Functional Magnetic Resonance Imaging of the Anterior Cingulate Gyrus and Medial Prefrontal Cortex during Visceral and Somatosensory Pain

A Thesis

Submitted to the Faculty of Graduate Studies

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

In Physiology

By

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DOCTOR OF PHILOSOPHY

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ABSTRACT

The Anterior Cingulate Gyrus (ACG) and Medial Prefrontal Cortex (MPFC) are part of the pain neuromatrix and therefore the multidimensional experience of pain. Although ACG and MPFC activity has been well documented in studies of somatosensory pain, little is known about the activity of these regions during visceral pain. This work uses Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) to characterize ACG and MPFC activation and deactivation patterns during visceral pain and draws comparisons with somatosensory pain. It also assesses ACG sub-regional responses to pain and attention tasks and investigates whether ACG sub-regional responses to pain can be modulated by a cognitive distraction task. Comparisons of ACG and MPFC activity during chronic visceral pain revealed a higher percentage of activation in the ACG of control subjects than in patients suffering from chronic visceral pain. Patient and control groups were further differentiated by a deactivation in somatosensory cortex and an MPFC deactivation that bordered on statistical significance. In a comparison of ACG and MPFC activity in visceral and cold pressor pain, midcingulate activity was most prominent during both pain modalities. Lateralization was not significant. ACG and MPFC activity to visceral pain resembled that of left-sided cold pressor pain and both differed from right-sided cold pressor pain. The study on ACG sub-regional modulation demonstrated that distraction from pain results in decreased activation of ACG sub-regions responsive to pain and increased activation in ACG sub-regions responsive to the distraction task. Together, these findings suggest that: 1. ACG and MPFC are key regions in the processing of visceral pain; 2. ACG and MPFC show similar patterns of response in different pain modalities; 3.

ACG and MPFC activation patterns may be used to differentiate between health and disease and 4. ACG sub-regional responses depend on the degree of attention directed towards the stimulus.

ABBREVIATIONS

ACG	Anterior Cingulate Gyrus
AC-PC	Anterior Commissure-Posterior Commissure
ANOVA	Analysis of Variance
B0	Main Magnetic Field
B1	Magnetic Field perpendicular to Main Magnetic Field
BA	Brodmann Area
BOLD FMRI	Blood Oxygen Level Dependent Functional Magnetic Resonance Imaging
CBF	Cerebral Blood Flow
CNS	Central Nervous System
CPT	Cold Pressor Test
DCP	Dorsal Column Pathway
DLPFC	Dorsolateral Prefrontal Cortex
DMPFC	Dorsomedial Prefrontal Cortex
DT	Distraction Task
EEG	Electroencephalogram
FOV	Field of View
GI	Gastrointestinal
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
MEG	Magnetoencephalography
MID	Midcingulate
MPFC	Medial Prefrontal Cortex

MRI	Magnetic Resonance Imaging
NIRS	Near Infrared Spectroscopy
PAG	Periaqueductal Gray
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PGC	Perigenual Cortex
ROI	Region of Interest
S1	Primary Somatosensory Cortex
S2	Secondary Somatosensory Cortex
SCT	Spinocervical Tract
SMT	Spinomesencephalic Tract
SPECT	Single Photon Emission Tomography
SPM99	Statistical Parametric Mapping 1999
SRT	Spinoreticular Tract
STT	Spinothalamic Tract
T	Tesla
T1	Longitudinal Relaxation
T2	Transverse Relaxation
TE	Echo Time
TR	Repetition Time
VAT	Verbal Attention Task
VMPFC	Ventromedial Prefrontal Cortex

GENERAL INTRODUCTION

I. Thesis Overview

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.

This definition is provided by the International Association for the Study of Pain (http://www.iasp-pain.org/terms-p.html). It is the most widely accepted definition of pain and emphasizes that pain represents a complex psychophysiological event. It involves the neurochemically complex interaction of sensory, emotional, motivational and cognitive systems. Although the psychophysiological mechanisms underlying the perception of pain are poorly understood, it is well accepted that complex processes such as pain are organized in widely distributed neural networks. Since the first functional imaging reports appeared (Jones et al., 1991; Talbot et al., 1991) that show that pain is represented cortically, we and others have proposed that the anterior cingulate gyrus (ACG) and medial prefrontal cortex (MPFC), which is further divided into dorsomedial [DMFC] and ventromedial prefrontal cortices [VMPFC]) are part of such a neural network including dorsolateral prefrontal cortices, somatosensory cortex, insular cortices, thalamus, periaqueductal grey matter and the cerebellum (Derbyshire et al., 2000; Frankenstein et al., 2001; Ingvar, 1999). The interplay between different components of this network as well as contributions of the ACG and MPFC to different modalities of pain, particularly pertaining to visceral and somatosensory pain processing, is not well understood. Therefore it is the overall objective of this thesis to use Blood Oxygen Level Dependent functional Magnetic Resonance Imaging (BOLD fMRI) to gain a better

understanding of ACG and MPFC involvement in visceral and somatosensory pain processing.

A growing body of research suggests that the ACG and MPFC are key players in pain processing. Indeed, there is evidence not only from anatomical work, but also from lesion, electrophysiological and neuroimaging studies. It has been shown that the ACG is composed of functionally heterogeneous sub-regions (Devinsky et al., 1995) playing a role in motor functions, cognition and emotion, all of which can be part of the pain experience. The heterogeneity of ACG function is supported by studies on neuronal cytoarchitecture and anatomical connections suggesting a neuroanatomical and functional segregation of specific subregions. The ACG has prominent reciprocal connections with other parts of the prefrontal cortex including the DMPFC, VMPFC and dorsolateral prefrontal (DLPFC) cortices, all of which have been implicated in various aspects of pain processing. The following sections provide a review of and general background information on areas relevant to the focus of this thesis.

II. Central Nervous System Regions involved in Pain Processing

From the peripheral receptor to the cerebral cortex, noxious visceral and somatosensory stimuli are processed by specialized neural pathways. It is important to demarcate the various spinal pathways and their projections as a means of understanding functional differences and similarities between cortical activations in visceral and somatosensory pain. Therefore it is the first objective of this review to provide the general anatomical and physiological background on the ascending pathways and brain regions involved in visceral and somatosensory pain processing.

II.1. Spinal cord pathways involved in the transmission of visceral pain

Visceral pain is dull, aching, diffuse and, thus, often poorly localized. These properties indicate that the representation of internal organs within the CNS is very imprecise. The factors contributing to this poor localization include the low number of afferent nerves carrying information from the viscera to the CNS (relative to the afferent innervation of somatic tissues), their greater rostrocaudal distribution in the spinal cord and convergence of visceral input on spinal cord dorsal horn neurons that also receive input from other cutaneous or visceral sites (Gebhart, 1995).

Research indicates that there are three distinct classes of nociceptive receptors that innervate visceral organs (Cervero, 1994; Cervero and Jaenig; 1992). High threshold receptors evoke nerve activity by stimuli that are entirely within the noxious range. Low threshold receptors have an encoding function that encompasses stimulation intensities in the innocuous to noxious range and silent nociceptors, which are normally unresponsive to stimuli become activated only in the presence of inflammation. Both, unmyelinated, slow C-fibers and myelinated-rapid Aδ fibers respond to pressure and mechanical stimuli. Responses to stimuli are then carried to the brain via spinal cord pathways that carry visceral nociceptive information. Traditionally it was believed that visceral pain is transmitted via the spinothalamic and spinoreticular tracts (Al Chaer, 1999). This view has recently been challenged by the discovery of a visceral pain pathway in the dorsal column of the spinal cord (Willis et al., 1999). The spinothalamic, spinoreticular and dorsal column pathways are reviewed below.

II.1.1. The spinothalamic tract (STT)

Although the majority of STT neurons code noxious cutaneous stimuli, spinal cord cells projecting to the thalamus have also been shown to respond to a wide variety of other stimuli including manipulation of joint or muscle and distention of the viscera (Al-Chaer et al., 1999). STT neurons have been classified into wide-dynamic range, if they respond to innocuous, but maximally to noxious stimuli, low-threshold neurons that respond only to innocuous mechanical cutaneous stimulation and nociceptive-specific, if they exclusively respond to noxious stimuli.

The spinothalamic tract is the most prominent ascending nociceptive pathway in the spinal cord. The cellular origin of the STT is located in the lumbar and sacral enlargements, specifically in cells in the marginal zone and neck of the dorsal horn in Rexed laminae I and laminae IV-VI (Willis et al., 1997). Visceral afferent fibres display a consistent pattern of central termination throughout the spinal cord with areas of projection in Laminae I and V but sparing the intermediate dorsal horn (Cervero, 1991). Dorsal horn and other spinal cord neurons can be classified into two main groups depending on the presence or absence of excitatory visceral input. Some neurons are not driven by visceral afferent fibres and can only be excited from their somatic receptive fields (somatic neurons). Other neurons have both somatic and visceral inputs resulting in convergent transmission. Whereas somatic neurons are mainly located in Laminae II, III and IV, viscero-somatic neurons are located in Laminae I, V, and the ventral horn. STT cells are also located around the spinal central canal known as lamina X.

Along with the spinomesencephalic and spinoreticular tracts the STT initially synapses ipsilaterally in the spinal cord grey matter and then ascends contralaterally in the anterolateral quadrant of the spinal cord white matter. At the brain stem the STT

divides into the paleospinothalamic (old) pathway and the neospinothalamic (new) pathway. The paleospinothalamic pathway is evolutionary older and signals dull and burning pain, receiving input from small, unmyelinated C fibers and the neospinothalamic pathway carries sharp and pricking pain via fast conducting myelinated A fibers.

The STT innervates several areas of the brain most importantly the thalamus and the limbic system. There are clear anatomical differences between STT cells projecting to the lateral thalamus and those projecting to the medial thalamus. The former are more likely to be situated in laminae I and V whereas medially projecting cells are more likely to be situated in the deep dorsal horn and in the ventral horn. In primates STT terminals have been located in the following medial and lateral thalamic nuclei: the caudal and oral parts of the ventral posterior lateral nucleus, the ventral posterior inferior nucleus, the medial part of the posterior complex, the central lateral nucleus and other intralaminar and medial thalamic nuclei. Whereas lateral thalamic nuclei project to somatosensory cortex, thalamic midline and intralaminar nuclei have been shown to project to the ACG.

II.1.2. The spinoreticular tract (SRT)

The SRT originates in the ventral horn and laminae VII and VIII. The SRT ascends in the anterolateral quadrant of the spinal cord and SRT nociceptive neurons in laminae VII and VIII terminate in the brainstem reticular formation, pontine and medullary regions as well as the medial thalamic nuclei. In monkey the SRT termination sites in the brainstem include the lateral reticular nucleus, the nucleus gigantocellularis, the nucleus interfasicularis hypoglossi, the nucleus pontis centralis and the sub-coeruleus (Kerr,

1975). The reticular formation is excited by noxious somatosensory and visceral stimuli and also receives descending projections from the periaqueductal grey matter which is one of the key structures in descending modulatory pain pathways (Reynolds et al., 1969).

Based on its termination sites and indirect connections with higher brain centers it has been suggested that the SRT mediates functions related to autonomic, motivational and affective responses to pain (Markenson, 1996, Melzack and Casey, 1968; Willis and Westlund, 1997). The latter is supported by the observation that the reticular formation sends projections to the medial thalamus and hypothalamus (Bowsher et al., 1975). These structures in turn provide input to limbic forebrain structures such as the ACG and hippocampal formation which have been shown to participate in attentional, motivational and affective networks (Bonica, 1990).

II.1.3. The Dorsal Column Pathway (DCP)

The DCP arises from neurons in lamina III of the dorsal horn as well as from a few cells lateral to lamina X (Rustioni, 1973; Bennett et al., 1983; Giesler et al., 1984). Most of the early reports indicate that the DCP conveyed innocuous information about tactile discrimination and position sense. However, in 1932 Foerster and Gagel observed that mechanical manipulation of the dorsal column caused awake human subjects to experience unbearable pain in the sacral region and perineum. More recently, Al-Chaer et al. (1996) found that visceral pain is largely mediated by the dorsal column pathway and that dorsal column neurons project to the lateral thalamus, which is part of the lateral pain system. Moreover, it has been shown that limited midline myelotomy is highly

effective in reducing intractable pelvic cancer pain in humans (Willis et al., 1999). And finally Willis et al., (1999) published their findings on a visceral pain pathway in the dorsal column. In primates they showed that lesions of the DCP, and not the STT, dramatically reduced neuronal responses in the ventral posterolateral thalamic nuclei to colorectal distention. Willis et al., (1999) supported their findings by fMRI studies on anesthetized monkeys and showed that sham surgery did not reduce thalamic activations in response to colorectal distention whereas DCP lesions did result in decreased activity in the lateral thalamus.

II.1.4. Summary

Although separate spinal cord pathways respond to noxious visceral stimulation and carry the information to the brain, several anatomical and functional similarities are evident. All pathways project either directly or indirectly to the thalamus. Viscerosomatic neurons are mainly located in Rexed laminae I and V of the STT. STT cells in these laminae project to the lateral thalamic nuclei, which in turn send projections to the somatosensory cortices. The DCP, with origins in dorsal horn lamina III, also projects to the lateral thalamus and lesions of the DCP result in reduced lateral thalamic activity to colorectal distention. Even though both pathways project to the lateral thalamus, a clear somatosensory cortical representation of visceral pain has not been demonstrated. The research cited above provides support for this somatosensory representation. SRT and STT cells located in the deep dorsal and ventral horns send projections to the medial thalamic nuclei. Based on these similar termination sites it is

likely that both pathways relay information to the ACG and are concerned with pain affect.

II.2. Spinal cord tracts involved in the transmission of somatosensory pain

The majority of what we know about pain processing derives from studies of somatosensory pain. An obvious reason for this relies on the fact that it is much easier to produce a painful somatosensory stimulus than a painful visceral stimulus.

There are several different classes of nociceptive afferent fibres that have been identified. Thermal or mechanical nociceptors have thinly myelineated, small diameter Aδ fibres that conduct about 5-30m/sec and result in sensations of burning or pricking pain. Polymodal nociceptors are activated by a variety of stimuli including mechanical, thermal (hot and cold) and chemical stimuli. They have small diameter unmyelinated C-fibres that conduct at a slower rate of about 0.5-2m/sec. Projection neurons in lamina I of the spinal cord receive direct input from Aδ fibres and indirect input from C-fibres through interneurons in lamina II. Lamina V also receives direct and indirect input from Aδ and C-fibres.

Nociceptive somatosensory information is relayed to the brain via several major ascending pathways that originate in different laminae of the spinal cord. These include the spinothalamic, spinoreticular, spinomesencephalic and spinocervical tracts. As the pain stimulus used in this thesis is an adaptation of the Cold Pressor Test (CPT), the focus of the spinothalamic review will be on the involvement of the STT in the transmission of cold pain. The spinoreticular tract mediates functions related to autonomic, motivational and affective responses that are common to both visceral and

somatosensory pain and is largely reviewed in the section on spinal cords tracts involved in the transmission of visceral pain. The spinocervical tract will only be reviewed briefly as a significant spinocervical tract has not been shown to exist in humans.

II.2.1. The spinothalamic tract (STT)

The cellular origin, cell types and supraspinal termination sites of the STT have been discussed under the spinal cord pathways involved in visceral pain. Briefly, STT cells are located in laminae I, IV-VI and X. Both somatic and viscero-somatic neurons have unique origins in the spinal cord grey matter. The tract initially synapses ipsilaterally, decussates, and terminates in several brain regions, most notably the thalamus and limbic system via the medial thalamic nuclei.

Apart from its involvement in transmitting visceral information, it is generally accepted that the STT is the main ascending pathway conveying sensory information related to pain and temperature sensation. Cold fibers are predominantly of the Aδ type (Raja et al., 1999), however, C-fiber cutaneous nociceptors have also been shown to respond to noxious cold stimulation in the rat (LaMotte and Thalhammer, 1982). Previous studies of the spinal cord have shown that many nociceptive neurons, both wide dynamic range and high threshold cells in the superficial and deep dorsal horn, are excited by cold stimuli, and can in addition to encoding the intensity of noxious heat stimuli, respond to noxious cold stimuli to 0 °C (Craig and Bushnell, 1994; Craig and Serrano, 1994; Kenshalo, 1982). Intense cold noxious stimulation of the rat hindpaw induces c-fos expression in lumbar spinal cord neurons (Abbadie et al., 1994) and internalization of substance P receptors in laminae I and II dorsal horn neurons (Allen et

al., 1997). Interestingly, noxious heat stimulation of the hindpaw induced expression of c-fos in laminae I and II cells with a distribution similar to that produced by noxious cold stimuli (Abbadie et al., 1994), suggesting that many dorsal horn neurons may process information from heat- and cold-sensitive nociceptors.

II.2.2. The spinoreticular tract (SRT)

As previously discussed in the spinal cord pathways involved in visceral pain, the SRT originates in laminae VII and VIII of the spinal cord grey matter. Termination sites of spinoreticular fibers in the brainstem include several nuclei of the parabrachial region, the Kolliker-Fuse nucleus, the nucleus gigantocellularis and the locus coeruleus, for example (Kerr, 1975). In turn, neurons of the parabrachial nuclei project to the amygdala, a major component of the limbic system, that has been suggested to contribute to the affective component of the pain experience. Indirect connections with higher brain centers such as the ACG are mediated through the reticular formation and medial thalamic projections.

II.2.3. The spinomesencephalic tract (SMT)

Together with the STT, the SMT ascends the spinal cord contralaterally in the anterolateral quadrant. SMT neurons are nociceptive, responding either exclusively or preferentially to noxious stimuli (Willis and Coggeshall, 1991).

In the primate, most SMT cells originate in Rexed's laminae I and IV-VI and can be classified as wide dynamic range or nociception-specific. Some of the SMT cells send projections to the lateral thalamus; whereas, others project to the mesencephalic reticular

formation, the deep layers of the superior colliculus, the parabrachial nucleus or the lateral aspect of the periaqueductal gray matter (PAG), all of which have been implicated in pain processing. The different projections of the SMT may have different functions in regards to the processing of painful stimuli. For example, the PAG has been implicated in the descending control of nociceptive input. Thus it has been shown that stimulation of the PAG causes inhibition of nociceptive dorsal horn neurons (Carstens et al., 1979) including spinothalamic tract cells (Hayes et al., 1979). Since the superior colliculus contains neurons which respond to noxious (Telford et al., 1996) as well as auditory (Gaese and Johnen, 2000) and visual stimulation it is possible that SMT input into the deep layers of the superior colliculus could play a role in the orienting response to a noxious stimulus.

II.2.4. The spinocervical tract (SCT)

A significant spinocervical tract has not been shown to exist in humans (Talbot, 1996). However, nociceptive SCT neurons have been identified in the spinal cord dorsal horn of the cat and monkey. In the cat, the SCT primarily originates from cells in lamina IV. From the dorsal horn the pathway relays in the lateral cervical nucleus (segments C1 and C3 of the spinal cord) (Molander and Grant,1995) from where it decussates and ascends to the thalamus. Reports of the lateral cervical nucleus thalamic projection sites vary slightly among studies and they include lateral thalamic nuclei including the ventroposterior- and ventromedial lateral divisions.

SCT cells have been studied in the spinal cord of cats using electrophysiological methods. Although many SCT cells respond to tactile stimulation, only some SCT

neurons have been shown to respond to noxious stimulation (Craig and Tapper, 1978), thus providing a potential pathway through which nociceptive signals can reach the lateral thalamus.

II.2.5. Summary

There is some overlap in the type of cells, the spinal cord pathways and supraspinal structures involved in somatosensory and visceral pain. Both the STT and SRT have been implicated in the transmission of both pain modalities. The reticular formation is excited by noxious somatosensory and visceral stimuli and receives descending projections from the periacqueductal grey matter which is one of the key structures in descending modulatory pain pathways (Reynolds et al., 1969). Transmission of responses to visceral or somatosensory pain to the ACG are mediated through the medial thalamic nuclei and may play a role in the affective component of the pain experience.

The STT contains somatic and viscero-somatic neurons that are classified based on the presence or absence of excitatory visceral input. Referred pain from the viscera to cutaneous sites is the result of convergent transmission where neurons have both somatic and visceral input. Both the STT and SMT have cells of origin in lamina I of the dorsal horn and are wide dynamic range or nociceptor specific. These cells project to the lateral thalamus. SMT cells also project to the PAG and stimulation of the PAG causes inhibition of STT nociceptive dorsal horn neurons including STT cells, however a relationship between visceral pain and the SMT has not been demonstrated. Although DCP lesions result in a dramatic reduction of lateral thalamic nuclei to colorectal

distention, a minor reduction in thalamic responses to colorectal distention has also been observed with STT lesions.

II.3. Brain regions involved in pain processing

At the supraspinal level, the neural network subserving pain processing has been divided into two theoretical systems (Vogt et al., 1993). Therefore brain regions involved in pain processing will be discussed as part of either the *lateral* or the *medial* pain systems. The distinction between the *medial* and *lateral pain systems* is based on the divergence of spinothalamic projections in the thalamus. The medial pain system includes the medial and intralaminar thalamic nuclei, which receive input from multiple ascending systems of the spinal cord and reticular formation and project to the anterior cingulate gyrus (Vogt et al., 1993). The ACG in turn projects to the prefrontal cortex including MPFC (including DMPFC and VMPFC) and dorsolateral prefrontal cortex (DLPFC). The lateral pain system receives input from lateral thalamic nuclei and projects to the somatosensory cortex. The lateral pain system is involved in the sensory discrimination and localization of a noxious stimulus, whereas the medial pain system plays a crucial role in the affective response to noxious stimulation. For both systems anatomical, lesion, stimulation and functional imaging research will be reviewed. Figure 1 is a simplified schematic depicting *some* of the major projection sites of spinal cord pathways in the brainstem, subcortical and cortical structures.

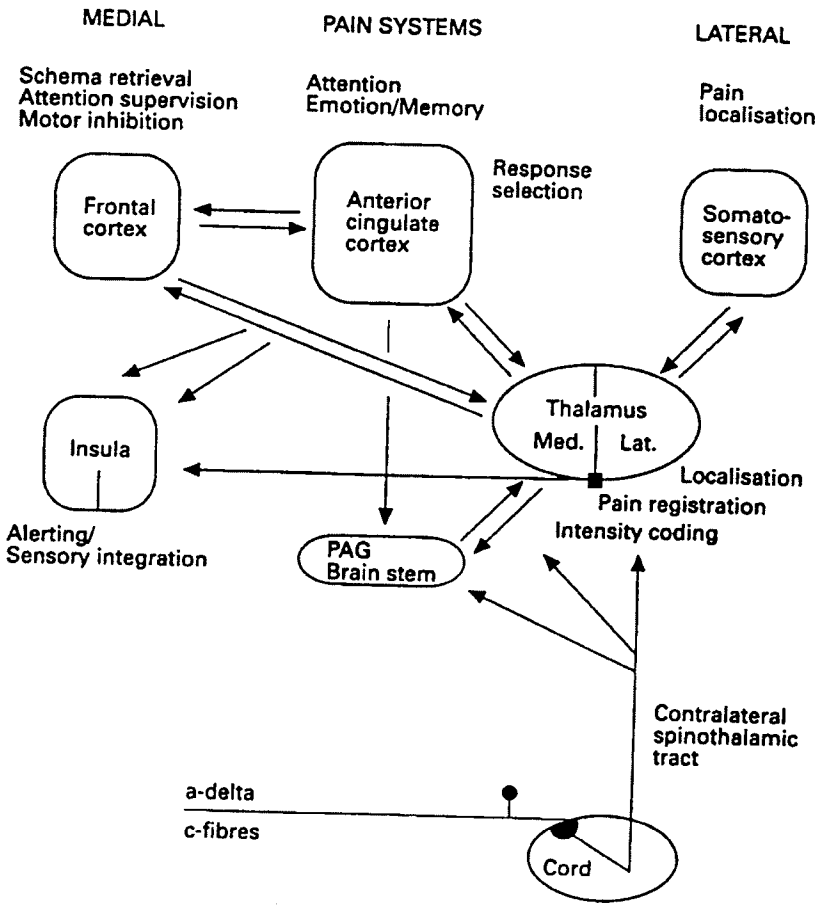

Figure 1

Figure Legend: Simplified schematic of the main components of the medial and lateral pain systems. The lateral pain system includes spinal cord projections to the lateral thalamus and somatosensory cortex. The medial pain system includes the anterior cingulate and frontal cortices and receive projections from the spinal cord via medial thalamic nuclei.

II.3.1. The medial pain system

The medial thalamic nuclei, anterior cingulate cortex and prefrontal cortex will be introduced as part of the medial pain system. Within prefrontal cortex the DMPFC, VMPFC and DLPFC will be briefly reviewed.

II.3.1.1. The medial thalamus

There are several anatomical and physiological lines of evidence implicating a role of the medial thalamus in pain processing. The STT projects to the lateral and medial thalamus and the SMT and SRT pathways relay in the reticular formation in areas that project heavily to the medial thalamic nuclei and intralaminar nuclei. Anatomical studies show that Rexed's spinal cord laminae I, V, VI, VII, and VIII contain neurons that project to the medial thalamus. 60% of lamina I, VII, and VIII STT projection neurons and 40% of lamina V and VI neurons terminate in the medial thalamus (Craig et al., 1989). Work in the primate, racoon and rat (Craig and Burton, 1981; Craig and Burton, 1985) has identified termination sites in the paraventricular, parataenial, submedial and ventromedial nuclei as well as the nucleus reunions. The medial thalamic nuclei in turn project to limbic cortical areas that subserve emotional reactions to noxious stimuli, including the ACG, orbitofrontal and insular cortex.

Based on similar response properties of medial thalamic and ACG neurons, Sikes and Vogt (1992) investigated whether noxious information is transmitted via midline thalamic nuclei to the ACG in the rat. Lidocaine was injected into midline thalamic nuclei and nociceptive neuronal activity was evaluated in cingulate cortex before and after these injections. Injections of lidocaine into the medial thalamus virtually abolished the nociceptor-evoked activity in ACG providing strong evidence that nociceptive input to the ACG derives from midline thalamic nuclei.

Electrophysiological studies show that neurons within the medial thalamus have large and often bilateral receptive fields preventing individual neurons from transmitting

detailed information about stimulus location. Moreover, neurosurgical lesions of the midline and intralaminar thalamic nuclei can alter responses to noxious stimuli in experimental animals and relieve chronic pain in humans. For example, lesions of the Cl-Pf complex in cats undergoing tooth pulp stimulation abolish escape behaviours (Kaelber et al., 1975). In humans, medial thalamic lesions (Young et al., 1995) alleviate chronic pain. These findings suggest that lesions of the medial thalamic nuclei either directly abolish conscious sensations associated with noxious afferents in the thalamus or that they deafferent higher levels at which such sensations occur in cerebral cortex (Vogt, 1993).

Thalamic responses to pain have been demonstrated with both Positron Emission Tomography (PET) and fMRI. Although thalamic activations have been observed in many neuroimaging studies, the spatial resolution of PET is insufficient to discriminate the separate neuronal regions within the thalamus (Peyron et al., 2000). Furthermore, general methodological issues of stimulus presentation and type make it difficult to compare thalamic activations from one study to the next. Nevertheless, thalamic activations to painful stimuli are frequently described as bilateral (Peyron et al., 2000). Bilateral thalamic activation also increases during vigilance and attentional processes suggesting that the thalamic response to painful stimuli may be part of an attentional network involved in pain processing. Comparisons between non-painful and painful stimuli show thalamic activation in the latter and an absence of activation in the former. Using PET, Iadarola et al., (1998) showed contalateral thalamic midline activity after capsaicin injection into the left volar forearm. Light brushing of this region did not result in midline or ventral thalamic nuclei activity, however the contralateral projection field in

S1 was clearly activated. Using fMRI, Davis et al., (1998) demonstrated activations in discrete regions of the thalamus in response to noxious cold stimulation. These were predominantly contralateral and within the lateral and/or posterior thalamus. Some medial thalamic activations were also observed. Similarly, Tracey et al., (2000) observed dominant contralateral thalamic activation during the application of noxious heat and cold stimuli.

