processing and statistical analysis was accomplished using the software package FSL 3.2 (Smith et al. 2004).

Subject level analysis

The following pre-processing steps were applied to each functional dataset: removal of non-brain structures, motion correction, spatial smoothing, "grand mean" intensity normalization and high pass temporal filtering (cut-off: 30 seconds). A general linear model (GLM) was applied on a voxel by voxel basis to these data (Worsley and Friston 1995) using FILM (FMRIB's improved linear model) (Woolrich et al. 2001) to model blood oxygen level-dependent (BOLD) signal intensity changes in response to mechanical stimulation. Regressor 1 was constructed by convolving a boxcar function (the stimulus input function: mechanical stimulation = 1; baseline = 0) with a gamma haemodynamic response function. The first temporal derivative of the regressor was included in the model as covariate of no interest. Voxel-wise parameter estimates (PEs)

were derived for regressor 1 using the appropriate contrast ([1 0]). For each patient, one statistical image was calculated for both the allodynic stimulation and control stimulation. The subject level statistical images were registered into MNI standard space using FLIRT (Jenkinson and Smith 2001). For patients with left-sided allodynia, raw functional images of the allodynic condition were mirrored about the midline before statistical analysis and registered to a symmetrical version of the MNI template. Similarly, raw functional images of control stimulation carried out on the left body side (i.e. for patients with right-sided allodynia) were mirrored about the midline and registered to the same symmetrical template.

Group level analysis

Group analyses were performed using FLAME (FMRIB's Local Analysis of Mixed Effects) (Beckmann et al. 2003). To identify brain activation associated with allodynic stimulation, a group statistical map called "allodynic pain" was calculated using one regressor that was constant across subjects. In a second group analysis brain regions in which activation increases linearly with subjective ratings of allodynic pain were identified. Patients' individual pain ratings of the allodynic scan were entered as a regressor. Two covariates of no interest were included in the model: one was constant across subjects to account for activation common to all subjects and ongoing pain ratings were included as the second covariate of no interest. The regressor of interest (i.e. allodynic pain ratings) was orthogonalised with respect to both covariates of no interest to ensure that respective activations were independent of ongoing pain ratings and of any activation that was common to all subjects

irrespective of their allodynic pain ratings. Using the appropriate contrast, a group map called "pain rating" was calculated. For the control condition, one statistical group map was calculated using one regressor that was constant across subjects. Voxel-based thresholds for the group statistical maps called "allodynic pain", "pain rating" and "control stimulation" were set to $Z > 2.3$ and significant clusters were defined according to spatial extent at $p < 0.01$ (corrected for multiple spatial comparisons according to Gaussian random field theory (Worsley et al. 1992)). The same analyses were performed using negative contrasts and hence tested for decreases in activation in response to allodynic pain and control stimulation respectively, and for brain regions in which activation decreases linearly with subjective ratings of allodynic pain.

Paired comparison

In order to account for the mechanical component of the allodynic stimulation, a paired t-test between allodynic stimulation and brushing of the unaffected contralateral homologous body side was performed (voxel-based threshold: $Z = 2.3$, cluster-level correction: $p < 0.01$).

Interaction analysis

In order to investigate the relation between allodynic and ongoing pain, an interaction analysis was performed in which regressor 1 corresponded to the allodynic pain ratings, regressor 2 to the ongoing pain ratings and regressor 3 represented the multiplicative interaction term of regressor 1 and regressor 2. The search volume was restricted to only those voxels that were activated by the allodynic stimulation. Contrasts were set to

investigate a positive as well as a negative interaction between allodynic and ongoing pain. Voxel-based threshold was set to Z = 2.3 and no cluster-level correction was applied in order to detect subtle interaction effects.

6.3 Results

6.3.1 Clinical and psychophysical data

According to BDI scores, two patients displayed mild symptoms of depression and in one patient there was an indication of moderate depressive symptoms (BDI score: 20). For the remaining patients, BDI scores were in the range of non-depressed values. All STAI scores, both for the trait and for the state scale, lay in the range of adult normative data.

Pain ratings of the allodynic stimulation in the imaging session ranged from 3.5 to 9 out of 10 (mean: 5.8, SD: 1.9) (Table 6.1). None of the patients experienced pain paroxysms during the experiment and two patients had no ongoing pain. Ongoing pain ratings of the remaining six patients did not differ before and after the allodynic scan and ranged from 3 to 8.5 out of 10 (mean: 5.4, SD: 1.9). Allodynic pain ratings and ongoing pain ratings were not correlated, although a non-significant association was present ($r = 0.6$, $p = 0.06$). Ongoing pain intensity was significantly and inversely correlated with age ($r = -0.9$, $p = 0.001$). Stimulation of the contralateral control site did not provoke painful sensations in any of the patients.

6.3.2 Imaging data

Cerebral activation in response to provocation of dynamic mechanical allodynia

A number of cortical and subcortical areas exhibited significant activation in response to provocation of allodynic pain (Table 6.2, Figure 6.1). Significant BOLD signal intensity increases were seen bilaterally in the anterior insula (peak in the rostral anterior insula), anterior cingulate cortex, a region around the upper bank of the Sylvian fissure consistent with S2, S1, inferior parietal cortex (BA40), supplementary motor cortex (SMA) and pre-motor area (BA6 and 8), lentiform nucleus and cerebellum. Unilateral changes were observed in the contralateral posterior insula (a cluster distinct from S2), contralateral thalamus, and contralateral prefrontal cortex (BA10). No significant decrease of the BOLD signal was found in response to provocation of allodynic pain.

Control stimulation

Control stimulation of the contralateral, unaffected body side resulted in broad bilateral activation of the supramarginal and angular gyrus and the upper bank of the Sylvian fissure spanning S2, BA39 and BA40 (Table 6.3, Figure 6.2). Activation in contralateral S1 was only observed in an uncorrected activation map where no cluster-level correction was applied, probably due to heterogeneity of stimulation sites leading to small focal activations in the highly somatotopically organised primary somatosensory cortex. No significant decrease of activation in response to control stimulation was detected.

Paired comparison between allodynic and control stimulation

The paired comparison between allodynic stimulation and non-painful mechanical stimulation of the unaffected body side revealed activation similar to the "allodynic pain" group map. However this activation exhibited lower statistical significance and hence peak Z-scores in several regions were only detected in an analysis without cluster-level correction. These regions are marked (*) in Table 6.2. No brain area showed significantly more activation in the control condition than in the allodynic condition.

Table 6.1 Subject baseline and clinical data

	Patient							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Age (yrs)	40	37	53	58	76	44	70	30
Sex	M	M	F	F	F	M	F	M
Handedness	R	R	R	R	R	R	R	R
Diagnosis	brachial plexus avulsion	DNP	surgical nerve lesion	traumatic nerve lesion	surgical nerve lesion	brachial plexus avulsion	surgical nerve lesion	sacral plexus avulsion
Disease duration (yrs)	14	6	15	5	2	10	16	6
Affected body site	left arm	feet bilateral (L > R)	right foot	left foot	right knee	left arm	right knee	right foot
Intensity DMA	4	9	6.5	7	4	3.5	5.5	7
Punctate HA	yes	yes	yes	yes	yes	yes	yes	yes
Lemniscal examination	Hypaes.	Hypaes., vibration sense ⇩	Hypaes.	normal	Hypaes., vibration sense ⇩	Hypaes., vibration sense ⇩	normal	Hypaes.
Spino-thalamic examination	abnormal	abnormal	abnormal	abnormal	abnormal	abnormal	abnormal	abnormal
OP intensity	5	6.5	3	5	n.p.	4.5	n.p.	8.5
OP quality (NPSI)	squeezing	squeezing	pressure	burning	n/a	pressure	n/a	burning
Medication	carba-mazepine, dis-continued	ami, discontinued	ami, discontinued	co-codamol, dis-continued	ami	none	doxepin, dis-continued	clonidine
Last medication intake before scanning	2 wks	2 wks	2 wks	1 wk	Night before	n/a	2 wks	Night before

Yrs, years; M, male; F, female; R, right; L, left; DNP, diabetic neuropathy; DMA, dynamic mechanical allodynia; HA, hyperalgesia; hypaes, hypaesthesia; OP, ongoing pain; n.p, not present; n/a, not applicable; ami, amitriptyline; wks, weeks.

Cerebral activation dependent on perceived intensity of allodynic pain

In this analysis, only brain regions where changes in BOLD signal intensity were associated with perceived pain intensity over and above the allodynic activation common to all subjects were investigated (statistical group map called "pain rating"). The magnitude of activation in caudal anterior insula showed a significant positive relationship with pain ratings (Table 6.4, Figure 6.3). No brain region showed a negative correlation with perceived pain intensity.

Interaction analysis between allodynic and ongoing pain

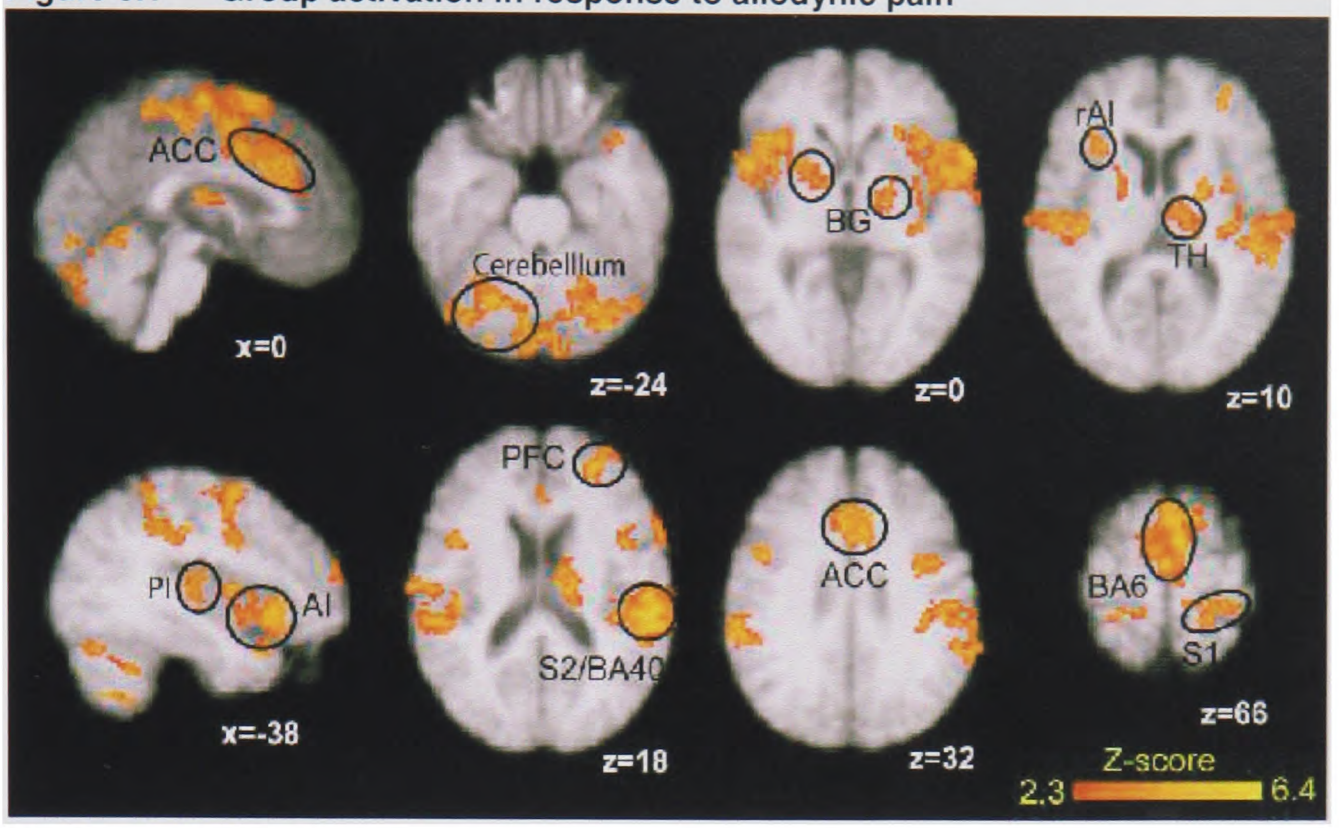
The uncorrected group statistical map (i.e. not corrected at cluster-level) showed positive interaction effects at the border between mid-ACC and perigenual ACC and negative interaction effects in perigenual ACC (Table 6.5).

