Imaging the neural correlates of cutaneous sensation and its modulation by psychological factors

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A Thesis submitted in partial fulfilment of the degree of Doctor of Philosophy

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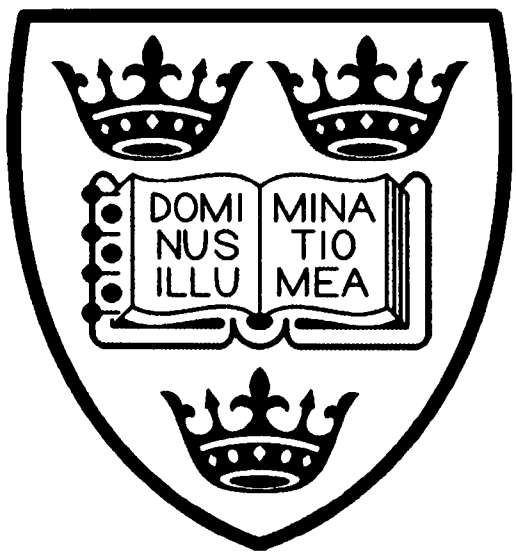



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ABSTRACT

This thesis looks at the central processing of two unpleasant sensations, itch and pain, and how these sensations can be modulated using psychological tools. The initial section focuses on the induction and modulation of thermal noxious stimuli and the way in which this is represented in the brain.

It is well known that pain can be modulated by distraction, and also which areas of the brain are frequently activated by noxious stimuli. The research reported here expands upon this knowledge. The first study found that when subjects were engaged in a cognitively demanding task (the Counting Stroop task) their attention was distracted away from thermal painful stimuli. They reported a significantly ($p=0.006$) lower pain intensity rating for stimuli during the interference (more cognitively demanding) task compared to the neutral (less cognitively demanding) task. The interference task was confirmed to be more difficult than the neutral task by significantly increased reaction times in the former compared to the latter. The reduced perception of painful thermal stimuli applied during the interference task compared with those felt in the neutral task was accompanied by a reduced activation in some key components of the pain matrix (insula, mid-cingulate, thalamus). Some

key pain processing regions showed an increase in signal during the interference task compared with the neutral task (perigenual cingulate, orbitofrontal regions).

The next experiment (Chapter 3 (development) and Chapter 4 (experimental) built upon the observed modulation of key pain processing areas by cognitive distraction seen in Chapter 2. The aim was to produce a parametric decrease in reported pain intensity (and corresponding modulation of the fMRI signal in key processing regions) with increasing task difficulty of the n-back task (a working memory task). A graded increase in task difficulty (progressive reduction in percentage accuracy from 1-back to 2-back task levels) was produced using this paradigm. There was a significant reduction in pain intensity in the 2-back task compared with the 1-back task and with the 3-back task compared with the 1-back task. There was a non-significant rise in perceived pain intensity from the 2 to the 3-back task. There was not a graded decrease in pain intensity with increase in task complexity. The n-back task may not be the most appropriate task with which to produce a parametric modulation in pain intensity but further study with larger subject cohorts may be indicated. Region of interest analyses revealed modulation of key pain processing regions as a result of cognitive distraction using the n-back task, but not in a parametric fashion. The central processing of pain and its investigation is more than a simple academic question since many people's lives are blighted by pain, and increasingly cognitive therapies are looked upon as an adjunctive therapy to complement existing pharmacological regimens. It would greatly inform our knowledge of the treatment of pain, and aid the development of novel pharmacological agents to combat pain if we had a greater knowledge about which brain areas can be modulated and how this is reflected in the behavioural reports of subjects.

Another vexing and highly prolific symptom of various disease and allergic states is itch or pruritis, for which, unlike pain, there is no specific drug available. This research addresses the development of a pure and pain-free itch stimulus and its adaptation for use in the MRI environment, ultimately using this paradigm to measure online itch ratings induced by allergen and histamine whilst correlating these behavioural ratings with the subjects' central activation.

Chapter 5 was concerned with the development of a reliable and sufficiently potent itch stimulus to achieve sufficient signal to noise ratio (SNR) to measure signal changes as a result of itch sensations in central areas using fMRI. Subjects' average

itch intensity profile (outside the scanner) was averaged over 3 sessions. This profile was used to correlate with the fMRI data acquired in the magnet in order to investigate which central regions were involved with the processing of itch intensity. Itch was produced using histamine prick test. We obtained significant activations in brain areas that correspond with regions reported in the literature in addition to further areas not previously identified. This study therefore demonstrated the ability of the histamine prick test to produce a robust itch stimulus and brain activation, results of which can be compared to the pain activations identified earlier.

Chapter 6 used laser Doppler fluxometry to measure blood flow changes in atopics as a result of challenge with their appropriate type I allergen and with histamine (both prick test administrations). Challenge with the atopic subjects' appropriate type I allergen produced a more protracted and greater increase in blood flow together with a more intense itch than challenge with histamine. A cohort of atopics were then challenged with their type I allergen using a prick test paradigm in the MRI scanner. fMRI data were collected together with on-line ratings of itch intensity throughout the experiment. The central regions displaying signal changes correlating with the itch intensity profile were revealed. Areas of activation found to correlate with the itch intensity profile included the bilateral cerebellum, bilateral BA 20/21, contralateral fusiform gyrus, contralateral BA19 and contralateral posterior cingulate cortex.

Chapter 7 used the online itch rating paradigm developed in Chapter 6 although this time non-atopics were used and challenged with histamine prick test only. FMRI data was correlated with the itch intensity profile produced from online ratings obtained during the allergen and histamine prick tests and compared. There were fewer central areas activated during allergen prick test, however, those found active were in regions known to be more involved in processing the aversive elements of somatosensation. One specific region activated in the allergen study yet absent in the histamine study was the posterior cingulate. This region processes unpleasant affective information. The sensation of allergen induced itch is quantitatively (different blood flow profiles) and qualitatively (more intense, more unpleasant) different to histamine induced itch.

Taken together these experiments have investigated the neuronal correlates of pain and used cognitive distraction paradigms to produce modulation in behavioural and central neuronal correlates of thermal pain intensity perception. A robust itch

stimulus (prick test of allergen or histamine) paradigm was developed. In conjunction with online ratings (used in the n-back and itch allergen and itch histamine studies), central regions involved in the processing of pruritic stimuli were isolated. Many similarities exist between the two unpleasant sensations investigated in this thesis, with regard to the central representation. Differences were noted, however, which might be explained based upon the different time courses of sensation perceived between the pain and itch paradigms as well as the potential inhibition of motor output differences generated from an urge to withdraw from a painful stimulus compared to an urge to scratch during the itch paradigm.

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OVERALL CONCLUSIONS

CHAPTER 1: The central processing of pain and the use of functional imaging to investigate the neuronal correlates.

Pain is a multi-faceted and essential human condition that leaves nobody entirely unscathed. However, there are still significant elements of the way in which the brain perceives and processes pain that continue to be enigmatic. Ongoing research into this dynamic field is of pivotal importance if we are to further elucidate the functional anatomy of the key central nervous system areas involved. This knowledge could be the most promising ammunition currently available in the war against suffering. This line of research will help advance the development of new and exciting analgesic methods both pharmaceutical and psychological. Functional imaging techniques have much to recommend them as exciting tools in the study of pain, providing an accurate, objective, and non- invasive tool with which to investigate human pain processing pathways.

Recent years have seen an exponential increase in the level of our understanding of pain mechanisms. Modern molecular, cellular and systems neurobiological techniques have all contributed to this. The results of pain imaging research can be applied to the clinical arena, allowing treatment options to be based upon a more thorough understanding of the contributory processes. Once the mechanisms of pain and the functional areas of the brain involved are better known, we can more accurately target clinical pain, and apply treatment and assessment options which are more tailored to the individual case concerned.

The research reported in this thesis aims to investigate the psychological modulation of pain perception and its functional central neuronal correlates.

1.0 Overview of pain processing

The sensation of pain is triggered by the activation of a division of specialised primary sensory afferent neurons, known as high-threshold nociceptors. Sherrington proposed the existence of this special type of primary sensory neuron in 1900 (Sherrington 1900). The basic molecular mechanisms underlying nociception are summarised thoroughly in a review by Julius and Basbaum (Julius and Basbaum,

2001). Various aspects of peripheral pain processing are also discussed in Craig's 2003 review (Craig, 2003). Sherrington defined nociception as sensory activity evoked by noxious stimuli that cause or threaten tissue damage (Sherrington, 1948).. Sinclair (Sinclair, 1967) later described how various aspects of bodily sensations such as their quality, intensity and spatiotemporal characteristics result from how the brain interprets the pattern and intensity of activity in the somatic afferent fibres. Primary afferent nociceptors were identified as a distinct entity in the 1960s and this is described by Perl (Perl, 1984). Selective nociceptive neurons were isolated in lamina I of the spinal cord dorsal horn in 1970 by Christiansen and Perl (Christensen and Perl, 1970). Nociceptors are selectively sensitive to noxious stimuli (mechanical or thermal) and different subtypes exist (Campbell, 1996). Some nociceptors are dormant or insensitive and respond only following actual tissue damage or inflammation (Torebjork, 2000).

The physiological properties of ad and C nociceptors are described by Andrew and Greenspan 1999(Andrew and Greenspan, 1999). Only the summated activation in C-nociceptive fibres causes the conscious perception of pain (Gybels et al., 1979). C fibres often discharge slowly (<1Hz) without provocation and this is apparently not consciously perceived (Campbell and Meyer, 1996).The mechanical thermal and polymodal thresholds of small diameter afferents extend across the pain threshold in all tissues (Cervero and Janig, 1992)

The primary protective role of these neurons means that they are *only* activated by intense and potentially harmful stimuli. As such, they are functionally distinct from low threshold sensory fibres, which are activated in response to innocuous, low intensity stimuli.

Nociceptors can be activated by a variety of stimuli: those that activate temperature sensitive receptor ion channels (i.e. vanilloid receptors); mechanoreceptors; stretch receptors; or chemoreceptors (activated by purines, protons, amines, peptides, growth factors, and cytokines released from damaged tissue or inflammatory cells). The precise mechanisms of activation and transduction fall beyond the scope of this discussion. Evidence supporting the unique properties of nociceptors has been furnished in part from electrophysiological studies demonstrating the existence of a sub-type of primary sensory neurons that could be excited by noxious heat, intense pressure or irritant chemical stimuli, but not by innocuous stimuli such as warmth or light touch (Burgess and Perl, 1967).

The afferent fibres innervating the head and body originate in the trigeminal and dorsal root ganglia of the spinal cord (Weidner et al., 1999). These fibres can be classified into three main groups. Aß primary sensory fibres are large, myelinated, fast-conducting afferents. Most of these fibres also respond to innocuous stimuli and do not contribute to the perception of pain, although the stimulation of such fibres can reduce the transmission of pain information (see later explanation of the gate theory).

Painful stimuli are transmitted by two main types of sensory afferent fibre, C-fibres and Aδ fibres. C-fibres are unmyelinated and slow-conducting and Aδ fibres are thinly myelinated and more rapidly conducting. The table below (Table 1.0) shows how both types of fibre contribute to pain perception.

Table 1.0 Table showing primary afferent fibres and their respective thermal thresholds (adapted from Julius and Basbaum (Julius and Basbaum, 2001)

Type of primary afferent axon	Thermal threshold
Aα and Aβ fibres	
• Myelinated	
• Large diameter	
• Involved in proprioception and light touch	None
Aδ Fibre	
• Lightly myelinated	53 °C Type I
• Medium diameter	43 °C Type II
• Nociception (mechanical, thermal, chemical)	
C-fibre	
• Unmyelinated	
• Small diameter	
• Innocuous temperature, itch	43 °C
• Nociception (mechanical, thermal, chemical)	

There are two types of Aδ nociceptor (Raja et al., 1999), both of which respond to intense mechanical stimuli, but are differentiated from each other by their

response profiles to intense heat or tissue injury. Most C-fibre nociceptors are also polymodal and respond to noxious thermal and mechanical stimuli (Julius and Basbaum, 2001), whilst other neurons are not responsive to mechanical stimuli, but do fire in response to noxious thermal stimuli. Most C-fibre nociceptors are also activated in response to noxious chemical stimuli such as acid (protons) or capsaicin.

Some nociceptors appear to be 'silent' or 'sleeping', and are only triggered under conditions of inflammation when they become sensitised by tissue injury in the surrounding area (Schmidt et al., 1995).

Nociceptors in different body organs manifest different response profiles. For example, visceral pain does not have a fast and a slow component, but is often described by the sufferer as diffuse, deep and dull. Such pain can occur in the absence of actual tissue damage, for example from the excessive stretching of tissue (i.e. the colon).

The details of receptor transduction are not discussed here in any depth, although it should be noted that the major excitatory neurotransmitter in nociception is glutamate (Glu). The neurochemistry of the nociceptors is highly complex, however, and other neurotransmitters are involved in pain transmission including the peptide substance P and nerve growth factor (NGF).

Primary sensory neurons involved in pain transmission are polymodal and respond to a wide range of stimulus modalities (i.e. physical, chemical). Harmful stimuli to the skin or subcutaneous tissue activate the peripheral endings of primary sensory neurons whose cell bodies are found in the dorsal root ganglia and trigeminal ganglia. There are three main classes of nociceptor, thermal, mechanical and polymodal. In contrast to the specialised somatosensory receptors involved in detecting touch and pressure, nociceptors are free nerve endings. The precise mechanism by which noxious stimuli depolarise free sensory endings and trigger action potentials is not known (Kandel and Schwartz). The membrane of the nociceptor contains proteins that convert the thermal mechanical or chemical energy of the noxious stimuli into a depolarizing electrical potential.

The receptive properties of nociceptors can also be modulated. Central sensitization occurs when the responsiveness of spinal cord neurons is increased pathologically. Peripheral sensitization occurs when the nerve terminals of nociceptors are surrounded by products released during tissue damage and inflammation (such as protons, arachidonic acid, serotonin, bradykinin, nucleotides,

NGF). This can be further exacerbated by the release of peptides and neurotransmitters such as substance P and ATP from stimulated nociceptors, which can then stimulate further production of inflammatory agents from nearby cells and blood vessels. The interested reader is referred to additional papers on the subject of pain and neurobiology referred to in the following review (Julius and Basbaum, 2001).

Information related to fast pain carried in the fast conducting, myelinated Aδ fibres enters the marginal layer and lamina V of the dorsal horn. C-fibres terminate in lamina II (sustantia gelatinosa).

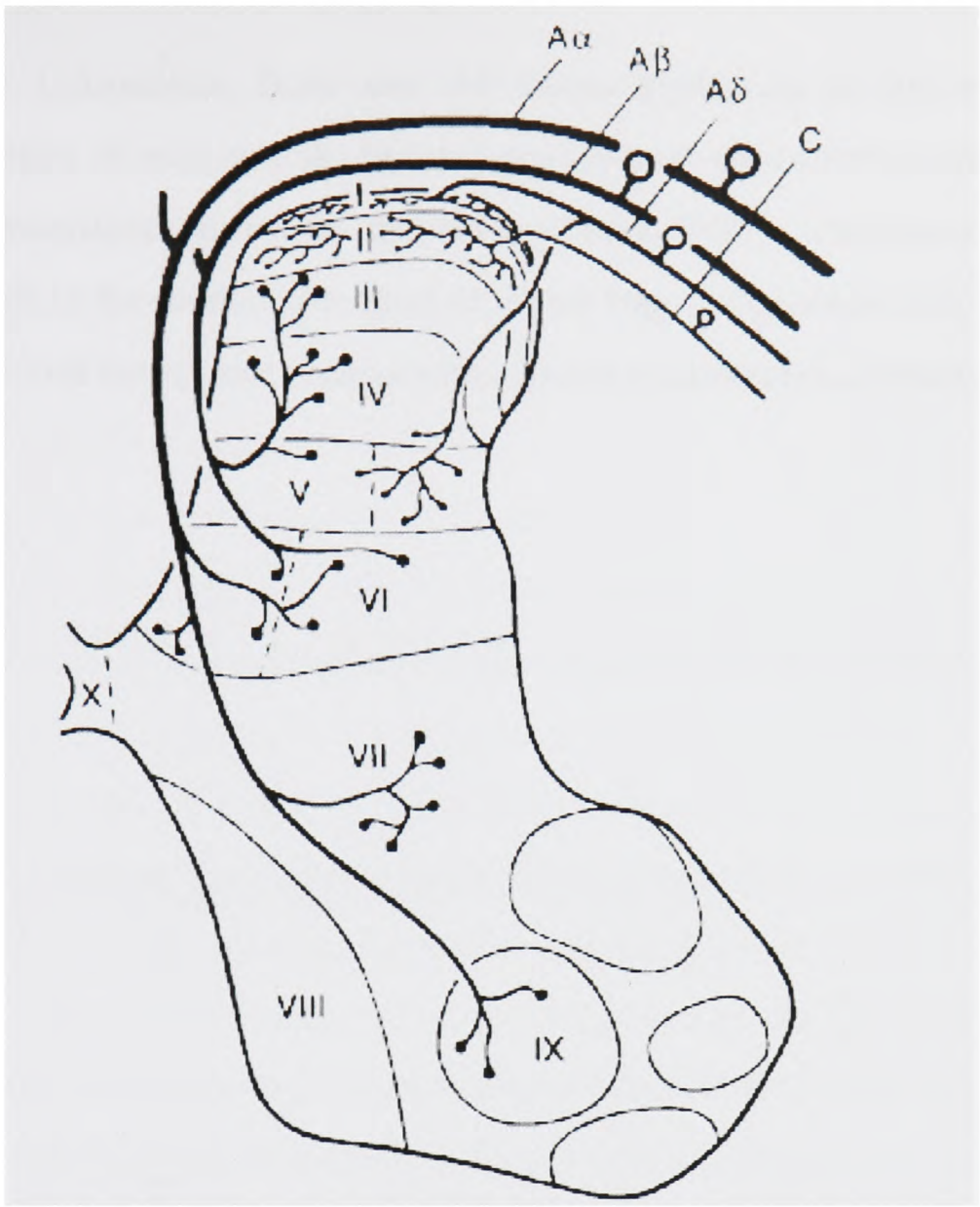


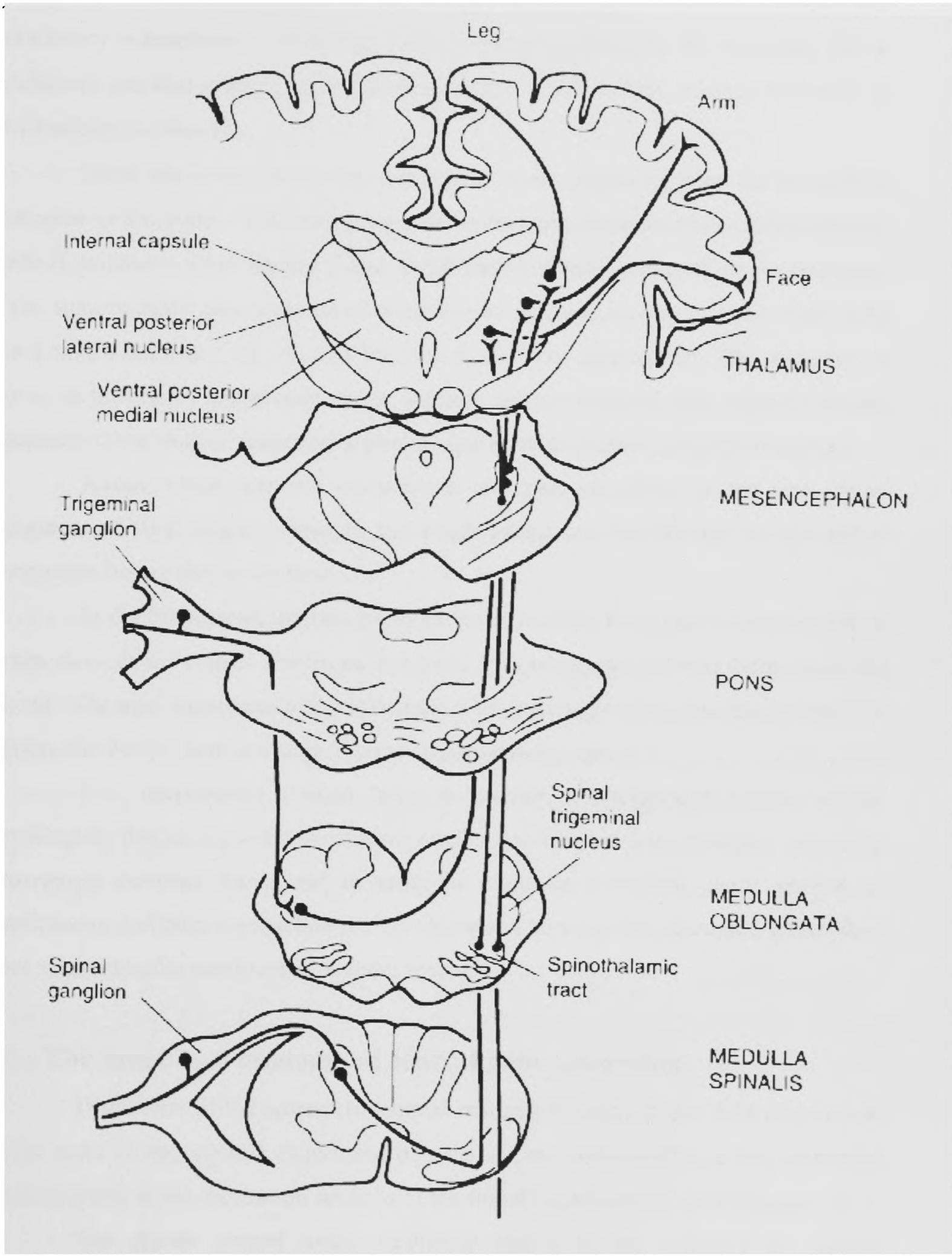
Fig. 1.0 Diagram of the dorsal horn of the spinal cord.

The terminal regions of the dorsal root fibres in the cord. The thickest myelinated fibres (A α, from muscle spindles and tendon organs) end deep in the dorsal horn and partly in the ventral horn. Thick, myelinated fibres from cutaneous mechanoreceptors (Aβ) end in laminae III-VI. The thinnest myelinated and unmyelinated dorsal root fibres (Aδ and C), many of them arising from nociceptors, end in laminae I, II, and parts of V. Taken from Brodal, P (1998).

The marginal zone consists of large neurons responsive to skin and muscle stimulation. The substantia gelatinosa (lamina II) receives information from C-fibre and Aδ neurons. After pain related information has been processed in the spinal cord, it ascends to the brain via long tracts in the white matter of the spinal cord. Most nociceptive information is conveyed in the spinothalamic tract. At this juncture, pain related information crosses through the central portion of the cord and ascends to the brain in the opposite side from where it entered the spinal cord. Pain information from the right side of the body ascends in the left sided tracts and vice versa. Information is thus conveyed to central areas for further processing.

Information from fast Aδ fibres pertaining to the sensory discriminative properties of pain ascends in the lateral spinothalamocortical tract, terminating in the somatosensory cortex of the brain (Craig, 2003). Information from the C-fibres ascends in the medial spinothalamic tract (fig. 1.1), terminating in the medial thalamic nuclei and limbic areas responsible for the motivational-affective elements of pain.

Fig. 1.1 Diagram of pathways involved in the processing of noxious stimuli.



There are three main types of pathway in the dorsal horn for the transmission of pain signals. Nociceptive afferents from the periphery may synapse directly with the neurons of the ascending anterolateral pathway. Afferents can also synapse with excitatory interneurons, which then relay nociceptive inputs to the ascending fibres. Afferents can also synapse with inhibitory inputs, which inhibit or block transmission of nociceptive impulses.

There are several ascending nociceptive tracts projecting from the dorsal horn laminae to the brain. The most prominent is the spinothalamic tract (Anterolateral), which originates form lamina 1 and V-VII neurons and terminates in the thalamus. The spinothalamic tract consists of second-order afferent sensory neurons originating in Rexed's laminae I, III, IV, and V of the dorsal horn grey matter. The tract crosses over to the contra lateral side in the anterior commissure and runs rostrally in two separate tracts: medial (anterior) spinothalamic tract and lateral spinothalamic tract.

Axons which transmit temperature and pain decussate within one spinal segment of their origin. Pressure and touch axons can rise through several spinal segments before they cross over.

In the brain stem, the two tracts merge to become the spinal lemniscus which runs close to the medial lemniscus and joins with trigeminal afferent fibres from the head. The tract terminates in the thalamus in the ventral posterior nucleus. From this point, third-order neurons project to the somatosensory cortex.

This introductory chapter aims to provide a background section to the subsequent descriptions of pain imaging studies described in later chapters. Pain is an extremely complex topic, and in order to minimise repetition, some aspects of processing and pain modulation are not discussed here but are discussed where they are relevant to the empirical data under review.

1.1 The functional anatomy of central pain processing

Discussion of the current functional imaging findings in the field of pain and their roles in the overall experience of pain are not discussed here but addressed subsequently in the discussion sections of the initial experimental work (Chapter 3).

The classic central areas frequently shown to be activated by painful stimulation (the 'pain matrix') include the thalamus, insula, somatosensory and motor cortex, basal ganglia, lateral parietal cortex, anterior cingulate cortex, and the

posterior cingulate cortex. Other areas more recently identified include the frontal cortex and the amygdalo-hippocampal region.

These activations are mainly bilateral, even as a result of unilateral stimulation. The exact nature of the functional central anatomy of pain processing is discussed later in this section.

1.2 The use of functional imaging in pain research

In 1890, the physiologist Arthur Sherrington first demonstrated empirically that direct stimulation of the brain resulted in a corresponding local increase in blood flow. He also found that the increase in total oxygen delivery to the local area exceeded the increase in oxygen consumption. The increased differential between oxygenated haemoglobin in the capillaries and the cell mitochondria allows tailoring of diffusion-limited transport mechanisms to the rate of oxygen uptake in the active area. Essentially, it is such activation induced changes in blood oxygenation and flow which form the basis of functional magnetic resonance imaging methodologies used today.

MRI emerged contemporaneously with PET. Felix Bloch from Stanford University (Bloch et al., 1946) and Edward Purcell's group from Harvard University (Purcell et al., 1946) were the first to discover the foundation principles of magnetic resonance, namely the behaviour of hydrogen atoms in a magnetic field. They jointly received the Nobel Prize in Physics in 1952 for their seminal discovery. Paul Lauterbur (Lauterbur, 1973) expanded upon this pioneering work, using magnetic field gradients to localise the MR signal. Researchers were excited by the information pertaining to anatomy, metabolism and physiology that MRI was able to provide. In 1976, Peter Mansfield (Mansfield and Maudsley, 1976) from the University of Nottingham developed echo planar imaging (EPI) which allowed the whole human brain to be rapidly scanned. It was subsequently discovered that changes in neuronal activity resulted in local changes in the amount of oxygen in the tissue (Fox and Raichle, 1986; Fox et al., 1988). This observation, in combination with much earlier work showing the magnetic properties of oxygenated haemoglobin and its ability to create local magnetic field inhomogeneities, and research by Ogawa *et al.* (Ogawa et al., 1990) confirmed that in vivo variations in local blood oxygenation could be detected using MRI. An abundance of studies looking at various brain regions

followed. The results of such studies confirmed that blood oxygenation level dependent (BOLD) signal changes were indeed linked to local task-induced activation in brain areas (Bandettini et al., 1992; Kwong et al., 1992; Ogawa et al., 1992). All of this work forms the foundation of fMRI, and triggered the exponential development of fMRI in neuroscience based research.

Before discussing further the benefits of fMRI, it is important to acknowledge the contribution of electroencephalography or EEG. The EEG involves placing electrodes over the surface of the scalp and making extracellular recordings from the brain region beneath the electrodes at various points of interest. The EEG represents a recording of a set of field potentials recorded by multiple electrodes placed on the surface of the scalp. Field potentials reflect the synchronised electrical activity (action potentials) of large numbers of neurons. Deep structures such as the hippocampus, thalamus, or grain stem (all structures involved in pain) do not contribute directly to the surface EEG. The EEG can give a representation of the nerve cell activity in various cortical areas at rest or during the completion of a task. It directly measures electrical activity in the neurons and does not use the indirect measure of changes in blood oxygenation as a result of neuronal firing.

The use of fMRI has increased dramatically in the ten years following the first published fMRI study that showed activation in primary sensory visual cortex in human volunteers (Belliveau et al., 1991). It has become an invaluable method with which to investigate the central neuronal correlates sensory, perceptual, cognitive and emotional processes.

Psychological and medical research has used psychophysical techniques to infer the neuronal processing resulting from the performance of a task or from the stimulation of a particular system (i.e. visual system). Valuable as this research undoubtedly is, it is not possible from such experiments alone to deduce the precise location in the brain in which this activity takes place. Other research strategies have utilised electrophysiological recordings in laboratory animals, and neurological examination of patients with lesions of the cortex that result in compromised neural processing. The resulting deficits can be assessed and hypotheses formed about the function of the damaged areas in the intact human brain.

It is not possible to use non-invasive techniques to look at the responses of single neuronal units in the human cortex, but various studies have led to the belief (Boynton et al., 1999) that behaviourally significant activity does not invariably occur

at the level of an individual unit but instead takes place at the level of networks of inter-connected neurones (Shadlen et al., 1996). This provides a potentially promising niche for fMRI studies to aid understanding of the central correlates of human behaviour, and the units and systems of the brain regions involved.

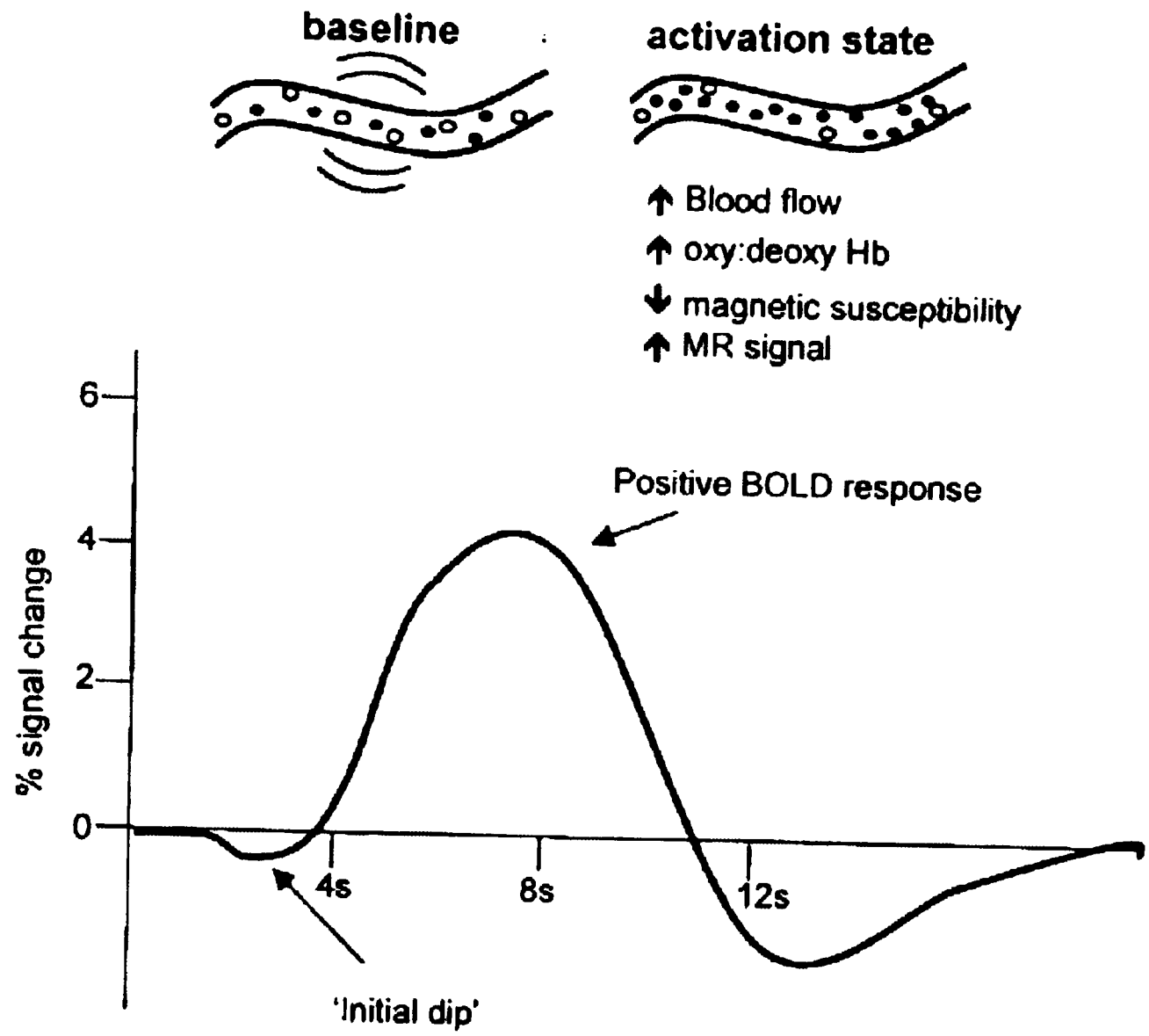
Functional imaging is a powerful tool with which to investigate the functional anatomy of the human brain. fMRI enables us to identify regions of the brain and their temporal relationships associated with performing a carefully designed task paradigm. In addition, fMRI allows us to accurately quantify the activation in the brain resulting from sensory stimulation such as pain and itch. Such quantitative analyses extend the utility of fMRI and its application, particularly in studies that are longitudinal or serial, and might include a treatment regime. Unlike positron emission tomography (PET), fMRI is non-invasive, and does not rely on the use of ionising radiation. fMRI therefore allows safe and rapid scanning of the intact and awake human brain many times, thus enabling serial, longitudinal study designs. Applied to the problem of pain processing, it can broaden our knowledge of the central neuronal correlates of pain.

fMRI benefits from good spatial resolution equal to an in-plane spatial resolution of 3mm or less. Spatial resolution can be defined as the ability to distinguish two points as separate and distinct. This is essentially dictated by voxel size. Small voxels give a high spatial resolution and therefore small structures are seen and distinguished easily, whereas large voxels result in low spatial resolution and small structures are more poorly resolved. The size of a voxel is itself determined by slice thickness, field of view and the size of the matrix. Reduced slice thicknesses allow better spatial resolution to be obtained. There is ongoing discussion into the extent to which the location of recorded changes in BOLD signal (discussed later) corresponds to the location of neuronal activity. Although the theoretical limit to the spatial resolution possible in fMRI is dependent upon the size of the smallest vessel able to act as an independent functional unit, the actual spatial resolution in a particular experiment is governed by a variety of other influences. Such factors could include the signal to noise ratio (SNR), the amount of motion artefact present (as a result of, for example, head movement and brain movement in cardio-respiratory cycle), the contribution of large vessel effects, and the location and size of metabolic and haemodynamic changes taking place alongside neuronal activation. There are methods of reducing the effect of these other factors, although they fall beyond the

scope of this thesis. To an extent spatial resolution is improved by the use of higher magnetic field strengths which permit increased signal to noise ratio.

A set of elegant experiments (Engel et al., 1994) designed to characterise the visual system function found that the fMRI signal could be localised to within 1mm at 1.5 Tessla (T). Spatial resolution is further increased by the use of high-field MRI scanners since they maximise the contribution of the microvasculature to the BOLD signal and allow a greater resolution to be achieved. Many methods can be applied to maximise the potential of fMRI to provide high spatial resolution images, and ultimately the barrier to further increases in resolution is likely to be the lateral connections in the horizontal layers of the cortex, and the vascular density, both of which vary by brain region (Zheng et al., 1991). The temporal resolution of fMRI is limited by a blurred intrinsic haemodynamic response. There is typically a lag of between four to six seconds between the onset of a stimulus and the peak of the haemodynamic response curve. It can be as little as 1 second for a short stimulus such as a flash of light to 8 seconds for a longer stimulus such as a flashing chequerboard (see Fig. 1.2)

Fig. 1.2 Diagram of the haemodynamic response curve



The BOLD response. In the baseline state (top left) there is a distortion of the magnetic field around a blood vessel. With increased neuronal activity (top right) there is an increase in blood flow greater than the increase in oxygen consumption, resulting in an increase in the ratio of oxy to deoxyhaemoglobin. This results in a decrease in magnetic susceptibility around the vessel and therefore an increase in fMRI signal. The positive BOLD signal is delayed by 4-6 seconds (graph bottom) from the onset of neuronal activity. However, there are also reports of a brief negative BOLD signal (the negative dip) possibly reflecting an initial decrease in the ratio of oxy to deoxyhaemoglobin.

Since the shape of the haemodynamic response can be accurately estimated, the lag does not in itself limit the temporal resolution of fMRI data, since the expected time course can be convolved with the expected haemodynamic response function (HRF). The upper constraint on the temporal resolution of fMRI is dictated more by

the variation in the haemodynamic lag between individuals (Aguirre et al., 1998), brain areas (Rajapakse et al., 1998), and with disease (Pineiro et al., 2002).

Temporal resolution is dependent upon the repetition time (TR) between the acquisitions of signal from the same slice. The scanner used also determines to a considerable extent the maximum temporal resolution that can be achieved. If the TR selected is very short and multiple slices are acquired then this places demands upon the scanner for very fast gradient switching.

Temporal resolution is of considerable import when carrying out the type of experiment detailed in this thesis. The experiments that form the basis of this work were primarily designed in an event-related fashion. The aim of such studies being to detect individual responses to single events separated in time (Buckner, 1998; Friston et al., 1999)

1.3 Functional Magnetic Resonance Imaging and the BOLD response

Functional magnetic resonance imaging (fMRI) is a rapid functional imaging technique that acquires images of the brain whilst subjects are performing a task and during rest.

In order to understand the mode of operation of fMRI it is necessary to have an understanding of the magnetic properties of the oxygen transporting molecule in the blood, haemoglobin. When haemoglobin binds and transports oxygen in the bloodstream it is known as oxyhaemoglobin. In this state the magnetic properties of iron are largely suppressed and the molecule is said to be diamagnetic. When there is no oxygen bound to the haemoglobin (deoxyhaemoglobin) the molecule is more magnetic (paramagnetic). Paramagnetic deoxyhaemoglobin creates local field inhomogeneity and leads to reduced signal in this area.

The brain is highly sensitive to oxygen concentrations in the cerebral vessels. When an area of the brain is active in response to a task, the cerebral vasculature increases the blood flow to that region. This in turn leads to a change in the proportions of oxyhaemoglobin to deoxyhaemoglobin with an increase of oxyhaemoglobin. Increased amounts of local oxyhaemoglobin manifest themselves by an increase in signal in that area. This allows us to design experiments to ascertain the brain regions which are involved in certain cognitive and behavioural phenomena.

The differential between oxygenated and deoxygenated haemoglobin is the basic physiological tenet causing differences in MR signal between activity and rest periods. This effect is known as the blood oxygenation level dependent (BOLD) effect. BOLD builds upon the different magnetic properties of oxyhaemoglobin and deoxyhaemoglobin. The increased proportion of oxyhaemoglobin present in the blood in areas of neuronal activity results from the combined effect of increased cerebral blood flow to that region and the very marginal or non-existent changes in local oxygen consumption during stimulation. Deoxyhaemoglobin is paramagnetic and so where there are high levels of deoxyhaemoglobin in the blood vessels this creates local inhomogeneities in the magnetic field and signal loss. When an area is active, the levels of deoxyhaemoglobin fall and the levels of oxyhaemoglobin rise. This results in a reduction in dephasing and therefore a signal increase. These changes correlate with local field potentials from groups of neurons rather than single spike activity of single neurons. .

The early fMRI studies (Belliveau et al., 1991) used exogenous contrast agents to investigate local cerebral blood volume changes in the visual cortex during photic stimulation. By injecting the paramagnetic agent, gadolinium-DPTA into the blood stream, local increases in magnetic susceptibility are generated around the vessels that leads to a loss of signal in that area that corresponds to the amount of agent present. Current fMRI studies do not require the use of such exogenous agents to generate contrast. Instead, the magnetic properties of oxy and deoxyhaemoglobin (as previously discussed) are used to achieve contrast. Deoxyhaemoglobin (deoxy-Hb) is more paramagnetic than oxyhaemoglobin (oxy-Hb) (Ogawa et al., 1990). Changes in blood oxygenation occur as a result of neuronal activity and thus the BOLD signal can be used as an indirect marker of neuronal firing. Activity of neurons creates increased metabolic demand in the local area which is met in the main by increased oxidative metabolism. The additional oxygen required to sustain the increased neuronal firing rate is provided by increased local blood flow. There are various reasons that could explain the mismatch between oxygen consumption and blood flow but they are not discussed in any detail in this thesis. It is known, however that the precise nature of the BOLD response varies between cortical areas to some extent. Further research continues to inform this area. BOLD signal changes concomitant with neuronal activity are fairly small, on average in the order of 2-5%. Ultimately, the signal

change is dependent upon the strength of magnetic field used, with signal changes at 4T being up to four times greater than those observed at 1.5 T (Turner et al., 1993).

Echo-planar (EPI) fMRI allows fast image acquisition which permits a very high temporal resolution. This is ultimately constrained by the blurred haemodynamic response and the signal to noise ratio.

From the early work by Ogawa (Ogawa et al., 1990) which showed that gradient echo MR images of a cat's brain exhibited signal loss around blood vessels during induced hypoxia to the present day, fMRI continues to be an exciting and dynamic research field, providing the ideal combination of a fast, non-invasive technique with good spatial and temporal resolution, rendering it both flexible and safe to use.

1.4 The basic principles of MRI

The signal detected in BOLD fMRI comes from hydrogen atoms which are present in abundance in the water molecules in the brain and brain tissue (Heeger and Ress, 2002). Hydrogen atoms have MR active nuclei because the mass number of hydrogen is odd (1). Because hydrogen possesses a lone proton, it has a comparatively large magnetic moment. This causes the nucleus to act as a small magnet that is able to align with an externally applied magnetic field (Westbrook and Kaut, 1998). In the absence of an applied external magnetic field, the hydrogen nuclei are would naturally arrange themselves randomly with respect to the orientation of their magnetic moments. When an external field is applied, some of the nuclei align themselves in parallel with the external magnetic field (B0), and some of them align themselves anti-parallel to it.

According to the laws of classical quantum physics, hydrogen nuclei only possess two energy states or populations, low and high energy. Low energy nuclei align their magnetic moments parallel to Bo and are known as *spin up* nuclei. High-energy nuclei line up in an anti-parallel fashion to Bo and are termed *spin down* nuclei. As the field strength increases, fewer nuclei possess sufficient thermal energy to line up in anti-parallel to the magnetic field of the external magnet. Therefore, the magnitude of the nuclear magnetic moment is greater at higher field strengths which ultimately results in an improved signal.

The hydrogen nuclei spin upon their axes. The presence of an external magnetic field produces an additional spin around B0 known as precession. The magnetic moments follow a circular path around B0. The speed at which the NMV wobbles around B0 is called the precessional frequency. When excitatory radio frequency (RF) pulses are applied to the hydrogen nuclei at their specific frequency (Larmour frequency of hydrogen), they absorb this energy and resonate.

The hydrogen atoms then absorb energy applied at a characteristic radio frequency (~ 64 MHz for a standard clinical 1.5 T scanner). After the RF excitation is applied the hydrogen atoms emit energy at the same radio-frequency until they gradually return to their equilibrium state. The scanner measures the sum of the emitted radio-frequency energy. The RF signal decays over time due amongst other things to an uneven magnetic field strength. This causes decreased image intensity because each atom experiences a different magnetic field strength and after a while their radio frequencies cancel themselves out

BOLD fMRI measures changes in the inhomogeneity of the magnetic field within each small volume of brain tissue that result in changes in blood oxygenation.

How does this link to the BOLD effect? fMRI measures the level of oxygen in the blood in the local area of the brain being studied. There is a robust relationship between changes in brain activity and blood flow and changes in fMRI signal is directly related to actual changes in neuronal activity. This was demonstrated by Logothetis *et al.* (Logothetis et al., 1999; Logothetis et al., 2001) during studies of monkey visual cortex. They obtained concurrent fMRI and electrophysiological (action potentials and local field potentials) whilst monkeys were shown a flashing visual checkerboard. They found an area of increased fMRI signal in the monkey visual cortex which corresponded directly to neural activity recorded from the visual cortical cells themselves.

The main determinant of fMRI signal was found to be local field potentials, which are the result of the input to and processing within neurons, rather than action potentials that more reflect the all or nothing firing of neurons. Therefore, it should be noted that if no fMRI signal is obtained in a study in a particular brain region, it does not necessarily mean that no information processing is taking place.

The human brain consumes a fifth of the total oxygen required by the body despite the fact that it represents less than 2 percent of the body by mass. The oxygen is used to break down glucose in order to provide oxygen for the brain.

Transient increases in activity and energy needs of a particular brain area are accompanied by heightened blood flow and glucose utilisation rates that greatly exceed the rise in oxygen consumption.

This is due to the fact that glucose is broken down anaerobically by glycolysis in order to provide energy for the neurons. Therefore an increase in oxygen in the local blood results and ultimately supply exceeds demand. Rising blood oxygen levels are essentially due to increased rates of glutamate utilisation in astrocytes following excitatory neurotransmission. Therefore, in summary the BOLD signal is a reflection of local field potentials in post synaptic neurons due to changes in neuronal firing rates.

1.5 The MRI environment

The MRI environment is unique. By comparison to the tightly controlled quiet rooms in which many behavioural experiments are carried out, it is very noisy, claustrophobic, subjects have a reduced visual field from their supine position within the scanner, and spontaneous communication is difficult except via headphones. Response modalities are also constrained because anything that generates movement of the body and more specifically the head generates undesirable motion artefact.

Visual fields of subjects can be limited to 15-20 degrees for stimulus presentation on projector screens. Subjects can view such stimuli via the use of prism glasses which can be fitted with prescription lenses. However, the small space inside the scanner and the limited field of view can render the atmosphere claustrophobic.

Safety is a major consideration. Subjects have to be thoroughly assessed so that it can be verified that they do not have any metallic magnetic substances either in their body due to medical or dental procedures, and that they do not carry any such items into the room on their clothing or person. Metal in the subject can reduce image quality, but more importantly create radio frequency heating or if the object is not secured it can act as a projectile.

The scanning room itself is insulated against external radiofrequency interference to reduce noise which would result in impoverished signal to noise ratios. All equipment that could act as radiofrequency antennae (i.e. physiological monitoring equipment) must be shielded from environmental radiofrequency noise or be entirely contained within the scanner room.

The environment is very noisy because the rapid gradient switching generates considerable acoustic noise. In a typical 3-4 T MRI scanner, the noise produced by gradient switching could reach 120 dB in some sequences. Subjects can communicate with the experimenter via high performance headphones and their ears are protected by the combined use of ear-plugs and headphones.

Whilst carrying out the experiment, subjects' physiological responses are monitored for additional information and for safety purposes. In the studies reported here, oxygen saturation and heart rate were measured but not used in the data analysis.

It is of particular benefit to be able to obtain behavioural data from the subject during the fMRI scanning session. This does ensure that the subject is performing the task but also provides real time measurements of reaction time and accuracy. Responses in the scanner are not verbal since any overt vocalisation necessarily produces some head movement and undesired movement artefact. Motor responses can therefore be measured using a button press with alternative choices for rating pain for example and for measuring reaction time to stimuli presented on the screen.

1.6 Experimental paradigm design and fMRI pain research

The main consideration in designing an fMRI study is obtaining sufficient signal to noise ratio (SNR). The changes in signal observed are typically small, and fMRI can only really be used to ascertain the relative signal change within a single imaging session.

Once a hypothesis has been generated as to the brain regions believed to be activated as a result of a particular task, the experimental variables can then be isolated. The idea is to design an experiment where various components of the same task will activate different processes and therefore brain regions in an independent fashion.

A factorial design is used here to investigate the central processing of pain stimuli.

Block designs are fairly easy to analyse as they produce a simple response function. The subject engages in alternating periods of different types of tasks interleaved with each other and sometimes with a rest state. The rest state can be artificial in that it is rare that the subject's mind is not engaged in any activity. The requirement for subjects to switch from one task to another or one task level to

another does present some demands on the subject that are additional to that of the study itself.

The block design is based upon a method of direct subtraction. It has formed the cornerstone of functional imaging research since the 1980's. The assumption is that if you balance the 'off' and 'on' blocks so that only one thing is altered, then you should be able to subtract the activation in the 'on' period from the 'off' period and be left with activation attributable solely to the experimental task. It is necessary to consider habituation and sensitisation. In olfactory experiments, for example the subject can become accustomed to the stimulus over time. In pain experiments, it is necessary to space out the noxious stimuli sufficiently in order to present sensitisation. If this occurs, then subsequent stimuli will appear increasingly painful, even when the same temperatures and timings are used.

In order to achieve a satisfactory signal to noise ratio, it is necessary to have enough repeats of each experimental block. For this study it is ensured that there are approximately seven painful stimuli per type of block since this yields a good signal to noise ratio.

It is also advantageous to control to as great an extent as possible for subjects' attention e.g. by presenting a fixation cross on the screen in between tasks and asking the subjects to focus on it and press a button if it moves.

The order of block presentation should ideally be randomised in order to prevent order effects. Where possible the order should be randomised between and within subjects. Where a complex cognitive task is employed, practice effects should be considered. Sometimes it is beneficial to have a practice or familiarisation session for subjects prior to the scanning session to avoid steep 'learning curves' in the scanner.

The assumption behind the block design is that neural structures supporting cognitive and behavioural processes combine in a simple and additive fashion. This assumes that an extra cognitive component can be added to the first without affecting the first. However, it is likely that processes combine in a non-additive or interactive fashion (Zarahn et al., 1997).

Factorial designs such as the ones used here, combine two or more factors within a task or tasks. The experiment is designed in order to look at the interaction between these two factors or the effect that one factor has upon the responses due to another.

Another design used is a parametric factorial design where the response of particular brain regions to increasing difficulties of cognitive tasks or stimulus intensity are investigated in response to the effect they have on the response to another task.

Specific to pain experiments, as previously discussed, sensitisation to successive pain stimuli can be minimised by thorough 'thresholding' of individual subjects prior to starting the experiment. Thermal painful stimuli are delivered and the subject gives them a subjective intensity rating. When the desired intensity is reached for that subject a set of stimuli can be delivered at the same inter-stimulus interval as in the experiment to verify that no sensitisation occurs.

In order to precisely synchronise data presentation to data acquisition, triggers are used. One way is to design an experiment using stimulus presentation software that incorporates times when the software waits for a scanner trigger. A protocol list or protlist is written and run on the scanner that defines when the triggers are sent out by the scanner (user triggers). This enables the stimuli to be time locked to the data acquisition. Protlist files control when the scanner will send out user triggers.

1.7 Preparing fMRI data: A brief overview

The ultimate aim in any fMRI experiment is to identify voxels in the regions of interest that manifest fMRI signal changes that vary with the experimental tasks. fMRI data are susceptible to motion artefacts arising from head movement of the subject and also to an extent from the movement of the brain during the respiratory or cardiac cycle. There are also some problems relating to reduced sensitivity in some areas of the brain. This is typically found near regions of magnetic susceptibility changes such as the sinuses.

In essence, fMRI data are noisy and various pre-processing steps are applied in order to minimise artefacts. When these steps have been carried out, the data can then be analysed to ascertain which voxels are significantly activated by the task paradigm.

During any fMRI experiment there will be some movement of the subject's head due to subject motion and also to physiological factors such as the cardio-respiratory cycle. This means that the signal from an individual voxel might not correspond to the same point of the brain throughout the experiment. Linear image

registration techniques are used to align all the volumes from an experiment with a single volume.

When the data has been corrected for motion, it is then spatially smoothed. The primary reason for doing so is to boost signal to noise. fMRI is used to detect a transient signal change that lasts for a limited time period in a small localised brain region. Signal detection theory states that if a signal is blurred in space and or time then this can enhance the signal to noise. Any noise in the data that changes faster in space or time than the signal of concern is 'flattened' more than the signal of interest. The other reason for carrying out spatial smoothing is that later analysis steps employed for the data in this thesis assume that the data are spatially smoothed.

Temporal smoothing can be carried out on the data. High pass filtering would remove low frequency noise such as cardio-respiratory effects or scanner-drift. The level of the high pass filter is selected to avoid removing variations related to the experimental paradigm. Generally this can be calculated by using a filter of one and a half times the paradigm frequency. Low pass filtering removes high frequency noise and aims to cancel out random fluctuations due to noise whilst preserving the signal changes of interest. The danger of this is that it can generate false positives to occur because the similarity between one time point and the next in terms of signal intensity is artificially increased reducing the amount of independent time points.

The statistical analysis steps necessary to identify areas of activation are then carried out. Such steps are described as part of the experiment that they refer to and are not discussed at this point in the thesis.

1.8 Advantages of fMRI for the study of central pain processing

Functional neuroimaging methods (PET, fMRI, MEG) show great promise as tools for central pain research. To date research using functional imaging has shown that there is a classic network of interconnected brain regions that reflects the central nervous system (CNS) response to noxious stimuli.

fMRI is able to furnish us with new information. It is non-invasive and allows the conduction of serially designed studies that can resolve the effects of modulators, such as cognitive distraction or drugs, on the response to pain in the brain.

Within the arenas of both clinical and experimental pain research, some of the difficulties in interpreting outcomes and results stem from the present paucity of

reliable and objective pain measurement methods. The sensation of pain is a multifaceted and heterogeneous entity. The treatment of pain relies upon self report and clinician led assessment. The importance of developing functional imaging techniques to help provide an objective and accurate method with which to assess the sensation of pain should not be underestimated in this regard.

The rating of pain is an inexact science, and this contributes to vast inter-subject and inter-patient variation in the scoring of pain in the experimental and clinical arenas respectively. In order to assess the efficacy of any therapeutic advance in the area of analgesia and palliative care, it is vital to be able to objectively measure the perceived pain in order to obtain accurate outcome measures. Functional imaging can be of significant importance in contributing to advances in pain measurement. The more that is understood about the precise functional mechanisms of pain perception, the more efficient and directed pain assessment can be.

As previously discussed, both the spatial and temporal resolution of fMRI is high. The latter is of particular relevance to pain imaging studies that tend to be based upon event related design paradigms (see earlier).

1.9 Methods in pain research

Before discussing the various methods of pain imaging research, it is important to define what type of stimulus should be used when carrying out experimental research. The stimulus used should be able to be used to produce quantitative measurements of the pain threshold. The intensity of such a stimulus should be controllable and measurable to a just noticeable difference (JND) in pain sensation. The subject should be able to discriminate differences in pain intensity throughout the effective range of the stimulus from threshold to 'ceiling' pain. The stimulus should cause minimal tissue damage at pain threshold and should be a minor hazard to subjects even at highest intensities. Any noxious stimulus used should ideally be able to evoke a single quality of pain such as burning or aching.

The induction of pain within the laboratory requires careful consideration of the ethical implications of causing pain in human or animal subjects together with the ecological validity of the pain induction techniques.

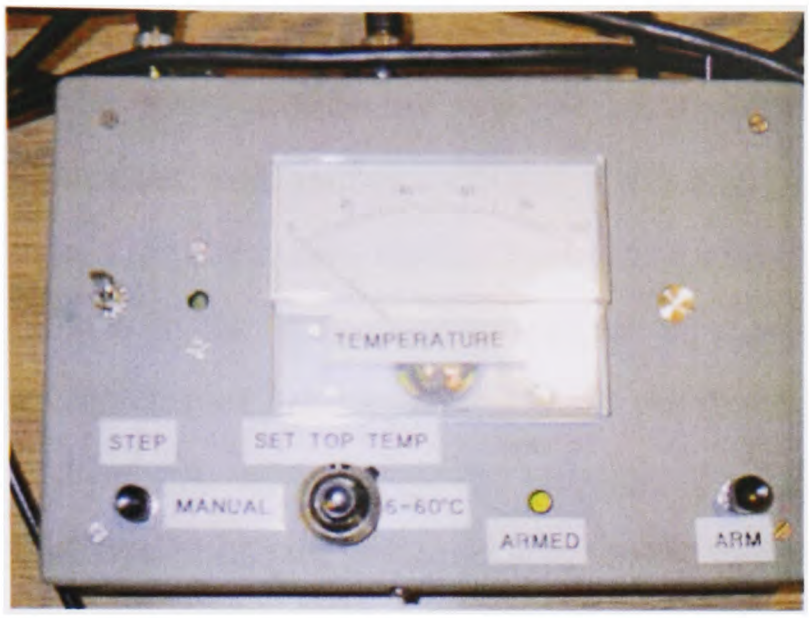
The degree to which mild laboratory induced pain is truly comparable to clinical pain in the late stages of cancer is tenuous in the extreme. This is not merely

by dint of the intensity of the pain, rather that additional cognitive and emotional issues are present in clinical pain. Clinical pain is inescapable whereas the subject in an experiment is free to leave at any point during the study. However, laboratory pain experiments including imaging experiments have the potential to provide invaluable insights into pain perception that can then be brought to the clinical arena and applied to clinical practice and treatment regimes.

There are four modes of pain induction: thermal (cold pressor, peltier thermode), electrical, chemical and mechanical. Electrical methods are common since they are easy to administer and quantify. Chemical methods use chemical stimuli at various tissue depths (cutaneous, subcutaneous, intraperitoneal and intramuscular), and can include ischemic methods which induce pain by local chemical changes. Mechanical stimuli use pressure applied in various ways.