II.3.1.2. The anterior cingulate gyrus (ACG)

The ACG is a functionally heterogeneous region that has been implicated in many behaviours including the experience of pain. As part of the medial pain system, the ACG contains regions subserving affect (Brodmann Areas [BA] 24,25, 32) and cognition (BA 24' and 32') (Devinsky et al., 1995), both of which partake in pain sensation. Initially, some anatomical research implicating the ACG in pain processing will be introduced. This will be followed by background information on the ACG's role in affect and cognition.

Studies of cytoarchitecture and anatomical connectivity demonstrate that the ACG is broadly divided into two sub-regions, the perigenual and the midcingulate ACG sub-regions. The perigenual region contains BA24, 25 and 32 and the midcingulate region contains BA24' and 32' (Devinsky et al., 1995). Whereas perigenual area BA24 receives most medial thalamic and amygdala afferents, BA24' has major reciprocal connections with the cingulate motor areas, pontine nuclei and the dorsolateral prefrontal cortices (Vogt et al., 1993). In the primate it has been shown that the rostralmost part (BA32, also known as the cingulofrontal transition cortex) of the perigenual region sends projections to the lateral prefrontal cortex, mid-orbitofrontal cortex and to the rostral

portion of the superior temporal gyrus, all of which have been implicated in pain processing (Pandya et al., 1981). In addition to projections from the amygdala, the perigenual affect division also has pronounced projections to the PAG, which has been implicated in descending analgesia mechanisms.

The midcingulate region (BA24' and 32'), on the other hand, projects to the spinal cord and red nucleus and has few connections with the perigenual region. This supports a dissociation between affective and cognitive regions within ACG. Recently it has been proposed to further sub-divide the BA24'midcingulate region into $BA24^1$ and $BA24^2$ (Derbyshire, 2000). The reasons for this further subdivision were threefold. First, amygdala projections to the perigenual cortex also encroach onto the more anterior section of BA24' ($BA24^1$) and amygdala involvement in affect has been well documented (Davidson et al., 2000). Second, $BA24^1$ has a higher density of opioid receptors than $BA24^2$ (Vogt et al., 1995). And third, $BA24^1$ has been shown to activate in portion to the degree of experienced unpleasantness during noxious stimulation (Rainville et al., 1997).

II.3.1.2.1. ACG and the affective dimension of pain

It has been generally considered that the perigenual ACG response to noxious stimulation reflects an affective "suffering" component of pain. The implication of the perigenual region in pain affect is supported by several lesion and electrophysiological studies as well as by recent functional imaging experiments.

The earliest lesion studies implicating ACG in pain processing were conducted by Brown and Schaefer (1888) who reported decreased pain sensitivity after cingulate lesions in monkeys. Pivotal evidence of ACG involvement in pain affect derives from

neurosurgical studies. Cingulumotomy has proven effective in human patients suffering from chronic cancer pain (Foltz and White, 1962). After the procedure patients report that they continue to feel the pain, but that it is no longer preoccupying. This implies that the affective quality of pain was in some way ameliorated. Electrical stimulation of the perigenual region can evoke fear and other emotional responses in human subjects (Bancaud and Talairach, 1992). For example, stimulation of perigenual region BA24 resulted in the patient's response: "I was afraid and my heart started to beat." This response supports the idea that the perigenual region of the ACG is involved in affect but also points to a possible role in autonomic function. In human neurosurgical patients, Pool and Ransohoff (1949) reported that stimulation of the rostral portion of the ACG resulted in blood pressure elevations, changes in pulse rate and respiration. Electrical stimulation studies in primates, dogs and cats have also demonstrated profound autonomic changes in blood pressure, respiration and heart rate (Smith, 1945; Kaada, 1951).

Functional imaging studies have shown that the perigenual region is activated during induced sadness (George et al., 1995) or anxiety states in healthy subjects, during the depressed phase of major depressive disorder and also during alcohol-induced euphoria. Similarly perigenual ACG activates during a large number of pain studies (Vogt et al., 1996; Derbyshire et al., 1998). There are also recent reports implicating the midcingulate region in pain affect. Rainville et al., (1997) demonstrated that hypnotic suggestions designed to enhance the unpleasantness of a tonic heat stimulus correlated with increased blood flow to the midcingulate region (BA24') corresponding to BA24[1] described above (Derbyshire et al., 2000). Similarly, Toelle et al., (1999) found that pain

unpleasantness was positively correlated with increased rCBF in caudal regions of the ACG. Hence, it appears that affective responses to noxious stimuli are not only part of the perigenual region, but may be relatively distributed including rostral and also caudal regions of the ACG.

II.3.1.2.2. ACG and the cognitive dimension of pain

The cognition division (BA24' and 32') is usually not implicated in affect, but rather in tasks involving complex information processing such as performance monitoring (Carter et al., 1998), response selection (Turken et al., 1999), willed acts as well as pain and attention (Davis et al., 1997; Derbyshire et al., 1998; Devinsky et al., 1995). This is supported by connection studies demonstrating major reciprocal connections of BA24' with DLPFC (BA46) which itself has been implicated in a variety of executive functions. Also, recent functional imaging studies have provided direct support for the cognitive division's involvement in pain and attention processes (Peyron et al., 1999; Rainville et al., 1997).

Pain-evoked responses have not only been directly demonstrated in individual BA24 neurons of animals (Sikes and Vogt, 1992), but have recently been reported using electrophysiological recordings in humans (Hutchison et al., 1999). Direct recordings from patients undergoing bilateral cingulotomy for the treatment of obsessive-compulsive disorder and chronic depression showed that individual neurons in BA24' responded to noxious thermal and mechanical stimuli, but not to innocuous stimuli. Interestingly, BA24' neurons activated both to actual noxious stimulation and observed, but not experienced, noxious stimulation actually administered to the experimenter. This suggests that attention to the stimulating event plays a role in this activation.

The contribution of ACG to cognitive processes has been demonstrated in a large number of studies. For example, BA32' activity has been observed during the Stroop task (Pardo et al., 1990), verbal fluency tasks or maze tasks (Davis et al., 1997; Petrovic et al., 2000), all of which have a strong attentional component. Furthermore ACG activity has been observed in a comparison of rCBF during routine and willed acts. Routine acts involved repeating words or responding with the previously rehearsed opposite, whereas willed acts required an open ended response that entailed a deliberate choice. Willed acts were associated with increased blood flow in DLPFC and to a lesser extent in ACG. Recently Carter et al., (1998) showed that ACG activity increased during tasks involving response competition once again suggesting that choice amongst possible responses plays a role in this activation.

Both PET and fMRI have been used to demonstrate that the ACG responds to tasks requiring attentional focus and to tasks involving the delivery of noxious stimuli. Separate attention and pain related activation sites have been reported in studies comparing the Stroop task and noxious thermal stimulation (Derbyshire et al., 1998) as well as in a study comparing an attention demanding silent word generation task and painful electrical stimulation (Davis et al., 1997). In Davis et al.'s (1997) study, attentional activation encompassed superior and anterior aspects of the ACG whereas painful stimuli activated inferior and posterior sites.

II.3.1.3. The prefrontal cortex

In the context of this thesis the prefrontal cortex will be broadly divided into the medial prefrontal cortex (MPFC), which includes ventromedial and dorsomedial

prefrontal cortices (VMPFC and DMPFC) as well as dorsolateral prefrontal cortices (DLPFC). The VMPFC is often referred to as the orbitofrontal cortex, and the terminology will be used interchangeably. The ACG may directly interact with each of these regions during pain processing and has strong reciprocal connections with the MPFC and DLPFC (Pandya et al., 1981). It has been suggested that frontal cortical regions may modulate the cognitive appraisal of pain-related affective signals from the limbic system (Hsieh et al., 1997) and that the ACG may inhibit activity in prefrontal cortex during noxious stimulation (Devinsky et al., 1995).

II.3.1.3.1. The ventromedial prefrontal cortex (VMPFC)

The VMPFC (also referred to as the orbitofrontal cortex) is a cytoarchitecturally complex region that has not been well-defined in humans. Traditionally VMPFC cortex includes BA 11, 13, 14 and 12/47 (Roberts and Wallis, 1999). The innervation of the VMPFC is primarily with limbic or paralimbic structures that have been implicated in emotional processing such as the amygdala, hypothalamus, lateral orbital cortex and ACG (Barbas and Pandya, 1981; Carmichael and Price, 1995). In the primate autoradiographic techniques have demonstrated that tracer injections into BA32 of the ACG result in labelling of orbitofrontal regions BA 11-13, whereas tracer injections into BA24 resulted in clusters of labelling in fronto-orbital region BA12 (Pandya et al., 1981).

It is generally known that stimulation of the VMPFC can produce cardiovascular, respiratory and other visceral reactions, presumably through projections to the hypothalmus and brainstem (Price, 2000). Electrical stimulation of the VMPFC and DMPFC results in analgesia in both primates and humans (Oleson et al., 1980; Thorpe et

al., 1983). In rat, neurons in ventrolateral orbital cortex respond to noxious colorectal distention (Follett and Dirks, 1995). The ventrolateral orbital cortex receives afferent input from the nucleus medius, itself a pain-receptive area (Craig and Burton, 1981) and ventrolateral orbital cortex neurons respond to a variety of noxious stimuli (Snow et al., 1992).

The orbitofrontal cortex has been ascribed a role in the inhibitory control, as well as the emotional control of behaviour (Roberts and Wallis, 2000). Damage to the orbitofrontal cortex in primates and humans can cause inflexibility, impulsiveness and emotional disturbance. The textbook example of the role of orbitofrontal lesions on human behaviour is that of Phineas Gage (Macmillan, 2000). After a large iron rod pierced his orbitofrontal cortex in a railroad accident, Phineas's social and emotional behaviour changed dramatically. Although surviving the accident, his personality changed exhibiting signs of behavioural disinhibition including emotional outbursts and impulsive behaviour. Since then similar social and emotional difficulties have been reported in animal studies and human patients with damage to this area of the brain (Damasio., 1994). In his book "Descartes' Error" Damasio (1994) highlights the VMPFC as the "intersect" of emotion and reason. He presents several case studies in which damage to this region is consistently associated with impairments of reasoning/decision making and emotion/feeling. Based on these and other anatomical and lesion studies it is probable that the VMPFC has a role in the emotional aspects of responses to pain.

Pain imaging studies have shown increases and decreases in signal intensity in VMPFC. For example, Vogt et al (1996) found a decrease in rCBF in VMPFC and

DMPFC regions during noxious electrical stimulation. Similarly, Porro et al (1998) reported decreased BOLD signal in VMPFC regions after ascorbic acid injections into the sole of the foot. Increases in VMPFC rCBF were seen during distraction from a painful stimulus (Petrovic et al., 2000) and a negative correlation has been reported between pain intensity ratings and VMPFC activations. Less activation in VMPFC is associated with higher pain ratings (Derbyshire et al., 1997).

These data suggest that increased VMPFC activity is associated with decreases in pain perception and vice versa. The exact mechanisms mediating this relationship remain to be determined.

II.3.1.3.2. The dorsomedial prefrontal cortex (DMPFC)

The DMPFC has intrinsic connections with different ACG subregions and also sends projections to medio-dorsal orbital regions. Isotope injections into BA32 resulted in labelled grains in areas BA24 and 25, but also rostral non-cingulate DMPFC regions BA9 and 10. In monkey, nearly all of the projections to the PAG arise from medial areas such as medial BA9. This area along with BA24 projects mainly to the lateral column of the PAG, which has been implicated in mediating analgesia. Studies in the rat have shown that stimulation of medial prefrontal regions results in increased pain thresholds during noxious stimulation (Hardy, 1985).

Concomitant rCBF increases in ACG (BA 24' and 32') and decreases in cingulofrontal transition cortex (BA32) have been observed in response to phasic heat pain (Vogt et al., 1997) These results were corroborated by fMRI findings of positive and negative BOLD signal changes in the same regions during ascorbic acid injection into the

sole of the foot (Porro et al., 1998). Decreased activity in emotion-related areas may reflect a relative reduction in the processing resources devoted to emotional evaluation or experience in the control task relative to the experimental task (Drevets and Raichle, 1998). In addition, it is likely that subjects are adaptively inhibiting emotional responsiveness to painful stimuli in the experimental context. FMRI of rat brain during capsaicin injection into the foot resulted in a similar pattern of increased and decreased BOLD signal in ACG and MPFC, respectively (Malisza et al., 2001). Recently, using fMRI, Ploghaus et al., (1999) showed that among other regions, anticipated rather than actual pain activated a more anterior region extending from the perigenual cortex to the frontal pole corresponding to the DMPFC.

II.3.1.3.3. The dorsolateral prefrontal cortices (DLPFC)

In the primate, the DLPFC (BA46) has major bilateral reciprocal connections with BA24' (Pandya et al., 1981) whereas the MPFC receives projections from the perigenual cingulate. There are also extensive reciprocal connections between dorsolateral areas BA9 and 10 and the ACG (Pandya et al., 1989). The DLPFC has been implicated in a variety of executive functions including the supervision of attentional processes, working memory (Bunge et al., 2000) and willed acts. DLPFC activations during noxious stimulation are therefore likely to mediate part of the cognitive dimension of pain processing.

There is evidence in the functional imaging literature that suggests that cingulate and prefrontal cortices work in concert during acute noxious stimulation and chronic pain syndromes. These studies suggest that pain-evoked ACG activation may result in

decreased activity in prefrontal cortex, possibly reflecting an inhibitory modulation of the ACG on prefrontal brain regions. For example, application of noxious thermal stimuli to the back of the right hand in patients with atypical facial pain and rheumatoid arthritis resulted in increased rCBF to the ACG but a decrease of rCBF to the ipsilateral DLPFC (Derbyshire et al., 1994). Right DLPFC decreases in rCBF have also been observed in normal control subjects undergoing painful esophageal extention (Aziz et al., 1997). On the other hand, DLPFC activity was shown to increase in response to tonic cold pain (Casey et al., 1996) and ethanol injections (Hsieh et al., 1995). In normal volunteers the application of phasic thermal pain to the right hand results in consistent activation of the ACG, right DLPFC, anterior insular and inferior parietal cortices (Derbyshire et al., 1994; Jones et al., 1991). Since the DLPFC has been associated with strategic planning for higher motor functions, activation may be related to the planning of withdrawal or escape from noxious stimuli.

II.3.2. Summary

The medial pain system is a theoretical construct designed to better understand the functional anatomy of the affective dimension of the pain experience. It receives input from multiple ascending systems of the spinal cord such as the SRT and STT, for example, and includes the medial and intralaminar thalamic nuclei which in turn project to the ACG. The ACG has connections with prefrontal regions including MPFC and DLPFC.

The involvement of medial thalamic nuclei in the affective dimension of the pain experience is largely supported by projections to limbic cortical areas that subserve

emotional reactions to noxious stimuli including the ACG and insular cortices. Lesion and electrophysiological studies have demonstrated similar response properties of ACG and medial thalamic neurons as well as the discontinuation of nociceptor-evoked activity in ACG after lidocaine injections into midline thalamic nuclei. Both, medial and lateral thalamic activity have recently been observed during noxious thermal stimulation using fMRI (Davis et al., 1998, Tracey et al., 2000).

The ACG contributes to the cognitive and affective dimensions of the pain experience. In addition to a large body of research linking the ACG to cognitive processes, recent evidence suggests that the cognition division is implicated in processes involving pain and attention. There are also recent reports implicating the cognitive division in pain affect (Rainville et al., 1997). Generally it is the perigenual region of the ACG that is associated with pain affect. This is based on landmark neurosurgical and ACG stimulation studies as well as recent functional imaging research demonstrating perigenual activity during a variety of emotional states.

The ACG has strong reciprocal connections with the prefrontal cortex. Whereas damage to the VMPFC may result in the compromise of social and emotional behaviour, stimulation of the same region results in analgesia in both primates and humans. Moreover, VMPFC activations tend to decrease with higher pain ratings. Similar observations were made in DMPFC where stimulation increases pain thresholds during visceral stimulation. Concomitant increases and decreases in ACG and MPFC activity have been reported during painful stimulation and may reflect adaptive inhibition of emotional responses to pain or a relative shift of processing resources devoted to the emotional evaluation of the painful stimulus. The DLPFC likely mediates part of the

cognitive dimension of pain processing as it has been implicated in attentional processes, working memory and willed acts.

II.3.3 The lateral pain system

The lateral pain system includes the ventral posterolateral and ventral posteromedial thalamic nuclei, which are the principal relay nuclei for nociceptive-derived information from the spinal cord and trigeminal complex neurons to the somatosensory cortices one and two (S1 and S2). In this section background information on the lateral thalamic and somatosensory involvement in pain processing will be reviewed.

II.3.3.1. The lateral thalamus

In comparison to the medial thalamus, the lateral thalamus is somatotopically organized (Ingvar, 1999). It receives input from a somatotopically organized ascending tract and sends fibers to the primary and secondary somatosensory cortices where refined localization and discrimination of stimuli occur (Ingvar 1999). This is supported by labeling studies that have shown that specific regions within the lateral thalamus project to specific regions in somatosensory cortex (Gingold et al., 1991). For example, ventral posterolateral neurons project to S1. STT neurons project to ventral posterolateral neurons mainly from Rexed laminae I and V although laminae VI, VII and VIII termination sites are also present in smaller numbers. The response characteristics of neurons in the ventral posterolateral are very similar to those of the STT projection neurons. Therefore both wide dynamic range and nociception-specific neurons have been identified in ventral posterolateral thalamus, although the majority of nociceptive neurons are wide dynamic range (Apkarian and Shi, 1994). Lateral thalamic nociceptive neurons

generally have small receptive fields and are capable of encoding stimulus intensity (Bushnell et al., 1993; Bushnell and Duncan, 1987). The ascending projections of the thalamus and the response properties, as well as the restricted receptive fields of many neurons within the lateral thalamus suggest that this region is involved in the sensory-discriminative aspects of pain.

Electrophysiological studies (Kenshalo et al., 1980) in the monkey have shown maximal responses of ventral posterolateral neurons to noxious stimulation. These responses were polymodal and largely wide dynamic range although some nociception-specific responses were also observed. In rat, ventrobasal neurons respond to noxious levels of thermal stimulation applied to the tail by increasing the number of cells responding to the stimulation as well as increasing their firing frequency (Peschanski et al., 1981).

As mentioned above the spatial resolution of many imaging studies has been insufficient to discriminate the separate functional activations of neuronal regions within the thalamus. However, using PET, Derbyshire et al., (1997) show that there is a clear positive correlation between VPL and medial thalamic activations with pain intensity. Two fMRI studies have reported medial and lateral thalamic activity during noxious heat and cold pain (Davis et al., 1998; Tracey et al., 2000). Tracey et al. (2000) and Davis et al. (1998) found that the location of thermal pain sites are consistent with the involvement of both medial and lateral thalamic nuclei. Thus, these studies provide evidence for a complex integration from multiple sites.

II.3.3.2. The somatosensory cortex

Anatomical evidence shows that STT neurons project to lateral and medial nuclei of the thalamus, which in turn, project to S1 and S2. These pathways transmit information about the intensity, duration and location of the noxious stimulus from the spinal cord to the cortex (Vogt et al., 1993).

Historically it was believed that S1 and S2 did not play a significant role in pain processing. Head and Holmes (1911) observed that "pure cortical lesions of S1 and S2 caused no increase or decrease in sensibility to measured painful stimuli" (p. 154). Penfield and Boldrey (1937) observed that electrical stimulation of the human cortex only rarely elicited painful sensations. Thus, it was concluded that S1 only plays a small role in nociception. However, more recent anatomical and physiological studies suggest that the somatosensory cortices do participate in the spatial discrimination of noxious stimuli and in the further elaboration of the sensory experience (Ingvar, 1999).

Mountcastle and Powel (1959) were the first to report that neurons in cytoarchitectonic area 3b of S1 in the primate respond to noxious stimulation. The number of nociceptive neurons in S1 of the primate is small but have response characteristics that parallel those observed in humans when experiencing pain. Ablation of S1 in the primate has been shown to impair discrimination among noxious thermal stimuli without altering the detection of these stimuli (Kenshalo et al., 1991).

Electrophysiological studies in rats and primates have shown that S1 contains neurons that are involved in the localization and intensity coding of noxious stimuli. Although they are found less frequently than neurons responding to tactile stimulation, their activity correlated with the duration and intensity of the noxious stimulus (Chudler

et al., 1990; Kenshalo et al., 1988; Lamour et al., 1983). The majority of neurons in these studies were of the wide dynamic range type and outnumbered nociceptive-specific neurons by a ratio of 100 to 4. Wide dynamic range and nociceptive-specific neurons have been identified in the rat, cat and monkey and have similar receptive field properties to those of nociceptive neurons in the ventral thalamus.

The functional imaging evidence on S1 involvement in pain processing in humans is inconsistent. The first functional imaging evidence showing that the cerebral cortex participates in pain perception was published in the early 1990s (Apkarian et al., 1992; Jones et al., 1991; Talbot et al., 1991). Applying repeated 5 second heat pain stimuli to different regions of the forearm, Talbot et al (1991) showed that rCBF increased in the contralateral S1. In contrast, Jones et al (1991) showed that the application of heat pain to the same area of the dorsal surface of the hand did not produce any significant changes in S1 activity. According to Jones et al., (1991) the differential activation observed in Talbot et al.'s (1991) study may be due to their use of a moving noxious stimulus on the forearm. This type of stimulus may contain a larger attentional component than stimulating the same area on the dorsal surface of the hand. Apkarian (1992), using a tonic three-minute hand submersion into hot water showed a decrease in rCBF to S1. On the contrary, Rainville et al., (1997), using the same stimulus, showed increased rCBF in contralateral S1. Hypnotic suggestion directed at increasing or decreasing the perceived intensity of the burning pain sensation modulated pain-related activity in S1. Hypnotic suggestion aimed at changing the perceived unpleasantness of the stimulus had no effect on S1 activity, but resulted in a modulation of activity in the ACG, which was directly correlated with the subjects' perception of unpleasantness. More recently, using a

different tonic pain stimulus consisting of ascorbic acid injection into the sole of the foot, Porro et al, (1997) demonstrated an increased BOLD response in the contralateral S1 foot area. Petrovic et al. (2000) published a study showing that tonic cold pressor stimulation resulted in robust activation of S1 and S2. In this study, distraction from cold pressor pain resulted in a modulation of rCBF flow in the orbitofrontal, periacqueductal grey matter and somatosensory association areas. Modulation of S1 activity in the somatotopic area for the hand in S1 was below the level of significance. Peyron et al., (1999), using fixed heat thermode stimulation showed no activation of S1, but a robust decrease in rCBF in the S1 region ipsilateral to the stimulated hand. The size and significance of decreased rCBF in the ipsilateral S1 area increased with the level of attention to pain and was assumed to reflect anticipatory processes.

II.3.4. Summary

Sensory discrimination and localization of noxious stimuli are managed by the lateral pain system. The lateral pain system receives input from the STT, the DCP, the SMT, as well as the SCT. These tracts carry visceral and somatosensory information and project to the lateral thalamus. The lateral thalamus projects to the somatosensory cortices. The lateral thalamic involvement in the localization of noxious stimuli is supported by its somatotopic organization showing that specific regions within the lateral thalamus project to specific regions in somatosensory cortex.

Somatosensory cortical neurons respond to noxious stimulation, but their numbers are small. The activity of these neurons correlates with the duration and intensity of the noxious stimulus. Functional imaging evidence has shown no changes, increases and

decreases in somatosensory activity due to noxious stimulation. These inconsistencies may be due to factors, such as different stimuli, acquisition methods, data analysis tools and experimental design.

III. Methodology: Review of Magnetic Resonance Physics and Blood Oxygen Level Dependent functional Magnetic Resonance Imaging

The objective of this section is to introduce the methodology used in this thesis. Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) was used to investigate the cerebral representation of visceral and somatosensory pain. In the following section the concepts of magnetism, precession, resonance, and relaxation will be introduced as building blocks to explain the BOLD fMRI contrast mechanism. We will elaborate on the reasons for choosing BOLD fMRI relative to other imaging techniques and discuss the advantages and disadvantages of currently used imaging methods.

III.1. MR physics

In 1952 Felix Bloch and Edward Purcell received the Nobel Price for discovering that the application of low-energy radio frequency pulses within a strong magnetic field caused atoms to give off unique radio frequency signals yielding characteristic information about the molecules' chemical make up (Bloch, 1946, Purcell, 1946). The possibility to localize these resonating nuclei in space was demonstrated by Paul Lauterbur in 1973. His work led to the first reconstruction of a proton spin density map using nuclear magnetic resonance. Since the first human MR scan, which was completed

in five hours (Damadian,1977), the field of MR imaging has advanced rapidly and today echo planar images (EPI) can be acquired in less than a second (Mansfield, 1977).

III.1.1. Magnetism and precession

The interaction between the magnetic field (B_0) and NMR nuclei is the basis for MRI. To be considered NMR active, nuclei must have either an odd number of protons, neutrons or both (Krause et al., 2000). The interest in the hydrogen nucleus in NMR is based on its abundance in the human body as well as its large magnetic moment. The magnetic moment allows the hydrogen nucleus to align its axis with an externally applied magnetic field.

The law of electromagnetism states that a charged particle in motion will create a magnetic field. Therefore, a positively charged proton will have a magnetic field. The hydrogen nucleus contains one proton, no neutrons and possesses a positive charge. In the presence of an external magnetic field, nuclear magnetic moments in this situation may adopt one of two possible orientations; alignment parallel or anti-parallel with B_0 (the main magnetic field). Anti-parallel alignment requires more energy and there is a slight excess of nuclei aligned parallel with B_0 (a few in a million, this is dependent on the B_0 field strength.). It is this small net magnetization arising from the difference in the ratio of parallel versus anti-parallel spins, which is detected by MR techniques.

In 1897 the Irish physicist Larmor showed that the application of any aligning force (torque) to a spinning object causes a circular motion (Meadows, 1999) known as precession. We may therefore expect a spinning magnetic moment (such as the hydrogen nucleus) subject to an externally applied magnetic field, to precess, and indeed it does. When in an external magnetic field, the proton does not only spin on its own axis, but

also moves/precesses around the axis of B_0. This is often likened to a spinning top. The path of individual protons is referred to as the precessional path. In order to detect an NMR signal from the random precessional paths a radiofrequency pulse is applied at the resonating frequency of the hydrogen protons.

III.1.2. Resonance

Resonance occurs when an object is exposed to an oscillating perturbation that has a frequency close to its own natural frequency of oscillation. In NMR resonance occurs through the application of a radiofrequency pulse at the resonating frequency. This frequency is determined by the Larmor Equation. In an external magnetic field, such as a 1.5 Tesla magnet, for example, the spinning nucleus precesses at a frequency determined by the Larmor Equation, where:

The precessional frequency $\varpi_0 = B_0 \times \gamma$

B_0 = the magnetic field strength of the MRI (1.5T or 3T, for example)

γ = the gyromagnetic ratio

The strength of a magnetic field is measured in Gauss (G) or Tesla (T) (10000 G = 1 T). The gyromagnetic ratio of hydrogen = 42.57 MHz, thus in a 1.5T magnet, a pulse of radio frequency energy applied at 63.86 MHz (42.57 x 1.5) will cause the hydrogen proton to resonate. The radio frequency pulse has an associated magnetic field known as B_1 . B_1 is perpendicular to B_0, the main magnetic field. The application of the RF pulse results in the alignment of hydrogen protons with B_1 and causes them to precess "in phase" for the duration of the pulse.