Table 6.2 Group activation in response to allodynic pain

Brain region	Brodmann area	side	x	y	z	Z-score
Mechanical allodynia						
Rostral anterior insula	13	cl	-26	28	2	3.6
		il	36	28	8	3.7
Posterior insula	13	cl	-40	-16	14	3.7
Anterior cingulate cortex	24	il	2	14	34	3.6
S1	3	cl	-30	-36	66	3.5
		il	28	-42	66	3.1
S2	-	cl	-60	-22	16	4
		il	56	-18	14	3.8
Prefrontal cortex	10	cl	-30	50	18	3.6
Inferior parietal cortex	39	cl	-58	-34	20	4
		il	52	-34	20	4.1
SMA/premotor cortex	6/8	cl	-42	0	52	3.8
		il	50	0	50	3.6
Thalamus	-	cl	-8	-10	14	3.7
Lentiform nucleus	-	cl	-20	-2	10	3.7
		il	24	14	-2	3.5
Cerebellum		cl	26	-64	-24	3.7
		il	-28	-70	-24	3.9
Mechanical allodynia > non-painful mechanical control stimulation						
Rostral anterior insula	13	cl	-26	26	-6	3.3
		il	32	24	-6	3.5
Posterior insula	13	il	40	-18	12	3.3*
Anterior cingulate cortex	24	cl	-10	12	34	3.5
S1	3	cl	-30	-36	68	3.4*
		il	34	-32	66	3.4*
S2	-	cl	-56	-18	20	6.4
		il	58	-18	22	5.8
Inferior parietal cortex	39	il	54	-56	22	3*
Prefrontal cortex	10	cl	-34	38	18	3.4*
SMA / premotor cortex	6/8		0	16	60	3.3
Thalamus	-	cl	-12	-10	8	2.6*
		il	12	-18	8	2.9*
Lentiform nucleus	-	cl	-20	-4	12	2.4*
		il	18	8	0	4.1
Cerebellum	-	cl	-44	-56	-36	3.3*
		il	30	-56	-24	3.4

Voxel threshold: $Z > 2.3$, cluster threshold: $p < 0.01$. * No cluster threshold applied. S1, primary somatosensory cortex; S2, secondary somatosensory cortex; SMA, supplementary motor cortex; cl, contralateral; il, ipsilateral.

Figure 6.1 Group activation in response to allodynic pain



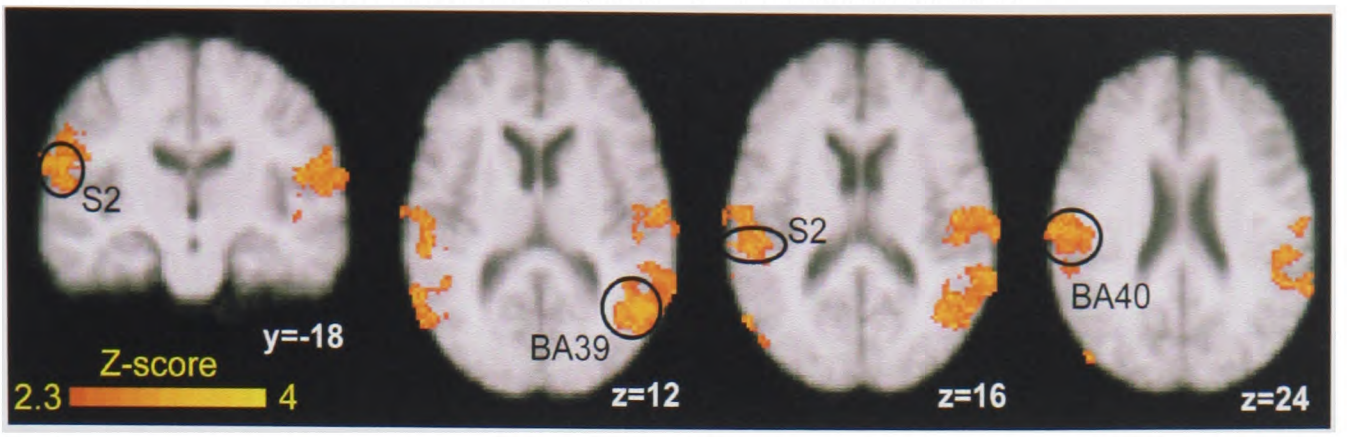
The figure depicts brain regions that were activated across the group in response to allodynic stimulation. Raw EPI images of patients with left-sided allodynia were mirrored about the midline; hence, left side corresponds to the side ipsilateral to stimulation. Voxel threshold was set to $Z > 2.3$ and cluster threshold to $p < 0.01$. ACC, anterior cingulate cortex; BG, basal ganglia; rAI, rostral anterior insula; TH, thalamus; PI, posterior insula; PFC, prefrontal cortex; BA, Brodmann area; S1, primary somatosensory cortex.

Table 6.3 Group activation in response to control stimulation

Brain region	Brodmann area	side	x	y	z	Z-score
S1	3	cl	-46	-40	56	3.2*
S2	-	cl	-50	-18	20	3.7
		il	60	-18	22	3.6
Inferior parietal cortex	39	cl	-56	-54	4	3.6
		il	52	-68	4	3.6
Inferior parietal cortex	40	cl	-46	-40	22	3.6
		il	58	-32	14	3.4

Voxel threshold: $Z > 2.3$, cluster threshold: $p < 0.01$. * No cluster threshold applied. S1, primary somatosensory cortex; S2, secondary somatosensory cortex; cl, contralateral; il, ipsilateral.

Figure 6.2 Group activation in response to control stimulation



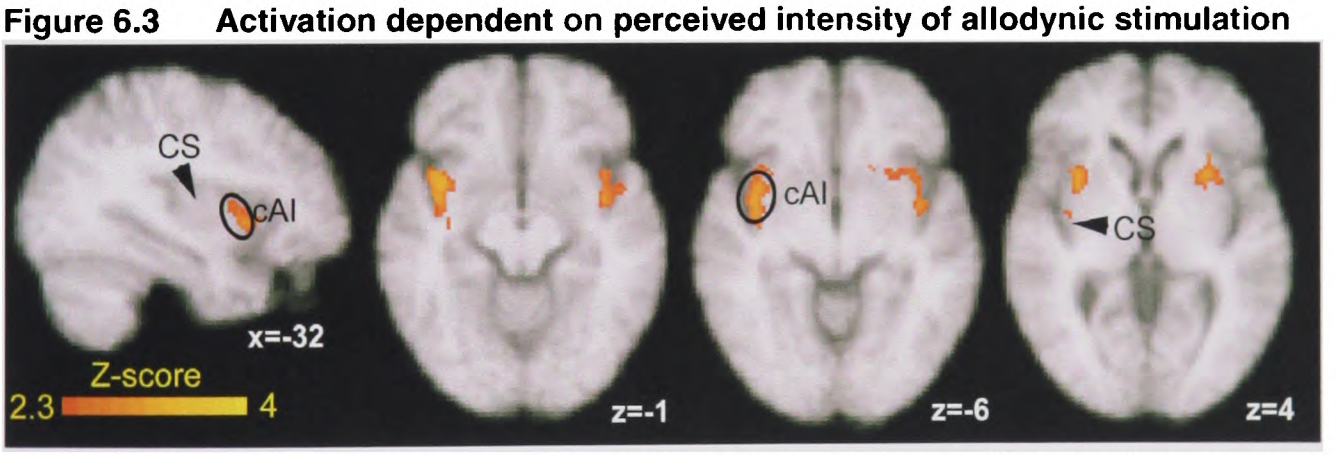
Brain regions that were activated across the group in response to control stimulation, i.e. brushing of the contralateral unaffected body side. Raw EPI images of patients who were stimulated on the left side (i.e. patients with right-sided allodynia) were mirrored about the midline; hence, left side corresponds to the side ipsilateral to stimulation. Voxel threshold was set to $Z > 2.3$ and cluster threshold to $p < 0.01$. S2, secondary somatosensory cortex; BA, Brodmann area.

Table 6.4 Group activation dependent on perceived intensity of allodynic stimulation

Brain region	Brodmann area	side	x	y	z	Z-score
Caudal anterior insula	13	cl	-30	12	4	2.9
		il	40	6	-8	3.1

Voxel threshold was set to $Z > 2.3$ and cluster threshold to $p < 0.01$.

Figure 6.3 Activation dependent on perceived intensity of allodynic stimulation



As allodynic pain and pain rating were modelled independently within the linear model, activation dependent on pain rating is activation beyond allodynic activation common to all subjects. Raw EPI images of patients with left-sided allodynia were mirrored about the midline; hence, left side corresponds to the side ipsilateral to stimulation. Voxel threshold: $Z > 2.3$; cluster threshold: $p < 0.01$. cAI, caudal anterior insula; CS, central sulcus (of the insula).

Table 6.5 Interaction between allodynic and ongoing pain intensities

Brain region	Positive interaction		Negative interaction	
	[x y z]	Z-score	[x y z]	Z-score
Mid-ACC	[-10 24 40]	2.6*		
Perigenual ACC			[4 40 18]	2.4*

Locations of peak Z-scores are given according to MNI coordinates. Voxel threshold was set to Z > 2.3; * no cluster threshold applied. ACC, anterior cingulate cortex.

6.4 Discussion

In addition to largely confirming the brain activation pattern previously described in studies of clinical allodynia (Lorenz et al. 1998; Maihofner et al. 2003; Petrovic et al. 1999; Peyron et al. 1998b; Peyron et al. 2000a; Peyron et al. 2004), this study shows that the perceived intensity of allodynic pain is encoded in the caudal anterior insula (cAI). This result was obtained by controlling for the intensity of ongoing pain and in addition, no interaction effects between the intensity of allodynic and ongoing pain were detected in the cAI. This is promising for the use of FMRI as a surrogate marker of perceived allodynic pain intensity that is independent of ongoing pain.