The research detailed in this thesis discusses thermal noxious stimuli. The experiments carried out use heat pain to investigate the central neuronal correlates of pain perception and processing. The noxious stimuli are administered to the dorsum of the hand using a thermal resistor (1.5x 2cm), designed and built in-house (see Fig. 1.3). This device delivers noxious stimuli with a short temperature ramp time (30-60° in 0.8s). It is controlled by in-house software and simultaneously measures skin temperature over the area stimulated. For safety reasons, it is fitted with an alarm which sounds whenever there is current passing through it and therefore whenever pain is being delivered.

Fig. 1.3 Photograph of the pain induction device used throughout the experiments in this thesis.



This photograph shows the thermal pain device used in our experiments. It was built in-house.

1.10 Cognitive strategies for pain modulation

As an introduction to the subsequent chapters describing experiments designed to modulate laboratory induced thermal pain using cognitive distraction, some psychological interventions for the modulation of pain will be discussed here. The interested reader is referred to Horn and Munafo (1997) for further information on this topic.

It is thought that simple techniques such as distraction have been used to help reduce acute pain for many years, indeed hypnosis-induced analgesia was employed long before the advent of modern pharmacological techniques (Horn and Munafo 1996). The development of non pharmacological techniques to alleviate pain has been developed significantly since the 1930s (Horn and Munafo 1996).

There are various roots behind the strategies employed to modulate pain by psychological means. In the 1960s Miller et al (1974) demonstrated that various autonomic nervous system functions could be modified by operant conditioning providing the basis for biofeedback, which is used today in pain control. Fordyce (1984) pioneered the use of operant conditioning for chronic pain.

It should be noted at this point that such psychological interventions for the treatment of pain gained popularity by showing efficacy against pain in patients whose conditions did not respond to more traditional methods or pharmacological approaches. Referral to a psychologist to address this was often seen as a last resort, and such patients labelled as suffering from neurotic conditions or psychogenic pain.

It is now known, that in some cases, even where no organic cause is revealed in medical examinations, pain can be modulated by psychological techniques.

Relaxation techniques are essentially based upon the fact that muscular tension associated with pain exacerbates it still further and this in turn can lead to immobility, which in turn causes further muscular tension and further pain. Jacobsen (1938) was a pioneer in this area. Jacobsen taught his subjects to relax 44 different skeletal muscle groups in turn, taking up to an hour per muscle group, so it was a lengthy process. He showed that with appropriate training, residual muscle tension could be abolished and that this technique was effective in the treatment of a variety of medical conditions including pain relief. Modern relaxation training methods are considerably shorter, and patients can use tapes of instructions to help with training in the technique and incorporate a calming mental exercise to complete after the muscle relaxation. Some such regimens involve controlled breathing to further deepen the level of relaxation. Some relaxation schemes specifically aimed at modulating pain involve some use of visual imagery in combination with the muscle relaxation. All such treatments must be carried out in a quiet comfortable environment and involve the progressive relaxation of skeletal muscles. These treatments must be adapted to suit the individual needs of the patient and to suit the condition involved. Relaxation has been shown to be effective in reducing the pain of tension headache, low back pain, and arthritis (Horn and Munafo 1996). Syrjala et al (1995) found that a combination of relaxation training and imagery was effective at reducing pain during cancer treatment. It is hard to carry out controlled studies of relaxation therapy by itself since it is difficult to provide a suitable control condition against which to evaluate its efficacy. Relaxation engenders a range of effects such as decreased autonomic arousal, decreased muscle tone, and focused attention so its mechanisms are complex. Further studies are required to confirm the beneficial effect of relaxation in the reduction of chronic pain (Carroll and Seers 1998) In order to accurately assess the efficacy of psychological techniques in the reduction of clinical pain, it is necessary to carry out condition specific studies because heterogeneous subject groups can confound the results (Nielson and Weir 2001).

Distraction using visual or sensory methods can result in the modulation of experimentally induced pain. This is addressed in more detail in Chapter 2. Johnson et al (1998) for example found that distracting tasks were more effective at reducing

experimental pain when attention was directed to external cues such as thermal detection tasks than where the strategy required positive or neutral imagination.

The use of visual imagery has been used to help reduce clinical pain. Miller et al (1992) found that burns patients reported lower levels of pain when they watched videos of beautiful scenery accompanied by relaxing classical music in conjunction with conventional analgesics compared with analgesics alone.

Another method of pain control is biofeedback. This works by providing the patient with information such as skin temperature, or muscle tension on a continuous basis, translated into an easily readable signal such as a flashing light or a series of clicks. This gives them information about the state of their body. This technique will not be discussed here at any length. Pain can be reduced by giving patients information relating to the state of tension in their muscles for example. Conditioned learning can then allow them to relax their muscles and this is reinforced by seeing information showing that muscle tension has reduced as a direct result of their actions. Chapman (1986) showed that biofeedback is effective in reducing headache pain. If pain is caused by excessive muscle tension or vasoconstriction then biofeedback should provide a method to aid the conditioning of corrective responses to alleviate the pain. This is made slightly more complicated by the fact that the focussing of attention on the output of the machine could be a distraction from the pain and that this mechanism could also contribute to the overall effect.

Hypnosis has also been used to modulate pain both clinically and experimentally. There is ongoing discussion and debate in this area and its application to pain control (Horne and Munafo 1997). Some see the nature of hypnosis as an altered state of consciousness and others see it as a sophisticated social interaction between hypnotist and patient (Wagstaff 1995). Hypnosis (therapist led) induces the successful recipient into a more passive state where there attentional focus is narrower and their arousal level is lower. This state is not dissimilar to drowsiness where the individual is less in touch with reality and more open to suggestion (Budzynski 1971). Laboratory studies of hypnosis have typically involved inducing pain with pressure or ice-water and then giving the suggestion of analgesia (Horne and Munafo 1997). The use of hypnosis as a treatment for pain in conditions such as surgery, labour, burns, cancer and headache has been documented. The reader is referred to Bromley (1985) for a full discussion. Suggestions are given to the patient concerning numbness or sensory transformation or dissociation for example.

Cognitive behavioural therapy has also been employed in the treatment of chronic pain. Fordyce (1984) describes pain behaviours (grimacing, crying, limping etc.) as operants which are sensitive to learning or conditioning effects from reinforcers in the environment. Positive reinforcement of pain behaviour occurs when pain behaviour results in extra care or attention being offered to the patient. Negative reinforcement results when pain behaviour allows unpleasant or aversive situations to be avoided by the patient. The effect of conditioning on pain behaviour depends upon the expectations of reinforcement from past experience, the extent to which the environment has potential for reinforcement and the length of the pain condition (longer duration pain will mean that there are more opportunities for reinforcement). Treatment involves therapy to eliminate or extinguish the maladaptive pain behaviours such as overuse of medication, excessive diminution of activity by non-reinforcement and also by reinforcing "well" behaviours by praising the patient. Some critics argue that cognitive behavioural therapy regimens merely moderate the outwards signs of pain but do not ameliorate the underlying pain experience. However patients often report a decrease in pain levels and a heightened sense of well-being as such programmes progress and activity levels increase (Horne and Munafo 1997). There are many reasons that could contribute to this effect: busy days offer distraction from the pain, increased activity rebuilds muscle and counteracts the effects of disuse. Coping skills are also developed which are very important in the battle against chronic pain. Cognitive behavioural therapy has been shown to be effective in children with acute pain and to help patients cope with painful medical treatments after burn injuries (Elliott and Olson 1983). Such treatments are hard to accurately evaluate due to the combination of factors involved and the difficulty of controlling for the different variables against a control condition. Some studies have shown that CBT is effective in a range of painful conditions but sometimes that the whole package of therapy is not more effective than some of its components such as relaxation and imagery (Horne and Munafo 1997). Possibly coping skills developed during CBT are implicitly conditioned as successful relaxation or imagery is attained and perceived to be effective in reducing the patients' pain.

This chapter only gives an introduction into the different cognitive strategies that can be employed to modulate clinical and experimental pain. Chapter 2 discusses one such technique in more detail- distraction.

The next chapter describes the modulation of pain through the use of a cognitive distraction task and the central brain areas involved in this modulation of perceived pain intensity. The hypothesis for this study is that the cognitive interference condition within the counting Stroop task would be more distracting than the neutral condition. This distraction task would distract the attention of subjects away from the thermal painful stimuli resulting in lowered perceived ratings of pain intensity. The hypothesis continues to suggest that there would be corresponding decreases in signal observed in pain processing regions such as the ACC which are involved in both pain processing and attentional processing.

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CHAPTER 2: The attentional modulation of pain

The previous chapter discussed the advantages of using fMRI to investigate the central processing of pain.

Current clinical and experimental literature strongly supports the phenomenon of reduced pain perception whilst attention is distracted away from noxious stimuli. This study used fMRI to elucidate the underlying neural systems and mechanisms involved. An analogue of the Stroop Task, the Counting Stroop, was used as a cognitive distraction task whilst subjects received intermittent painful thermal stimuli. Pain intensity scores were significantly reduced when subjects took part in the more cognitively demanding interference task of the Counting Stroop than in the less demanding neutral task. When subjects were distracted during painful stimulation brain areas associated with the affective division of the anterior cingulate cortex (ACC) and orbitofrontal regions showed increased activation. In contrast, many areas of the pain matrix (i.e. thalamus, insula, cognitive division of the ACC) displayed a reduced activation, supporting the behavioural results of reduced pain perception.

This experimental chapter explores how pain can be modulated by attention, and the functional anatomy of the central areas involved.

2.0 Introduction

The behavioural-affective, and sensory-discriminative elements of pain perception are reduced in situations such extreme trauma experienced in battle, via feedback information from higher order cognitive areas (Melzack and Casey, 1968). It is found that patients report more intense post surgical pain when they are required to attend to the pain (Miron et al., 1989), and methods such as listening to music can significantly reduce postoperative pain in patients (Good et al., 1999). Current research suggests that unless subjects' attention is actively directed elsewhere, painful stimuli will take precedence over competing non-painful ones (Eccleston and Crombez, 1999). Psychological state is therefore able to modulate the perception of ecological and experimentally induced pain. Psychophysical examples of this include work by Miron et al. (1989), showing that directing attention to a painful stimulus increased its perceived intensity and unpleasantness compared to when subjects were asked to direct their attention away from the stimulus. Pain ratings were reduced when a cognitive task was carried out at the same time as administration of cold pressor pain (Hodes et al., 1996). In addition, a thermal laser study found reduced human electrocortical evoked potentials when attention was diverted away from the painful stimuli (Siedenberg and Treede, 1996).

It is clear that attention can modulate pain, but our understanding of the precise cognitive mechanisms remains poor. To date, the focus of which brain areas are involved with attentional modulation of pain has centred on the anterior cingulate cortex (ACC). The ACC has a pivotal role in executive processes, motivation, allocation of attentional resources, premotor functions, and error detection (Turken and Swick, 1999). The ACC is activated by moderate to intense painful stimulation (Casey et al., 1994) and PET studies have revealed large concentrations of opiate receptors in this region (Jones et al., 1991). It has been suggested that attention and pain activate separate sites within the ACC (Vogt et al., 1992; Davis et al., 1997). Hsieh et al. (1995) have shown that areas of the ACC activated by painful stimulation lie in adjacent parts of the ACC to those activated by attention demanding tasks. During a distraction paradigm using a maze task, orbitofrontal cortical areas were found to be activated in situations of increased cognitive load, such as when the pain interfered with completion of a cognitive task (Petrovic et al., 2000).

The present study aims to address the function of other brain areas that may also play a significant role. This information is important to determine if novel therapies based upon attentional manipulations are to be developed. The original Stroop Colour Word Task (Stroop, 1935) is a cognitive interference task, used as a sensitive clinical test of executive function (Peterson et al., 1999). A problem with using this task in functional magnetic resonance imaging (fMRI) is that it necessitates speech, which produces head movements and image artefacts on functional imaging data sets. An analogue of the Stroop Task developed specifically for use with fMRI is the Counting Stroop Task (Bush et al., 1998). In this task subjects are required to respond via a button press with the number of words presented regardless of the word itself (speech is therefore obviated). As the Counting Stroop activates a wide network of brain regions, produces significant cognitive interference and provides on-line behavioural data, it is an ideal task to use for studies investigating the attentional modulation of pain. The hypothesis is that during the interference condition, the painful stimulation will be perceived as less intense due to the effect of being distracted by the attention demanding cognitive task, and that this will be reflected in corresponding modulation of blood oxygen level dependant (BOLD) response. This would enable us to find out which areas of the brain are involved in the lowering of pain scores by cognitive interference.

Painful stimulation alone activates many areas within the brain, often defined as the "pain matrix" (Melzack and Wall, 1965; Tracey et al., 2000). Areas activated include somatosensory and motor cortices, anterior cingulate cortex (ACC), parietal and prefrontal cortices, as well as the insula, thalamus and cerebellum. Some of the areas identified during painful stimulation, such as the posterior parietal cortex, the ACC, dorsolateral prefrontal cortex and the thalamus, are also reported as belonging to attentional networks (Peyron et al., 1999). The cognitive-evaluative component of pain therefore includes attentional processes, anticipation and memory for past experiences of pain (Peyron et al., 1999). FMRI is an excellent tool to study pain mechanisms in the brain. Owing to its high spatial and temporal resolution, sophisticated paradigm designs are possible, allowing individual components of the pain matrix to be characterized and dissociated. Using fMRI and a differential pain-conditioning task, Ploghaus et al. (2000)

examined the predictions of attentional (Pearce and Hall, 1980; Mackintosh, 1975) and non-attentional (Recorla and Wagner, 1972) learning theories. Attention was manipulated by varying the degree to which painful stimulation, or its omission, was surprising or expected. The omission conditions were included in order to separate attention to the noxious stimulation site from attention-induced changes in pain perception. This accounts for the absence of activations in some areas of the "pain matrix" observed by Ploghaus et al. (2000). The present study, in contrast, examines attention-induced changes in pain perception. We therefore hypothesize that distraction will modulate regions of the "pain matrix", and our analyses will focus on these areas.

2.1 Materials and methods

2.1.0 Subjects

Eight healthy right-handed volunteers (six males, 2 females; mean age 30 ± 9 years) were recruited. Subjects gave informed consent in accordance with full ethical approval by the Central Oxfordshire Research Ethics Committee (COREC). Subjects underwent a comprehensive verbal screen to ensure that they did not meet any exclusion criteria for MRI experimentation.

2.1.1 Functional MRI

Subjects were scanned in a 3T human MRI scanner (Oxford Magnet Technology), using a bird cage radio frequency coil and reduced bore gradient coil (Magnex SGRAD MK III). Foam padding was placed around the subject's head to minimise movement. An MR compatible pulse oximeter (9500 Multigas Monitor, MR Equipment Corporation) was attached to record heart rate and blood oxygen saturation throughout. Subjects wore earplugs and MR compatible electrostatic headphones (MRC Institute of Hearing Research) to attenuate the noise of the scanner and facilitate communication with the experimenter.

A localiser scan served as a reference for axial slice selection for functional imaging data covering the entire brain volume. Echo planar imaging (EPI) continued throughout the pain and attention paradigm and used the following parameters: TE = 30

ms, TR = 2.5 s, flip angle = 90°, FOV = 256 x 256 mm, matrix = 64 x 64, slice thickness = 6 mm, 21 slices. A 3D Turbo Flash T1-weighted (axial) high resolution anatomical scan was taken for each subject using the same slice prescription as used for functional imaging data. This was used for co-registering scans from different individuals to a common standard.

2.1.2 Psychophysical details

Visual stimuli

Stimuli were generated using in-house software and back projected onto a screen (In Focus LP1000) viewed by subjects wearing prism glasses. A home built, hard-wired, MR compatible, four-button box was used to obtain simultaneous recordings of subjects' button press responses and reaction times throughout.

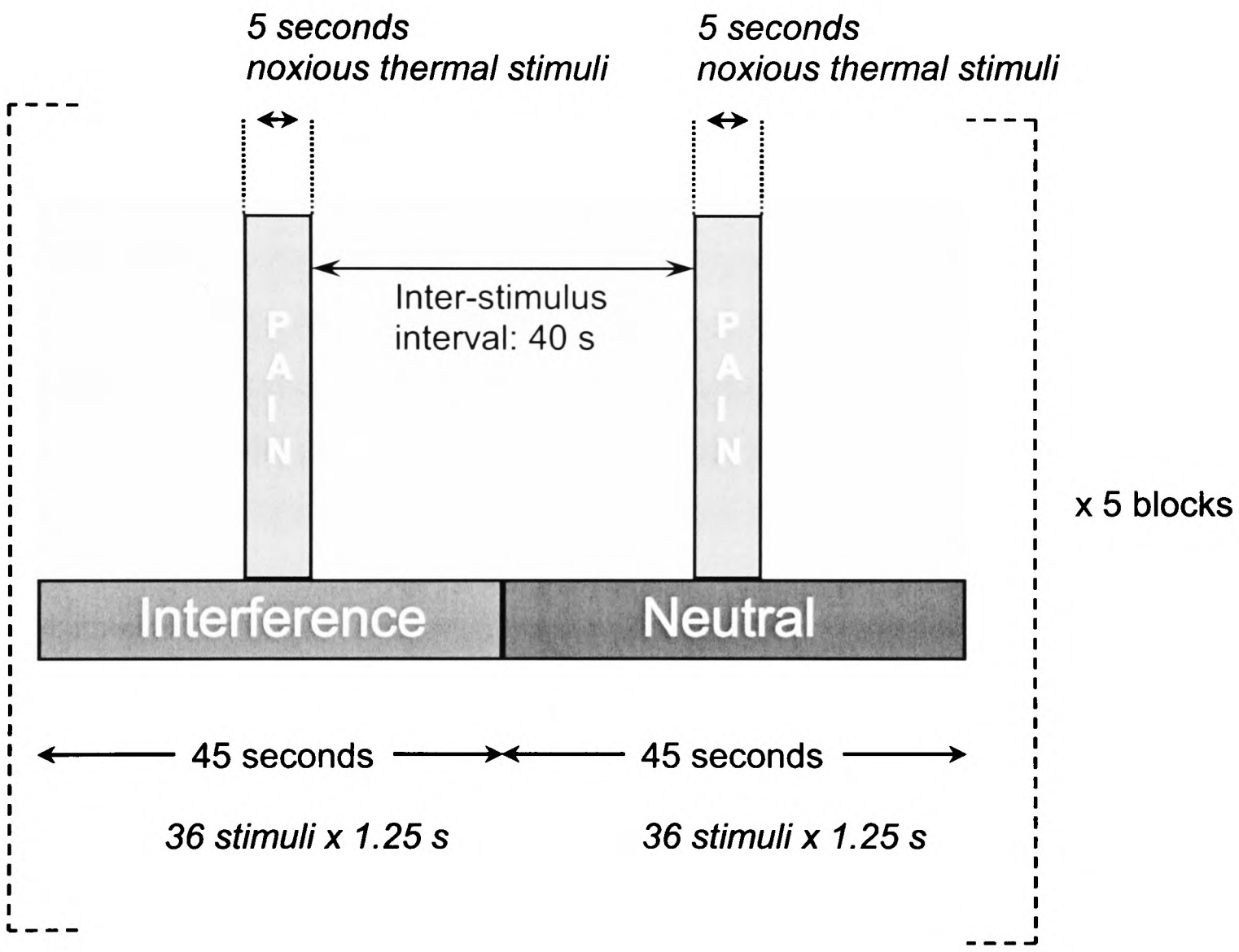
Noxious thermal stimuli

Thermal noxious stimuli (temperature range across subjects 50-53.5 ° C, of 5 s duration) were administered to the dorsum of the subject's left hand using a thermal resistor (1.5 cm x 2 cm), designed and built in-house. This device delivered noxious stimuli with a short temperature ramp time (30-60 ° C in 0.8 s). It was controlled by in-house software, and simultaneously measured skin temperature over the area stimulated.

2.2 Experimental design

Each subject attended one scanning session of approximately 45 minutes duration. Thermal noxious stimuli were administered to the left hand to 'threshold' each individual to a temperature to which they consistently attributed a pain scale rating of '8' out of 10 or 'strong pain'. An outline of the timings utilised throughout the paradigm can be seen in Fig. 2.0.

Fig. 2.0 The stimulus paradigm used in this study. Interference and neutral blocks of stimuli were alternated in sequence. Five blocks of each type were presented, during each of which there were 36 counting Stroop stimuli of 1.25 s duration (45s in total). A painful thermal stimulus of 5 s duration was administered in each block concurrently with the counting Stroop stimuli. The temperature of the painful stimulus was the same across all blocks, with 40 s between each painful stimulus to prevent sensitization to the stimulus.



This study used a modified version of the Stroop Task (Stroop, 1935), the Counting Stroop (Bush et al., 1998). Subjects were informed that they would see sets of between 1 and 4 identical words presented vertically below each other on the screen that would change every 1.25 seconds. They were asked to record the number of words presented on the screen (regardless of the word itself) using the button corresponding to the number of words presented, as quickly and accurately as possible, with strong emphasis placed on

not sacrificing accuracy for speed. In order to try to prevent subjects making the task easier by blurring the words on the screen they were asked to internally verbalise the word once, at the same time as pressing the relevant button. The 'interference' block (I) stimuli consisted of the number words 'one', 'two', 'three', and 'four'. During the 'neutral' blocks (N) the stimuli consisted of the animal names 'cat', 'fox', 'tiger', and 'frog'. Five 45 second 'interference' blocks alternated with five 'neutral' blocks of the same duration, starting with an interference block. Both stimuli sets in the two experimental block types comprised words in the same semantic category and were balanced for word length (i.e. the word 'cat' is the same length as 'one' and 'frog' the same length as 'four'). For each of the five 'neutral' and five 'interference' blocks, the individual word stimuli were presented in pseudorandom sequence in each case, and the order of stimuli within blocks was different in all of the five trials for each condition. There were no congruent trials in the interference condition, i.e. one presentation of the word 'one'. The reader is referred to Fig. 2.1, which details the presentation of the stimuli.

Fig. 2.1 Stimuli used for the counting Stroop paradigm. The counting Stroop is a distraction task in which subjects press buttons as fast and as accurately as possible to indicate the number of words presented. The neutral task contained animal words. The interference task was more complex, with incongruence between the number of words presented (required response) and the word meaning. For example, four presentations of the word 'one' would require the subject to press button 4.

Neutral task

Cat **Cat** **Cat**	*36 stimuli, 1.25 s duration, pseudorandom presentation. Subject required to press button as quickly and accurately as possible. Reaction times were recorded.* **In this example the correct response is button 3.**

(5 blocks)

Interference task

One **One** **One** **One**	*36 stimuli, 1.25 s duration, pseudorandom presentation. Subject required to press button as quickly and accurately as possible. Reaction times were recorded.* **In this example the correct response is button 4.**

(5 blocks)

In the middle of each of the ten blocks a five second painful thermal stimulus of constant temperature was applied to the subject's left hand at the same temperature previously rated as '8' by the subject. The visual stimuli carried on throughout and therefore 4 stimuli were shown whilst the pain continued. Subjects were told that they should pay

attention to the task throughout but maintain awareness for the rating they would give to the thermal stimuli. They would then be asked to compare pain intensity for conditions I and N at the end of the experiment using the pain scale presented during thresholding.

2.3 Statistical analysis

Behavioural data

The mean reaction time between stimulus presentation and button presses, across interference blocks and neutral blocks was calculated across subjects and a Student's one-tailed t-test was carried out to assess the significance of the difference between the two groups of reaction time means. The pain scores across subjects were similarly analysed and a one tailed Student's t-test carried out to compare the significance of the difference between the interference versus the neutral pain intensity scores.

Imaging data

Analysis of the fMRI images to identify regions exhibiting significant changes in blood oxygen dependent signal (BOLD) (Ogawa et al., 1992) was carried out via a multi-stage process using the image analysis package, FEAT (www.FMRIB.ox.ac.uk/fsl), an extension of MEDx (Sensor Systems). The following pre-statistics processing was applied from within the FEAT package; motion correction using the SPM realign procedure (Friston et al., 1995a); spatial smoothing using a Gaussian kernel of full width half maximum (FWHM) 5.0 mm; mean-based intensity normalisation of all volumes by the same factor; non-linear high pass temporal filtering (Gaussian-weighted LSF straight line fitting, with high pass filter cut-off 80.0 s. The statistical portion of the analysis was carried out from within the FEAT package using a general linear modelling (GLM) approach (Friston et al., 1995b). This allows a description of the experimental design to be made; then a model is created that is fitted to the fMRI data, indicating where the brain has activated in response to the stimuli. In FEAT, the GLM method is known as FILM (FMRIB's improved linear model) (Woolrich et al., 2000, Woolrich et al., 2001) (www.fmrib.ox.ac.uk/ analysis.techrep/tr01mw/tr01mw). FILM uses a robust and accurate nonparametric estimation of time series autocorrelation to prewhiten each

voxel's time series; this gives improved estimation efficiency compared with methods that do not pre-whiten.

The study was designed in a factorial way. Two explanatory variables were proposed to model each subject's fMRI time-course data on a voxel by voxel basis. The explanatory variables were the Stroop condition and the painful stimulation, and were modelled within the General Linear Model (GLM) both independently and as interactions between the two variables. Each explanatory variable results in a parameter estimate image. This estimate indicates how strongly that waveform fits the fMRI data at each voxel; the higher it is, the better the fit. Areas showing a significant positive interaction between variables are more active, as measured by the fMRI signal change, when the pain and Stroop are occurring simultaneously than would be predicted by the simple addition of the responses to both stimuli alone. In contrast, the negative interaction indicates a reduction in activity during simultaneous stimuli. To convert from a parameter estimate to a t statistic image, the parameter estimate is divided by its standard error, which is derived from the residual noise after the complete model has been fit. The t image is then transformed into a z statistic, 'activation map' via standard statistical transformation.

A fixed effects group cluster analysis (a second level analysis) was carried out on the subjects' activation maps to produce representative group results. Four contrasts were formed. The first compared interference blocks with neutral blocks, the second compared pain with non-pain, the third reported the positive interaction between pain and interference and a fourth looked at the negative interaction between the pain and the interference task. The thresholding parameters used for the fixed effects analysis were a z-score threshold of 2.0 and a probability value of $P = 0.05$ for cluster significance (Friston et al., 1994; Worsley et al., 1992; Forman et al., 1995).

2.3.1 Quantification of pain modulation by distraction

The inclusion of interactions in the model for the fMRI signal provides the flexibility to identify those voxels in which the activation in response to painful stimulation is increased (positive interaction) or decreased (negative interaction) by the

interference condition. Similarly, the examination of such interactions reveals the effects of pain on the activation in attentional areas.

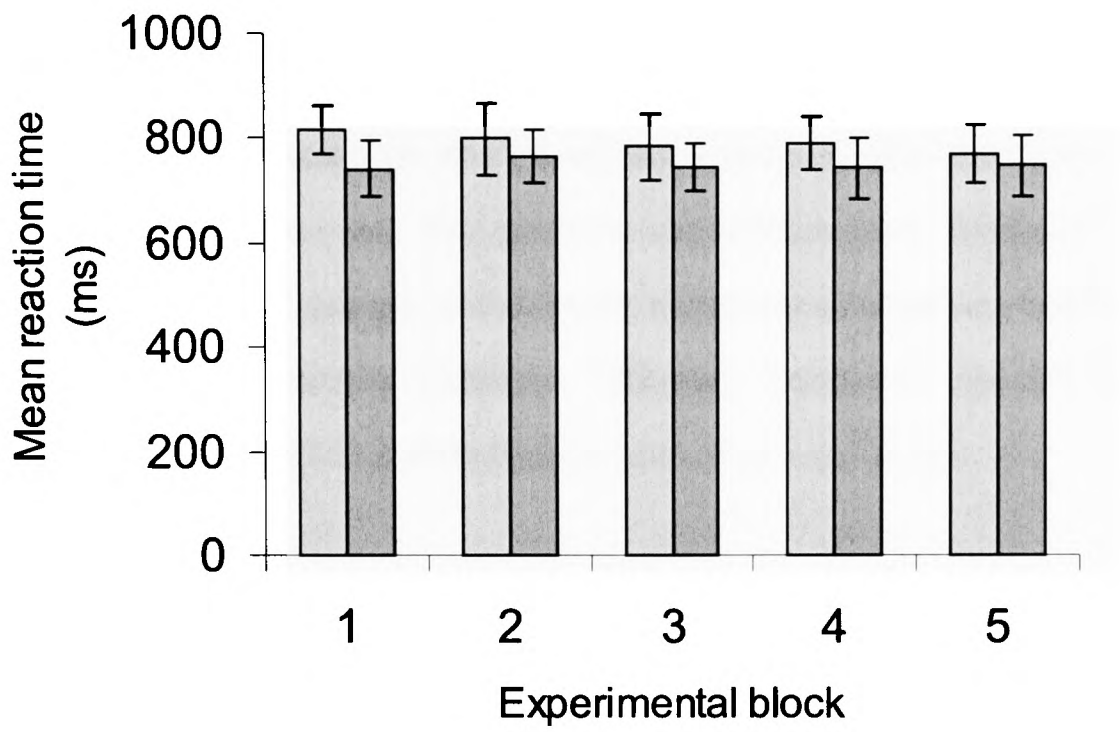
Using this model, the magnitudes of fMRI signal responses to the painful stimulus in the interference and non-interference condition were identified. These responses are described here as parameter estimates from the model fitting, in arbitrary units, representing the fMRI signal changes. Having manually defined regions of interest of the pain matrix from anatomical scans, the mean values of those parameter estimates (fMRI signal changes) were calculated over those regions. This provided a measure of regional pain activity during the two different cognitive load conditions. A group mean activity across subjects was thus established allowing a test of significance between the interference and neutral conditions (paired t-test) to be carried out for each brain region.

2.4 Results

2.4.1 Behavioural results

For the Counting Stroop task, the overall mean reaction time (RT) for all interference blocks (I) was significantly greater than the mean reaction time for all neutral blocks (N), mean RT 794 ms ± 55 (I); mean RT 748 ms ± 52 (N) ($P = 0.0008$, Student's t-test). This is displayed graphically in Fig. 2.2. This gives behavioural evidence that the subjects were engaging in the task, and also that the distraction task was more cognitively demanding than the neutral task. The measure of reaction time was taken to show the difference in cognitive difficulty level between the two tasks. Subjects were asked to prioritise accuracy over speed throughout the experiment.

Fig. 2.2 Mean RTs by experimental block for both interference (light grey) and neutral (dark grey) tasks. The mean RT for the interference task was significantly increased compared with the mean RT for the neutral task (P= 0.0008). ANOVA revealed no significant practice effects with time.



There was no indication of practice effects. This was important to ascertain because if the task became easier across the experiment then there would be a reduction in the strength of the distraction task. A one-way analysis of variance (ANOVA) showed that there was no significant difference in reaction times over time from blocks 1 to 5 (N: P = 0.998, F= 0.0317; I: P = 0.986, F = 0.087). Pain intensity ratings were significantly lower during the interference blocks than during neutral blocks, mean intensity 6.6 ± 0.2 (I); mean intensity 7.3 ± 0.3 (N); (P = 0.006, Student's t-test). Subjects reported that the pain felt less intense whilst they were engaged in the more demanding interference task than in the neutral task.

2.4.2 Functional imaging results

The group analysis for the Counting Stroop alone showed activation in previously reported areas (anterior cingulate cortex, medial frontal gyri, premotor and primary motor

cortex, inferior temporal gyrus, and the superior parietal lobule) (Bush et al., 1998). The group analysis for noxious thermal stimulation confirmed that subjects show activation in all brain regions associated with the "pain matrix" (Peyron, et al. 1999). The talairach coordinates for the noxious stimulation alone are shown in Table 2.2.

Areas activated in this study are consistent with this typical activation resulting from noxious stimulation and include insular cortex, bilateral thalamus, anterior cingulate, and sensory cortex. These findings confirm the validity of our modelling and its capacity to dissociate pain and Stroop activations within the brain.

Group analysis for the positive interaction (see Methods) reveals activity in bilateral orbitofrontal cortex, and bilateral perigenual cingulate. Table 2.0 shows the areas activated in this contrast together with the Talairach coordinates for these areas. Illustration of the cingulate activation is shown in Fig.2.3 (a).

Table 2.0 Positive interaction: This table gives the regions activated in the positive interaction with their Talairach coordinates and mean and maximum Z statistic values

Brain region	Laterality	Talairach coordinate	mean *Z*	max *Z*
Orbitofrontal cortex	L/R	18, 44, 2	2.31	3.80
Perigenual cingulate	L/R	-10, 32, -2	2.25	3.05

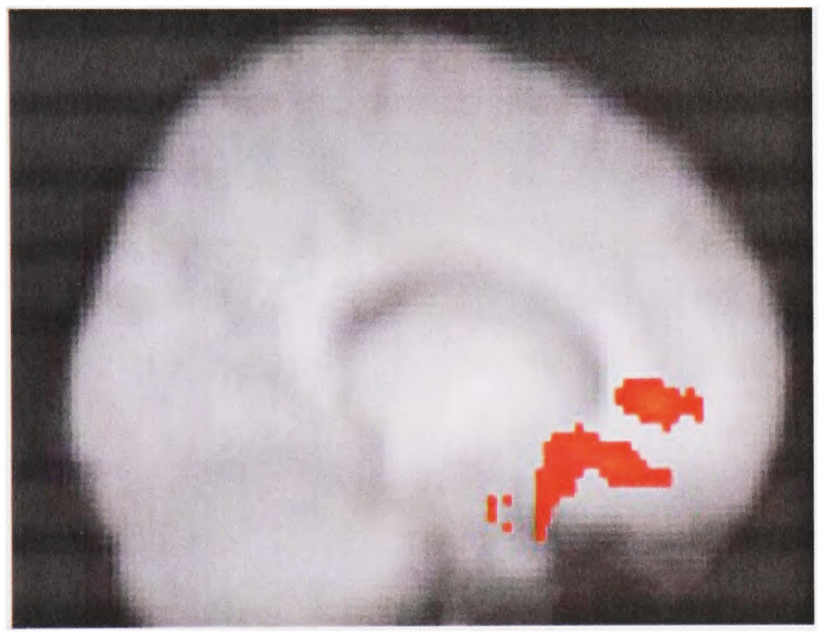
Group analysis for the negative interaction, reveals activation in areas including the contralateral insula, midline cerebellum, medial thalamus, left superior and medial temporal gyri (BA 21/22), contralateral hippocampus/caudate, bilateral posterior cingulate cortex, bilateral midcingulate, and midcingulate / premotor areas (see Table 2.1 for areas activated together with their corresponding Talairach coordinates). Illustration of the midcingulate activation is shown in Figure 2.3 (b).

Table 2.1 Negative interaction: This table details the areas of the brain activated in the negative interaction.

Brain region	Laterality	Talairach coordinate	mean *Z*	max *Z*
Insular cortex	L	-36, 6, 0	2.34	3.78
Cerebellum	L/R	8, -78, -16	2.36	3.25
Thalamus	L/R	2, -14, 12	2.44	3.71
Medial/superior temporal gyrus	L	-54, -10, 0	2.27	3.48
Hippocampus/caudate	L	-26, -34, 6	2.17	2.83
Midcingulate	L/R	-4, -20, 32	2.38	3.60
Posterior cingulate	L/R	0, -36, 28	2.17	2.70
Midcingulate/premotor cortex	L/R	6, -2, 50	2.29	3.40

Fig. 2.3 (a) Perigenual cingulate activation associated with the positive interaction. The explanatory variables of the Stroop task and pain were modelled within GLM independently and as a non-linear interaction between the two variables. The positive interaction reveals areas that are more active when pain and Stroop are occurring simultaneously than would be predicted by the simple addition of the responses to both stimuli alone. (b) Midcingulate activation associated with the negative interaction. The negative interaction reveals areas that are less active when the pain and the Stroop task are occurring simultaneously than would be predicted by the simple addition of the responses to both stimuli alone.

(a)



(b)

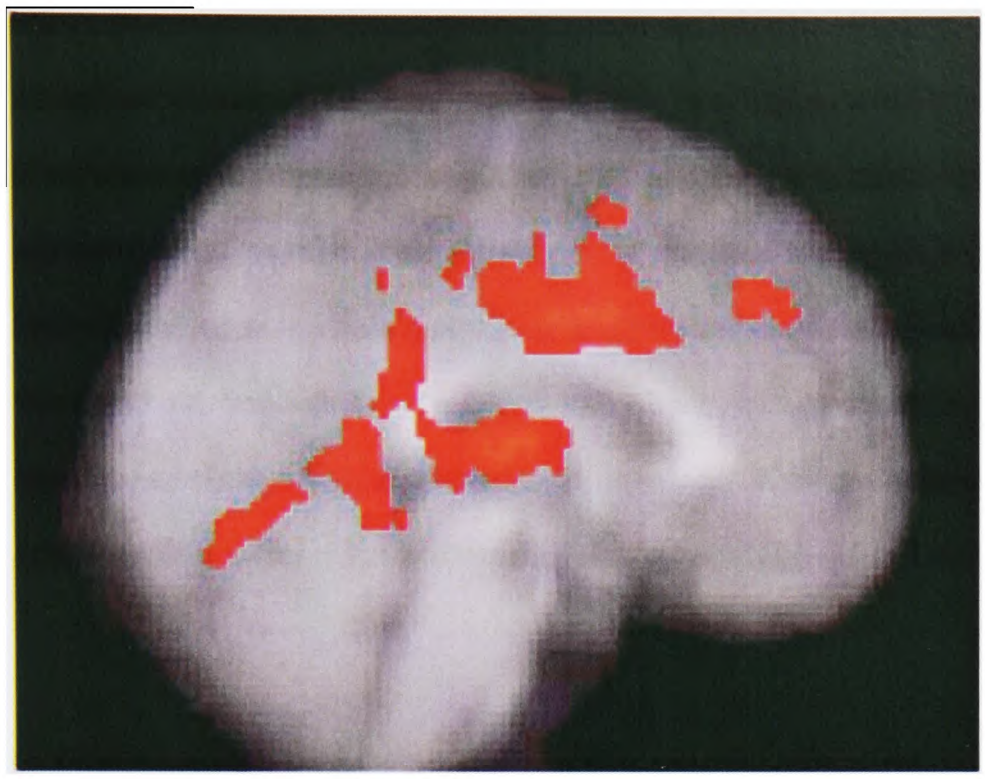
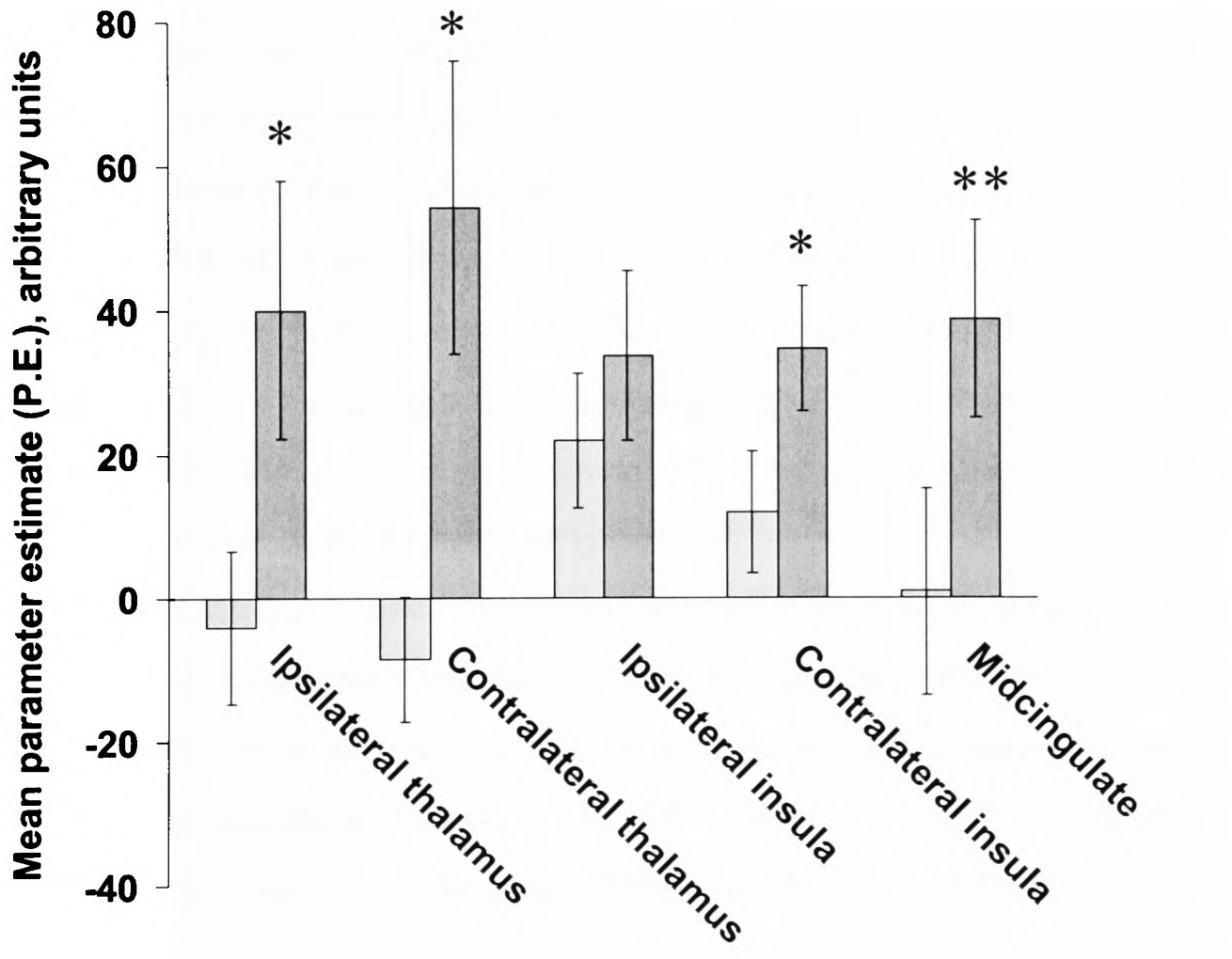


Table 2.2 Talairach coordinates for the painful thermal stimulation alone.

Coordinates (XYZ)			Brodmann's Area	Region/Hemisphere
40	19	-14	38	
4	-76	-14		cerebellum
42	14	-12	38	Bilateral
46	22	-10	47	Predom. Right
-40	22	-8	47	Left
46	38	-4	10/47	Right
42	-22	2	medial temporal gyr 22/21	Predom. Right
36	-26	0	insula	Predom. Right
-38	-18	0	insula	Left
4	-16	2	thalamus	Bilateral
4	-6	4	pulvinar	Bilateral
-8	-6	6	pulvinar	Bilateral
2	-46	8	ACC 30/23	Midline
0	24	28	ACC 24/32	Bilateral
4	-32	28	ACC 23/31	Bilateral

Fig. 2.4 Mean parameter estimates within key areas of the pain matrix during painful stimulation in the interference task (white) and the neutral task (black). The group results show mean ± 1 SEM regional activation, and significance levels are indicated for P < 0.05 (*) and P < 0.005 (**). The parameter estimate is the factor by which the linear signal model is scaled to best fit the fMRI time-course data. Parameter estimates are measured in arbitrary units and are proportional to fMRI signal changes. The ipsilateral thalamus, contralateral thalamus, contralateral insula and midcingulate all showed a significant drop in pain activation during the distracting interference condition compared with the neutral condition.



The group results (mean ± SEM) of regional activation or signal changes in the "pain matrix" during interference and neutral conditions are illustrated in Fig. 2.5. The ipsilateral thalamus, contralateral thalamus, contralateral insula, and ACC all show a

significant drop in pain activation during the distracting interference condition compared to the neutral condition. This is discussed more fully in the discussion section of this paper.

2.5 Discussion

Our experimental results showed decreased behavioural pain ratings whilst subjects were occupied in a more distracting cognitive interference task than when they were performing a neutral less demanding task. These ratings were obtained as an average rating at the end of the experiment for pain perceived during the interference task and pain perceived during the neutral task. Pain ratings obtained during an experiment are optimal since they negate the need for subjects to remember average pain intensities. However during complicated cognitive paradigms it is not always desirable to introduce other confounds such as rating pain during baseline blocks between Stroop conditions as this might invalidate the fundamental Stroop task itself.

We believe that we have minimized any potential bias in reporting pain intensities in this experimental paradigm via the use of naïve subjects and careful attention to the instructions given to participants. Future studies could potentially benefit and reduce any bias still further by carrying out one non-imaging session in the scanner where pain would be rated during the cognitive paradigm or where subjects would carry out the paradigm on the bench outside of the scanning environment.

Painful stimuli take precedence over non-painful ones unless a concerted effort is made to direct attention elsewhere. It is thought that frontal regions including the orbitofrontal cortex play a key role in modulating pain processing during attentional manipulation paradigms (Miron et al., 1989; Davis et al., 1997; Derbyshire et al., 1998; Peyron et al., 1999; Petrovic et al., 2000; Ploghaus et al., 2000). Peyron et al. (1999) suggest that an intensity coding matrix (anterior insula, SII, contralateral thalamus) is overlaid on an attentional matrix (bilateral thalami, posterior parietal and prefrontal cortices, and anterior cingulate gyrus). Although the "pain matrix" is well characterised, studies to date have not entirely clarified the nature of its modulation by attention leading to a drop in perceived pain intensity. Our experiment combines high field fMRI with a

novel attentional paradigm ideally suited for functional imaging (Bush et al., 1998). To look at this in detail, our imaging data was analysed using the general linear model (GLM) (Friston et al., 1995b), a sophisticated analysis tool able to discriminate interactions between competing events, such as pain and Stroop.

This discussion is confined to the positive and negative interactions, as they reveal specifically how attention modulates pain activation, and vice versa. Fig. 5 shows the dramatic ability of cognition, and more specifically attention, to directly reduce the BOLD signal in response to the same painful stimulation in areas known to be involved with pain processing, such as the insula, thalamus and midcingulate.

2.5.1 Negative interactions

Insula

The insular cortex is involved with the intensity encoding of painful stimuli (Peyron et al., 1999), interacting with the autonomic nervous system, and playing a role in somatosensory processing (Coghill et al., 1999). The area receives inputs from spinothalamically activated posterior thalamic nuclei and has links with the amygdala, the temporal pole, the hippocampus, the premotor cortex, the prefrontal cortex, and the anterior cingulate cortex. Decreased activation in this area is therefore consistent with decreased reported pain intensity.

Thalamus

The medial thalamic nuclei have been found to show increased activation as a result of painful stimulation (Tracey et al., 2000). Studies documenting increased populations of nociceptive neurons in the medial intralaminar thalamus lend credence to its role in the transmission and processing of noxious stimuli (Kwan et al., 2000). A relative decrease in activation found in this study could reflect reduced processing of the noxious stimuli resulting in a lowered perception of pain. Peyron et al. (1999) found bilateral increased activation when subjects actively attended to noxious stimuli, supporting existing research (Portas et al., 1998). If attention is distracted away from the stimuli, one might expect to find a relative fall in activation, such as found in our study.

The use of a high field scanner such as the 3T one used in this study might generate sufficiently greater signal to noise than lower power scanners that bilateral activation in the thalamus could be picked up more readily. If for example other researchers detect contralateral thalamus with weaker ipsilateral thalamic activation this may be missed at the analysis thresholds used for the fMRI data analysis. Bilateral thalamic responses have been reported (Peyron et al., 2000) in response to noxious stimuli which is thought to reflect generalised arousal in response to painful stimulation. Processing of painful and non painful somatosensory information is thought to be carried out by contralateral brain regions to the stimulated region (Coghill et al., 2001). However the same author found bilateral thalamic activation as a result of noxious thermal stimulation. Other regions of the pain "matrix" such as the somatosensory cortex and insular cortex exhibited contralateral stimulation.

It is intriguing that the thalamus (the 3rd neuron in the pain processing pathway) is shows reduced activation in this study when subjects were distracted from the painful thermal stimuli by the interference condition of the counting Stroop task.

The thalamus is known to be an important gateway to cortical areas where selection amongst sensory input channels might take place (Posner and Raichle, 1994). Posner and Raichle (1994) propose that the thalamus might block inputs to the cortex from sensory systems that are currently unattended due to the subject being distracted from them. Attention can thus alter the way the brain processes information in sensory areas. The Stroop effect produced in the counting Stroop task (an adaptation of the original Stroop task for fMRI) induces conflict and provides an ideal means of studying the effects distracting attention away from sensory stimuli. The interference task is much more difficult than the neutral task (see earlier discussion). Such tasks require high level attentional control and areas such as the ACC is more active during interference parts of the Stroop task than the neutral condition (Posner and Raichle, 1994)

The thalamus is known to be involved during attention and arousal in humans (Portas et al., 1998), and paying attention to experimental stimuli has been shown to increase thalamic activation (Peyron et al., 2000).

One possible explanation for reduced thalamic activation during the interference task would be the interaction of higher cortical regions with the descending pain control system. Activation of the descending pain control system would lead to release of endogenous opioids at the dorsal root ganglia in the spinal cord which would then reduce the amplitude of the pain signal traveling up the spinothalamic tract to the thalamus and lead to reduced activation in this region. It is possible to block the perception of pain, and attention is known to be able to powerfully modulate subjective responses to noxious stimuli (Tracey et al., 2002). Experimental research into stimulation produced analgesia (SPA) supports the role of the PAG as a key region involved in the modulation of peripheral painful stimuli via descending activity from higher cortical regions (Tracey et al., 2002). Tracey et al (2002) used fMRI to ascertain the involvement of the PAG in the modulation of pain by distraction. They found that the PAG showed a significant increase in activation associated with a noxious thermal stimulus specifically in the condition where the subjects were distracted away from the painful stimuli. This difference directly related to the perceived pain intensity reported by the subjects.

The slice thickness selected in this study was insufficiently thin to readily detect activation in the PAG. Direct stimulation of the PAG does produce analgesia (Reynolds 1969 cited by Tracey et al 2002). Decreased thalamic activation (accompanied by reduced perception of pain intensity) in the interference part of the counting Stroop distraction task could be a result of an antinociceptive effect mediated through endogenous opioids released by the rostral ventromedial medullary system or the PAG. This would reduce the pain input to the thalamus resulting in a reduced signal in this region. The interested reader is referred to Tracey et al's 2002 study which discusses possible mechanisms in further detail (Tracey et al., 2002).

It is known that attentional areas of the brain can be modulated by selective sensory attention (Johansen-Berg and Matthews, 2002). Paying attention to various sensory stimuli has been shown to have widespread effects throughout the sensory system and motor cortex. Distracting subjects from painful stimuli could result in the pain information traveling up the spinothalamic tract to the thalamus and being projected on to the sensory areas. They could exert reciprocal inhibitory influences on the thalamus.

Another theory is that the thalamus is involved in general arousal and attentional processing (Portas et al., 1998)and therefore if it is already involved in this type of processing then processing of painful stimuli by the thalamus could be somehow "inhibited" by the attentional processing being carried out. The very fact that arousal increases thalamic activation would fit with a model of reduced arousal or orienting to painful stimuli when subjects are distracted away from them. This would result in reduced activation of the thalamus in this case. Manipulation of subjects' attention to sensory (vibrotactile) stimuli has been shown to modulate ongoing processing within the brain (Johansen-Berg and Lloyd, 2000)and so this could be another mechanism for altered activation in the thalamus in this experimental paradigm.

Hippocampus

There is evidence to suggest that the hippocampus has a role in pain processing (Lathe et al., 2001). For example, Wei et al. (2000) demonstrated that the amplitude of excitatory postsynaptic potentials of hippocampal CA1 pyramidal cells is positively related to the intensity of nociceptive stimulation. Ploghaus et al. (2000) demonstrated that hippocampal responses to pain vary as a function of attention. In this study, attention to the noxious stimulation site was manipulated directly by varying the degree to which painful stimulation, or its omission, was surprising or expected. The hippocampus was activated whenever a mismatch between pain expectation and experience led to increased attention. The present finding shows that the hippocampus is activated when attention is not distracted away from the pain, which is consistent with the observation by Ploghaus et al. (2000) that the hippocampus is activated during attention to the noxious stimulation site.

Midcingulate

The ACC has a variety of specialised subdivisions processing cognitive, sensory, and motor information (Devinsky et al., 1995). Further subdivisions of the anterior cingulate cortex (Bush et al., 2000), based on function, neuronal projections and cytoarchitecture (Devinsky et al., 1995), include a caudal 'cognitive' division

(midcingulate), Brodmann's areas 24b', 24c' and 32', and a rostral 'emotional' division (perigenual cingulate) (BA24 a-c, 32, 25, 33). The midcingulate sends projections to the lateral prefrontal cortex (BA 9/46), and the premotor and supplementary motor areas. It forms part of a distributed attentional network and is activated by cognitively demanding tasks such as the Stroop (Devinsky et al., 1995).

During cognitively demanding tasks, an increase in signal in the midcingulate occurs and interestingly a decrease in signal is observed in the perigenual cingulate at the same time (Whalen et al., 1998; Drevets and Raichle, 1998). This phenomenon is reciprocal with the perigenual cingulate able to inhibit and decrease activation in the midcingulate (Drevets and Raichle, 1998; Whalen et al., 1998). In other experiments, the midcingulate shows increased activation during cognitively demanding tasks but is 'deactivated' by intense emotional states (Bench et al., 1992), and in the anticipation of pain in the laboratory (Drevets et al., 1995).

A relative decrease in activation compared with what would be expected from the simple sum of activation in the pain and the Stroop conditions was found in the mid cingulate. This could explain the fall in pain intensity scores reported by subjects as it is known to be involved pain processing (Rainville et al. 1997). In addition, the reduction of mid cingulate activation is probably via reciprocal inhibition due to the increased perigenual cingulate activation found in our study (see positive interaction).

The midcingulate region of the ACC is commonly activated in all pain studies. It is involved in attentional processes common to both attention and pain (Davis et al., 1997; Kwan et al., 2000). In addition, increased activation in rostral parts of the ACC is thought to reflect anticipation or orienting to the painful stimulus, whilst pain itself leads to activation in the midcingulate region (Ploghaus et al., 1999).

2.5.2 Positive interactions

Orbitofrontal cortex

The orbitofrontal cortex (BA 10, 11) is a heterogeneous prefrontal region with strong links to the hippocampus and other medial temporal lobe structures, posterior cingulate cortex, retrosplenial cortex, sensory areas (SI and SII), amygdala, thalamus and

insula. These connections point to a possible involvement with context dependent memory and pain processing roles. In addition, the orbitofrontal cortex contains neurons responsive to negative emotional stimuli, and studies have shown that electrical stimulation of the orbitofrontal and medial prefrontal cortices results in analgesia in both primates and non primates (Thorpe et al., 1983; Oleson et al., 1980). Increases in orbitofrontal activation were found during the modulation of laboratory pain in situations of increased cognitive load (Petrovic et al., 2000). There is a negative correlation between experimental pain intensity ratings and activation of the orbitofrontal cortex, thus lower activation in this area occurs with higher pain ratings (Derbyshire et al., 1997). In our study, we found increased activation of orbital frontal cortex as subjects rated pain lower, thereby confirming these earlier findings. The increased activation in the orbitofrontal cortex as a result of increased cognitive load during the interference Stroop task may possibly inhibit, for example, insular cortical, parietal or midcingulate areas leading to diminished perception of pain intensity and reduced pain ratings by subjects.

Perigenual cingulate

Our study found a relative increase in activity in the perigenual cingulate compared to what would be predicted by adding the signal increases in the pain and the Stroop condition together. This indicates a synergistic effect of pain and Stroop on this area leading to more activation in the perigenual cingulate when pain and Stroop interference occur together, perhaps accounting for the reduced midcingulate activation found (see above) via the reciprocal inhibition observed in previous studies.

The perigenual cingulate has links with the nucleus accumbens, the amygdala, the anterior insula, the hippocampus, the orbitofrontal cortex, and the periaqueductal grey (PAG). Other studies have shown that associated limbic areas show decreases in activation alongside the perigenual cingulate when this area is inhibited by the midcingulate (Shulman et al., 1997). Interestingly, in our study, the negative interaction observed in the midcingulate was reflected in a corresponding decrease in activation in the contralateral hippocampus and insular cortex.

This experiment focuses on the ability of cognition, specifically distraction of attention whilst subjects engage in a cognitively demanding task relative to a less demanding neutral one, to reduce the subjects' perception of the intensity of painful thermal stimuli. This is reflected in modulation of key regions known to be selectively involved in thermal pain processing. Pain, however, is a multiplex of cognitive and sensory attributes, which could also interact and be modulated by attention (or distraction). We can therefore only interpret our results as an interaction between levels of attention (or distraction) and painful thermal stimuli. Future studies to extend our observations would investigate the ability of attention to modulate other painful stimuli that are non-thermal and any corresponding brain activation changes; thereby controlling for these potential other components of this painful stimulus.