III.1.3. Relaxation times

When the RF pulse is removed, the hydrogen protons relax in a process called *relaxation*. During *relaxation* the net magnetization vector gives up absorbed RF energy and returns to B_0. Relaxation results in recovery of magnetization in the longitudinal plane (T1 relaxation/recovery) and decay of magnetization in the transverse plane (T2 relaxation/decay). T1 recovery is also referred to as spin lattice relaxation. This description is based on nuclei giving up their energy to the surrounding environment or lattice, the release of which causes recovery of magnetization in the longitudinal plane. T2 decay is caused by nuclei exchanging energy with neighboring nuclei, this is also often referred to as spin spin relaxation. Spin spin relaxation results in the decay of transverse magnetization. Differences in T1 and T2 relaxation times in different tissues are the source of image contrast in MR. Thus fat has a short T1 and T2 time whereas water has a long T1 and T2 time. T1 weighted images in brain are therefore characterized by bright fat (white matter) and dark water (grey matter). T2 weighted images are characterized by bright water (grey matter) and dark fat (white matter). Finally, $T2^*$decay, which is very sensitive to the BOLD effect, is faster than T2 decay since it is a combination of T2 decay and dephasing due to magnetic field inhomogeneities.

III.1.4. The blood oxygen level dependent functional magnetic resonance signal

It is well established that neuronal activation is normally associated with increases in glucose and oxygen consumption via the so-called neuro-vascular coupling of cerebral blood flow (CBF) and oxygenation. These localized changes in blood flow and metabolism are the basis of neuroimaging techniques such as Positron Emission Tomography (PET) and (Single Photon Emission Chromatography) SPECT. These

techniques require the use of exogenous contrast agents and have been superseded in this regard by the BOLD fMRI method that allows mapping of hemodynamic changes in the brain without invasive contrast agents. The feasibility of using the MRI technique based on BOLD contrast for indirectly mapping neuronal activation was first demonstrated in the human brain during a simple visual perception task (Kwong et al., 1992; Ogawa et al., 1992) and a motor task (Bandettini et al., 1992).

The BOLD imaging technique takes advantage of the fact that the oxygenation state of hemoglobin affects its magnetic properties. Pauling and Coryell (1936) were the first to note that the magnetic susceptibility of hemoglobin and deoxyhemoglobin differed slightly and in 1990 Ogawa et al. demonstrated that MRI could be used to detect these small changes. Thus, the BOLD technique does not measure tissue perfusion or flow, but the MR signal on T2 and $T2^*$ - weighted images is influenced by the oxygenation state of the blood. The presence of paramagnetic deoxyhemoglobin modulates the rate of loss of proton spin phase coherence and results in susceptibility-induced T2* signal loss. On the other hand, an increase in oxygenated arterially delivered blood in response to local neuronal activation will result in more oxygenated iron in the capillary and venuous beds thereby creating a relatively longer T2* and thus image intensity increase. It is therefore the localized increase in oxygenated blood that accompanies synaptic activity that increases spin coherence and results in a signal intensity increase relative to regions with less oxyhemoglobin.

For example, visual stimulation produces rapid neuronal activation in occipital cortex. This activation in turn increases CBF, cerebral blood volume (CBV) and oxygen delivery. CBF increases more than CBV and oxygen delivery exceeds local oxygen

demand resulting in a larger ratio of oxygenated to deoxygenated hemoglobin in the capillary and venuous beds. As the relative proportion of deoxyhemoglobin to oxyhemoglobin decreases so does paramagnetically induced dephasing. Thus, the decrease in this relative proportion will result in a longer $T2^*$ and therefore increased signal on the $T2^*$ weighted images. This signal intensity increase is referred to as "activation" and used as an indirect reflection of neuronal activity/excitability.

Other interesting BOLD phenomena that have received increasing attention in recent years are related to decreases in BOLD signal intensity. Although frequently observed these decreased signal intensity changes are more difficult to interpret. The two more commonly accepted BOLD signal intensity decreases include the initial dip and the post-stimulus undershoot. The third is a paradigmatic decrease of BOLD signal below resting level that is negatively correlated with the activation paradigm.

Using EPI, the initial dip was first demonstrated in a study using photic stimulation (Menon et al., 1995). This negative response reached a maximum at 2 sec after stimulus onset and had a peak amplitude of 1%. These results were in good agreement with optical imaging data in which a deoxygenation phase has been shown to occur in specific cortical columns (Malonek and Grinvald, 1996). Since then this response has been documented in a large number of fMRI studies (Yacoub et al., 2001). The initial dip is explained as reflecting the initial activity of neurons which draw oxygen out of the capillary network, resulting in a local increase in paramagnetic deoxyhemoglobin and hence a decrease in MR signal intensity.

The post-stimulus undershoot was initially noted in one of the first human fMRI studies (Kwong et al., 1992). The authors described that after stimulus-induced

activation the BOLD signal dipped below baseline levels and remained depressed for a considerable amount of time (seconds to up to a minute). Although not always evident, numerous examples can be found in the fMRI literature (Buxton et al., 1999). The origin of this undershoot is not well understood, however, it is thought to reflect a complex interaction between CBF, CBV and CMRO2. The two leading theories of the source of the undershoot are, that either CMRO2 or CBV remain elevated after CBF has returned to resting levels.

The least understood of all BOLD signal intensity decreases is the paradigmatic decrease of BOLD signal, which is commonly known as a "deactivation". Throughout this thesis we will use the term deactivation to refer to BOLD signal that drops below baseline at the onset of the stimulation condition and *remains* depressed until the end of the stimulation condition. These decreases are indicative of an increased ratio of deoxyhemoglobin to oxyhemoglobin at the onset of stimulation condition and a return of oxyhemoglobin levels towards the end of stimulation. There are several hypotheses attempting to explain this decreased signal. Firstly, it is possible that blood is shunted from an area of the brain that is not required for the task when another active brain region requires more blood. Shunting would be most probable when activations and PDBs are detected in close proximity. Secondly, it has been suggested that BOLD signal decreases could be the result of a decrease of neuronal firing rate significant enough to affect blood flow or glucose utilization (Hutchinson et al., 1999). And thirdly, it was postulated that BOLD signal decreases could be the result of neuronal disinhibition (Rauch et al., 1997).

In summary, BOLD fMRI is a valuable tool in neuroscience that provides the opportunity to study human brain function without the use of invasive contrast agents.

Due to the inherent magnetic properties of blood, fMRI is able to detect changes in cerebral hemodynamics indicative of both active and inactive brain regions. Whereas BOLD activations are widely accepted in the neuroscientific community, the source of decreases in BOLD signal intensity remain to be clarified.

III.1.5. Reasons for choosing BOLD fMRI

This section briefly reviews some of the advantages and disadvantages of using fMRI versus Positron Emission Tomography (PET), Near Infrared Spectroscopy (NIRS) or Electroencephalography (EEG) and Magnetoencephalography (MEG). Activation of neuronal networks can be detected by the direct measurements of electrical activity with electroencephalography (EEG), measurements of magnetic fields with magneto-encephalography (MEG) and indirectly by measurement of changes in blood dynamics (PET, NIRS and fMRI).

PET has been the gold standard of functional brain imaging and has been extensively used since the early 1970s. PET data is often used to cross-validate fMRI results and the data are comparable. SPECT imaging is similar to PET, however, the spatial and temporal resolutions are poorer and the availability of tracers limited. There are several advantages using BOLD fMRI relative to PET. First, contrary to PET which uses labelled isotopes as contrast agents, fMRI does not require the use of radioactive isotopes to generate a signal. The fMRI signal is obtained from endogenous BOLD contrast that relies on the deoxyhemoglobin/oxyhemoglobin ratio. Secondly, as no exogenous contrast agents are used in fMRI, stimulus presentation can be repeated more rapidly and repeatedly. There is no risk of overexposure to radioactive isotopes in fMRI. Thirdly,

the temporal and spatial resolution of fMRI is superior to the temporal and spatial resolution of PET. PET scanners have reached a high level of spatial and temporal resolution in the order of 5 mm and 40 s, respectively. EPI images can be acquired in less than a second with a spatial resolution in the 1 mm range. On the other hand, an exciting advantage of PET is, that using appropriate tracers allows not only the measurement of regional cerebral blood flow (rCBF) and metabolic rate (rCMR), but also the identification of receptor densities and sub-nanomolar neurotransmitter levels. These measurements are currently the exclusive domain of PET.

NIRS is a noninvasive optical method that allows *in vivo* measurement of relative concentrations of different substances. Its relevance to functional imaging is that it is able to differentiate oxygenated and deoxygenated hemoglobin by using their different light absorption spectra. Relative to fMRI the spatial resolution of NIRS is poor and has, at best, a penetration depth of 7 mm not allowing measurements of activity in deeper cortical or subcortical brain regions. The main advantages of this technique are its low cost and ease of application.

The main advantage of electrophysiological methods such as EEG and MEG is their excellent temporal resolution. Neuroimaging methods that rely on measurements of the hemodynamic response are limited by the time scale of vascular events. This, of course, limits the temporal resolution of functional neuroimaging studies. Whereas neuronal activity occurs on a time scale of milliseconds, the metabolic and vascular events occur on a time scale of seconds. Since EEG and MEG directly measure the electrical source currents in the brain, these methods can monitor real-time activation patterns of the cerebral cortex. The main disadvantage of electrophysiological techniques relates to the

fact that only weighted averages of electrical brain activity can be recorded. These are difficult to define in terms of spatial extent and precise location of an activated brain region.

Various brain imaging techniques have been developed to study the function of the human brain *in vivo*. Each of these techniques has inherent limitations. In the past decade, imaging techniques have been combined to improve the accuracy and reliability of brain imaging data. Although fMRI has many advantages relative to other imaging techniques, the combination of fMRI with EEG, MEG, PET or NIRS will provide a more complete picture of human brain physiology than any technique on its own.

IV. Specific Objectives and Strategies

The objective of this thesis is to use Blood Oxygen Level Dependent functional Magnetic Resonance Imaging (BOLD fMRI) to better understand the role of ACG and MPFC in the processing visceral and somatosensory pain.

It is well known that the ACG and MPFC are involved in somatosensory pain processing. The relative lack of information on sub-regional functions, as well as the paucity of information on ACG and MPFC activity during visceral and chronic pain raises several fundamental questions about the role of these regions in pain processing. Three studies were conducted to address some of these issues.

IV.1. Specific objective 1

The paper (Bernstein et al., 2002) in part I of this thesis examines visceral pain. The impetus for this work was based on the lack of information in regards to the

following issues. First, although visceral pain is a common form of pain produced by disease and one of the most frequent reasons why patients seek medical attention (Cervero 1999), much of what we know about the mechanisms of pain derives from experimental studies of somatosensory pain. Second, at the onset of this work there were no known reports on the cortical representation of enteric visceral pain. Third, as the ACG and prefrontal cortex have been identified as key structures in only somatosensory pain (Porro et al., 1998; Vogt et al. 1996), we were interested in documenting their activity during visceral pain. Fourth, visceral pain in IBS patients appears to be psychogenically maintained (Blomhoff et al., 2000), whereas IBD has a definite inflammatory component. With the exception of our paper (Bernstein et al., 2002) there are no known reports on the differential cortical representation of these two gastrointestinal diseases.

In this context the question in the first paper was: does cortical activity due to noxious visceral stimulation differ between control subjects and patients suffering from chronic visceral pain? As there were no experiments comparing gastrointestinal pain in control subjects and patients with gastrointestinal disorders, the first part of this thesis was designed to examine cortical responses to visceral sensation and pain in control subjects and in patients suffering from Inflammatory Bowel Disease (IBD) and Irritable Bowel Syndrome (IBS) by means of BOLD fMRI. Particular interest was directed at comparing BOLD responses in ACG and PFC of clinical and control groups. We hypothesized that, relative to control subjects, patients with gastrointestinal disorders show aberrant cortical activation patterns.

Functional data were acquired using Fast Low Angle Shot (FLASH) on a 3 T whole body system. The data were analyzed using parametric and nonparametric methods contained within Stimulate and EvIdent software packages, respectively. Pixel counts were compared for different regions of interest as well as clinical and control groups.

IV.2. Specific objective 2

At the onset of this thesis, the ACG and MPFC have been implicated as key structures in processing painful somatosensory stimulation. Since then, the cortical representation of visceral pain has become the focus of several PET investigations (Aziz et al., 1997; Silvermann et al., 1997). Although separate studies using either visceral or somatosensory stimulation have reported activations and deactivations within cortical regions (Aziz et al., 1997; Michelic et al., 1999; Porro et al., 1998, Vogt et al., 1996), there are no direct comparisons of the cerebral representation of visceral and somatosensory pain within the same subjects. As little information exists about the differences and/or similarities of cerebral processes involved in these different pain modalities our objective was to use a single set of subjects, measure subjective pain ratings and directly compare the cerebral representation of these two pain modalities. Comparisons across studies are difficult as subjects, acquisition methods, analysis tools, type of stimulation and pain ratings differ from study to study.

Again, we focussed on the ACG since it is one of the most frequently reported active brain regions during any type of pain study (Ingvar, 1999). The ACG sends projections to the MPFC and has been implicated in the affective and cognitive dimensions of the pain experience, which are common to both, visceral and

somatosensory pain. Based on these reasons as well as the paucity of studies conducting direct comparisons of visceral and somatosensory pain, we sought to focus on these structures.

Experiments were designed to compare the patterns of ACG and MPFC activation arising during visceral and somatosensory pain. These experiments were also designed to establish the consequences of the lateralized presentation of the somatosensory stimuli and the midline stimulation inherent to visceral pain. Studies were conducted on a 1.5 T whole MR body system. Pain ratings were obtained for both, visceral and somatosensory pain. Functional images were acquired using echo planar imaging, analyzed with Stimulate (Strupp) and normalized pixel counts were entered into a repeated measures Analysis of Variance (ANOVA).

IV.3. Specific objective 3

The paper in third part of this thesis is based on the simple observation that focusing one's attention on pain usually results in an increase in its perceived intensity. Accordingly, distraction from pain often reduces the perceived intensity of pain. As pain includes sensory-discriminative, affective-motivational and cognitive-evaluative dimensions, we believed that the cognitive-evaluative dimension interacts with and modulates the pain experience by the amount of attention directed towards the painful stimulus. Therefore, our basic objective was to determine whether cognitive distraction reduces the perception of pain and investigate whether this behaviour pattern is reflected in brain physiology.

Although it was known that pain and attention tasks activate separate sub-regions of the ACG, (Davis et al., 1997; Derbyshire et al., 1998) it was not known whether there was a relationship between these different sub-regions during pain processing. In view of these points, selection of the ACG as a region of interest was motivated by a large body of evidence implicating the ACG in the processing of painful stimuli as well as by reports of separate ACG sub-regional involvement during pain and attention tasks (Davis et al., 1997; Derbyshire et al., 1998). To address the possibility of sub-regional ACG modulation, we initially sought to replicate these previous reports of separate pain and attention activations in ACG. We then hypothesized that distraction from pain would result in decreased activation of ACG sub-regions responsive to painful stimulation and increased activation in ACG sub-regions responsive to the attention/distraction task. To meet this goal separate pain, attention and distraction tasks were designed and acquired using Echo Planar Imaging on a 1.5 T whole body MR system. Individual pain ratings were obtained and functional imaging data was analyzed using Statistical Parametric Mapping 1999 (SPM 99).

Part I

Cortical mapping of visceral pain in patients with gastrointestinal disorders using functional magnetic resonance imaging

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Abstract

We sought to identify central loci that activate in response to noxious visceral stimuli (stool and pain). We had a particular interest in observing the anterior cingulate gyrus (ACG) and frontal cortex in normals and in patients with intestinal disease, including inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Subjects underwent rectal balloon distention to a sensation of stool and to a sensation of pain while undergoing BOLD fMRI. Experiments were conducted in a Magnex 3.0 Tesla whole body magnet with a Bruker Biospec console, and a quadrature head coil. Four contiguous 5.0 mm oblique axial slices designed to optimize coverage of areas believed to be responsive to noxious stimulation were acquired. Activations were detected by using cross correlation maps ($p<0.001$) for individual subjects. The experimental groups were compared using both an analysis of variance and profile analysis. A significantly higher percentage of pixels activated in the anterior cingulate gyrus over both pain and stool conditions for the control group than for the IBS group and for the IBS group than for the IBD group ($p<0.035$). Deactivation of left somatosensory cortex was greater for the IBS group than for the IBD group and greater for the IBD group than for the controls ($p<0.0065$) in the boxcar condition. Frontal deactivation in controls compared to disease groups bordered on statistical significance ($p<0.08$). Profile analysis of the three groups across six regions of interest revealed that the control and IBD groups were distinguished by different profiles of response ($p<0.005$). Non-parametric evaluation of the data suggest that, amongst the pixels in the anterior cingulate activating to pain, there are two patterns of response to pain – on/off and graded. This was true for both controls and disease groups. Normal controls and subjects with IBD and IBS share similar loci of

activations to visceral sensations of stool and pain. Both activation and deactivation of particular regions of interest differentiate the three groups, as do profiles of patterned response across six of the regions of interest for the control and IBD groups.

Introduction

The role of brain function in visceral pain transmission remains poorly understood even though links between the nervous and digestive system are well known (Mayer et al., 1995). Networks activating in response to visceral pain involve cortical structures receiving input both from cognitive and emotive centers, and from afferent transmission originating in the gut. Although there is an evolving body of work exploring cortical processing of noxious visceral (Aziz et al., 1997; Mertz et al., 2000; Silvermann et al., 1997;) there are no published data that directly contrast cortical responses to visceral stimulation in the two dominant bowel disorders, inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

Evidence of altered sensory processing in IBD and IBS patients was shown previously (Bernstein et al., 1996; Chang et al., 2000). Rectal balloon distention in patients with ileal Crohn's disease and in patients with IBS revealed altered referral patterns of the sensation of discomfort compared with normal controls. This suggests that both conditions are associated with altered processing of visceral sensory afferent information (Bernstein et al., 1996). Crohn's disease patients however, were hypoalgesic to ramp rectal balloon distention whereas IBS patients were hyperalgesic to phasic rectal balloon distension (Bernstein et al., 1996). Hence, although the manifestation of abdominal cramps or pain may be described similarly by patients with IBS or IBD, or even by some normal controls, it is possible that the neural processing of painful stimuli may be distinct among different patient or control groups, including at a cortical level.

Other evidence suggests that the neural processing of noxious stimuli may be different between patients with IBD and IBS. For example, differential autonomic

nervous system functioning has been observed in these disease groups (Jorgensen et al., 1993). Patients with IBD have also been shown to have increased somatosensory thresholds for noxious stimuli (Lindgren et al., 1993). The groups are differentiated psychologically as well. A greater placebo effect is observed in IBS patients compared to IBD patients (Ilnyckyi et al., 1997; Klein, 1988). Furthermore, stressful life events have been more readily associated with exacerbation of symptoms in IBS (Ford et al., 1987) and behavioral strategies can be beneficial therapeutically (Guthrie et al., 1991). Our current studies investigate the possibility of using functional magnetic resonance imaging (fMRI) to characterize the brain centers activating to noxious visceral stimulation. In addition, we aimed to determine whether the patterns activation of cortical structures in IBD, IBS, and in normal controls differentiate the groups.

Functional imaging has been used in a number of studies to identify the structures activating in response to painful stimulation (Tank et al., 1992; Roland, 1992; Jezzard et al., 1995). The majority of these studies employ a modality of somatosensory pain. Results implicate a variety of structures including anterior cingulate gyrus (ACG), prefrontal cortex, thalamus, somatosensory cortex and periaqueductal grey matter (Roland, 1992). Evidence that the ACG is involved in pain processing is extensive (Devinsky et al., 1995). Surgical cingulectomy has shown the potential for using cingulate lesions for the relief of pain (Brown and Schaefer, 1888; Vaccarino and Melzack, 1989; Pillay and Hassensbusch, 1992). Still, uncertainty regarding the central representation of pain, particularly of visceral pain, remains (Roland, 1992).

In a PET study of esophageal sensations (Aziz et al., 1997) the authors showed that the ACG, among other structures, activated in response to pain. This study also

revealed multiple areas of decreased blood flow. Another PET study investigated the functional neuroanatomy of visceral pain in IBS patients versus normal subjects (Silvermann et al., 1997). The authors asserted that the ACG was activated by visceral pain in normal subjects but not in patients with IBS, and that the left dorsolateral prefrontal cortex was stimulated in IBS during the anticipation of pain, but not in normal subjects. In the first published work using fMRI, IBS subjects were reported to have greater activation at the ACG to painful rectal stimulation than normal controls (Mertz et al., 2000). Thus, further exploration of cortical activation is necessary to help reconcile these contradictory findings in IBS. Furthermore, adding a second comparative group other than normal controls, that of IBD subjects, may allow for a more in depth understanding of the implications of cortical activation patterns in IBS.

Our study utilizes blood oxygenation level-dependent (BOLD) fMRI at high field (3.0 Tesla) during rectal balloon distention. BOLD fMRI is non-invasive, and has greater sensitivity, and spatial resolving power than PET. BOLD fMRI possesses the ability to acquire meaningful data on individual subjects such that the range of individual differences can be determined. The added sensitivity of imaging at 3.0 Tesla results in an increase in contrast to noise as compared to standard 1.5 Tesla systems. It also improves the tissue specificity of activations so that it is more likely that they derive from parenchyma than from larger vessels. The added contrast to noise allows for spatial resolution superior to that of standard fMRI images.

We hypothesize that different patterns of brain response will be found to noxious and painful stimuli in controls, IBS patients, and in IBD patients. Defining overlapping and pathognomonic areas of brain activation in IBD and IBS will enhance our

understanding of central pain processing in these conditions. Furthermore, it is possible that identifying loci of differential central pain processing may even serve as a diagnostic test for patients with known diagnoses of IBD who have complex abdominal pain out of proportion to their inflammatory changes.

Materials and Methods

Subjects

Twenty volunteers were recruited who either had no gastrointestinal complaints (n=6), had IBD (n=6, 4 Crohn's disease and 2 ulcerative colitis patients) or pain predominant IBS (n=6). Two subjects were excluded due to noticeable head motion during the imaging sessions. IBD patients participated in the study at a time of symptomatic remission and were considered as a single group in this first probative experiment because of the small sample size. Crohn's disease and ulcerative colitis are both inflammatory disorders and together form an important and useful comparison group to IBS patients. The range of clinical disease activity scores was 0-1 using the Harvey-Bradshaw score for Crohn's disease and the Powell Tuck score for ulcerative colitis. All studies were performed at the National Research Council's Institute for Biodiagnostics (Winnipeg, Canada). Ethics approval for the study was obtained from both the University of Manitoba Ethics Committee for Human Studies as well as the National Research Council's Ethics Committee. Subjects gave informed consent and were free of exclusion criteria for MRI (Shellock and Kanal, 1996). All six of the control subjects were males. For both the IBS and IBD groups two of the subjects were male and four females. All subjects were right-handed. The mean age for the control

group was 33.8 ± 9.4 years; for the IBS group mean age was 40.2 ± 6.6 years; and, for the IBD group mean age was 38.2 ±7.4 years (NS).

Protocol

A Fleet™ enema was administered to all subjects. After rectal emptying, a latex balloon (MAK-LA Company, Thousand Oaks, CA) was placed in the rectum and the tubing extending from the balloon was secured with tape to the buttock. The tubing was connected to a 60 ml syringe. One investigator (CNB) conducted all distention experiments with manual balloon inflation. A test protocol was conducted in the preparation room. The balloon was inflated until the patients sensed an urge for a bowel movement (stool), held inflated for 90 seconds and then further inflated until the patients sensed pain and held inflated for 90 seconds. For the rest condition the balloon was deflated. This paradigm was repeated again to ensure the patient understood the paradigm as well as to allay anxiety as to what to expect from the distention protocol. Manual ramp inflation was used to allow for imaging immediately after the subject signaled sensations of stool or pain, as opposed to computer-driven phasic distentions that rely on subject recall of sensations post imaging (Silvermann et al., 1997; Mertz et al., 2000).

Imaging

Experiments were conducted in a Magnex 3.0 Tesla whole body magnet with a Bruker Biospec console, and a quadrature head coil. Imaging was done with subjects supine. Subjects were imaged supine and their heads were immobilized with a vacuum

bag. One investigator remained at the foot of the magnet to conduct the balloon inflation experiments. Subjects were instructed to neither move nor speak during the experiment but to hand signal when the sensations of stool and pain were perceived. Seven images of each condition were obtained in the following sequence: 1) rest, 2) stool, 3) pain, and 4) rest. There was a pause between conditions and the first image in every condition was dropped so longitudinal magnetization could reach equilibrium. Thus, each block started from the same magnetic baseline. A T1-weighted scout image was acquired just off the midline in the left hemisphere. It was used to define four contiguous 5.0 mm oblique axial slices designed to optimize coverage of the anterior cingulate gyrus (ACG) and frontal cortex. An example of the slice positions used is shown in Figure 1. Four T1-weighted anatomic scout images were obtained with an inversion-recovery prepared turbo-FLASH (TR/TE =14/7ms, (α=20°, 256 x 256 matrix, 25cm FOV) in four interleaved steps with an inversion time of 0.9 seconds. T2*-weighted images for functional imaging were acquired using a flow compensated FLASH sequence (TR/TE =56/40 ms, α=15°, 256x128 matrix, 25 cm FOV). Both the long echo time and low flip angle were used to ensure that the obtained T2* weighted functional images emphasize signal from parenchyma rather than vessels. Following acquisition the images were processed to remove respiratory and cardiac artifacts.

Data Analysis

Data analysis was divided into two parts. The first part identified those pixels that bore a statistically significant relationship to the stimulation paradigm. Images were analyzed using the Stimulate image analysis program (Strupp, 1996). The goal of this

phase of image analysis was to select pixels demonstrating a statistical relationship with the paradigm conservatively. Three correlations were run that tested for pixels activating to stool alone (stool specific), pain alone (pain specific), or to both pain and stool (boxcar). The first image in every condition was excluded to allow longitudinal magnetization to reach equilibrium. Pixels were selected for further processing if their time-courses correlated with one of the reference time courses ($p<0.001$). Pixels that satisfied this criterion were processed further in two ways. The first was to correct for the large number of independent comparisons. False positives that occur by chance were reduced by using a criterion of anatomical connectedness that is used as a more anatomically motivated form of Bonferroni correction (Xiong et al., 1995). Pixels were passed only if they were connected to five other active pixels. In addition, pixels satisfying both of these criteria were filtered so only pixels changing signal intensity between 1 and 5 per cent were used. Change of this magnitude is consistent with that expected from parenchyma at 3.0 Tesla. The exclusion of larger levels of change increases the likelihood that the activations reported are from tissue rather than vessels. This, of course, improves the spatial specificity of the results. These results were used to identify the number of pixels activating in the various regions of interest for all of the subjects individually. These results formed the basis of the group analysis.

The pixel counts for each of the regions of interest reflect both specific responses to stimulation and factors that appear to vary from subject to subject which influence the total number of active pixels. Pixel count data was normalized by expressing the data for each ROI as a percentage of the total number of active pixels. Thus, for example, the meaning of 20 active pixels in a ROI is different for a subject with a total of 80 active

pixels than for a subject with 200. The percentage data for each subject was calculated as was the average percentages for each group. These were used to examine how well fMRI could differentiate the three groups in two ways. To test whether the relative activation or deactivation of specific ROIs differentiates the groups the percentages were analyzed with a group by ROI analysis of variance corrected for the non-independence of the percentage scores. Profile analysis (Rencher, 1995) was used to determine whether the overall patterns of activation and deactivation differed across groups.

Finally, the non-parametric fuzzy clustering algorithms in the EvIdent image analysis software were used to study activations probatively. Pixels that satisfy a particular parametric test may have different time-courses. Fuzzy clustering is a technique that allows for the differentiation of the various patterns of temporal coherence that all satisfy the same parametric test (Baumgartner et al., 2000; Scarth et al., 1995).