6.4.1 Brain activation in response to mechanical stimulation

Brushing of the unaffected control site resulted in activation of bilateral S2, which is known to be involved in somatosensory processing and strongly activated by moving tactile stimuli (Disbrow et al. 2000), and parietal association areas (BA39 and 40). Application of the same stimulus to the affected body site provoked additional activation in a number of brain regions that are all known to be involved in pain processing (Apkarian et

al. 2005; Peyron et al. 2000b), reflecting the painfulness of the allodynic stimulation. Activation in prefrontal areas, insula, ACC, inferior parietal areas, S1 and S2 is demonstrated quite consistently in imaging studies of clinical (Lorenz et al. 1998; Maihofner et al. 2003; Petrovic et al. 1999; Peyron et al. 1998b; Peyron et al. 2000a; Peyron et al. 2004) and experimentally-induced allodynia (Baron et al. 1999; Iadarola et al. 1998; Lorenz et al. 2002; Maihofner et al. 2004b; Witting et al. 2001; Zambreanu et al. 2005). Minor differences between clinical studies might be due to the fact that the patient groups investigated vary considerably, for example in both symptoms and aetiology. Another source of heterogeneity is the varying degree of deafferentation encountered in neuropathic pain patients. It could be hypothesized that the impairment of small fibre and lemniscal pathways (see Table 6.1 for patients of this study) should lead to reduced brain activation in response to allodynic stimulation in areas that are (partly) deprived of their input, such as posterior insula, S2 and S1. Indeed, a previous study found decreased contralateral S2 and posterior insula activation in response to stimulation of the allodynic compared to the control site (Peyron et al. 2004). However, other studies of clinical allodynia (Petrovic et al. 1999; Peyron et al. 1998b) and the present study do not uniformly observe decreased activation in these areas. Differences between studies are likely to arise from different relative contributions of deafferentation and various factors that enhance activation in S1, S2 and posterior insula compared to control stimulation. These factors include reorganisation along afferent pathways and in cortical areas (Flor et al. 1995; Maihofner et al. 2004a), the presence of nociceptive neurons in somatosensory cortices and posterior insula (Craig 1995; Dong et al.

1989; Ostrowsky et al. 2002) and increased somatosensory activation when attention is directed towards a stimulus (Burton et al. 1999; Bushnell et al. 1999; Johansen-Berg et al. 2000) – based on the possibility that subjects attend more to the painful than to the non painful stimulus. This illustrates that the net brain activation observed in studies of clinical allodynia is composed of and influenced by the impairment of lemniscal and spinothalamic pathways, peripheral and central reorganisation, the perception of pain, and associated emotional and cognitive processes.

6.4.2 Encoding of perceived allodynic pain intensity

The perceived intensity of brush-evoked allodynia was reflected by the magnitude of FMRI signal in the cAI. In contrast, the peak of insular activation in the allodynic condition when perceived intensity was not modelled was located in the rAI. This result is in good agreement with a previous imaging study of brush-evoked allodynia in neuropathic pain patients (Petrovic et al. 1999). These findings further support a functional sub-division of anterior insular cortex as suggested in Chapter 4. The encoding of perceived allodynic pain intensity in cAI matches the portion of the insula where encoding of perceived intensity of experimental pain in healthy volunteers is consistently found (Coghill et al. 1999; Craig et al. 2000; Derbyshire et al. 1997; Peyron et al. 1999). Hence, cAI activation seems to reflect the perceived intensity of pain, irrespective of whether pain is the result of normal nociceptive processing or due to aberrant processing of a normally innocuous stimulus as in neuropathic pain. This might indicate that intensity perception of clinical allodynia is sub-served by the same neuronal population as intensity perception in healthy

volunteers. Because this study deliberately controlled for the intensity of ongoing pain, the result reflects the perceived intensity of allodynic pain alone and not of the ongoing pain component. A patient study that correlated "total" pain intensity (composed of provoked and ongoing pain) across scans without stimulation and scans in which allodynia was provoked found perceived intensity to be represented in the rAI (Petrovic et al. 1999). It is possible that intensity encoding in that study was shifted to the rAI by the ongoing pain component. In the present study, though, no interaction between allodynic and ongoing pain intensity was found in the insula, above the chosen cluster threshold.

6.4.3 Interaction between allodynic and ongoing pain in cingulate cortex

The mid-ACC has been suggested to be important for the integration of basic nociceptive information with pain perception and attentional processes (Buchel et al. 2002). This role fits the idea that the ACC is involved in response selection and goal-directed behaviour (Devinsky et al. 1995), including skeletomotor responses mediated by cingulate motor areas (CMA). Thus, the activation found in this study in mid-ACC, which extended into CMA, together with activation in SMA, premotor cortex and cerebellum, could represent selection and initiation of (suppressed) motor responses to painful stimulation.

Processing of ongoing pain is not only known to engage the ACC (Hsieh et al. 1995) but its intensity is also reflected by the magnitude of ACC activation (Willoch et al. 2003). In healthy volunteers, parts of the ACC have been found to reflect experimental pain intensity (Bornhovd et al.

2002; Buchel et al. 2002; Coghill et al. 1999). Controlling for the intensity of ongoing pain, combined with the fact that allodynic and ongoing pain intensities were related ($r = 0.6$, $p = 0.06$), could explain why allodynic pain intensity in the present study was not correlated with the ACC signal. This is supported by the observation that allodynic and ongoing pain ratings showed a positive interaction in the ACC. However, in healthy volunteers, pain intensity encoding is not consistently found in the ACC (Iannetti et al. 2005a; Peyron et al. 2000b). The theory that the ACC signal represents an integrated component that is different from the early component in the operculo-insular (i.e. insula and S2) and / or S1 region (Garcia-Larrea et al. 2003; Ploner et al. 1999; Treede et al. 1999), which was put forward in studies focussing on temporal aspects of pain processing, might explain the inconsistent results of studies in healthy volunteers and might also provide an explanatory framework for the interaction of provoked clinical and ongoing pain.

Some studies have observed signal *decreases* in response to allodynic stimulation in the perigenual ACC (Petrovic et al. 1999; Peyron et al. 1998a). This was not confirmed by the present study, but the observation that allodynic and ongoing pain showed a negative interaction in this region might offer an explanation for diverging results of studies investigating patient groups that differ with respect to the intensity of ongoing pain. To clarify the role of the ACC in clinical pain further, it might be beneficial to investigate neurophysiological interactions between the processing of provoked and ongoing neuropathic pain and their time courses in detail.

6.4.4 Methodological considerations

Whereas the perceived intensity of experimental pain in healthy volunteers is reflected by activation in several brain regions, only the cAI was found to encode perceived allodynic pain intensity. Several reasons could contribute to this. First, the intensity of ongoing pain was controlled for, which probably also removed some of the variance of allodynic pain as the intensities of ongoing and allodynic pain were non-significantly related. Second, allodynic pain processing could interfere with the processing of ongoing pain and might therefore be reflected by fewer brain regions than experimental pain. Third, the analysis in the present study is different in that pain intensity encoding was investigated over and above activation common to all subjects, whereas studies of experimental pain commonly use only one regressor weighted by pain ratings. Lastly, insufficient statistical power due to the small number of patients could contribute to false negative results. It is therefore necessary that the results are replicated by a study of a larger patient population. In contrast, the observation that cAI encodes the perceived allodynic pain intensity should be subject only to a type I error because estimation of the inter-subject random effects component of the variance within the employed mixed-effects analysis is considered accurate for groups greater than six subjects (Woolrich et al. 2004).

6.5 Conclusions

The FMRI signal, specifically in the cAI, might have the potential to provide a more direct read-out of perceived clinical pain intensity than commonly used pain rating scales. This would help to differentiate between the subjective experience of pain and the subjective pain report. Because pain rating is only an indirect report of the pain experience and the rating behaviour itself introduces another source of variance, a direct read-out of the subjective experience could be more sensitive in detecting alterations of the pain experience. Advantages offered by a direct and more sensitive marker of the subjective pain experience are manifold and include usage in non-verbal patient populations or in patients with difficulties in pain rating and detection of subtle treatment effects, which would be especially useful in drug development. The next chapter expands on the need of the pharmaceutical industry for a sensitive measure of potential analgesic drug efficacy in humans and illustrates how FMRI might be used in drug development.

CHAPTER 7: Modulation of pain processing in neuropathic pain patients by commonly used analgesics

7.1 Introduction

Although a range of conventional (e.g. opioids) and unconventional (e.g. anti-depressants) analgesic drugs are available to date, some chronic pain conditions remain poorly treated with inadequate pain relief being achieved (Collins and Chessell 2005; Harden and Cohen 2003). Billions of pounds have been spent on analgesic drug research by the pharmaceutical industry, government funded research councils, charitable organisations and a variety of patient bodies over the past decades. In recent years, novel molecular targets have been identified in pre-clinical animal models and several substances (e.g. neurokinin NK1 receptor antagonists and adenosine A1 receptor agonists) have been investigated in a variety of patient groups, including those with migraine, neuropathic pain, osteoarthritis or rheumatoid arthritis. However, these large clinical studies have often shown poor clinical outcomes (Hill 2000; Seymour et al. 1999). Several reasons might cause the difficulties encountered in clinical trials.

Firstly, pain as a symptom is immensely heterogeneous. This means that selection of a completely homogeneous group of patients is very difficult. This is in complete contrast to *in vivo* pre-clinical studies, where for example a homogenous rodent population has the same injury applied at approximately the same time. As a result, a potential beneficial effect of the new substance might not be detected due to large statistical variances

in the patient population in a clinical trial.

Secondly, the predictive value of animal models might be limited. This could be due to differences between pre-clinical species and the human organism with regard to the significance of a particular target for the processing and subsequent perception of pain. Also, differences in bioavailability of a particular compound due to different pharmacokinetics, such as differences in the permeability of the blood brain barrier, could contribute to a reduced predictive value of animal models. The dose evaluated in clinical studies that investigate the potential efficacy in patients (i.e. Phase II studies) tends to be below the "maximum well tolerated dose" established in Phase I in young healthy volunteers. This dose may not be sufficient to achieve the right concentration, at the right site, for the right length of time to produce the desired pharmacodynamic effect in humans. Indeed, in pre-clinical animal models the dose is sometimes escalated in an acute setting to produce an analgesic or anti-hyperalgesic effect. This may be possible because the ability to assess safety and tolerability in lower species is very limited compared to humans (e.g. nausea cannot be measured in rats and mice).

Some of these difficulties could be addressed with a tool that allows sensitive evaluation of the potential efficacy of a novel compound *in humans* at an early stage of drug development. Pharmacological functional magnetic resonance imaging (phFMRI) has the potential to measure a functional effect of a drug in specific brain regions in relatively small numbers of patients (n = 10 to 12) (Salmeron and Stein 2002; Stein

2001; Tracey 2001) and recent phFMRI studies have demonstrated the utility of this methodology for assessing drug effects in a variety of diseases (Honey and Bullmore 2004; Koeppe et al. 2004). With regard to pain, the neural correlates of pharmacological analgesia in the brain have been described by two studies in healthy volunteers (Tracey et al. 2005; Wise et al. 2002).

As FMRI signals appear to reflect pain intensity ratings across patients (Chapter 6), the present study investigated if FMRI has the potential to serve as a marker of longitudinal within-subject alterations of clinical and experimental pain perception in neuropathic pain patients. To mimic the clinical situation, the patients' regular (co-) analgesic medication was modulated to induce changes in their clinical pain. FMRI measurements of pain processing were performed at two time points, before and after drug alteration.