2.6 Conclusions

Significantly lower pain intensity scores were reported whilst subjects were engaged in the more cognitively demanding interference Stroop task than in the neutral condition. This experiment used functional neuroimaging to look closely at the neural correlates of this phenomenon. The reduced perception of painful stimuli applied during the interference task compared to those felt during the neutral task was accompanied by reduced activation in some key components of the pain matrix including the insula, midcingulate and thalamus. In conjunction with this, the perigenual cingulate and orbitofrontal regions involved in cognitive tasks and attention were found to show increased activation during cognitive interference coupled with pain. We should like to build upon the results of this acute experimental pain study to characterise the modulation of chronic clinical pain by cognitive modulation of attention. Our results suggest which brain areas might be subject to modulation and specific hypothesis can now be generated and tested. A greater knowledge of the central neural components orchestrating the modulation of pain by attention is vital if we are to continue to make informed choices in the selection of cognitive behavioural strategies for the alleviation of pain within the clinical arena.

This experiment manipulated attention and resulted in a behavioural modulation in pain intensity. From the preliminary data provided by this study, it was ascertained that this reduction in the perceived intensity of pain was accompanied by significant reductions in signal in some key areas of the pain matrix (thalamus, hippocampus and midcingulate). All of these areas are known to be involved in pain processing and the midcingulate is known to show a modulation in activity as a result of cognitive paradigms such as the Stroop task. Since diverting attention from a painful stimulus was found to reduce pain intensity and also modulate activation in areas processing pain the Stroop task or other cognitive tasks of this nature requiring sustained concentration and monitoring of stimuli could be useful to reduce short stimuli of a similar nature to those employed during this experiment. It is hard to accurately extrapolate from this data to the clinical arena since the nature of clinical pain is not sufficiently similar to that of experimental pain. Clinical pain can be chronic and there can be no escape from it (unlike in the laboratory). The prolonged duration of some chronic pain states carries with it an increased aversive component. It is not known from this experiment whether or not this component of pain can be modified by a distracting task in the same way as pain intensity. Activity in some regions involved with the processing of the emotional component of pain (OFC, perigenual cingulate) suggest that this could be so however. The measurement of the aversive component of pain in an experimental setting is something that would need to be assessed with this paradigm to see whether or not the unpleasantness of thermal pain as administered here could be reduced by the use of the Counting Stroop Task.

The previous study shows the ability of a cognitive distraction task (the Counting Stroop) to modulate the perception of experimentally induced thermal pain intensity. This behavioural effect was further supported by corresponding decreases in fMRI signal in key regions of the pain matrix. It is not advisable to undertake warm and painful thermal stimuli in the same experimental session since some sensitisation tends to occur to the stimulus, and also it is necessary to have at least 7 thermal stimuli or events in order to achieve a satisfactory signal to noise ratio. This would make the cognitive component of

the experimental session too long for the subjects with the warm stimuli interleaved with the painful stimuli. However it is necessary to ascertain that cognitive distraction did not have any significant effect upon warm (non painful) thermal stimuli. The next experiment was carried out in exactly the same was as the painful Counting Stroop experiment, in every regard. The ONLY difference to the study was that stimuli were used which were rated by the subjects in the study as consistently WARM and NOT painful.

2.7 The effect of distraction on the perceived intensity of non-painful, warm stimuli: A follow-up fMRI study.

This experiment aims to build upon the previous findings that perceived pain intensity of thermal pain is able to be modulated by engaging subjects in a cognitively demanding task. This study uses an identical experimental paradigm and methodology but substitutes the painful thermal stimuli with warm, non-painful thermal stimuli.

2.7.1 Introduction and methods

The experimental design used for this study was identical to that used in the previous study. This applies also for the scanning parameters used. In order to obtain a warm but non painful stimulus, subjects were thresholded so that the temperature administered to the dorsum of the left hand was consistently rated by the subject (on individual basis) as corresponding to an intensity rating of 2-3 or 'warm'. The temperature range for this rating was 40-43 °C.

2.7.2 Behavioural results

The reaction time data for this experiment were not analysed. The data was recorded as a means of obtaining behavioural data which corroborated the fact that the subjects were engaged in the cognitive task throughout. Button presses were recorded and subjects had again been told that they should focus on the task but maintain awareness for the thermal stimuli. They were told that they should not sacrifice accuracy

for speed as before. All seven subjects rated the interference and the neutral thermal stimuli as NOT being perceptibly different from each other in terms of intensity. Subjects were unable to find a difference in the intensity ratings between the more demanding and the neutral cognitive distraction task in this study.

2.7.3 Imaging results

The purpose of the analysis was to ascertain whether, despite the lack of any behavioural modulation of the thermal intensity ratings, there was any central modulation in the areas activated by a warm thermal stimulus. The analysis was the same as the previous analysis for the painful stroop experiment. Three experimental variables (EVs) were used:

1. Interference versus neutral
2. Warm stimuli
3. Positive and negative interaction between EV1 and EV11

It would then be also possible to compare the activation obtained as a result of the pain and as a result of the warm activations.

In order to validate the study it was decided to check that the activation as a result of the Counting Stroop task was the same in the warm and the painful experimental paradigms. Both of these aims were met by carrying out a group feat multi session second level analysis which compared the warm Stroop (Group A) results obtained in this current study with the painful Stroop (Group B) results obtained in the previous study. This was carried out at a statistical threshold of $Z=2.3$. The analysis always runs as test minus control for each experimental contrast. This would be B-A in this case. Any activation seen on the group result for this analysis would therefore show any areas which were more active in the control group (warm Stroop) than in the test group (painful Stroop).

Using this analysis technique the activation for all contrasts (Stroop positive contrast, Stroop negative contrast, warm positive contrast, warm negative contrast, positive and negative interaction) 1-6 for the warm could be subtracted from those in the

painful Stroop (since all parameters were the same and therefore variance was controlled for) to ascertain which if any areas showed more activation in the painful Stroop than in the warm Stroop.

The EVs modelled for the warm Stroop were the same as those for the painful Stroop and the imaging parameters and methods used were identical. The EV modelled for the interaction between the warm and the Stroop (EV3) did not show any activation reaching threshold for either the positive or negative contrast at z=1.8.

2.7.4 Initial group results

Table 2.7 Talairach coordinates for the warm Stroop group result (fixed effects group analysis) at z=1.8, p=0.05

Talairach coordinate	Region	Z score
54, 16, -20	38 BA 21/medial temporal gyrus	2.10
44, 18, -4	BA 10/47	2.09
42, 14, 0	Ipsilateral Insula	2.16
46, 20, 6	BA 45	2.18
42, 10, 8	BA 6/44 prefrontal cortex	2.20
54, 20, 8	BA 45/46	2.14

The activation observed during the warm stimuli was different and also reduced by comparison to the pain activation (see previous experiment).

2.7.5 Discussion and conclusion

This experiment was carried out in order to ascertain whether or not there would be modulation of a warm sensation by cognitive distraction. The results of this suggest that painful thermal stimuli can be modulated by cognitive distraction tasks but that warm, non-painful stimuli are not susceptible to such modulation. The study also shows

that the activation in the Stroop task is consistent across our studies and is commensurate with the activation obtained in other imaging studies using the same task. However, the means currently available in terms of statistical analysis applications are limited in their ability to make accurate and valid comparisons across different sessions, and so the extent to which conclusions can be drawn from this comparison between the warm and the noxious Stroop experiment is limited. It is safe to conclude that the activation as a result of the Stroop task itself is similar in the warm and in the noxious thermal stimuli experiments. These results do show that there is no positive interaction between the 'warm' condition and the Stroop.

2.7.6 References

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CHAPTER 3: The use of a parametric working memory (n-back) task to modulate pain perception: background and development

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This experiment builds upon previous work (Chapter 2) that employed a cognitive distraction task (the Counting Stroop task) to modulate the perceived pain intensity of experimentally induced thermal pain. The ability of this distraction task to reduce the behavioural perception of experimentally induced thermal pain and the associated decreased activity in pain related areas renders this area worthy of further research. The n-back experiment used in this experiment will aim to discover if it is possible to demonstrate an incremental modulation of perceived intensity and an associated parametric reduction in central pain activation at different difficulty levels of the n-back task.

The experiment is described in chapter 4 of this report, whilst the background, development of the task, and behavioural data is discussed in this section of the thesis.

3.0 Introduction

Current clinical and experimental literature strongly supports the phenomenon of reduced pain perception whilst attention is distracted away from noxious stimuli. Both the behavioural-affective and sensory-discriminative elements of pain perception are reduced in situations such as battle or intense excitement, via descending feedback mechanisms from higher order cognitive areas (Melzack and Casey, 1968). Methods such as listening to music can significantly reduce postoperative pain in patients (Good et al., 1999b). Experimental examples of the ability of psychological state to modulate the perception of laboratory induced pain are becoming more numerous. Miron *et al.* (Miron et al., 1989) showed that directing subjects' attention towards a painful stimulus increased its perceived intensity and unpleasantness compared with when subjects were asked to direct their attention away for the stimulus.

The previous cognitive distraction paradigm (Bantick et al., 2002a) used the Counting Stroop (Bush et al., 1998a) to distract subjects from thermal painful stimuli, as discussed in chapter 2. In this paradigm, the counting Stroop was used as a cognitive distraction task whilst subjects received intermittent painful thermal stimuli. Pain intensity scores were significantly reduced when subjects took part in the more cognitively demanding interference task of the counting Stroop than in the less demanding neutral task. When subjects were distracted during painful stimulation, brain areas associated with the affective division of the anterior cingulate cortex (ACC) and orbitofrontal cortical (OFC) regions showed increased activation. By contrast, many areas of the pain 'matrix': thalamus, insula, cognitive division of the ACC showed a relative reduction in signal. An observed reduction in pain intensity scores whilst subjects were distracted from the pain by a demanding cognitive task was reflected in a significant correlated reduction in signal in key areas of the pain matrix. I would like to build upon this study with the current experiment in order to establish if the use of a parametric cognitively distracting task (working memory) can result in a parametric reduction in pain perception with increased task complexity, and, if so, whether this corresponds to changes in brain activity in pain processing regions.

3.1 Functional anatomy and FMRI of the N-back working memory task

Barch (Barch et al., 1997) carried out a study that aimed to functionally dissociate the brain regions involved with task difficulty from those involved with working memory processes per se. Generally, when working memory tasks are employed, the increased working memory demand is concurrent with an increase in task difficulty, therefore it is hard to separate activations as a result of task difficulty form those arising from involvement in working memory processes.

Barch's study (Barch et al., 1997) used two separate tasks to manipulate both task difficulty and working memory independently. The working memory task employed was the continuous performance test (CPT). This task involves the continuous monitoring by the subject of a stream of singly presented letters for a target probe. They should then respond when the target probe follows a predetermined cue. The delay between the cue and the target probe can be

manipulated and increases in this delay period result in concomitant increases in working memory demand.

The task that was used to investigate the activation as a result of increased task difficulty in the absence of increased working memory load was the same, but rather than manipulating the delay period between cue and target probe, visual degradation of the stimuli was employed. Previous studies (Barch et al., 1997) have shown that the use of visually degraded stimuli significantly reduces performance levels to an equal extent in the long and short delay conditions. The task is rendered more difficult as a result of altered perceptual requirements but the degradation of stimuli does not interact with memory demands.

The results of their experiment suggest that the network of regions involved in verbal working memory includes the dorsolateral prefrontal cortex (DLPFC), Broca's area, and an area within the left parietal cortex. These regions have frequently been shown to show activation as a result of tasks engaging working memory (Braver et al., 1997). These areas were activated here as a result of the manipulation and increase in working memory load as the delay period between target and cue increased. The precise functional significance of this network has not yet been identified. Working memory is a complex process involving a number of sub-component processes such as active maintenance, updating of information in working memory, and comparison operations (Baddely and Hitch, 1974). The region of DLPFC activated in Braver's study (Braver et al., 1997) is not the same as that activated in n-back working memory tasks (Cohen et al., 1997). However, the n-back task requires the encoding of sequence order information, not required by the CPT task.

In terms of task difficulty, the main area activated by increased task difficulty was the ACC. The ACC is often activated in working memory and related task paradigms, particularly when task complexity is manipulated during the course of the experiment (D'Esposito et al., 1995). It has been hypothesised (Barch et al., 1997) that the ACC could play a role in the motivational or affective response to task difficulty which would be consistent with its connections with the limbic structures of the brain (Devinsky et al., 1995).

When carrying out a study which looked at working memory task performance in schizophrenics and matched healthy controls, Perlstein (Perlstein et al., 2001) found that activation in the DLPFC increased in direct proportion to working memory load.

fMRI has provided an ideal method with which to investigate the regions activated during working memory tasks. It also makes possible the use of more sophisticated paradigms which can look at parametric increases in working memory load (manipulation of the amount of information held in memory and the time it has to be maintained there). The DLPFC has been shown in monkey lesion studies to be of crucial importance in the active maintenance component of working memory (Funahashi et al., 1993), and now it has been shown using event- related functional magnetic resonance imaging designs that the lateral PFC is engaged during the active maintenance of information over short delays (Zarahn et al., 1999).

Very few cases exist of patients suffering focal PFC lesions and any associated decrement in performance on delayed-response tasks. This is another reason why the use of fMRI has been of considerable benefit in unravelling the functional anatomy of working memory.

Some patients with PFC lesions are impaired in their performance of delayed-response tasks, and that these difficulties were more pronounced when the task included distraction during the delay period (D'Esposito et al., 2000). Generally, when such lesions were lateral, performance on typical digit span tests was normal.

The lateral PFC is not vital for the mere simple working memory storage of information. DLPFC is employed in the manipulation of information and may make contributions to the maintenance of information when it has to be protected from distracting stimuli occurring in delay periods. The DLPFC is also involved in the encoding processes involved in working memory (D'Esposito et al., 2000).

The letter n-back task is a variant of the n-back task (Braver et al., 1997; Cohen et al., 1997; Smith and Jonides, 1997). The version used throughout the experiment described here requires subjects to respond by pressing a button when the letter presented on the screen (the target) matches a letter previously presented (cue stimulus). This requires the subjects to retain and permanently update the relevant information in working memory. Load and mental manipulation within this type of working memory task can be increased step-wise by using three levels of the n-back task. The least demanding level used in the study, the 1-back task, requires the maintenance of 1 piece of information in the working memory store within the interval between the cue and the target stimulus. The 2 and 3-back tasks involve the interposition of one or two letters (distractors) between the cue and target stimuli respectively. Depending upon the level of task being carried out, the subjects should

respond when the current letter matches the one seen 1, 2, or 3 letter presentations before it.

The use of the parametric n-back task working memory paradigm makes it possible to get a more accurate picture of the functional areas involved and maximise the temporal and spatial resolution benefits of fMRI.

A method of investigating which brain regions are involved in carrying out a particular cognitive task is to subtract activity generated by a control task from that seen in an activation task. The aim of this approach is to design a control task that utilises all the same processes as the activation task itself apart form the particular process of interest. When the control image activation is subtracted from the activation in the experimental condition, this should leave activity that is associated with the process of interest only. One major limitation of the subtraction method in fMRI studies is that it assumes that (McClelland, 1979) the processes common to both tasks which are subtracted out are performed in the same way in both tasks. This also requires the activity common to both tasks to be carried out in the same regions in both tasks. This is not necessarily the case, since the higher levels of complexity of a given cognitive task might result in subjects employing different strategies and could alter processes that are associated with the task concerned but which are not directly related to the task itself.

One way to address these limitations is to adopt a parametric approach to investigating the process of interest, such as working memory in this case. Different levels of task difficulty of the same task are presented, meaning that although other processes might still show variation across conditions, it is less likely that these associative processes will show the same pattern of response as that predicted for the process of interest. Greater selectivity is enabled in the measurement of the brain activation produced by the task concerned.

Parametric designs have been used in neuroimaging studies to ascertain which brain regions are involved in carrying out a particular task (Braver et al., 1997). fMRI is an ideal technique with which to combine a parametric approach. fMRI is fast and non invasive and allows the acquisition of large numbers of whole brain images from many task conditions within a single imaging session.

Braver (Braver et al., 1997) found that increasingly demanding levels of the letter n-back task resulted in parametric increases in working memory load which were in turn reflected in increases in signal in the mid dorsolateral area and the left

inferior region (Broca's area). The study found a 'dose-response curve' relationship with a progressive and linear increase in signal with each increment of load. This was not observed in the ACC or the left anterior inferior regions. In fact activity in these areas decreased across the load conditions. The discussion and implications of these results are not discussed here but later in this report (see Discussion). The current study utilises the n-back task in a parametric design because it gives three levels of task difficulty which have been shown in neuroimaging studies to be accompanied by corresponding increases in signal (and decreases or no change in some areas) in some key areas involved in working memory.

3.2 Overview of working memory

Jonides defined working memory as, "..the short term retention of information that is no longer accessible in the environment, and the manipulation of this information, for subsequent use in guiding behaviour" (D'Esposito et al., 2000). This has considerable functional significance, since it permits humans to keep limited amounts of information in an 'active' or 'on-line' state for a short duration of time, and to be able to use or manipulate this information for this time period (Smith and Jonides, 1998). An everyday life example of the basic processes involved would be using verbal directions to create a mental map in order to locate a particular place. Even this relatively simple task itself entails both maintenance of the verbal information in working memory, and the manipulation of this information to generate the map. It is precisely because the working memory system is dynamic, maintaining information prior to its mental manipulation that renders it of such pivotal importance in the execution of higher order cognitive processes such as reasoning, decision-making, problem solving, and the comprehension of language (D'Esposito et al., 2000; Jonides, 1995).

3.3 Working memory: a non unitary system

It is currently believed that the type of working memory system varies depending on the exact nature of the information involved. Indeed, the weight of the behavioural, neuropsychological, and neuroimaging literature supports the existence of two separate working memory systems for verbal and spatial information (Jonides et al., 1996). Functional imaging studies have revealed distinct patterns of brain

activation when subjects perform either verbal or spatial working memory tasks (Smith and Jonides, 1998).

3.4 Components of the working memory system

In essence, working memory can be conceptualised as comprising three distinct functional components, each driven by different neural correlates.

a) Pure storage component

The contents of the pure storage component decay very fast and are lost.

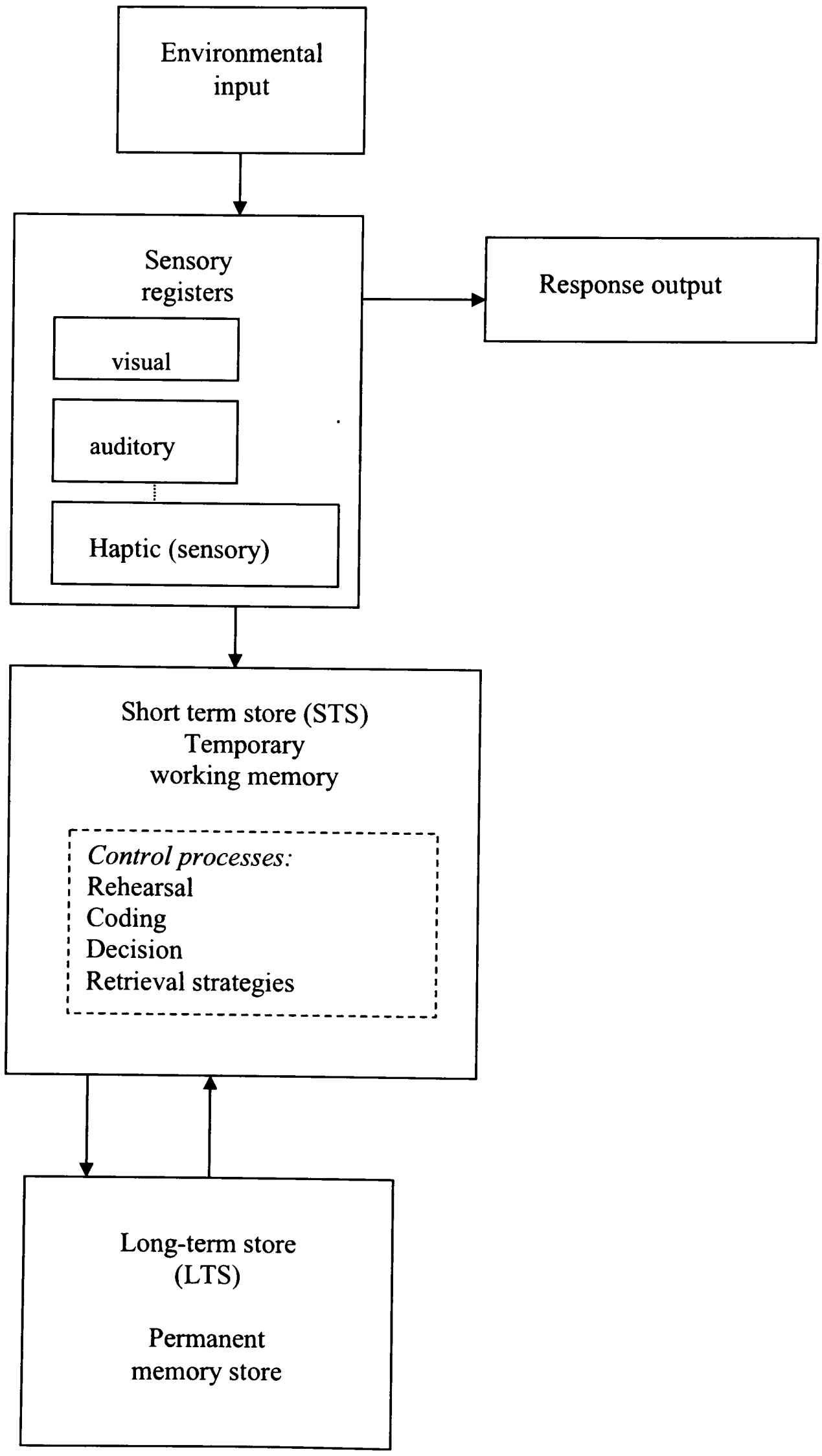
a) Rehearsal component

This component is able to reactivate and prevent the rapid decay of information contained within the pure storage component.

b) Executive component

This component manages the processing of information held within working memory. This part of working memory could depend on the same brain regions irrespective of the nature of the information being manipulated (verbal or spatial), which is not true for the preceding working memory components.

Fig. 3.0 Structure of memory proposed by Atkinson and Schriffin (1968)
Taken from Baddely, Wilson and Watts (1995)



This influential model shows how working memory fits into the overall framework of memory; introducing the concept that memory is not a unitary entity. Environmental information enters a sequence of brief sensory registration stages, which in turn pass this information on to a short-term store (STS). This part of the system is crucial, as it is only via the STS that information can be consolidated into the long-term store (LTS).

This model, however, implies that learning and therefore transfer into LTS is directly proportional to the amount of time information is held in the STS. This proved to be a serious limitation for the model, but kindled the research of others who sought to find out the nature of the relationship between short and long term memory.

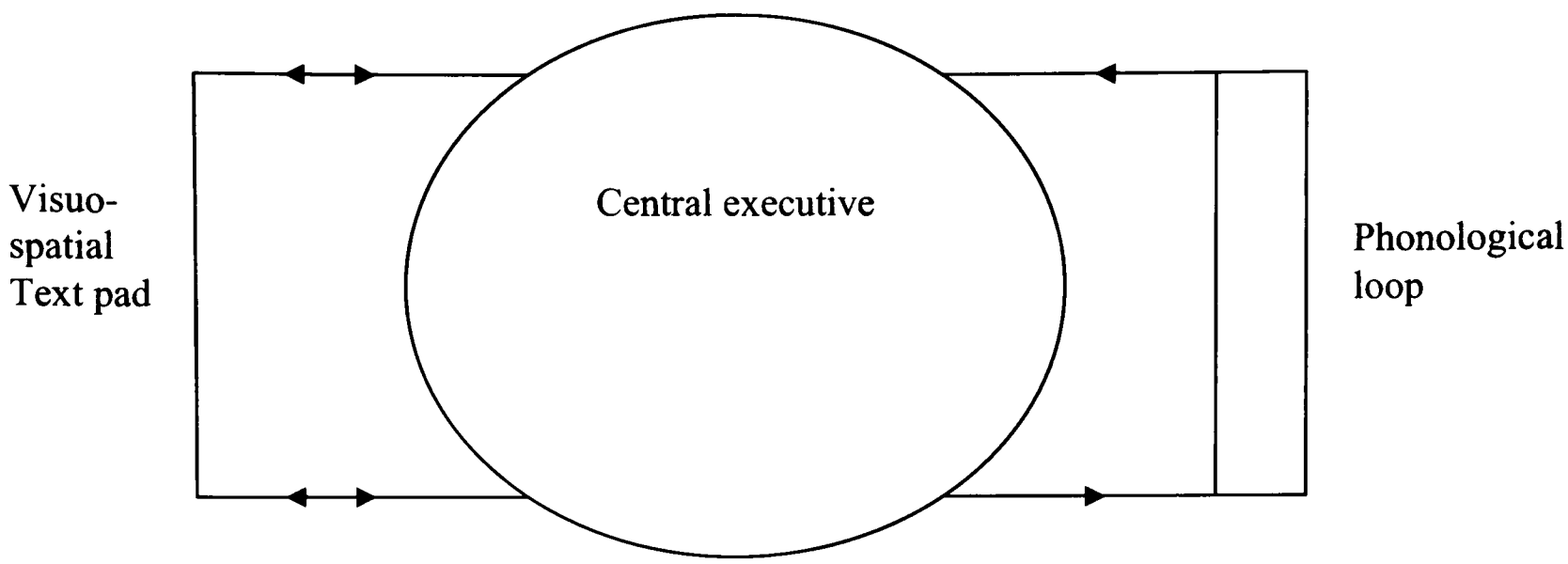
Atkinson and Shriffin's model (Atkinson and Shriffin, 1968) (see figure 3.0) shows how working memory fits into short term memory as a whole. Baddeley and Hitch (Figure 3.1) moved away from the idea of a unitary short-term store and towards the idea of a multi-faceted working memory (Baddely and Hitch, 1974).

In this model there is a central executive (attentional control) component of the system that interfaces between long term memory and two or more slave systems. Information is stored in a temporary storage area but its maintenance and registration from then on is governed by an active set of control processes.

The visuo-spatial scratchpad or sketchpad is specifically concerned with the maintenance of visuo-spatial information, and verbal information is maintained in the phonological or articulatory loop.

The central executive's function is to select and execute strategies and to maintain or switch attention as required. The central executive is functionally located within the frontal lobes of the brain. Damage to this area has been shown in the clinical environment to be linked to what is referred to as the dysexecutive syndrome (Baddeley, 1986). Baddeley suggested that the supervisory attentional system conceptualised by Norman and Shallice (1986) as a foundation for the central executive. This model states that habit structures or schemata govern routine behaviour. If a novel action is needed in a given situation, for example to override a habit in an emergency, the supervisory attentional system (SAS) can override the schemata. Other functions of the SAS included the planning and coordination of responses.

Fig. 3.1 The working memory model proposed by Baddeley and Hitch (1974) Taken from Baddely, Wilson and Watts (1995).



Essentially, Baddeley and Hitch's model (Baddely and Hitch, 1974) comprises three structures: a central executive, a phonological loop, and a visuo-spatial sketchpad. These structures each have entirely distinct characteristics and roles. In the early versions of this model, the central executive is described as having a limited capacity, with its resources allocated accordingly to either processing or storage depending on the current demands imposed upon the system. Subsequently Baddeley himself moved away from the idea that the central executive served as a flexible workplace for the storage and processing of information, although many other models of working memory are still based upon Baddeley's earliest model.

The other two parts of the working memory model were viewed as limited capacity slave systems for the transient storage of information. These systems did not alter the function of the central executive. The visuo-spatial sketchpad stores small amounts of visual or spatial information and the phonological loop is concerned with the maintenance of small quantities of verbal information in serial order and for limited time periods.

Recent research evidence has led to discussion as to whether or not there are indeed distinct slave systems for visuo-spatial and verbal information (Farrand and Jones, 1996; Shah and Miyake, 1996). Other researchers have also questioned the validity of having a separate central executive and slave system model (LaPointe and Engle, 1990; Lehto, 1996). This has stimulated research into a new generation of working memory models.

From this point onwards, this discussion of working memory for the purposes is focussed upon the manipulation of cognitive load using working memory tasks, and not upon working memory processes *per se*. Such topics are currently beyond the remit of this study.

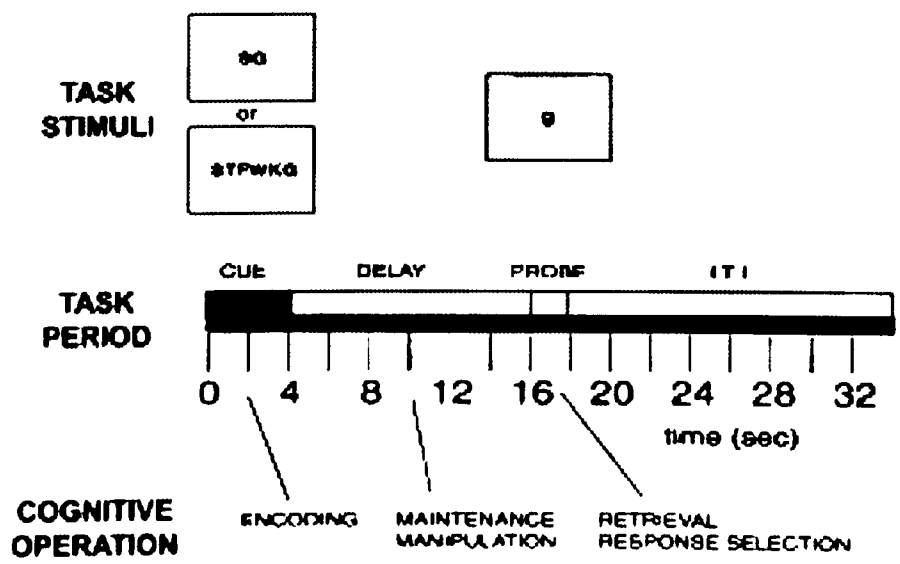
3.5 A functional anatomical outline of working memory

Work by Perlstein et al (2001) suggests that the dorsolateral prefrontal cortex is strongly implicated in working memory function. Other authors confirm this(D'Esposito et al., 2000; Fuster, 1997; Manoach et al., 1997) This is consistent with the fact that working memory also encompasses attentional and goal directed behavioural components (also located within the frontal lobes).

Further evidence to support this comes from a range of sources: human neuropsychological studies (Baddeley, 1986), brain lesion and functional imaging studies (Braver et al., 1997; Cohen et al., 1997; Courtney et al., 1997), and lesion and neurophysiological data from non human primates (Fuster, 1997; Goldman-Rakic, 1987).

Initial data supporting the neural correlates of working memory was gathered from animal studies (Owen et al., 1999). In studies carried out on conscious monkeys, delayed response tasks have been used to examine the functional anatomy of working memory (see figure 3.2 for a diagram of a typical task).

Fig. 3.2 Schematic illustration of a prototypical delayed-response task, together with the proposed cognitive processes that may be engaged during each task period. ITI=inter-trial interval. Taken from D'Esposito et al. (2000).



Electrophysiological studies have revealed that neurons within the lateral prefrontal cortex (PFC) are active during the short delay period whilst the monkey is actively maintaining the stimulus information before making a motor response which is dependent upon this target related information. Indeed, this evidence is corroborated by lesion study evidence, which has shown that working memory task performance is compromised in animals with lesions of the lateral PFC (Funahashi et al., 1993; Rainer et al., 1999).

Human fMRI studies have provided further evidence for the pivotal involvement of the lateral PFC during the active maintenance of information in working memory during delayed-response cognitive tasks (Courtney et al., 1997; Zarahn et al., 1999).

Active storage of information within the working memory system is associated with bilateral activation in the posterior parietal cortex (Honey et al., 2000). Prolonged response latencies are associated with protracted maintenance of information in an active state in the working memory system, and this in turn was found to result in increased activity in the aforementioned area of the brain. This region has been implicated in the storage of phonological information (Honey et al., 2000; Paulesu et al., 1993).

The functional central correlates of working memory are not specifically discussed here; although where they relate to the results of the cognitive parametric distraction study they will be discussed later in this thesis (Chapter 5). More relevant to this report is the concept of working memory load. The study aims to increase the cognitive load of the task (in this case a working memory n-back task) in order to establish if there is a corresponding parametric reduction in perceived pain intensity with increasing cognitive load.

3.6 Experimental development of the n-back paradigm

Clinical (Good et al., 1999a), experimental (Miron et al., 1989) and anecdotal evidence supports the ability of cognitive distraction tasks to modulate perceived pain intensity. This thesis investigated the ability of the Counting Stroop task (Bush et al., 1998b) to distract subjects from experimentally administered thermal pain stimuli. This experiment is addressed in chapter 3, and therefore only briefly discussed within

this section. Subjects reported a reduction in the perceived intensity of their pain whilst engaged in the interference (more difficult) task compared with the neutral and non distracting task (Bantick et al., 2002b). This observation corresponded to a decrease in MRI signal in some key pain matrix areas within the brain (ipsilateral thalamus, contralateral thalamus, ipsilateral insula, contralateral insula and midcingulate cortex). These findings suggest some central neuronal candidates for the behaviourally observed fall in pain ratings whilst subjects were distracted away from the pain stimuli using a cognitive distraction task. This task only permitted us to investigate the reduction of pain perception whilst subjects were engaged in an interference task compared to an easy, neutral task, and left some interesting questions unanswered.

This experiment aims to build upon earlier work by investigating the modulatory ability of cognitive distraction on thermal pain intensity using a parametric working memory task. Ideally, such a task would have three parametric levels of difficulty, which would in turn result in differing levels of distraction related changes in pain intensity ratings. The behavioural indices collected from this experiment in order to verify that the task (n-back) does produce different levels of distraction induced pain intensity modulation are pain intensity ratings (measured in real time during the task) and reaction times (measured using button press responses throughout the experiment). The study aims to ascertain which brain regions are involved in the behavioural reduction in perceived pain intensity when subjects perform a parametric cognitive distraction (working memory) task. The hypothesis is that some key regions of the pain matrix will show a parametric reduction in MRI signal in response to pain with greater levels of task difficulty. Further, not all pain processing regions within the brain will show the same response to pain in conjunction with a parametric increase in difficulty in the cognitive distraction task (n-back in this case). The task has to be one which could be used within the MRI environment, and therefore one that required non-verbal responses by subjects (since speaking causes head movements and therefore undesirable movement artefacts).

3.7 The choice of cognitive distraction task

The task chosen for this experiment was a parametric working memory task, the verbal n-back task, which has three levels of difficulty.

Most fMRI studies looking at the regions of the brain involved with working memory have shown activation In the bilateral frontal and parietal areas of the brain whilst subjects are engaged in such tasks (Smith and Jonides, 1997).

When the working memory load is increased this has been shown to result in associated increases in activation in these areas (Braver et al., 1997). Within the N-back task, there are three processing load conditions. The 0-back is a vigilance task, and the 1-back and 2-back tasks are mild and moderate processing load conditions respectively (McAllister et al., 1999).

The n-back task involves the subject seeing a series of letter presented upon a screen and responding using a button press when the target letter appears. The task has three levels of difficulty in this case. In the 1-back task the subject must monitor the sequence of letters on the screen and make a response when the letter currently shown upon the screen is identical to the letter shown immediately before it (or one letter back). The 2-back and 3-back task levels are progressively harder to complete since in these cases the subject has to respond when the letter on the screen exactly matches the letter 2 stimuli and 3 stimuli back respectively. This is shown graphically in figure 3.4.

3.8 Methodological difficulties

It is important to ensure that subjects do not use a task strategy for remembering the sequence of letters that involves vocalising or speaking the letters to themselves. This could cause motion artefact problems as speaking inevitably causes some associated head movements. The subjects are asked to stay as still as possible and to try to avoid actually speaking the letters. They can sub-vocalise or read them in their head but not aloud. The subject's head movement can also be minimised by thorough packing around the head in the magnet using foam pads.

It is vital that subjects maintain their concentration on the task throughout the experiment. Although they are specifically asked to do so, it is also emphasised during the pre-scanning instructions that they prioritise accuracy over speed of response. It is desirable that subjects make the correct response and concentrate, even at the expense of slightly increased reaction times.

Subjects are required to attend a short training session outside of the scanner to check that they are confident at performing the task and making the button box

responses required. A mock-up task identical to the scanner phase of the experiment in every detail except for the noxious thermal stimuli and the pain rating scales was designed and each subject performed this prior to carrying out the experiment in the MRI scanner. This avoids the scenario wherein the subjects are still improving at the task, and developing their task strategies throughout the experiment. This would add an unwanted additional variable, altering their degree of distraction from the pain stimuli, as they adjusted their method of dealing with the task. Other research groups have also found practice sessions to be necessary when using such a task for cognitive experiments in the scanner (Jansma et al., 2000).

Table 3.0 Traditional n-back task levels

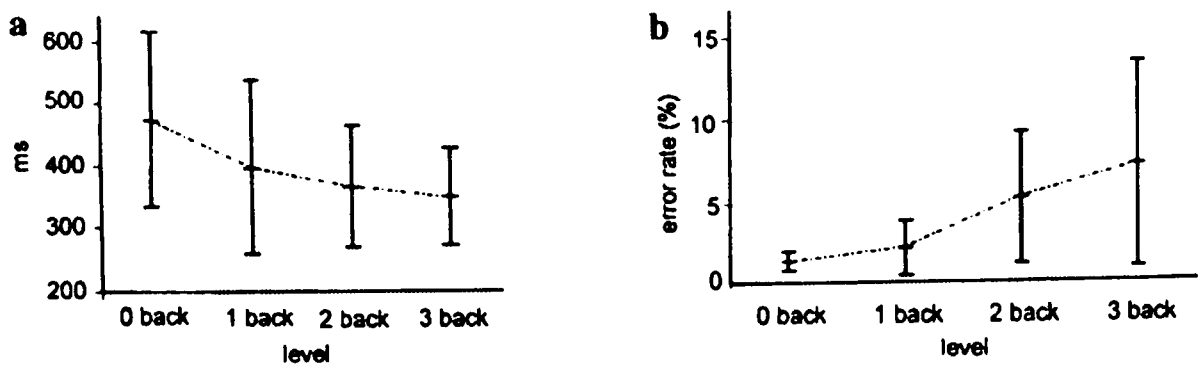
0-back: Control task. The response is made directly following the stimulus
1-back: The response is made with a delay of 1 stimulus
2-back: The response is made with a delay of 2 stimuli
3-back: The response is made with a delay of 3 stimuli

A button box is used in this study to record reaction times and pain intensity ratings (see method section later in this chapter)

It is then possible to obtain behavioural measures of reaction times and error rates. The task requires constant vigilance and monitoring on behalf of the subject in order to maintain and update information in working memory. The information presented in each trial is the same as are the demands upon response selection and execution.

Error rate is a measure of workload or performance and not reaction time. With the exception of the control 0-back task, subjects know what the next response will be before they are allowed to respond as it is determined by the preceding stimulus. Therefore the emphasis is placed upon accuracy rather than speed. A study carried out to demonstrate this showed no effect of workload level on reaction time (Jansma et al., 2000).

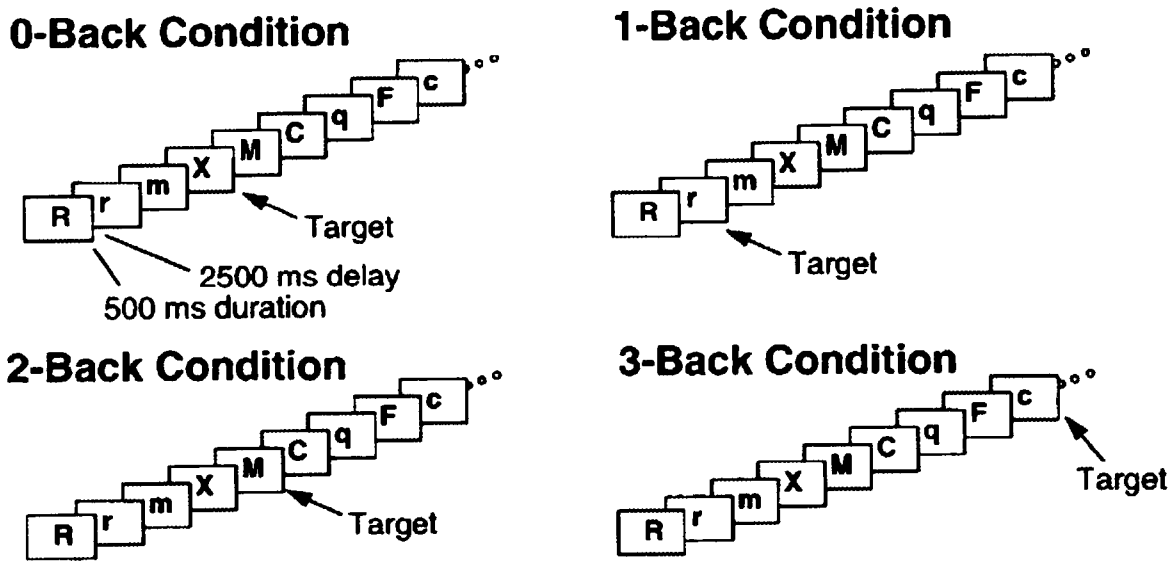
Fig 3.3. (Taken from Jansma et al. 2000)Reaction times in n-back levels



(a) Reaction times averaged over correct response trials per level for eight subjects recorded separately from scan sessions. Results show that in the used version of the n-back test, reaction time is not correlated with level, indicating that speed of response execution and selection is not affected by workload in the spatial n-back test. Error bars represent standard deviations.

(b) Error rate per task level during scans. Calculated within subject as the percentage errors and omissions during the whole scan session. Results are averaged over all subjects. Error bars represent standard deviations.

Fig. 3.4 Illustration of the verbal n-back task (taken from Jansma et al. 2000)



For the purposes of this experiment, we are using the n-back task to elicit a reduction in perceived pain perception by distracting subjects from the noxious thermal stimuli. This study was designed to investigate the central neural correlates of such a behavioural effect. This requires three levels of task difficulty in order to analyse the data for trends in the differential effect of the task upon the pain intensity scores and the link between this and the signal changes in pain processing brain regions. The task itself is discussed in the methodological section of this experiment.

3.9 Methods and Design

The design for this experiment draws upon the work of Braver *et al.* (1997) and Jansma *et al.* (2000) to which the reader is referred for background information and full details of their work.

As a result of initial bench experiments, we were able to adapt previous n-back paradigm designs and render them suited to our study objectives

Three levels of the n-back task, one back, two back, and three back were used in this experimental paradigm. In early pilot bench experiments, subjects reported that they did not perceive a sufficient difference in difficulty levels between the 0-back, 1-back and 2-back as used in other experimental paradigms. Our objective was to attain a reproducible (between subjects) and adequate difference in the difficulty level between the n-back levels chosen. A further objective was that such that a difference in task difficulty level would be reflected in differing perceived pain intensity according to the degree of distraction of the task in hand.

This was not the case when 0-back, 1-back and 2-back tasks were used. Researchers in this area of experimental research have used the 3-back task (Braver et al., 1997) as the most complex task level, and our bench experiments using a combination of 1-back, 2-back, and 3-back tasks yielded much better behavioural data. Subjects felt that there were distinct steps up in difficulty from 1-back to 2-back, and from 2-back to 3-back, as well as reporting a behavioural difference in pain intensity levels perceived in the different levels, to the extent that the pain in the 3-back felt less intense than that in the 1-back. The data is not presented here, but final bench data acquired prior to running the fMRI experiment is reported in chapter 5.

The experiment designed by Braver *et al.* (1997) comprised 4 task level sets (10 repetitions of each stimulus type) comprising 25 consonant letter stimuli (mixed case), each presented on the screen for 0.5 seconds with a 2.5 second gap between each stimulus. They used four levels of difficulty, 0-back (control vigilance task), 1-back, 2-back, and 3-back. The 0-back vigilance task involves pressing the button press whenever a letter 'X' appears on the screen, and therefore does not involve any working memory load. The working memory load increases incrementally between the 0-back to the 3-back condition. The stimuli were presented centrally on a screen in pseudorandom sequence and random variation in letter case (capital non-capital).

Subjects were required to respond to the appearance of a target by pressing a button with their dominant hand. Thirty three percent of the letters in each trial were targets in each case. Each condition block was approximately 72 seconds duration. The order of task type was randomised across both blocks and subjects. There was a ten second delay between each block as a rest and to allow recovery of the haemodynamic response.

Only capital letter consonants were used in our study, mainly due to the increased ease of distinction between letters and the constraints of the restricted visual field within the scanner environment.

A paradigm was used where five repetitions of each task difficulty level (three different letter sequences existed for each n-back task level) were presented in random order across blocks and subjects. Using this paradigm, subjects reported that they became confused due to the difficulty of rapid switching between different cognitive sets required for the three types of task. This had the undesirable effect of forcing some subjects to make undue amounts of errors, and even giving up from time to time, which meant that they were not distracted from the painful stimuli.

An introductory screen to each block is presented at the beginning of each task block denoting the task type currently underway. In addition, each letter in the sequence has 1, 2, or 3 dots underneath it depending upon whether the task is 1-back, 2-back, or 3-back respectively (see figure). Even with these changes, and reminders of the type of task being carried out, subjects found that the rate of task switching was too rapid for them to engage accurately in the task.

Fig. 3.5 Diagram of an example instruction screen for the 1-back task.

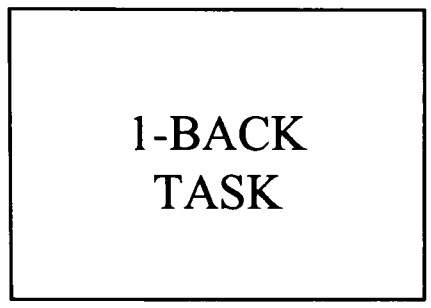
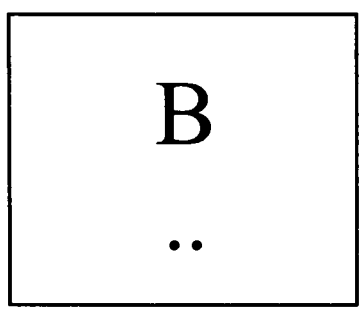


Fig. 3.6 Diagram of an example letter stimulus screen for the 2-back task



A design was trialled, which consisted of three long blocks formed from five blocks of each task type in a long string. The order of the three blocks was randomised across subjects although subjects never started with the hardest, 3-back task. This highlighted one of the main problems requiring attention with paradigms where painful thermal stimuli are used, namely sensitisation.

During the experiment, five second thermal painful stimuli were administered to the back of the left hand at an intensity which the individual subject consistently attributed a rating of strong pain. There were six thermal pain stimuli per task block type. Noxious thermal stimuli were always at least sixty seconds apart from each other to avoid sensitisation to the stimulus. If this happened, subjects would tend to rate each subsequent stimulus as more painful than the previous one, despite the temperature being held constant throughout the experiment. A sixty-second inter-stimulus interval prevents such sensitisation from occurring. The experiment was run without any visual stimuli to verify that the temperature remained constant throughout with the noxious stimuli spaced sixty seconds apart.

In order to ascertain what temperature to use throughout the experiment, subjects were individually "thresholded" in the scanner, immediately prior to commencing the scanning session. This involves administering five second stimuli of gradually increasing temperature sixty seconds apart until a temperature is reached to which the subject consistently attributes a temperature pain rating scale of '7'.

Fig 3.7 Pain rating scale used in this experiment

0	1	2	3	4	<5>	6	7	8	9	10
Usual					mild pain					Unbearable pain

This scale is operated using a button press. The scale always displays with the default setting of '5' being selected with the brackets. The button box allows this to be adjusted to reflect the subject's perceived pain intensity score.

A few stimuli after each pain stimulus, an 11 point pain rating scale (see above) was presented on the screen for 4.5 seconds, and subjects were asked to rate the intensity of the pain perceived, using a button box. The scale utilises bitmap files, which are set up so that the initial scale shows a rating of 5 (denoted by the position of the brackets around the number) in each case.

The rating was made by pressing the right button to make the scale move upwards (pain greater than five) or the left button to make the scale move downwards (pain less than five) until the appropriate pain scale rating number was highlighted by the bracket. This rating of the pain was only carried out during the time the scale was visible on the screen. Subjects were able to view the screen from their supine position within the magnet via the use of prism glasses. Prescription prism glasses were available should the subject normally wear prescription glasses to view distant objects.

Within the experimental design, these pain scales were projected about three letter n-back stimuli after the pain was administered. This was to avoid subjects knowing that the scale signalled the end of the block and therefore that as soon as the pain stimuli was perceived they did not have to focus on the task any longer. When the scales were presented a few seconds after the pain itself, this enabled the task to continue regardless of the pain. The distance was sufficiently short that subjects did not report difficulties in remembering the pain intensity over the short gap of 3 stimuli. On the fly ratings of pain stimuli are necessary to avoid subjects having to rely on their memory of the pain whilst engaging in a working memory task that would only confound the results.

It is necessary to control for visual stimulation and motor-button presses so that the activation in the brain as a result of the motor activity in pressing the buttons was controlled across task type. This was achieved by ensuring that there was always a 33% target rate amongst the letters of each trial block.

3.10 Behavioural results from the final pilot study

Before the experiment was piloted in the MRI scanner, the new final design was tested on six subjects (see behavioural data section) to verify that we were able to obtain the desired behavioural effect.

Results from the interim bench experiments are not discussed in this report, merely the changes they stimulated in the design of the experimental paradigm. However, this section of the report will thoroughly summarise the results of the behavioural data obtained by carrying out the final experimental design on six healthy naïve volunteers.

The volunteers were instructed that they should not speak the letters aloud as they saw them on the screen. All volunteers were fluent English speakers. They were instructed to respond as quickly but as accurately as they could when they saw the targets. They were told that the experiment was looking at reaction times and error rates and that as such, the main task to concentrate on was the n-back task. This was fully explained until they were confident with the task. They were then told that there would be some thermal stimuli of various intensities. They were given some thermal stimuli of various intensities and after a full explanation of the rating scale used, asked to rate their intensity. The temperature which was then used throughout the bench session was the same throughout but individually tailed to each subject so that it was a temperature to which they consistently attributed a score of '7' or moderate pain. They were told that they would be asked to rate the stimuli soon after they felt them but that they should focus their attention on carrying out the task as accurately as possible.

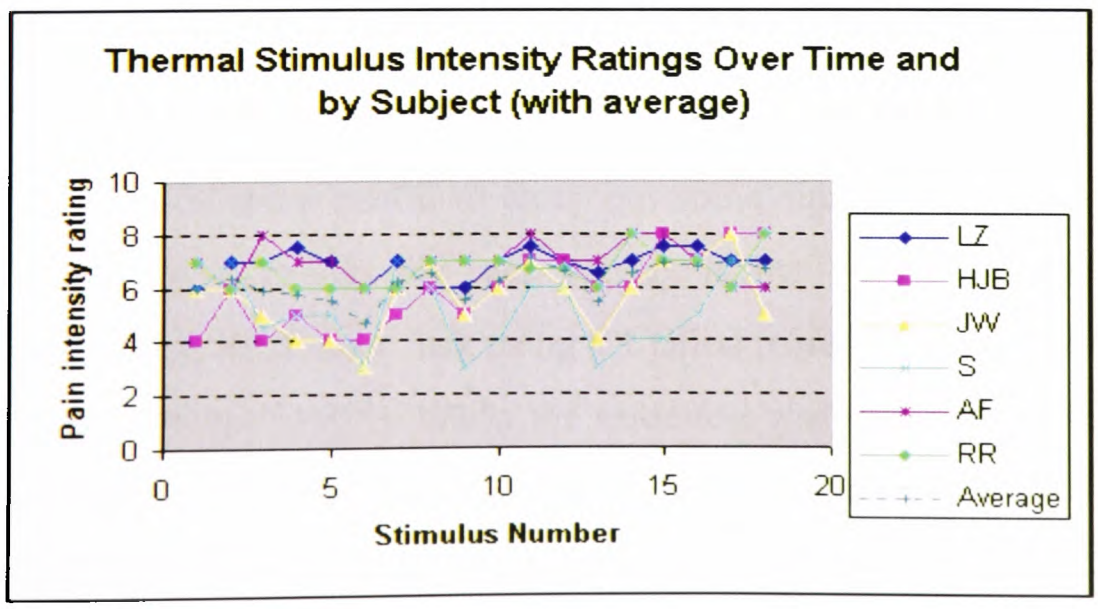
Fig. 3.8 Pain ratings across the experiment

This graph shows that there was no significant trend towards sensitisation to the thermal stimuli. This is an issue in pain research, as if the stimuli are too painful and too close together, the subjects become sensitised so that the subsequent stimuli are reported as more painful than the previous ones, even if they are in fact the same temperature thermal stimuli.

	Subject ratings						
Thermal Stimulus	LZ	HJB	JW	S	AF	RR	Average
1	6	4	6	6	7	7	6
2	7	6	6	7	6	6	6.33333
3	7	4	5	4.5	8	7	5.91667
4	7.5	5	4	5	7	6	5.75
5	7	4	4	5	7	6	5.5
6	6	4	3	3	6	6	4.66667
7	7	5	6	7	6	6	6.16667
8	6	6	7	6	7	7	6.5
9	6	5	5	3	7	7	5.5
10	7	6	6	4	7	7	6.16667
11	7.5	7	7	6	8	6.6667	7.02778
12	7	7	6	6	7	6.6667	6.61112
13	6.5	6	4	3	7	6	5.41667
14	7	6	6	4	8	8	6.5
15	7.5	8	7	4	8	7	6.91667
16	7.5	7	7	5	7	7	6.75
17	7	8	8	7	6	6	7
18	7	8	5	6	6	8	6.66667
Average	6.861111	5.888889	5.666667	5.083333	6.944444	6.685189	

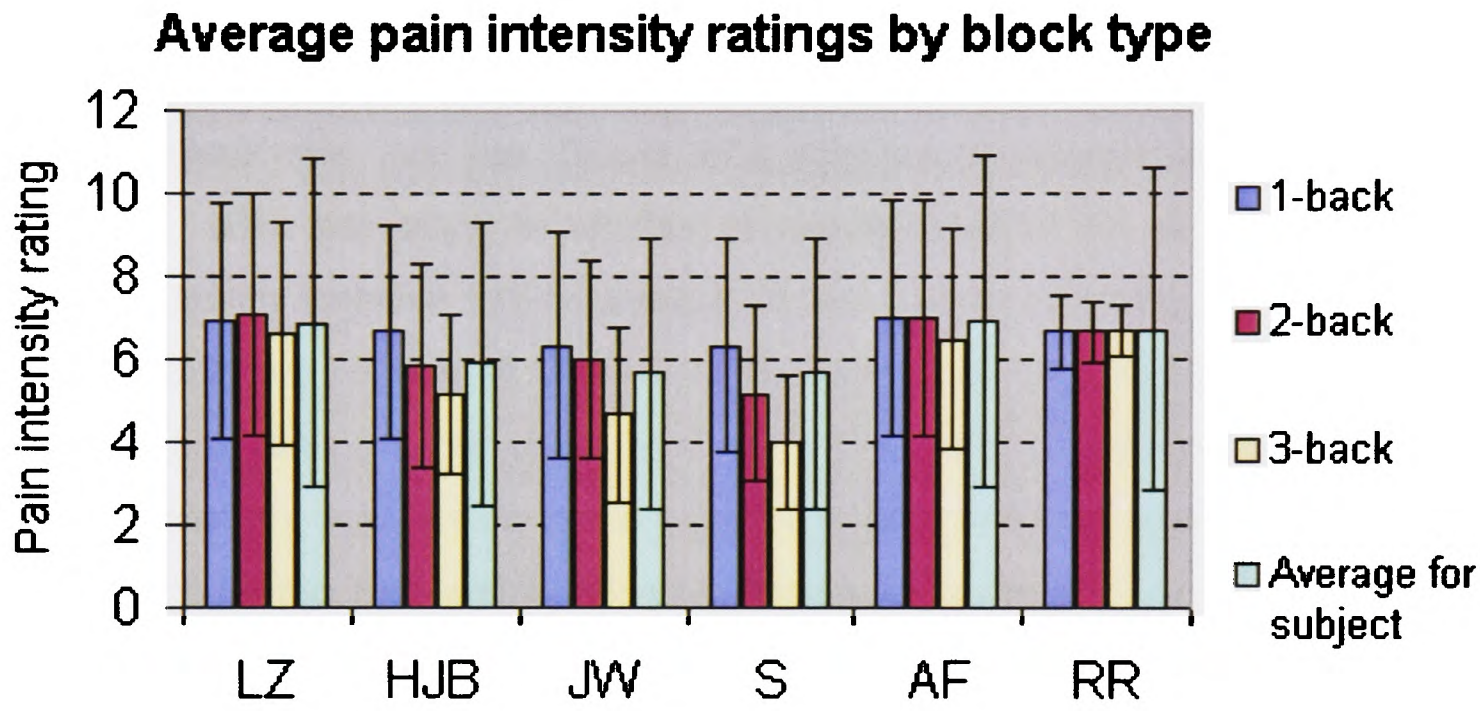
The scores in the table above (Fig. 3.9) show the behavioural pain intensity ratings given by each of the six subjects in real time as they received the stimuli during the experiment. This is shown graphically in the following figure (Fig. 3.9).

Fig. 3.9 Graph of real time pain intensity ratings by subject across time during the n-back experiment.



It can be seen that the pain intensity ratings attributed to the stimuli remained fairly constant throughout the experiment, without an obvious trend towards sensitisation to the stimuli. However, subjects did not rate pain stimuli in the different blocks as always being the same, hence the variation in intensity ratings across time.