Results

Pixels satisfying the three selection criteria (i.e. $p<0.001$, part of a connected set of five pixels, and between 1 and 5% change in signal) were found in number of cortical areas. Both activations and deactivations were found. The regions activating or deactivating to stool and pain conditions include frontal cortex (Brodmann Areas {BA} 9, 10 and 11) and left and right dorsolateral prefrontal cortex (L/RDLPC) (BA 46). For purposes of analysis the cingulate was divided into three parts. The most anterior part included cingulo-frontal transition cortex (BA 32) and perigenual (BA 24). Our middle area included caudal ACG (BA 24' and 32') and our most posterior area included BA 23 and 31. Somatosensory cortex also demonstrated both activation and deactivation. The

raw pixel counts for each ROI were converted to a percentage of the total number of pixels activating or deactivating. The results for the three groups are displayed in Figure 2.

Our primary interest was in determining if any of the ROIs would clearly differentiate the groups. A significantly higher percentage of pixels activated in the anterior cingulate gyrus over both pain and stool conditions for the control group than for the IBS group and for the IBS group than for the IBD group ($p<0.035$). Examples of this activation and time-course are presented in Figure 3. The groups were also differentiated by a deactivation. Deactivation of left somatosensory cortex was greater for the IBS group than for the IBD group and greater for the IBD group than for the controls ($p<0.0065$) in the boxcar condition. Examples of this activation and time-course are presented in Figure 4. The incidence of frontal deactivation in controls was more extensive than for disease groups. These differences bordered on statistical significance ($p<0.08$). Thus, both activation and deactivation of particular ROIs differentiate the groups even with relatively few subjects.

The profile of percentages for each group was analyzed both for activations and deactivations separately for pain specific, stool specific, and box car time-courses. The results show that controls and the IBD group were differentiated for both the boxcar and stool specific activations ($p<0.05$). The profile of the IBS group did not differentiate from either the control or the IBD groups. A second profile analysis over six responses – RDLP, LDLP, anterior cingulate, and mid-cingulate activations; and left somatosensory and anterior frontal deactivations – for the boxcar time-course again differentiated the control and IBD groups. However, the value of Hotelling's T2 was more robust

($p<0.005$). We believe that this is important because clinically relevant discrimination of groups requires statistically robust differentiation.

Finally, the results of the non-parametric analysis were interesting. It was designed to identify patterns of temporal coherence that might be overlooked on the basis of the parametric analysis alone. One of the areas that appears to be most active in this study and many other studies of pain is the anterior cingulate. We were interested in the finding that the anterior cingulate in another fMRI study did not show pain specific activation (Mertz et al., 2000). Thus, we analyzed only those pixels that satisfied the three criteria for pixel selection to the pain specific and boxcar time-courses for control subjects. The results are presented in Figure 5. Clearly, Cluster A is pain specific in that they appear not to change signal intensity in the stool condition. Cluster B demonstrates graded activation in that they are active in the stool condition but more active in the pain condition. These two patterns of activation were found in 5 of 6 control subjects. They suggest that amongst the pain sensitive pixels in the anterior cingulate there are two patterns of response to pain – on/off and graded. This finding may help to resolve some apparent discrepancies between studies.

In summary, we have found a widely distributed network of structures that activate and deactivate to two levels of noxious visceral stimulation. These structures are largely consistent with other reports of visceral stimulation and also for studies involving other modalities of pain. The three groups are differentiated by both activation and deactivation of particular ROIs – anterior cingulate activation and left somatosensory deactivation. The overall patterns of activation differentiated two of the three groups. In addition, non-parametric analysis demonstrates that several patterns of temporal

coherence exist in pixels that satisfy particular analytic criteria and may offer insight beyond that provided in purely parametric analyses.

Discussion

We have provided evidence that high-resolution fMRI may be used to identify structures responsive to noxious visceral stimulation. These structures are consistent with other studies of response to noxious visceral and to other modalities of painful stimulation. We have shown that the three experimental groups are differentiated by activity in particular ROIs. We have demonstrated that responsiveness may take the form of activation or deactivation and that both patterns of activation and deactivation differentiate the control and IBD groups. Studies of the response to noxious visceral stimulation have generally overlooked the existence of deactivations. There is increasing recognition of the importance of deactivations (Aziz et al., 1997; Hutchinson et al., 1999) and it is noteworthy that they both exist and in some cases differentiate the groups.

We have employed profile analysis to test whether the global profile of activation and deactivation differentiate the groups. There is general agreement that complex processes such as the experience of pain are instantiated in widespread distributed networks. As pain involves sensory, nociceptive, attentional, and emotional dimensions, data analysis should be testing whether the overall pattern of activations and deactivations differentiate experimental groups with multivariate methods. Finally we have shown that non-parametric analysis may complement parametric analyses in ways that are especially useful at this early stage of neuroimaging research. For example, the finding that there are not pain sensitive pixels in the anterior cingulate of control subjects

that is based on pixel counts (Mertz et al., 2000) may be confounded by the existence of graded activations.

Our data are distinct from another report of fMRI responses to noxious visceral stimulation in IBS patients. This report suggested that patients with IBS had an activation at the ACG that was greater than normal controls (Mertz et al., 2000). This report, however failed to assess graded activations. Furthermore, it used a comparison of numbers of pixel counts in any one area ignoring the possibility that overall pixel counts may have been vastly different and that therefore proportionally pixel counts in one area may have different implications than simply absolute numbers in that area. The use of the overall activation as a means of expressing each activation as a percentage of the total was instituted in our study to overcome global autonomic influences such as blood pressure which might vary between groups.

The study by Mertz et al also used phasic computer driven balloon distentions. The advantage of this approach is to introduce identical stimuli to each subject. We chose to use a manual balloon inflation technique (simulating more of a ramp distention) because our interest was to establish imaging at the moment the subject experienced a sensation of stool or discomfort. This could be accomplished by the subject cueing the investigator at the moment the balloon volume was reached that simulated a stool or painful sensation. Ultimately, a comparison to computer driven phasic distentions with that of manual balloon distentions will be pursued to determine if method of stimulus delivery affects central loci of activation.

Our data showed that the IBD patient group could be more distinctly differentiated from controls than the IBS group. We acknowledge that group differences

in gender composition may account for some portion of the observed differences (Paulson et al., 1998). We chose the IBD group to serve as another comparison group to the IBS group. Patients with Crohn's disease and ulcerative colitis are not a homogeneous group, even if all patients were in clinical remission. Nonetheless, this group is a patient population with intestinal inflammation and thus serves as a novel comparison to IBS. No general conclusions can be made for IBD since IBD is comprised of these two distinct disorders. It may be that Crohn's disease and ulcerative colitis patients do in fact have a consistent fMRI pattern of response to noxious visceral stimuli but this will require testing in larger groups of Crohn's disease and ulcerative colitis patients, respectively. However, these are the first central fMRI data in IBD patients to be reported to date in the literature.

Based on our understanding of the role of emotive and memory centers in IBS we considered a priori that control responses could be more easily differentiated from IBS than IBD. This did not prove to be the case. Both disease groups suffer from chronic or recurrent pain, however, it may be that the combination of chronic pain and intestinal inflammation has a greater impact on neural plasticity and hence brain activations as identified by fMRI.

We found that controls had greater ACG activation and greater frontal deactivation than IBS patients. It may be that IBS patients who experience repeated episodes of abdominal pain demonstrate more diffuse cortical activations such that proportionally fewer ACG pixels met the rigorous statistical criteria employed in the present study. This may result from the fact that there are more diffuse affective and attentional activations in these subjects. Frontal deactivations were prominent particularly

in controls, and rarely observed in IBS. These findings are consistent with the areas of decreased flow in studies of painful esophageal stimulation of normals (Aziz et al., 1997). Frontal cortical regions may modulate the cognitive appraisal of the pain-relevant affective signals from the limbic system (Hsieh et al., 1995). Deactivation may reflect the intentional control of these responses. Myocardial ischemia studies also support our finding of visceral pain involving the frontal cortex. Angina has been associated with a diffuse pattern of activation and deactivation including deactivation of the rostral cingulate (Rosen et al., 1994). It is possible that the deactivation in a stressful experimental situation and chronically in the angina patients reflects a modulatory role for the frontal cortex in which emotional responses to pain are suppressed. Removal of the prefrontal cortex and cingulate cortex have been associated with a lack of 'emotional' response to pain in man (Folz and White, 1962; White et al., 1960). Some have suggested at least partial segregation of function between pain affect and sensation with ACG activity reflecting the emotional experience that provokes our reaction to pain (Rainville et al., 1997). The emotional response to visceral pain anticipation in the experimental setting among IBS and IBS patients may have suppressed frontal deactivations.

Figure 1 – Typical oblique axial slice orientation.

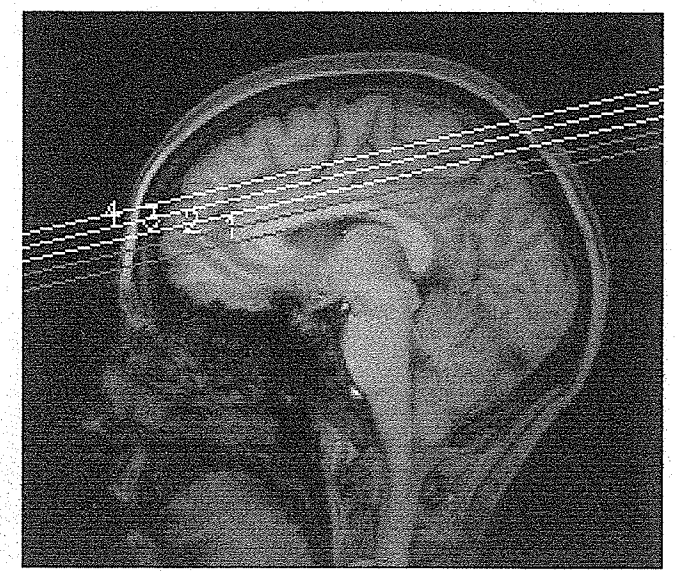
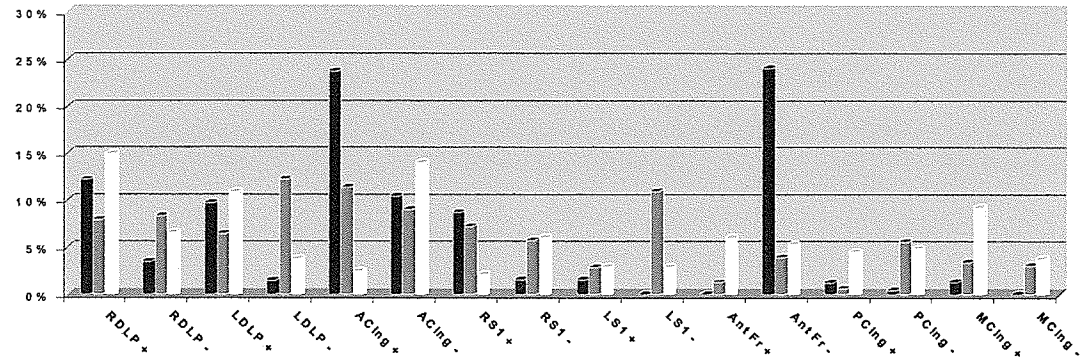
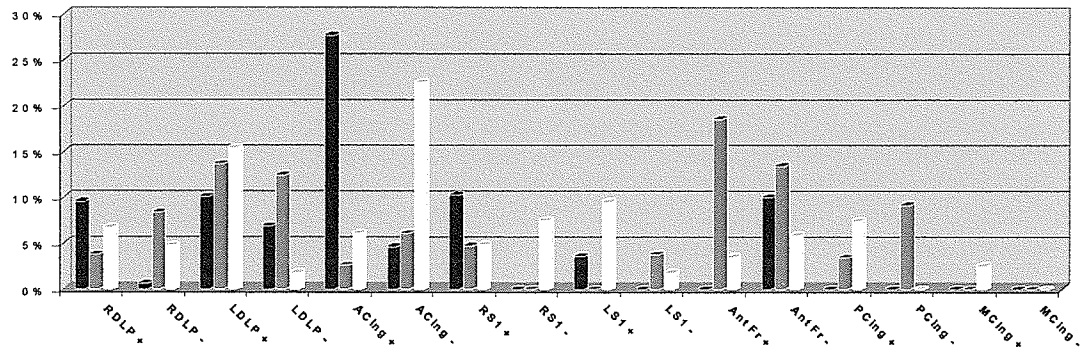


Figure 2 – Percent of the total of paradigm sensitive pixels responding (i.e. activating {+}or deactivating {-}) for each of the regions of interest studied for each of the three experimental groups. A presents data for pixels activating for both stool and discomfort (Boxcar); B presents data for pixels activating only to pain; and, C presents data for pixels activating only to stool. RDLP=right dorsolateral prefrontal, LDLP=left dorsolateral prefrontal, A Cing=anterior cingulate, RS1=right somatosensory I, LS1=left somatosensory I, AntFr= anterior frontal, P Cing=posterior cingulate, M Cing= mid cingulate.

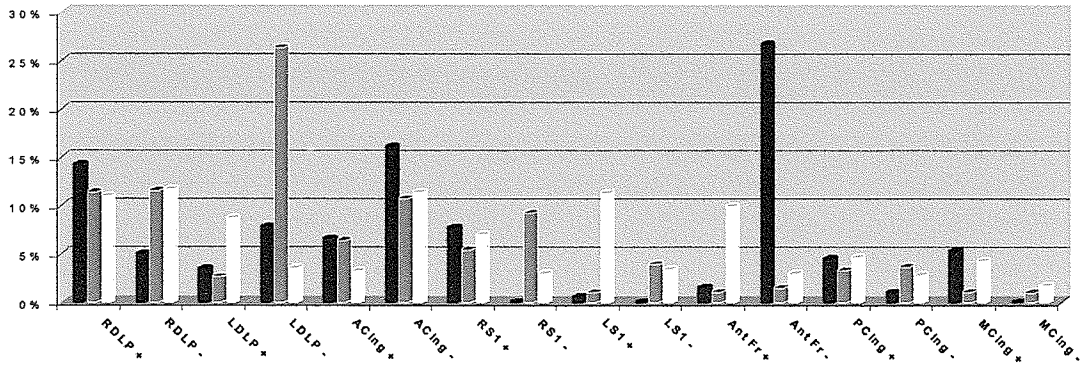
A. Boxcar Correlation



B. Pain Specific Correlation



C. Stool Specific Correlation



Legend

Figure 3 – Comparative cingulate activations and time-courses across both pain and stool for A. typical Control subject and B. typical IBS subject.

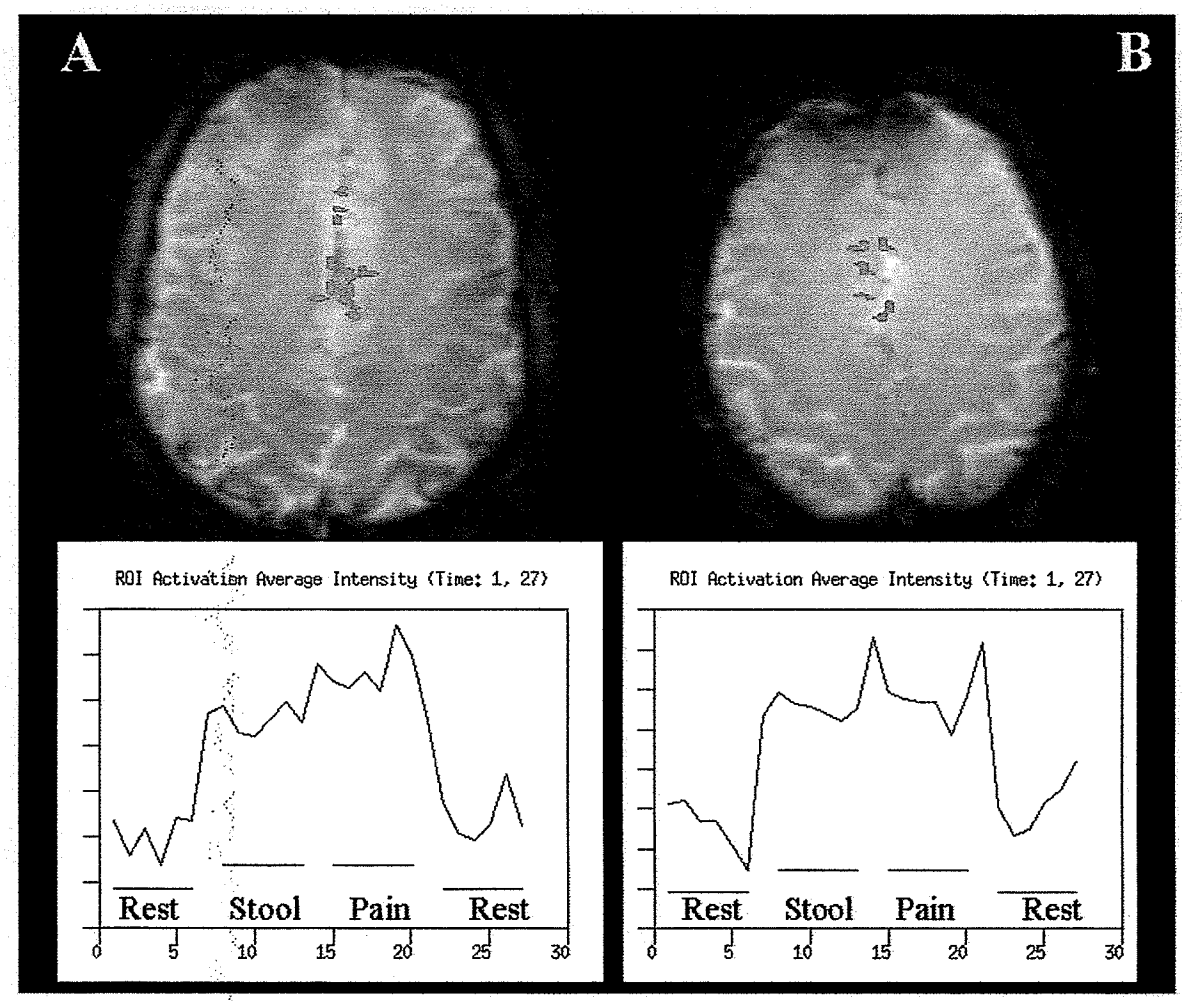

A
B
ROI Activation Average Intensity (Time: 1, 27)
Rest
Stool
Pain
Rest
0
5
10
15
20
25
30
ROI Activation Average Intensity (Time: 1, 27)
Rest
Stool
Pain
Rest
0
5
10
15
20
25
30

Figure 4 – Representative somatosensory deactivation and time-course for an IBS subject.

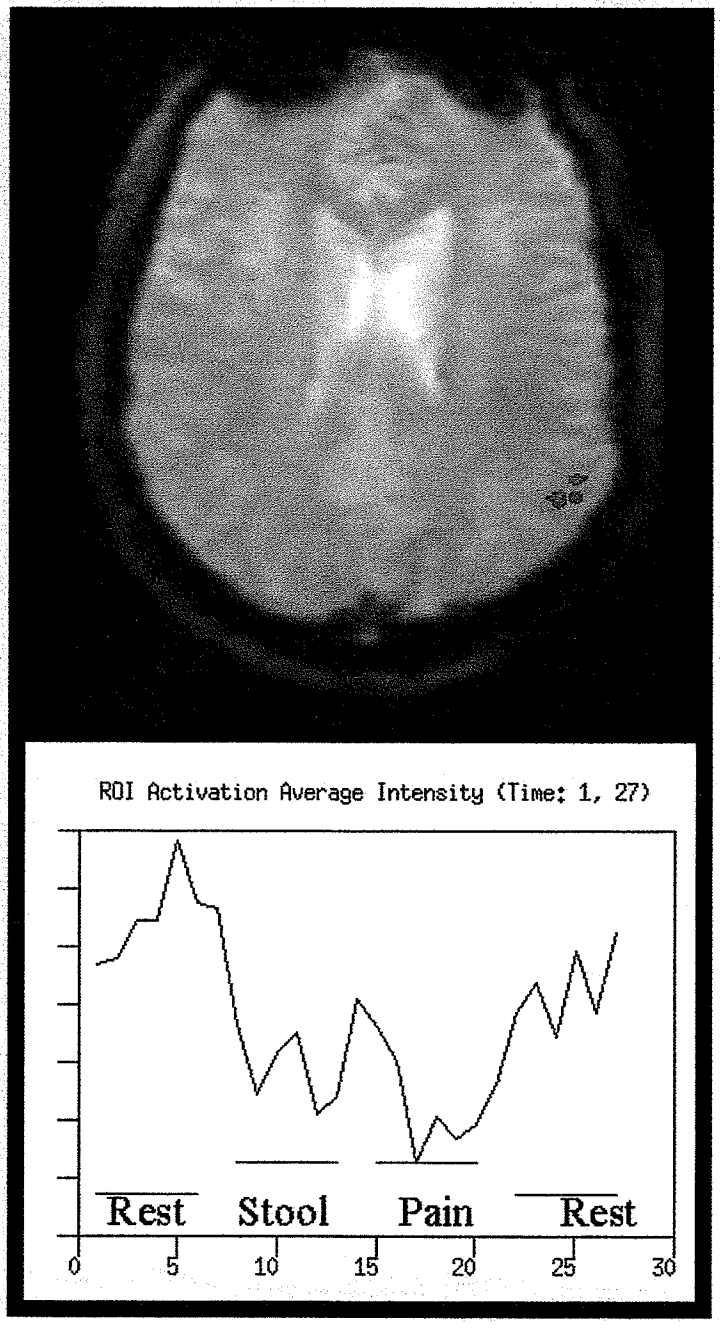
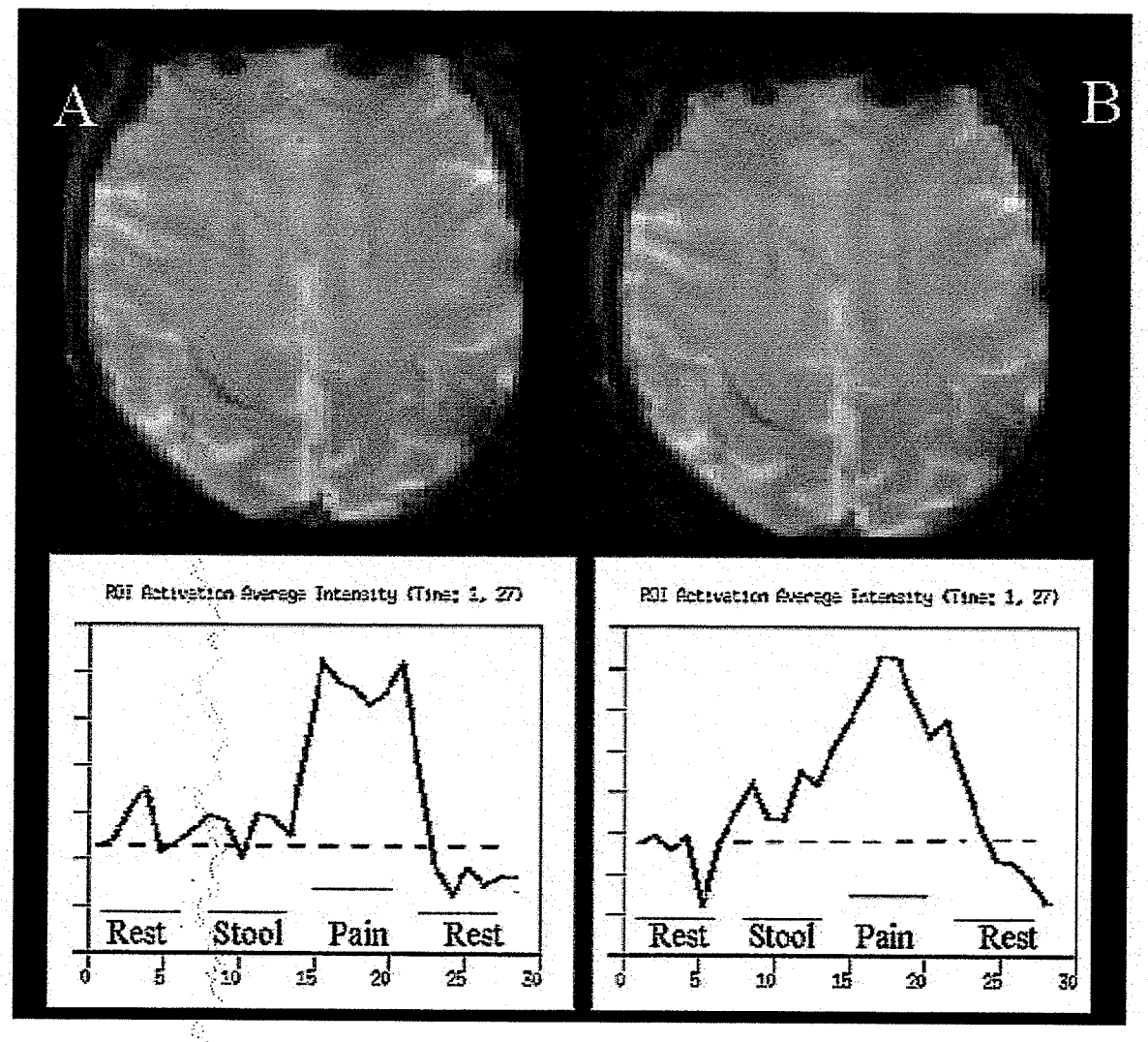

ROI Activation Average Intensity (Time: 1, 27)
Rest
Stool
Pain
Rest
0
5
10
15
20
25
30

Figure 5 – Results of non-parametric analysis demonstrating the existence of two forms of pain responsiveness (pain specific and graded activations) in the cingulate of a typical control subject.


A
B
ROI Activation Average Intensity (Time: 1, 27)
Rest
Stool
Pain
Rest
0
5
10
15
20
25
30
ROI Activation Average Intensity (Time: 1, 27)
Rest
Stool
Pain
Rest
0
5
10
15
20
25
30

Part II

Anterior Cingulate Gyrus and Medial Prefrontal Activity during Visceral and Somatosensory Pain

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Gastroenterology, submitted 02-0313-00.

Abstract

Direct comparisons in the same subjects of the cerebral representations of somatosensory and visceral pain are non-existent. Our aim was to use the high spatial resolution of functional Magnetic Resonance Imaging (fMRI) to compare anterior cingulate and medial prefrontal cortical activation patterns that arise during both pain modalities. These areas have often been implicated in the functional networks subserving pain. We hypothesized that differences in the overall patterns of activity would arise because of the lateralized presentation of somatosensory stimuli and the midline stimulation inherent in visceral pain. FMRI was performed on twelve healthy subjects undergoing painful rectal balloon distention and a 0-2°cold pressor test on left and right hands. Behavioral data was obtained to ascertain that both pain modalities were equivalently intense. Imaging data were submitted to a pixel-by-pixel statistical analysis ($p \leq 0.001$). Normalized pixel counts were entered into a repeated measures ANOVA. Pain ratings were not significantly different between modalities ($p \leq 0.585$). Midcingulate responses were most predominant regardless of pain modality ($p \leq 0.037$). A significant 3-way interaction was observed between task, activation and deactivation and laterality ($p = 0.017$). The brain's activation and deactivation pattern to visceral pain resembles that of left-sided somatosensory pain and both differ from the response to right-sided pain. We conclude that the midcingulate region is of particular importance in all modalities of pain and that the overall pattern of ACG and medial prefrontal activity during visceral pain is similar to left-hand somatosensory pain.