7.2 Methods

7.2.1 Patients

FMRI and psychophysical data were obtained from ten neuropathic pain patients whose clinical details are outlined in Table 7.1. Patients were recruited from the Oxford Pain Relief Unit and selected according to the following inclusion criteria: (1) presence of neuropathic pain of peripheral origin or caused by plexus avulsion, (2) presence of pronounced mechanical allodynia, (3) constant ongoing pain or no ongoing pain, (4) stable medication, (5) willingness to alter pain medication and (6) absence of any major psychiatric disorder or systemic disease. To ensure a

constant background of ongoing pain during the FMRI experiments, patients who experienced frequent pain paroxysms (every hour) or who perceived that the intensity of their ongoing pain fluctuated over a one hour period were excluded from the study.

7.2.2 Drug modulation

Each subject underwent two functional imaging sessions: one on the regular pain medication and the other on reduced medication. Analgesic drugs were gradually discontinued according to the drug's characteristics. If participants were taking more than one agent, the decision which drug would be stopped was taken according to the following priority list: (1) anti-depressants or anti-convulsants, (2) opioid-containing compounds if patient was not taking any drug of type (1), (3) others (non steroidal anti-inflammatory drugs). All but one patient stopped one analgesic agent completely. The remaining patient reduced the daily dose of amitriptyline from 50 mg to 25 mg (for details see Table 6.2). For simplicity, the sessions are called "OFF" and "ON" drug session. The order of the two sessions was randomised and fully balanced. Elapsed time between imaging sessions was five weeks on average.

7.2.3 Clinical assessment

All subjects underwent detailed neurological examination on a separate day prior to the first imaging session to ascertain the diagnosis of neuropathic pain. During the course of the examination, three types of mechanical allodynia were tested (Table 7.1): dynamic mechanical allodynia using a soft brush (Somedic, Hörby, Sweden), punctate

hyperalgesia using von Frey filaments (Stoelting, Illinois, U.S.A) and deep pressure allodynia using a semi-quantitative, MR-compatible, home-built pressure device with a round rubber tip of 1 cm diameter. The type of mechanical allodynic stimulation to be used in the imaging session was determined as follows. If brushing elicited a pain rating greater than 2/10 on the NRS, dynamic mechanical allodynia was considered suitable for the study. If rated equal or less than 2/10, punctate hyperalgesia was assessed for suitability. If punctate hyperalgesia was rated below 2/10, the pain intensity elicited by deep pressure was assessed. When deep pressure provoked a pain rating greater than 2/10, deep pressure was chosen as the stimulus for the study. If none of the mechanical stimuli elicited a pain rating greater than 2/10, the patient was excluded from the study. The most painful body site upon the respective mechanical stimulation was marked and photographed to allow easy recognition in the imaging sessions. The described procedure to determine the stimulus for the imaging session resulted in six patients in whom mechanical allodynia was provoked by brushing. Punctate hyperalgesia and deep pressure were each used in two patients.

At the second imaging session, patients were asked how they felt overall compared to the first imaging session (patient global impression of change, PGIC). Patients chose one of seven response categories (very much worse, much worse, worse, no change, better, much better, very much better), which were associated with numbers from +3 to -3.

7.2.4 Experimental design

Four functional experiments were performed in each imaging session: two pain experiments (allodynic stimulation and noxious heat stimulation of an unaffected body site) and two control experiments (mechanical stimulation contralateral to the affected side and visual stimulation). Allodynic stimulation and mechanical stimulation contralateral to the affected side were always carried out in the first two functional experiments; the order of these two scans was randomised and balanced across subjects. These two experiments were always followed by the thermal experiment after which the visual stimulation was carried out. At the end of the imaging session, a structural scan was acquired.

Mechanical allodynia was provoked as determined during the first visit. Location of stimulus application was retrieved by means of a photograph taken at the clinical examination. Prior to imaging, the location and intensity of allodynic stimulation was verified and the extent of the site to be stimulated during the experiment was marked on the skin. During the functional experiment, three mechanical stimuli were delivered during a 6 second long stimulation block. Each stimulation block was preceded by a 24 second long rest period and repeated 15 times. The last stimulation block was followed by an 18 second long rest period. An average rating of the pain provoked by the mechanical stimulation was obtained immediately after the scan. In addition, a rating of ongoing pain was obtained before and after the allodynic scan.

The noxious temperature that elicited the same pain rating as the allodynic

stimulation was determined directly before the start of the heat pain experiment. During the heat pain experiment 15 brief (3 seconds) stimuli were delivered to the right medial forearm (not affected by the neuropathic pain condition in any of the patients) with inter-stimulus intervals of 54 to 66 seconds. An average pain rating of the thermal pain was obtained immediately after the scan.

Two additional scans were performed to test for non-specific drug effects between sessions: non-painful mechanical stimulation of the homologous body side contralateral to the site of allodynic stimulation was carried out using the same stimulus and according to the same experimental paradigm as on the affected side. This area was unaffected in eight patients and less affected in two patients (Table 7.1). A visual control scan (checkerboard flashing at 8 Hz) was acquired using a block design (30 s OFF, 15 s ON; 7 blocks). Subjects were instructed to keep their eyes closed, except for the visual scan, and to keep as still as possible during image acquisition.

In the second imaging session, an identical experimental design was used. The physical properties of the allodynic stimulus remained constant between the two imaging sessions and the same temperature as used in the first imaging session was employed for the heat pain experiment. For provocation of dynamic mechanical allodynia, special care was taken to stimulate the same area in both imaging sessions (the extent of the stimulation site had been marked on the skin and measured) as the extent of the area stimulated is the property of a dynamic mechanical allodynic

stimulus that most influences the perceived intensity (Samuelsson et al. 2005).

7.2.5 Data acquisition

FMRI data were acquired on a 1.5 Tesla Siemens Sonata MRI scanner using the protocol outlined in 2.7.4.

7.2.6 Analysis of psychophysical data

For each patient, the raw change on the NRS (ratings $_{\text{OFF drug session}}$ minus ratings $_{\text{ON drug session}}$) and the percentage change (100 * raw change / pain rating $_{\text{OFF drug session}}$) were computed (Farrar et al. 2001). Raw changes were calculated for allodynic, thermal and ongoing pain whereas percentage change was only calculated for allodynic and thermal pain as three patients rated their ongoing pain as "0" in the OFF drug session. Pearson's product moment correlation coefficient ($p < 0.05$, two-sided test) was used to test if raw changes or percentage changes of allodynic, thermal and ongoing pain were correlated.

One of seven response categories was chosen by the patients in the second imaging session to indicate their global impression of change compared to the first imaging session. To represent the overall well-being in the OFF compared to the ON drug session, the response categories "very much better", "much better" and "better" of the PGIC had to be changed to their negative counterparts, and vice versa, for patients who underwent their first imaging session OFF drugs. Pearson's product moment coefficient ($p < 0.05$, two-sided test) was used to determine the

association between PGIC and raw change of allodynic rating, between PGIC and raw change of ongoing pain rating and between PGIC and the sum of raw allodynic and ongoing pain rating changes. For allodynic ratings, the correlation between percentage change and PGIC was also calculated.

7.2.7 Analysis of imaging data

All processing and analysis of FMRI data, except region of interest (RoI) analysis, was performed using the software package FSL 3.2 (Smith et al., 2004). Data from each patient were analysed separately. The following pre-processing steps were applied to each functional dataset: removal of non-brain structures, motion correction, spatial smoothing, "grand mean" intensity normalization and high pass temporal filtering. For high pass filtering, a cut-off of 50 seconds was used for scans in which thermal stimulation was performed. A high pass filter with a cut-off of 30 seconds was used for scans in which allodynic or mechanical control stimulation was performed. For scans in which visual stimulation was performed, a high pass filter with a cut-off of 45 seconds was used. A general linear model (GLM) was applied on a voxel by voxel basis to this data (Woolrich et al. 2001; Worsley and Friston 1995) using FILM (FMRIB's improved linear model) (Woolrich et al. 2001) to model blood oxygen level-dependent (BOLD) signal intensity changes in response to stimulation. One regressor was constructed by convolving a boxcar function (the stimulus input function representing allodynic, thermal, mechanical control or visual stimulation: stimulation period = 1, baseline = 0) with a gamma haemodynamic response function. To allow for imperfect timing, the first

temporal derivative of the regressor was included in the model as covariate of no interest. Voxel-wise parameter estimates (PEs) were derived for the regressor using the appropriate contrast (stimulation versus baseline). Four statistical PE maps were calculated for each patient: allodynic stimulation, thermal pain, mechanical control stimulation and visual stimulation.

7.2.8 RoI analysis

Choice of RoIs was based on brain areas known to play a role in pain processing and included the following regions: anterior cingulate cortex, anterior and posterior insular cortex, thalamus, putamen, cerebellum, periaqueductal grey (PAG), primary and secondary somatosensory cortex (S1 and S2), and prefrontal cortex (PFC). Based on the apparent functional sub-division described in Chapters 4 and 6, the anterior insula was sub-divided into rostral anterior insula (rAI) and caudal anterior insula (cAI). Anatomical RoIs were drawn on the high resolution image of each individual and converted into the functional space of the respective PE map (allodynic, thermal, mechanical control or visual stimulation). Within each RoI, the top 20% of voxels with the highest PE were selected and the mean PE of these voxels was calculated as an index of activation (in the following: "mean PE"). This approach has been shown previously to provide good sensitivity for the detection of drug-induced effects (Iannetti et al. 2005b). Paired t-tests ($p < 0.05$, two-sided) between mean PEs in the ON and OFF drug session were performed for all RoIs for all experiments. The rational to do this for the control experiments is described in Section 7.2.12. For the pain experiments, no systematic

effect between ON and OFF drug session was expected because of the variety of the modulated drugs in conjunction with the observation that the pain ratings were not uniformly decreased in all patients in the ON drug session.

7.2.9 Correlation analysis of RoI data and psychophysical data

To investigate if the change of pain ratings between sessions is reflected by the imaging data independently of the direction of change, a correlation analysis between the FMRI signal change and pain rating change was performed. To this end, the same procedure that was performed for the pain ratings was applied to the mean PEs of each RoI: raw change between sessions (mean PE $_{\text{OFF drugs}}$ minus mean PE $_{\text{ON drugs}}$) as well as percentage change (100 * raw change / mean PE $_{\text{OFF drugs}}$) were calculated. Raw changes of pain ratings were correlated with raw changes of mean PE and percentage changes of pain ratings were used together with percentage changes of mean PE (Pearson's product moment correlation coefficient, $p < 0.05$, two-sided tests).

7.2.10 Magnitude FMRI signal change versus pain rating change

To investigate how the magnitude of the FMRI changes compares to the magnitude of the pain rating changes, the equations of the linear regression for the percentage changes were calculated according to $y = a^*x + b$. Y corresponds to the percentage change in the FMRI signal; x to the percentage change in pain rating; therefore a coefficient $a > 1$ would signify that the fractional change in FMRI signal is greater than the respective fractional pain rating change.

7.2.11 GLM analysis

To investigate whether the results of the RoI analysis can be replicated using a different methodological approach, a GLM analysis was performed on a voxel by voxel basis into which the pain ratings were incorporated. For this analysis, the search volume was restricted to the regions in which signal intensity change significantly reflected pain rating change in the RoI analysis to reduce the number of voxels compared to a whole-brain analysis. No correction at cluster-level was applied and the voxel-based threshold was set to $Z = 2.3$. Pain ratings of the OFF drug session were entered as regressor one and pain ratings of the ON drug session as regressor two. In addition, one extra regressor was entered for each subject to model each subject's mean effect as covariate of no interest. By using the contrast [1 -1], significant voxels reflect that a greater change of pain ratings between the two sessions is accompanied by a greater change of the magnitude of activation.