Fig. 3.10 Pain intensity ratings by subject and level of n-back task.
The figure shows the pain intensity ratings by subject and by level of n-back task. It can be seen that the perceived pain intensity is lower in many subjects in the 2 and 3-back tasks than in the 1-back task.



When the average pain intensity ratings for each type of block and by subject throughout the experiment are calculated, it can be seen (Fig. 3.10) that on average, there is a reduction in perceived pain intensity between blocks 1 and 3. These are the two extremes of the experiment, being the least and most demanding respectively. There is also a tendency for the average pain rating to be greater in the 1 back than in the two back and for the two back ratings to exceed the three back ratings.

It is more useful to carry out some simple statistics on the data. When the average pain ratings for each subject in the 1-back task were compared to those obtained in the 2-back task using a 1 tailed paired Student's t-test, the results were not significant ($p= 0.078$). When the same test was carried out comparing the mean pain intensity scores reported by subjects in the 2-back task with those reported in the 3-back task, the result was significant ($p= 0.009$) at the 5 and 10% levels. This shows that the pain reported in the 3-back task was significantly less than the perceived pain

reported in the 2-back task. Finally the same Student's t-test comparison was made between pain ratings given in the 1-back and the 3-back tasks. This was significant at the 5% level (p= 0.02). The average pain ratings for 1-back, 2-back, and 3-back respectively were 6.7, 6.3, and 5.6 respectively when all subjects scores by block were averaged together.

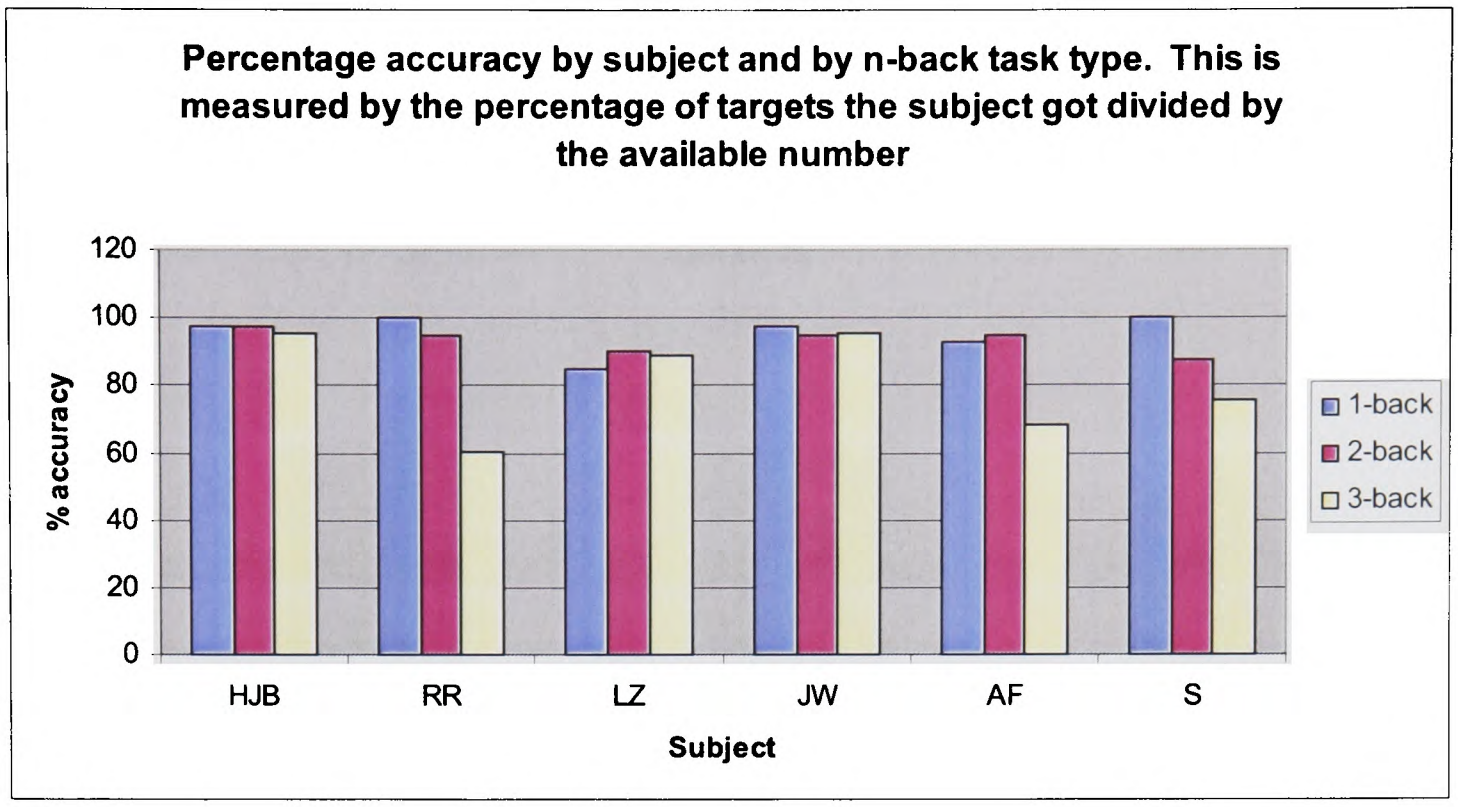
In terms of assessing the cognitive load, previous researchers have used either individual subject reaction time per stimulus block or error rates or both to reflect working memory load. Reaction time data was recorded but not discussed in this thesis. All that was of note was that there was a graded increase in reaction times from the 1-back task to the 3-back task. This is reassuring in that it does indicate that subjects have to take longer to make decisions in the more demanding tasks and were concentrating on accuracy over speed.

Instead error rate was chosen as a behavioural outcome measure of task difficulty. This was taken as number of targets detected out of the number of available targets recorded as a percentage. It was decided that only one measure of task difficulty was required to simplify the analysis at this stage, and also because the focus of this study was never intended to be the cognitive aspects of the n-back working memory task. Rather it was intended to look at the task simply to ascertain its ability to distract from and therefore modulate pain intensity.

The graph below shows how the percentage accuracy varies by task (Fig 3.11)

Fig. 3.11 Error rates by n-back block

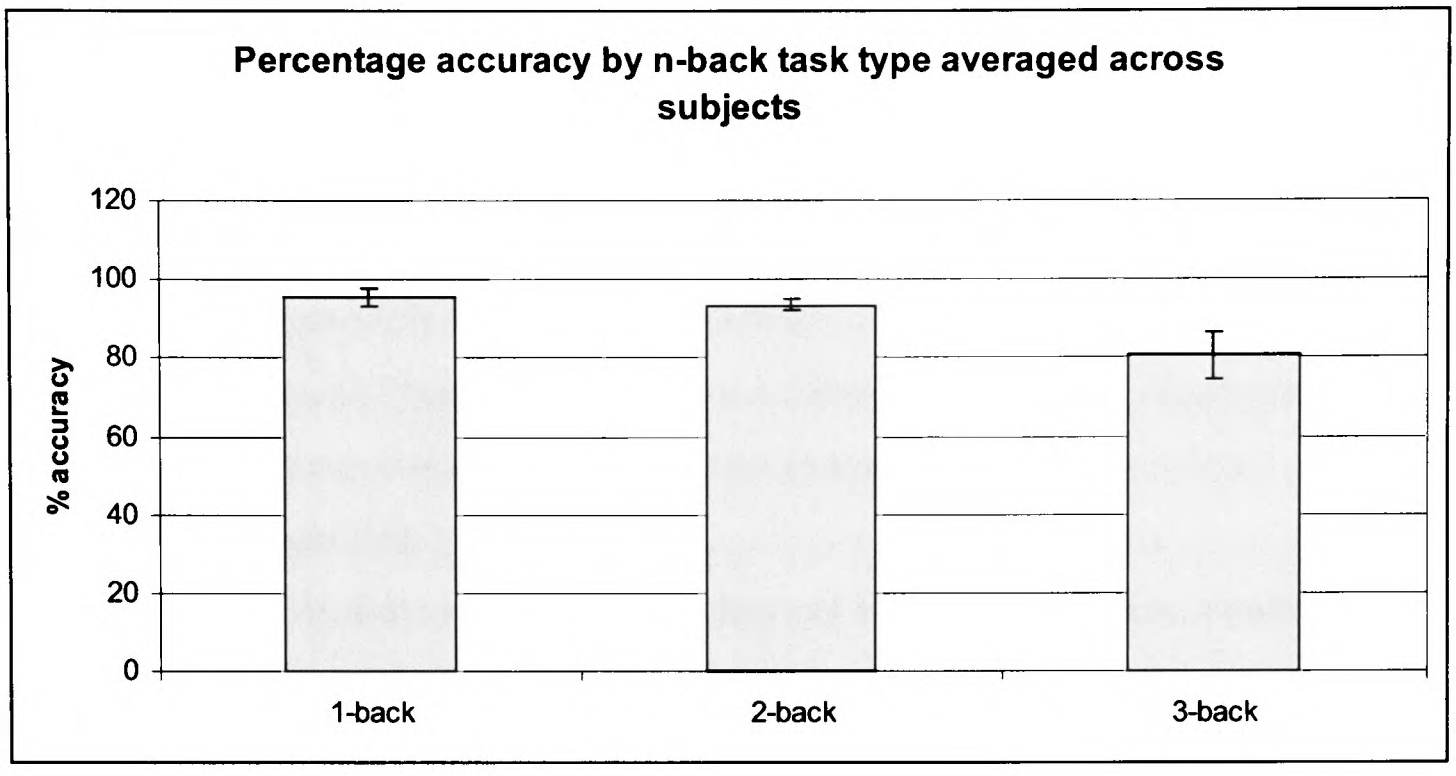
This graph shows the error rates (percentage of targets identified) by n-back block and by subject. Standard error bars are not shown.



From this graph it can be seen that the percentage accuracy rate for the 3-back task is in 4 of 6 cases less than that in the 1-back task (not statistically significant). The 1-back task is performed at around 100% accuracy with the two back at the same or slightly lesser rate of accuracy overall. The error rate for three back is greater than the two back and the one back in 4 of 6 cases. When statistical analysis was carried out on the percentage accuracy data, the following results were obtained. There was no significant difference between the 1-back and 2-back percentage accuracy levels (paired Student's t-test, 1-tailed, p= 0.23). There was a significant difference at the 5% level between the percentage accuracy level in the 2-back and 3-back tasks and between the 1-back and 3-back tasks (paired Student's t-test, 1-tailed, 0.042, 0.043 respectively).

Fig 3.12 Overall mean accuracy by task block

This figure shows the overall average accuracy percentage by task block obtained using the means of all subjects. It can be seen that the accuracy rate is highest in the 1-back task and then slightly reduced in the 2-back task but less in the 3-back task.



This behavioural bench data supports our assumption that the 3-back task is significantly more cognitively demanding than the 1-back task or the 2-back task. There is not a significant difference in cognitive load as measured by percentage accuracy between the 1-back and 2-back tasks.

The pain intensity data support these findings as well, indicating that the greatest reduction in pain intensity is found between the 1 and 3 back tasks and not between the 1 and 2 back tasks.

The experiment was then tested in the fMRI environment in order to see whether or not these behavioural results manifested themselves in imaging changes in key pain matrix areas of the brain.

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This experiment demonstrated a behavioural reduction in perceived pain intensity using the n-back task paradigm. The next experiment will go on to address the central functional nature of this reduction in pain ratings.

CHAPTER 4: The use of a parametric working memory task to modulate perceived pain intensity: An fMRI study

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The previous sections have outlined the background behind the modulation of pain by attention (also see chapter 2), the nature of working memory as it relates to cognitive load, and the reasons for choosing the N-back task (Chapter 3). This experiment aims to modulate the perceived intensity of noxious thermal stimuli using a graded or parametric distraction task. The initial pilot behavioural data are also summarised in the previous chapter.

This chapter will describe the imaging experiment carried out at 3T to ascertain whether there is a parametric decrease in central pain areas with an increase in n-back task difficulty when the n-back task is used as a distraction task from the noxious stimuli. The pilot behavioural data shows that there is a subtle reduction in pain intensity with increased level of n-back difficulty. This fMRI experiment aims to find the central neuronal correlates of this phenomenon.

The nature of working memory and the n-back task is fully discussed in the previous chapter (Chapter 3).

4.0 Introduction

The aim of this study is to parametrically manipulate cognitive load across three conditions that are identical apart from the cognitive complexity involved. During all the levels of the task, painful thermal stimuli will be administered and rated by the subject. Building upon previous findings showing that distraction via the use of a cognitive task can reduce the perceived intensity of experimentally induced pain, this study was designed to ascertain whether or not a parametric reduction in pain across the three levels of the n-back task can be produced in the scanner. If this result can be replicated in the scanner (based upon successful behavioural results described in Chapter 3, then experiments will further aim to discover whether this behavioural modulation would again (Bantick et al., 2002) be reflected in a corresponding

reduction in activation in key pain processing regions and whether this modulation behaves in a linear or non-linear fashion across different brain regions.

4.1 Materials and Methods

Subjects

Informed consent was obtained from eight healthy right-handed volunteers (7male, 1 female, mean age 33.5 +/- 7.8 years). Subjects also underwent a comprehensive verbal screen to ensure that they did not meet any exclusion criteria for MRI experimentation.

fMRI Scanning Parameters

The reader is referred to Chapter 2 of this Thesis where full details of scanning parameters used in this study are given.

Experimental paradigm and subject preparation

As discussed previously (Chapter 3), three levels of the letter n-back task were used (1-back, 2-back, and 3-back) as a distraction task that carried on throughout the experiment.

There were 3 presentations of each stimulus per type of task (1-back, 2-back, and 3-back). In total there were 125 letter stimuli in each type of stimulus block, although the actual block length varied between experimental blocks. The block length of individual blocks, regardless of block type always remained in the region of 2 minutes duration. There was a break lasting ten seconds between each block of stimuli.

The stimulus letters were presented in pseudo-random sequence on the screen for half a second's duration each with a two and a half second's inter stimulus interval between subsequent letter presentations. Each letter used during the experiment was a capital letter consonant. Out of the total amount of stimuli per task type (125) 33% were targets (41 letters).

The order in which each type of stimulus block was presented was randomised across blocks. No two blocks of the same type were the same, and there were five versions of each stimulus type and two versions of the entire paradigm.

For the purpose of scanning, an extra ten minutes was required for scanner setup at the beginning of the experiment and an additional ten minutes at the end of the session in order to take a structural high resolution scan.

The subjects all performed a practice session before they completed the experiment in the MRI scanner. This practice session was identical in every way to the experiment that they would ultimately take part, in except that the letters were different (1 letter had been added to the original letter so R became S and so on), and there were no noxious thermal stimuli or associated pain intensity rating scales. The structure and timings of the paradigm were identical.

In the middle of each block throughout the experiment, thermal stimuli were administered at a temperature that had been found by the individual subject concerned to correspond to a pain intensity rating of 7 out of a possible 10. This corresponded to a rating of moderate pain. This temperature was ascertained through a comprehensive individual thresholding procedure for each subject. They were told that they would receive a variety of thermal stimuli, which they would be asked to rate. During the thresholding process, stimuli of a variety of temperatures from warm to painful were randomly administered so that subjects were anticipating a range of temperatures, had practice rating the stimuli and had experienced a range of temperatures prior to starting the experiment.

A rating scale would be presented upon the screen two letter stimuli after each thermal stimulus in each block. During this time the subject was asked to rate the intensity of the stimulus immediately preceding the scale using the same criteria as in the thresholding session. This was done by clicking a button on the button press (left to move the scale down to show decreased intensity and right to move the scale up on the screen) displaying the number chosen by the subject as they altered it so that it represented their perception of the stimulus. The default position of the scale pointer was always 5. Refer to Chapter 3 for further details of this rating scale.

Subjects were asked to concentrate on the task and to try not to sacrifice accuracy for speed. They were asked to press any button on the button press when they saw the target letter on the screen. They should do this as quickly and accurately as possible.

The first letter of each level of the task was always preceded by a screen detailing which level was about to begin. Throughout the experiment, letters forming

part of the 1-back task were distinguishable by a single dot underneath the letter, 2-back task letters by two dots and the 3-back task by 3 dots to act as a constant cue to the subject which level they were taking part in.

The reader is referred to Chapter 3 for a detailed account of the experimental paradigm and diagrams to illustrate the nature of the task.

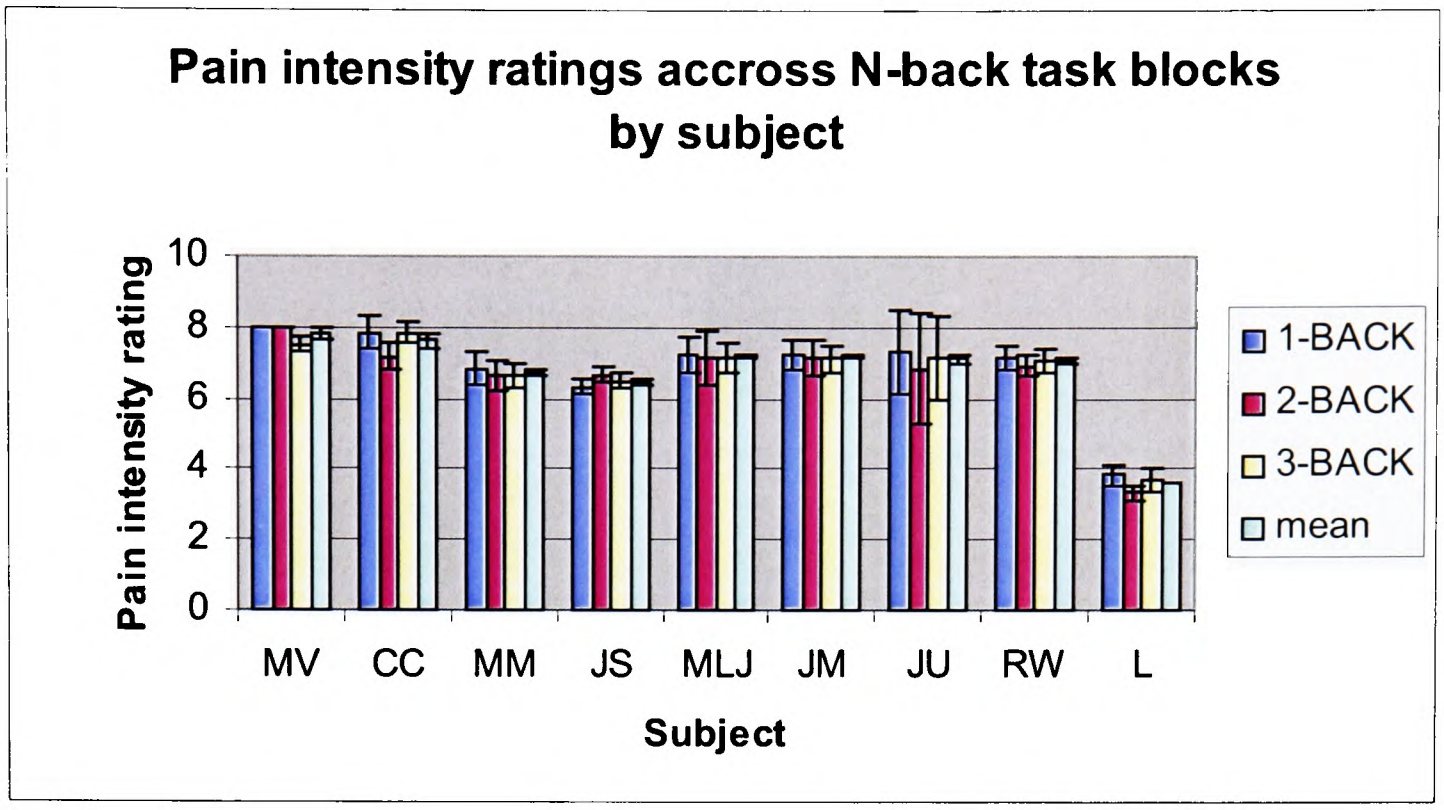
4.2 Behavioural Results

The behavioural data from the fMRI experiment were analysed in terms of perceived pain intensity during each of the n-back task levels, and error rates between levels.

4.2.1 Perceived pain intensity

Subjects' pain intensity ratings were obtained online throughout the imaging experiment using a button press in response to a pain scale presented after each pain stimulus. The subjects had been told that there would be various thermal stimuli of different intensities throughout the experiment that they would be asked to rate but that they should focus on the n-back task throughout the study. The temperature used corresponded to a consistent intensity rating of '7' for each individual subject, and did not cause sensitisation (when the temperature is too high relative to the gap between thermal stimuli, the subject can become sensitised, resulting in subsequent stimuli appearing to be more painful than the previous ones). The temperature used for each subject did not vary throughout their scanning session. The individual subjects' perceived pain intensities by subject block are shown in Fig. 4.0.

Fig. 4.0 Individual subjects' perceived pain intensity ratings across experimental block.



Paired 1-tailed Student's t-tests were carried out to compare the pain intensity ratings in the three n-back task levels using the mean for the three blocks for all subjects.

Table 4.0 Mean group pain intensity ratings by experimental group. P values obtained from Student's 1-tailed paired t-test.

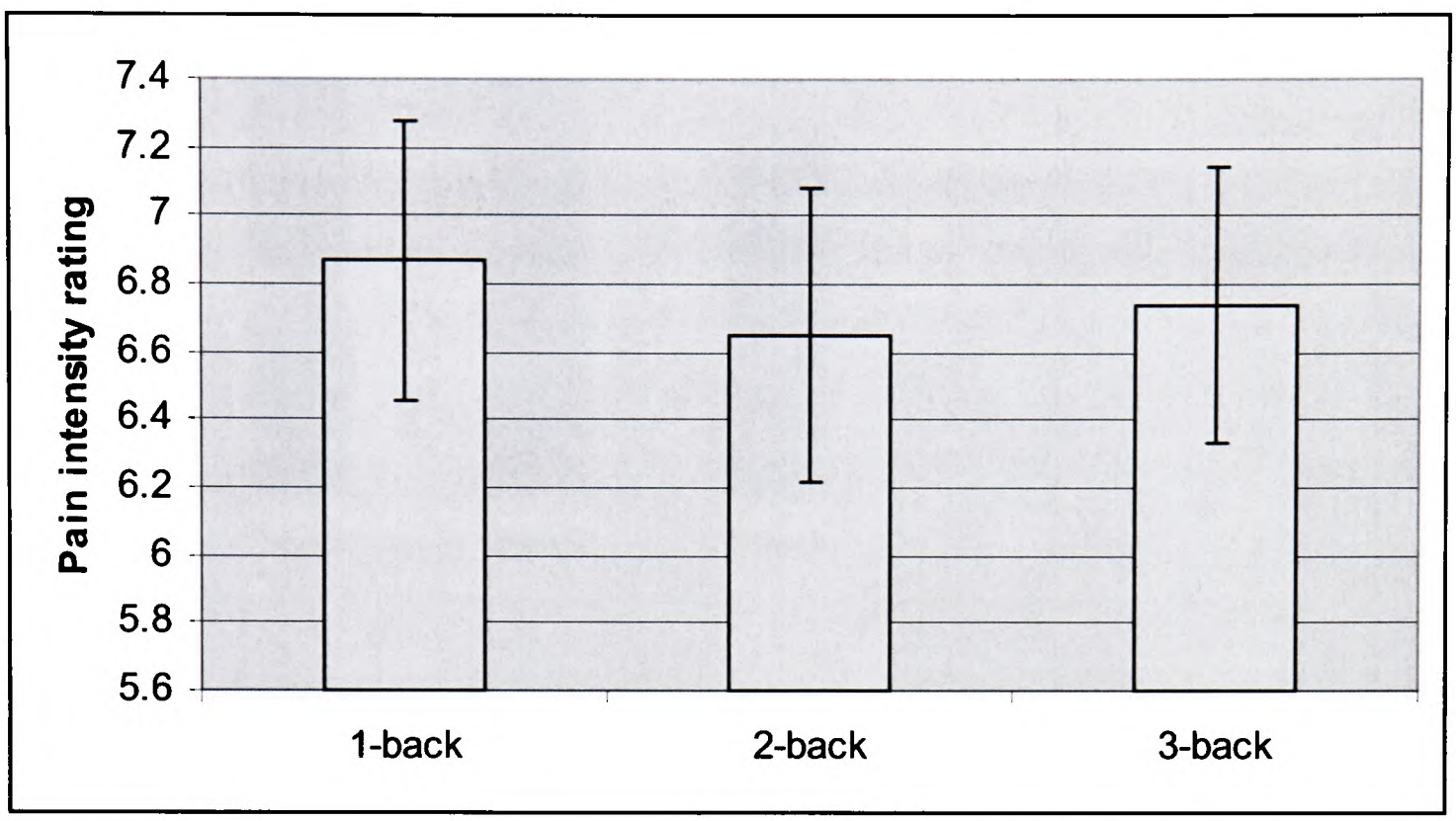
Group comparison	P value	Significance level
1-back pain v 2-back pain	0.031	*
1-back pain v 3-back pain	0.031	*
2-back pain v 3-back pain	0.222	NS

Key: non significant (NS), p = < 0.05 (*)

There is a significant reduction in the 3 back pain compared to the 1 back pain (p= < *0.05*), and between the 1 back and the 2 back pain (p= < *0.05*). The results show a trend of reducing perceived pain intensity with increasing task difficulty of the n-back task, although the difference in pain intensity between the 2 back and the 3 back task

levels did not reach significance. The results are displayed graphically in Fig. 4.1 below.

Fig. 4.1 Graph of group mean perceived pain intensity ratings across n-back task block.

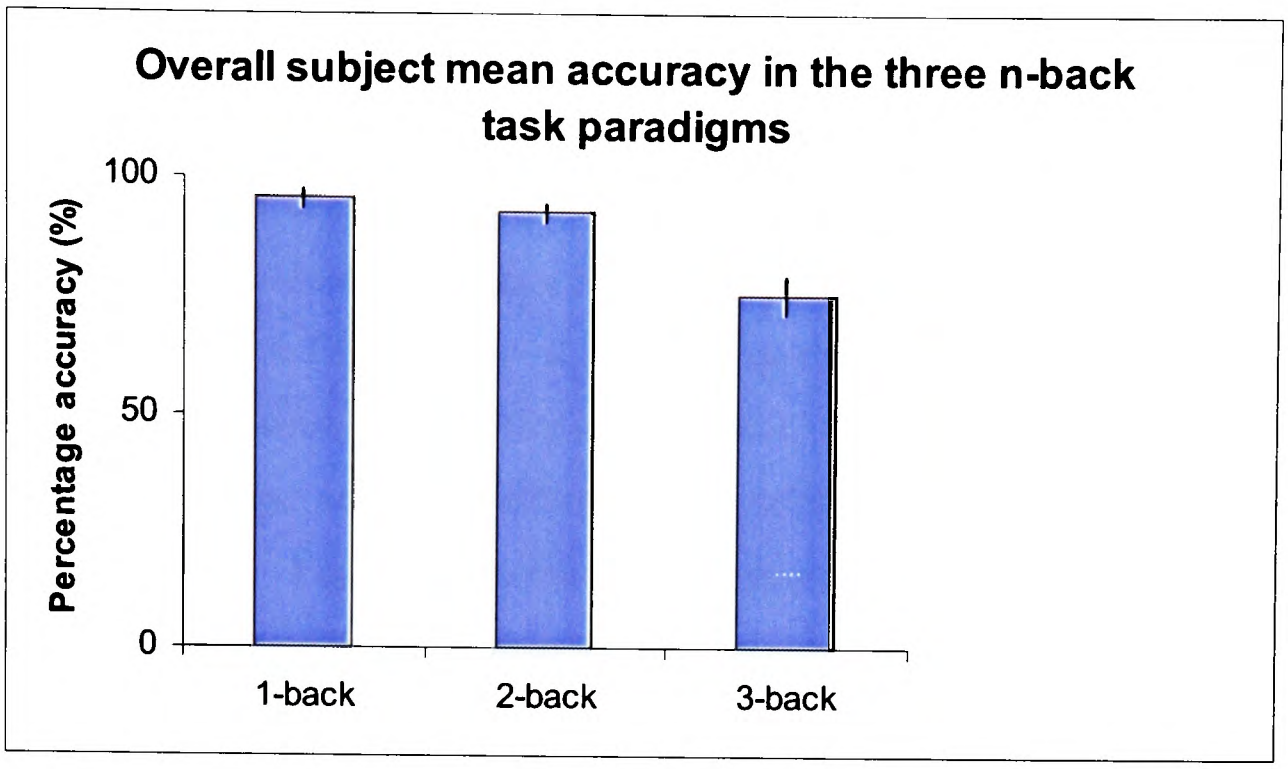


1 back vs 3 back pain $p<0.05$, 1 back vs 2 back $p<0.05$, 2 back vs 3 back NS

4.2.2 Error rates

The error rate recorded here is a percentage, calculated from the number of targets correctly identified, as a percentage of the possible number of targets. The mean accuracy across all subjects for the three n-back task levels is shown in Fig. 4.2

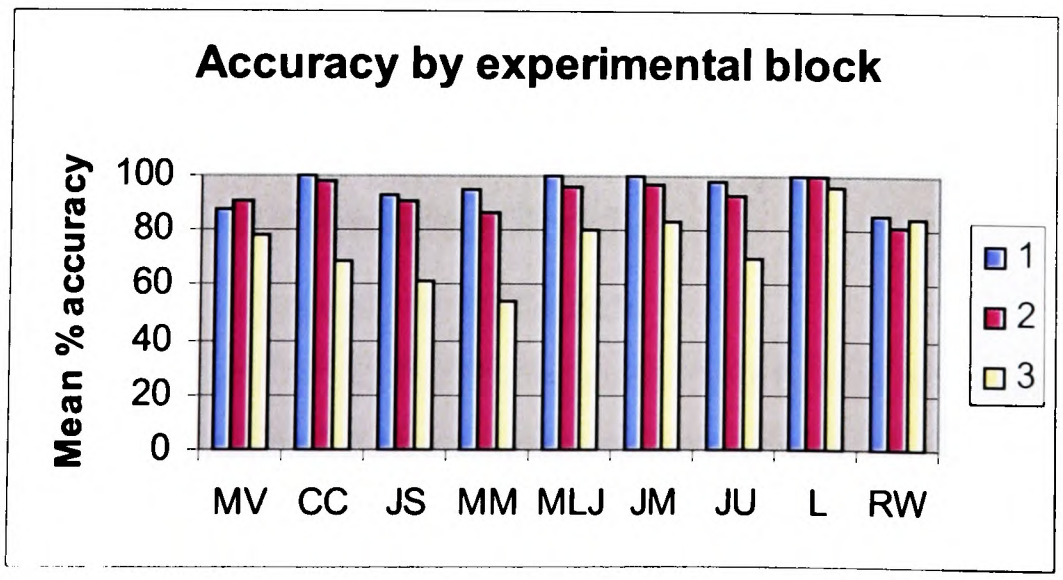
Fig. 4.2 The mean percentage accuracy by task block across subjects.



The same data presented by each individual subject shows the same trend, with more targets being missed in the three back than in the one back task.

The error rate in the 3 back task is significantly greater than both the 2-back and 1-back tasks (paired 1-tailed Student's t-test, p=0.001 for both comparisons). The error rate in the two back task is greater than in the 1-back task but this difference is less marked (paired 1-tailed Student's t-test, p= 0.01).

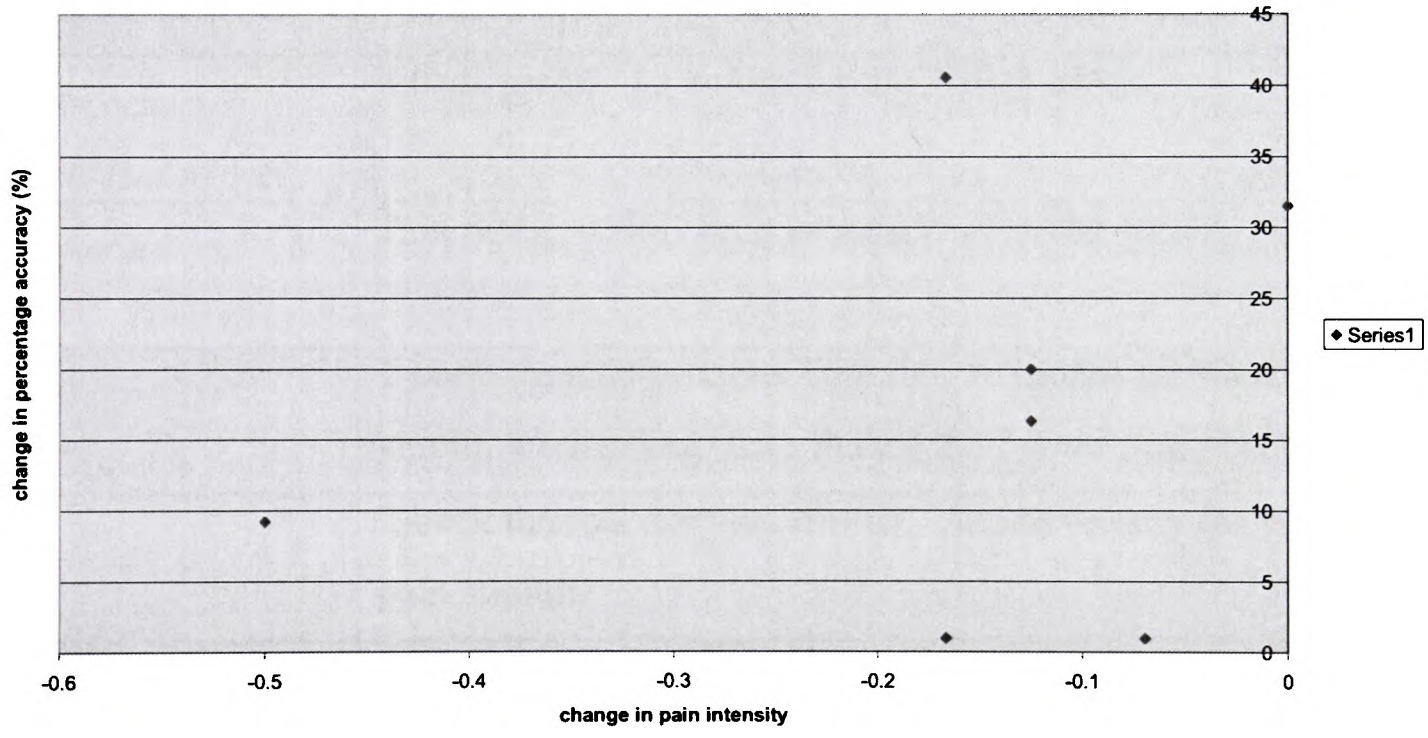
Fig. 4.3 Individual experimental subjects' percentage accuracy across n-back block level.



4.2.3 Correlation between reduction in perceived pain intensity ratings (from 1-back to 3-back) and percentage accuracy (between 1-back to 3-back.

When the average reduction in pain intensity rating from 1-back to 3-back for each subject for each n-back block was compared using a correlation analysis to the reduction in percentage accuracy from the 1-back to the 3-back blocks there is a weak correlation (T= 1.12, df 7, p = 0.15) between the two indices. This shows that there is some relationship between the reduced pain intensity and task difficulty (measured using percentage accuracy) although this relationship does not achieve statistical significance. Fig. 4.4 (below) displays the relationship between perceived pain intensity and percentage accuracy.

Fig. 4.4



4.3 Functional Imaging Results

Statistical analysis of the fMRI data

Analysis of the fMRI images to identify regions exhibiting significant changes in blood oxygen dependent signal (BOLD) (Ogawa et al., 1992) was carried out via a multi-stage process using the image analysis package, FEAT

(http://www.fmrib.ox.ac.uk/fsl), an extension of MEDx (Sensor Systems). This process is described in detail in Chapter 2.

The study was designed in a factorial way. Nine explanatory variables were proposed to model each subject's fMRI time-course data on a voxel by voxel basis. The explanatory variables were as follows (see table 4.1).

Table 4.1 Explanatory variables (EVs) used in the n-back experiment to model the fMRI data.

Explanatory variable	Description
1	Introduction screens- these denote which type of task is about to commence. Information is purely visual.
2	1-back block. This models the type of task being undertaken throughout the experiment. In this case this models the one-back task, including all the data from when the 1-back task is taking place.
3	2-back block. As above for the 2-back block.
4	3-back block. As before for the 3-back block.
5	Pain rating scales being presented upon the screen.
6	1-back thermal noxious stimuli. A model of when the pain stimuli were taking place during the *1-back task ONLY.*
7	2-back thermal noxious stimuli. As above only for the 2-back pain stimuli.
8	3-back thermal noxious stimuli. As before only for the 3-back pain stimuli.
9	Button press responses. This models *every* button press made by the subject and is derived from the button press data acquired in the MRI scanner.

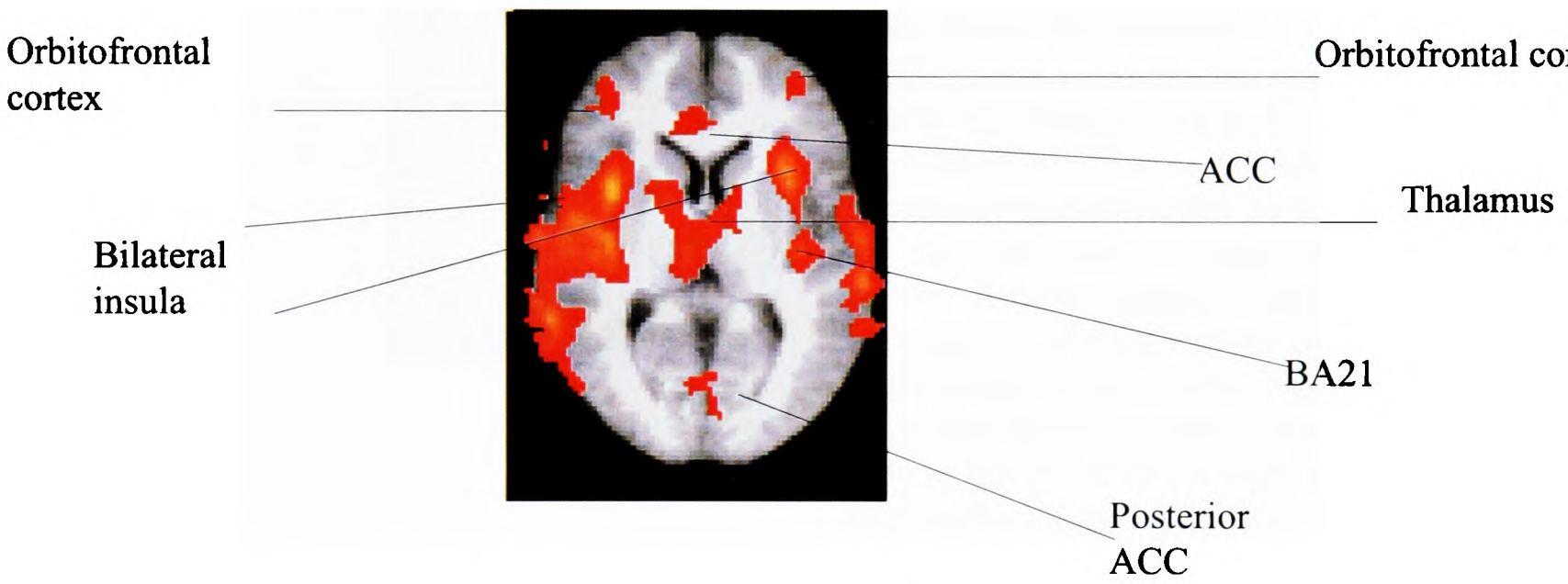
The timings for this model were derived from the behavioural data acquired concurrently with the fMRI data. The timings used were from individual subjects' behavioural data acquired real-time during the scanning experiment.

These EVs were then entered into the General Linear Model (GLM) as independent variables. Please refer to Chapter 2 for full details of the statistical analysis carried out on the raw fMRI data.

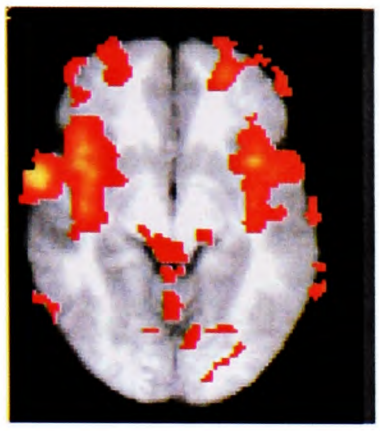
fMRI images depicting pain activation in the different n-back task levels

Figures 4.5, 4.6, and 4.7 depict the group mean activation during the 1, 2, and 3-back tasks as a result of the painful thermal stimuli.

Fig. 4.5 Image showing areas of activation during the 1-back noxious thermal stimuli. This illustration shows activation in bilateral orbitofrontal cortex, ACC, bilateral insula, thalamus, BA21/37, and posterior ACC

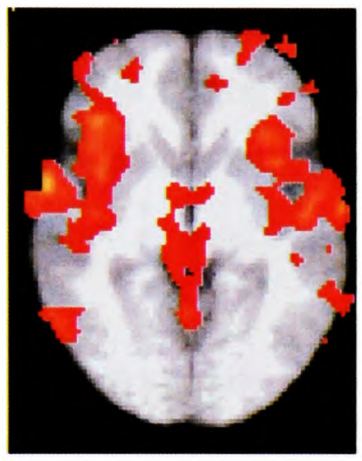


4.6 Image to illustrate the areas of activation during the 2-back noxious thermal stimuli



At the top of the image bilateral orbitofrontal activation is seen. Also lower down bilateral insula activation. There is some posterior cingulate activation seen in the middle/bottom of the picture and the middle of the image depicts thalamic activation

4.7 image to illustrate the areas of activation during the 3-back noxious thermal stimuli



At the top of this image left prefrontal cortex activation is seen on the top right. Below this bilateral orbitofrontal activation is seen though this is less distinct than in the previous figure. There is strong bilateral insula activation seen on the left and right middle edges of the picture. Anterior cingulate activity is seen (posterior region in the middle of the image with an area of thalamic activation above it. Some activation is seen in BA 21 on lower right outside edge of the image (left sided activation) and also some activity in area 40 (posterior attentional system) on the left side of the image (right side of the brain)

4.4 Quantification of pain modulation by distraction

Precise modelling of all the explanatory variables within the experiment allows the GLM analysis method to be applied to the fMRI data. This method can ascertain which brain areas are activated in response to noxious stimulation whilst the subject is engaged in the each of the three distinct levels of the n-back task. The central activation as a result of the painful stimuli during each n-back task level (1, 2, and 3-back) can then be compared.

fMRI signal changes as a result of painful stimulation can be identified for all three n-back task conditions. The fMRI signal change is expressed as parameter estimates derived from the GLM model fitting and is measured in arbitrary units. Regions of interest within the pain matrix were defined anatomically using high resolution fMRI scans. These high resolution scans were used to create "masks" defined using the outer limits within which the particular region or structure of interest is located (i.e. ACC, insula, thalamus, primary somatosensory cortex (SI). This means that the signal change due to pain in all three task levels can be calculated by region of interest.

The mean parameter estimate was calculated for each region of interest in all three task levels, allowing comparisons to be made of signal change by task level in the regions of interest. Mean parameter estimates for the pain activation in each region of interest and in each task level can be calculated for each subject, together with mean above zero values, minimum and maximum PEs and the location of the voxel where the maximum PE is recorded. Parameter estimates are derived from the COPE images created during the statistical analysis of fMRI data using the FEAT analysis package. A positive COPE value means that the signal in that voxel increases during the stimulus. A negative COPE value represents a drop in signal during the stimulus.

A group mean activity across subjects for each anatomically defined region of interest was three conditions (paired t-test) to be carried out for each brain region. The different ways in which the data was interrogated are described below.

4.5 Initial analysis of mean fMRI signal data

The mean fMRI signal change for each region and in each n-back level condition were analysed for each subject. A group mean and standard error (SE) for the signal change as a result of the noxious thermal stimuli were calculated by region of interest. Raw data (group means by task condition) are shown below (Table 4.2)

Table 4.2 Group mean parameter estimates

Mean fMRI signal

	1-back pain	2-back pain	3-back pain
ACC	16.73	33.64	14.78
L.Insula	49.34	55.10	37.88
R. Insula	16.73	16.04	33.64
L. SI	-41.53	-16.44	-35.59
R. SI	27.61	12.53	34.93
L. Thalamus	26.73	26.25	32.50
R. Thalamus	31.19	35.66	32.17

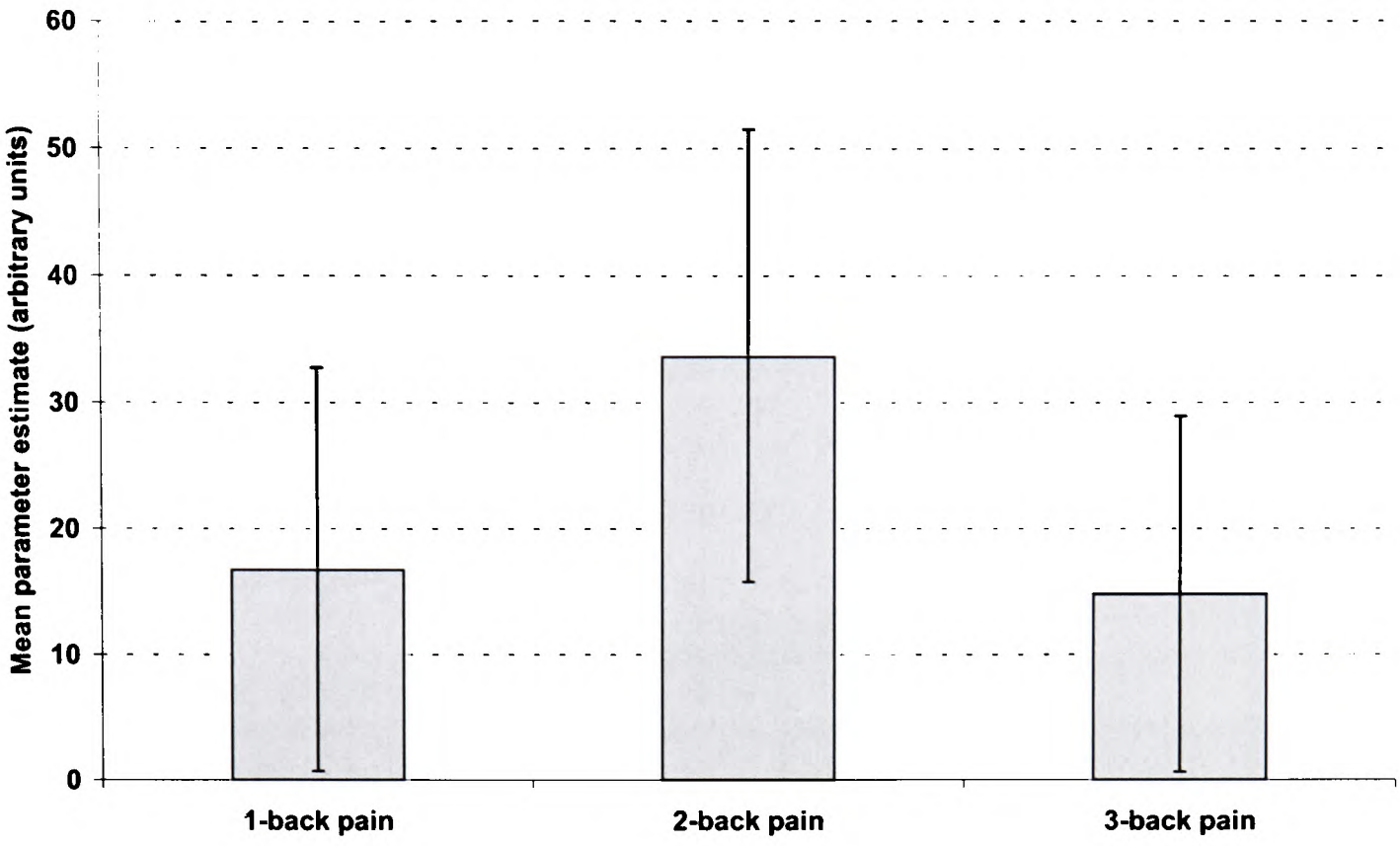
Noxious thermal stimuli were applied to the left hand so right lateralised brain regions are contralateral to the stimulus.

1-tailed paired Student's t-tests were carried for the mean fMRI signal by region of interest and by n-back task level. The key results are now described.

Anterior cingulate cortex (Fig. 4.8)

There is a trend level increase in the 2-back pain activation compared with the 1-back pain activation. There is a significant ($p = < 0.01$) reduction in the mean parameter estimate in the 3-back pain compared to the 2-back pain. The decrease in the mean parameter estimate from the 1-back to the 3-back task did not reach significance.

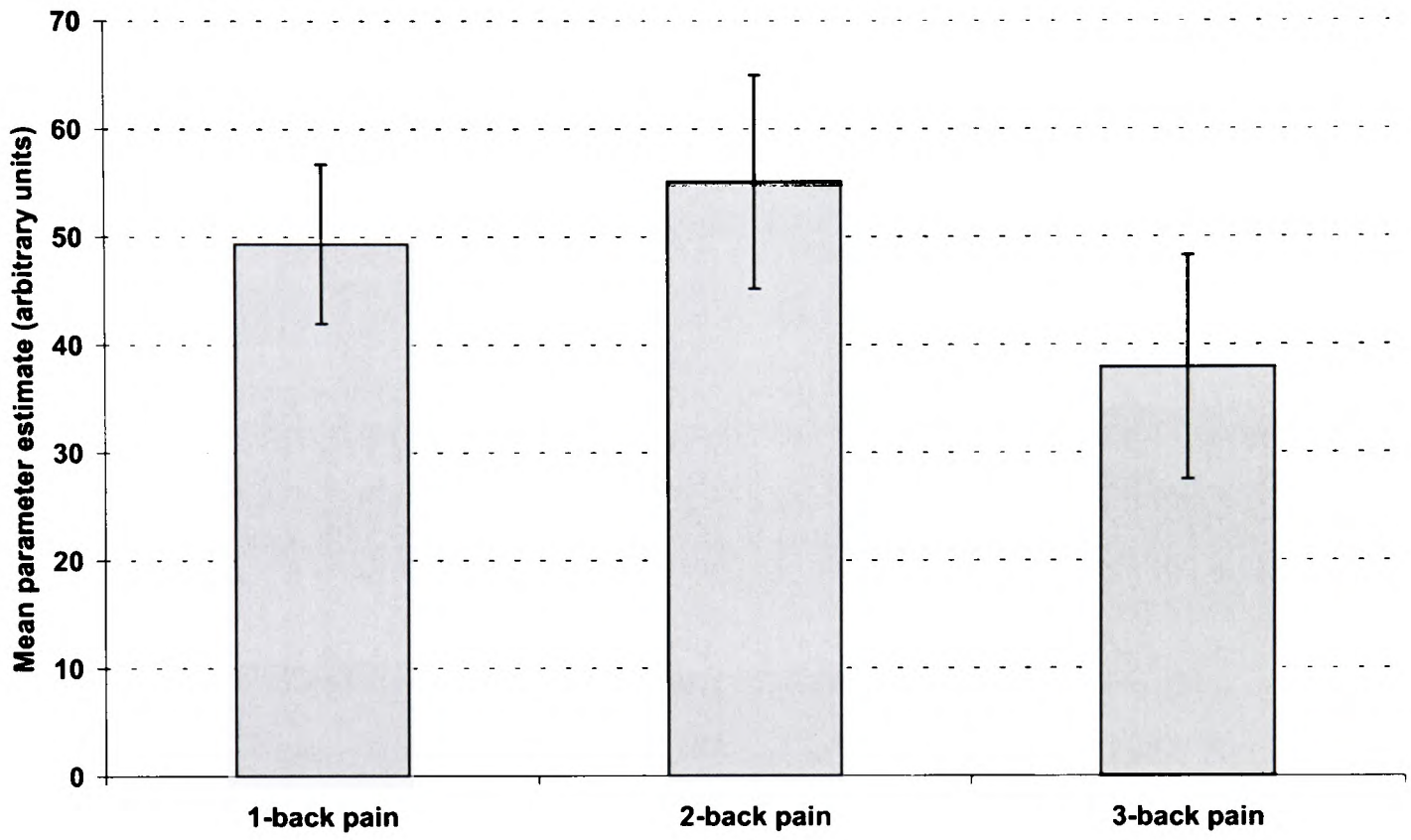
Fig. 4.8 Graph of group mean parameter estimates by n-back task block from Anterior Cingulate



Left insula (Fig. 4.9)

There is trend level increase in activation from the 1-back level to the 2-back level pain. There is a significant reduction ($p < 0.01$) in the mean parameter estimate in the three back pain compared with the 2-back pain. The reduction in the mean parameter estimate between the 1-back and 3-back tasks was not significant.

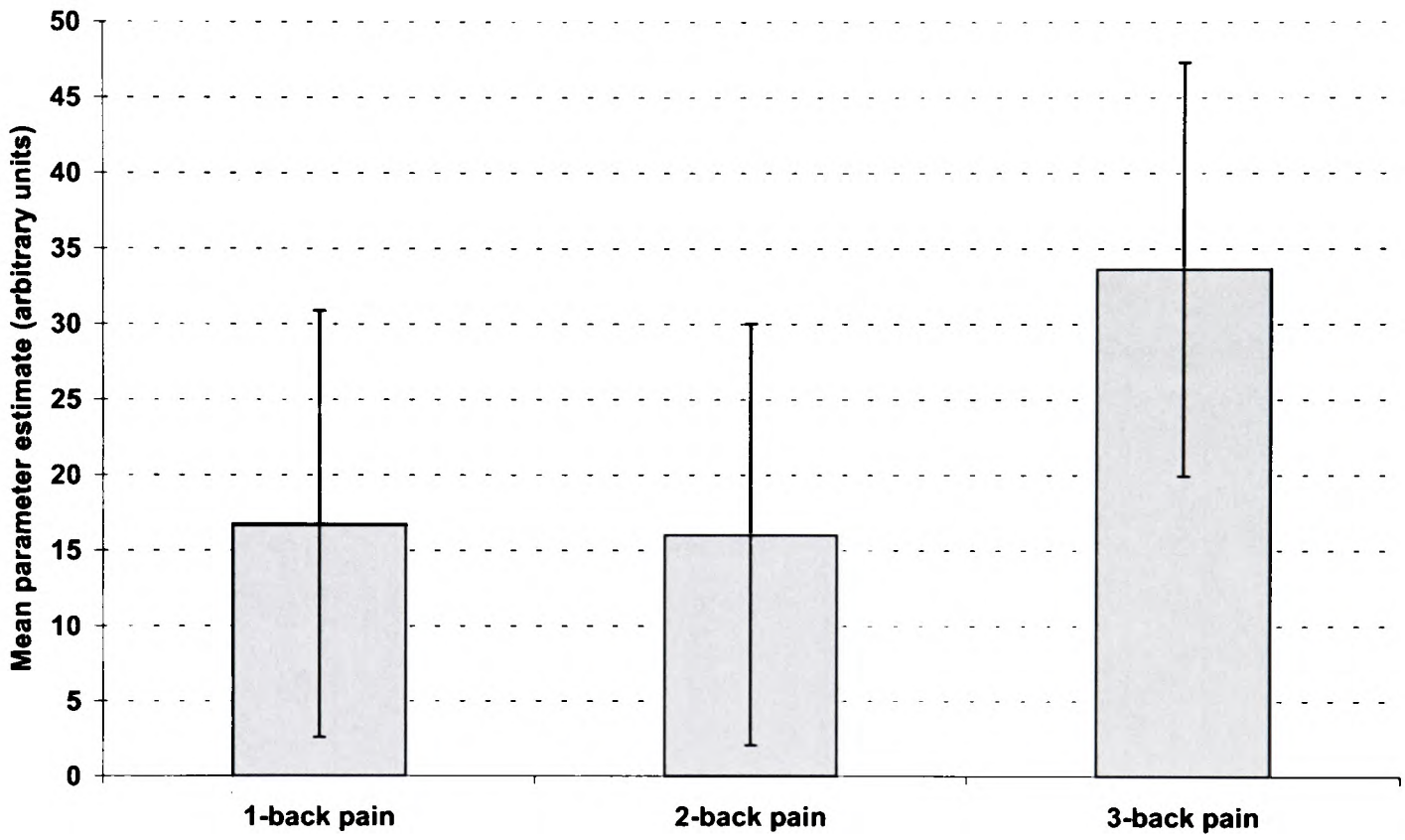
Fig. 4.9 Graph of group mean parameter estimates by n-back task block from left insula



Right insula (Fig. 4.10)

There is no significant difference in the mean parameter estimate between the 1-back and 2-back pain conditions. There is a significant ($p< 0.01$) increase in activation between the 2-back and 3-back pain levels. There is a significant ($p< 0.05$) increase in the mean parameter estimate for the 3-back pain activation compared with the 1-back activation.