Introduction

Numerous neuroimaging studies have documented the brain's response to noxious somatosensory stimulation. These studies have generally distinguished between direct spinothalamic projections and activation in parts of the cortex associated with more general attentional, emotional, and nociceptive processing. Although imaging of internal organ pain is less well-developed, important similarities have emerged between the cerebral representations of somatosensory and visceral pain (Aziz et al., 2000; Ladabaum et al., 2000; Baciu et al., 1999). The aim of the present experiment is to investigate these similarities by means of Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) in a single set of subjects undergoing both noxious somatosensory and visceral stimulation. Comparisons across studies are difficult as subjects, acquisition methods, analysis tools, type of stimulation and pain ratings differ from study to study. By using a single set of subjects it is possible to measure pain ratings and directly compare the cerebral representation of these two pain modalities within the same subjects.

Some studies on visceral pain suggest that unlike somatosensory pain, which has a homoncular representation in primary somatosensory cortex, visceral pain is primarily represented in the secondary somatosensory cortex (Aziz et al., 2000) Other studies have shown pain-induced activations in several brain regions, including prefrontal cortices, somatosensory cortices, thalamus, insular cortices and anterior cingulate gyrus (ACG) (Baciu et al., 1999; Bernstein et al., 2002; Mertz et al., 2000; Silvermann et al., 1997; Vogt et al., 1996) consistent with those observed during somatosensory pain. Similarly, decreased signal has been reported during visceral and somatosensory pain in dorsolateral

prefrontal cortex, medial prefrontal cortex and specific ACG regions (Aziz et al., 1997; Bernstein et al., 2002; Mertz et al., 2000; Porro et al., 1998; Vogt et al., 1996) Thus it is apparent that brain activity during both pain modalities is represented in paralimbic and limbic structures including the prefrontal cortex and ACG.

Our group has recently reported that patterns of increased and decreased BOLD signal intensity of particular brain regions during visceral pain differentiate control subjects from patients suffering with gastrointestinal disorders (Bernstein et al., 2002). In that study visceral pain–specific activations were predominantly observed in perigenual and midcingulate regions of the ACG and BOLD signal intensity decreases were consistently observed in medial prefrontal cortex and perigenual regions. These observations parallel regional cerebral blood flow changes and BOLD responses reported during some somatosensory pain studies (Porro et al., 1998; Vogt et al., 1996). Although separate studies have reported similar brain activity during visceral and somatosensory pain, there is only one study documenting the brain's response to visceral and somatosensory sensation within the same subjects (Schnitzler et al., 1999) In this work the authors determined the cortical areas activated by non-painful electrical stimulation of visceral afferents in the distal esophagus and somatosensory afferents in the median nerve and lip using magnetoencephalography. As the majority of studies investigating the functional neuroanatomy of nociception have employed either positron emission tomography or fMRI, it is the objective of the present study to extend comparisons of visceral and somatosensory pain using fMRI within the same subjects. The present experiment focuses on the activations in the medial wall of the frontal lobe where previous investigations of both somatosensory and visceral pain have reported activation

and deactivation (Bernstein et al., 2002; Michelic et al., 1999; Porro et al., 1998; Vogt et al., 1996) Since the ACG is one of the most frequently reported active brain regions during any type of pain study (Ingvar, 1999) sends projections to the medial prefrontal cortex (Pandya et al., 1981) and has been implicated in the affective and cognitive dimensions (Melzack and Casey, 1968) of the pain experience which are common to both, visceral and somatosensory pain, we sought to focus on these structures. We hypothesized that ACG and medial prefrontal cortex activation patterns would arise during visceral and somatosensory pain but that differences in the overall patterns of activation would arise because of the lateralized presentation of the somatosensory stimuli and the midline stimulation inherent in visceral pain.

Materials and Methods

Subjects

Twelve right-handed volunteers (mean age: 32.7 years [range: 20-45 years], 8 males) without gastrointestinal complaints were recruited from the general population as normal subjects. Two subjects were excluded due to noticeable head motion during the imaging session. All studies were performed at the Magnetic Resonance Imaging Suite at the Health Sciences Centre in Winnipeg, Manitoba, Canada. Ethics approval for the study was obtained from both the University of Manitoba Ethics Committee for Human Studies and the National Research Council's Ethics Committee. Subjects gave informed consent and were free of exclusion criteria for MRI.

Tasks

Subjects were asked to close their eyes for all experiments. All experiments consisted of an initial 150-second (-s) rest condition followed by a 150-s stimulation condition and a final 150-s rest condition. During the rest condition subjects were asked to relax and listen to the sound of the machine.

Cold Pressor Test

Foot or hand immersion into 2-6°C water is a standard test for pain threshold evaluation and is known as the Cold Pressor Test (CPT). We used a unilateral cold compress (0-2°C) on the palmar surface of either the right or left hand. A trial run was conducted before each experiment to determine the water temperatures acceptable to the subjects. All subjects reported the 0-2°C cold compress as bearable but painful. At the end of each experiment subjects were asked to rate the pain from 0 (no pain) to 10 (worst pain imaginable).

Visceral Pain Task

A Fleet™ enema was administered to all subjects. After rectal emptying, a latex balloon (MAK-LA Company, Thousand Oaks, Ca) was inserted into the rectum. The tubing attached to the balloon was connected to a 60ml syringe that was used for the manual balloon inflations. As opposed to computer-driven phasic stimulation that relies on subject recall of sensations post-imaging, balloon inflations were conducted manually to allow for imaging at the instant the subject hand-signaled the sensation of pain. Prior to entering the magnet, the distention protocol was tested and subjects were asked to

hand-signal when the sensation of pain was met. This reduced anxiety and ensured that the subject could consistently identify the inflation pressure corresponding to pain. At the end of each experiment subjects were asked to rate the pain from 0 (no pain) to 10 (worst pain imaginable).

Imaging Parameters

Experiments were conducted on a 1.5 Tesla whole body magnet (General Electric Signa Horizon LX) and a homogeneous quadrature head coil. Contiguous whole-brain echo planar images (EPI) were acquired parallel to the AC-PC line (anterior/posterior commissure, Talairach and Tournoux, 1988) with a slice thickness of 4mm. Thirteen single-shot blipped gradient echo planar images were acquired for each rest and stimulation condition (TR/TE = 1000/50 ms, $\alpha = 60°$, 64 x 64 matrix, 25 cm FOV). Whole-brain T1-weighted high-resolution anatomical images were obtained for the overlay of functional statistical maps.

Data Analysis

To meet our first objective and identify significant brain activity in our regions of interest (ROIs), data were submitted to a pixel-by-pixel statistical analysis (Strupp, 1996) Signal intensities of the stimulation conditions versus rest were correlated to a boxcar function ($P \leq .001$). The first image of every condition was excluded to ensure that only those images were analyzed where longitudinal magnetization had reached steady state. Pixels that satisfied this criterion were processed further in two ways. False positives that could occur by chance were reduced by using a criterion of anatomical connectedness

that is used as a more anatomically motivated form of Bonferroni correction (Xiong et al., 1995). Pixels were passed only if they were connected to at least 3 other pixels. We then filtered the data so that only pixels between 1 and 5% signal intensity were analyzed.

For all results presented, pixel count data was normalized by expressing the data for each ROI as a percentage of the total number of active pixels in all ROIs. The percentage data for each subject was calculated, as were the average percentages for each ROI. Pixel counts for each ROI reflect both specific responses to stimulation and factors that vary between subjects which influences the total number of pixels. To determine whether there is a significant difference between pain ratings during the different tasks, pain ratings were recorded for eight subjects and assessed using a Student's *t*-test.

The next objective of the data analysis was to compare the number of activated and deactivated pixels between the different experimental conditions as well as between regions of interest within tasks. "Deactivation" in this study refers to pixels that have a negative correlation with the stimulation paradigm. Pixel counts were also assessed for significant differences in lateralization. For this purpose, the normalized data was submitted to a repeated measures ANOVA.

To assess main effects and interactions, the normalized data for each participant was submitted to a 3 (task) x 2 (percentage data on activations and deactivations) x 3 (brain region) x 2 (laterality) repeated measures ANOVA with Greenhouse-Geisser corrections where appropriate.

ROIs were drawn according to the cortical regions displayed in mid-sagittal and axial sections in Figure 1. With all ROIs, regions below the AC-PC line were excluded from analysis based on the inhomogeneity of T2*-weighted axial MR images in the

orbital regions of the brain. Six ROIs were identified on high-resolution axial T1-weighted anatomical images. These included the left and right medial prefrontal cortices (MPFC) (BA 9, 10 and some of BA 8 and 6 at the most dorsal aspect of the ROI), left and right perigenual cortex (PGC) (BA 24 and 32) and left and right midcingulate cortex (MID) (BA 24' and 32'). Identification of ACG sub-regions was based on Talairach and Tournoux ordinates in the z direction (Talairach and Tournoux, 1988) and approximated to cytoarchitectural areas superimposed onto a flat map (Vogt et al., 1995) MPFC ROIs were drawn from z = 0mm - 46mm. ROIs of the PGC region were drawn from z = 0mm - 16mm. ACG regions located caudal to these regions, also referred to as midcingulate (MID) or the caudal aspect of the ACG were drawn from z = 20mm - 46mm. To show anatomical locations of the different activations, functional maps were superimposed on high-resolution T1-weighted images taken from individual subjects.

Results

Behavioral Results

Subjective pain ratings during the somatosensory pain conditions (left and right hand CPT) were not significantly different from the visceral pain condition. Average ratings of pain intensity during the left-hand CPT condition were 5.94 (range: 1 – 9, S.D. = 3.19), 6.13 (range: 1 – 9, S.D. = 3.23) for the right-hand CPT condition and 5.38 (range: 2. – 6.5, S.D. = 1.46) for the visceral pain condition. Paired t-test significance levels on pain ratings were $p < 0.197$ for the left and right-hand CPT comparison, $p < 0.464$ for the right-hand CPT and visceral pain comparison and $p < 0.585$ for the left-hand CPT and visceral pain comparison.

FMRI Results

The first objective was to determine significant brain activity in our regions of interest. Only pixels satisfying our three selection criteria ($p = 0.001$, part of a connected set of 3 pixels and between 1 and 5% signal change) were selected for report here. During both, the visceral and somatosensory pain conditions significant activations and deactivations in BOLD signal intensity were observed in all ROIs investigated ($p = 0.001$). Although specific regions responded selectively to the different experimental conditions, significant signal intensity changes were observed in MPFC (BA 9, 10) PGC (BA 24, 32) and MID regions (BA 24', 32') during all experiments. Figures 2 and 3 show CPT and visceral pain activation and deactivation patterns from a representative subject (activations are depicted in green, deactivations in red). Figures 2A and B show that during the CPT, ACG activations tend to be lateralized to the contralateral side whereas deactivations tend to be lateralized to the ipsilateral side. Although there were PGC activations (z = 8 mm - 16mm) during both, the left and right-hand CPT, more activations appeared to be located in MID regions (z = 20 mm – 46 mm). Figure 3 shows that activations during visceral pain were lateralized on the right side and deactivations were more prominent on the left side. Similarly to the CPT, there were more activations in the MID region. To further investigate these observations, ROI pixel counts were normalized and the grouped percentage data submitted to a repeated-measures ANOVA (see below).

The second objective of the data analysis was to compare the number of activated pixels between tasks and deactivated pixels between tasks as well as the different regions within tasks. Using a repeated measures analysis of variance (ANOVA) on the grouped

percentage data, we did not observe a significant difference between the percent of activated or deactivated pixels between tasks in our regions of interest. Thus, the amount of activation and deactivation was task-independent. Moreover, there was no significant main effect for activations and deactivations in the 3x2x3x2 repeated measures ANOVA suggesting equivalent percentages for both activations and deactivations. However, within tasks, MID activations were significantly larger than PGC and MPFC activations. This difference was significant for the MID regions during gastrointestinal pain ($p \leq 0.0102$) and similarly for the MID regions during the right and left hand CPT ($p \leq 0.023$, $p \leq 0.037$). A similar pattern was observed for decreases in BOLD signal intensity. The largest deactivations were observed in MID regions during the left and right hand CPT ($p \leq 0.007$, $p \leq 0.0028$, respectively) and visceral pain ($p \leq 0.022$). These results were further supported by a significant main effect for brain region in the 3x2x3x2 repeated measures ANOVA ($P = 0.000072$). Thus, the MID region displayed the most task dependent activity independently of the direction of signal change.

Lateralization of Signal Intensity Changes during Visceral and Somatosensory Pain

Our third objective was to examine the patterns of lateral asymmetry in the data. The 3x2x3x2 repeated measures ANOVA revealed a significant main effect for laterality ($p = 0.0267$). To investigate this effect we further examined the repeated-measures ANOVA within brain regions and within tasks. For visceral pain, the null hypothesis was that visceral stimulation would elicit bilateral responses. In fact this was the case, however, visceral and left-hand CPT pain had a larger percentage of activations in the

right hemisphere including MPFC, PERI and MID regions, when compared to right-hand CPT pain (Figures 4A-C). However, this lateralization was not statistically significant. For the right-hand CPT, activations were larger in the contralateral hemisphere, but again, the lateralization was not statistically significant. Thus, it appears that there is a tendency for the activations elicited by visceral pain to resemble left-hand thermal pain.

The percentage of deactivated pixels in right versus left ROIs is depicted in Figures 4D-F. Deactivations during visceral and left-hand CPT pain (Figures 4D-E) were more prominent in the left hemisphere and the pattern of deactivations was more similar then when compared to the deactivation pattern during the right hand CPT (Figure 4F). During visceral pain there was no significant lateralization in MPFC, PGC and MID regions. However, left MID deactivations were significantly larger than PGC ($p \leq 0.001$) and MPFC ($p \leq 0.022$) deactivations. Left-hand CPT decreases showed a similar pattern in that left MID deactivations were significantly larger than MPFC ($p \leq 0.007$) and PGC ($p \leq 0.00073$) deactivations. Left MID deactivations were also significantly larger than right MID decreases ($p = 0.0014$). There was no significant lateralization of decreases during right-hand CPT pain. Thus, there also appears to be a tendency for the deactivations elicited by visceral pain to resemble those elicited by left-hand pain.

That the pattern of lateralization for midline visceral pain appeared to resemble that of unilateral left-sided thermal pain was surprising. Thus we decided to examine it further. To study this tendency further, we examined the interaction effects in the 3x2x3x2 ANOVA. The ANOVA revealed a significant 3-way interaction between the type of task, activations and deactivations and laterality ($p \leq 0.017$). This interaction is displayed in Figure 5. The interaction between laterality, condition, and direction of

signal change can be seen by virtue of the fact that the visceral and left-hand conditions are nearly parallel to one another. In addition, those of the right-hand condition are nearly orthogonal to the other two conditions. This is the case both for activations and deactivations. Thus, we conclude that the functional neuroanatomy of visceral pain in the medial wall of the frontal cortex closely resembles that of left-hand somatosensory pain.

Discussion

Before discussing the fMRI findings the behavioural data deserve comment. There were no significant differences in pain ratings between the CPT and visceral pain. Thus neuroanatomical responses analyzed were to subjectively equivalent levels of discomfort. Since our pain ratings did not vary by task, we did not expect any significant differences in the number of activated and deactivated pixels between visceral and somatosensory pain, which was, indeed, the case. PGC activations are implicated in the affective/suffering component of the pain experience and higher pain ratings during either pain modality may have been reflected in differential activation of the ACG. Indeed, it has been demonstrated that ACG activations correlate with pain ratings (Coghill et al., 1999; Davis et al., 1997; Frankenstein et al., 2001; Porro et al., 1998)

In this experiment we investigated similarities and differences in the brain's response to lateralized somatosensory pain and to visceral pain in the medial frontal structures implicated in pain processing. We first determined that all of the regions of interest activated to the three stimulation paradigms. We also determined that the MID was the most reactive ROI before investigating task-specific patterns of response. The MID responses were larger than MPFC or PGC responses regardless of pain modality

and direction of signal change. The preponderance of activations in the MID is consistent with numerous other studies that have demonstrated a predominant BA24' response in the MID (Davis et al., 1997; Frankenstein et al., 2001, Hutchison et al.,1999; Kwan et al., 2000). The significant main effect for brain region is consistent with the interpretation that the MID region is of particular importance in all modalities of pain.

An interesting result was the absence of significant lateralization in our regions of interest during both visceral and somatosensory pain. Even though we observed a tendency of more contralateral activation during CPT pain and right activations during visceral pain (Figures 2, 3) significant differences within MPFC, PGC and MID ROIs were not observed. Bilateral ACG activations were shown during esophageal stimulation (Aziz et al., 1997) Another group using esophageal stimulation showed that the cerebral activation pattern was related to the strength and quality of the stimulus (Binkofski et al., 1998) Use of a tonic painful esophageal stimulus, as opposed to a low intensity phasic stimulus, resulted in bilateral activation of primary and secondary somatosensory cortices as well as the right premotor cortex and right ACG. Activation of the right ACG was also demonstrated with single photon emission tomography during rectal distention (Bouras et al., 1999). For somatosensory pain, immersing the left hand into a 6°C water bath for 105 seconds, produced significant activation in the contralateral ACG (Casey et al., 1996). In a very recent study on thermal pain, it was found that stimulation of either the right or left hand produced significant right predominant ACG activations Brooks et al., 2002). Our group has previously shown significant contralateral ACG activation when the CPT was applied to the foot (Frankenstein et al., 2001). It may well be that the differences between the present study and the results obtained by other studies derive

from the type and level of painfulness of the stimulation. In our experiment a cold compress was used on the palmar surface of the hands (Frankenstein et al., 2001). The other studies used either heat or cold water immersion to the wrist (Casey et al., 1996; Brooks et al., 2002). In the cold water immersion study pain ratings were much higher resulting in a significant contralateral ACG activation. We also suspect that subjective pain ratings at the upper limit of heat used (49°C) would be higher than those measured in the present experiment and thus resulted in a significantly lateralized activation.

Most of the fMRI literature examines increases in signal intensity or activations (Davis et al., 2000; Davis et al., 1997; Kwan et al., 2000; Mertz et al., 2000). One very surprising finding was that the number of pixels demonstrating increased signal was not different from the number of pixels demonstrating signal decrease or deactivation. The second intriguing finding in this regard was that deactivations were also more prominent in MID cingulate regions regardless of the type of stimulation. This latter finding is not consistent with our previous observations of prominent MPFC deactivations or reports of other groups demonstrating decreased signal in medial prefrontal regions (Bernstein et al., 2002; Frankenstein et al., 2000a,b; Frankenstein et al., 1999; Porro et al., 1998; Vogt et al., 1996 In contrast to these studies, our analyses did not include areas below the AC-PC line and therefore excluded medial orbital areas in the analysis (see methods) and may thus have resulted in a lower percentage of deactivation in this region. The result of equivalent percentages of activations and deactivations might suggest that the deactivated pixels appear because of hemodynamic capture. This implies that blood would be diverted away from the deactivated pixels to activated pixels. If this were the case one would expect that deactivations would be penumbral to the activations. This was not the

case. In large measure, the deactivations were contralateral to and, thus, hemodynamically independent of activations.

The interaction analysis further captured the activation/deactivation relationship in this study (Figure 5). When activations were increased during left-hand CPT pain and visceral pain in the contralateral/right hemisphere, deactivations in that same hemisphere were decreased. The same relationship between activations and deactivations was observed for right-hand CPT pain in the left hemisphere. If deactivations are the result of a hemodynamic steal phenomenon, then we could expect areas of increased activations to also show increased deactivations. Again, this was not the case. Rather, our results suggest a hemispheric inverse relationship between the percentage of activations and deactivations during noxious visceral and somatosensory stimulation. Previous studies have demonstrated the involvement of deactivations in the response to noxious visceral and somatosensory stimulation (Aziz et al., 2000; Michelic et al., 1999; Porro et al., 1998; Vogt et al., 1996) The present data suggest that the existence and potential impact of deactivations should reflect regularly in the analysis of painful stimulation. We believe that this becomes especially important when fMRI is used to study pharmacological effects that are designed to reduce activity in particular areas.

Comparisons of pixel counts (Figure 4) and the interaction results indicate that the brain's response to visceral pain resembles the response to left-sided somatosensory pain and that both differ from the response to right-sided pain. This was the case for both activations and deactivations. Although the significance of these observations is not clear, some studies have documented the involvement of right ACG during visceral pain (Binkofski et al., 1998; Bouras et al., 1999). It has been suggested that limbic structures,

such as the ACG for example, become engaged when the visceral sensation becomes unpleasant or painful (Binkofski et al., 1995). Whether this limbic response is lateralized to the right ACG is not known. Although many studies report contralateral activations, it has recently been demonstrated that the right ACG activates to painful heat regardless of the side of stimulation (Brooks et al., 2002). To date, the differences in somatosensory and visceral activation patterns appear to be at the level of the somatosensory cortex (Aziz et al., 2000). Whereas visceral sensation appears to be clearly represented in second somatosensory cortex, somatosensory stimulation shows the involvement of primary somatosensory cortex (Schnitzler et al., 1999).

In conclusion, we have found that pain mechanisms beyond the primary and secondary somatosensory cortices activate and deactivate bilaterally. Although activations and deactivations are bilateral, they are not symmetric. We have found that midline visceral pain activation and deactivation patterns resemble left-sided CPT pain. Differences in this regard with other studies focusing on the same regions may plausibly derive from variations in the size of the ROIs and differences in the stimulus used and also the perceived pain intensity. With specific temperatures, inflation volumes or pressures there are differences in subjective response. What is painful for one subject may be merely uncomfortable for another. Moreover, comparisons across experiments that use subjectively different levels of pain are difficult. It is therefore necessary to conduct studies that equate the subjective ratings of pain within subjects across modalities. The use of a standard stimulus is also problematic; studies that systematically vary the level of pain within experiments are needed. Our group has previously demonstrated the existence both of pixels that activate in a pain-specific manner and also

of pixels that activate in a graded manner to discomfort and to pain (Bernstein et al., 2002). Studying the homogeneity of response on a pixel-by-pixel basis may shed light on the patterned differences in responses to different levels of stimulation.

However, the reasons why frontal medial wall activation and deactivation patterns of visceral pain should resemble left-sided somatosensory pain remain to be determined.

Figure Legend: Figure 1

Schematic drawing of representative sagittal and axial sections modified from the Talairach and Tournoux atlas, depicting the location of the three regions of interest (divided into three left and three right ROIs). The axial drawings are at the level of 8mm, 24mm and 45mm. ROIs span from z = 0 - 46mm. The characterization of ACG subdivisions corresponds with that described by others (Derbyshire et al., 1998; Talairach and Tournoux, 1988; Vogt et al., 1995). The sagittal drawing at G/left 3mm was added to provide an overview of the ventral/dorsal aspect of the ROIs. The AC-PC and Vca and Vcp lines are indicated. MID, mid-cingulate gyrus; MPFC, medial prefrontal cortex; PGC, Perigenual Cortex.

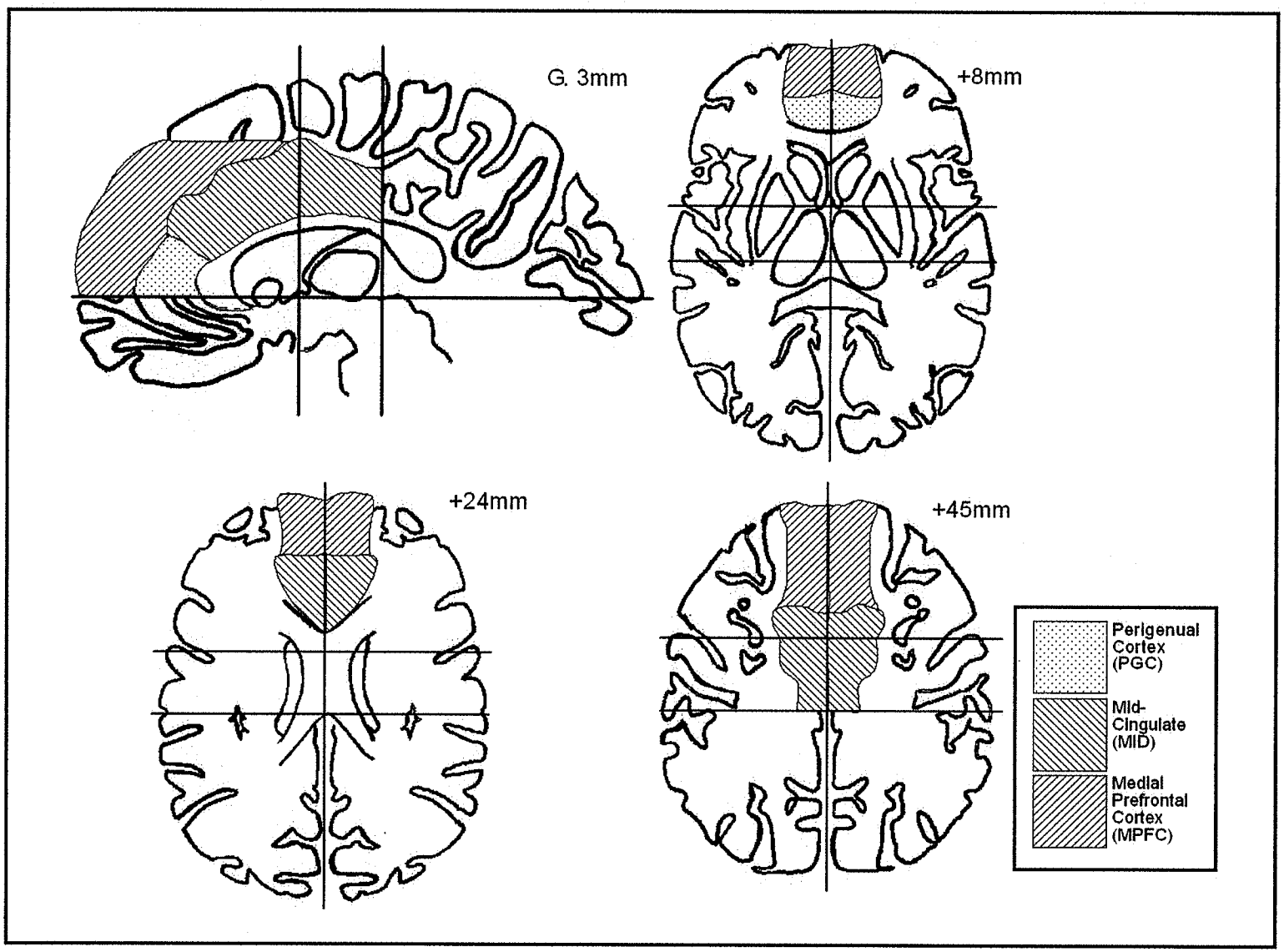

G. 3mm
+8mm
+24mm
+45mm
Perigenual Cortex (PGC)
Mid-Cingulate (MID)
Medial Prefrontal Cortex (MPFC)

Figure Legend: Figure 2

Activation maps of BOLD signal increases and decreases in ACG and MPFC during (A) the cold pressor test (CPT), (consisting of a cold compress at 0-2 °C on the palmar surface of the left hand) and (B) the CPT on the right hand. Both activations maps were thresholded at a *p* value of 0.001. The activations are colour-coded, green corresponding to activations and red to deactivations. Activation maps are displayed on a high resolution T1-weighted anatomical image. Images are displayed according to radiological convention, the left hemisphere is on the right and the right hemisphere on the left side of the image. The plane of the displayed brain regions is indicated on the top of each column, spanning from z = 8mm to z = 44mm.