7.2.12 Control experiments

Paired t-tests ($p < 0.05$, two-sided) between mean PEs in the ON and OFF drug session were performed for all RoIs for the mechanical and the visual control experiment to test for non-specific drug effects. An additional RoI in the primary visual cortex was used for the visual control experiment.

Table 7.1 Subject baseline and clinical data

	Patient									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Age (years)	40	37	53	58	60	44	70	22	59	65
Sex	M	M	F	F	M	M	F	F	M	M
Handedness	R	R	R	R	R	R (since accident)	R	R	R	R
Diagnosis	brachial plexus avulsion	painful DNP	surgical nerve lesion	traumatic nerve lesion	traumatic nerve lesion	brachial plexus avulsion	surgical nerve lesion	traumatic nerve lesion	traumatic nerve lesion	painful DNP
Disease Duration (yrs)	14	6	15	5	3	10	16	6	5	3
Affected body / stimulation site	left arm	feet bilateral (L > R)	right knee	left foot	left hand	left arm	right knee	left abdomen	left thigh	feet bilateral (L > R)
Dynamic mechanical allodynia	yes	yes	yes	yes	no	yes	yes	no	no	yes
Punctate hyperalgesia	yes	yes	yes	yes	no	yes	yes	yes	no	yes
Deep pressure allodynia	no	yes	yes	no	yes	no	yes	yes	yes	no
Stimulus used for FMRI	brush	brush	brush	brush	pressure	brush	brush	punctate	pressure	punctate

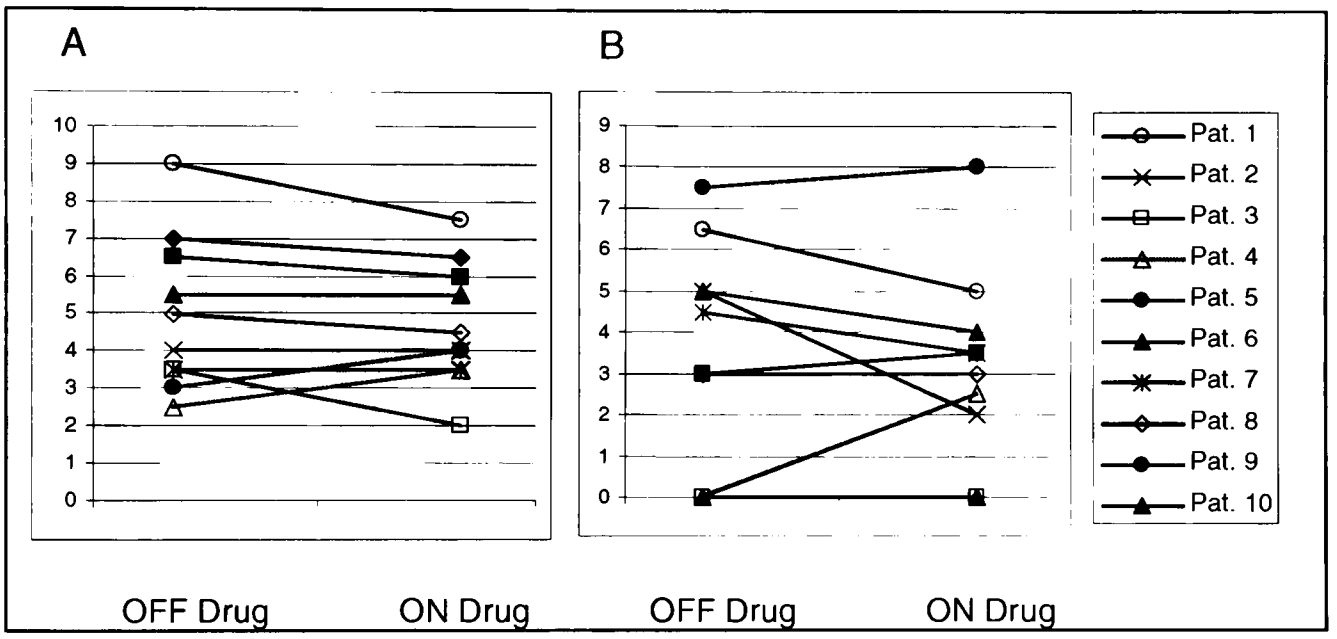
In case of bilateral disease, the more affected side was chosen as stimulation side. M, male; F, female; R, right; L, left; DNP, diabetic neuropathy; FMRI, functional magnetic resonance imaging.

7.3. Results

7.3.1 Behavioural data

Intensity ratings of allodynic, ongoing and thermal pain in the two sessions, as well as raw and percentage changes between ON and OFF drug session are reported in Table 7.2. Figure 7.1 depicts individual allodynic and ongoing pain ratings in the ON and OFF drug sessions.

Figure 7.1 Pain ratings in OFF and ON drug sessions



(A) Rating of allodynic pain in OFF and ON drug sessions on a numerical rating scale (NRS) from 0 ("no pain") to 10 ("worst pain imaginable").
(B) Rating of ongoing pain in OFF and ON drug sessions on the same NRS as in (A).

Allodynic pain

5 out of 10 patients rated the allodynic pain in the OFF drug session higher than in the ON drug session, 3 experienced the same pain upon allodynic stimulation and 2 reported lower allodynic pain in the OFF drug session than in the ON drug session. The absolute values of raw change on the NRS ranged from 0 to 1.5 (mean: 0.65, SD: 0.58), which relates to a percentage changes from 0% to 43% (mean: 16%, SD: 16%). Only one

patient achieved an improvement greater than 30%, which is considered to be clinically meaningful (Farrar et al. 2001). The medication of this patient with painful diabetic neuropathy was gabapentin, which provided 43% pain relief.

Thermal Pain

5 patients rated the thermal pain lower in the ON drug session, 3 patients rated it higher in the ON drug session than in the OFF drug session and 2 reported the same pain rating.

Ongoing pain

Ratings of ongoing pain obtained before the functional experiment did not differ from those obtained after the experiment. 5 of the 8 patients suffering from ongoing pain experienced less ongoing pain in the ON drug session; 3 patients rated their ongoing pain higher in the ON drug session than in the OFF drug session. 2 of the latter also rated the allodynic pain higher in the ON drug session.

Relationship between allodynic, thermal and ongoing pain

Inter-session changes of thermal pain perception were negatively correlated with changes in allodynic pain intensity, both for raw changes ($r = -0.71$, $p = 0.02$) and percentage changes ($r = -0.65$, $p = 0.04$). Neither changes of thermal pain ratings nor changes of allodynic pain ratings were correlated with inter-session changes of ongoing pain ratings.

Table 7.2 Drug alteration

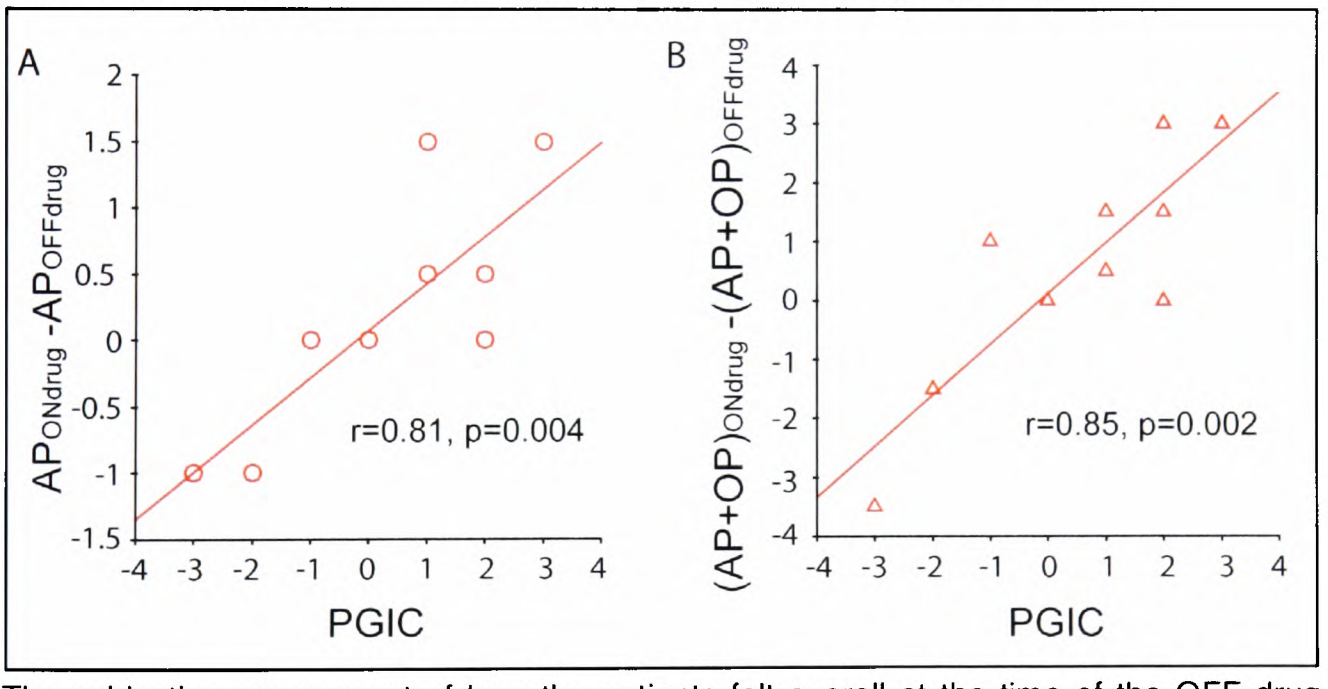
	Patient									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Drug modulated	carba	ami	ami	cocod	diclo	diclo	TCA	coprox	cocod	gaba
Stimulus	B	B	B	B	PR	B	B	PU	PR	PU
$AP_{ON\ drug}$	4	7.5	6	6.5	4	3.5	5.5	3.5	4.5	2
$AP_{OFF\ drug}$	4	9	6.5	7	3	3.5	5.5	2.5	5	3.5
Raw change AP	0	1.5	0.5	0.5	-1	0	0	-1	0.5	1.5
Percent change AP	0%	17%	8%	7%	-33%	0%	0%	-40%	10%	43%
Thermal Pain $_{ON\ drug}$	4	7	5	6	0	3.5	7	6	3.5	6
Thermal Pain $_{OFF\ drug}$	4	6	4.5	7.5	3	4	7	6.5	4	5
Raw change TP	0	-1	-0.5	1.5	3	0.5	0	0.5	0.5	-1
Percent change TP	0%	-17%	-20%	20%	100%	13%	0%	8%	11%	-20%
PGIC	+2	+3	+2	+2	-2	-1	0	-3	+1	+1
$OP_{ON\ drug}$	2	5	3.5	4	8	3.5	0	2.5	2.5	0
$OP_{OFF\ drug}$	5	6.5	3	5	7.5	4.5	0	0	3	0
Raw change OP	3	1.5	-0.5	1	-0.5	1	n/a	-2.5	0.5	n/a

Patients' regular medication was modulated between the two sessions. The rows in the table correspond to the stimulus used for provocation of mechanical allodynia in the FMRI experiment; allodynic pain rating in the ON drug session ($AP_{ON\ drug}$); allodynic pain rating in the OFF drug session ($AP_{OFF\ drug}$); thermal pain rating in the ON drug session (Thermal $Pain_{ON\ drug}$); thermal pain rating in the OFF drug session (Thermal $Pain_{OFF\ drug}$); ongoing pain rating in the ON drug session ($OP_{ON\ drug}$); ongoing pain rating in the OFF drug session ($OP_{OFF\ drug}$) and patient global impression of change (PGIC). PGIC is reported for OFF drug session compared to ON drug session and numbers from +3 to -3 correspond to categories "very much worse", "much worse", "worse", "no difference", "better", "much better", "very much better". Carba, carbamazepine; ami, amitriptyline; cocod, co-codamol (codeine and paracetamol); diclo, diclofenac; TCA, tricyclic antidepressant (doxepin); coprox, co-proxamol (dextropropoxyphene and paracetamol); gaba, gabapentin; B, brush; PR, pressure; PU, punctate; AP, allodynic pain; TP, thermal pain; PGIC, patient global impression of change; OP, ongoing pain; n/a, non applicable.