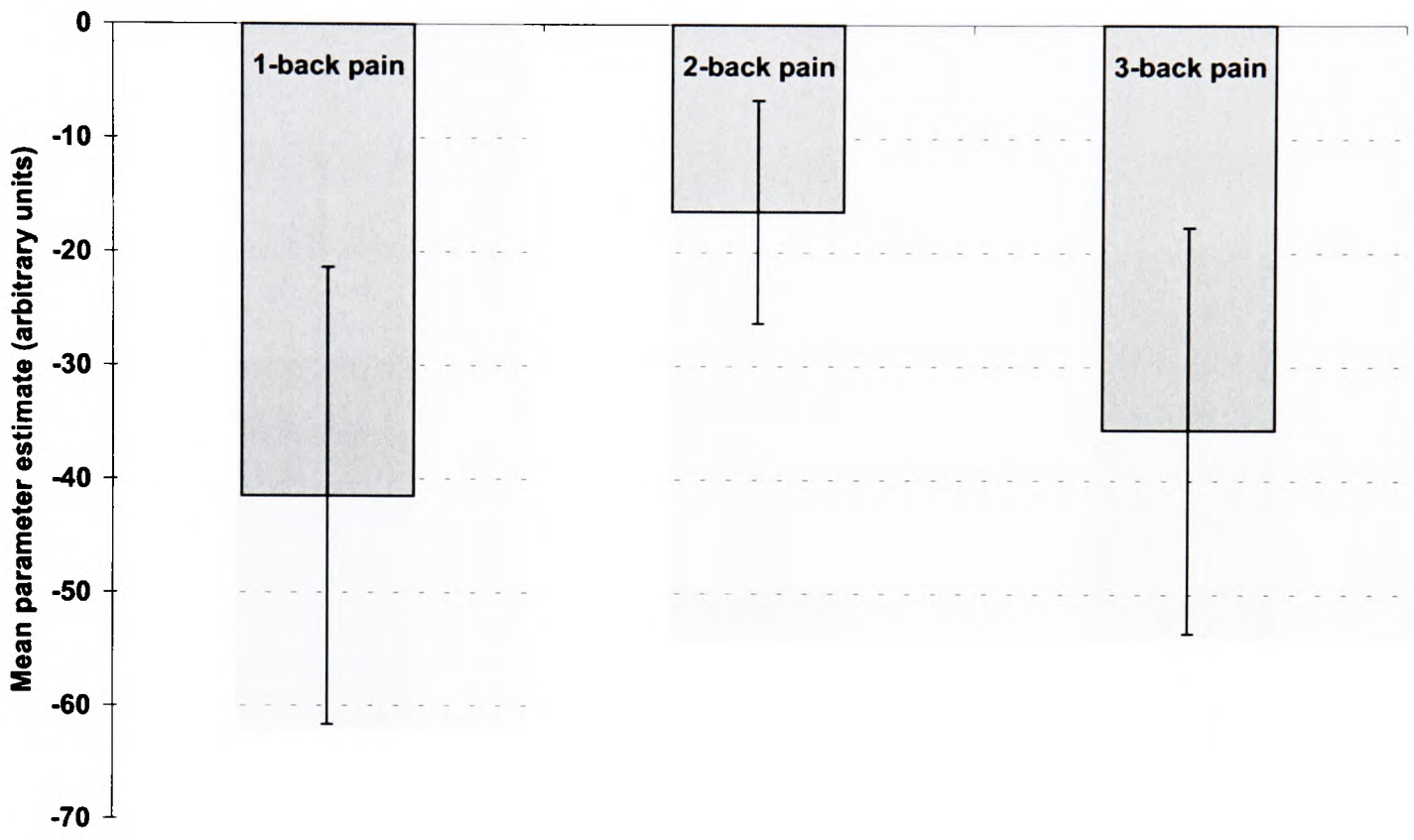
Fig. 4.10 Graph of group mean parameter estimates by n-back task block from right insula



Left SI (Fig. 4.11)

There is a significant ($p < 0.05$) increase in mean parameter estimate between the 1-back pain and the 2-back pain. There is a significant ($p < 0.05$) reduction in mean parameter estimate in the 3-back pain compared to the 2-back pain. There is no significant difference between the mean parameter estimate for the 1 back task compared with the 3-back task.

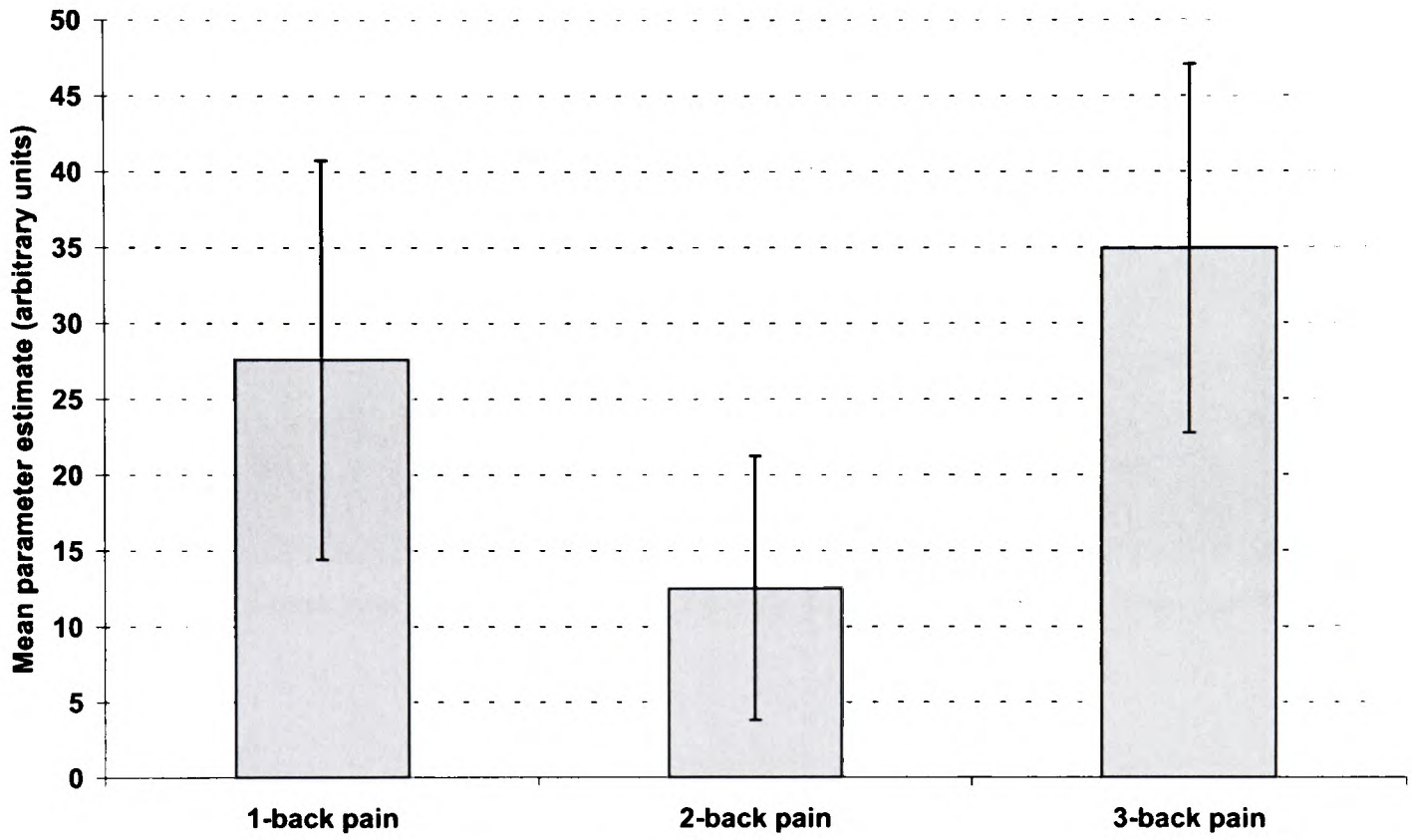
Fig. 4.11 Graph of group mean parameter estimates by n-back task block from left S1



Right SI (Fig. 4.12)

There is a significant ($p < 0.05$) decrease in pain 2-back activation compared to pain 1-back activation. There is a significant ($p < 0.05$) increase in the 3-back pain activation compared to the 2-back activation. The increase in 3-back the mean parameter estimate compared with the 1-back task did not reach significance.

Fig. 4.12 Graph of group mean parameter estimates by n-back task block from right S1



Thalamus

None of the between-level comparisons of mean parameter estimates in this region reached statistical significance.

Fig. 4.13 group mean parameter estimate by n-back task for the left Thalamus

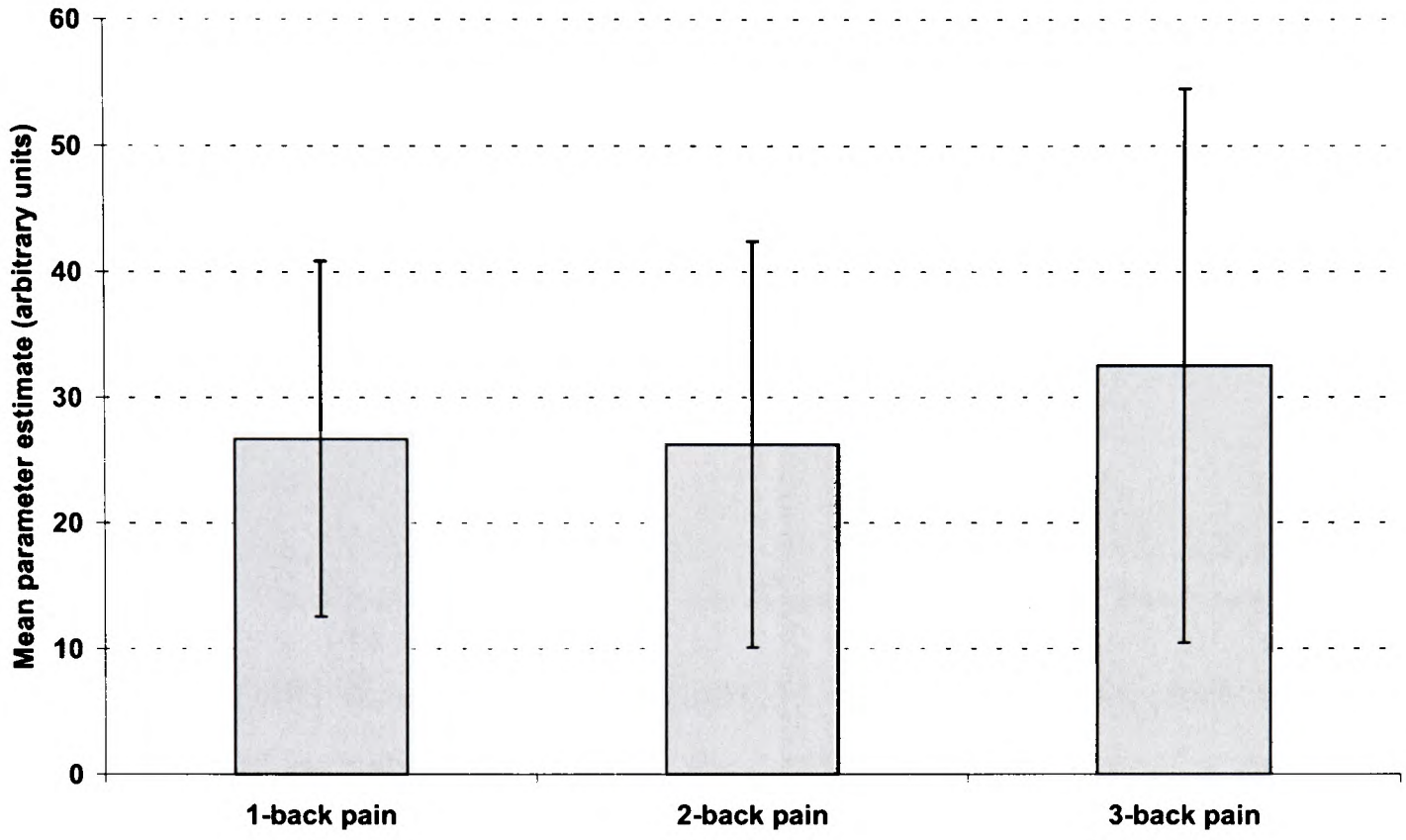
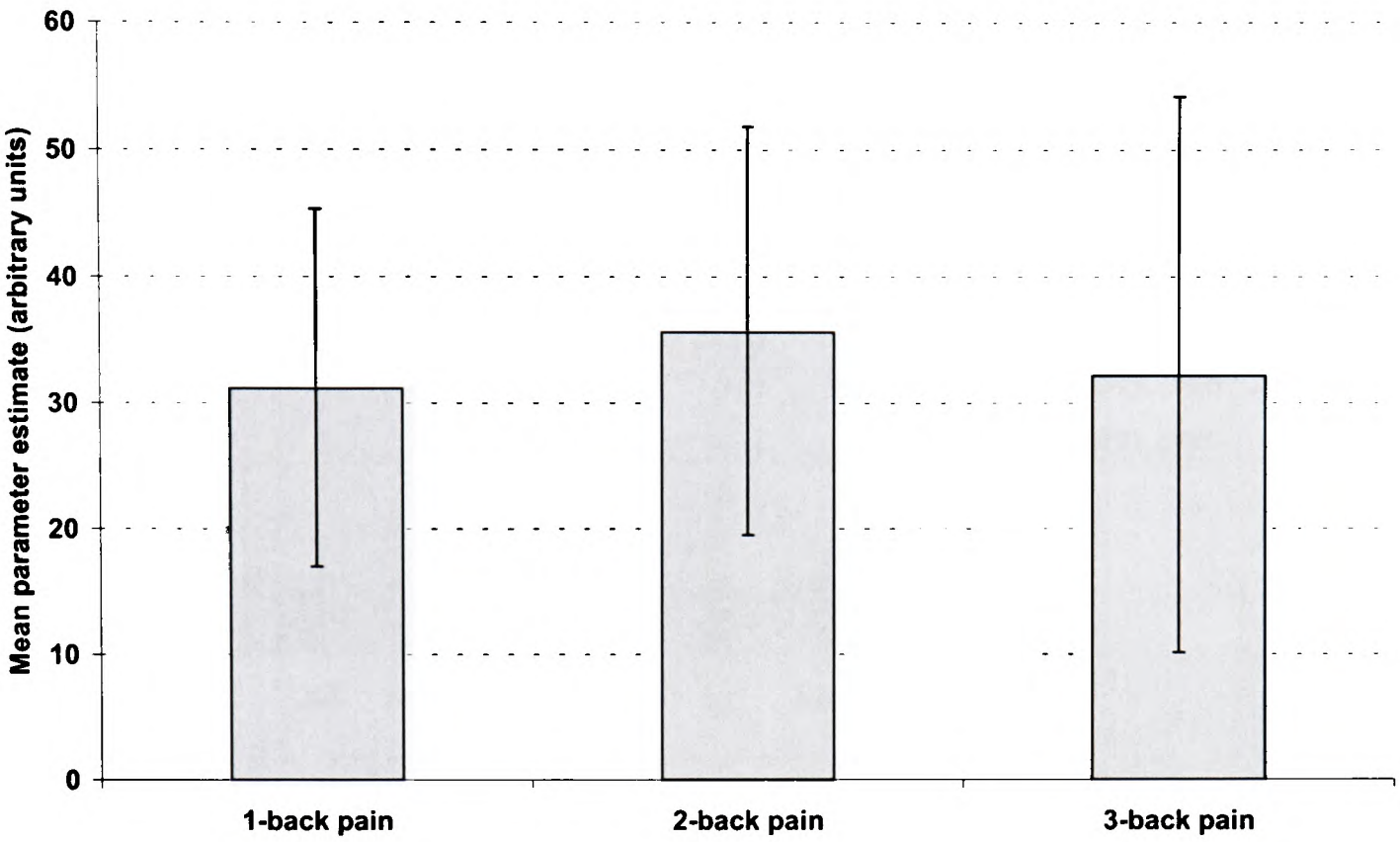
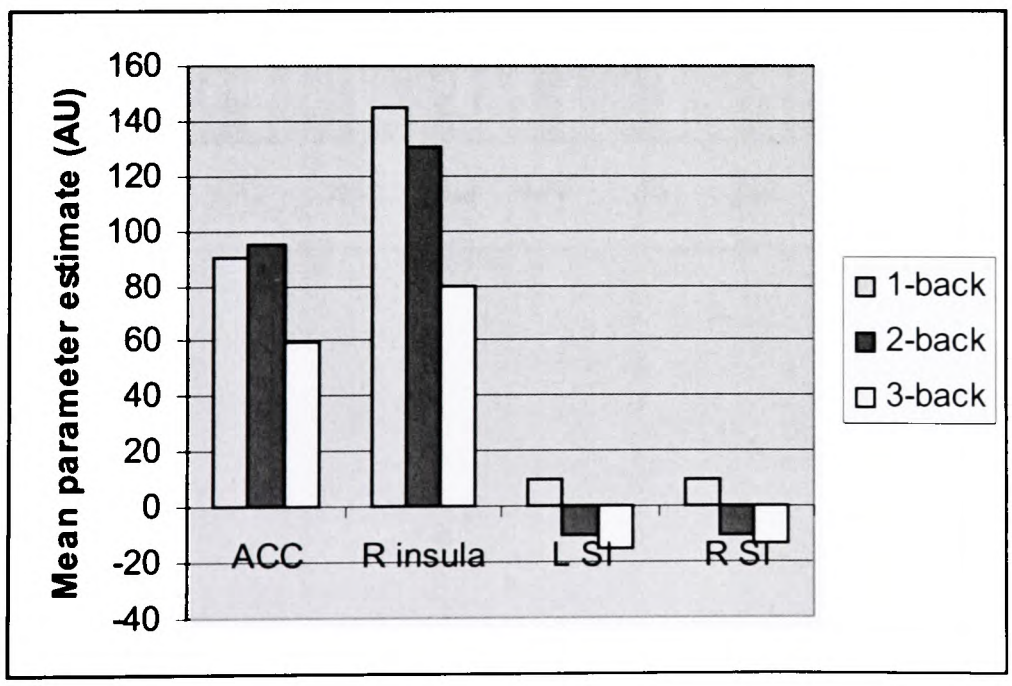


Fig. 4.14 Graph of group mean parameter estimates by n-back task block for right Thalamus



When fMRI data (parameter estimates) were analysed for each individual subject, it could be seen that three of the eight subjects did show a decrease in activation across the n-back task level in some key pain processing areas. The graph below (Fig. 4.15) illustrates data from subject 'CC' that did show a decrease in fMRI signal with increasing n-back level difficulty.

Fig. 4.15 Mean parameter estimates for subject CC by region and n-back task level



The following graphs show data for all subjects showing fMRI signal changes by region and by level of n-back task.

Fig. 4.16 Mean parameter estimates for each subject in the ACC by n-back level

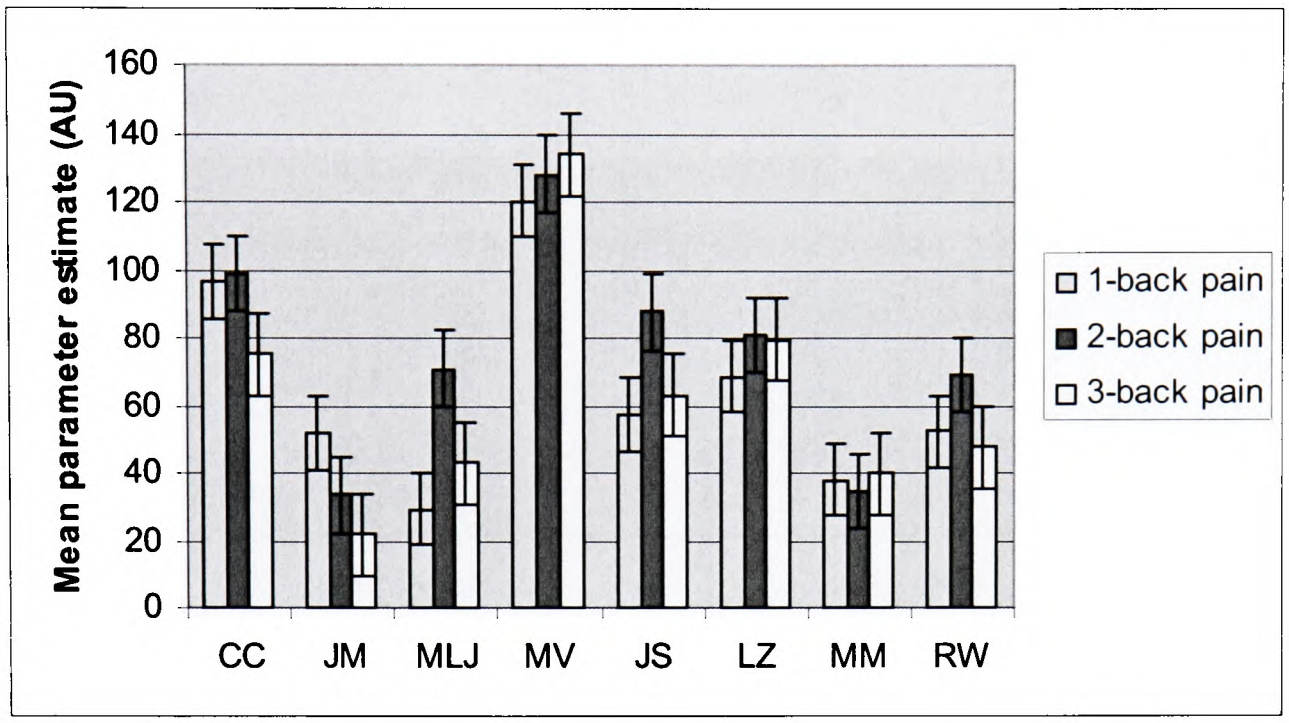


Fig. 4.17Mean parameter estimates for each subject in the left insula by n-back level

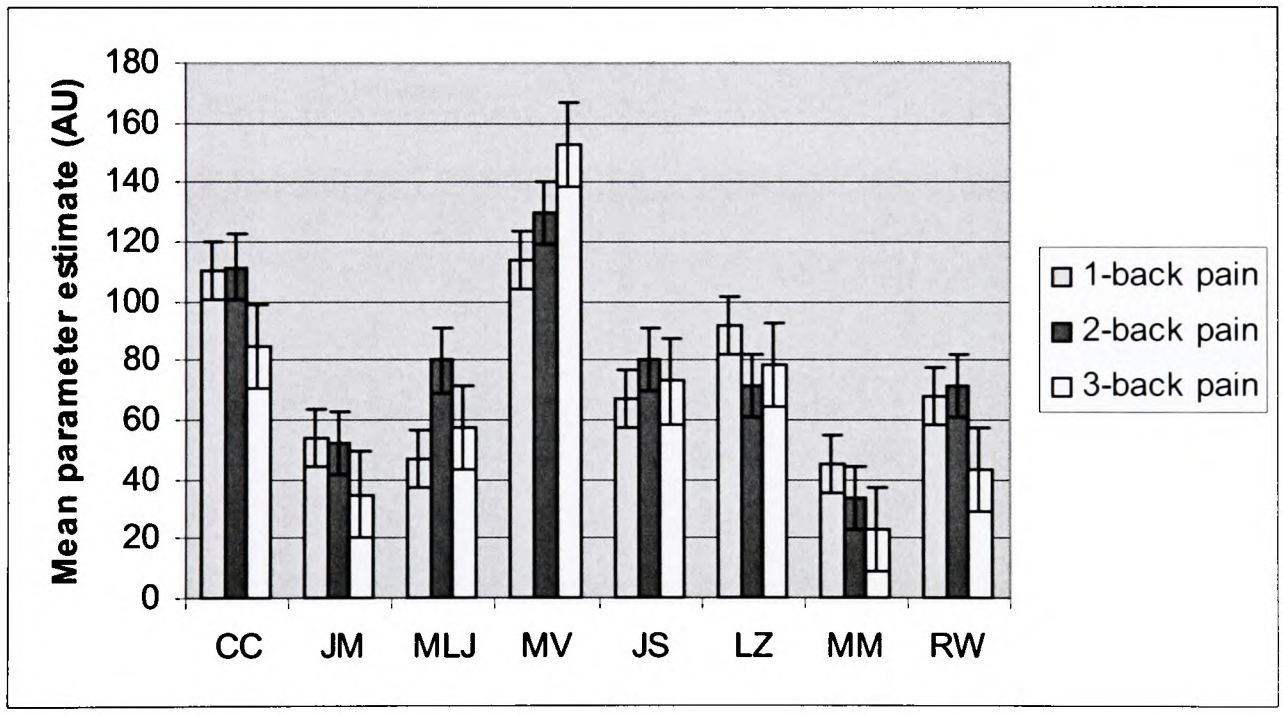


Fig. 4.18 Mean parameter estimates for each subject in the right insula by n-back level

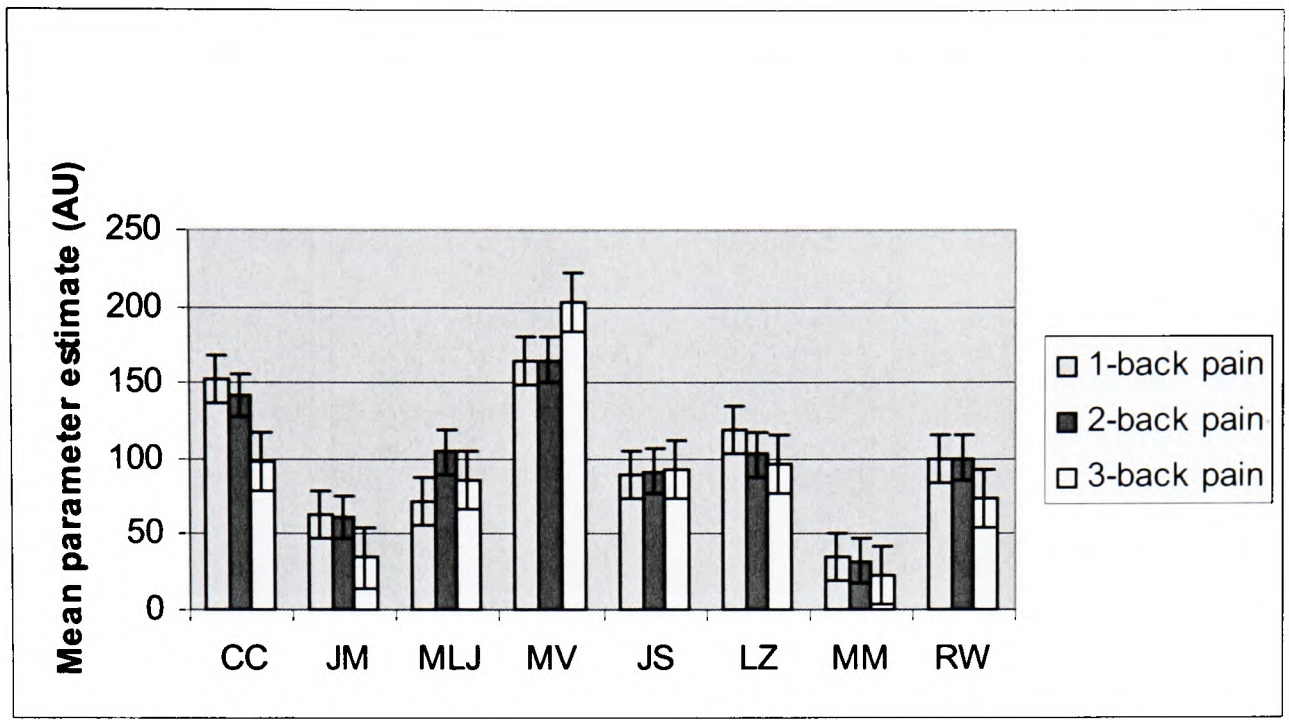


Fig. 4.19 Mean parameter estimates by subject and by n-back level in the right SI

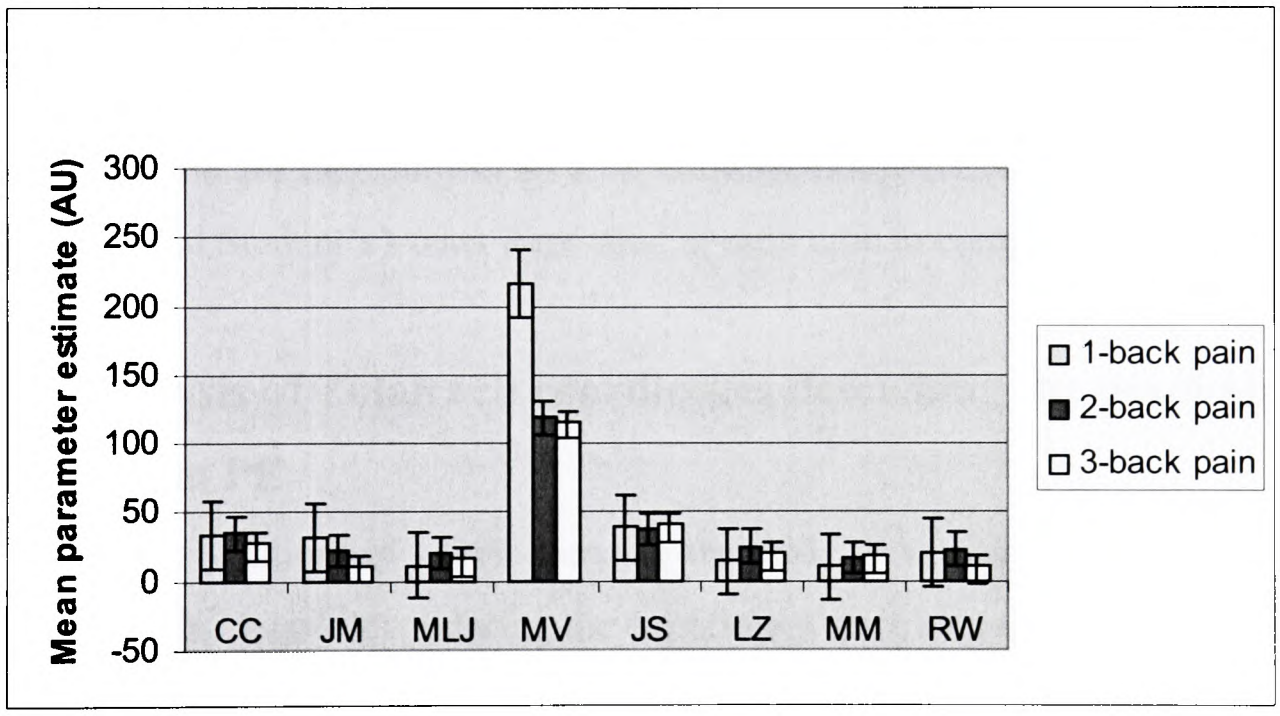
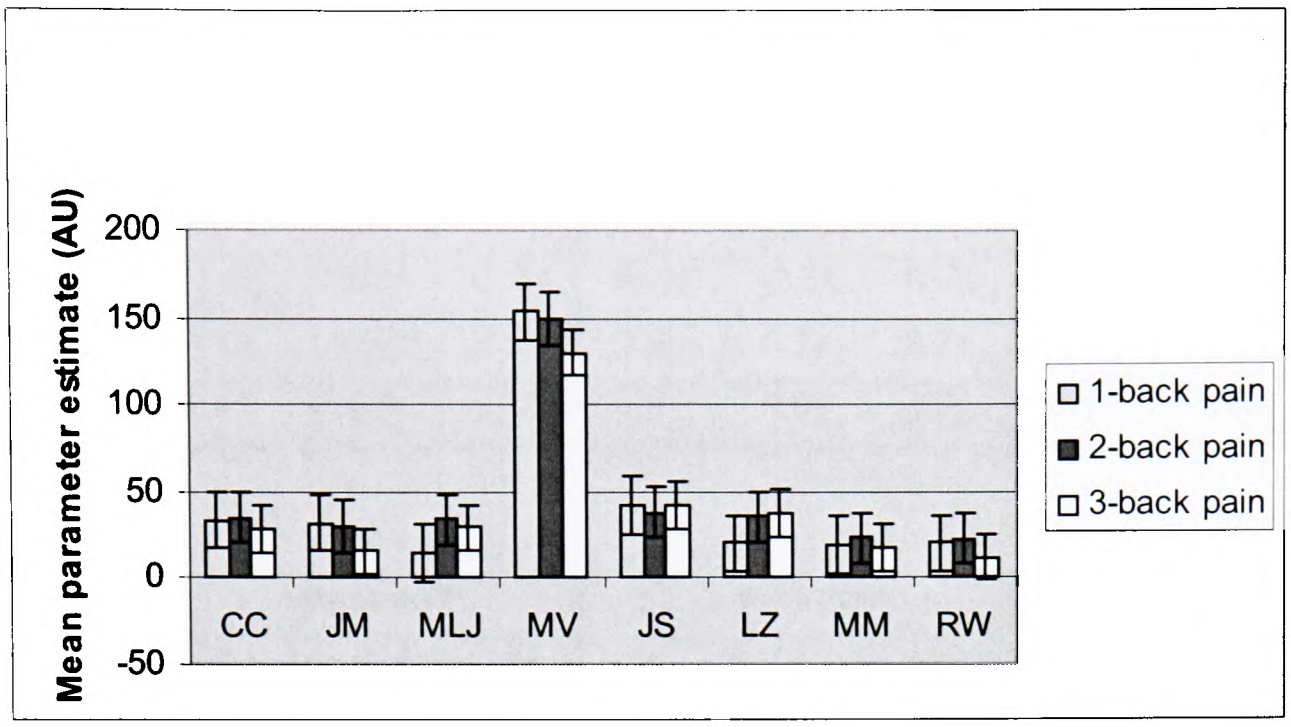


Fig. 4.20 Mean parameter estimates for each subject by n-back level for the left SI



The fMRI data was then analysed to establish whether or not the location of the peak PE resulting from pain activity in the chosen regions of interest altered with the n-back level.

The x y and z Talairach coordinates (measured in mm) at the location of maximum PE for each subject by area and condition were recorded (Table 4.3). One-tailed paired Student's t-tests were used in each case to compare the means.

4.6 Analysis of Talairach coordinates describing the voxel of maximum PE

The locations of voxels showing the peak PE values for each region of interest for each n-back task level during the experiment were averaged across subjects. These values show great consistency across task condition, showing that the location of maximum signal does not alter noticeably by n-back task level. The results of this analysis are tabulated below (table 4.3).

Table 4.3 Mean Talairach coordinates describing the location of the voxels showing the maximum PE value in each condition of the n-back task by anatomical region of interest.

Anterior Cingulate Cortex

	1-back pain			2-back pain			3-back pain		
	x	Y	z	x	y	z	x	y	z
mean	31.63	33.88	12.13	32.13	32.88	12.75	30.88	33.88	12.63
SD	1.19	6.83	2.53	2.30	5.33	1.75	1.25	5.46	2.50
SE	0.42	2.42	0.90	0.81	1.88	0.62	0.44	1.93	0.89

Left Insula

	1-back pain			2-back pain			3-back pain		
	x	Y	z	x	y	z	x	y	z
Mean	11.08	14.37	5.18	11.74	13.36	5.04	10.85	13.76	5.34
SD	17.80	17.03	6.07	17.67	16.99	6.70	17.34	17.51	6.36
SE	6.29	6.02	2.14	6.25	6.01	2.37	6.13	6.19	2.25

Right Insula

	1-back pain			2-back pain			3-back pain		
	x	Y	z	x	y	z	x	y	z
Mean	11.72	12.48	4.47	11.89	12.12	4.70	11.44	12.49	4.65
SD	5.78	5.75	2.06	5.71	5.59	2.19	5.63	5.77	2.14
SE	2.04	2.03	0.73	2.02	1.98	0.77	1.99	2.04	0.76

Left SI

	1-back pain			2-back pain			3-back pain		
	x	Y	z	x	y	z	x	y	z
Mean	6.52	6.75	2.42	6.54	6.56	2.55	6.35	6.76	2.52
SD	4.88	5.29	1.89	4.98	5.14	1.99	4.77	5.30	1.97
SE	1.73	1.87	0.67	1.76	1.82	0.70	1.69	1.87	0.70

Right SI

	1-back pain			2-back pain			3-back pain		
	x	Y	z	x	y	z	x	y	z
Mean	4.37	4.64	1.66	4.43	4.51	1.75	4.27	4.64	1.73
SD	2.43	2.50	0.90	2.44	2.44	0.95	2.37	2.51	0.93
SE	0.86	0.89	0.32	0.86	0.86	0.34	0.84	0.89	0.33

Left Thalamus

	1-back pain			2-back pain			3-back pain		
	x	Y	z	x	y	z	x	y	z
Mean	2.56	2.68	0.96	2.58	2.60	1.01	2.49	2.68	1.00
SD	1.76	1.88	0.67	1.79	1.83	0.71	1.72	1.88	0.70
SE	0.62	0.67	0.24	0.63	0.65	0.25	0.61	0.67	0.25

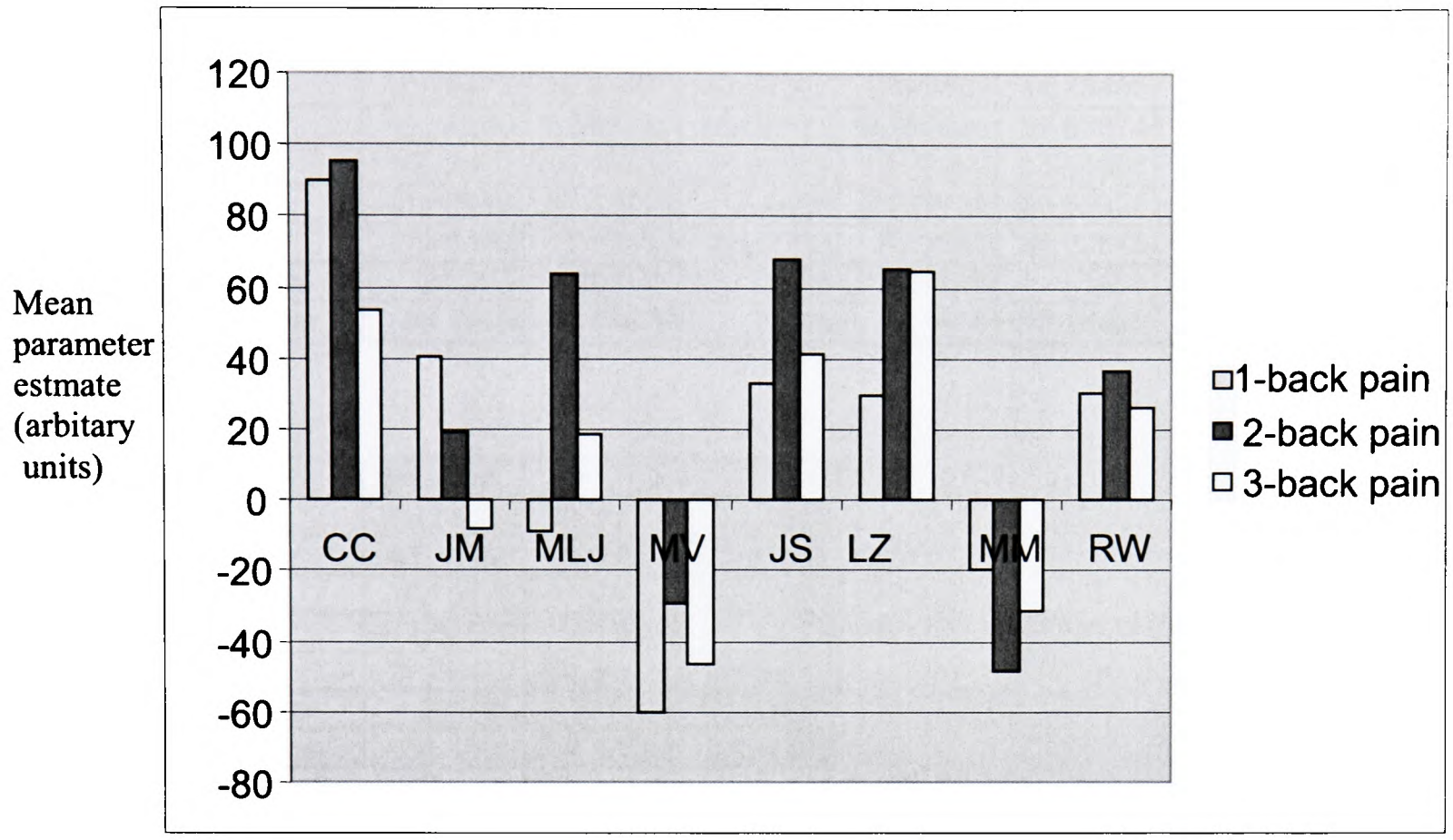
Right Thalamus

	1-back pain			2-back pain			3-back pain		
	x	Y	z	x	y	z	x	y	z
Mean	1.65	1.74	0.62	1.67	1.69	0.66	1.61	1.74	0.65
SD	0.97	1.01	0.36	0.98	0.98	0.38	0.95	1.01	0.38
SE	0.34	0.36	0.13	0.35	0.35	0.14	0.34	0.36	0.13

4.7 Analysis of percentage change from baseline

There are some subjects who produced 'outlier' data. The mean signal values obtained from analysis of their fMRI data do not fit in magnitude with the rest of the group. This can be illustrated by looking at an example set of mean data for a brain region (see fig. 4.21). This has a considerable impact upon the statistics applied to this data, and therefore it was decided to make the data more proportional to each other for greater validity of comparison.

Fig. 4.21 mean parameter estimates by subject for the anterior cingulate region in the three n-back task levels. Observe that there is an outlier (CC) and considerable variation in the data set.



In order to address this problem, the mean PE values for each subject (all regions) for the 2-back and 3-back tasks were compared to the 1-back values taking 1-back as the baseline. The calculations and raw data used to prepare Fig. 4.22 are found in Table 4.4.

Table 4.4 Raw data used for Fig. 4.22

Group mean Parameter estimates (AU) - Raw Data for Graph

	1-back pain		2-back pain		3-back pain	
Region	mean	SE	mean	SE	mean	SE
ACC	16.73479	16.03527	33.64387	17.84183	14.78465	14.16773
L.Insula	49.34369	7.396281	55.09721	9.895981	37.87674	10.38521
R. Insula	16.73479	14.15223	16.03527	13.98892	33.64387	13.68279
L. SI	-41.52519	20.13718	-16.4408	9.799737	-35.59325	17.82284
R. SI	27.60442	13.15612	12.52793	8.73583	34.92545	12.16064
L. Thalamus	26.725	14.16071	26.25	16.16848	32.5	22.05108
R. Thalamus	31.1925	14.19976	35.66	18.1556	32.16925	21.98284

Region	*Baseline*	*2*	*3*
ACC	16.73479	101.0415	-11.6532
L.Insula	49.34369	11.66008	-23.23895
R. Insula	16.73479	-4.180044	101.0415
L. SI	-41.52519	60.5	14.29
R. SI	27.60442	-54.61624	26.5212
L. Thalamus	26.725	-1.777362	21.60898
R. Thalamus	31.1925	14.32235	3.131362

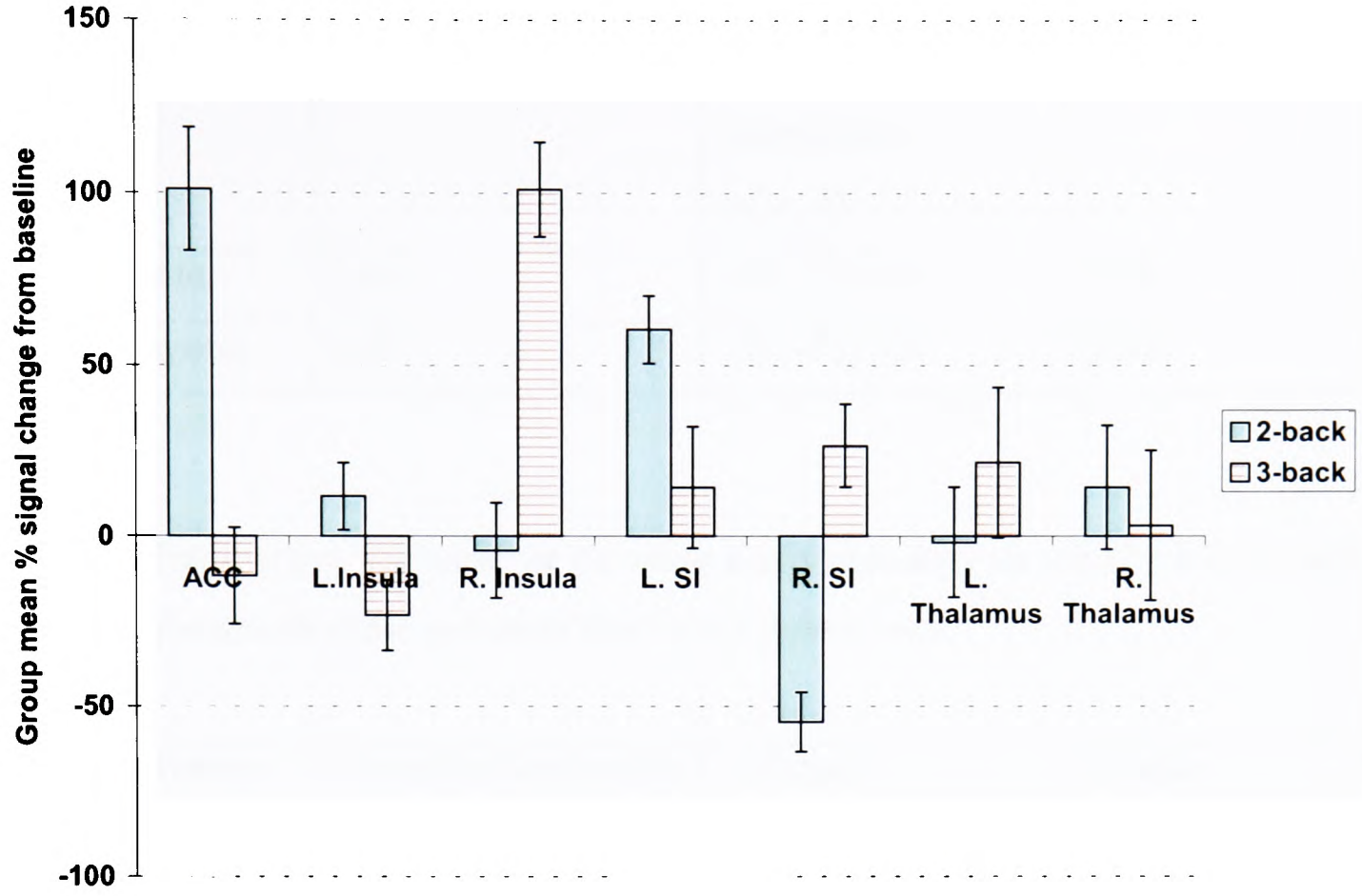
Formulae for calculations

2-back column (2-back - 1-back)/1-back x 100

3-back column (3-back - 2-back)/1-back x 100

A graph summarising the results obtained from this "normalisation of fMRI data" can be seen in Fig. 4.22

Fig. 4.22 Graph to show mean percentage change in pain activation in the 2-back pain and 3-back pain relative to baseline (1-back pain) by brain region



The significant result (p<0.05) in the above graph are as follows:

Rise in L and fall in R SI from 1-back to the 2-back task. The rise in signal in R.SI from 1 to the 3-back task was also significant.

4.8 Central activation during the fMRI experiment

The Talairach coordinates of the areas activated during the n-back task are consistent with other research data in this area. The following tables give the Talairach coordinates for the n-back task activation. This data is not discussed further in any detail. The data in this experiment were consistent with the existing n-back literature (see Chapter 3).

Table 4.5 Talairach coordinates of main areas activated during the n-back task. This table documents those activated during the 1-back task.

Area of activation	Laterality/hemisphere	Talairach coordinates (x, y, z)	Z value
BA4, premotor	Left	-30, -20, 44	2.30
BA 1,2 premotor	Left	-38. -26, 56	3.30

Table 4.6 Talairach coordinates of the main areas activated during the n-back task. This table documents those activated during the 3-back task.

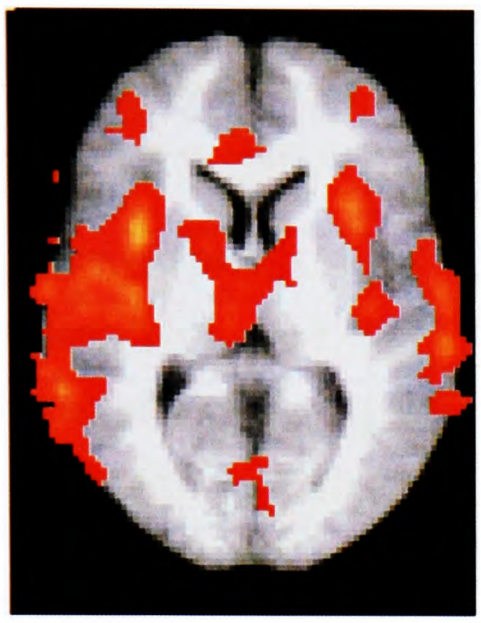
Area of activation	Laterality/hemisphere	Talairach coordinates (x, y, z)	Z value
Cerebellum	Left	-48, -60, -40	4.58
Midline cerebellum	Midline	0, -82, -30	2.04
BA 21/37	Right	60, -52, 6	2.50
BA 39	Left	-36, -52, 24	2.42
BA40	Right	56, -52, 24	2.70
BA 40	Left	-52, -54, 44	4.02
BA4 premotor	Left	-36, -20, 62	2.50

Again, to show that pain activation was consistent with the previous data, a table of pain coordinates (this table derived from the pain in the 3-back task) is shown by Table 4.7.

Table 4.7 Talairach coordinates of the central pain areas activated during the 3-back task.

Area of activation	Laterality/hemisphere	Talairach coordinates (x, y, z)	Z value
Cerebellum	Left	-36, -62, -36	3.30
Cerebellum	Right	48, 10, -16	3.03
BA11/prefrontal cortex	Bilateral	48, 10, -16 -48, 10, -16	3.03 2.98
Cerebellum	Midline	2, -72, -16	2.90
BA21/37	Right	2, -72, -16	2.90
Insula	Bilateral	34, 12, 2 -34, 10, 2	6.70 7.90
BA 10/46 orbitofrontal cortex	Bilateral	42, 46, 6 -34, 52, 6	2.12 2.30
ACC	Midline	4, 38, 6	3.01
Putamen	Bilateral	14, -2, 6 -14, 2, 6	3.28 2.09
ACC (BA31)	Midline	4, -30, 26	4.40
Thalamus	Bilateral	10, -20, 0 -10, -20, 0	3.80 3.20
BA40	Bilateral	58, -40, 24 -56, -30, 24	6.20 2.55
ACC (24/32)	Midline	2, 8, 44	2.50
BA7 (Precuneus)	Bilateral	2, -72, 44 -6, -50, 56	2.20 3.70
BA6 Premotor	Right	14, 6, 68	4.04

Fig. 4.24 Image to illustrate areas of activation during the 1-back pain stimuli
This illustration shows activation in bilateral orbitofrontal cortex, ACC, bilateral insula, thalamus, BA21/37, and posterior ACC



4.9 Results- summary

Pain intensity (Fig. 4.1)

There was a significant decrease in the level of perceived pain intensity with an increase in task difficulty from the 1-back to the 2-back pain. There was a non significant rise in the level of perceived pain intensity from the 2 to the 3-back task (increasing task difficulty from 2 to 3-back). The fall in perceived pain intensity from the 1 to the 3-back task was significant.

Percentage accuracy (Fig. 4.2)

It is noticeable here that the standard error of the group mean is very small. The error rates do not vary much between individual subjects. The percentage accuracy did not fall below 70% which reflects a good engagement in the n-back task by the subjects.

There is a non significant decrease in percentage accuracy between the 1 and the 2-back tasks and a significant fall in accuracy between the 2 and 3-back tasks. The drop in percentage accuracy observed in the 3-back task compared with the 1-back task is also significant.

Pain and % accuracy

There is no significant correlation between % accuracy and perceived pain intensity (Fig. 4.4). However, the following table (Table 4.8) illustrates the trends that exist within the behavioural data for pain and percentage accuracy.

Table 4.8 Behavioural data trends

	1 vs 2 back	2 vs 3 back	3 vs 1 back
Perceived pain intensity	↓ Significant	↑ NS	↑ Significant
% accuracy	↓ NS	↓ Significant	↑ Significant

The significant fall in perceived pain intensity from the 3 to the 1-back tasks (opposite ends of the difficulty spectrum from easiest to most challenging level) is associated with a significant decrease in % accuracy from the 3 to the 1-back task.

There is a trend decrease in accuracy from the 1-back to the 2-back task which in turn is associated with a significant reduction in perceived pain intensity.

Imaging data- Brain region signal changes and the change in perceived pain intensity

Table 4.9 Table of brain regions with pain intensity and accuracy by n-back level

	1 vs 2 back	2 vs 3 back	3 vs 1 back
Pain	↓ Significant	↑ NS	↑ Significant
% accuracy	↓ NS	↓ Significant	↑ Significant
ACC	↑ NS	↓ Significant	↑ NS
Ipsilateral insula (L)	↑ NS	↓ Significant	↑ NS
Contralateral insula (R)	↓ NS	↑ Significant	↓ NS
Ipsilateral SI (L)	↑ Significant	↓ Significant	↓ NS
Contralateral SI (R)	↓ Significant	↑ Significant	↓NS
Ipsilateral thalamus	↓ NS	↑ NS	↓ NS
Contralateral thalamus	↑ NS	↓ NS	↓ NS

4.10 Discussion

Background and selection of task

The experiment built upon the results of former work (Chapter 2) that confirmed the ability of the Counting Stroop to reduce subjects' perceived pain intensity ratings. The current study used a parametric working memory task (the N-back task) as a distraction task with three increasing levels of difficulty (1-back, 2-back and 3-back). Noxious thermal stimuli were administered during all three levels of the n-back task and pain intensity ratings were obtained from subjects during the experiment.

Studies have shown a parametric signal increase in key brain regions involved in working memory (i.e. the prefrontal cortex) with increasing levels of difficulty (and therefore working memory load) of the n-back task (Braver et al., 1997). Braver et al (1997) found a linear relationship between fMRI signal in the dorsolateral and left inferior regions of the PFC and working memory load. They used four levels of the letter n-back task (see Chapter 3), 0-back, 1-back, 2-back and 3-back. There was a

significant increase in reaction time with increased task difficulty from the 0-back task, which served as a control as it demanded no working memory processes, to the 3-back task. When memory load was plotted against percentage increase in fMRI signal in the dorsolateral PFC and BA 44/5, a significant positive correlation with working memory load was revealed. This study reinforces the validity of employing the n-back task as a means of producing parametric increases in working memory load with increasing task difficulty levels. The n-back task is used here to produce parametric increases in the level of distraction from painful thermal stimuli with increasing n-back task level. It is not employed as a working memory task per se.

The fMRI results from this study confirm that the central regions activated by the n-back task are broadly consistent with previous working memory imaging literature. The areas of activation include premotor areas, cerebellum, and prefrontal cortex.

Ability of the n-back task to produce graded increase in task difficulty (fall in % accuracy)

Behavioural data acquired during the fMRI experiment are also consistent with the results of Braver *et al.* (Braver et al., 1997). As a measure of task difficulty, percentage accuracy achieved in the three n-back task levels was used. This is calculated by finding the percentage of targets identified by the subject out of the total available targets in that block. Percentage accuracy rates (Fig. 4.2) were generally acceptable (>60%) and provide confirmation that the subjects understood the task and were engaging in it with a sufficient degree of attention. The percentage accuracy levels fell significantly ($p< 0.01$, Student's 1-tailed paired T test) from the 3-back to the 2-back task and from the 3-back to the 1-back task. A fall in accuracy was also observed between the 2-back and the 1-back task ($p < 0.05$). This shows a trend in reduction in percentage accuracy from the 1-back to the 3-back task and therefore it is likely that the task difficulty shows an increase from the 1-back to the 3-back task accordingly using this measure of accuracy. A correlation analysis between % accuracy and pain perception (Fig. 4.4) did not reach significance.

The data obtained from this study confirm the ability of the n-back task to produce graded increases in task difficulty. Taking percentage accuracy as a measure of task difficulty, it was found that task difficulty decreased significantly from the 1 to

the 3-back tasks as reflected in the significant fall in accuracy from the 1-back to 2-back to 3-back tasks.

Effect of the n-back task on pain intensity ratings

The n-back task was used to distract subjects from thermal painful stimuli. When the perceived pain intensity ratings (Fig. 4.1) are considered, the results show a significant fall in perceived pain intensity from the 1-back to the 2-back and from the 1-back to the 3-back tasks, although there is a slight increase in pain intensity from the 2 to the 3-back task which did not reach statistical significance (summary Fig 4.8). The reason for this slight increase in perceived pain intensity from the 2 to the 3 back tasks is not clear.

When pain intensity and increase in task difficulty (decreased percentage accuracy) are related to one another, the key finding is that the significant decease in pain intensity seen from the 1 to the 3-back task is associated with the significant increase in task difficulty from 1 to 3-back. This suggests that the increase in n-back task difficulty from the easiest (1-back) to the hardest (3-back) task increases the degree of distraction from the thermal painful stimuli by a sufficient extent to produce a reduction in subjects' reported pain intensity. There is also a significant decrease in pain intensity from 1 to 2-back which is accompanied by a significant increase in task difficulty in the same case.