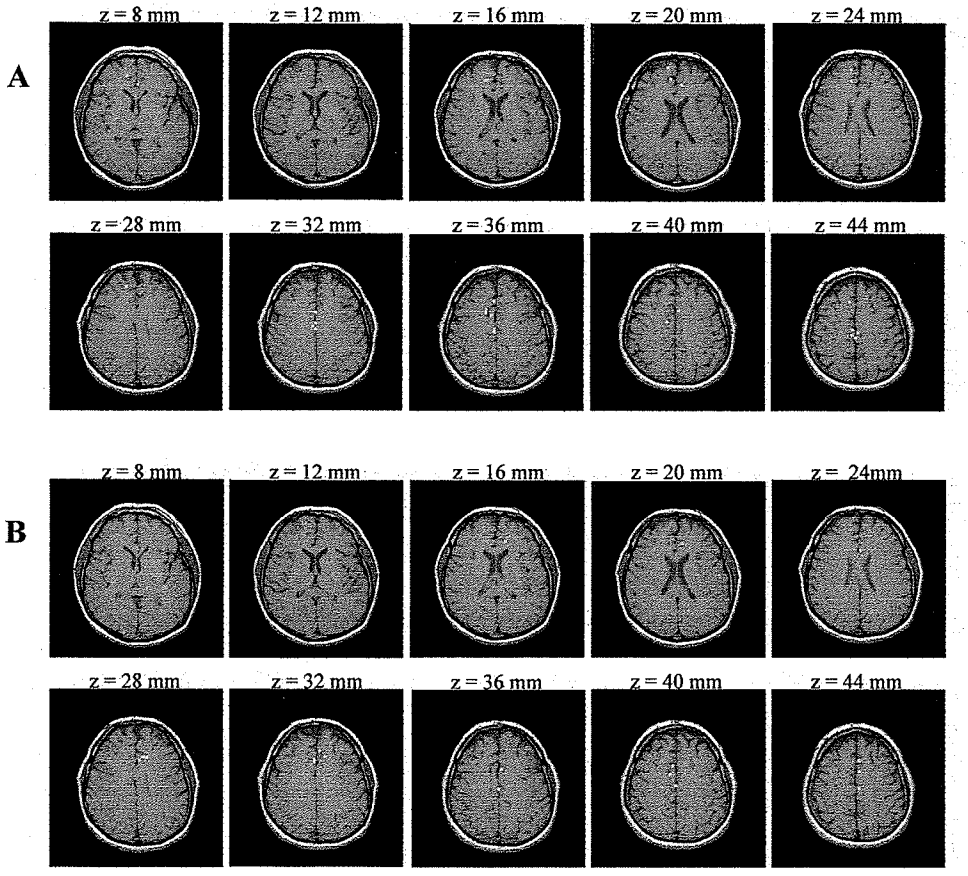

A
z = 8 mm
z = 12 mm
z = 16 mm
z = 20 mm
z = 24 mm
z = 28 mm
z = 32 mm
z = 36 mm
z = 40 mm
z = 44 mm
B
z = 8 mm
z = 12 mm
z = 16 mm
z = 20 mm
z = 24mm
z = 28 mm
z = 32 mm
z = 36 mm
z = 40 mm
z = 44 mm

Figure Legend: Figure 3

Activation maps of BOLD signal increases and decreases in ACG and MPFC during visceral pain. The activation map was thresholded at a *P* value of 0.001. The activations are colour-coded, green corresponding to activations and red to deactivations. The plane of the displayed brain regions is the same as in Figure 2 and indicated on the top of each image. Activation maps are displayed on a high resolution T1-weighted anatomical image. Images are displayed according to radiological convention, the left hemisphere is on the right and the right hemisphere on the left side of the image.

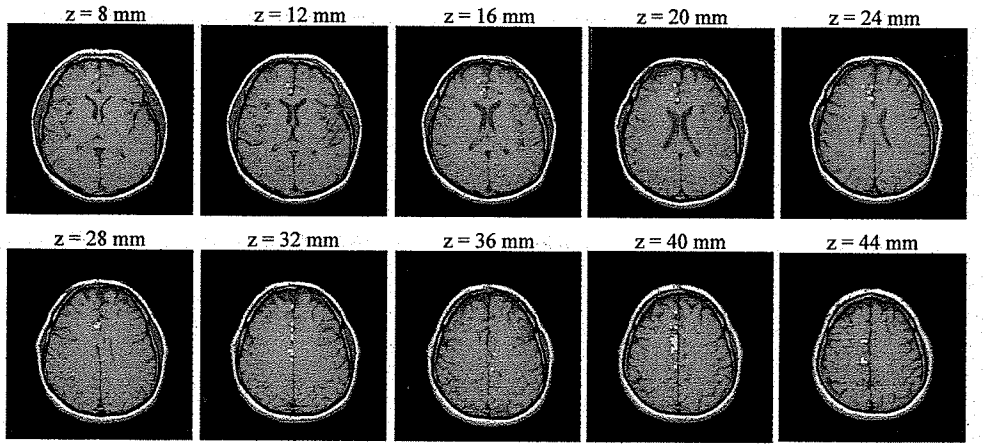

z = 8 mm
z = 12 mm
z = 16 mm
z = 20 mm
z = 24 mm
z = 28 mm
z = 32 mm
z = 36 mm
z = 40 mm
z = 44 mm

Figure Legend: Figure 4

Histogram of the normalized percentage data. (A) Visceral pain activations; (B) Left-hand CPT activations; (C) Right-hand CPT activations; (D) Visceral pain deactivations; (E) Left-hand CPT deactivations; (F) Right-hand CPT deactivations. Brain regions are shown on the horizontal axis: R/LMPFC, right and left medial prefrontal cortex; R/LPERI, right and left perigenual cortex; R/LMID, right and left midcingulate. The vertical axis represents the data for each ROI as a percentage of the total number of active pixels in all ROIs. The pattern of activation and deactivation is more similar for visceral (A,D) and left hand pain (B,E) than for right-hand pain (C,F).

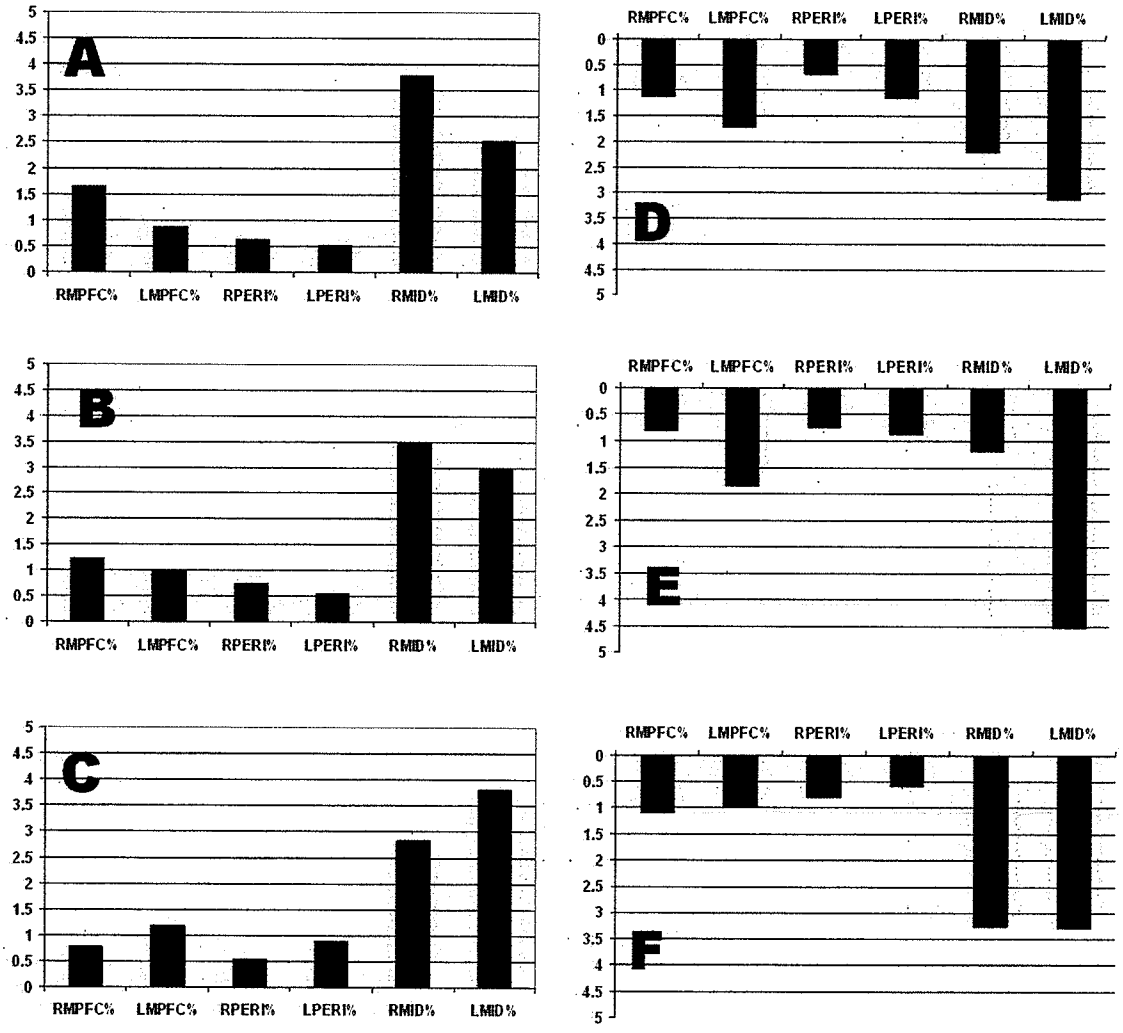
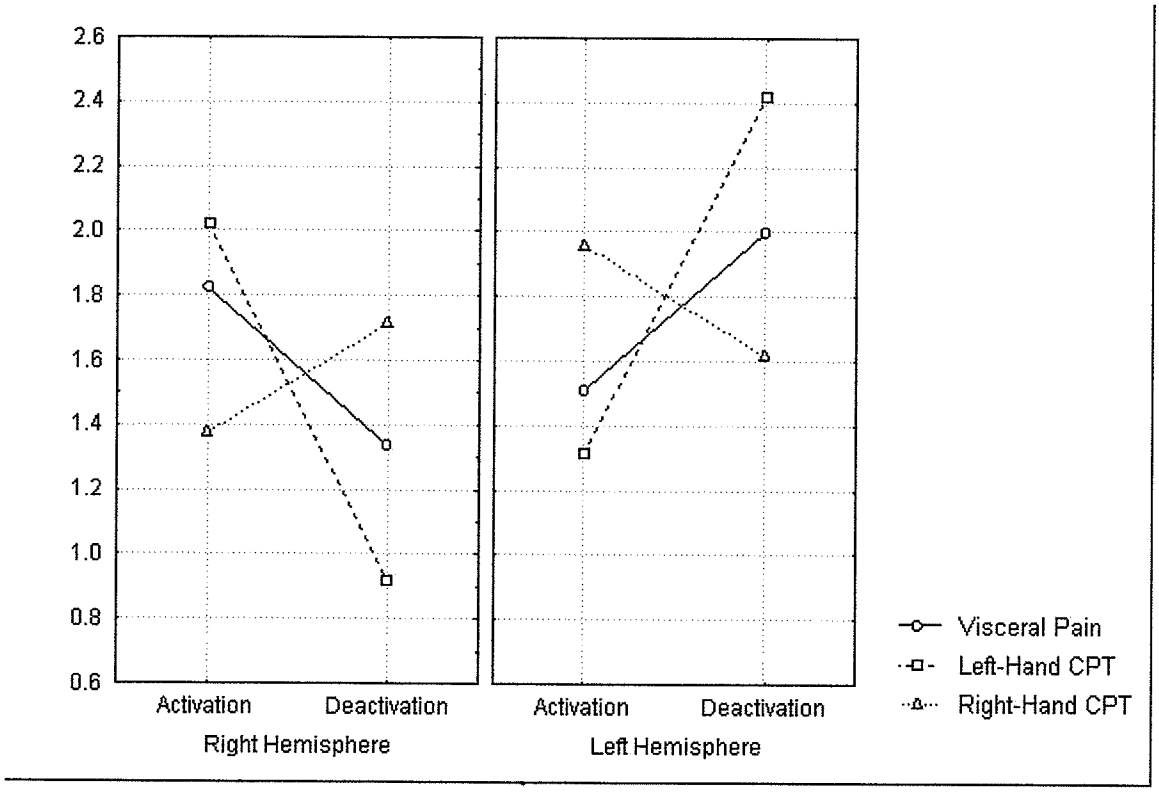

A
B
C
D
E
F
RMPFC%
LMPFC%
RPERI%
LPERI%
RMID%
LMID%

Figure Legend: Figure 5

Three-way interaction ($F(2,18) = 5.15$; $P = 0.071$) between task, activations and deactivations and left versus right hemisphere. On the left side, activations and deactivations are displayed for the right hemisphere. On the right side, activations and deactivations are displayed for the left hemisphere. For both the left and right hemisphere activation and deactivation patterns are similar for visceral and left-hand CPT pain and different form right-hand CPT pain. The vertical axis is in percent, the horizontal axis displays activations and deactivations.


2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
Activation
Deactivation
Right Hemisphere
Activation
Deactivation
Left Hemisphere
Visceral Pain
Left-Hand CPT
Right-Hand CPT

Part III

Distraction modulates Anterior Cingulate Gyrus Activations during the Cold Pressor Test

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Abstract

The Anterior Cingulate Gyrus (ACG) is part of a neural network implicated in attention- demanding tasks including the experience of pain. It has been shown that separate ACG sub-regions respond to cognitive demands and to painful stimulation. Since directing attention away from a painful stimulus is known to reduce the perceived pain intensity, we hypothesized that distraction from pain would result in both decreased activation of ACG sub-regions responsive to painful stimulation and increased activation of ACG sub-regions responsive to the distraction task. BOLD fMRI has relatively high spatial resolution and allows for better identification of ACG sub-regional responses than other neuroimaging techniques. Twelve subjects were tested using the Cold Pressor Test (CPT), a Verbal Attention Task (VAT), and a Distraction Task (DT) (a combination of the CPT and VAT). Analysis was performed on a voxel-by-voxel basis using a general linear model as implemented in SPM99. In addition to ACG activations common to both the CPT and VAT, we identified one CPT-specific cluster which was lateralized in an area corresponding to BA24'. The modulation effect of distraction on pain was assessed by contrasting (CPT-DT) and (DT-CPT). In support of our hypothesis, contrast (CPT-DT) revealed a decrease in BA24' during the DT and contrast (DT-CPT) showed increased activation in BA32/32'. These data suggest that distraction from pain and concomitant low pain ratings are reflected in distinct ACG sub-regional responses.

Introduction

The experience of pain is composed of sensory-discriminative, affective-motivational, and cognitive-evaluative dimensions (Melzack and Casey, 1968; Melzack and Katz, 1999). The sensory-discriminative and affective-motivational dimensions are involved in the quality, location, and perceived unpleasantness of the painful stimulus. Several studies suggest that the cognitive-evaluative dimension may modulate the pain experience by determining the amount of attention directed towards the stimulus (McCaul and Malott, 1984; Miron et al., 1989). Accordingly, it is a common experience that cognitive demands distract from the perceived intensity of painful stimuli. The exact brain areas that interact in this modulation are not well established. Thus, it is the aim of the present experiment to document attentional modulation of anterior cingulate gyrus (ACG) responses to painful stimuli using spatially-resolved fMRI.

Classic evidence for the involvement of the ACG in pain perception derives from frontal cingulumotomy (transection of the cingulum) patients who experience pain, but surprisingly report it as less bothersome (Foltz and White, 1962). Furthermore, electrophysiological studies in the rabbit (Sikes and Vogt, 1992) and human (Hutchison et al., 1999) have demonstrated neuronal activity in response to painful stimulation in the caudal aspect of the ACG. This region is consistent with Brodmann's Area (BA) 24' (Devinsky et al., 1995; Vogt et al., 1995). In addition, numerous imaging studies established that the ACG activates to subjectively painful stimuli (Casey et al., 1996; Derbyshire et al., 1998; Hutchison et al., 1999; Ingvar et al., 1999; Vogt et al., 1996). A recent attempt has been made to define the cognitively responsive regions of the ACG (Devinsky et al., 1995). This review argues that the cognitive division includes "caudal

BA24'and BA32', the cingulate motor areas in the cingulate sulcus, and the nociceptive cortex." These areas of the ACG are engaged in tasks involving either response selection associated with cognitive activity or the selection of a response to a noxious stimulus (for review see Devinsky et al., 1995; Ingvar, 1999; Kwan et al., 2000; Vogt et al., 1996). That these areas are involved in cognitive aspects of the response to noxious stimulation should not be taken as evidence that there are purely nociceptive responses in these areas.

Some functional imaging studies have attempted to link responsiveness in more purely cognitive tasks and responsiveness to painful stimuli in the same experiment. Both Positron Emission Tomography (PET) and Blood Oxygen Level Dependent functional Magnetic Resonance Imaging (BOLD fMRI) have been used to demonstrate that the ACG responds to tasks requiring attentional focus and to tasks involving the delivery of noxious stimuli. Separate ACG attention and pain related activation sites have been reported comparing the Stroop task and noxious thermal stimulation (Derbyshire et al., 1998) as well as an attention demanding task consisting of silent word generation and electrical stimulation (Davis et al., 1997). In the latter study attentional activation encompassed superior and anterior aspects of the ACG whereas painful stimuli activated inferior and posterior ACG sites. Likewise, Derbyshire et al. (1998) showed that individual analyses revealed only minimal overlap between sites processing pain or attention.

Recently, two PET studies have investigated the modulation of the brain's haemodynamic response to a painful stimulus occasioned by distraction tasks. One group (Petrovic et al., 2000) reported that the presentation of a distractor significantly modified

responses of somatosensory association, periaqueductal gray, midbrain and lateral orbitofrontal regions. ACG activity was not significantly altered. The other group (Peyron et al.,1999) studied how turning attention away from or toward a painful stimulus affected brain activation patterns. This study also investigated how the intensity of the stimulus (painful or not) affected brain activations. Areas in the ACG did not activate selectively to the painful stimulus compared to the non-painful stimulus. However, ACG sub-regions did respond selectively when attention was directed to the painful stimulus compared to when attention was directed away from the painful stimulus. The experiment (Peyron et al., 1999) showed that ACG activity pertained to the attentional neural activity triggered by the pain. These findings are contrary to observations made by other groups, who showed that ACG activity is selectively involved in the perception of thermal pain (Craig et al., 1996) and is also modulated by the perceived unpleasantness of the stimulus (Rainville et al., 1997).

The present fMRI study was designed to investigate the hypothesized modulation of ACG activity that occurs when a distraction task was superimposed onto a pain task. We were interested in exploiting the high spatial resolution of fMRI to describe ACG sub-regional BOLD responses to Cold Pressor Test stimulation when subjects focussed on the noxious stimulus or were distracted from it. A verbal attention task was used for the distraction task. This task has been shown to activate ACG sub-regions distinct from those processing noxious stimuli (Davis et al., 1997). The first objective of this experiment was designed to replicate separate ACG sub-regional responses to pain and attention tasks (Davis et al., 1997; Derbyshire et al., 1998). Then, we were further interested in documenting ACG sub-regional response modulation during distraction

from pain. Since directing attention away from a painful stimulus is known to reduce the perceived pain intensity (Hodes et al., 1990; McCaul and Malott, 1984; Petrovic et al., 2000; Peyron et al., 1999), we hypothesized that distraction from pain would result in decreased activation of ACG sub-regions responsive to painful stimulation and increased activation of ACG sub-regions responsive to the distraction task.

Materials and Methods

Subjects

Twelve healthy volunteers (all right-handed, mean age: 23.2 years [range: 19 – 39 years], 6 females) were recruited from the general population. Two subjects were excluded from the analysis due to excessive head movement. Studies were performed at the Health Sciences Center MR facilities in Winnipeg, Manitoba, Canada. Ethics approval was obtained from the National Research Council's Human Research Ethics Board. Subjects gave written informed consent and were free of exclusion criteria for MRI.

Tasks

Subjects were asked to close their eyes for all experiments. All experiments consisted of an initial 30-second (s) rest condition, followed by a 90-s stimulation condition and a final 60-s rest condition. To reduce within and between subject variability during the rest condition subjects were asked to relax and listen to the sound of the machine. Although controlling for intra and inter-subject variability during the rest

condition, non-linear changes may have been introduced from the experimental to the control task.

Cold Pressor Test (CPT)

Foot or hand immersion into 2-6°C water is a standard test for pain threshold evaluation and is known as the Cold Pressor Test. We used a unilateral cold compress (0-2°C) on the dorsal surface of the right foot to avoid the movement associated with traditional ice water immersion of the hand or foot. A trial run was conducted before each experiment to determine the water temperatures acceptable to the subjects. All subjects reported the 0-2°C cold compress as bearable but painful. At the end of each experiment subjects were asked to rate the pain from 0 (no pain) to 10 (worst pain imaginable). Reports of discomfort typically increased 10-30-s after application of the CPT.

Verbal Attention Task (VAT)

Silent word generation was used for the VAT. Subjects were asked to generate as many words within a given category of proper names (musicians, athletes...) or objects (fruits, vegetables...) without articulation. This task was chosen because it has previously been shown to activate regions in the ACG distinct from ACG regions responsive to pain (Davis et al., 1997). Subjects were told the word category immediately after the initial rest condition.

Distraction Task (DT)

The DT consisted of a combination of the CPT and VAT. After an initial rest condition the cold compress was applied to the foot. Distraction from the CPT was achieved by cueing subjects with a given word category (VAT) 45 s after the initiation of the CPT. This time point was chosen because of subject reports of increased discomfort after 10-30- s (see above) of cold compress application. The DT concluded with a final rest condition in which the cold compress was removed from the foot and the subject was asked to discontinue the VAT. As in the CPT subjects were asked to rate their pain on a scale from 0 (no pain) to 10 (worst pain imaginable).

Imaging Protocol

MRI experiments were conducted on a 1.5 Tesla whole body magnet (General Electric Signa Horizon LX) with a homogeneous birdcage coil. Eight 6mm contiguous axial-oblique slices were referenced and acquired parallel to the AC-PC line (anterior/posterior commissure, Talairach and Tournoux, 1988). Based on the eight slice acquisition limitation and individual differences in shape and size of the ACG, slice positions were moved parallel and up from the AC-PC line to optimize coverage of the ACG regions of interest. Sixty single-shot blipped gradient-echo planar images were acquired (TR/TE = 3000/60 msec, α = 80°, 64 x 64 matrix, 25 cm FOV). High-resolution T1-weighted gradient-echo images were obtained for the overlay of functional activation maps. Each experiment required a three-minute imaging period.

Data analysis

One objective of the analysis was to compare activations during the different stimulation conditions (VAT, CPT and DT) in relation to rest. This was achieved by a simple contrast analysis. A second objective was to compare activations common to both the VAT and CPT. This was achieved by masking the CPT activations with the VAT activations. To investigate the hypothesized ACG modulation effects due to the distraction task we used the contrasts (CPT-DT) and (DT-CPT). Contrast (CPT-DT) allowed for the identification of brain activations increased during the CPT as compared to the DT and contrast (DT-CPT) allowed for the identification of brain activations increased during the DT when compared to the CPT.

Image preprocessing and statistical analyses were all performed using Statistical Parametric Mapping 99 (SPM99) software (Friston *et al.*, 1994). In every time-series, the first scan was discarded prior to preprocessing, resulting in 59 volumes in each series. Then, for each subject and each session, all EPI volumes were realigned to the first volume in order to correct for three-dimensional movement of the head during time-series acquisitions. To allow group analysis, realigned images were spatially normalized to the MNI (Montreal Neurological Institute) brain template (SPM99) using affine and non-linear transformations. During normalization, all scans were re-sampled to $2mm^3$ isotropic voxels using sinc interpolation. Finally, all images were spatially smoothed using an 8-mm full-width-at-half-maximum isotropic Gaussian kernel, to improve signal-to-noise ratio and to account for residual inter-subject differences.

Statistical analysis was performed on a voxel-by-voxel basis, using a general linear model approach (Friston *et al.*, 1995), as implemented in SPM99.

First, a within-subject analysis was performed, with identical models across subjects (balanced design). For model estimation, individual data were temporally smoothed using a convolution with the haemodynamic response function. Also, a temporal high-pass filter (cut-off = 222 s) was applied in order to remove low-frequency confounding effects, such as cardiac and respiratory artifacts.

For each individual, the three different sessions (VAT, CPT and DT) were all included in the same design matrix in order to look at both simple and modulation contrasts, resulting in 81 degrees of freedom (177 scans) in the single-subject analyses.

Each session was identically modeled with two conditions (box-car convolved with the haemodynamic response function), corresponding respectively to the first 15 scans and the last 15 scans of the stimulation period. This model was used for the following reasons: in the DT experiment, the distraction from the CPT starts after 45-s (15 scans), so the distraction effect was analyzed only during the second condition (last 15 scans). Then, in the CPT experiment, only the second half (15 scans) of the CPT stimulation was taken into account, to be able to directly compare it to the DT (same epoch length and same "feeling of pain"). As we used an epoch-related design, the VAT was also analyzed only during the second half (15 scans) of the stimulation period.

Group analysis was performed using a random-effects procedure (Holmes and Friston, 1998). This procedure takes into account both random effects (within and between subjects components of variance) and fixed effects (activation due to a particular task) in order to provide a better generalization to a population effect. For this purpose, for each of the different contrasts of interest defined above, one contrast image per subject was entered into a one-sample *t* test across the 10 subjects. The resulting SPM

maps show the t statistics in every voxel for the group and for each effect of interest. Activations observed in the simple contrast analyses were thresholded at $P < 0.01$ uncorrected. The modulation effects were significant at a threshold of $P < 0.05$ uncorrected. An extent threshold of 10 voxels was used for all contrasts. We also report P-values corrected for multiple comparisons at the cluster level. Small volume corrections (SVCs) (Worsley et al., 1996) were performed on SPMs (using no extent-threshold) to assess the significance of some of the ACG activation foci when a priori hypotheses existed about well-defined coordinates of functional activity and corresponding anatomical areas. Also, pain ratings were entered as covariates of interest in a linear regression model to analyze both the CPT and DT activations as a function of the pain intensity.

In order to show anatomical locations of the different activations, SPM maps were superimposed on a high-resolution T1-weighted image taken from one of the 10 subjects. This T1-weighted image was first coregistered to the functional scans of the same subject and was then spatially normalized to the MNI brain template. Identification of ACG sub-regions was based on MNI coordinates and approximated to cytoarchitectural areas superimposed onto a flat map by Vogt et al. (1995). Thus, activations in the perigenual regions of the ACG were designated as BA32 and 24. ACG regions located caudal to these regions (also referred to as midcingulate or the caudal aspect of the ACG) were designated as BA24' and BA 32'. As well, areas 32 and 32' (the cingulofrontal transition cortex) form a dorsal rim around area 24 and 24'. Clusters with local maxima that were not clearly located in one cytoarchitectonic area were identified as such, for example BA32/32'.

Results

Behavioral Results

Subjective pain ratings were reduced during the DT condition when compared to the CPT condition. Average rating of pain intensity during the CPT was 5.8 (range: 2.5 – 8.5, S.D. = 1.8) and 4.5 (range: 1.5 –7, S.D. = 2.0) during the DT. This difference was significant at $P < 0.013$ (paired t-test on pain ratings). During the CPT trial run subjects also consistently reported increased discomfort after the first 10 – 30-s into the CPT.

FMRI Results

Table 1 summarizes the cluster size in voxels, the approximated anatomical location and MNI coordinates (x,y,z) of the peak voxels, as well as t values for local maxima of activations and corrected P values at the cluster level for the following analyses: 1. The simple contrasts VAT, CPT and DT ($t = 2.82$); 2. The masked analysis (CPT masked with VAT) ($t = 2.55$); and 3. The modulation contrasts (CPT - DT) and (DT - CPT) ($t = 1.83$). In each task and contrast, ACG activations are listed first in Table 1 and displayed as SPMs in Figures 1 and 3. Initial visual inspection of SPMs suggests substantial functional overlap of ACG activations between the VAT, CPT and DT (Figure 1A – C) as confirmed by the masked analysis of the CPT with the VAT (Figure 1D).