Relationship between PGIC and rating of neuropathic pain

PGIC scores, which reflect patients' global impression of change, correlated with the change of neuropathic pain ratings (raw change allodynic pain: $r = 0.81$, $p = 0.004$ (Fig. 7.2), raw change ongoing pain: $r = 0.68$, $p = 0.03$). The inter-session change of the sum of allodynic and ongoing pain ratings even explained a higher variance of the PGIC ($r = 0.85$, $p = 0.002$; $R^2_{adjusted} = 0.69$) (Fig. 7.2). PGIC was also correlated with the percentage change of allodynic pain ratings ($r = 0.75$, $p = 0.012$). Although changes in allodynic pain rating were correlated with changes in thermal pain rating, thermal pain rating changes were not correlated with PGIC.

Figure 7.2 Correlation between neuropathic pain rating and PGIC



The subjective assessment of how the patients felt overall at the time of the OFF drug sessions compared to the ON drug session (patient global impression of change, PGIC) correlates with the change of neuropathic pain rating between OFF and ON drug session. (A) Correlation of PGIC (x-axis) and raw change of allodynic pain rating. (B) Correlation of PGIC (x-axis) and raw allodynic plus ongoing pain rating change. r: Pearson's product moment correlation coefficient.

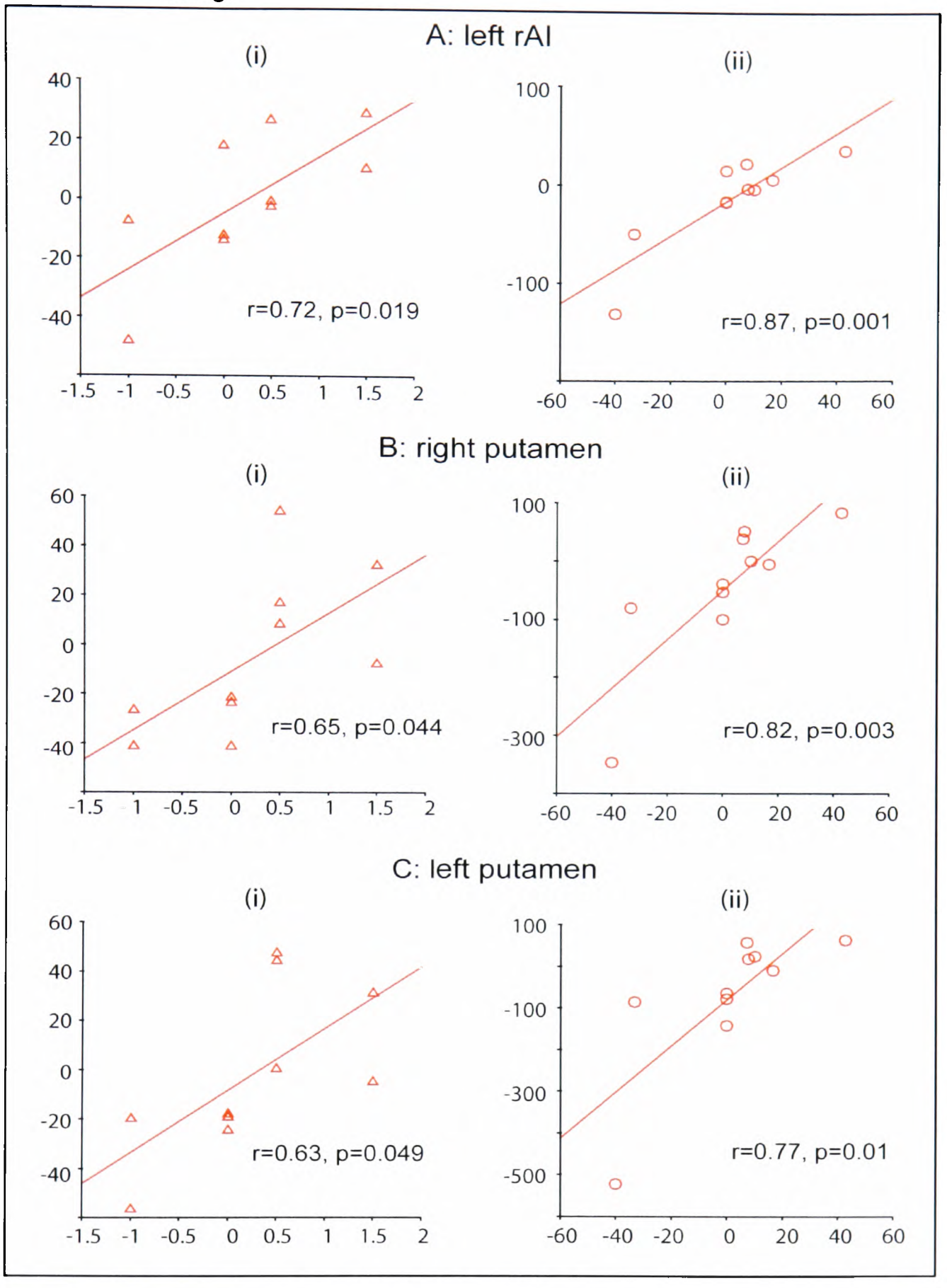
7.3.2 Relationship between imaging and psychophysical results

The FMRI signal change between the ON and OFF drug sessions reflected the pain rating change between sessions, both for allodynic and experimental thermal pain, in a subset of brain regions. For allodynic stimulation, FMRI signal change correlated with pain rating change bilaterally in the putamen and left rAI (Figure 7.3). Correlation coefficients for raw changes were: left rAI: $r = 0.72$ ($p = 0.019$), right putamen: $r = 0.65$ ($p = 0.044$), left putamen: $r = 0.63$ ($p = 0.049$). Analysis using percentage changes yielded similar results, the respective correlation coefficients were: left rAI: $r = 0.87$ ($p = 0.001$), right putamen: $r = 0.82$ ($p = 0.003$), left putamen $r = 0.77$ ($p = 0.01$). As can be seen from Figure 7.3 A(ii), B(ii) and C(ii), one extreme value was found in each correlation using percentage changes. To ascertain that the significant correlations are not false positive results caused by this value, correlation analysis was repeated after excluding this subject. Results for the left putamen were no longer significant but those for the left rAI ($r = 0.86$, $p = 0.003$) and right putamen ($r = 0.75$, $p = 0.019$) were similar to those with the subject included.

For noxious heat stimulation, raw signal change correlated linearly in left and right cAI with the raw change of pain ratings ($r = 0.64$, $p = 0.048$ and $r = 0.75$, $p = 0.012$) (Figure 7.4). For percentage changes, the left and right cAI ($r = 0.83$, $p = 0.003$ and $r = 0.9$, $p < 0.001$, respectively) and the left rAI ($r = 0.75$, $p = 0.013$) correlated with pain ratings. As the data point of one subject was an extreme value for the heat pain analysis in which

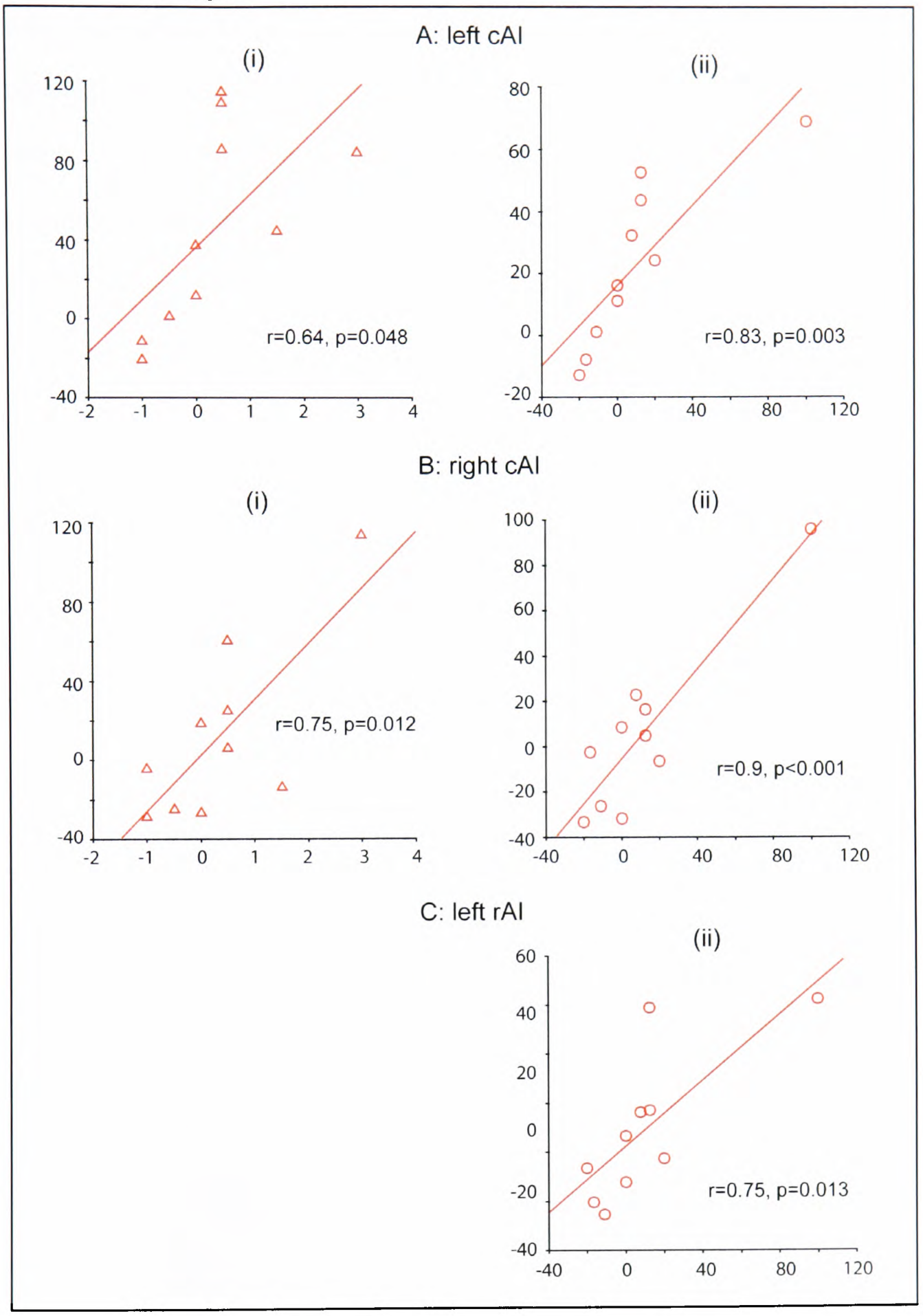
percentage changes were used, correlation analysis was repeated without this subject. Signal change in the left cAI still correlated with change of pain ratings ($r = 0.88$, $p = 0.02$), but correlations in left rAI and right cAI were no longer statistically significant ($p = 0.6$ and $p = 0.1$, respectively).