However, the desired gradual diminution in perceived pain intensity with increasing task difficulty was not seen. Clear and significant pain modulation was only seen from the 1 to 2-back and 1 to 3-back but not in between the 2 and 3-back tasks. This might suggest that using the n-back task does produce a gradual increase in working memory cognitive load but that this is not reflected in a graded decrease in perceived pain intensity ratings. The n-back task appears from these initial results not to be suited to producing a parametric modulation of pain intensity. Larger subject numbers of potentially even a greater number of increments of increasing task difficulty could produce different results but from this initial study it would be unwise to over interpret the research findings.

Pain ratings were recorded on line to avoid reliance on subjects' memories of pain intensity. However changes in pain perception due to attentional modulation can

be small (see Chapter 2) and so this experiment might warrant repeating with a larger cohort of subjects to see if the same results are obtained.

The fact that pain intensity increase from the 2-ack to the 3-back task (non significant) could be due to the fact that from the 1 to the 2-back task there was an increased task difficulty by subjects were still sufficiently engaged in the task (not too difficult) as to be distracted and report lower pain intensities. The 3-back task is considerably more difficult to perform and subjects sometimes made mistakes and stopped and then restarted the task later on. This interrupts the continuity of the distraction task and might mean that pain intensity was not modulated as much as if they were constantly distracted by taking part in the 2-back task. Frustration may also come into play as the 3- back task is so difficult to aster. Subjects all had practice sessions to familiarise themselves prior to the fMRI study session.

The n-back task does not appear from this data to be the best task to produce a parametric decrease in pain intensity, despite its ability to produce graded increases in task difficulty across the 3 different n-back levels (Fig. 4.2). The n –back task was chosen because previous studies (see Chapter 3) and bench work (Chapter 3) illustrated its ability to produce a graded increase in task difficulty. There was fairly large inter subject variation which is another reason to support the repetition of the study with a larger group of subjects. It would also be interesting repeat the study without obtaining any pain ratings. This would minimise signal changes associated with decision making as to the pain ratings as well as motion artefact confounds form the data set thereby allowing comparisons to be made purely on signal changes in key pain matrix areas between n-back levels.

In a previous study (Chapter 2) subjects distracted from pain using a cognitive task reported a reduction in pain scores (Bantick et al., 2002). The key regions showing significant reduction in signal (similar analysis to that carried out on the imaging data in this study) were the ACC, insula and thalamus. Within these regions, the most significant reductions in signal were observed in the ACC followed by the contralateral insula, contralateral thalamus and ipsilateral thalamus. Decreased activation in the 3-back task relative to the 1-back task was observed in the ACC, contralateral thalamus, and contralateral insula. The n-back task did not produce graded reductions in pain intensity with increased task difficulty although it was able to produce a reduction in pain intensity scores from the 1 to the 2 back and from the 1 to the 3 back tasks.

The next task was to ascertain whether or not this behavioural effect of pain modulation with increasing task difficulty corresponded to changes in fMRI signal in key areas of the pain matrix (see Chapter 2).

fMRI activation as a result of thermal stimuli in this study

During the experiment, the pain activation seen in the fMRI data was consistent with our former work and with the current literature. Areas of activation include the cerebellum, anterior cingulate cortex, prefrontal cortex, insula, thalamus, putamen, area 40, and some premotor areas (Talairach coordinates given earlier in the report). This provides evidence that the n-back task was not producing atypical pain activation. From the key areas of the pain processing matrix discussed here, regions of interest were selected. The regions chosen for this quantification of the fMRI data were the thalamus, insula, ACC, and SI. Such analysis involves looking at the mean fMRI signal values (mean parameter estimates) for each region and for each n-back condition to ascertain if any particular region or regions exhibits a fall in fMRI signal which correlates with the behavioural observation of reduced perceived pain intensity whilst subjects were distracted to an increasing extent with the increasing n-back difficulty from the 1-back to the 3-back task.

The mean location (Talairach coordinates) of the voxel exhibiting the maximum PE in the three n-back conditions by region of interest remained fairly consistent across n-back condition (Table 4.3).

fMRI signal in key pain processing areas by n-back task level

When the fMRI signal values (mean PE values) are averaged to provide mean values for each region by n-back task level, the following observations can be made. In order to make valid comparisons across subjects and take account of inter-subject variation, the mean signal scores were standardised by recording the percentage change in the 3-back condition PEs compared to the 1-back condition (Fig. 4.22). Thus any percentage increase or decrease in signal in the 3-back block PEs relative to the 1-back block PEs was recorded as a percentage change. Such comparisons are backed up by the data for group mean signal in all 3 conditions (depicted by n-back task level rather than as a percentage change from baseline).

Change in signal from 1 to 2 back task (Table 4.9)

There is a significant reduction in perceived pain intensity from the 1-back to the 2-back task accompanied by a decrease in percentage accuracy (and therefore an increase in task difficulty). There are four pain processing regions which demonstrate increased signal from the 1 to the 2-back task (Table 4.9), the ACC, the ipsilateral insula, the ipsilateral SI and the contralateral thalamus. If the regions mentioned above, which showed increased activation from the 1 to the 2-back task, are increasingly engaged by the increased task difficulty then their ability to process pain intensity at the same time would potentially be reduced thus leading to the observed reduction in reported pain intensity.

The ipsilateral thalamus showed a non significant decrease in signal from the 1 to the 2-back task. The aforementioned regions exhibited increased signal, which could represent a manifestation of increased cognitive load with increased task difficulty. However, the contralateral thalamus did not behave in the same fashion, showing a rise in signal from the 1 to the 2-back task. Thalamic activation can be difficult to interpret, and larger subject cohorts may reduce the effect of poor signal to noise in this region. The medial thalamic nuclei are often activated by noxious stimulation (Tracey et al., 2000) and have a role in the processing of painful information. A reduction in activity in the thalamic region would be consistent with a reduced perception of pain intensity as found in this experiment with increased task difficulty. There was a reduction in activation in the ipsilateral thalamus between the 1 and 2-back tasks. The thalamic data might be slightly prone to noise, since the subjects do not all perform the tasks to the same degree of accuracy. There is some degree of inter subject variation, although none of the subjects performs any level of the experiment to such a degree of inaccuracy that one could argue that they are not sufficiently engaged in the task

Moving on to discuss the ACC, this region is involved with the affective-motivational component of pain (Rainville et al., 1997). Increased activation here as a result of increased involvement with the distracting task might impair the ability of the region to simultaneously process affective pain intensity. Since the ACC processes both cognitive and affective pain related information it might be that the effect of the n-back task on pain related signal might be different in the affective and cognitive divisions of this region. The mask used was fairly wide and encompassed both functional regions thus diluting any differential effect in the cognitive and

affective processing regions of the ACC. IT would be interesting to use the literature to create two different masks for the two regions and repeat the analysis to ascertain any difference in behaviour in the two areas of the ACC. The division of the ACC is discussed in more detail in Chapter 2 of this Thesis.

The ipsilateral insula and ipsilateral SII as well as the contralateral thalamus showed a decrease in signal from the 1 to the 2-back tasks. The only region for which this change reached significance was the ipsilateral SI. The contralateral SI showed a fall in activation from the 1 to the 2-back task. This supports Bushnell's findings (Bushnell *et al.* 1999). However, activation in the contralateral SI region did not fall from the 2-back to the 3-back task. This is reflected in the behavioural data. The pain ratings did not decrease from the 2 to the 3-back task.

Change in signal from the 2-back to the 3-back task (Fig. 4.9)

The pain intensity ratings did not show a significant fall from the 2 to the 3-back task, despite an increase in task difficulty reflected in a decrease in percentage accuracy. This study did not clearly demonstrate a reduction in pain intensity with increase in task difficulty from the 2-back to the 3-back task. In fact, the pain intensity showed a slight increase (non significant) from the 2 to the 3-back task. One possible explanation of this could be that the 3 back task is so difficult to perform (despite subjects achieving satisfactory accuracy rates exceeding 60%) that the ability of the task to distract volunteers from the thermal pain stimuli is interrupted periodically as they abort the particular train of the n-back task and resume later because they have got "lost" or become confused. If this happens then subjects will stop doing the task briefly, and restart again. This might therefore result in a reduced ability of the 3-back task to distract subjects away from thermal painful stimuli relative to the 2-back task. Some subjects may become very frustrated by the 3-back task which may also increase their perception of the pain stimuli as they find that the stimuli interfere with their ability to take part in the task. Neither of these suggested explanations has been empirically proven, however.

In terms of mean parameter estimate changes from the 2 to the 3-back task, there were significant falls in signal in the ACC, ipsilateral insula and ipsilateral SI. It is hard to interpret these in the light of the non-significant increase in pain ratings between these two conditions. There may have been insufficient difference in task difficulty between the two conditions to produce sufficiently greater cognitive

distraction to produce a reduction in perceived pain intensity and a corresponding modulation in central pain processing areas.

The contralateral insula, contralateral SI and ipsilateral thalamus showed an increase in mean parameter estimates from the 2 to the 3-back task. The contralateral SI is implicated in the processing of painful stimuli as is the contralateral insula. However, further results would be needed on a larger subject pool in order to be able to accurately ascertain the relationship between the imaging and behavioural data for this particular comparison.

Change in signal from 3 back to 1-back task (Fig.4.9)

In terms of the behavioural data, the reported mean pain intensity ratings were significantly higher in the 1-back task than in the 3-back task. This was accompanied by significantly higher percentage accuracy (a reflection of a significantly lower task difficulty) in the 1-back task compared with the 3-back task. The n-back task used in this context was able to produce a significant reduction in perceived pain intensity from the least to the most complex n-back task level.

A lower parameter estimate in the 3-back task compared with the 1-back task was observed in the following regions: ACC and the ipsilateral insula. A higher mean parameter estimate in the 3-back task compared with the 1-back task was observed in the following regions: contralateral insula, bilateral SI, bilateral thalamus. None of the comparisons for change in mean parameter estimate between the 3-back and the 1-back task reached statistical significance in this study.

By some mechanism, possibly related to involvement with the processing of an increasingly difficult distraction task, the contralateral insula, bilateral SI and bilateral thalamus showed an increase in activation in the 3-back versus the 1-back task. This corresponded with a significant fall in pain perception. The modulation of pain perception could therefore have been mediated by altering the load involved in processing the more complicated 3-back task compared with the 1-back task. The thalamus, insula and SI areas are key areas in the processing of painful stimuli and so if they are unable to process the stimuli as effectively due to their engagement in a distraction task then it could be predicted that the sensation of pain could therefore be reduced. It is not yet clear why there is a difference in behaviour in terms of change in mean parameter estimate between the 1 and 3-back tasks between the ipsilateral and contralateral thalamus.

The SI and SII regions are involved in pain processing in humans (Timmermann et al., 2001) and both the contralateral SI and bilateral SII have been shown to be significantly positively correlated with sensory stimulus intensity. The relationship between the SI and stimulus intensity is exponential, and very closely reflects subjects' pain ratings. This suggests that a main role of the SI region is in the discriminatory processing of pain intensity. Bushnell *et al.* (Bushnell et al., 1999) report a focus in SI activation in the contralateral SI. The SI region is a prime candidate for modulation by cognitive manipulation and distraction (Bushnell et al., 1999). Such modulation of SI activation by cognitive paradigms occurs where the paradigm alters the perceived pain intensity and not the unpleasantness alone. The SI area is activated by a variety of noxious stimuli including capsaicin and is involved in the discriminatory processing of noxious stimuli.

The areas of the pain matrix demonstrating a fall in activation from the 1 to the 3-back task were the ACC and the ipsilateral insula. As mentioned previously, there are no obvious reasons to explain the different behaviour pattern of the contralateral and ipsilateral insula in this case and caution should be exercised in over interpreting this finding. The insular cortex is involved in the intensity encoding of painful stimuli (Peyron et al., 1999). There are connections between the insula and the emotional processing centres in the brain which could suggest a role in the emotional aspect of pain processing. There is a highly significant correlation between activation in the contralateral insula (middle/posterior) and stimulus intensity (Craig et al., 2000), suggesting the region is involved in the contralateral cortical representation of discriminative thermal sensation. The largest correlation found in Craig *et al.*'s study (Craig et al., 2000) between reported pain intensity ratings and fMRI signal was with the contralateral anterior insula. Although the correlation between the stimulus intensity in ipsilateral and contralateral insula and stimulus intensity were not significantly different from each other, the authors comment that the correlation with pain ratings was significantly stronger in the contralateral anterior insula than in the ipsilateral insula.

Since the ipsilateral and contralateral insula behave in opposite ways caution is advised in interpreting the implications of these findings. Craig et al found a positive correlation between activity in the ipsilateral insula and temperature of thermal stimuli (Craig et al., 2000) which supports the role of this region in the processing of pain intensity information as it pertains to thermal noxious stimuli.

The fall in activation in the ACC is more difficult to interpret. This is because there are two functional areas within this region (see Chapter 2), one primarily processing affective information and one processing cognitive information. The masks used in this study to investigate signal changes in this region would have encompassed both regions. In order to accurately discern changes in the ACC with task level in this paradigm the mask would need to be refined so that both the cognitive and affective regions could be analysed separately. The ACC is likely to be involved with the processing of the N-back task as well as the processing of pain intensity information.

It is worth mentioning here the distinction between pain intensity and affective unpleasantness. The sensory discriminative component of the pain sensation involves the evaluation of the pain intensity. Processing of information pertaining to intensity information or sensory-discriminative aspects of pain is believed to take place in the somatosensory cortex with information travelling in the spinothalamocortical pathway terminating in this region (Davis *et al.* 1997). The lateral pain system (including SI and SII) is involved primarily with the processing and integration of information pertaining to the spatial discrimination and intensity aspects of noxious stimulation (Ingvar *et al.* 1999). Activity in the primary somatosensory cortex has been shown to correlate with the intensity of a painful thermal stimulus (Ingvar *et al.* 1999). Pain intensity therefore is a sensory discriminative component of the overall pain experience and is linked to the amplitude or strength of the stimulus. The ACC may also have some involvement in the processing of pain intensity (Ingvar *et al.* 1999). Pain intensity is simpler to measure psychophysically and increasing stimulus intensities of for example thermal stimuli produce robust and reproducible response curves using visual analogue scales.

The affective unpleasantness component of the pain sensation is generally associated with the medial pain system including the thalamus and limbic areas involved with pain processing. Pain unpleasantness, though integral to the sensation of pain, is harder to measure objectively. Scales using various words to describe the emotional quality of pain can be used and subjects asked to chose the one that best describes the quality of the pain. This is not so reproducible or easy to carry out and differs with the type of stimulus used and with context. Experimental pain is by its nature escapable, not linked to the progress of a disease process, and of limited duration and tends not to have the huge negative emotional quality of long-term

clinical pain. The unpleasantness of pain is linked also to its meaning and therefore with the cognitive-evaluative component of pain. The unpleasant nature of pain is associated with negative affect or emotion and a strong desire to escape or reduce the pain. Psychophysical studies have shown that although both unpleasantness of affect and intensity or sensory information contribute to the overall experience of pain they are also two distinct dimensions with different relationships to nociceptive stimuli. Longer stimuli are often associated with higher ratings of unpleasantness than shorter ones where the ratings of intensity and unpleasantness are similar. Price (2000) suggests psychological or cognitive interventions may differentially affect the two components of the pain experience. The affective component may be modulated more by psychological factors such as distraction than the intensity component where processing occurs at an earlier stage within the nervous system. The ACC (part of which is strongly involved in the processing of affective information relating to the pain experience) is readily modulated by cognitive distraction paradigms suggesting that it may be the affective element of pain which should be the focus of such psychological modulation therapies.

Unlike other sensory modalities such as vision, pain is by its very definition, unpleasant at its threshold. A very bright light could be painful, or a very hot object. Pain can be triggered by various sensory modalities, and is a protective mechanism since it indicates actual or potential tissue damage to the individual. Unpleasantness is the subjective correlate of a drive to escape and avoid or limit tissue damage. It is also intended in the case of an injury to drive the individual to rest or immobilise the injured part in order that recovery and recuperation can take place. It is the unpleasantness that is often most significant in the clinical arena to the patient. The negative affective property of pain places a motivational demand to escape upon the individual. The relationship of unpleasantness to intensity is still not entirely resolved (Fields 1999).

The dichotomy between sensory-discriminative and affective-motivational is possibly over simplistic although it has become fairly widely accepted within pain research. This is despite a relative lack of research into the accuracy of the hypothesis (Fields 1999).

There is still debate as to the merit of the distinction between the intensity and affective components of the pain experience. Fields (1999) terms it an artificial separation and a "roadblock to understanding". The neural circuits mediating

unpleasantness are partially separate from those involved in pain intensity processing (Fields 1999). There is no specific neural process sub-serving the two different aspects of sensation and no totally independent processing pathways responsible for their processing. Fields (1999) suggests that unpleasantness and intensity (termed here "algosity") are two distinct intensity-based sensory discriminations and that any attempt to pigeonhole unpleasantness as sensation or affect is invalid.

4.11 Conclusion

This study shows that the n-back parametric working memory paradigm was unable to produce a graded modulation of perceived pain intensity. There was a significant reduction in pain intensity from the 1 back to the 2-back and from the 1-back to the 3-back although from the 2 to the 3-back task there was a slight but non significant increase in pain intensity. Although the n-back task successfully replicated experiments documented in the literature by producing a graded increase in task difficulty (as seen by a reduction in percentage accuracy from 1 back to 3-back), this did not correlate with a graded decrease in pain intensity ratings.

From quantification analysis of the individual pain matrix regions, there is no clear pattern of modulation between regions. Further analysis is indicated with a larger subject cohort in order to investigate this further. The experiment does back up a previous study (Chapter 2) in that cognitive distraction tasks can produce a reduction in perceived pain intensity. The desired parametric decrease in pain intensity with a graded increase in task difficulty was not produced in this study.

The pain processing area which showed a pattern of activity in terms of mean parameter estimate which followed the behavioural pain intensity ratings reported was the contralateral SI in which a significant drop in mean parameter estimate value was found from the 1-back to the 2-back task and then a significant increase from the 2 to the 3-back task. This is the same pattern as seen in the behavioural results. A reduction in activation in the contralateral SI in the 2-back task compared with the 1-back task would suggest a reduced perception of intensity and this is what is seen in the behavioural results with lower pain intensities being reported in the 2-back compared with the 1-back task. The significant rise in pain intensity between the 2-back and 3-back tasks is accompanied by an increase in mean parameter estimate score in the contralateral SI.

These results are still early findings and merit more extensive study. For example, it would be interesting to devise a mask to separate the cognitive from the affective regions of the ACC and also a posterior cingulate mask to ascertain the pattern of activity with different n-back level in these regions.

The data should not be over analysed, but provide a basis upon which to develop further cognitive modulation paradigms. The various trends in the data were discussed in the previous section but an overall trend was not established here.

4.12 References

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Chapter 5: Investigating the central neuronal processing of itch using FMRI

So far this thesis has focused upon the induction, central processing and cognitive modulation of thermal pain. There are some similarities between itch and pain, yet compared with the range of pharmacological approaches available to treat pain, there remains a lack of specific systemic antipruritic medications. Antihistamines can be taken orally or applied topically to relieve itch where it is mediated by histamine and the sedative antihistamines can be of use to patients suffering from severe itch because they have been shown to reduce itch as well as aiding with sleep. This set of itch experiments aims to apply the results from pain research to the problem of itch modulation via counter stimulation or with cognitive modulation. There are two main challenges to the advance of research into itch: the development of a reliable itch stimulus, and the development of an appropriate imaging paradigm with which to further examine its neuronal correlates.

Itch sensation has been documented since ancient times. As early as 450 BC, Herodotus, a writer of travel guides in ancient Greece, described the considerable efforts made by the Egyptian people to avoid the itchiness caused by mosquito bites.

Itch and pruritic skin conditions represent a major clinical problem and the central mechanisms involved are not entirely understood. Itch is also a highly unpleasant side-effect of opiate analgesic medications. It is therefore not a purely academic search to elucidate the neural correlates of itch, since it is of major importance for patients who suffer from itch which is resistant to treatment; the latter being a common sequel of atopic dermatitis, liver disease, and chronic renal disease.

5.0 Introduction

Hafenreffer (1660) wrote the first documented medical definition of itch, defining it as, ".an unpleasant sensation with the urge to scratch". Much research has been carried out in the field since then and thus the definition is in need of refinement. Itch is the single most significant subjective symptom of dermatological disease. It is also a distressing side-effect of various pathologies and systemic drugs (Thomas et al., 1993). Work continues towards unravelling the neural basis of itch so that it can be alleviated within the clinical arena.

Various researchers have investigated the peripheral pathways involved in the processing of itch (Shelley and Arthur, 1957; Tausk et al., 1993). The receptor population implicated is a sub-group of chemosensitive unmyelinated C-fibres. The reader is referred to the following research works (Carstens, 1997; Forster et al., 1995; Handwerker et al., 1991; Schmelz et al., 1997; Schmidt et al., 1995) which discuss current and future work in this area, which falls outside the remit of this thesis. Limited research so far exists into the central processing of pruritic stimuli, and it is upon this work that this experimental research work is based.

5.1 Some theoretical explanations of the processing of the itch sensation

The reader is also referred to Tuckett 1982 and Schmelz 2001 for further information. The specificity theory suggests that there are a group of primary afferent nerve fibres that respond specifically to pruritic stimuli. Activation of such fibres results in the sensation of itch. Such fibres would be amongst the small diameter fibres including some unmyelinated afferents. Specificity theory requires the existence of a 'labelled line' within the central nervous system dedicated to the processing of pruritogenic stimuli. Experimental evidence to date seems to indicate that only a few spinal neurons are dedicated to the processing of pruritogenic stimuli. Further studies of a human microneurographic nature are required to clarify this further (Mc Mahon and Kolzenberg 1992).

A small percentage of polymodal nociceptive C fibre receptors respond to pruritic stimuli such as histamine. This response is fairly weak and does not match the prolonged itch sensation caused by histamine stimulation seen behaviourally. Schmelz et al (1997) have uncovered histamine sensitive receptors that are supplied by C fibres. Itch sensation is not lost by peripheral nerve compression until C fibre conduction is also impaired (Magerl 1990).

These primary afferent neurons appear to be itch specific, and insensitive to mechanical stimuli (Schmelz et al 1997). Such neurons respond to histamine in a dose dependent fashion with a time course similar to that of the itch sensation and have a low conduction velocity and are unresponsive to mechanical stimuli. They do not show spontaneous activity (suggesting tonic inhibition by spontaneously active wide dynamic range or nociceptor cells) and project preferentially to the lateral

thalamic nuclei. All of these characteristics render these histamine sensitive 'itch' neurons distinct from traditional nociceptors. This suggests that they form a separate system for itch processing (Schmelz 2001, Andrew and Craig 2001).

Histamine sensitive afferents are also activated by painful stimuli such as capsaicin injection. Injection of capsaicin causes pain and suppresses itch via the coactivation of nociceptors mediating painful sensations. It is not contradictory for secondary neurons to be itch specific despite their primary afferents also responding to capsaicin to produce pain sensations. All this suggests is that selective activation of a particular neuronal pathway is a prerequisite in order to produce itch rather than pain for example. The systems pertaining to the processing of itch and pain interact in a dynamic fashion in order to determine the emerging sensation. The specificity theory of itch gains further credence from the fact that a group of secondary afferents exist which show typical histamine responses. These might be likely targets for antipruritic therapy in the future, yet they require further detailed investigation.

Another such finding (Andrew and Craig 2001) was that these histamine responsive neurons were initially insensitive to mechanical stimuli. However, when histamine was administered prior to brush or pinprick stimuli, such stimuli evoked itch (Atanassoff et al 1999).

This finding, that there are two separate channels for processing itch and pain has crucial relevance to pruritic conditions in the clinical arena. Painful stimuli can abolish itch and painful chemical or electrical stimulation can reduce histamine induced itch for hours (Nillson et al 1997, Brull et al 1999). Histamine induced itch can be enhanced and prolonged if the pain of injection is abolished by lidocaine (Atanassoff et al 1999).

Pruritic stimuli cause activation of one sub-population of central neurons and noxious stimuli another. It is suggested within this framework that noxious stimuli cause specific inhibition of the populations activated by the pruritic stimulus.

5.2 Conduction of the itch sensation to the central nervous system

The projection of the itch and pain sensations to the central nervous system from their induction in the periphery is via the spinothalamic tract in both cases and both sensations appear to display somatotopic organisation within the somatosensory

cortex (Kolzenburg et al 1993). The exact nature of the central processing of itch is not entirely known (Mc Mahon and Kolzenburg 1992).

5.3 Central processing of itch

So far, little is known about the central and supraspinal processing of itch in humans. Animals react with a stereotypical behavioural repertoire when challenged with pruritic or painful stimuli, making it hard to develop animal itch models of itch.

Itch, like pain, is a complex and multidimensional subjective experience, which is also imbued with a negative emotional valence.

Although there has been a recent explosion in the number of studies using imaging techniques (PET, FMRI) to study the central processing of pain (Davis, 2000), this has not yet been paralleled in the field of itch research. To date only three such studies have been carried out, and much still remains to be learnt about the functional areas implicated. By contrast to the wide spectrum of analgesic medication available, there are no specific drugs to target itch, and research into the central areas involved in itch could hasten development in this area.

It was formerly believed that itch was merely a subliminal form of pain, rather than a sensation in its own right. It is now known that itch and pain are two distinct sensations, but they do interact and share some common features with one another. Functional imaging studies of pain and itch and the relationship between them will ultimately help increase our level of understanding of both phenomena.

Itch and pain do share some features (Teofoli et al., 1996). Both can be induced by the administration of noxious chemical stimuli (Greaves and Wall, 1996; McMahon and Kolzenburg, 1992), both result in attentional arousal, both have a negative affective aspect, and both lead to the initiation of motor responses (Drzezga et al., 2001b). Neither itch nor pain is a one-dimensional experience but comprises quantitative (intensity), qualitative (unpleasantness), and motor (withdrawal, scratch) components.

However, some fundamental differences between itch and pain are evident. Whilst morphine effectively inhibits pain, it causes itch in humans and scratch responses in animals (Thomas et al., 1993). Itch can be inhibited by the counter administration of painful stimuli, such as noxious heat and vibration (Nilsson et al., 1997; Ward et al., 1996). The motor output components of itch and pain are different.

Itch results in a scratch reflex (to remove any particles from the skin), and pain leads to a withdrawal response. The autonomic reactions to pain, such as tachycardia are observed to a much lesser extent, if at all, in itch.

Of the three existing studies carried out using imaging techniques (PET) to elucidate the central processing of itch, the following broad findings can be seen. The ACC is found to be activated in all three studies (Darsow et al., 2000b; Drzezga et al., 2001b; Hsieh et al., 1994a). Hsieh *et al.* (Hsieh et al., 1994a) linked this activity with the sensory-affective component of itch. Drzezga's study (Drzezga et al., 2001b) showed a correlation between increased histamine concentration and increased ACC activation. The ACC is activated by painful stimuli (Peyron et al., 2000) and cognitively demanding paradigms (Davis et al., 2000; Nakamura et al., 2000; Turken and Swick, 1999). Activation in the prefrontal cortex was found to be correlated with the unpleasantness ratings of the itch sensation (Drzezga et al., 2001b). The orbitofrontal cortex has been found to activate in response to unpleasant stimuli (Lotze et al., 2001) in some pain studies, and was also found to show activation in an experimental itch imaging study (Darsow et al., 1996).

Motor and premotor areas are activated in response to administration of histamine and pruritic stimuli. An early study found ipsilateral activation within motor areas concerned with intention to act and motor programming, such as the SMA and premotor cortex (Hsieh et al., 1994a). This contrasts with activation found in a pain imaging study by Coghill *et al.* which was predominantly contralateral in nature (Coghill et al., 1994a). The same study also found activation in areas of the motor association cortex, cerebellum and ipsilateral red nucleus. However, the two most recent studies have found activation associated with the motor system (motor intention and planning of movement) to be contralateral- reflecting the intention to use the opposite limb to scratch with. A study by Drzezga (Drzezga et al., 2001b) found activity in the contralateral motor areas to correlate with the degree of unpleasantness of the itch sensation. In all studies, subjects are asked not to scratch the site of administration to histamine, even though it is itchy and they feel the urge to scratch it. Scratching would produce huge movement artefact errors in the images acquired. By contrast, recent studies indicate that particular regions of activation within the motor system include the ipsilateral primary motor cortex which represents the limb stimulated, the contralateral SMA and contralateral motor cortex, together with some ipsilateral hemisphere premotor activation (Darsow et al., 2000b; Drzezga

et al., 2001b). However, there are methodological differences in the method of histamine concentration between the first and two subsequent studies mentioned here. Darsow *et al.*'s (Darsow et al., 2000b) study did reveal bilateral motor area activation although it was predominantly contralateral. It was revealed in the subtraction analysis between saline and histamine activation, showing it to be involved with itch processing although activity in the contralateral motor cortex (premotor cortex, supplementary motor area) was also found to correlate with itch intensity and histamine concentration.

Significant activation was found in the contralateral ACC (BA 24) which is thought to represent the unpleasant emotional nature of itch and its processing. The same area is believed to process affective information pertaining to painful stimuli (Peyron et al., 2000). A subsequent study (Darsow et al., 2000b) found ipsilateral activation of the ACC to be correlated with the redness (wheal) resulting from histamine prick test administration. The most recent imaging study in fact found a highly significant correlation between ratings of itch unpleasantness and ipsilateral ACC activation (Drzezga et al., 2001b). The ACC was found in a subtraction analysis with saline to be involved with the processing of itch. A non-significant correlation between itch intensity and activation in the posterior cingulate was also found in this study. Activation of the contralateral ACC (BA24) is proposed as the correlate of the unpleasant affective nature of itch (Hsieh et al., 1994a), and has been found to be almost solely contralateral in nature.

In terms of somatosensory cortical activation, the earliest imaging study (Hsieh et al., 1994a) failed to show any activation in the primary or secondary somatosensory cortices. Drzezga *et al.* (Drzezga et al., 2001b) found activation in the contralateral somatosensory cortex representing the right limb. This was correlated with intensity of itch and histamine concentration. The same study found that there was a strong link between activity in the contralateral somatosensory cortex and the unpleasantness ratings of the itch sensation. Darsow's study also found bilateral somatosensory cortical activation which correlated with ratings of itch intensity (Darsow et al., 2000b).

Posterior insula activation was found contralaterally and was shown to correlate with itch unpleasantness ratings (Drzezga et al., 2001b) and in another study with itch intensity (Darsow et al., 2000b).

Activation correlated with itch unpleasantness was found in the contralateral dorsolateral prefrontal cortex (BA 9/10) (Darsow et al., 2000b; Drzezga et al., 2001b; Hsieh et al., 1994a) in subtraction analyses between histamine and saline control. Such analyses also revealed activation in the contralateral orbitofrontal cortex (Darsow et al., 2000b).

Within the inferior parietal cortex (BA 39/40), activation was found to correlate with histamine concentration (Darsow et al., 2000b; Drzezga et al., 2001b) and itch intensity (Drzezga et al., 2001b). Activity as a result of itch was also found in this brain region by Hsieh *et al.* (Hsieh et al., 1994a).

This study aims to further explore the functional areas involved in the perception of itch intensity using the higher resolution fMRI technique. Since itch and pain are separate sensations, we hypothesise that a map of areas activated by itch will not correspond exactly to those activated by painful stimuli. However, existing studies suggest that some areas of the brain which are activated by painful stimuli, and which correspond to the unpleasant nature of pain are also activated in itch (posterior part of the anterior cingulate). We are interested in isolating the central areas involved in the perception of pure itch and ultimately in the relationship between pain and itch processing (analogies and differences).

5.4 Itch and pain

For many years, itch was regarded merely as a subliminal form of pain rather than a sensation in its own right. The contention that itch is merely a subliminal form of pain is inaccurate. Rather it seems that the two sensations are distinct but interrelated.

One reason for initial suggestions of itch being a form of pain arose from the clinical literature which documented the abolition of itch sensations alongside those of pain when patients underwent cordotomies disrupting the lateral spinothalamic tract (Nathan et al 1990). Pruritic stimuli do induce weak activation in a subpopulation of polymodal C fibre nociceptors (Tucket et al 1987).

The evidence for itch and pain being dissociable into two entities comes from clinical evidence that the administration of opiates in humans decreases the pain felt but frequently causes itch as a side-effect (Hales 1980). Itch is also strongly inhibited by painful stimuli (Ward et al 1996).

Itch can be a consequence of opiate administration, being reported in 8.5% of opiate analgesia and 45.8% of intraspinal opioid analgesia cases (Ballantyne et al 1988). When opiates are injected into the intrathecal space they reach the medullary dorsal horn of the brain stem where there are many opiate receptors. Injection of morphine into the medullary dorsal horn of the brain stem causes long lasting intense and facially directed itching in monkeys and rats (Thomas and Hammond 1995). Systemically administered naloxone (opiate antagonist) relieves this itching acting at the medullary dorsal horn receptors. Systemic morphine also alleviates the itch by activation of endogenous antinociceptive systems.

Historically, Muller's theory of specific nerve energies did not consider itch as a separate sensory entity. Later, itch spots were described as localised areas where tactile stimulation could result in itching sensations. Initially, itch was thought to be caused by weak stimulation of pain spots whereby stronger stimulation of the same area would cause pain to be perceived. In 1943, Bishop (McMahon and Kolzenburg 1992) found that electrical stimulation of such sites caused itching to be felt.

In the 1950s studies were carried out looking more closely at 'itch spots' and they drew attention to the importance of chemical mediators in the generation of itch sensations. Some studies (Shelley and Arthur 1957) used spicules from the pruritogenic agent cowhage (mucuna pruriens), which contains a proteinase that causes itch. The study correlated histologically verified injection depths of itching powder spicules with subjects' reports of itch and it was found that the highest sensitivity to the spicules, and the greatest itch, corresponded to injection in the basal cell layer of the epidermis.

In pain research, models have been developed using tightly controlled experimental conditions and using a variety of stimulus modalities. By contrast, itch studies had been held in check because of the continuing development of an itch-inducing stimulus, which is both reliable and potent as well as producing a pure itch sensation.

It is not really possible to develop appropriate animal models, since most animals respond with the same or similar stereotypical behaviour repertoires whether challenged with noxious or pruritic stimuli. Humans, however, are able to dissociate between the two sensations of itch and pain on a reliable basis and thus the majority of evidence has been compiled from clinical and experimental studies on humans.

The best candidate for producing a pure sensation of itch to date is histamine since it is a natural mediator of itch. This is the most commonly used stimulus to date in the development of itch models. The sensation of itch usually starts 30-60 seconds after administration of histamine by iontophoresis and reaches a peak after 2-3 minutes. The itch sensation typically lasts approximately 10 minutes. Histamine cannot cross the horny outer skin barrier when it is unbroken, and therefore the main methods for administration involve the intracutaneous injection of a defined amount of histamine or a histamine releasing substance.

5.5 Induction of pruritic stimuli

Progress in the study of itch has therefore been impeded in the past by the difficulty of developing a reliable, potent itch-inducing stimulus. The skin has a horny outer epidermal layer which is relatively impervious to aqueous solutions on its surface. Previous studies have used intracutaneous injection of histamine (Hsieh et al., 1994a) which has been validated by other experimental models of itch (Hagermark, 1974). The main disadvantage of this approach is that it necessarily causes some pain and local inflammation thus potentially confounding the results of an itch experiment. This study used histamine prick tests (Pepys, 1975) administered to the foot to produce an itch sensation. This method has been validated experimentally and is used widely in dermatological clinics. This technique has been shown to produce a pure and pain free itch sensation (Drzezga et al., 2001b). This technique delivers histamine to the area of the skin (dermal-epidermal junction) where the greatest concentration of itch receptors is found (Drzezga et al., 2001b).

5.6 Histamine prick testing

As said above, one means of applying histamine is via the skin prick test, which is the method used in this study. This method is used in routine allergy testing (Malling 1993). It is not really possible to accurately quantify the amount of histamine delivered in this fashion however. The typical quantity penetrating the skin via this technique is approximately 10^{-6} ml (Pepys 1975). The depth of the skin prick test is such that it is able to deliver histamine to the superficial dermal-epidermal junction that contains the highest concentration of pruriceptive receptors. This

method minimizes contamination of the pure itch sensation by painful stimulation, such as that encountered via delivery through injection.

Prick tests are used in routine allergy testing in dermatological clinics. A single drop of the substance to be tested (in this case, histamine in an aqueous solution) is dropped onto the skin after cleansing it with an alcohol swab. A superficial puncture is made into the region of skin underneath each drop using a sterile prick test lancette. These are standardised and so penetrate a set distance through the horny outer layer of the skin and into the superficial layers of the skin. The histamine is deposited via this technique of application into the dermal - epidermal junction of the skin. The use of saline allows a control test to be carried out. Water is not used for this as it can cause pain when injected beneath the skin. Delivery of histamine via the prick test method does not cause pain other than a slight pricking feeling. Injection routes for the delivery of histamine were considered but they would result in pain and some local inflammatory reactions that would confuse the interpretation of results adding to the itch responses that we are focusing on in this study.

5.7 Iontophoretic Delivery of Histamine

The method of iontophoretic delivery of histamine is not discussed in any depth here. Initial pilot experiments in this laboratory to try to use iontophoresis in order to induce an itch sensation did not show the desired results. In our 3T environment, we were unable to generate a sufficiently intense stimulus to enable us to achieve an adequate signal to noise ratio. The main sensory observation was that the current delivering the histamine could be felt as an uncomfortable strong tingling sensation. Further experiments within this group may address this issue further.

Iontophoresis is a means of inducing ionic (charged) drug solutions to pass into the skin by use of small electric currents. When a low voltage is applied to a drug solution contained in a chamber the ions of the drug conduct electricity as they pass into the skin. This technique is non invasive, and it is possible to precisely control the doses delivered. The drug solution is contained in a reusable perspex chamber, attached to the skin using adhesive disks. The quantity of ions crossing into the skin depends on the current density and the amount of time the current flows but not upon the concentration of the drug in the chamber. The application of histamine via

iontophoresis has been used by Magerl et al (1990) to investigate dose related effects to histamine in both volunteers and patients.

This method enables precise quantification of the substance being administered and it is non invasive. However, some authors have found that it is not necessarily a pure itch sensation that results, since, especially at higher currents some sensation is perceived as the current is applied. This can serve as a centrally mediated inhibition of the itch via counter irritant inhibition that is induced by the iontophoretic current (Darsow et al 1996).

5.8 Experimental development of a pruritic stimulus

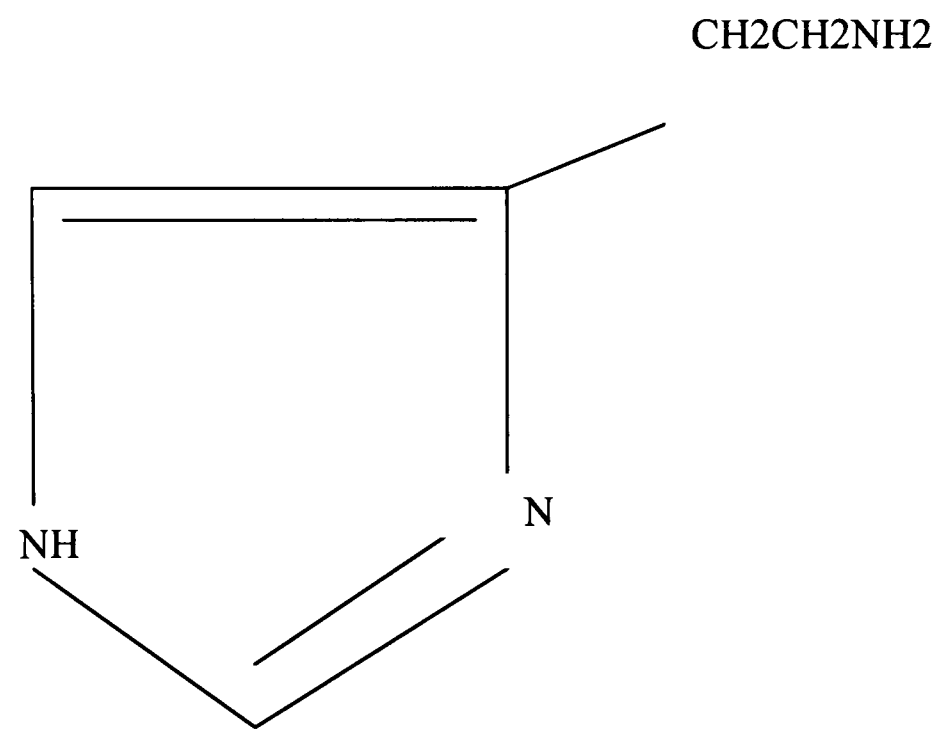
Prior to the study being undertaken by any one individual, thorough allergy screening is undertaken (see appendix) to avoid possible side effects such as anaphylaxis or severe allergic reactions. Subjects with a history of allergies, atopic eczema, asthma or other dematological disease were excluded form the study. Each subject participated 3 times in bench studies whereby histamine was administered via a prick test (see below) and the intensity of the itch was measured every 30 seconds for 12 minutes to mirror the study which would take place in the MRI scanning environment. Three sessions were used to ensure that a reliable and reproducible itch profile was obtained to correlate the behavioural data with the functional imaging data that would be collected later. The average profile of itch intensity for each subject over time was found from this data.

Sites for use of the prick test were looked into. The volar aspect of the forearm is traditionally favoured within the dermatological setting. This area is very hard to access in the MRI scanner and therefore the back of the hand was chosen. Sites were chosen near the thumb joint where there were minimal blood vessels and the skin was not too hairy.

Initially allergen extracts were going to be used as they were found to induce a greater intensity and duration of itch than that resulting from the administration of histamine using prick test techniques. However, they were disregarded since they resulted in an impure sensation, and different people react to different allergens and therefore it would be hard to control for different subjects receiving different allergenic substances. It was also considered more of a risk for anaphylaxis.

The histamine solution used contained 10 mg/ml of histamine dihydrochloride. The structure of histamine is shown in the diagram below

Fig. 5.0 Diagram of the structure of histamine



It can be verified that the histamine has penetrated the superficial layers of the skin since it produces an area of raised skin (wheal) and redness surrounding this (flare).

An itch scale was used with two anchors from 5 (no itch- itch threshold) to 10 (maximum itch). Using this scale bench work was carried out to ascertain the time course of the itch resulting from histamine prick testing. It was found that using a few drops of histamine and pricking the skin twice at one time elevated the level of itch and prolonged the time course sufficiently for our experimental purposes. The skin itches virtually immediately upon prick testing with histamine and the sensation builds with a peak at around 90-120 seconds. The skin remains itchy for an average of 8 minutes and then this sensation falls back towards baseline and most subjects did not report any itch after 12 minutes.

This data is consistent with other behavioural data obtained via histamine prick tests (i.e. Drzezga et al 2001), who found a latency of 25 s to elicit an itch sensation, reaching peak intensity at 120s after application. The duration of the itch

sensation is dose-dependent and ranges from 4-12 minutes. Prick testing is a standardized method to ensure a long-lasting, stable and intense itch sensation. It does not induce pain or any other sensations apart from pure itch (Darsow et al 1996). These ratings were obtained outside the scanner for a behavioural profile of the itch sensation that we were going to elicit using the same methodology within the scanner.

5.8.1 Behavioural experiments

Subjects were recruited and their written informed consent obtained. There is an outside risk of anaphylaxis during the administration of histamine. In accordance with our ethical approval for these studies, a medically qualified person is always present throughout. This person is familiar with the procedure in the case of anaphylaxis and with the location of all emergency contact procedures and where to locate the relevant emergency drugs.

A comprehensive screening is carried out on each subject, and this is checked with the medically qualified person. Subjects are asked about relevant medical history and allergies (see appendix for form used) and those with a history of severe allergy or asthma are excluded from the study.

Standardised histamine and saline skin prick tests were administered outside of the scanner environment in a quiet room (21°C). All skin testing was carried out in the same season to avoid variations in the thickness of the stratum corneum outer layer of the skin. The purpose of the saline prick tests was to verify that the administration of saline via this route did not elicit any sensations other than the initial prick sensation.

The histamine skin prick test was carried out so that the skin was pricked twice over the same drop of aqueous histamine solution. Subjects were asked to focus on the sensations they might feel in their hand and asked to rate the intensity of the itch every 30 seconds. For the purposes of this experiment a score of '5' denoted no itch and '6' was the itch threshold.

The scale used was as follows:-

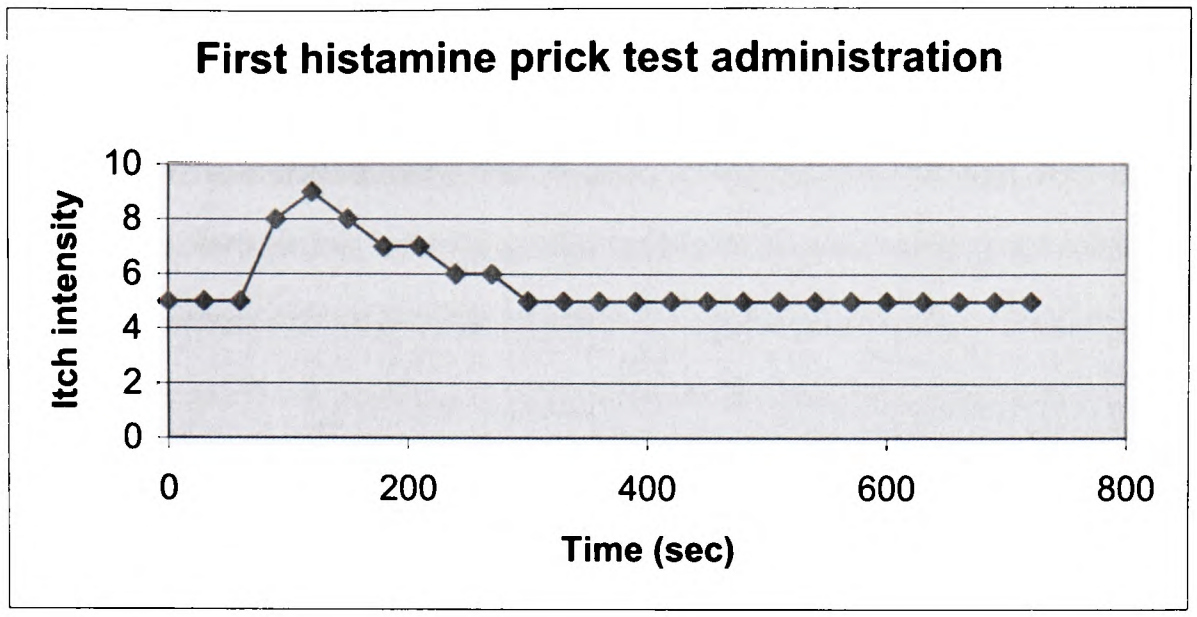
Itch Rating Scale

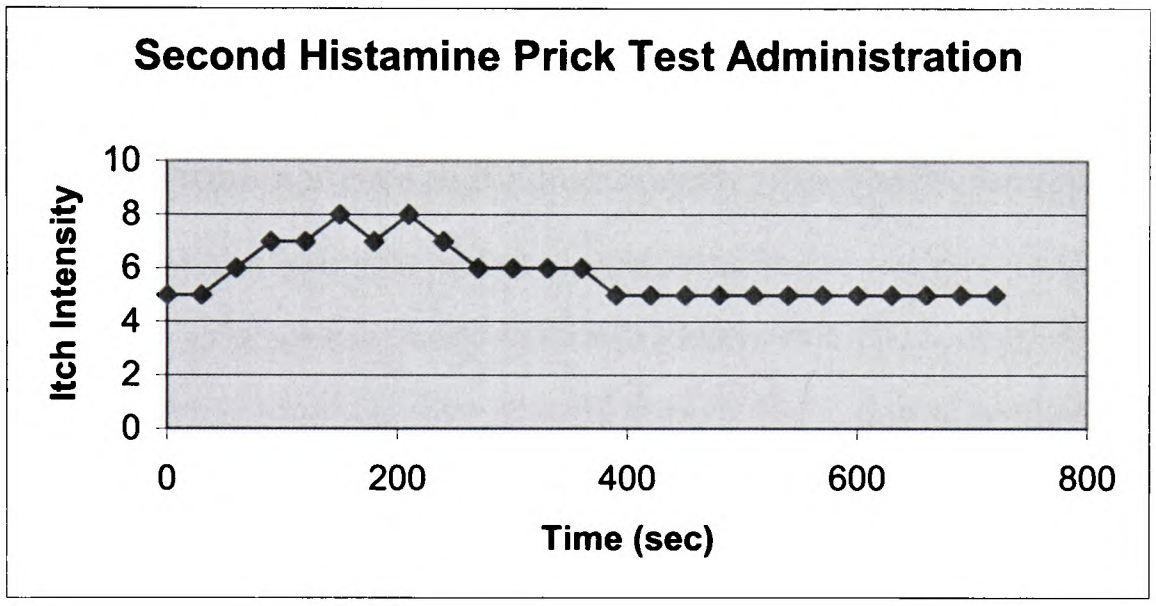
5	no itch
6	itch threshold
7	mild itch
8	moderate itch
9	strong itch
10	the most intense itch imaginable

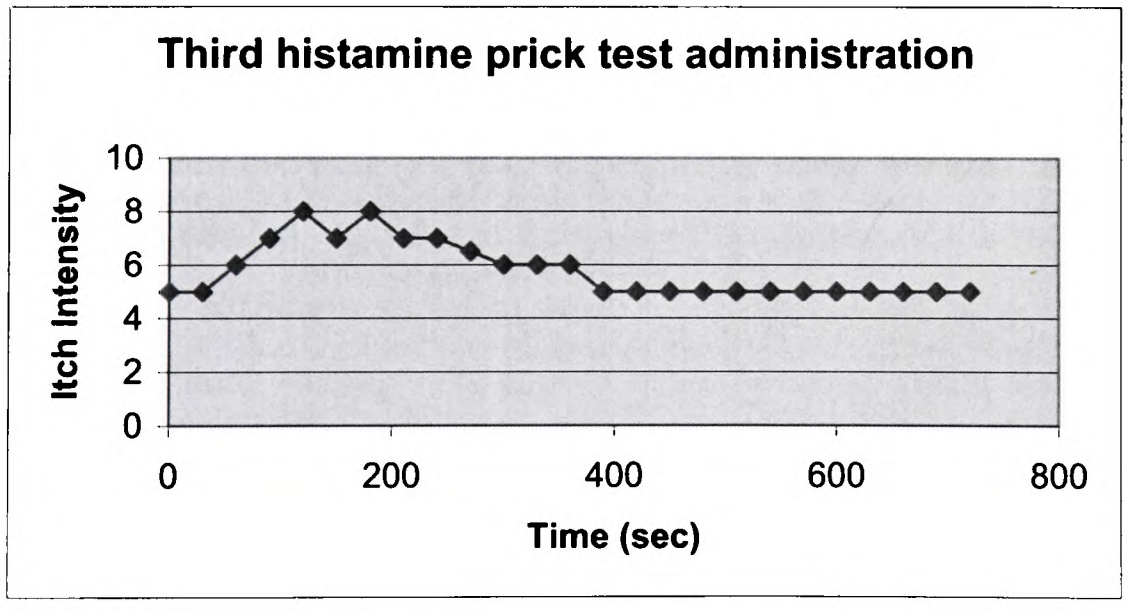
The skin prick test with histamine was carried out three times on each subject using the same hand but on separate occasions. These sessions were a minimum of 2 and a maximum of 4 days apart.

This data was used to plot graphs of itch intensity over time on all three occasions and calculate an average itch intensity profile for each individual subject.

Fig. 5.1 Graph for subject DL to show itch intensity over time

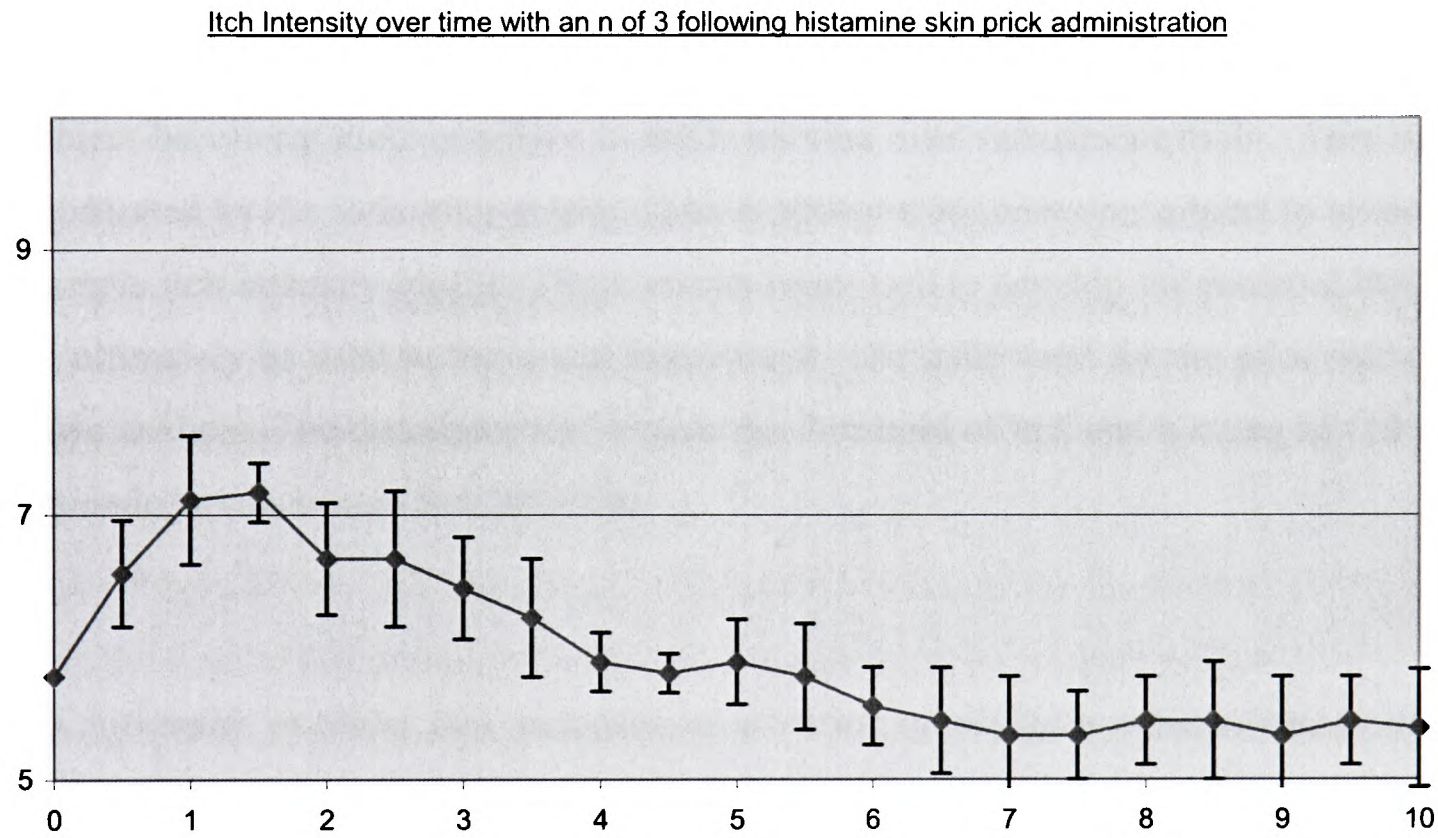






The average itch intensity profiles are for each subject were combined to obtain an overall average intensity curve for the time course of itch intensity produced (see fig. 5.2 below).

Fig. 5.2 Graph illustrating the itch intensity profile of histamine skin prick induced itch. The data is the average time course for an individual subject (i.e. mean of 3 sessions).



Building on the initial behavioural data it was decided that single events were not ideal to model within the fMRI data analysis environment due to reduced signal to noise. When carrying out pain experiments using noxious thermal stimuli, ten pain events are needed, distributed throughout the course of the scanning session, in order to generate sufficient signal to noise. This would not be possible for this particular study but bench studies were carried out to optimise multiple event paradigms for use in the scanner.