Verbal Attention Task

ACG activations during the VAT spanned approximately 1.2 cm at the level of (z =18 – 30mm). Within the activation cluster ($P < 0.002$ corrected), there were three local peaks (Table 1, Figure 1A [arrows]). One local maximum (-4, 32, 28) was left-lateralized in a superior and anterior region of the ACG and was located between BA32/32' (t = 9.11). Two additional more inferior ACG local maxima, were located medially (0, 22, 20) (t = 4.48) and left (-8, 18, 22) (t = 4.99) corresponding to BA24/BA24'. Comparing the group ACG activations in the three different tasks, it is clear that cluster size and significance were larger in the VAT and DT as compared to the CPT (Table 1, Figure 1).

Cold Pressor Test

During the CPT, ACG activations spanned a region of 1.4 cm at the level of (z = 18 – 32 mm). There were three, small, separate clusters (Table 1, Figure 1B arrows) in BA24. Two were located in more inferior regions of the ACG with local maxima at (0, 24, 20) and (12, 28, 22) (t = 3.41; t = 4.85, respectively) and one superior and left-lateralized with a local maximum at (–8, 10, 32) (t = 3.58). The two inferior CPT clusters (arrows in Figure 1B) spatially overlap with the VAT cluster in Figure 1A. To further assess this spatial overlap, we masked the CPT activations with the VAT activations ($P <$ 0.01) (Figure 1D). This analysis revealed two local ACG maxima at (-2, 22, 20) (t = 3.90) and (10, 26, 20) (t = 3.42) (Figure 1D, arrows). There are common regions of activation mostly medial (z = 20mm) and right – lateralized up to (z = 28 mm). Based on these results we were furthermore interested in assessing the significance of the two inferior CPT clusters, which are common to both, the VAT and CPT. Therefore a volume

of interest around the VAT local maximum (-8, 18, 22) was defined to perform a small volume correction (SVC) on the inferior CPT activations. After SVC, the two clusters did not remain significant ($P = 0.2$).

The superior left-lateralized CPT cluster in BA24' (Figure 1B arrow at level z = 32mm) was not present in the masked analysis suggesting a CPT-specific activation. To further assess the significance of this cluster an SVC was performed. According to electrophysiological recordings by Hutchison et al. (1999), we chose a small volume of interest around (-4, 8, 32) corresponding to BA24'. With this correction, the superior cluster was significant at $P = 0.031$. Moreover, in a correlation analysis of the signal intensity change in this voxel with individual pain ratings, there was a significant linear correlation between pain ratings and signal intensity ($r = 0.7$ and $P < 0.012$) (Figure 2).

Distraction Task

Two significant ACG local maxima, belonging to the same ACG cluster (Figure 1C [arrows], Table 1), were observed during the DT ($P < 0.0001$ corrected). Both were located in more superior planes, one left-lateralized at (-6, 20, 30) ($t = 6.05$) and the other right-lateralized (12, 30, 28) ($t = 6.99$) corresponding to BA32/32'. No local maxima were identified in BA24' although a very small activation can be observed at z = 32 mm in this region.

Modulation Effects

Figure 3 depicts the modulation contrasts (CPT-DT) and (DT-CPT). In the contrast (CPT – DT) (activations in blue-green color scale), a modulation effect was

observed in an ACG cluster with a local maximum at (-12, 0, 34) (t = 2.18) corresponding to BA24' (Table 1, arrow in Figure 3 at level z = 34mm). This local maximum shows increased activations during the CPT when compared to the DT. To further assess the significance of this cluster a small volume of interest still centered at (-4, 8, 32) (Hutchison et al., 1999) was used and led to a corrected $P = 0.16$.

Increased ACG activation due to the distraction task was assessed with contrast (DT - CPT) (activations in red-yellow color scale). With this contrast we identified one ACG cluster with two local maxima located in BA32/32' (Table 1, Figure 3 at levels z = 26mm and z = 30mm). As the activations in this contrast should be VAT specific, a volume of interest around the VAT maximum (-4, 32, 28) was chosen to perform the SVC. When corrected, the cluster was significant at $P = 0.012$.

Other modulation contrasts such as (DT - (VAT + CPT)) and ((VAT + CPT) - DT) were also tested on the group but did not show any significant clusters in the ACG ($P < 0.05$ uncorrected).

Discussion

The aim of the present experiment was to investigate the modulatory responses of ACG sub-regions when a distraction task was superimposed onto a pain task. Within our acquisition volume, we observed pain-related activations in prefrontal cortex, somatosensory association areas, the insula, and ACG. Selection of the ACG as a region of interest was motivated by a large body of evidence implicating the ACG in the processing of painful stimulation as well as by reports of separate ACG sub-regional involvement during pain and attention tasks (Davis et al., 1997; Derbyshire et al., 1998). Consistent with these previous neuroimaging studies on pain and attention (Davis et al.,

1997; Derbyshire et al., 1998) our results indicate that separate ACG sub-regions respond to cold pressor pain and a verbal attention task. In addition we also identified an ACG sub-region that commonly activated during both, the CPT and VAT. A surprising finding was the remarkable difference in the size of ACG activations between the CPT and VAT. Similar observations were made by Davis et al (1997), who reported that VAT-related ACG activations were typically larger than TENS-elicited pain activations. These results draw attention to the variability of ACG activations between tasks and furthermore emphasize caution when comparing ACG sub-regional responses from different pain studies using different noxious stimuli, imaging modalities and analysis methods. A cold compress was used in our study to avoid the movement associated with traditional submersion of the hand into ice water. Although inducing pain in all subjects, pain intensity ratings ranged from 2.5 to 8.5. Thus, stimulus type and individual differences in intensity ratings may account for the small ACG activations observed during the CPT. Nevertheless, consistent reports of pain and ACG activations during the CPT allowed for the further investigation of modulation responses during the distraction task. In contrast to other neuroimaging studies investigating the modulatory role of attention on pain (Petrovic et al., 2000; Peyron et al., 1999) our results show that BA32/32' activation is increased during the distraction task whereas BA24' activations are reduced.

Anterior cingulate cortex activations during the verbal attention task

One large cluster with three local ACG maxima was identified during the VAT. The localization of our VAT local maxima in BA32/32' and BA24/24' corresponds with those reported by Davis et al (1997) using the same verbal attention task. Since the VAT

is a component of the DT the majority of activations observed in the VAT were also observed in the DT (Figure 1A and C).

Anterior cingulate cortex activation related to pain

The ACG is a functionally heterogeneous region (Devinsky et al., 1995; Paus et al., 1993; Vogt et al., 1992) involved in motor and cognitive tasks and is the cortical region that is activated in almost every study of elicited pain (Ingvar, 1999).

The localization of pain activations observed in the group is consistent with electrophysiological recordings of ACG activations in humans (Hutchison et al., 1999) as well as other functional imaging studies of pain (Davis et al., 1997; Kwan et al., 2000; Porro et al., 1998; Vogt et al., 1996). The cold compress used in our study covered much of the dorsal surface of the foot and elicited average pain ratings of 5.8. Concerning the rather small size of ACG activations in the group, apart from the individual variability in pain ratings, activation size may be influenced by the type of stimulus used. Indeed differences in activation size have been reported when comparing phasic versus tonic pain (Derbyshire and Jones, 1998a) or small versus large contact surfaces (Apkarian et al., 2000). As well differences in stimuli such as heat versus cold or mechanical versus electrical stimulation may influence the amount of activation observed. Furthermore different imaging techniques (PET, SPECT, or fMRI) and analysis methods used to study pain make it difficult to precisely compare activation sizes between experiments.

The two PET studies (Petrovic et al., 2000; Peyron et al., 1999) investigating rCBF changes due to attentional processes in pain did not show any significant ACG activation when subtracting a non-painful thermal (N) condition from a painful thermal

condition (P). Our study was not designed to perform this contrast, but as expected, ACG activations were present in the simple contrast results. In an attempt to repeat the (P-N) contrasts we subtracted the first 15 scans during the CPT (reports of pain intensity are very low, see Methods) from the last 15 scans during the CPT (peak reports of pain-intensity) and found an absence of ACG activations similar to Peyron et al. (1999) and Petrovic et al. (2000). With an elegant experimental design, Peyron et al. (1999) demonstrated that ACG activity did not pertain to a pain-intensity coding network, but rather to the attentional neural activity triggered by the pain. This observation is consistent with electrophysiological findings made by Hutchison et al. (1999), who show that ACG neurons do not only fire in response to painful thermal stimulation, but also fire in response to "the watching of a painful stimulus" administered to the experimenter. The coordinates of these neurons correspond precisely with our CPT-evoked BA24' activations providing support for the idea that this region may be involved in nociceptive and attentional aspects of the pain experience.

It is also interesting to note that ACG activations are correlated with pain ratings (see also Coghill et al., 2000; Davis et al., 1997; Porro et al., 1998;). Indeed, the correlation analysis revealed an ACG cluster ($P<0.001$ corrected) in left BA24', close to the pain-specific activation during CPT (see also Figure 2). However, a positive correlation of brain activation with pain intensity does not exclude a possible attentional component that may be part of the ACG activations observed in BA24'.

Common areas of ACG activation during the CPT and VAT

A novel finding in our study was that the inferior ACG local maxima of the VAT appeared to spatially overlap with the inferior local maxima during the CPT (compare Figure 1A and B at level z = 20 mm and z = 22 mm). The MNI coordinates of these inferior local maxima were nearly identical (Table 1). To assess common regions of activation we performed a masked analysis of the CPT with the VAT which revealed activations in BA24/24' and the left insula. Contralateral insula activation was also shown by Peyron et al. (1999) who suggested that insula activation subserves the sensory-discriminative dimension of the pain experience. Using grouped analysis Derbyshire et al. (1998) reported common areas of ACG activation (midcingulate) during pain and attention tasks. However, individual analyses revealed widespread and independent areas of ACG activations, suggesting that the identification of common areas of activation using grouped analysis should be interpreted with caution. The common ACG activations during the VAT and CPT in our study were located in more inferior regions than those observed in the grouped analysis by Derbyshire et al (1998), placing them into the perigenual region, not the midcingulate. These perigenual activations may be involved in autonomic functions and/or affective dimensions common to both tasks (George et al., 1995; Pool and Ransohoff, 1949; Lewin and Whitty, 1960)

Modulation of anterior cingulate cortex activation during the distraction task

As hypothesized, the distraction task resulted in a decrease of ACG activations responsive to pain and an increase of ACG activations when the VAT was used as a distraction task.

During the distraction task Peyron et al. (1999) observed a dissociation between pain ratings and activation in BA24. We observed a similar dissociation in the (DT-CPT) contrast (Figure 3). However the local maximum of this cluster (-6, 26, 30) was located anterior and superior to the local maximum (-10, 16, 26) reported by Peyron et al., (1999) placing it in BA32/32'. The silent word generation task (VAT) used in our study may account for this difference. The local maxima of both the VAT (Figure 1A) and the (DT-CPT) contrast (Figure 3) seem to belong to the same ACG region (arrows in Figure 1A and 3) suggesting a VAT-specific response.

Furthermore, the (CPT-DT) contrast revealed a left–lateralized BA24' activation at (-12, 0, 34) (Table 1, Figure 3). The significant decrease in signal intensity in this voxel during the DT suggests decreased activations in BA24' during distraction. Using hypnotic suggestion, this type of modulation was demonstrated by Rainville et al. (1997) who observed that pain-related activity in ACG closely paralleled a selective change in the perceived unpleasantness of the stimulus. Likewise, distraction from a painful stimulus, such as the CPT, may alter the perceived unpleasantness of the painful stimulus and therefore result in a reduction of activations in BA24'. To further analyze this cluster, we chose the same volume of interest for a small volume correction as for the CPT (-4, 8, 32) which led to a low corrected P value of 0.16. In Figure 3 (also see Table 1), it can be seen that the (CPT-DT) local maximum is located superior and more lateral than the CPT- specific local maximum, however, both clusters correspond to BA24'. Three subjects were not effectively distracted (pain intensity ratings stayed the same during the CPT and DT), which may tend to reduce the significance of the distraction effect in the group. Although a detailed discussion of the individual results is beyond the

scope of this study, it is appropriate to note that six out of ten subjects showed a significant modulation in BA24'.

Conclusion

The ACG is part of a large number of neuronal areas involved in the multidimensional experience of pain. It should be emphasized that our focus on ACG sub-regional responses was *by no means* an investigation of a 'pain center', but was an effort to more precisely examine ACG sub-regional responses, which are part of a larger neuromatrix involved in the perception of pain. Our study identified common and selective ACG sub-regional responses to separate pain and attention tasks as well as modulatory ACG sub-regional responses when an attention task was superimposed onto a pain task. The results from this study suggest that distraction from pain is reflected in a modulation of activity in distinct ACG sub-regions involved in the processing of painful and cognitive stimuli. Lowered pain intensity ratings during the distraction task may relate to activation of ACG regions specific to the verbal attention task and the attendant reduction of activity in BA24'.

Figure Caption: Table 1

The table displays the cluster size, anatomical location, MNI coordinates (x,y,z), and *t* values of peak voxels, as well as corrected *P* values at the cluster level during the VAT, CPT and DT tasks (thresholded at $t = 2.82$, $P < 0.01$ uncorrected), the CPT masked by the VAT (thresholded at $t = 2.55$, $P < 0.01$) and the contrasts (CPT - DT) and (DT – CPT) (thresholded at $t = 1.83$; $P < 0.05$ uncorrected). ACG activations are highlighted in grey. SVCs are indicated with a '*'. X represents the left-right axis from midline (negative = left), Y represents the front-back axis (negative = posterior to the anterior commissure) and Z represents the up-down axis (negative = ventral to the AC-PC line).

Foci of Significant BOLD Signal Increases during the VAT, CPT, CPT masked by VAT, DT, (CPT - DT) and (DT-CPT)

Cluster size	Anatomical location of peak voxel	Coordinates (mm) x	y	z	*T* Value	Corrected *P* Value
	Verbal Attention Task (VAT)					
532	ACG BA32/32'	-4	32	28	9.11	**0.002**
	BA24/24'	-8	18	22	4.99	**0.002**
	BA24/24'	0	22	20	4.48	**0.002**
804	Broca's Area BA45	-46	24	16	5.83	**0.000**
	Supplementary Motor BA6	-40	0	34	4.41	**0.000**
43	Dorsolateral Prefrontal BA10	-36	54	8	4.16	0.768
36	Parietal Cortex BA7	-28	-68	48	4.06	0.793
13	Insula	34	30	10	3.52	0.956
	Cold Pressor Test (CPT)					
64	ACG BA24/24'	12	28	22	4.85	0.200 *
23	BA24'	-8	10	32	3.58	**0.031** *
15	BA24/24'	0	24	20	3.41	0.200 *
213	SS/Supram. Gyrus BA2/40	52	-26	24	6.11	**0.050**
	Supram. Gyrus BA40	62	-18	20	5.22	**0.050**
328	Insula	40	0	18	4.94	**0.010**
	Insula	34	12	12	4.26	**0.010**
	Claustrum	28	-12	18	4.47	**0.010**
64	Dorsolateral Prefrontal BA10	-34	50	14	3.77	0.540
18	Primary Motor BA4	-58	-12	38	3.38	0.938
	CPT masked by VAT					
75	ACG BA24/24'	-2	22	20	3.90	0.506
	BA24/24'	10	26	20	3.42	0.506
15	Insula	-30	20	16	3.02	0.932
	Distractor Task (DT)					
845	ACG BA32/32'	12	30	28	6.99	**0.000**
	BA32/32'	-6	20	30	6.05	**0.000**
1093	Dorsolateral Prefrontal BA10/46	-30	46	10	6.19	**0.000**
	BA10	-36	14	28	5.32	**0.000**
	Broca's Area BA44/45	-48	20	16	4.55	**0.000**
82	Putamen	20	4	14	4.05	0.451
	Thalamus	16	-6	16	3.43	0.451
84	Parietal Cortex BA7	-38	-62	44	3.96	0.433
20	Supram. Gyrus BA40	-24	-50	40	3.85	0.912
	Modulation (CPT - DT)					
15	ACG BA24'	-12	0	34	2.18	0.160 *
12	Mid Cingulate BA 24'	14	-12	34	2.30	1.000
778	SS/Supram. Gyrus BA2/40	52	-22	24	6.96	**0.049**
369	Supram. Gyrus BA40	-52	-24	30	3.15	0.341
27	Supplementary Motor BA6	-60	4	22	3.15	0.998
	Modulation (DT - CPT)					
120	ACG BA32/32'	12	30	26	4.35	**0.012** *
	BA32/32'	-6	26	30	2.57	**0.012** *
28	Posterior Cingulate BA31	4	-40	36	2.21	0.998
114	Caudate	4	12	14	3.86	0.918
622	Dorsolateral Prefrontal BA9	-40	22	32	3.79	0.100
129	Parietal Cortex BA7	-28	-54	46	3.08	0.889

Figure Caption: Figure 1

Statistical parametric maps of BOLD signal increases in ACG during (A) the verbal attention task (VAT), (B) the cold pressor test (CPT, consisting of a cold compress at 0-2 °C on the dorsal surface of the foot), (C) the distraction task (DT) (consisting of a combination of tasks (A) and (B)) and (D) the CPT masked by the VAT (thresholded at $P < 0.01$). A-D were thresholded at an uncorrected P value of 0.01. The activations are colour-coded from red to white corresponding to the t value (bar code on the right). SPMs are displayed on a single subject T1-weighted anatomical image transformed into MNI space. Images are displayed according to neurological convention, the left hemisphere is on the left and the right hemisphere on the right side of the image. The plane of the displayed brain regions is indicated on the top of each column, spanning from z = 20mm to z = 34mm. The arrows identify the local ACG maxima discussed in the text (also refer to Table 1).

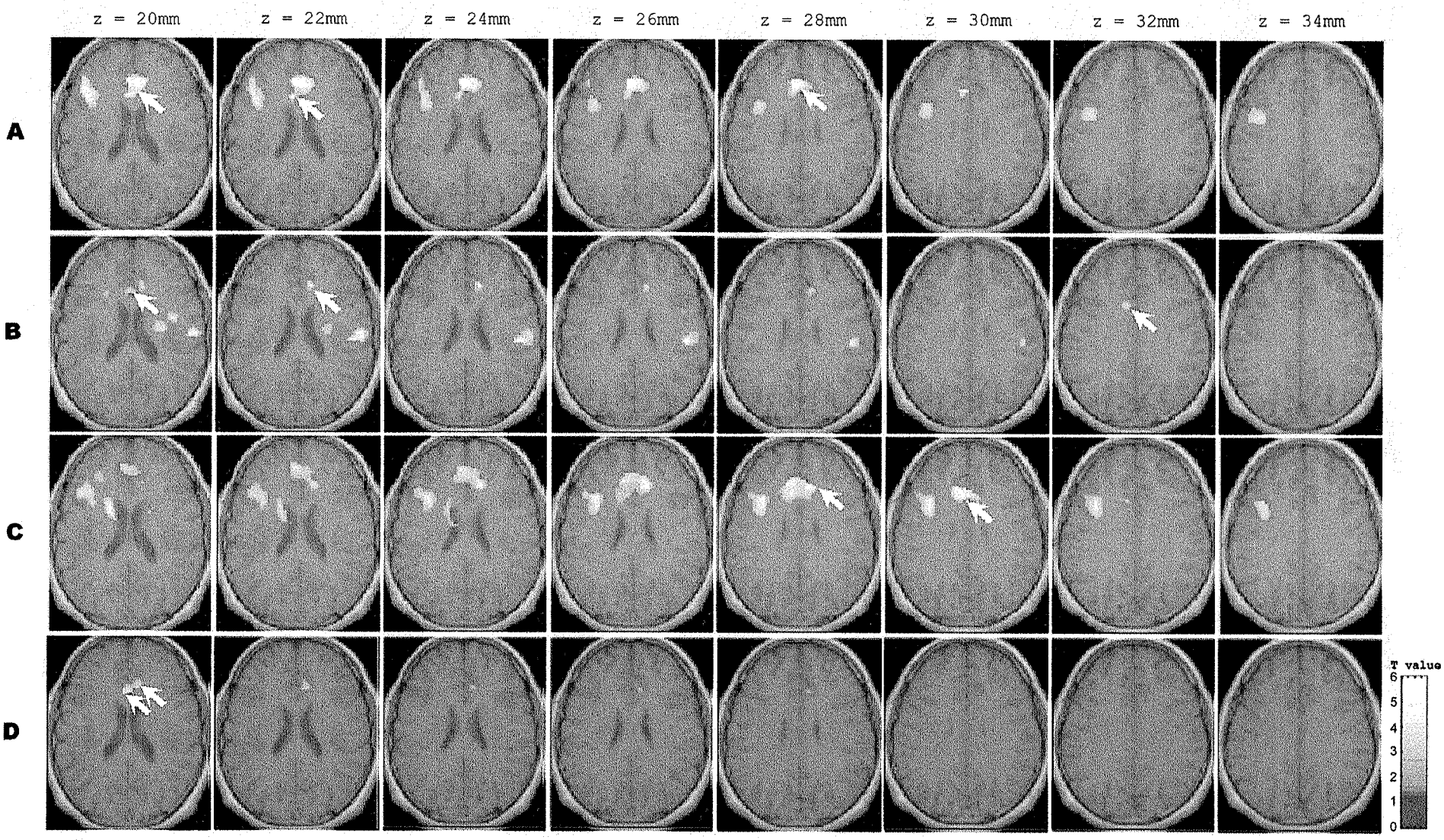

z = 20mm
z = 22mm
z = 24mm
z = 26mm
z = 28mm
z = 30mm
z = 32mm
z = 34mm
A
B
C
D
T value
6
5
4
3
2
1
0

Figure Caption: Figure 2

Correlation between pain ratings and adjusted fMRI signal changes during the CPT, as evaluated with the individual time-courses at voxel (-8,10,32) (CPT local maximum). The correlation is significant at $P<0.012$.

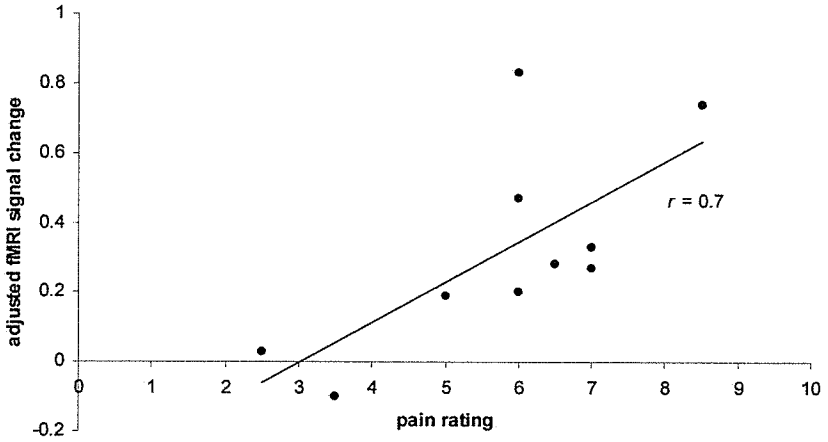
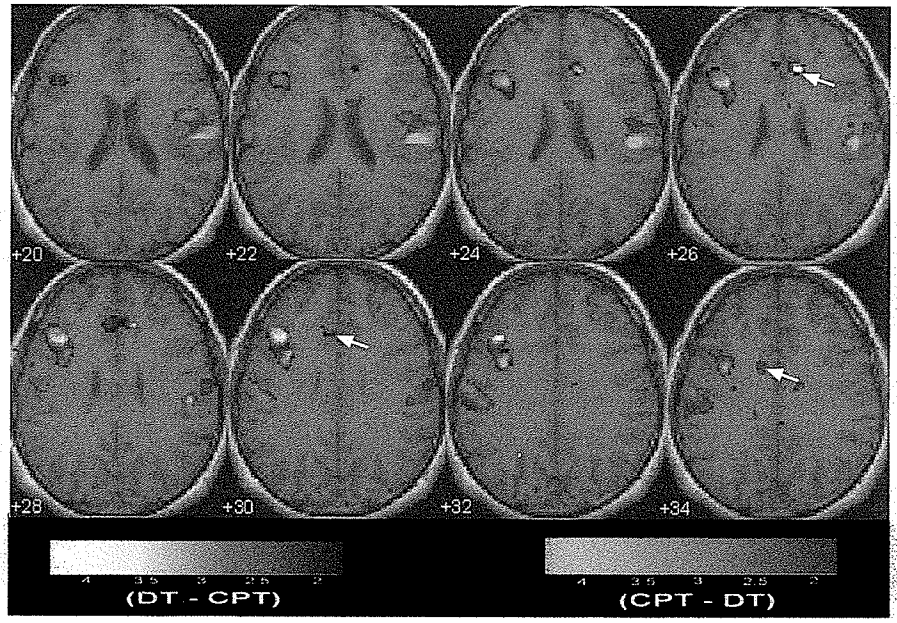

adjusted fMRI signal change
pain rating
r = 0.7
1
0.8
0.6
0.4
0.2
0
-0.2
0
1
2
3
4
5
6
7
8
9
10

Figure Caption: Figure 3

The modulation contrasts (CPT – DT) and (DT – CPT) were thresholded at an uncorrected *P* value of 0.05. The plane of the displayed brain regions is the same as in Figure 1 and indicated on the left-hand bottom of each image. Activations in the blue-green colour scale represent the modulation contrast (CPT – DT) and activations in the red-white colour scale represent the modulation contrast (DT – CPT). The arrows identify the local ACG maxima.


+20
+22
+24
+26
+28
+30
+32
+34
4 3.5 3 2.5 2
(DT - CPT)
4 3.5 3 2.5 2
(CPT - DT)

GENERAL DISCUSSION

The neuromatrix for pain expands beyond the ACG and MPFC to include the dorsolateral prefrontal cortices, somatosensory cortices, the periacqueductal grey matter, thalamus as well as the insula, motor, and inferior parietal cortices. Thus Melzack's original concept that the experience of pain is composed of sensory, affective and cognitive components has been confirmed by neuroimaging studies (Derbyshire 2001). Since the first PET reports of ACG activation during phasic heat pain (Jones et al., 1991; Talbot et al., 1991) the ACG, (Ingvar, 1999) together with the MPFC, has become one of the key brain regions in somatosensory pain processing. Relatively little is known about the cortical representation of these regions during visceral pain processing.

Therefore it was the objective of this thesis to further characterize ACG and MPFC activity during visceral and somatosensory pain using spatially resolved BOLD fMRI. A number of experimental designs were used to address basic questions regarding ACG and MPFC activity during both pain modalities in normal and clinical populations as well as the role of ACG sub-regional activity in pain and attention processes.

Our investigations of brain activity during visceral pain show pain-specific and graded responses in several cortical areas and also suggest that ACG activation and MPFC deactivation patterns can be used to differentiate between control subjects and patients suffering from gastrointestinal disorders (Bernstein et al., 2002). Furthermore, ACG and MPFC activation patterns are not unique to visceral stimulation, but also reveal similarities in comparison with cold pressor pain (Frankenstein et al., 2002 submitted; McIntyre et al., 2002). Attention plays a pivotal role in acute and chronic pain. Our replication of separate ACG sub-regional involvement in pain and attention tasks and

moreover, the demonstration that ACG sub-regional pain responses are decreased during a distraction task provide insight into how cognitive processes can modulate physiological brain responses to painful stimulation (Frankenstein et al., 2001).

Collectively, these studies show that MPFC and ACG sub-regional responses may proof to be useful in differentiating between health and disease (Bernstein et al., 2002). In addition, ACG and MPFC response patterns are not unique to visceral pain (Frankenstein et al., 2002, submitted). Finally, it is important to recognize, that ACG sub-regional responses to pain are influenced by the degree of attention directed towards the stimulus (Frankenstein et al., 2001) and may therefore have important implications on studies of pain in different patient and control groups.