Figure 7.3 Correlation between allodynic pain rating change and FMRI signal change



The change of pain rating of allodynic stimulation between the OFF and the ON drug sessions (x-axes) correlated with the pertinent FMRI signal change (y-axes) between OFF and ON drug sessions in left rAI, right and left putamen. A(i), B(i) and C(i) depict the correlations when raw changes are used; A(ii), B(ii) and C(ii) when percentage changes are used. rAI, rostral anterior insula; r: Pearson's product moment correlation coefficient.

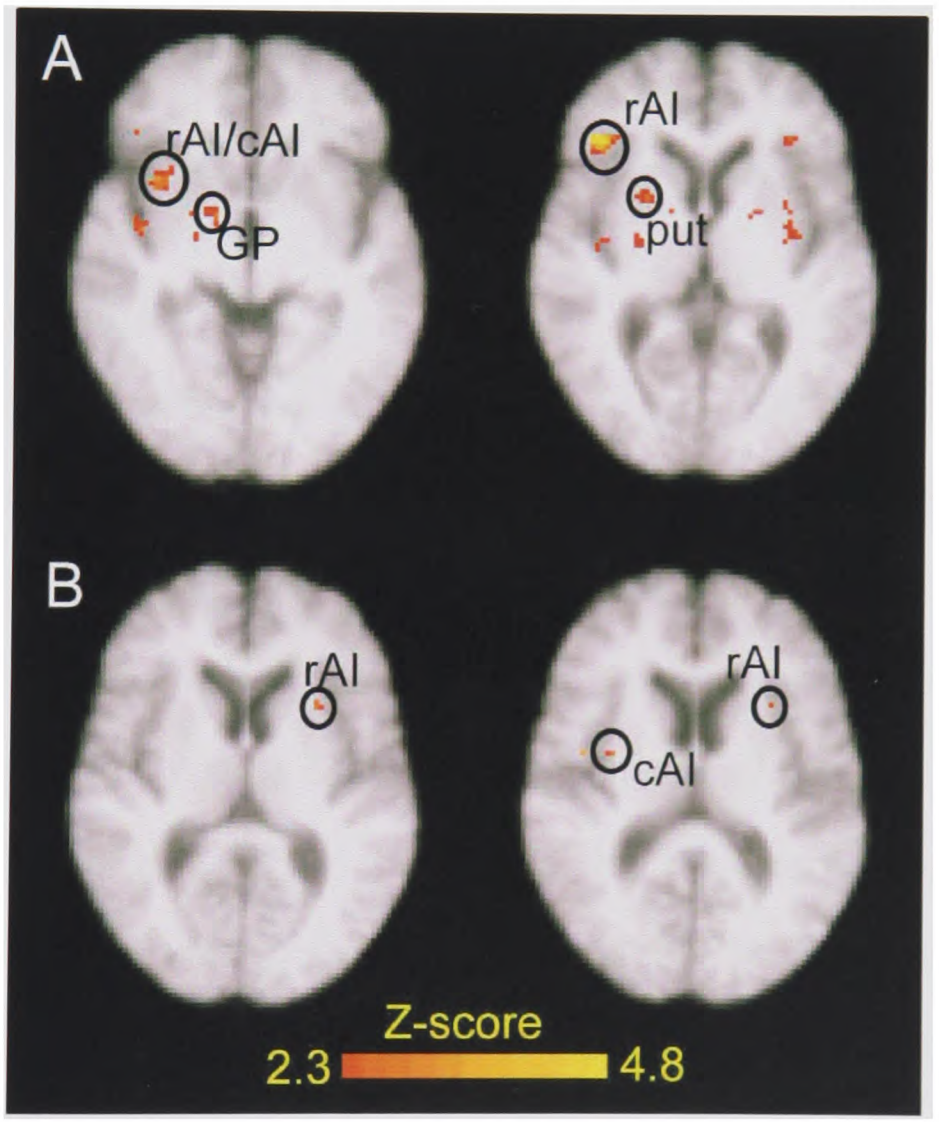
Figure 7.4 Correlation between thermal pain rating change and FMRI signal change



Correlation analysis between the change of pain rating of thermal stimulation between the OFF and the ON drug sessions (x-axes) and the corresponding FMRI signal change (y-axes) between OFF and ON drug sessions. A(i) and B(i) depict the correlations when raw changes are used, A(ii), B(ii) and C(ii) when percentage changes are used. When the extreme value was taken out of the analysis using percentage changes, only left cAI was significant. cAI, caudal anterior insula; rAI, rostral anterior insula; r: Pearson's product moment correlation coefficient.

GLM analysis largely confirmed the findings of the RoI analysis (Figure 7.5). For allodynic stimulation, voxels in which FMRI signal change between the two sessions correlated significantly with inter-session pain rating changes were found bilaterally in rAI, cAI and lentiform nucleus, i.e. putamen and globus pallidus (Figure 7.5A). For heat pain stimulation, voxels representing the pain rating change were found in rAI and cAI (Figure 7.5B).

Figure 7.5 Correlation between FMRI signal change and pain rating change investigated with GLM analysis



In essence, GLM analysis (uncorrected, voxel-based threshold: Z = 2.3) could confirm the results of the RoI analysis. For allodynic pain, voxels in which the FMRI signal change between the ON and OFF drug sessions reflects the change of pain rating between the two sessions were found in rostral and caudal anterior insula, putamen and globus pallidus (panel A). For thermal pain, rostral and caudal anterior insula contained voxels representing the difference in thermal pain rating between the two sessions (panel B). rAI, rostral anterior insula; cAI, caudal anterior insula put, putamen, GP, globus pallidus. Images are in radiological convention; i.e. left hemisphere is on the right side of the image.

7.3.3 Magnitude of FMRI signal change versus pain rating change

To compare the magnitude of FMRI signal changes with the magnitude of pain rating changes, the respective linear regression equations were determined. The regression equations were calculated after exclusion of the extreme value found in the percent changes for allodynic as well as thermal stimulation. The fractional FMRI signal change was greater than the respective pain rating change in all regions examined as indicated by a coefficient a > 1. Equations were as follows (y: FMRI signal change; x: pain rating change): allodynic pain, right putamen: y = 2.4*x - 25; left rAI: y = 1.1*x – 7.5; thermal pain, left cAI: y = 1.4*x +17.

Non-specific drug effects

As expected, no difference in activation between ON and OFF drug session was found for the thermal or allodynic pain experiment. To test nevertheless if a non-specific drug effect on the BOLD response exists, the stimulus-related FMRI signal of the control experiments was compared between ON and OFF drug session. No difference in activation between ON and OFF drug session was found for the mechanical control experiment. An indication of a non-specific effect of the drugs decreasing the BOLD signal change was found in the visual control experiment. Activation in the visual cortex was significantly higher in the OFF drug session than in the ON drug session. As this study did not investigate a change of FMRI signal between ON and OFF drug session but the relation between FMRI signal change and pain rating change, the results should

be unaffected by this non-specific effect, particularly as pain ratings did not change uniformly between ON and OFF drug session.

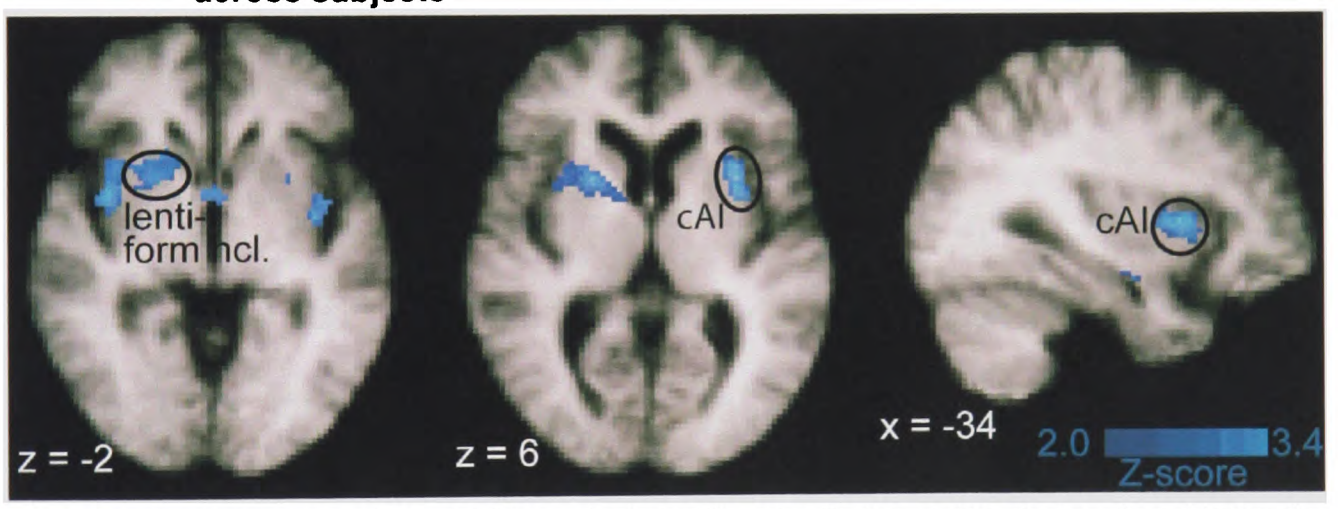
7.4 Discussion

Mimicking the situation commonly experienced in clinical trials, a heterogeneous group of ten neuropathic pain patients who differed with respect to underlying disease, disease duration, type of mechanical allodynia, age, sex and medication was investigated in this study. The regular analgesic medication was stopped (or reduced in one patient) to induce changes in neuropathic pain intensity. Most patients experienced less allodynic and/or spontaneous ongoing pain when taking their regular medication. As patients were not blinded to the reduction of their medication, it is possible that those who reported more pain OFF drugs did so partly because they were expecting to experience more pain. In a minority of the patients, reduction of their analgesic medication failed to increase the intensity of their clinical pain and they experienced less allodynic or ongoing pain without medication. This illustrates once more that the experience of pain is complex and does not only depend on peripheral and central neuropathological mechanisms targeted by the respective drugs, but is heavily influenced by other factors, such as the psychological state of the subject or contextual and environmental factors. Yet, the FMRI signal change between the two sessions was found to reflect the changes in pain perception in several brain regions irrespective of the reason underlying the perceptual change and despite the heterogeneity of the patient group. This suggests that FMRI might be useful as a read-out that reflects subjective pain perception.