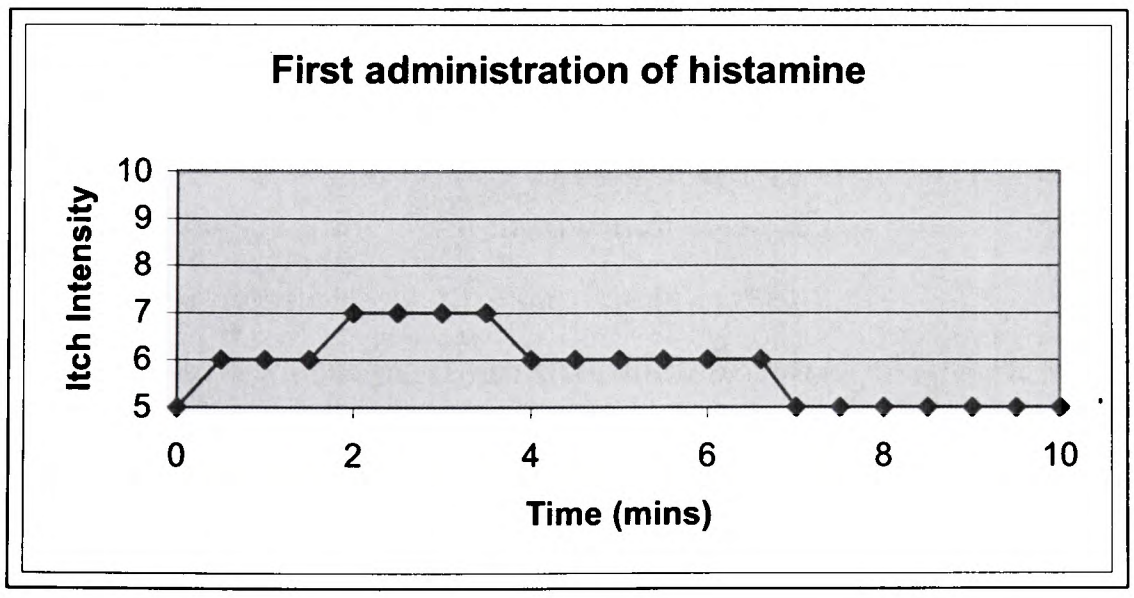
Due to potential movement artefact confounds it was decided that using the foot (area between the toes) for administration of histamine was preferable to the hand. This area is more accessible and therefore less likely to be susceptible to movement due to the experimenter administering the histamine. It can be secured

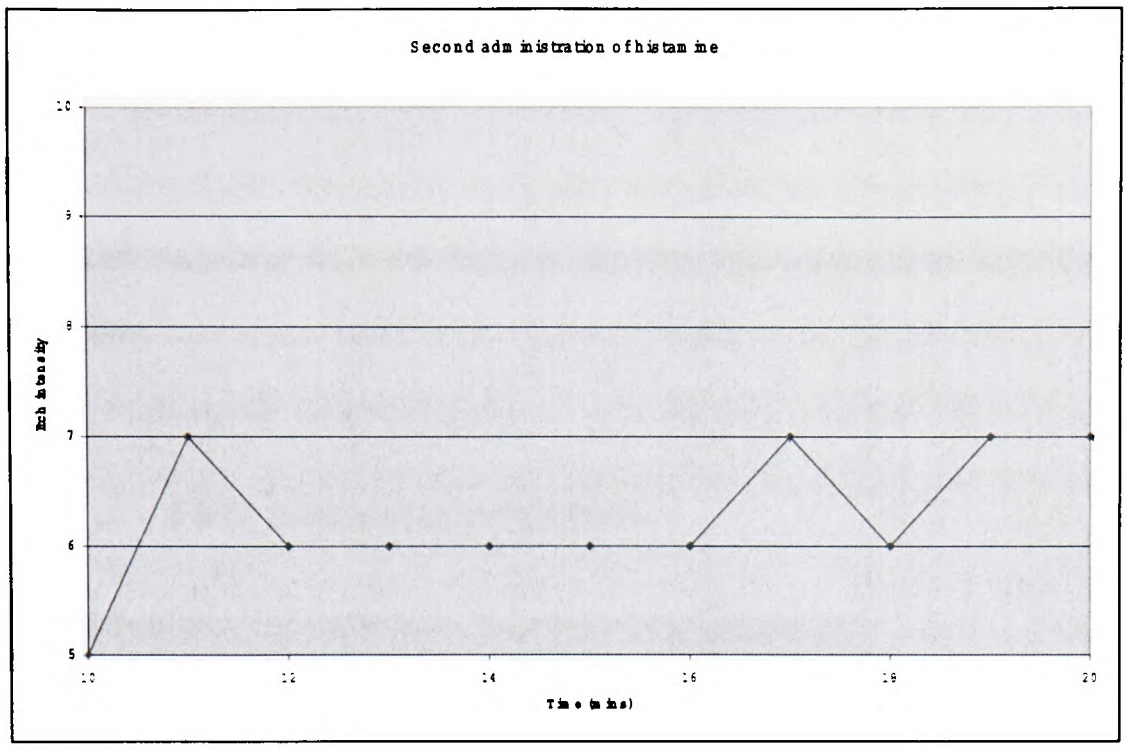
during the experiment also to minimise movement. Subjects are always instructed not to scratch the itchy area during the experiment.

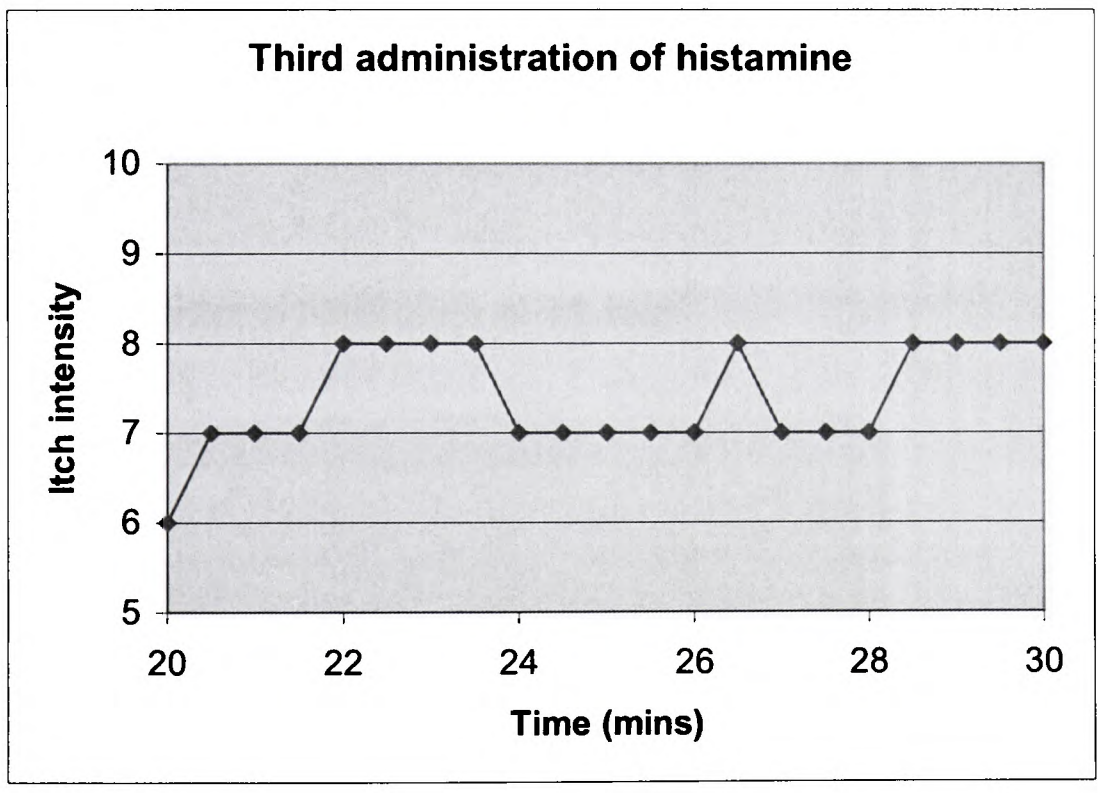
Multiple histamine administration sites on the dorsum of the hand were investigated whereby a subject received a single histamine prick test in one area and itch intensity was recorded for 10 minutes (itch sensation usually lasts less time than this). At this point a further prick test was carried out in a separate area of the dorsum of the hand and intensity measured for 10 minutes again. At this point a third and final prick test was carried out and itch intensity measured until it reached near baseline levels.

Data from this experiment showed that the sensation of itch 'summates' with the subject becoming more sensitive to the histamine over subsequent trials. This is best illustrated by the following graphs. Data is shown from only one subject to show an example itch intensity profile. These results were used to develop the protocol that would ultimately be used in the actual experiment. The scale used for the pilot study used two anchors. The descriptor for '5' was the threshold of itch and a rating of '10' corresponded to maximum itch intensity.

Fig. 5.3 Graphs to show itch intensity as a result of multiple administration of histamine to the dorsum of the right hand





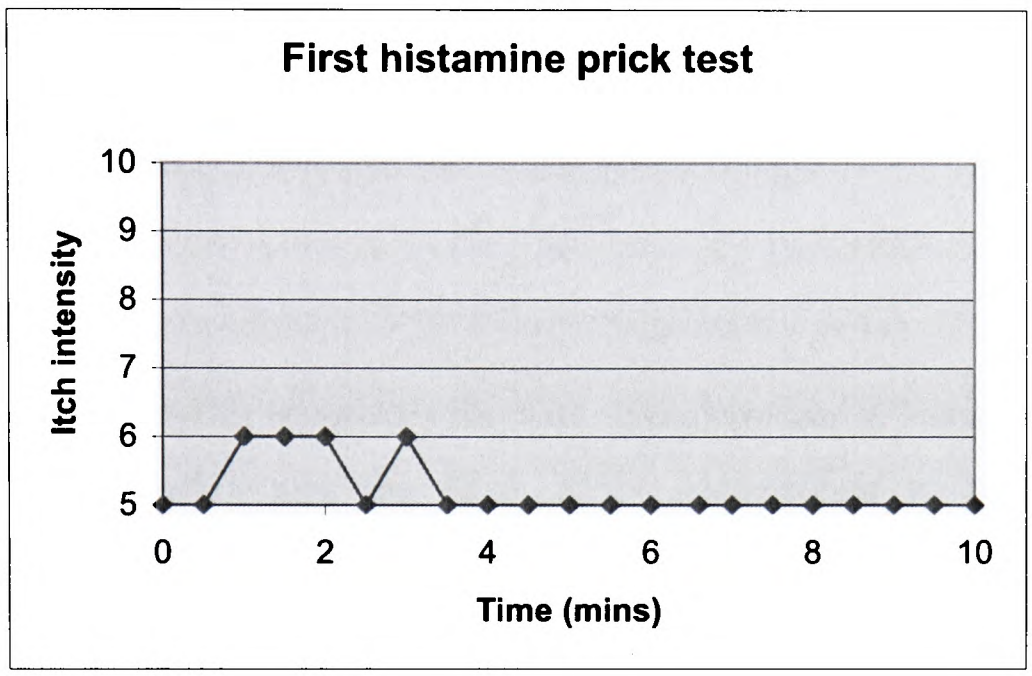


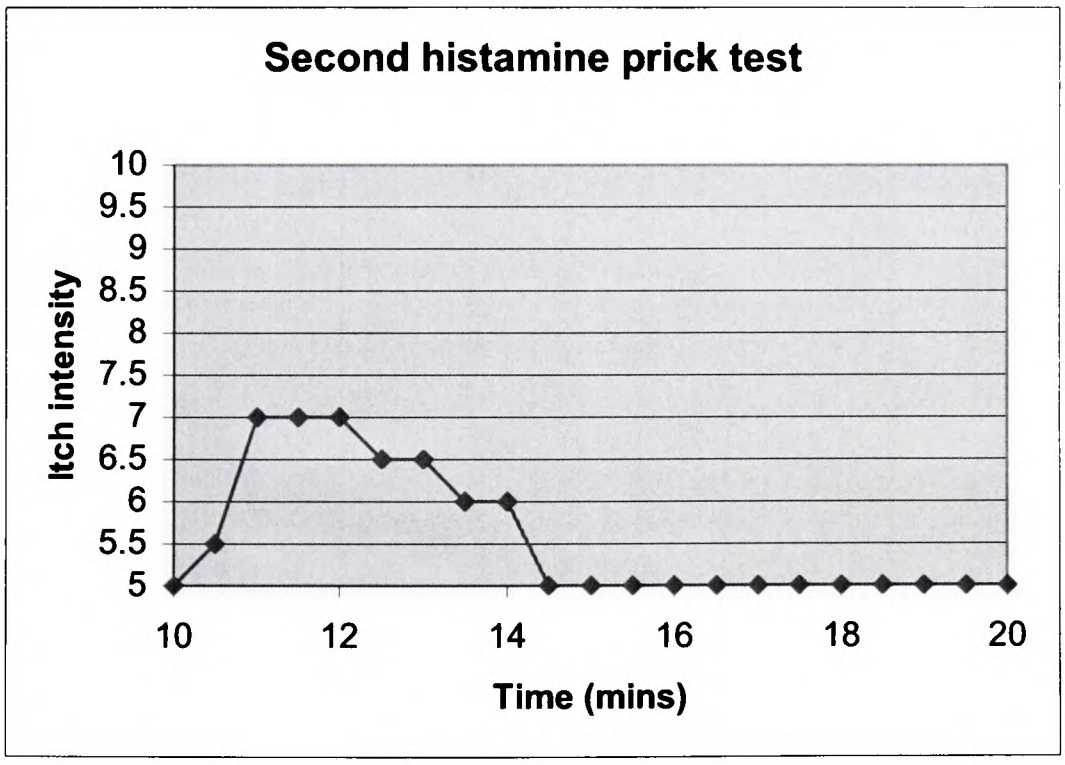
The first series represents the first administration of histamine, the second the second administration and the third series corresponds to the final administration of histamine. This was then later carried out on the foot following the same protocol. Data from this are seen below although this subject was less itchy following histamine administration

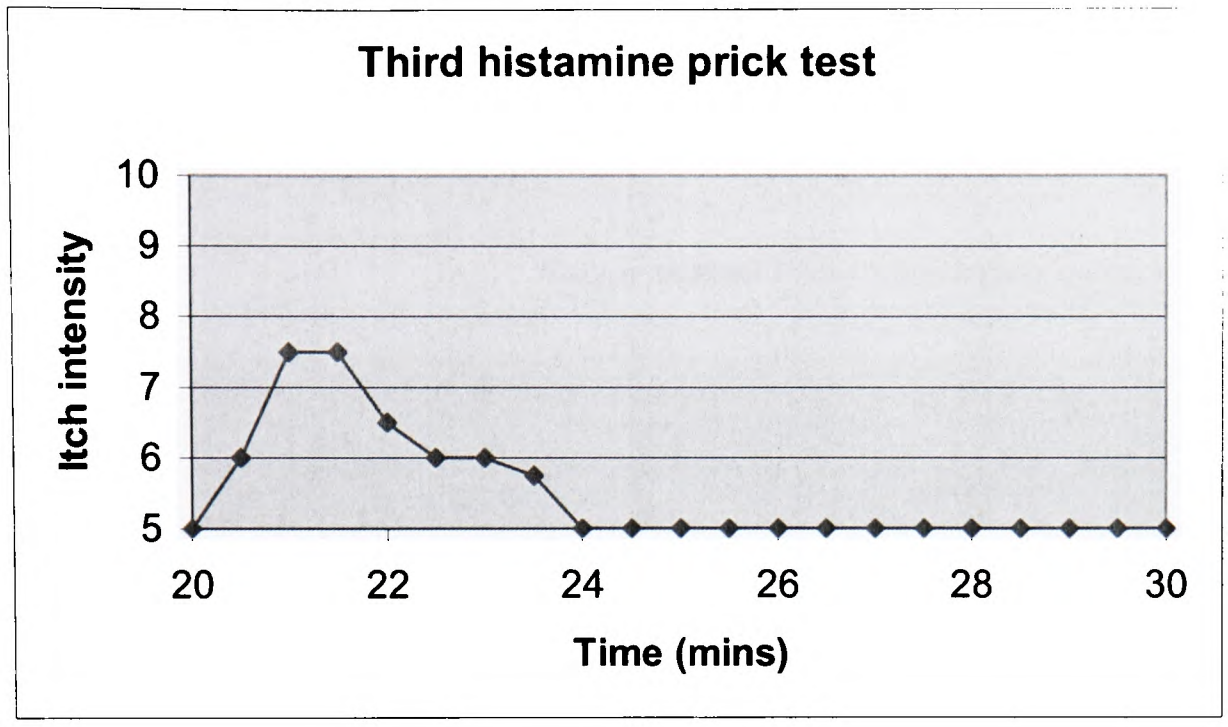
A six point itch intensity rating scale (5= itch threshold, 10= maximum itch) was used to carry out the same protocol on the right foot. Once again data is only

shown from one subject prior to the main experimental data collection phase in the actual experiment.

Fig. 5.4 Graph to show itch intensity following multiple administration of histamine to the right foot



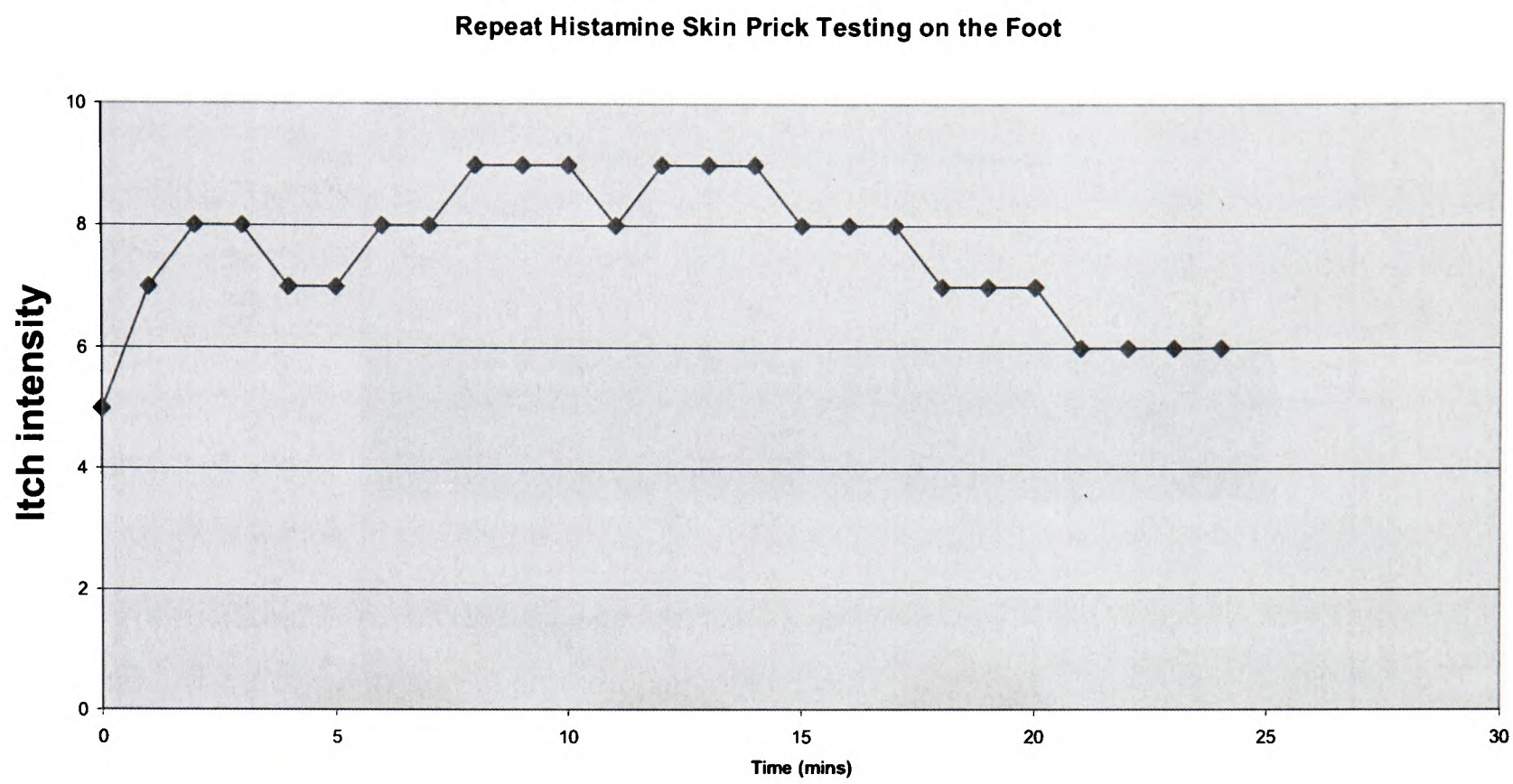




The first series represents the first administration of histamine, the second the second administration and the third series corresponds to the final administration of histamine.

The protocol was then altered further to follow the following structure. This was carried out on two subjects initially (AP and RW). The data for RW is shown below. Average profiles are not shown since it is likely that subject's individual profiles will be used to correlate their own behavioural data in the scanner with their imaging data.

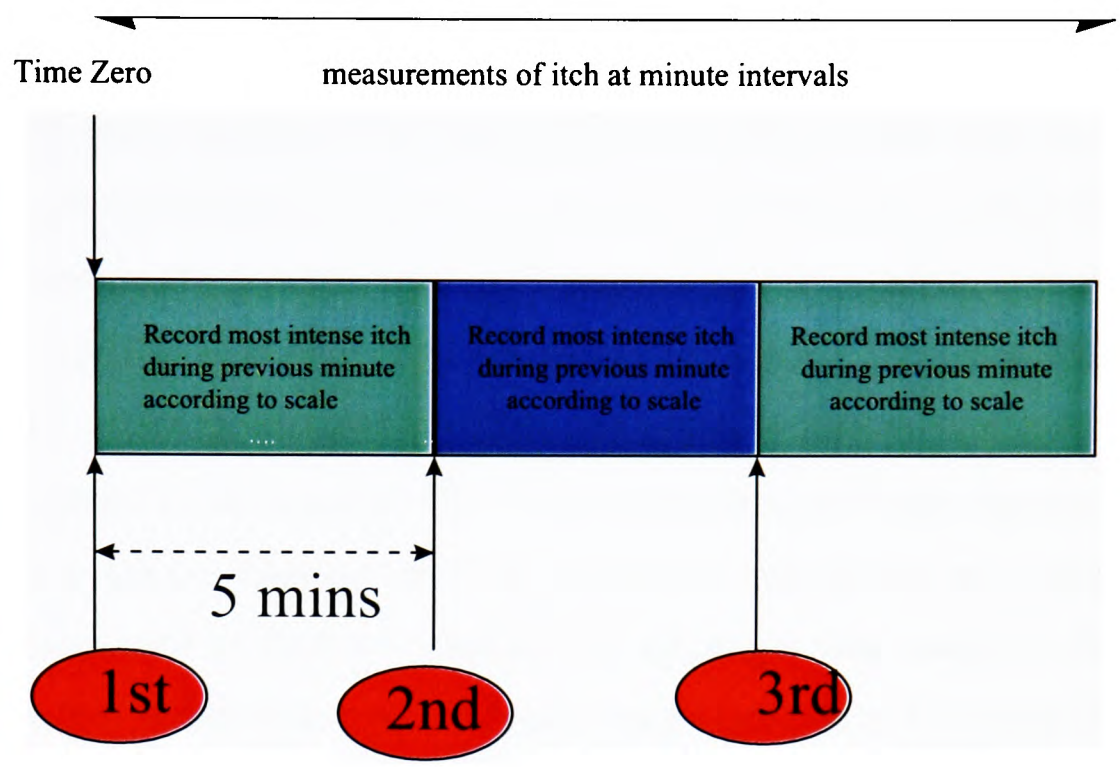
Fig. 5.5 Graph to show itch intensity from multiple administration of double prick test of histamine on the foot.



The prediction for this protocol was that higher differences in itch intensity would be beneficial to see changes in the imaging data and multiple events would help increase signal to noise ratio. Histamine is applied at 0 minutes, 5 mins, and 10 minutes.

5.9 Designing the FMRI protocol

Fig.5.6 Diagram of experimental protocol



With this protocol itch intensity is measured during the experiment in the magnet every minute with the itch sensation not having returned to baseline levels. Baseline recordings would be recorded at the beginning of the experiment. At another session, saline data would be obtained for each subject.

For each administration areas are chosen which are close to each other but outside the red flare response of the other administrations of histamine.

5.10 Aims of the current research

The aims of the study and current research are to further develop a model of human pruritis and an objective and reliable way to measure the itch sensation behaviourally and psychophysically.

The use of fMRI to find the central neuronal correlates of the subjective perception of itch intensity could help further our understanding of such an unpleasant clinical symptom.

5.11 Pilot functional imaging analysis of histamine induced itch

Histamine was administered to the right foot at time zero, 5 minutes and 10 minutes using two prick tests over the same histamine drop in each case. Itch intensity ratings (at 0T) were obtained at 1 minute intervals throughout and for 15 minutes. This bench experiment was carried out 3 times on each subject during sessions separated by at least 1 day outside the MRI scanner. The scanning environment is different, being noisier, more confined and more distracting than in the laboratory room used for the bench experiments. However, since the itch intensity profiles for each subject did not very greatly between sessions it was decided to create an average itch intensity profile over the 3 sessions for each individual subject. This was then taken as approximately reflecting the profile of the itch intensity that subject would feel in the fMRI experiment in the scanner. This is an assumption only and was used as a pilot to ascertain the nature of activation in the central nervous system as a result of histamine induced itch, and to find out whether this paradigm was viable in the scanner and allowed sufficient signal to noise for valid imaging results to be obtained.

The average itch intensity profile obtained during the bench experiments was used to find out which central regions showed activity that correlated with the fMRI data, and therefore which areas of the brain showed changes in activation associated with changes in the perception of itch intensity. The experimental paradigm used in the scanner was identical in terms of timing and administration of histamine to that of the bench experiments. The itch intensity profile is assumed in this case to be sufficiently similar outside the scanner to inside the scanner to allow valid modelling to be carried out. During the analysis of the fMRI data set, the individual subject's average itch intensity profile would be correlated with signal changes during the fMRI prick test paradigm. The itch intensity profile would represent one of the experimental variables analysed when the fMRI data set was evaluated. Future experiments would then be carried out if this pilot study yielded satisfactory results. Such studies would ideally use ratings of itch intensity obtained in the scanner environment itself to enhance the accuracy of the results.

Subjects were randomly allocated to saline first or histamine first scanning sessions.

During the histamine imaging sessions the protocol was as follows. Baseline data were collected for 60 seconds. Histamine was applied to the right foot using a

double prick over the same histamine drop at 1 minute, 6 minutes and 11 minutes after the start of the experiment and fMRI data were acquired throughout at 3T.

5.12 fMRI Pilot Experiment: An fMRI investigation of the central neuronal correlates of the itch sensation in humans

5.13 Introduction

The sensation of itch induces a strong urge to scratch but subjects in these experimental paradigms are specifically instructed not to scratch. This in itself is unnatural and there may be areas where activation represents a repression of the natural urge to scratch. This could be investigated further in future studies by allowing the subject to scratch at predetermined times at some points in the study and comparing the central activation resulting from the times when scratching was permitted with that when scratching was not permitted. It is pleasurable to relieve itch by scratching and so some pleasure related central activations might result. This is not addressed in this study. However, it would allow interesting comparisons to be made between the motor responses to itch (scratch) and pain (withdrawal). Ipsilateral motor activations could be a result of focusing on the limb or area of the limb where itching is felt. The majority of studies so far have found contralateral motor activation which is thought to correspond to the urge to scratch and therefore motor planning relating to moving the opposite side limb across to scratch the itchy area and bring relief. Indeed the amplitude of activation seems to correlate in many cases with the intensity of the itch and the concentration of the histamine applied.

Itch, like pain is not a unitary sensation, and different components make up the overall itch experience. The intensity of itch can be rated using a scale where there is a clear itch threshold and a maximum itch rating. These ratings are subjective and reflect the individual's assessment of the itch intensity. They are linked to the motor response to itch, i.e. the desire to itch because that is the threshold for itch which is readily measurable. Pain experiments can require subjects to rate when a stimulus just feels painful rather than warm (when thermal stimuli are used). The unpleasantness of itch is, as it is with pain, more difficult to measure. Verbal descriptor scales can be used although words signify different things to different people and the choice of word can alter the results obtained. However, subjects could be asked to rate how

unpleasant they feel the itch stimulus using words such as unpleasant, very aversive, and unbearable for example. Drzezga et al (2001) obtained ratings of itch unpleasantness (they do not describe the scale or method used) and intensity during their fMRI study of histamine induced itch. However, the author refers to a validated scheme described in Darsow et al. (2000) where a 100mm visual analogue scale is used to record perceived itch intensity. Drzezga et al (2001) only entered unpleasantness and the log of histamine concentration into their analyses of fMRI data as experimental variables. They did not enter itch intensity together with unpleasantness because there is a high correlation between itch intensity and itch unpleasantness which may render such analyses invalid (Drzezga et al 2001).

Significant activation was found in the contralateral ACC (BA 24) which is thought to represent the unpleasant emotional nature of itch and its processing. The same area is believed to process affective information pertaining to painful stimuli (Peyron et al., 2000). A subsequent study (Darsow et al., 2000b) found ipsilateral activation of the ACC to be correlated with the redness (wheal) resulting from histamine prick test administration. The most recent imaging study in fact found a highly significant correlation between ratings of itch unpleasantness and ipsilateral ACC activation (Drzezga et al., 2001b). The ACC was found in a subtraction analysis with saline to be involved with the processing of itch. A non-significant correlation between itch intensity and activation in the posterior cingulate was also found in this study. Activation of the contralateral ACC (BA24) is proposed as the correlate of the unpleasant affective nature of itch (Hsieh et al., 1994a), and has been found to be almost solely contralateral in nature.

In terms of somatosensory cortical activation, the earliest imaging study (Hsieh et al., 1994a) failed to show any activation in the primary or secondary somatosensory cortices. Drzezga *et al.* (2001) found activation in the contralateral somatosensory cortex representing the right limb. This was correlated with intensity of itch and histamine concentration. The same study found that there was a strong link between activity in the contralateral somatosensory cortex and the unpleasantness ratings of the itch sensation. Darsow's study also found bilateral somatosensory cortical activation which correlated with ratings of itch intensity (Darsow et al., 2000b).

Posterior insula activation was found contralaterally and was shown to correlate with itch unpleasantness ratings (Drzezga et al., 2001b)and in another study with itch intensity (Darsow et al., 2000b).

Activation correlated with itch unpleasantness was found in the contralateral dorsolateral prefrontal cortex (BA 9/10) (Darsow et al., 2000b; Drzezga et al., 2001b; Hsieh et al., 1994a) in subtraction analyses between histamine and saline control. Such analyses also revealed activation in the contralateral orbitofrontal cortex (Darsow et al., 2000b).

Within the inferior parietal cortex (BA 39/40), activation was found to correlate with histamine concentration (Darsow et al., 2000b; Drzezga et al., 2001b) and itch intensity (Drzezga et al., 2001b). Activity as a result of itch was also found in this brain region by Hsieh *et al.* (Hsieh et al., 1994a).

This study aims to further explore the functional areas involved in the perception of itch intensity. Since itch and pain are separate sensations, we hypothesise that a map of areas activated by itch will not correspond exactly to those activated by painful stimuli. However, existing studies suggest that some areas of the brain which are activated by painful stimuli, and which correspond to the unpleasant nature of pain are also activated in itch (posterior part of the anterior cingulate). We are interested in isolating the central areas involved in the perception of pure itch and ultimately in the relationship between pain and itch processing (analogies and differences).

5.14 Materials and Methods

Skin prick tests (Pepys, 1975) were used to induce an itch sensation. In order to achieve a longer lasting itch sensation necessary to allow sufficient SNR in the scanner, three successive administrations of histamine were required. The itch sensation was greater when two pricks were delivered over the same histamine drop site. Additionally, when repeat prick test administrations succeeded each other at 5 minute intervals, behavioural data showed that successive administrations of histamine elicited greater itch intensity than the initial one.

Five female and three male volunteers were scanned at 3T (TR=3.0, TE=30ms, 21x6mm axial slices, 228 volumes, flip angle 90 degrees). Each subject underwent a

thorough medical screen to exclude highly allergic individuals, and medical cover was present throughout.

The subject was given two prick tests on the same site and this was repeated twice more at 5 minute intervals. The subject rated itch intensity on the 5 point scale at 30 s intervals throughout. Pilot studies revealed the itch intensity to be relatively consistent across sessions and so only one bench session was used.

Subjects then underwent an identical protocol in the scanner at a later session. 12 seconds of dummy scans were acquired, followed by 60 seconds of baseline data prior to the first prick test. The histamine was then applied as previously described.

Subjects underwent the same experiment using a saline control that was identical in every other respect to the histamine paradigm (randomised order of administration).

5.15 Image Acquisition

A T1-weighted high-resolution scan was taken for anatomical overlay of activation maps (see later).

Behavioural data acquired outside the scanner in an exact replica of the scanning experiment were used to generate a graph of itch intensity over time for each individual subject. This graph was then input as a model to find out which brain areas showed activation correlating with the changes in itch intensity over time. The prick test was not painful but was still a sensory stimulus, and therefore the timings of this and its duration were put in as another variable into the experimental model to look for any activation attributable to the prick test itself.

The data acquired in the bench experiment (an itch intensity profile for each subject comprising of an average profile from data obtained across 3 experimental sessions at 0T) is considered for the purposes of this experiment to be sufficiently similar to enable a pilot study to be carried out in order to assess the validity of the paradigm. It proved harder than anticipated to generate a sufficiently intense and long lasting pruritic stimulus and it was necessary to establish that sufficient signal to noise had been obtained in order to produce reliable imaging results at 3T. In order to carry out initial pilot investigations to establish that the paradigm was suited to the scanner individual subjects' average itch intensity ratings (obtained at 0T) were used to see which areas showed signal changes correlating with the itch intensity profile derived from the bench data. It was always anticipated that following the outcome of this

experiment, online ratings would be developed in order to obtain real-time behavioural ratings of itch intensity in the scanner. The environments subjects encounter in the scanner and outside the scanner are different. However, the profile obtained on successive sessions revealed that itch intensity was fairly reproducible between sessions by subject. Because of this robustness of the data, the assumption was made for the purpose of the pilot study to use the profiles obtained outside the scanner as an experimental variable to interpret the fMRI data acquired in the scanner.

5.16 Statistical Analysis

Analysis of the fMRI images to identify regions showing significant changes in BOLD signal (Ogawa et al., 1993)was carried out with a multistage process using the image analysis package FEAT (www.fmrib.ox.ac.uk/fsl), an extension of MEDx (Sensor Systems, Sterling, VA., USA). The following pre-statistics processing was applied from within the FEAT package: motion correction using the SPM realign procedure (Friston et al., 1995); spatial smoothing using a Gaussian kernel of full-width half-maximum 5.0 mm; mean-based intensity normalization of all volumes by the same factor.

The statistical portion of the analysis was carried out using the FEAT package using the general linear modelling (GLM) method (Friston et al., 1995). A description of the experimental design is modelled, and this is then fitted to the fMRI data that indicates where the brain has shown activation in response to the stimuli within the paradigm. Within FEAT, the GLM method is known as FILM (FMRIB's improved linear model) (Woolrich et al., 2001). Two explanatory variables were used to model the time course data for each subject on a voxel by voxel basis. These were the itch intensity (derived from the identical bench experiment) and the prick tests (see diagram of experiment). These were modelled within the GLM independently. Each explanatory variable results in a parameter estimate image. These show the strength of fit of the to the fMRI data at each voxel. This parameter estimate image can be converted to a t-statistic image by dividing it by its standard error (derived from the residual noise after the complete model has been fitted to the data). A standard statistical transformation enables the t-image to be transformed into a Z statistic which is an activation map, more clearly showing areas of activation as a result of the paradigm variables (itch intensity and the prick test itself).

A fixed effects group cluster analysis (second level analysis) was carried out on the subjects' activation maps to produce representative group results. The thresholding parameters used for the fixed effects analysis were a Z-score threshold of 2.3 and a probability value of P= 0.05 for cluster significance (Worsley et al., 2002)

5.17 Results and Discussion

Behavioural data

All subjects (Mean age 28 years +/- 3.2) reported a pure itch sensation (itch ratings from mild to moderate/strong itch) and no pain was reported by any subject for the administration of the histamine by prick test administration. No subjects reported an itch sensation following administration of the control saline solution prick test.

Imaging data

The central activation which correlated with the itch intensity time course over time for the histamine prick test paradigm is shown in Fig. 1, and the location of these areas in table 1.

Fig. 5.7 Activation as a result of histamine induced itch

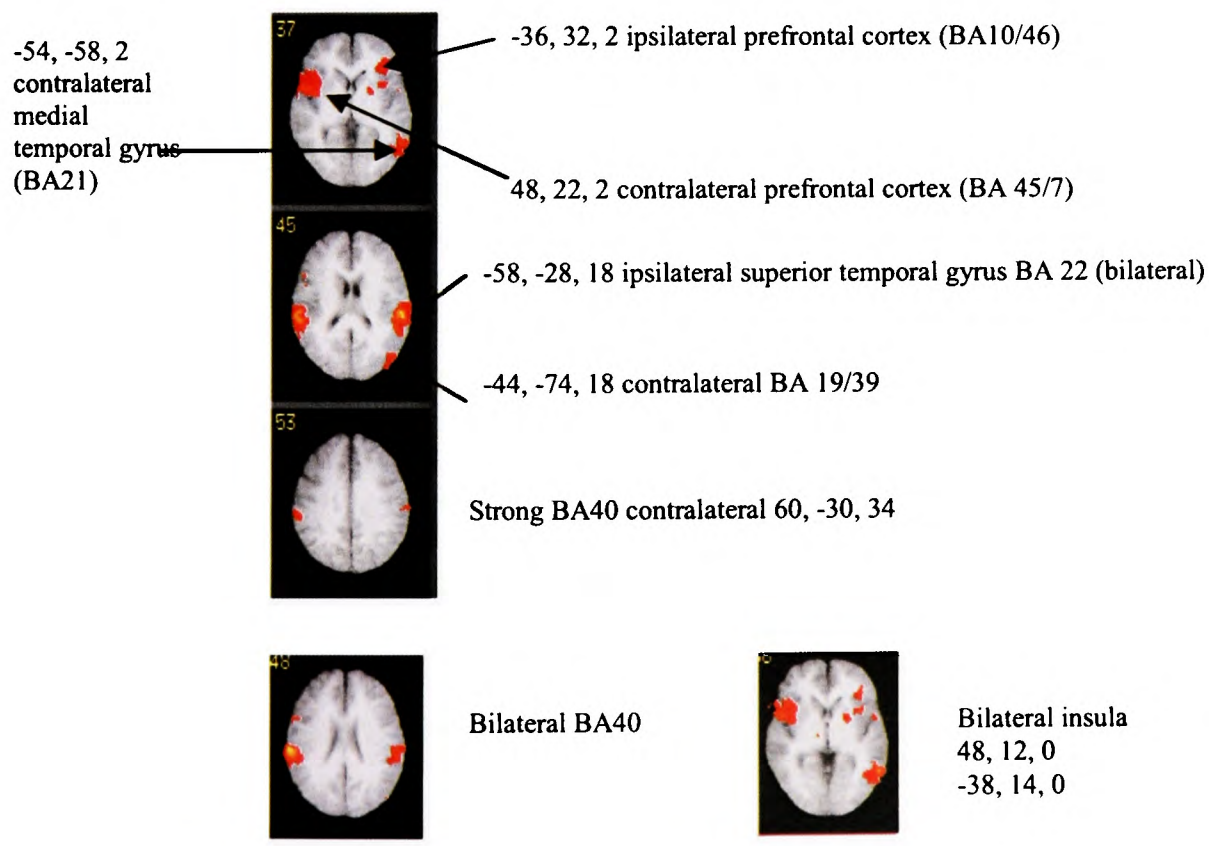


Table 5.0: Talairach coordinates to show location of areas activated by itch

Brain region	*Brodmann's area (BA)*	*Talairach co-ordinates (x, y, z)*	*Z-score*
BL prefrontal cortex	11	-4, -60, -14	3.79
		12, 45, -14	3.91
CL posterior cingulate	25	-4, -16, -10	2.87
BL caudate nucleus		12, 16, 0	3.90
		-10, 16, 0	3.90
BL ACC/orbitofrontal cortex	10/32	0, 58, 2	2.70
		-8, 60, 2	2.8
BL orbitofrontal cortex	10	6, 62, 8	2.84
		-20, 56, 8	2.9
CL Cuneus	18	-4, -78, 16	2.9
BL medial occipital gyrus	18/19	20, -92, 16	2.92
		-32, -86, 16	3.8
CL medial temporal gyrus	19/39	-56, -66, 20	3.65
IL superior temoral gyrus	22	-58, -28, 18	2.80
BL posterior parietal cortex	40	-34, -56, 40	2.9
CL precuneus	7	-18, -80, 44	4.79
BL insula		48, 12, 0	3.2
		-38, 12, 0	3.17

* IL = ipsilateral, CL= contralateral, BL = bilateral

s can be seen from the tables, group fixed effects analysis showed activation in the contralateral cerebellum, bilateral prefrontal cortex, contralateral hippocampus, contralateral basal ganglia, bilateral orbitofrontal cortex, contralateral ACC, bilateral medial temporal gyrus, contralateral BA40 (posterior parietal cortex). From these fMRI data the activation corresponding to the sensory input to the foot (ipsilateral activation) is likely to be that found at Talairach coordinates 6, 62, 8 although this data did not identify this. Activation in this area is likely to correlate to the intensity of the stimulus as perceived by the subject.

The itch intensity time course was plotted as a graph and used to model the itch intensity. This same graph was also used to model the saline paradigm. No brain areas were found to show activation at the threshold (z=2.3) using this model for the saline control data.

The activation attributable to the prick test itself was also modelled for both histamine and saline. The talairach coordinates for both are shown by tables 2 and 3, respectively.

Table 5.1: Talairach coordinates for the activation caused by the prick test alone for histamine

Brain region	*Brodmann's area (BA)*	*alairach co-ordinates (x, y, z)*	*Z-score*
IL* medial temporal gyrus	21	-54, -58, 2	2.97
IL orbitofrontal cortex	10/46	-36, 32, 2	3.01
CL prefrontal cortex	45/7	48, 22, 2	3.90
BL sup. temporal gyrus	22	-58, -28, 18	5.80
		60, -26, 18	3.80
BL posterior parietal cortex	40	60, -30, 34	2.96
		-60, -22, 34	3.85
BL insular cortex		48, 12, 0	3.80
		-38, 14, 0	2.9

* IL = ipsilateral, CL= contralateral, BL = bilateral

Table 5.2 Talairach coordinates for the activation caused by the prick test alone for saline

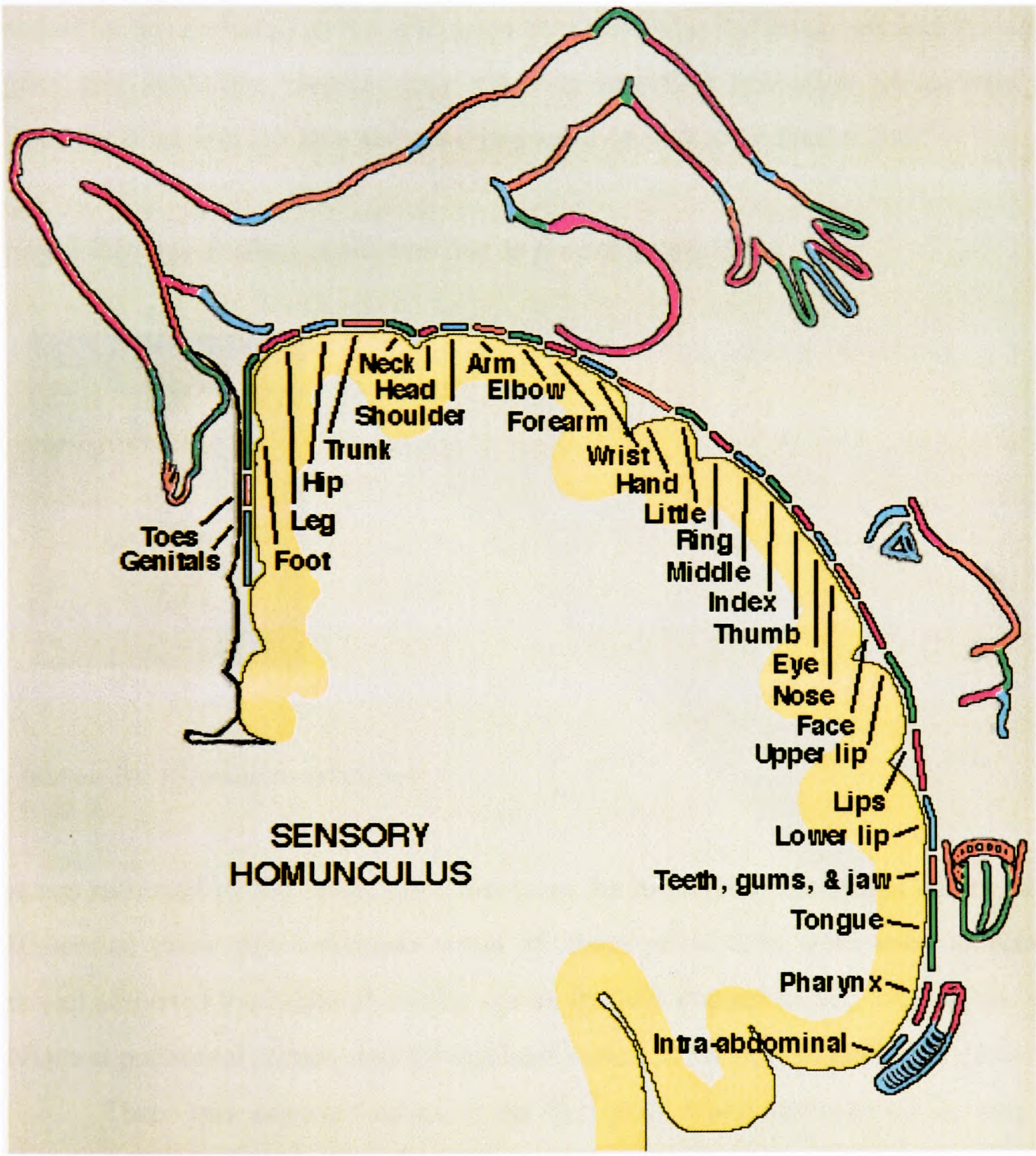
Brain region	*Brodmann's area (BA)*	*Talairach co-ordinates (x, y, z)*	*Z-score*
orbitofrontal cortex (midline)	10	6, 50, 2	3.14

It can be seen in the above table (table 5.2) of the activations caused by the prick test alone for saline that the only activation reaching significance was found in the orbitofrontal cortex (BA10). The Talairach co-ordinates for this region show it is ipsilateral to the site of the prick test (6, 50, 2) and the z-score confirms that it is strong activation (z=3.14). The same area (6, 62, 8) showed activation in response to histamine-induced itch (in this experiment) as shown in table 5.0. This is interpreted this as corresponding to the orbitofrontal cortex although it is equally probable, given the ipsilateral nature of the activation, that this activation represents the sensory itch sensation corresponding to activation in the foot area (where the histamine was applied). This activation was not found to be activated in the analysis of areas corresponding to the prick test alone for histamine. However, it is hard to separate activation due to the prick test alone from that due to the histamine. There is always a background itch sensation in each prick test after the first prick test where the histamine is applied. This makes it hard to get definitive correlates of the prick test alone in the histamine condition because in the 2nd and 3rd prick tests the area is

already very itchy and so some activation may be due to the histamine-induced itch as well at that time point and therefore confound the results.

The diagram below shows the sensory homunculus. The foot region is represented in an area located close to the recorded activation found at 6, 50, 2 and therefore ipsilateral activation here as a result of the prick test may represent the sensory input to the foot during the prick test itself.

Fig. 5.8. Sensory homunculus



In general, the somatosensory cortex and motor cortex maintain a representation of the human body. This representation reflects the sensitivity or the ability of the body in that domain. Thus, in somatosensory cortex those areas which are most sensitive to touch or to pressure, have the largest representation and those areas of the body which are relatively insensitive have very small representation.

fMRI images of the prick test activation for the saline and histamine paradigms show that there are differences between the two. The activation attributable to the prick test itself in the saline control part of the experiment should be the same throughout the experiment as there is no underlying additional itch sensation to confound the activation. However, for the itch paradigm it was decided to see what the activation was like from the first prick test alone.

The overall model gives activation that uses information from all three prick tests. This means that the underlying increases and decreases in the background itch sensation are included in the activation attributable to the prick test and therefore the prick test activation contrast may not truly represent activation which only results from the prick test but also show a component of activation due to itch.

Fig. 5.9 Image to show activation due to the saline prick test



Midline BA 10 (orbitofrontal cortex)
6, 50, 2

Areas activated by the saline prick test were the bilateral orbitofrontal cortex (BA 10). Histamine prick test activation when all three prick tests were used to create the model activated the bilateral insular cortex (mostly contralateral), contralateral BA40, bilateral prefrontal cortex, and bilateral activation in the medial temporal gyrus.

There was more activation in the first prick when analysed on its own, which was the reverse of the original hypothesis. There is likely to be underlying activation in the second and final prick tests due to the ongoing itch sensation which is waxing and waning in the background to the prick tests and as such it is not easy to get pure activation which arises solely from the histamine prick test and has no component of itch contributing to it.

5. 18 Discussion

The main aim of this initial pilot fMRI study was to ascertain whether it would be possible to create a reliable, non painful, and sufficiently potent itching stimulus to enable it to be used in the magnet with an adequate signal to noise ratio. There were also issues of the site of application of the itching stimulus. This study confirms that the site of application of histamine to the foot was favourable and that the prick test application of histamine (the most common mediator of itch naturally found in the human body) was an ideal stimulus for producing a non-painful and sufficiently intense pruritic stimulus. The paradigm we had developed with the three successive pricks of histamine in the foot area allowing summation of itch and a sustained sensation for about 12-15 minutes appears to be valid for the detection of central neuronal correlates pertaining to the itch sensation. The approach of using a model for the itch intensity over time generated outside the magnet to correlate with the signal obtained inside the magnet also appears to be valid.

The next main aim of the pilot study was to ascertain reproducibility with the findings of the existing imaging literature in the field of itch, and see how this activation compared with that elicited from noxious thermal stimuli used in our previous studies.

Some studies (e.g. Darsow et al., 2000) have reported activation in the ACC, following histamine induced itch. This region is also a key component of the pain 'matrix', and would represent a similarity in the central processing areas between the two sensations. The area is believed to be concerned with the sensory affective component of the itch, as it is in pain processing. It would be useful in that case, however subjective this may be, to obtain some concurrent measure of itch unpleasantness during the experiment. However, this study did not find any activation in this region. It is possible that this area may only be activated when the itch intensity exceeds a certain threshold and may be paradigm dependent. We plan to develop more sophisticated imaging studies and will see if this region is activated in this case. However, activation was found in the OFC which is close to the ACC.

The prefrontal cortex is a region that is frequently activated in itch imaging studies (Darsow et al., 2000a; Drzezga et al., 2001a). Both authors showed a correlation between activation in the prefrontal cortex with the unpleasantness of the

itch sensation. Unpleasantness of itch was not measured in this study although it shows our activation is consistent with the current findings.

The current study did not reveal significant activity in any pre motor or motor areas as found by Coghill *et al* (1994). Such activation is thought to represent the intention to scratch, and is most commonly observed in the contralateral hemisphere as a consequence of this. Further, such activation is proportional to the intensity of the itch and the desire of the subject to relieve the sensation by scratching. Subjects are asked not to scratch the itchy area in the fMRI experiments since this would cause unacceptable motion artefact and confound the results. However, the urge to scratch is still present and can manifest itself in pre motor or motor activation in the contralateral hemisphere.

The other area which has been activated in some studies but is by no means a consistently activated area is the somatosensory cortex in the contralateral hemisphere, which was found by Drzezga *et al* (2001) to correlate positively with the concentration of histamine. Activation was not found in this study, although since we did not analyse activations as a function of histamine function (due to the limitations of prick test histamine administration where it is not possible to administer accurately increasing histamine levels), it is possible that this area is involved with the processing of itch intensity. Prick testing delivers a consistent amount of histamine to the dermal-epidermal junction and therefore we cannot validate their paradigm using our current study design. This could represent the discriminatory or qualitative element of itch processing. In pain processing this area is thought to be linked to the discriminative processing of pain and activation here is also related to the degree of unpleasantness of the stimulus. The area is particularly amenable to modulation by cognitive manipulations (Bushnell et al., 1999). It would be useful to manipulate clinical itch using a cognitive distraction task, and see whether or not this area is also modulated in itch as it is in pain by psychological tasks.

The posterior insula is activated contralaterally in some studies (Darsow et al., 2000a), and we find bilateral activation in our study, although the contralateral area is more strongly activated. Bilateral activation is frequently observed in the pain research literature (Craig, 2002). Activation of the insula in an itch paradigm is thought to correlate with the unpleasantness of the itch sensation, where its role is similar to that played by the region in the processing of pain.

The final area which is activated in this study and in other imaging studies of itch is the inferior parietal cortex with bilateral activation in this area. Other authors (Darsow et al., 2000a; Drzezga et al., 2001a) have shown this to be linked with the concentration of histamine used and the intensity. The earliest itch study using imaging (Hsieh et al., 1994b) reports activation in this area. The significance of this area in itch processing will be discussed further later in this thesis.

This histamine prick test paradigm successfully elicited a pure and pain free itch sensation that was repeated three times with five minute intervals between each administration.

Behaviourally, the itch ratings obtained in this pilot study showed that there was some temporal summation of the itch response to successive doses of histamine being amplified with respect to the preceding ones. The fMRI data were not analysed to ascertain whether or not this summation and increasing intensity with successive histamine prick test administration was reflected in greater signal changes across the paradigm. The summation and increase in itch intensity was useful because it allowed a sufficiently sustained and intense itch stimulus to be generated to achieve a high enough signal to noise ratio. This was vital in order to acquire imaging data relating to central areas activated by histamine induced itch.

The areas activated by prick test administration in this study were consistent to a large extent with other imaging studies of itch that lends credence to the validity of this paradigm.

It seems clear that some areas activated by itch are strongly dependent upon itch intensity and therefore it might be more accurate to obtain an online rating of itch during the actual experiment to enhance the ability to dissociate the regions involved with the processing of itch intensity.

5.19 Conclusion

This pilot study has shown that the employed itch stimulus produces a sufficiently intense and sustained sensation that it can be used in the fMRI environment. The data are broadly consistent with existing itch imaging literature pertaining to the areas involved with the processing of histamine-induced itch.

A protocol has been developed which seems to generate sufficient signal to noise to enable the investigation of the regions of the brain showing signal changes correlating with changes in perceived itch intensity.

The results should however be interpreted with some caution and used only as a pilot study on which to base future research. The analysis of the fMRI data assumed that the itch intensity profile generated outside the scanner would be sufficiently similar to that obtained in real time inside the scanner to allow correlations to be made with changes in fMRI signal in the central brain areas.

Online rating of itch intensity within the scanner is the next logical step and allows closer coupling of the fMRI signal to its behavioural correlates.

5.20 References

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CHAPTER 6: Investigations into atopic itch: Laser Doppler and functional magnetic resonance imaging studies

The previous chapter was concerned with the development of a valid, reliable pruritic stimulus and with describing the central correlates of itch perception using fMRI. The experiment used individual subjects' average itch intensity ratings, acquired outside the scanner environment, immediately prior to the study, to correlate with the imaging data. This initial experiment validated our basic paradigm for the induction of itch using histamine prick testing, and provided evidence for its applicability to the MRI environment. Our imaging findings were consistent with existing fMRI itch studies.

This study uses these results to further investigate the link between the perception and underlying physiology of the itch sensation at a central level.

In addition, this experiment was designed to ascertain the link between the perception of itch and the changes in local regional blood flow to that region of the skin. This was done using laser Doppler fluxometry.

Another question to address was the quality and intensity of allergen induced itch and thus this chapter looks at the difference between itch parameters (intensity and blood flow) in atopic and non-atopic individuals using histamine, allergen, and saline. Later on, allergens were used to discover whether or not the different quality and intensity of allergenic itch could be detected using fMRI to probe the central neuronal correlates.

6.0 Introduction

In many developed countries over one fifth of the population are affected by one or more atopic allergic disorders (Sheikh, 2002). Many studies have revealed that the prevalence and severity of eczema, seasonal rhinitis, and asthma is on the increase (Fleming and Crombie, 1987; Jarvis and Burney, 1998; Shamssain and Shamssian, 1999). Various hypotheses have been suggested to explain this rise in the incidence of atopy and some researchers have implicated environmental factors to its aetiology (Sheikh, 2002). However, there is a tendency for cases of allergy to show a cluster distribution within individuals and within families that suggests that genetic factors

also play a significant role. Allergic eczema can cause severe itch, and it is the commonest cause of dermatitis in developed countries, affecting approximately 20% of the general population (Kay et al., 1994).

This evidence presents a convincing argument for extending research into the field of allergic itch. Allergic itch and itch as a serious and debilitating side effect of medications, such as opiates, and as a sequel of various disease states (Schmelz, 2001) causes considerable anguish and impacts significantly upon patients' quality of life. In the laboratory, the main focus of itch research has been itch mediated by histamine. Various chemicals can induce itch (Schmelz, 2001), but histamine is the best known inducer of itch in human skin. It is also easier to manipulate the magnitude of the stimulus and carry out a controlled experiment with histamine rather than attempting to modulate existing clinical itch where there are a greater number of confounds and factors beyond the control of the researcher.

Thus, the quest for increased knowledge as to how the brain processes itch seems now to be beyond an academic question, and more a matter of humanitarian urgency, afflicting as it does such a large number of people. There is still a tragic shortfall, or even existence of any specific anti-pruritic drug. The development of a model of allergic itch is addressed here

In the allergy clinic, histamine prick tests (described in Chapter 5) are a routine screening procedure for the evaluation of the response to allergens in comparison to a histamine positive control response. In the research reported here the histamine prick test is employed to reliably generate a reliable pruritic stimulus. In chapter 5 it has been shown that it is possible to induce a pure, pain free and intense itch using this method. The prick test technique delivers histamine to the area of greatest density of histamine receptors, the dermo-epidermal junction of the skin.