ACG and Prefrontal Responses during Visceral Pain

The first part of this thesis (Bernstein et al., 2002) was designed to better understand cortical processing of visceral pain in control subjects and patients suffering from IBS or IBD. It was hypothesized that different patterns of brain response would be found in control subjects and patients with gastrointestinal disease undergoing noxious visceral stimulation. The main findings of this work are discussed below in reference to the latest imaging literature on visceral pain in general.

First, we were interested in determining whether any ROI would clearly differentiate patient and control groups. Using pixel counts we showed a higher percentage of ACG activation in response to stool and painful stimulation in control subjects and to a lesser degree in IBS and then IBD groups. In the first visceral pain study using PET, Silverman et al., (1997) showed a left predominant ACG activation in

response to simulated and actual painful rectal balloon distention in control subjects. This activation was not observed in IBS patients, however, the left prefrontal cortex activated in IBS subjects during the anticipation of rectal distention. The total absence of ACG activation in the IBS group is not consistent with the observations made by our group (Bernstein et al., 2002). However, a recent report by the same group (Naliboff et al., 2001) is consistent with our findings. The authors showed that IBS patients did activate the ACG in response to innocuous rectal distention and its anticipation, but to a lesser degree than control subjects. They argue that the decreased activation of the IBS patients in the perigenual region of IBS subjects is consistent with their previous paper documenting an absence of ACG activity during actual painful stimulation. In terms of other differences between their two studies, the authors point to smaller sample sizes, several methodological differences, including the less invasive nature of the balloon placement in the earlier study. Based on these results it can be concluded that IBS patients show an overall reduced ACG response to visceral stimulation. Our findings are the first to extend these observations to subjects suffering from IBD.

In comparison to control subjects, Naliboff et al. (2001) showed that IBS patients consistently showed greater activation in a specific sub-region of the ACG, which they call the rostral portion of the ACG. Our ACG ROIs were not specifically selected to investigate the rostral portion of the ACG. In our study, activation in this region may have been part of the ACG ROI, which included both rostral and perigenual regions. The rostral portion of the ACG borders the midcingulate, a region found to correlate with pain unpleasantness ratings (Rainville et al., 1997). These findings are intriguing as IBS patients also show hyperalgesic responses to rectal distention (Bernstein et al., 1996).

These hyperalgesic responses may be reflected in higher unpleasantness ratings and thus greater activation of rostral ACG. Interestingly, in our paper on ACG activation during distraction from CPT pain (Frankenstein et al., 2001), we found a reduction in BOLD signal intensity in a similar area of the rostral ACG as Naliboff et al., (2001) and Rainville et al., (1997). We postulated that this reduction is due to the decreased feeling of unpleasantness accompanying distraction from pain, which was supported by lower pain ratings during this task. Further investigations of these ACG sub-regions during visceral pain may shed some light on the functional specialization of these specific sub-regions.

The first fMRI study (Mertz et al., 2000) on painful rectal distention showed findings contrary to those observed by us (Bernstein et al., 2002) and Silvermann et al. (1997). Mertz et al.'s (2000) results showed that IBS patients had increased ACG activations when compared to control subjects, whereas our group (Bernstein et al., 2002) and others (Silvermann et al., 1997) reported decreased activity in this region. The authors report that IBS patients had a greater number of pixels activating in the ACG and reported more intense pain at 55 mm/Hg rectal distention than control subjects. Again, one of the reasons for the apparent discrepancy between Mertz et al.'s (2000) and our results is the different specification of ROIs. Mertz's group investigated BA24, whereas our group separated the ACG into perigenual (BA24/32) and midcingulate (BA24'/32') sub-regions. It is also difficult to determine whether Mertz et al.'s results (2000) correspond with those of Naliboff et al., (2001). Without knowing the precise location of specific ACG ROIs in different studies it is impossible to draw direct comparisons. However, we cannot rule out the possibility that direct comparisons of the same BA24

regions between studies may yield similar results. A further difference between Mertz et al.'s and our study is that we normalized ROI pixel counts. This was done by expressing the data for each ROI as a percentage of the total number of active pixels. This was done to take into account that the meaning of 20 active pixels in an ROI is different for a subject with a total of 80 active pixels than for a subject with 200 active pixels. This consideration was not made when comparing differences in pixel counts in the Mertz et al. (2000) study.

In our paper (Bernstein et al., 2002), patient and control subjects were further differentiated by a deactivation in somatosensory cortex and an MPFC deactivation that bordered on statistical significance. IBS patients showed the largest deactivation in left somatosensory cortex, followed by the IBD and control subjects. The deactivation in MPFC was largest for the control subjects. Relevant to this observation was another study where esophageal distention also activated the ACG (Aziz et al., 1997). This study reported concomitant decreases in rCBF in right MPFC during innocuous and noxious esophageal stimulation. The authors suggest that the reduction of rCBF in this region represents a central response to cope with the esophageal stimulation and the emotional impact associated with it. Similarly, our group has argued that the BOLD signal intensity decreases observed in our studies of visceral and somatosensory pain may reflect active behavioural inhibition of emotional processes during a painful experimental condition (Bernstein et al., 2002). Therefore it is probable that the relative decrease in MPFC deactivations observed in both IBD and IBS patients may represent aberrant activity in areas associated with emotional processing. This is furthermore supported by a large number of anatomical, electrophysiological, lesion and functional imaging studies

implicating MPFC in the affective dimension of the pain experience (see general introduction).

Finally, we found that the ACG shows different activation patterns to noxious visceral stimulation. These include graded responses to the sensation of stool and pain as well as pain-specific on/off responses. Mertz et al., (2000) conclude that "in control subjects there was no significant increase in activation of these areas with pain when compared to the sensation of stool." Again, these results are contrary to our observations of pain-specific and graded ACG activity in control subjects as well as in patient groups. Using only pixel counts as a dependent variable in Mertz et al.'s (2000) paper may overlook the presence of different patterns of response in ACG. Although the number of activated pixels may not differ significantly between pain and the sensation of stool, the activation to the sensation of stool might be at a lower level than the activation to pain. Graded activation has been previously reported in the fMRI literature for motor and GI pain (McIntyre et al., 1996; 1997)

Reports on ACG and PFC activity in other chronic pain states are few. A frequent finding is that alleviation from chronic pain results in an increase of thalamic activity after relative hypoperfusion of this area before treatment. When compared to IBS patients, a greater thalamic response has been reported in control subjects experiencing innocuous visceral stimulation (Naliboff et al., 2001). Experiments are underway in our laboratory to further investigate thalamic and other subcortical and cortical responses to visceral pain.

A somewhat similar comparison to our study on IBD and IBS has been conducted by Jones and Derbyshire (1997). The authors compared brain activation patterns in

patients suffering from rheumatoid arthritis, which has a definite inflammatory component and patients suffering from psychogenically maintained atypical facial pain. Rheumatoid arthritis patients showed significantly reduced ACG (BA24) and cingulofrontal transition cortex (BA32) responses to standardized heat pain when compared to control subjects and patients with atypical facial pain. Although we used a noxious visceral stimulus, we also showed that IBD patients, who have a definitive inflammatory disease component, showed reduced ACG (BA24/32) activity when compared to control and IBS subjects. IBS patients activated more than IBD, but less than control subjects. As discussed above, it is probable that reductions in ACG activity in patient groups reflect adaptive behavioral responses designed to cope with the painful stimulation.

Methodological Considerations: Imaging Visceral Pain

Several methodological concerns should be kept in mind when performing functional imaging experiments on visceral sensation and pain. Recruitment of control subjects willing to participate in experiments that require balloon distentions of the esophagus or rectum, is difficult. Patients with gastrointestinal disease have a more personal interest in participating in this type of research and are more likely to volunteer. Control subjects are difficult to obtain. A number of control subjects in these experiments have medical or scientific backgrounds. It may be that these subjects display unusually composed responses to an arduous experimental paradigm. The difficulties in subject recruitment is a factor in the design and interpretation of data. We have designed experiments, for example by using each subject as their own control, to avoid some of the

inherent difficulties deriving from subject recruitment. Normalization is another accommodation.

The administration and experience of a visceral pain stimulus is complex. Balloon insertion into the rectum or esophagus requires a specialist. In our case, all balloon insertions were conducted by a gastroenterologist or his research nurse. Before the actual imaging experiment, subjects received a fleet enema. Then a test protocol was conducted to ensure that the subject understood the paradigm and to ally anxiety in terms of what to expect from the distention protocol. Anxiety and stress are part of the experience of a pain in general, but may be more so in an uncomfortable situation of experimental visceral pain. Although we did not observe differences in terms of pain intensity ratings between somatosensory and visceral stimuli, we did not specifically assess the differences in discomfort/anxiety levels between the visceral and somatosensory pain conditions. Pain/anxiety rating scales that are better designed to point out these differences should proof useful. Interestingly, in our comparison between visceral and somatosensory pain (Frankenstein et al., 2002, submitted) we did not see any differences in the activation of perigenual or midcingulate regions suggesting that there may have been no differences in unpleasantness or discomfort between the two pain modalities in our study.

A further consideration in performing functional imaging experiments on visceral sensation and pain is whether to use computer-driven balloon distentions relative to manual balloon inflations. The majority of studies examining visceral sensation and pain use the former method (Mertz et al., 2000, Naliboff et al., 2001, Silvermann et al., 1997). The advantage of phasic computer driven balloon distentions is that they introduce

identical stimuli to each subject. We chose to use a manual balloon inflation technique (simulating more of a ramp distention) because our interest was to establish imaging at the moment the subject experienced a sensation of stool or pain. This was accomplished by the subject alerting the investigator at the moment the balloon volume reached a level that simulated a stool or painful sensation. Table 2 provides an example of the different inflation volumes needed to produce the sensations of stool and pain in different subjects. This table underscores that using the exact same inflation volumes from subject to subject may yield different sensations in different subjects. What may be a painful stimulus in one subject may be merely a slight sensation in another. Ultimately, our group will compare computer driven phasic distentions and manual balloon distentions to determine if the method of stimulus delivery affects activation patterns.

Subject #	Stool/cc	Pain/cc
S1	115	215
S2	100	205
S3	80	140
S4	45	95
S5	55	115
S5	89	140
S7	140	180
S8	147	281
S9	146	206
S10	40	140

Table 2

ACG and Prefrontal Activity during CPT and Somatosensory Pain

There have been many studies examining the cortical representation of a variety of somatosensory stimuli. These include tonic or phasic delivery of stimuli to the skin using heat, cold, electricity and injection of capsaicin or ascorbic acid. ACG responses to noxious somatosensory stimuli are primarily contralateral, but also often bilateral (Peyron

et al., 2000). In our papers (Frankenstein et al., 2001; Frankenstein et al., 2002, submitted) we used the CPT, a tonic cold stimulus, and observed significant contralateral ACG activation in one study (Frankenstein et al., 2001) and bilateral activation in the other (Frankenstein et al., 2002). In the latter study, more activation was observed on the contralateral side; however, the differences in activation between the contralateral and ipsilateral sides did not reach statistical significance. These results are generally consistent with other neuroimaging studies employing tonic cold pain (Casey et al., 1996; Kwan et al., 2000; Petrovic et al., 2000). For example, Casey et al. (1996) reported robust contralateral midcingulate activation in response to phasic heat and tonic cold pain whereas Petrovic et al. (2000) observed bilateral ACG activation during CPT pain.

In part II of this thesis (Frankenstein et al., 2002, submitted) we compared ACG and MPFC activation patterns during CPT pain to activation patterns observed during visceral pain in the same subjects. It was hypothesized that ACG and MPFC activation patterns would arise during visceral and somatosensory pain but that differences in the overall patterns of activation would arise because of the lateralized presentation of somatosensory pain and midline stimulation inherent in visceral pain.

Although we expected our visceral stimulus to be more uncomfortable and painful, we did not observe any significant differences in pain ratings between CPT and visceral pain. Based on this behavioural result and the demonstration that cortical activation to pain has been shown to correlate with pain ratings (Coghill et al., 1999; Frankenstein et al., 2001) we did not expect any significant difference between the percentages of activated or deactivated pixels between the two pain modalities, which was the case. The amount of activation and deactivation was task-independent. This

result gave us the opportunity to compare activation and deactivation patterns in ROIs that were due to equivalently intense stimuli. Our study is the first comparison of activation patterns due to visceral and somatosensory pain in the same subjects with equivalent pain ratings.

During both, CPT and visceral pain, midcingulate activation and deactivation was most prominent. In our previous study (Bernstein et al., 2002) the largest percentage of activation was observed in the ACG ROI that included perigenual and the rostral ACG bordering the midcingulate. The variation between these observations may result from different slice orientations and numbers. In the CPT/visceral comparison slices were acquired parallel to the AC-PC line and slices below the AC-PC line were not included in the analysis. In Naliboff et al.'s (2000) study, perigenual activations were most prominent during anticipation and sensation of the visceral stimulus. Images were not acquired during actual painful stimulation. Therefore it is not possible to determine the involvement of midcingulate regions during actual painful visceral stimulation in that study. However, in our study, the preponderance of activations in the midcingulate region is consistent with BA24' activations observed during CPT pain in part III of this thesis.

A further novel finding in this study was the lateralization tendency of activation and deactivation during visceral and CPT pain. There was no significant lateralization for visceral and somatosensory pain, however, the ACG and MPFC activation and deactivation pattern to visceral pain resembled that of left-sided somatosensory pain and both differed from right-sided somatosensory pain. The interaction analysis revealed that activation during left-hand CPT and visceral pain increased in the right hemisphere and

deactivation decreased in that same hemisphere. The opposite pattern was apparent for right-hand CPT pain. There is debate in the literature regarding the origin of deactivations. Our results do not support "a steal phenomenon", as one would expect areas of increased activation to also show increased deactivation. Rather our data suggest a hemispheric inverse relationship (i.e. as activation increases in one hemisphere, deactivation decreases) between activation and deactivation during both pain modalities.

Part III (Frankenstein et al., 2001) was designed to investigate the role of attention in pain and determine whether attention to and away from a noxious stimulus can modulate subjective responses to pain. We hypothesized that a cognitive task that is presented during a painful stimulus will result in reduced activation in ACG regions responsive to pain and increased activation in ACG regions responsive to the distraction task. At the onset of this project there were no known reports on the effect of cognitive tasks on the cerebral representation of pain. Our work resulted in the first fMRI publication (Frankenstein et al., 2001) investigating ACG sub-regional modulation during distraction from pain. After the replication of separate ACG sub-regional responses to pain and attention tasks, we were able to test our hypothesis by performing subtraction analyses of the CPT and DT tasks (for discussion see below: complementarity of data analysis methods; SPM99). First, we noted that pain intensity ratings were reduced upon distraction from pain. Moreover, we provided support for our hypothesis that distraction from pain resulted in decreased activation in BA24' and increased activation in BA32/32'. We also identified common areas of activation in the attention and pain tasks.

During the course of our experiment two PET studies were published on the effects of distraction tasks on the cerebral representation of pain (Petrovic et al., 2000;

Peyron et al., 1999). These are discussed in detail in the discussion of part III. Peyron et al., (1999) reported a dissociation between pain ratings and activation in BA 24 and Petrovic et al., (2000) did not report a significant modulation in ACG. Since our work, two fMRI studies were published on the topic (Bantick et al., 2002; Tracey et al., 2002). Tracey et al.'s (2002) work focused on the PAG and showed that activation in the PAG was significantly increased during the distraction task. ACG activation was not assessed. Bantick et al. (2002) showed that distraction from pain resulted in increased activation in brain regions associated with pain affect such as the perigenual ACG and orbitofrontal cortex. Reduced activation was observed in several regions of the pain neuromatrix including the thalamus, insula and midcingulate. The reduced activation in the midcingulate region seems to be in general agreement with our result of decreased BA24' activity during distraction from pain.

Methodological Considerations: Imaging CPT Pain

There are both, simple and complex issues that should be kept in mind when using the CPT as a somatosensory pain stimulus. In our studies we used a version of the CPT adapted for administration within the magnet. The traditional CPT requires immersion of the hand or foot into a bucket of 0-2 degree ice water. Obviously, this test is associated with a lot of movement by the subject. To avoid this problem, we used a 0-2 degree cold compress that could be applied to either the hand or foot. Application of this compress was effective in reducing movement as there was no automatic grasping reflex associated with CPT application to the foot.

The CPT is not a very precise pain stimulus, although a stimulus consisting of a 0-2 degree ice water compress on the hand or foot reliably produces ACG activation in sub-regions responsive to pain. Additional experiments with event related designs would require a thermode with fast rise and fall times. The CPT goes through stages of cool to very cold to painfully cold. In our experiments the subjects typically rated the stimulus as painfully cold after 30 seconds of application. These responses have to be kept in mind when conducting data analysis.

Methodological concerns of a more complex nature come into play when considering the physiology of the CPT in more detail. The CPT procedure is generally referred to as the "cold pressor" test due to its ability to evoke substantial cardiovascular reactivity, especially elevations in blood pressure (Allen et al., 1992). CPT pain leads to afferent stimulation of the spinothalamic and spinoreticular tracts. The spinoreticular tract projects to reticular neurons, which have an intimate association with cardiovascular-regulating areas in the medulla. The excitation of neurons in this area causes increased cardiac output and vasoconstriction in the skin and, hence, increased blood pressure (Lovallo, 1975). Although the brain has auto-regulatory mechanisms, both modest and more extreme elevations in blood pressure can result in increases of cerebral blood flow in particular cortical areas (Tuor et al., 1986). Therefore, it is probable that activations during CPT pain, with its corresponding increase in blood pressure, may be affected by concurrent increases in blood flow and venuous oxyhemoglobin levels. Differences in blood pressure may also partly explain the large range of individual differences in BOLD fMRI signal intensities observed in different studies. To date it is unclear whether activations due to CPT pain are purely due to the noxious aspects of the stimulus.

Carefully designed experiments aimed at separating "purely" autonomic aspects from nociceptive components of the response may shed light on this issue.

Thus, there are factors other than neuronal activity that may indirectly influence the BOLD fMRI signal in functional imaging experiments. For example, under conditions of hypercapnia (increased blood levels of CO_2), marginal (Bock et al., 1998) and significant (Kastrup et al., 1999) increases in BOLD signal intensity have been observed. Different brain areas appear to differ in the magnitude of BOLD responsiveness. For example, the cerebellum and visual cortex show the highest and the frontal cortex shows the lowest signal intensity changes. The effects of vasoldilation-mediated blood flow increases on activation related signal intensity changes may be simply additive (Corfield et al., 2001), however, more research is necessary to substantiate these findings.

Complementarity of fMRI Data Analysis Methods

The ultimate step in fMRI data analysis is the generation of brain activation maps. These activation maps identify those brain regions in the image in which the signal changes upon stimulus presentation. Many of these analysis methods model a time-course and determine how well each pixel's temporal response fits this model. Before the actual analysis can be performed there are several preprocessing steps. These generally include subject motion correction, image registration and may include physio-correction methods as well as standardization into the Talairach and Tournoux or Montreal Neurological Institute brain templates (SPM99, Friston et al., 1995, 1994). Although standardization allows for group analysis and comparisons, it is at a loss of

spatial resolution. Therefore, depending on the objective of the study, the pros and cons of individual relative to grouped analysis should be evaluated.

Data analysis methods more typically used include parametric analyses such as the Student-t-test and cross-correlation. Non-parametric methods used for exploratory data analysis are data- and not model-driven. Voxel time-courses are segregated on the basis of temporal coherence using clustering methods.

This section contains brief descriptions of some of the current analytical approaches and reasons why the methods were used in different parts of this thesis.

Stimulate

Stimulate (Strupp, 1996) uses parametric methods for the generation of brain activation maps. These are in the form of cross correlations or Student t-tests, for example. Using correlation, a specified model can be designed for correlation against the intensity time course of each voxel in the functional image. The most standard way of performing correlation analyses is to specify a box-car function that corresponds to the experimental paradigm of control and stimulation conditions.

In part I and II of this thesis we used Stimulate's cross correlation algorithms for data analysis. In part I, one of the objectives was to determine the brain regions that activated differentially to visceral sensation and pain. To identify these regions we ran three correlations that tested for pixels activating and deactivating to stool alone (stool-specific), pain alone (pain-specific) and to both, pain and stool (boxcar). In part II we compared pain-specific responses due to visceral and somatosensory stimulation. The correlation function in Stimulate provides a brain activation map as a measure of

correspondence of these reference time-courses (stool-specific, pain-specific and boxcar) with the functional MR signal intensity changes.

EvIdent

EvIdent (EVent IDENTification) is an exploratory data analysis tool that detects anticipated and novel events in functional images that form a temporal set in volumetric 3D data (Baumgartner et al., 2000; Scarth et al., 1995). The basic idea behind exploratory data analysis is that the analyst must let the data speak for itself, without externally imposed assumptions about the experimental structure or statistical aspects (Jarmasz and Somorjai, 2002). Therefore looking for the unexpected in the data is the major purpose of exploratory data analysis. Exploratory data analysis is data- and not model-driven, as are the more commonly used neuroimaging analyses methods. Therefore the main advantage of the exploratory data analysis method is that it avoids the experimenter's preconceived notions about the activation paradigm by not specifying a model. Both, anticipated and unexpected events are identified using an analysis technique called fuzzy clustering.

Fuzzy clustering partitions time-course data and represents the intensity value of voxels over several discrete instances in time, into a user-specified number of fuzzy clusters. Unlike hard clustering techniques, fuzzy clustering separates the data by assigning a weight, referred to as a membership, for each time-course to each of the clusters. Thus pixels will be grouped into clusters that are most similar to the cluster centroid. The cluster centroid is a weighted average of the temporal time-courses in the

functional data and therefore represents the time-courses that belong strongly to the cluster.

We were able to exploit this data analysis method in several ways. Non-parametric analysis was used to identify patterns of temporal coherence that might be overlooked on the basis of the parametric analysis alone. Fuzzy clustering identified several unexpected brain activation and deactivation patterns in part I of this thesis. For example, pain-specific and graded BOLD responses were detected (Bernstein et al., 2002). Amongst the pixels in the ACG activating to sensation and pain, both, on/off and graded responses were observed. This was true for both controls and disease groups. A recent fMRI study reported that there were no pain sensitive pixels in the anterior cingulate of control subjects experiencing noxious visceral stimulation (Mertz et al., 2001). This study was based on pixel counts that correlate with a boxcar function and may have been confounded by the existence of graded activations. A further example of an unexpected response pattern was a deactivation in VMPFC and DMPFC regions during visceral sensation and pain. During this deactivation, BOLD signal dropped below baseline at the onset of the stimulation condition and remained depressed until the end of the stimulation condition (Frankenstein et al., 2002, submitted). Initially we became aware of deactivations in fuzzy clustering analyses of motor paradigms (McIntyre et al., 1996). Since then we and others have observed deactivation in several paradigms involving sensory and cognitive tasks (Frankenstein et al., 2000, 1999; Malisza and Docherty, 2001; Porro et al., 1998; Rauch et al., 1998). The location of deactivation observed in our pain studies corresponds with rCBF decreases observed with PET (Vogt et al., 1996).

Statistical Parametric Mapping 99(SPM99)

In SPM (Friston et al., 1995; Friston et al., 1994) analysis is performed on a voxel by voxel basis using a general linear model approach. In simple terms the general linear model is a mathematical equation that relates what one observes to what one expected to see by expressing the observations (response variables) as a linear combination of expected components and some residual error. The response variable observed in fMRI is the signal change at the voxel level due to the experimental stimulus.

There were several reasons for choosing SPM for the analysis in Part III of this thesis. First, in order to be able to compare the location of activations due to pain and attention to a large number of other studies, it was necessary to transform the data into a standardized stereotactic space. In SPM99, data from each subject is transformed into the Montreal Neurological Institute template, which is based on the Talairach and Tournoux co-ordinate system. The advantage of using the Montreal Neurological Institute template is that it is based on an average of 305 brains whereas he Talairach and Tournoux atlas is based on a single human brain. A further advantage of this method is that it allows averaging across subjects, however, this is at the expense of spatial resolution. Using this transformation method we found that the location of our pain and attention activations were in general agreement with the literature. These results allowed to further investigate our hypotheses on modulation responses in pain and attention sub-regions of the ACG during the distraction task. Secondly, we had a precise a priori hypothesis in regarding the expected CPT activation co-ordinates. These co-ordinates were based on electrophysiological reports in neurosurgical patients (Hutchison et al., 1999). Using a small volume correction it was possible to use these precise electrophysiological co-

ordinates to determine the correspondence with the BOLD signal intensity changes observed during our CPT task. Thirdly, in order to investigate our hypothesis that distraction from pain would result in decreased activity of ACG sub-regions responsive to pain and increased activation of ACG sub-regions responsive to the distraction task, a subtraction analysis seemed appropriate. Subtraction designs involve selecting an activation task that engages the component of interest and a baseline task that engages all but the component of interest (Price and Friston, 1997). In our study the activation task was the distraction task, which consisted of the CPT and the concomitant distraction which was the verbal attention task. The baseline task consisted of the CPT only. Therefore subtracting the DT from the CPT (CPT-DT) allowed identification of ACG sub-regions that were more strongly activated during the CPT, whereas (DT- CPT) subtraction allowed for the identification of areas that were more strongly activated during the DT. Finally, initial observations of the CPT and VAT statistical parametric maps suggested spatial overlap of inferior ACG (perigenual) local maxima. To investigate this observation, SPM99 has a masking function that allows the identification of common areas of activation between different tasks. Masking the CPT activations with the VAT activations we identified nearly identical MNI co-ordinates for BA24/24' activations during the VAT and CPT tasks which may be involved in autonomic or affective dimensions common to both tasks (George et al., 1995; Pool and Ransohoff, 1949; Lewin and Whitty, 1960).

Conclusions and Future Directions

The identification of cell assemblies and neuronal networks underlying various aspects of human behaviour is one of the fundamental tasks of functional human brain mapping. The cerebral representation of pain is complex as it involves sensory, affective and cognitive systems. As part of the pain neuromatrix the ACG and MPFC are not only key regions in somatosensory pain processing, but also visceral pain processing. In addition to demonstrating similar patterns of response during both pain modalities, ACG and MPFC activation patterns may be used to differentiate between subjects suffering from gastrointestinal disorders and controls. The modulation of ACG activity in response to a distraction task demonstrates an intricate relationship between pain and attention and provides support for the notion of a functional, interdependent pain neural network.

Based on our studies, several issues should be kept in mind for future research. Firstly, in terms of documenting activation in ACG or other brain regions it is important to have a good understanding of the functional anatomy of these sub-regions. The majority of neuroimaging studies, particularly in visceral pain, report overall ACG activity with little consideration for sub-regional specificity or laterality. Standardization of ACG ROIs between studies may facilitate the interpretation of ACG activation observed in different studies. In terms of localization, Talairach and Tournoux and Montreal Neurological Institute co-ordinates are the gold standards for comparing activation between subjects and studies. Although this standardization allows comparisons of co-ordinates between studies, it necessitates the warping of images into a standard space, thus compromising spatial resolution. This may be of lesser importance when analyzing larger neural network activation, but may figure prominently in sub-regional comparisons of perigenual versus rostral or midcingulate, for example. The

normalized pixel counts in within subjects designs in conjunction with group analyses may be the best means of dealing with these "trade-offs."

Secondly, non-parametric analyses should be used to complement standard parametric methods. Non-parametric analyses may identify unexpected patterns of brain response and avoid experimenters' preconceived notions about the activation paradigm.

Thirdly, our data suggest that the existence and potential impact of deactivation should reflect regularly in the analysis of painful stimulation. We believe that this is especially important when fMRI is used to study pharmacological effects in particular brain regions.

Fourthly, pain has an attentional component and focusing towards or away from a painful stimulus modulates activation patterns. We controlled our studies by specifically instructing subjects to pay attention to the painful stimulus.

Finally it is important to consider factors other than the stimulation paradigm that may influence the BOLD fMRI signal, such as autonomic responses during the CPT. Carefully designed control conditions may help separate purely autonomic from nociceptive components of the pain response.

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