7.4.1 Relationship between verbal pain report and FMRI signal

Chapter 6 showed that the perceived intensity of clinical allodynic pain is reflected across patients by the FMRI signal in cAI. In the present study, within-subject variation of the reported pain intensity was also found to be encoded in the cAI; but in addition, signal changes in rAI and lentiform nucleus (i.e. putamen and globus pallidus) reflected pain rating changes. Based on these results, the GLM analysis of Chapter 6 was repeated at a lower statistical threshold (voxel-based threshold: $Z = 2.0$, cluster-level correction: $p < 0.05$). In this analysis, signal intensity in lentiform nucleus did reflect allodynic pain ratings across subjects (Figure 7.6) but no correlation was found in rAI.

Figure 7.6 Activation correlating with perceived intensity of allodynic stimulation across subjects



Lowering the threshold of the GLM analysis in Chapter 6 showed that also signal intensity in lentiform nucleus (putamen and globus pallidus) correlates positively with allodynic pain ratings across patients. Voxel-based threshold: $Z = 2.0$, cluster-level correction: $p < 0.05$. ncl., nucleus; cAI, caudal anterior insula.

This suggests that the FMRI signal might reflect perceived pain intensity both within and across subjects in more brain regions than detected in the analysis of Chapter 6 and the present study. Also studies in healthy volunteers suggest that perceived pain intensity is widely reflected in a distributed network within the human brain (Coghill et al. 1999). Although

the studies in this and the previous chapter suffer from relatively low statistical power and hence, probably report false negative results in some brain regions, they show that the FMRI signal does reflect perceived clinical pain intensity in neuropathic pain patients and its modulation. Remarkably, this can be detected using a small cohort of patients.

To the best of our knowledge, the present study shows for the first time that the FMRI signal reflects intra-individual alterations of the perception of allodynic and experimental pain intensity in neuropathic pain patients. It should be emphasized that the FMRI signal did not encode whether the patient was ON or OFF drugs. Rather, the pain rating change across sessions was encoded in the FMRI signal change. This was also true if the pain rating went the "wrong way", i.e. when subjects rated the allodynic pain higher in the ON drug session than in the OFF drug session.

Allodynic pain is clinically relevant, as emphasized by the finding that it significantly reflects the subjective well-being. Hence, FMRI provides the possibility of monitoring clinically relevant pain of individual patients longitudinally in a more direct way than using behavioural measures, which might be confounded to a larger extent by memory and time elapsed between visits. FMRI would have a distinct advantage if it provided a more sensitive measure of pain perception than commonly used pain rating scales. Based on the consideration that it measures the activation associated with the subjective pain experience independent of pain labelling behaviour, i.e. without the biases that influence verbal report, which is most likely another source of variance, FMRI might have

the potential to be a more sensitive read-out of the pain experience than commonly used pain rating scales. The design of this study does not allow an investigation of whether FMRI is a more sensitive measure than pain rating because it does not permit us to estimate the error associated with each measure within subject. Nevertheless, the observation that the magnitude changes of the FMRI signal were greater than the magnitude changes of pain rating might provide a hint that the FMRI signal might indeed be a more sensitive marker of the pain experience. This is supported by results of previous studies of other disease entities (e.g. Alzheimer's disease, schizophrenia) that have indicated that FMRI could provide a more sensitive tool to study drug effects than the respective clinical measures (Honey et al. 1999; Rombouts et al. 2002). A sensitive measure of pain perception would be helpful in treatment monitoring, therapeutic decisions and drug dose adjustments in clinical practice. It would also make FMRI attractive for use in drug development, especially for proof-of-concept studies in the early phases of drug discovery (Phase II) and particularly when behavioural clinical measures are too crude to detect subtle drug effects. If phFMRI allowed demonstration of a potential functional effect with a novel drug in specific brain regions in small numbers of patients, it could be used to evaluate many substances in clinical settings quickly. There is a great diversity of potential targets and an even greater number of new chemical compounds to evaluate in patients, therefore the benefit to the pharmaceutical industry would be to get a sensitive read-out early so that reliable "go" or "no-go" decisions of drug development can be made.

An additional advantage of imaging methods that provide detailed spatial information, such as FMRI, as measures of pain perception is their potential to assess the multidimensionality of pain and to separate treatment effects on different aspects of the pain experience. Some pain rating tools use multidimensional scaling, e.g. pain intensity and unpleasantness, but these methods are based on the assumption that subjects can dissect their overall sensation of pain into its constituent elements. Provided that different aspects of the pain experience are processed in different neuronal assemblies, brain imaging could help to assess the different constituents of the pain experience and might even allow quantification of their relative contributions. Again, this would be useful as a diagnostic tool in clinical practice and would allow targeting drugs more specifically to individual symptoms of pain.

7.4.2 Relationship between PGIC and neuropathic pain ratings

The tight relationship between the perceived intensity of neuropathic pain and the general well-being of the subject was demonstrated by the finding that the patient global impression of change (PGIC) correlated significantly with the change of allodynic and ongoing pain ratings. This finding is not unprecedented in the literature. In a meta-analysis of ten treatment studies of neuropathic pain investigating more than 2700 patients in total, Farrar et al. found that pain ratings reflected significantly the PGIC (Farrar et al. 2001). In the studies included in the meta-analysis, pain was rated on an 11-point numerical rating scale (as in this study). However, separate ratings for evoked and spontaneous pains were not provided and there is no indication which type of pain influences the PGIC more. In our sample,

ongoing pain explained less variance of PGIC that allodynic pain. Simple summation of allodynic and ongoing pain ratings showed an even higher correlation with PGIC than each of the two types of pain alone.

7.4.3 Drug modulation of pain perception

Two patients reported increased allodynic and spontaneous ongoing pain in the ON drug session, one of whom was taking diclofenac as analgesic. Although this drug is traditionally considered to be ineffective in neuropathic pain, several animals studies suggest that allodynia and hyperalgesia might be partly mediated by spinal prostaglandins, which can be inhibited by unselective cyclooxygenase-inhibitors (Ghilardi et al. 2004; Yaksh et al. 2001; Zhang et al. 2001). Even if ineffective, the drug should not lead to higher neuropathic pain in the ON drug session. This could be explained either by the complexity of the pain experience or by random variation of pain ratings.

All patients who stopped an opioid-containing compound rated the pain intensity of the invariant thermal stimulus lower in the ON drug session, which is in accordance with opioid-receptor agonists influencing processing of nociceptive pain. The other patients rated the invariant thermal stimulus higher or the same in the ON drug session. Higher ratings could be due to random variation in pain rating scores. But interestingly, within-subject changes of thermal pain ratings correlated negatively with within-subject changes of allodynic ratings. It is purely speculative why patients rated the same thermal stimulus higher when they experienced less allodynic pain. It could be explained by a re-

calibration of the subjective rating scale or by attention that was redirected to the thermal stimulus when allodynic pain was less. Attention is known to increase pain perception (Miron et al. 1989). Future studies should investigate if ratings of invariant thermal stimuli do indeed depend on the change of neuropathic pain intensity to assess if thermal pain offers itself as an indirect measure to assess modulation of neuropathic pain.

7.5 Conclusions

It is shown here that within-subject FMRI signal changes between two imaging sessions reflect the alteration of allodynic and experimental pain perception in neuropathic pain patients. In addition, the magnitude of the FMRI signal change was greater than the pain rating change in all regions that encoded the perceptional change. This is a promising result as it emphasizes the potential of FMRI to detect subtle changes in pain processing. Such a tool could improve monitoring of treatment and disease progression of chronic pain patients with positive implications for therapeutic decisions and drug management. Equally, phFMRI could be useful for the pharmaceutical industry as an early indicator of the potential efficacy of a new compound, which would lead to more time- and cost-efficient drug development.

CHAPTER 8: General conclusions and outlook

This thesis demonstrates that a common network of brain structures is involved in the processing of the clinical pain that is characteristic for patients with neuropathic pain or with RA, of experimental pain in these two patient groups and of experimental pain in healthy volunteers. The substantial overlap of FMRI activation patterns indicates that clinical pain is on the whole processed in the same regions as nociceptive pain and that supraspinal pain processing is not substantially altered in chronic pain patients, at least in conditions in which the primary pathology is located outside the central nervous system. Ascending pain pathways that originate from spinal cord neurons responding only to supra-threshold stimuli under normal conditions and showing an aberrant response to sub-threshold stimuli in clinical pain conditions (Dubner 1991; Palecek et al. 1992; Price et al. 1994) and that therefore transmit pain-related information both in acute nociceptive pain and in clinical pain could account for the similarities found between the supraspinal correlates of experimental and clinical pain.

Although no brain region is specific to the processing of clinical pain, the neural representations of experimental and clinical pain are not identical. Rather, alterations in FMRI activation patterns manifest themselves in more subtle ways, such as spatial displacement or stronger activation. By these measures, FMRI can be used to demonstrate objectively that the experience of clinical pain is different from the experience of experimental pain. Theoretically, supraspinal representations of clinical pain *should* differ from those of experimental pain. The fact that clinical pain is more

strongly associated with negative affect and requires different coping strategies than experimental pain has to be encoded somewhere in the human brain. In addition, animal models of clinical pain conditions strongly suggest that facilitatory processes occur above the level of the spinal cord. Evidence for both arguments was found in this thesis. Brainstem structures that are known from animal experiments to be involved in descending facilitation were found to be activated by experimental pain only in neuropathic pain patients and not in healthy control subjects, as demonstrated in Chapter 3. Support for the notion that negative emotions and attentional processes interfere with pain processing more in pain patients than in healthy control subjects is provided in Chapters 3 and 4. In Chapter 3, this support comes from stronger activations observed in two classical limbic areas, i.e. the anterior insula and the amygdala. In Chapter 4, a spatial shift of activation *within* the anterior insula towards neural representations of highly negative emotions and the conscious perception of bodily sensations was detected for clinical pain. These two observations reflect that clinical pain processing differs from experimental pain processing. Moreover, both brain regions meet the neuroanatomical and functional pre-requisites to actively modulate the processing and the perception of pain. Cognitive and emotional determinants of the pain experience are likely to exert their influences via these and related brain structures. The medial prefrontal cortex, for instance, may mediate an influence of depressive symptoms on the perceived intensity of clinical pain, as shown in Chapter 5, perhaps via a circuit that contains the amygdala. New imaging techniques in conjunction with FMRI offer the opportunity to study details of the inter-connectivity and inter-dependences

of these pain modulatory networks in the future. Diffusion Tensor Imaging (DTI) allows the characterization and quantification of white matter tracts *in vivo* and can be used to sub-divide brain regions according to their neuroanatomical connection patterns (Rushworth et al. 2005). Functional spinal cord imaging, which is currently under development (Giove et al. 2004), will help to attribute different aspects of pain modulation to their respective sites of effect and will thereby possibly discriminate pain modulatory processes that exert their effects via descending networks from those alterations of the pain experience that are caused by intra-cerebral processes.

Brain FMRI itself might have the potential to provide a more direct and sensitive measure of pain perception than the commonly used verbal pain report. This thesis showed that the FMRI signal in several brain regions reflects perceived pain intensity in patients, both within- and between-subjects. Patient samples investigated in this work were relatively small and it is necessary that the results are replicated by larger investigations, but if the results are shown to be reproducible, FMRI would hold great promise as a tool for drug development. Ultimately, FMRI might be able to dissect the experience of clinical pain into its many contributing mechanisms, including peripheral and central sensitization, brainstem facilitation, affect-induced amplification and reduction by coping strategies. If the pain experience could be dissected on the single-subject level, FMRI could play a role as a diagnostic tool that enables clinicians to target therapeutic manipulations in chronic pain patients more specifically.

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