To date, there has been a paucity of research as to the time course nature of the response to prick test induced itch. Also building upon this research it is important to try to elucidate the link between the subjective report of itch intensity and any underlying blood flow changes. This has not been carried out to the best of the author's knowledge. It would of interest to further investigate the nature of any link between the perception of itch and the blood flow changes.

Histamine injection into the skin produces a typical wheal and flare reaction developing within minutes in the affected area of skin (Van Neste, 1990). Recently some researchers have utilised laser Doppler fluxometry (Serup and Staberg, 1985), to

evaluate the skin reaction after intradermal injection of histamine. Any changes observed at the site of histamine injection are mediated by a mixture of responses. One aspect of the response is generated by the microtrauma of the prick test itself, the other by the oedematous wheal reaction (which is histamine specific). At a distance from the prick test site, axon reflex dependent vasodilatation is recorded. It is for this reason that it is essential to draw any comparisons from studies where the measurement areas are in agreement and thus where the data are comparable. It is hard to differentiate between the saline control and histamine responses in the centre of the wheal but good discrimination is possible at 1cm from the injection site (Serup and Staberg, 1985). The prick test is a good method by which to ensure standardised delivery in terms of amount and depth of histamine and is also a well validated and safe method used in allergy clinics on a daily basis.

Vasodilatation is an essential feature of any inflammatory response (Ryman, 1973). The initial response of the skin to the allergen is the rapid release of potent mediators with vascular targets resulting in erythema which has frequently been used to assess the extent of allergic inflammation (Rook et al., 1978). This response is expressed in the skin by a wheal and flare reaction (Mygind, 1986). The wheal is caused by the oedema which is the result of a direct effect of histamine on the local blood vessels. The flare reaction is the result of a reflex-mediated vasodilatation. This reflex has been hypothesised to act partly via the release of neuropeptides from sensory nerve endings (Hagerfelt et al., 1978). Despite its role as a main target for the proinflammatory mediators released in the initial stages of an allergic reaction, the magnitude and time course of blood flow during allergic skin reactions is not yet totally clear. Laser Doppler represents a means with which to investigate the microcirculation in various tissues (Olsson et al., 1988). This technique confers the invaluable opportunity to measure blood flow changes non-invasively in intact skin and on a continuous basis.

Various researchers have studied the change in blood cell flux following provocation of the skin with histamine and allergen (Bisgaard and Kristensen, 1984; Juliusson and Bende, 1987; Olsson et al., 1988; Serup and Staberg, 1985; Staberg et al., 1984). Following histamine provocation the blood cell flux in the wheal area increases rapidly and subsides during the first hour. Allergen provocation results in a more sustained response, with blood cell flow remaining elevated for several hours in the wheal area. The time course of the inflammatory response was found to be similar

following histamine and allergen provocation (Evilevitch et al., 1999). The difference is potentially attributable to the wide range of mediators released in response to allergen provocation.

During skin test with an atopic challenge, the allergen is driven through the skin surface using a small disposable prick test lancette delivering it to the dermal-epidermal junction (Smith, 1992). If the patient is sensitive to the allergen in question then interaction of the allergen with the IgE coupled to high affinity receptors on mast cells will trigger the release of histamine and other inflammatory mediators. In conjunction with neuronal reflexes, the mediators will cause the formation of a wheal and flare reaction which peaks within 15 minutes and may in highly atopic individuals be accompanied by local inflammation 6-12 hours later.

The intensity of an allergic reaction depends upon various factors such as the number of molecules of specific IgE bound to the mast cell membrane, the number of mast cells present in the skin, the ease with which these cells cause the release of chemical mediators, the reactivity of the skin to the mediators themselves, particularly histamine, and the amount of antigen administered (Ownby, 1988).

6.1 The laser Doppler technique

The laser Doppler technique measures blood flow in the very small vessels of the microvasculature. The tissue thickness sampled is typically in the region of 1 mm, the capillary diameters about 10 microns and the velocity spectrum measurement ranges between 0.01 to 10 mm/sec.

The technique is based upon the Doppler principle whereby low power light from a monochromatic stable laser such as a Helium Neon gas laser or a single mode near infrared laser diode is scattered by moving red blood cells and as a consequence its frequency is broadened. This technique has been used to measure blood flow in neural tissue, muscle, skin, bone and intestine. The frequency broadened light, together with laser light scattered from static tissues, is detected and the resulting data travels via optic fibres to an analyser where it is processed to give a measurement of blood flow. The Doppler shift frequency range is typically about 20Hz to 20 KHz for a well perfused region of the microcirculation such as the fingertips. The relationship between the flow meter output and the flux of the red blood cells is linear.

Laser light can be directed to the tissue service via an optic fibre or as a light beam. We used a fibre optic monitor that terminates in an optic probe that can be attached to the surface of the skin. One or more light collectors also terminate in the probe head and these transmit a proportion of the scattered light to a photo detector and the signal processing electronics. Normal fibre separations in the probe head are a few tenths of a millimetre so blood flow is measured over a tissue volume of about one millimetre cubed or smaller.

When a larger volume of tissue is stimulated to vasodilate (as seen in the experiments described in this thesis), the measured blood flow changes in the small tissue volume measured directly are taken to be representative of the larger volume.

The term used to describe blood flow measured using the laser Doppler technique is 'flux' which is a quantity proportional to the product of the average speed of the blood cells and their number concentration (blood volume). This is expressed in arbitrary 'perfusion units', and is calculated using the first moment of the power spectral density.

The objectives of this experiment are outlined below:

- To establish the time courses of itch and blood flow increase in the reactions to histamine and type I allergens
- To compare the intensities of response to histamine exhibited by atopic and non-atopic individuals
- To compare the intensities of response to histamine and type I allergens in atopic individuals

6.2 Methods

Subjects were recruited and written informed consent was given by all volunteers. Approval for the study was given by South Buckinghamshire Local Research Ethics Committee. The study was conducted from January to March 2002, and therefore was outside of the main seasonal rhinitis season. Skin thickness is a factor in carrying out prick tests so the relatively narrow time frame within which the study was carried out allows us to control for this to a certain extent.

No subject had a past or present history of skin disease or current systemic disease and none were taking any current medication.

The experimental groups were split as follows:

- Atopics – Healthy females with a current history of mucosal atopy to grass pollen/house dust mite, verified by preliminary prick testing (n = 14; age range 20 - 58 years, mean age 39 years)
- Non-atopics – Age matched, healthy females, negative on prick testing with standard battery of type I allergens (n = 14; age range 21 - 57 years, mean age 40 years)

Atopics were challenged with their specific allergen, histamine and a saline control on three separate occasions. Non-atopics were challenged with saline and histamine. All the prick tests were done blind and the order of conditions was randomised across subjects to avoid expectancy and order effects respectively.

Subjects were given a thorough medical screen before commencing the experiment, and all tests were carried out in the proximity of qualified medical staff.

The test substances were administered via a standardised prick test using a single use prick test lancette. A small cut was made in the laser Doppler probe pad so that the shoulder of the prick test lancette could rest against the centre of the probe allowing for standardised site of delivery. The laser Doppler probe was secured on the skin surface so that it was in place prior to the prick test so that recording of physiological parameters could start at the same time as the prick test. The probe ends were secured to avoid movement artefacts.

The site for prick test injection was the volar aspect of the forearm. Since the difference in blood flow changes between the prick test site and the flare are not that different, we measured in the flare region, approximately 1 cm from the site of prick test. Subjects faced away from the trace recorder in order to avoid biofeedback. They were seated with their test arm (left arm) resting upon a cushion on a chair arm and the other hand operating the sliding VAS scale. The prick test substance was measured (5ml) using a calibrated needle. The site was 0.75 centimetres from the prick test site and 1cm outside the flare region.

Blood flow and skin temperature were measured for twenty minutes following a single prick test.

A computerised visual analogue scale was used to measure the subjective itch intensity ratings for each subject. This was calibrated before each experiment. On this scale the desire to scratch threshold corresponded to a rating of '3'. The scale was operated by sliding a scale up and down to correspond to the amount of itch felt. The maximum score was '10', which represented the most intense itch ever. Each subject was allowed a few minutes to practice using the scale prior to the experiment to get familiar with the scale and how much movement was required. Itch intensity was rated for 20 minutes. Subjects were prompted to rate the itch intensity every 30 seconds for 15 minutes, and then only if they noticed a change in intensity. The time of test was recorded after a baseline recording period prior to the prick test.

All experiments took place in the autumn and the ambient temperature was 21 degrees in each case.

Fig. 6.0 Photograph of the experimental layout



The laser Doppler fluxometry equipment used was a DRT4 laser Doppler perfusion monitor system and associated software (Moore instruments Ltd, Axminster, Devon,

UK, http://www.moore.co.uk). This enabled continuous recording of blood flow (x2/sec) for 2-5 minutes prior to prick testing and 20 minutes post prick testing.

6.3Testing procedures

Skin prick testing on atopics was carried out on the volar forearm using grass pollen, histamine or house dustmite extracts as well as a negative control (Bio-Diagnostics, Upton-upon Severn, Worcestershire, UK). Skin testing on non-atopics was carried out using histamine and negative (saline) control only. Prick test lancettes were sterile single use lancettes from Bio Diagnostics employed for sub-epidermal penetration of solution. The testing was carried out on consecutive days for different solutions, and the room temperature was maintained at 22 degrees throughout.

The measurements were taken from the flare region, 1cm distal to the actual prick site. Prior to using the blood flow measurements, baseline correction (log 10) was made to the laser Doppler fluxometry measurements to produce relative change values.

Itch was reported by subjects using a linear visual analogue scale from 0 to 10 (0= no itch, 3= desire to scratch, 10= worst itch imaginable). Continuous electronic recording of itch rating for 20 minutes following prick testing was carried out using a scale slider operated by the subject's opposite hand to that being tested. The data was analysed by the DRT4 monitor.

6.4 Statistical analysis

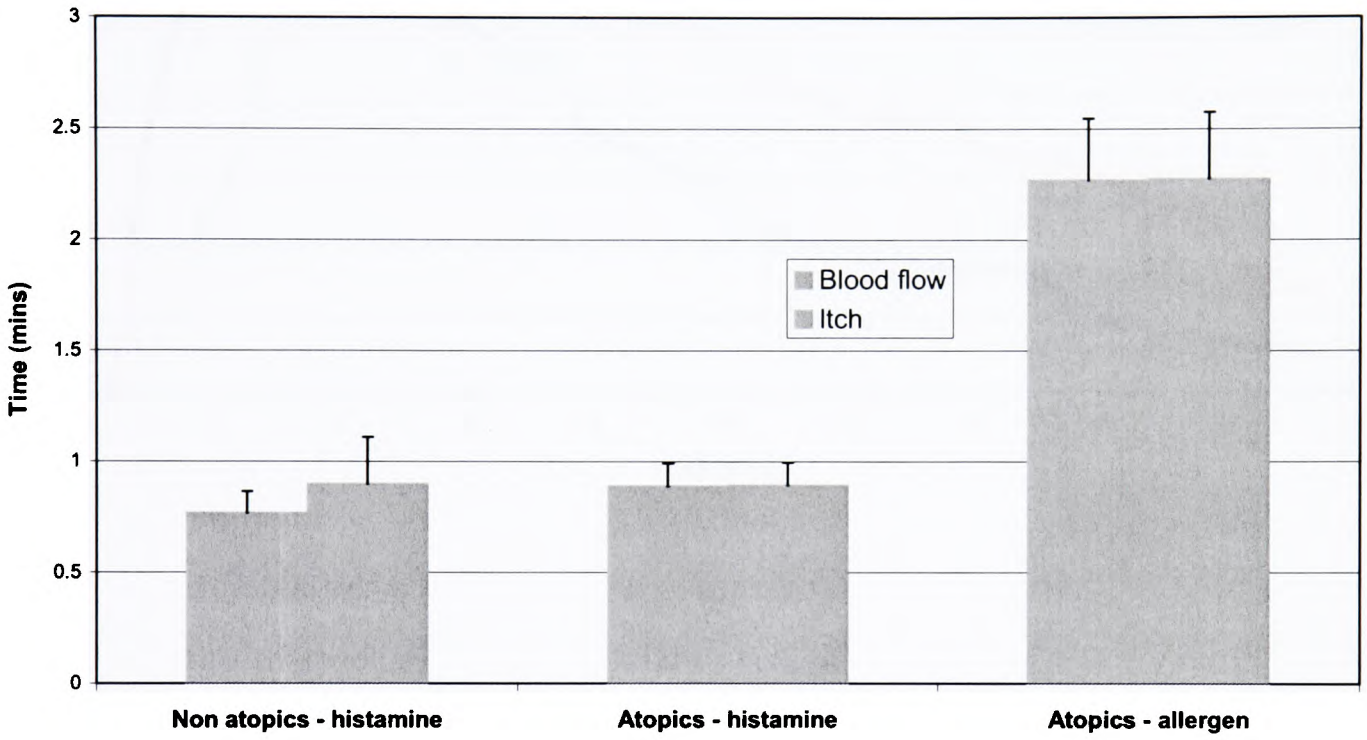
Within Excel (Microsoft Office software) a macro was written to summate data values to every 15 seconds. Mean values and standard deviations were calculated for each cohort and each prick test solution. Grass pollen and house dust mite data were combined to produce the 'type 1 allergen group'. An analysis of variance (ANOVA) for repeated measurements was carried out using Statistica (Statsoft, N.C., USA) software. The significance level chosen was $p < 0.05$.

6.5 Results

The data from the laser Doppler fluxometry and the behavioural data from the VAS itch ratings were collected and analysed.

The main findings are now discussed. The onset of itch and increased blood flow occurred significantly more rapidly with histamine (< 1 minute) compared to the type 1 allergens (> 2 minutes), ($p < 0.0001$). This is illustrated in the following graph.

Fig. 6.1 Time of commencement of response. This graph shows the time taken for a change in blood flow and itch intensity rating to occur from the time of prick test for the atopic group (allergen and histamine) and non-atopic group (histamine).



Itch and increased blood flow begin simultaneously for both histamine and type I allergens.

It can be seen that the responses of atopics and non-atopics exhibit similar responses to histamine (see figs 6.1, 6.2, and 6.3).

Fig. 6.2 Itch response following prick testing. This graph shows the profile of itch intensity rating changes over time for the two subject groups in response to different prick test challenges.

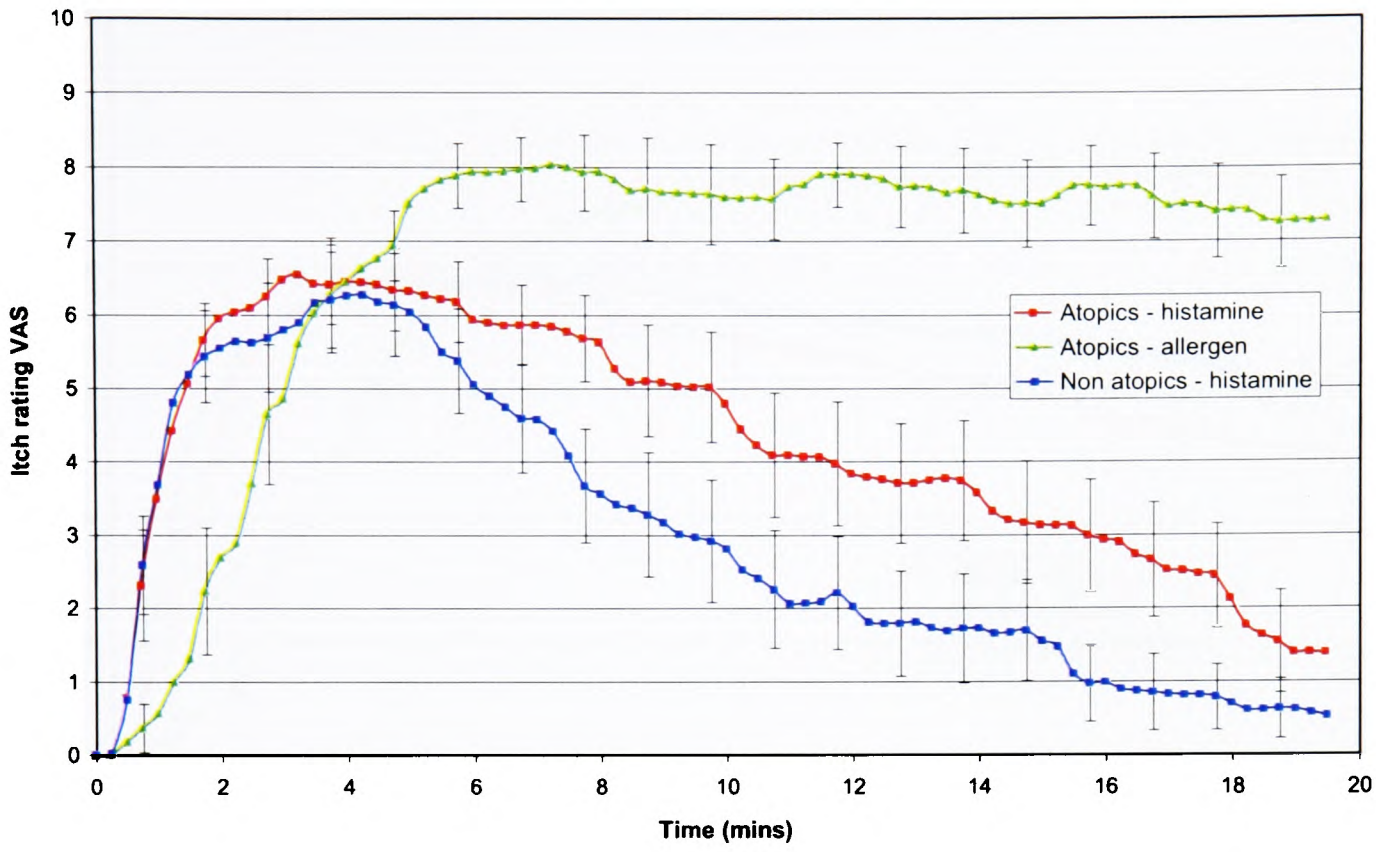
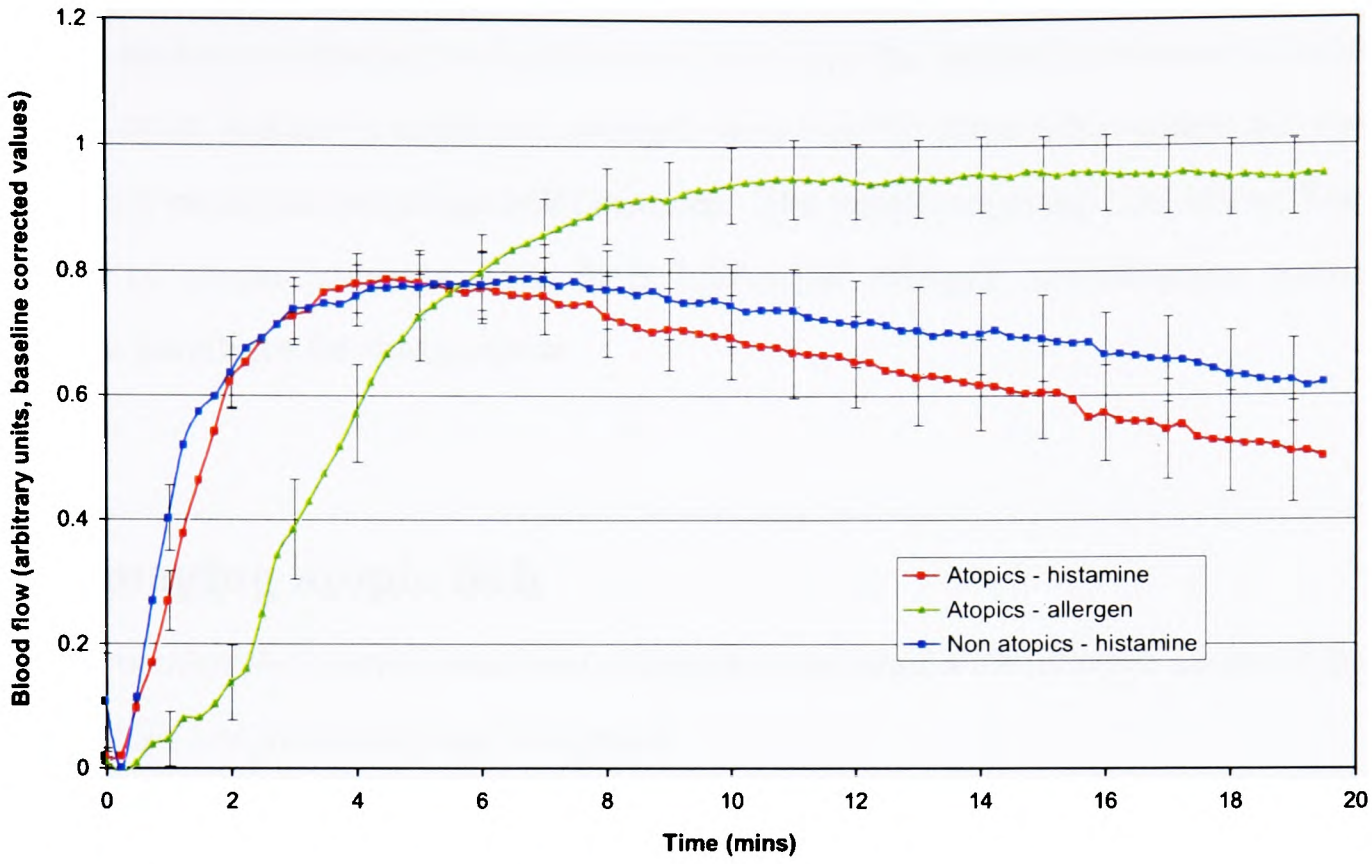


Fig. 6.3 Blood flow changes following prick testing. This graph illustrates the changes in skin blood flow to the flare region in atopics and non-atopics in response to their different prick test challenges.



The intensity of the response to type I allergens seen in atopics was significantly greater in magnitude to their response to histamine, both in terms of the degree of itch, and the increased blood flow ($p < 0.005$). This can be seen in figs 6.3 and 6.4.

The response to type I allergens seen in atopics was of a significantly more protracted duration than their response to histamine ($p < 0.005$).

Negative control solution produced neither a significant rise in blood flow nor any sensation of itch in either experimental group. The data is not shown.

6.6 Discussion and Conclusion

This study has shown that the prick test responses manifested in atopics to histamine and to their appropriate type I allergen vary significantly, both in terms of intensity of itch and blood flow increase, and in time course. The slightly delayed initiation of response seen following the administration of allergen almost certainly relates to the time taken for the molecular interaction between allergen and IgE-bearing dermal mast cells to take place. The subsequent degranulation of mast cells is

known to lead to the release of a number of pharmacological agents capable of inducing itch, in addition to histamine itself, including platelet activating factor, prostaglandins and proteases (Greaves, 1998). It is, presumably, this more potent combination of substances which gives rise to a more intense and prolonged itch.

Of importance in this study is the selection of a test suitable for use in future studies on brain activation in relation to itch where the sensation induced should have a rapid onset and be of sufficient strength to be readily discernible within the stressful and noisy environment of the MRI scanner. The results reported here show that prick testing of atopic subjects with their individual allergen undoubtedly provides a suitable paradigm for this purpose.

6.7 Imaging atopic itch

In this section the central neuronal changes associated with allergen induced itch are using fMRI are measured and discussed..

6.7.1 Methods

Eight volunteers were recruited and gave their informed consent to take part in the study. All the volunteers were atopic individuals and they all completed a thorough medical screening questionnaire prior to taking part in the study. A medically qualified person was present throughout the screening process and the experiment itself.

All subjects were challenged with their specific allergen (this information was confirmed via allergy testing prior to the experiment itself and had been given as part of the allergy screening interview), and with a control (saline). The subjects were blind to the condition and the order was randomised across subjects to avoid order and expectancy effects.

There were five female and three male healthy right handed volunteers, mean age 44 years ± 3.4 years.

Each subject was asked to lie as still as possible in the magnet, where one drop of the allergen or control substance was dropped onto their right foot in between the toes of the foot and a prick test lancette was used to prick the drop through the horny outer layer of the skin. Two pricks were made over the same drop. The subject was

then asked to rate the itch sensation every 30 seconds when a scale was presented on the screen asking them to rate the itch intensity. The subjects were asked to assign a rating score from 1 (no itch) to 5 (worst possible itch) whilst the rating scale was present on the screen. The ratings of itch intensity were therefore obtained during the experiment at 30 second intervals.

fMRI data were collected throughout and for 20 minutes after the prick test at 3T (TR=3.0, TE=30ms, 21x6mm axial slices, 360 volumes, flip angle 90 degrees). A T1-weighted high resolution scan was taken for anatomical overlay of activation maps (see later).

6.7.2 Statistical Analysis

Analysis of the fMRI images to identify regions showing significant changes in BOLD signal (Ogawa et al., 1993) was carried out with a multistage process using the image analysis package FEAT (http://www.fmrib.ox.ac.uk/fsl), an extension of MEDx (Sensor Systems, Sterling, VA., USA). The following pre-statistics processing was applied from within the FEAT package: motion correction using the SPM realign procedure (Friston et al., 1995) spatial smoothing using a Gaussian kernel of full-width half-maximum 5.0 mm; mean-based intensity normalization of all volumes by the same factor.

The statistical portion of the analysis was carried out using the FEAT package using the general linear modelling (GLM) method (Friston et al., 1995). A description of the experimental design is modelled, and this is then fitted to the fMRI data which indicates where the brain has shown activation in response to the stimuli within the paradigm. Within FEAT, the GLM method is known as FILM (FMRIB's improved linear model) (Woolrich et al., 2001). Three explanatory variables were used to model the time course data for each subject on a voxel by voxel basis. These were the itch intensity (derived from on line ratings) from the prick tests, and the itch ratings using the button press. The EVs used in this experiment to model the activation in the brain are: EV1 activation from the prick test itself, EV2 activation from the button press responses as subjects rated the itch every 30 seconds, and EV3 the curve of itch intensity rating itself. These were modelled within the GLM independently. Each explanatory variable results in a parameter estimate image. These show the strength of fit of the waveform to the fMRI data at each voxel. This

parameter estimate image can be converted to a t-statistic image by dividing it by its standard error (derived from the residual noise after the complete model has been fitted to the data). A standard statistical transformation enables the t-image to be transformed into a Z statistic which is an activation map, more clearly showing areas of activation as a result of the paradigm variables (itch intensity and the prick test itself). In order to analyse the activation which correlated with the itch intensity ratings given by subjects the raw ratings obtained in the scanner were interpolated in order to obtain the same number of time sampling points as there were in the fMRI data. The time structure of the scanning protocol was the acquisition of 12 seconds of dummies and 60 seconds of baseline with the substance being injected via a double prick test at 1 minute 12 seconds in each case.

A fixed effects group cluster analysis (second level analysis) was carried out on the subjects' activation maps to produce representative group results. The thresholding parameters used for the fixed effects analysis were a Z-score threshold of 2.0 and a probability value of P= 0.05 for cluster significance (Worsley et al., 2002).

6.7.3 Imaging results

The group level fixed effects analysis results of the fMRI data are illustrated with some fMRI images (Fig. 6.4).

Fig. 6.4 Series of fMRI images showing group activation (z= 2.00, p = 0.01). Areas shown are detailed in the table of Talairach coordinates (Table 6.0).

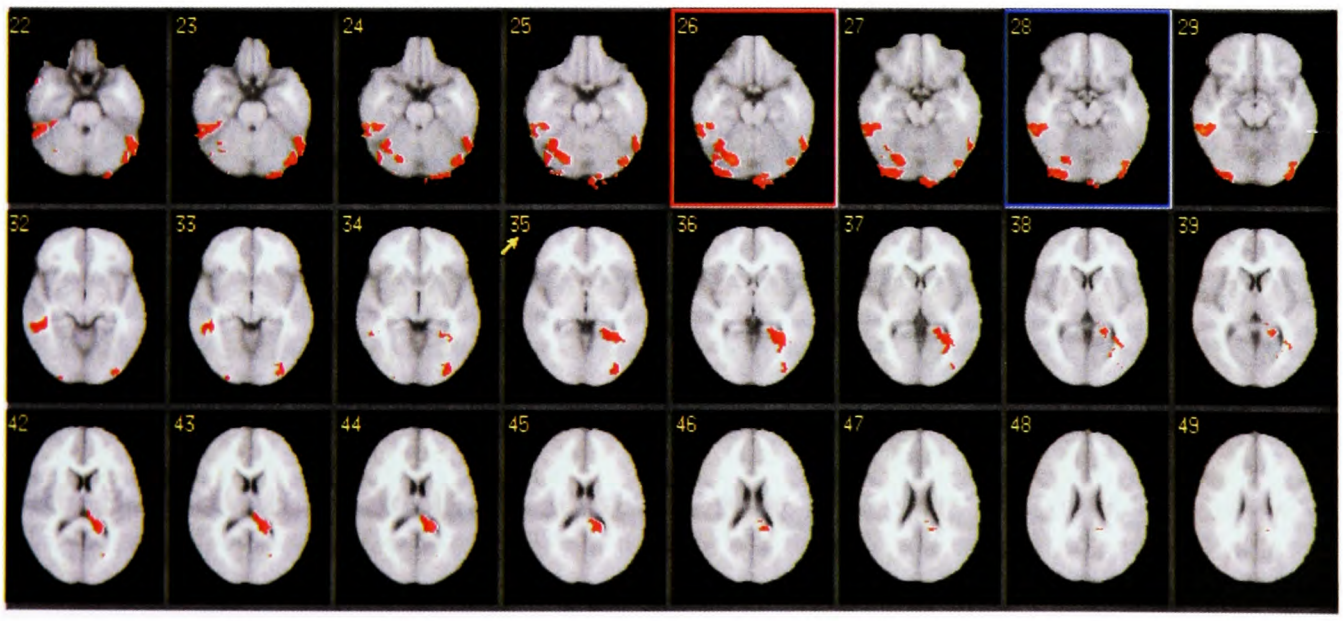


Table 6.0 Talairach coordinates and z score values for the regions of the brain found to be activated in the group level fixed analysis of the allergen data at z= 2.0, p= 0.01.

Laterality	**Talairach co-ordinates**	**Region**	**z-score value**
Bilateral	-34, -66, -22 -52, -52, -22	Cerebellum	3.40 3.12
Right	56, -40, -16	BA20 inferior temporal gyrus	5.06
Right	56, -38, -12	BA20/21medial temporal gyrus	5.22
Left	-34, -88, -2.0	BA18, fusiform gyrus	3.42
Left	-22, -52, 2	BA19, near lingual gyrus	2.60
Left	-16, -40, 16	BA30/23 posterior cingulate cortex	4.29

6.7.4 Discussion

The initial experiments looking at the correlation between blood flow and itch perception revealed differences between the responses of allergic and non allergic individuals. The sensation induced by challenge with the individual's specific allergen is more intense and longer lasting than the response of non atopics to histamine. Atopic individuals also respond differently to histamine than do non atopic individuals.

From subjective reports obtained whilst carrying out the experiment, the itch sensation produced when atopics were challenged with their own allergen is more intense in nature and not such a pure itch sensation as that elicited by histamine. Some subjects reported a prickling sensation that was very unpleasant and difficult to ignore. Various atopic individuals rated their itch as the maximum itch intensity bearable using our itch sliding scale. One shortfall of the data collection in this study is that no qualitative information was obtained as to the nature of the itch sensation and the unpleasantness induced by the itch sensation. It is difficult to accurately

measure the unpleasantness of a noxious sensation because it is so subjective and different individuals assign different meanings to descriptors of the emotional side of the sensation. One way to do this might be to simply have a scale of unpleasantness with not unpleasant or neutral up to highly unpleasant and use a five point scale with two anchors at the bottom and top to rate unpleasantness of the stimuli. This would help differentiate between the sensations of itch found as a result of histamine prick test versus challenge with allergen. Future experiments could employ such measurements. This would also help the interpretation of any differences in central activation between histamine and allergen induced itch.

Future studies could also record empirically the difference in the nature of the atopic itch compared with the histamine induced itch. Various scales measuring the intensity of different qualities (stinging, pricking, pain, aversiveness) that formed part of the itch experience could be measured and recorded. The itch caused by provocation with allergen tends to be reported as more intense and long lasting and pricking and strongly aversive and more unpleasant than the purer sensation caused by prick testing with histamine.

According to intensity ratings of histamine by atopics and non-atopics the intensity of the itch induced by histamine prick test was not more intense in atopics compared with non-atopics. The difference was seen in the response to allergen by the atopics which was more intense and more protected in nature.

The data were analysed using three EVs to model activation. The first was the activation due to the prick test, the second activation attributable to the itch rating using the button box. The third EV modelled the itch intensity rating obtained by using the online ratings obtained in the scanner and interpolating them so that the same numbers of rating points were available as there were volumes of FMRI data whilst keeping faithful to the shape of the itch intensity profile.

It is clear that fMRI investigation of allergen-induced itch warrants more attention, and further study. The initial pilot study shows that the areas activated by allergen-induced itch include the cerebellum, which may relate to intention to scratch or to some actual minor motion related to the itching sensation. By using the foot, instead of the hand, it was easier to immobilise the foot so that it did not move and also purely for logistical reasons, it was easier to access the foot as it is possible to reach it when the subject is in the scanner. Subjects were asked not to move throughout the experiment, but by the nature of the prick test, some movement does

occur and mostly this can be modelled out during the motion correction phase of the data preparation.

Other areas of activation include the contralateral medial and inferior temporal gyri (BA 20 and 21). Activation was also found in the ipsilateral fusiform gyrus although no clear hypothesis as to the function of this area in itch processing has been developed in this Thesis. One potential role would be a motor preparation role, but considerable further investigation would be required to elucidate the function of this area further.

The fusiform gyrus has been shown to activate in response to emotionally loaded visual cues (Geday et al., 2003)and does have links to the prefrontal cortex which is involved with the processing of unpleasant sensory information. When subjects were required to rate the unpleasantness or pleasantness of pictures this correlated with strong activation in the left fusiform gyrus (Taylor et al., 2003). Activation in the opposite hemisphere was found by Lang and Bradley (Lang et al., 1998)when participants were shown emotional pictures. This would suggest a possible role for the fusiform gyrus in the processing of the unpleasant emotional component of the itch sensation.

The fusiform gyrus has been shown in some studies to be involved with memory processing, particularly recognition memory (Onitsuka et al., 2003). This could fit in with the results of this experiment as the subjects had to rate the itch intensity and would need to recall how the itch felt in the time before they made their itch rating using the computerised scale.

The other activation found in this study, which is of interest, is activation in the ipsilateral/midline posterior cingulate cortex (BA 30/23). This region has a role in the processing of the unpleasant emotionally laden element of information pertaining to noxious thermal stimuli, and has recently been found to activate during itch studies (see Chapter 5).

Analysis of the saline data did not produce any results significant at the group level. The activation as a result of the prick test was also investigated but again no data exceeded threshold at the group level. When these results are compared to the results from histamine in Chapter 5, it is clear that only a few regions commonly activate across these two studies: posterior cingulate and mid-temporal gyrus. Despite the increased perception of itch from the allergen compared to histamine prick (Figure 6.3) found in this study, the imaging data from the two studies suggests a larger

recruitment of brain regions to process histamine-induced itch occurs when compared to allergen-induced itch. However, if the Z-scores for the brain areas commonly activated across the two studies are compared, it is clear that for both areas the Z-score is higher in the allergen study. These studies were not matched, because of time constraints of the atopic subjects, therefore this latter comparison should not be over-interpreted but does support further work being done in this area.

6.7.5 Conclusion

Extension of the work performed in Chapter 5 to an investigation of atopic sufferers with their allergen induced itch was carried out so that a more intense itch sensation could be developed that might yield stronger fMRI results. In addition, it was surmised that the quality of the itch sensation derived from histamine versus the allergen in atopics might be sufficiently different to manifest in measurable differences in the fMRI data. The results reported here do indeed suggest that there are regions within the brain common to the processing of both histamine and allergen. There is a stronger activation in the allergen study compared to the histamine study; a result reflected in the higher itch rating for the allergen-induced itch. In addition, several other brain areas were activated in histamine-induced itch that did not activate in the allergen study. The precise meaning of this is not clear but might relate to the quality differences noted by subjects but not rated as there are no scales for such subjective descriptions.

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CHAPTER 7: Correlating subjective perception with central neuronal activation in histamine-induced itch

In Chapter 5, a paradigm was developed for inducing a pure, robust and pain-free itch sensation suitable for fMRI experimentation. Histamine prick tests were used to induce the pruritic stimulation. Following the work in the previous chapter (Chapter 6), which looked more closely at the relationship between the perception of itch intensity and the underlying blood flow changes, it was decided to develop our earlier fMRI paradigm to incorporate on-line ratings of the itch sensation.

7.0 Introduction

The previous chapters have shown the development of a pain-free itch paradigm suitable for use in an MRI environment. The reader is referred to Chapters 5 and 6 for the background to the study, and reasons for undertaking it. From these experiments, it was predicted that analysis of the fMRI data would be more meaningful and robust if the subjective perception of itch was determined on-line and used as the input function within FEAT. This would better allow the specific correlation of individuals' neuronal activation changes with subjective perception at that time. Here, the measurement of perceived itch intensity is collected online during fMRI data collection with concurrent histamine-induced itch and therefore provides for a more rigorous data analysis procedure.

7.1 Methods

Nine healthy right-handed subjects (six male, 3 female, mean age 34.4 years ± 2.3) were recruited. Full written informed consent was obtained and a thorough medical screen carried out prior to the study to determine suitability for the experiment and MRI compatibility.

Subjects were asked to remain as still as possible during the experiment. They were given histamine via a prick test to the right foot at time zero, five and ten minutes, using the final optimised procedure described in Chapter 5.

The scale was explained to the subjects and they were told to rate the intensity of the itch they felt when they saw a rating scale projected on the screen. They could view this screen from their supine position in the scanner via prism glasses that could be fitted with corrective lenses where necessary. The itch rating scale used was 0 (no itch) to 5 (worst imaginable itch). They used a button press to rate the itch they felt whenever the scale was projected. Ratings were obtained every 30 seconds.

The reader is referred to Chapter 5 for the background information pertinent to the study. This study is an extension of the pilot study described in full in Chapter 5, and the methods used are identical in every way apart from the measurement of the itch sensation. This is also described in Chapter 6 where we used online ratings to investigate the response to allergen-induced itch.

7.2 Image Acquisition

A T1-weighted high-resolution scan was taken for anatomical overlay of activation maps (see later).

Behavioural data were acquired during the experiment in the form of itch intensity ratings. These were then interpolated so that they contained the same number of rating scale data points as fMRI data points. The itch intensity ratings were used as an EV in the modelling of the fMRI data. This graph was included as a model to find out which brain areas showed activation correlating with the changes in itch intensity rating over time. The prick test was not painful but was still a sensory stimulus, and therefore the timings of this and its duration were included as another variable into the experimental model to look for any activation attributable to the prick test itself.

All subjects were scanned at 3T, using similar protocols as previously described (TR=3.0, TE=30ms, 21x 6mm axial slices)

7.3 Statistical Analysis

Analysis of the fMRI images to identify regions showing significant changes in BOLD signal (Ogawa et al., 1993) was carried out with a multistage process using the image analysis package FEAT (http://www.fmrib.ox.ac.uk/fsl), an extension of MEDx (Sensor Systems, Sterling, VA., USA). The following pre-statistics processing was applied from within the FEAT package: motion correction using the SPM realign

procedure (Friston et al., 1995); spatial smoothing using a Gaussian kernel of full-width half-maximum 5.0 mm; mean-based intensity normalization of all volumes by the same factor.

The statistical portion of the analysis was carried out using the FEAT package using the general linear modelling (GLM) method (Friston et al., 1995). A description of the experimental design is modelled and fitted to the fMRI data that indicates where the brain has shown activation in response to the stimuli within the paradigm. Within FEAT, the GLM method is known as FILM (FMRIB's improved linear model) (Woolrich et al., 2001). Three explanatory variables were used to model the time course data for each subject on a voxel by voxel basis. These were the itch intensity (derived from the online itch intensity rating score profile) the prick itself, and the button press motor responses. These were modelled within the GLM independently. Each explanatory variable results in a parameter estimate image. These show the strength of fit of the waveform to the fMRI data at each voxel. This parameter estimate image can be converted to a t-statistic image by dividing it by its standard error (derived from the residual noise after the complete model has been fitted to the data). A standard statistical transformation enables the t-image to be transformed into a Z statistic which is an activation map, more clearly showing areas of activation as a result of the paradigm variables (itch intensity and the prick test itself).

A fixed effects group cluster analysis (second level analysis) was carried out on the subjects' activation maps to produce representative group results. The thresholding parameters used for the fixed effects analysis were a Z-score threshold of 2.0 and a probability value of P= 0.05 for cluster significance (Worsley et al., 2002)

7.4 Functional imaging results

7.4.1 Saline

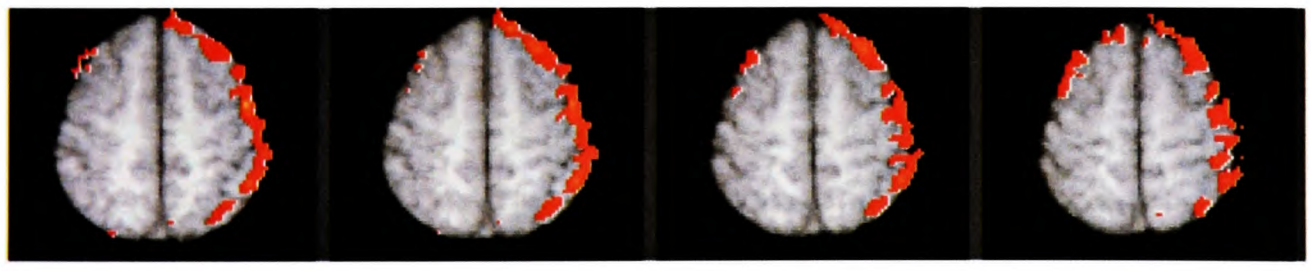
The group fixed effects analysis of the fMRI data showing activation as a result of saline did not reach significance even at a statistical threshold value of Z = 1.8. This would suggest that any activation attributable to administration of saline is not significant, which confirms its value as a control substance. In order to analyse the activation as a result of saline prick testing, the curve obtained for the same

subject's histamine itch intensity results was put in to the model for the saline. This approach has been used in recent pharmacological studies (Wise et al., 2002).

7.4.2 fMRI results from histamine group fixed effects analysis

One of the problems encountered with this study was motion artefact in the imaging results. This arises partly due to the nature of the paradigm (the prick test although not painful does produce some very minor response) and partly due to the nature of the stimulus. The pruritic stimuli necessarily induce the urge to scratch the affected area. This second factor could result in pre-motor areas becoming active. In order to address the motion problem, the importance of NOT moving in the scanner was emphasised to subjects, and their head was packed in securely with foam and a tape across the forehead. The following analysis was carried out with an additional step to remove motion artefact. During the motion correction pre-analysis stage of the fMRI data, motion correction parameters are obtained which can then subsequently be entered into the GLM and modelled out of the data. Any data that faithfully matches the profile of the motion correction parameters can then be removed, resulting in cleaner and more valid data to perform statistical analyses upon. There is a classic shape that is commonly indicative of motion artefact and we do see some of this occurring in our results, despite our best efforts to eliminate it from the data. Figure 7 (below) illustrates the appearance of such motion-artefact on the image.

Fig. 7 An example of motion artefact in fMRI data: This data is from the group fixed effects analysis. Note the ringed appearance of the activation.



The main results of the fixed effects group analysis at Z = 2.0, p= 0.05 are shown below (table 7), as a table of Talairach coordinates.

Table 7 Table of Talairach coordinates describing the activation arising in the brain as a result of histamine-induced itch (Fixed effects Group analysis).

Laterality	Talairach coordinates	Brain Region	Z score
Left	-12, -78, 40	Precuneus	3.50
Bilateral	46, 20, 50 -48, -10, 52	Premotor BA 8	3.91 3.94
Right	32, -78, 42	Fusiform gyrus	4.70
Left	-12, -74, 36	Precuneus BA7	4.87
Left	-20, 54, 32	BA9, frontal gyrus	6.46
Left	-62, -10, 26	Premotor areas/postcentral gyrus	5.30
Left	-32, 60, 18	Orbitofrontal cortex BA 10	3.88
Left	-64, -46, 14	Superior temporal gyrus	5.99
Bilateral	-14, 22, 4 14, 22, -2	Thalamus	3.56 6.27
Left	-62, -34, -6	Medial temporal gyrus	5.90
Left	-30, 44, -10	Prefrontal cortex	6.16
Right	50, -52, -20	Cerebellum	5.30

These coordinates can be seen illustrated in a series of FMRI activation images, which are rendered onto a group average structural image in Talairach space.

Fig. 7.1 fMRI data from a Group fixed effects analysis of histamine-induced itch.

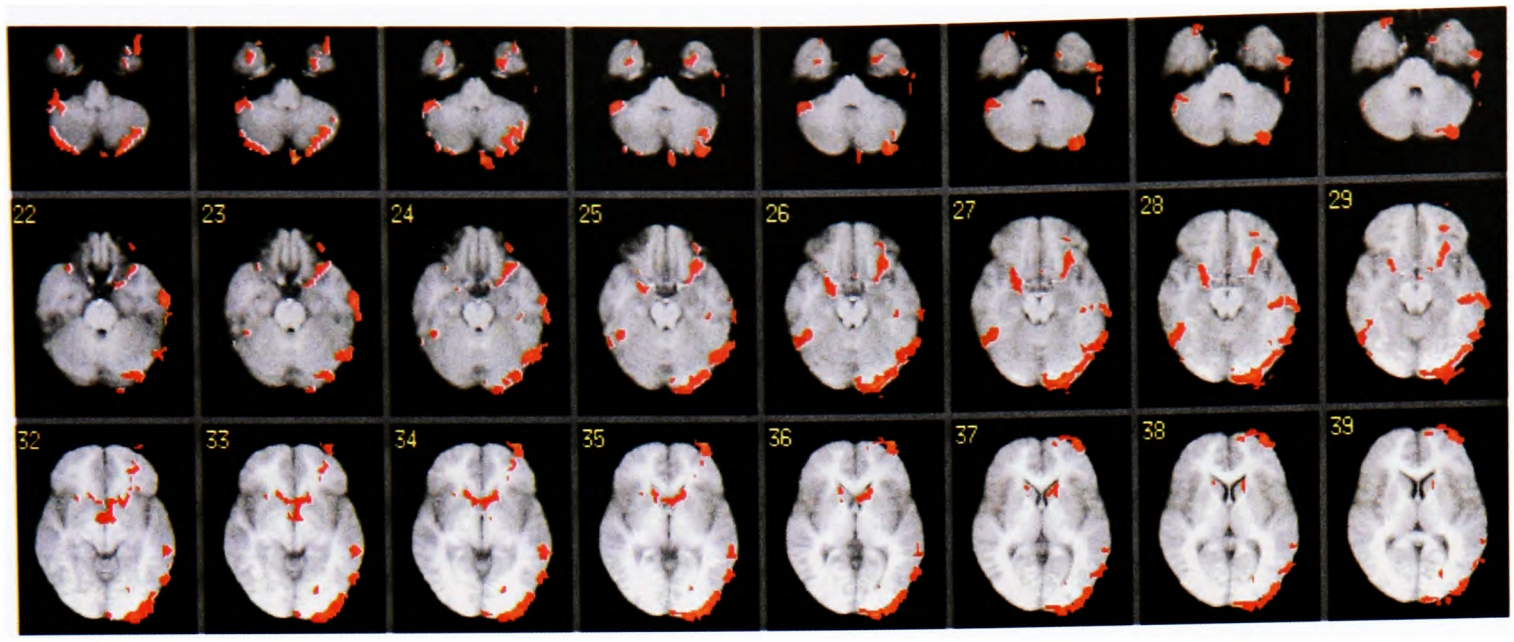


Table 7.1 Comparison of activations as a result of online and pre-fMRI VAS ratings

On-line rating of histamine-induced itch	***Bench rating of histamine-induced itch***
C/lateral (L) precuneus	*C/lateral (L) precuneus*
I/lateral (R) fusiform gyrus	*C/lateral (L) fusiform gyrus*
C/lateral (L) OFC	*Bilateral OFC/ACC*
C/lateral (L) PFC	*Bilateral PFC*
C/lateral (L) mTG	*C/lateral (L) mTG*
C/lateral (L) sTG	*I/lateral (R) sTG*
Bilateral premotor area	
C/lateral (L) premotor/post central	
C/lateral (L) frontal gyrus	
Bilateral thalamus	
I/lateral (R) cerebellum	
	Bilateral caudate
	Bilateral insular cortex
	C/lateral posterior cingulate
	Bilateral posterior parietal lobe
	Bilateral medial occipital lobe

Abbreviations: PFC prefrontal cortex; OFC orbitofrontal cortex; sTG superior temporal gyrus; mTG medial temporal gyrus; inf TG inferior temporal gyrus

Table 7.2 Comparison of histamine-induced central activation (on-line ratings) with activation as a result of allergen induced itch

Histamine-induced itch activation (online)	***Allergen-induced itch activation (online)***
I/lateral (R) cerebellum	*Bilateral cerebellum*
C/lateral (L) fusiform gyrus	*C/lateral (L) fusiform gyrus*
C/lateral (L) mTG	*I/lateral (R) mTG*
	C/lateral (L) posterior cingulate
	I/lateral (R) inf TG
C/lateral (L) sTG	
C/lateral (L) precuneus	
Bilateral premotor cortex	
Bilateral thalamus	
Bilateral OFC/ACC	
Bilateral PFC	
C/lateral (L) post central motor	
C/lateral (L) frontal gyrus	

Abbreviations: PFC prefrontal cortex; OFC orbitofrontal cortex; sTG superior temporal gyrus; mTG medial temporal gyrus; inf TG inferior temporal gyrus

7.5 Discussion

The results of this study, which used the online itch rating paradigm developed in the allergen-induced itch cohort, further validate our results. The central brain

regions found to be activated in response to histamine-induced itch show considerable overlap with those activated in our initial pilot study. This experiment builds upon previous work reported in this Thesis, using behavioural itch intensity profiles obtained outside of the scanner to correlate with the fMRI data pertaining to the processing of pruritic stimuli. This study takes this further by using on-line rating of the itch sensation through out the experiment, providing behavioural ratings of itch intensity acquired during the scanning session. The results of this study are broadly consistent with the central activations found in the allergen-induced itch study (Chapter 6) and with existing imaging studies of the central correlates of itch processing.

There are some similarities and differences between the central activations found in the online itch study and the pre-fMRI VAS studies. There were broad similarities in terms of activations in both studies in the precuneus, fusiform gyrus, orbitofrontal cortex and temporal gyri. This chapter goes on to discuss the functional significance of the activations found. The differences between activation using the online and pre-fMRI VAS paradigms is now discussed.

Most of the areas found to be active as a result of histamine induced itch in the online paradigm that were not observed in the pre-fMRI VAS study were motor areas including the cerebellum. These motor areas could also be involved in the intention to scratch to relieve the itch. The fact that the motor activation reaches threshold in the online study but not in the pre-fMRI VAS study could be explained by the additional motor component of the online rating. This can be explained by the fact that subjects were preparing to rate using the button press each time the rating scale was displayed upon the screen. There is likely to be some motor activation associated with the preparation to use the hand to rate the intensity of the itch. The premotor activity detected using the online paradigm is therefore an artefact of the method used. One way to begin to investigate whether or not these regions were activated as a result of motor planning in the rating of the itch would be to carry out the experiment without asking the subject to rate the itch intensity in one instance and another experiment where they were asked to rate the itch. It may then be possible to subtract the activation from the rating study away from the non rating study.

The online rating study revealed activation in the thalamus that was not observed in the bench rating study (pre-fMRI VAS). This could mean that the presence of activation was detected in the online study because the signal to noise

ratio in this paradigm was greater than in the other study. This region is fairly noisy and it can be difficult to detect activation with insufficient signal to noise ratios.

Fundamentally the same itch sensation was produced in both studies. In the online rating study the central correlates of this sensation were assumed to be time-locked to a one-off itch intensity profile as a result of the itch sensation occurring at the same time as the rating took place in the scanner. The pre-fMRI VAS study used an itch profile derived from the average itch intensity calculated over 3 separate study sessions. In both studies the analysis of the fMRI data centred on detecting those areas activated in the perception of itch intensity, since it was itch intensity profiles that were entered into the analysis model. This assumes that the model will pick out areas involved in itch processing that are activated and that follow the itch intensity profile. In both studies, our ability to detect the areas involved in the processing of itch is biased according to the algorithm chosen for analysis of the fMRI data. In both the offline and online studies it is assumed by using the itch intensity profile to pull out brain regions involved in the processing of itch, that the perception of itch intensity is time locked to the fMRI data. The fact that the thalamus is found to activate in the online study but not in the pre-fMRI VAS study could be proof that the online method is more accurate and more optimal for finding out which brain regions are involved with itch processing. However, this is not likely to be the case. From the current experimental data it is not possible to say which set of fMRI results is right. In both cases the sensation input to the brain is the same and it is likely that the regions involved in the processing of itch are drawn from both studies and comprise areas seen in by using both itch paradigms used in this Thesis. Using the online rating activation that is detected would have to be activation that is temporally locked to the fMRI data. When the average itch intensity profile from 3 sessions is used as a model to link to the fMRI data, this blurs the profile but does not necessarily make it a less reliable algorithm for the itch sensation. When the assumption is made, as it is in both cases, that the changes in signal in the fMRI data as a result of itch are time locked to the itch intensity profile then this model will only enable the detection of those regions where the activation is time locked to this profile. It could also be that a one-off rating session (i.e. the online rating study) is less reliable in some respects as it only provides a snap shot of the itch activation on that day. A composite profile averaged over 3 sessions could be more reliable. There are therefore limitations to the

model and it is not really possible to state the results from one or other of the two approaches is categorically right or wrong.

Activation in the insula, posterior cingulate and posterior parietal regions was found in the bench (pre-fMRI VAS) study but not in the online rating study. Again it is possible that such areas for some reason did not reach threshold in the online study. The posterior parietal regions are involved in attentional processing and could represent attention to the stimulus. The other regions are really involved in affective processing of unpleasant sensation. Again it is difficult to postulate the significance of this because this model would blur the input of the itch intensity profile and may enable the detection of areas that are not time locked to the fMRI data but which are involved in itch processing.

The consistent elements of central processing found in this study in response to histamine-induced itch that overlap with those activated by allergen-induced itch are the cerebellum (perhaps representing the urge to scratch), the inferior temporal gyrus and medial temporal gyrus and the fusiform gyrus.

One possible explanation for the activation found in the fusiform gyrus as a result of pruritic stimulation was discussed in the previous chapter (Chapter 6). The fusiform gyrus was activated when subjects were asked to rate the unpleasantness or pleasantness of pictures suggesting a possible role in the processing of unpleasant stimuli such as itch or evaluation of such stimuli for their relative pleasantness or unpleasantness (Taylor et al., 2003). This area is also activated by the presentation of emotional pictures (Lang et al., 1998) and given its links with the prefrontal cortex this could suggest a role for the fusiform gyrus in the processing of the unpleasant emotional component of itch stimuli. The prefrontal cortex is known to be involved in the processing of unpleasantness information pertaining to itch sensations (Drzezga et al., 2001).

The noticeable difference found in the histamine-induced itch compared to the allergen-induced and the earlier histamine-induced itch is that there is no activation in the posterior (or anterior) cingulate cortex in this cohort of subjects. The absence of cingulate activation could reflect a lower itch rating derived from histamine compared to allergen-induced itch (see ratings in Chapter 6). A heightened unpleasantness is perhaps associated with this stronger itch sensation. The posterior cingulate does play a role in the processing of unpleasant stimuli, reflecting the affective component, as

well as some pre-motor functions, which in itch could be related to the desire to scratch.

The other contrast is that there are a greater number of areas activated in the histamine-induced itch experiment compared to the allergen-induced itch. The pattern shows some similarities but the functional imaging results of these two studies indicate that the sensations produced when challenged with histamine and when challenged with one's specific allergen are distinct sensations, each warranting further research.

This study looked at the central correlates of histamine-induced itch and did not show any activation in the ACC, as others have found (Darsow et al., 2000; Drzezga et al., 2001; Hsieh et al., 1994). Activation in this region has been found in pain studies to correlate with the intensity of the stimulus (Coghill et al., 1994) and it is possible that the stimulus was insufficiently intense to generate activation in this area, and that the threshold for activation was not reached in this region. Imaging studies of itch have also linked activation in the ACC with the concentration of histamine, suggesting that activation in the ACC is related to intensity of itch as it is to pain (Drzezga et al., 2001).

This study showed that the prick test induction of pruritis using histamine results in activation in premotor areas which would be involved in the preparation for motor action, i.e. to scratch the affected area. This is consistent with other studies. The role of the fusiform gyrus and precuneus could also be linked to this intention to scratch but their significance is not known. The precuneus is close to supplementary motor areas (SMA) such as BA 8. Therefore activation here could be related to preparation to scratch. BA7 or the precuneus is located within the parietal lobe which is responsible for sensory processing and integration of sensory input, particularly visual input. This might mean that activation here in this study could be a result of visual cues when subjects were rating the intensity of the itch using the scales projected upon the screen. Another possibility to explain activation here is found I the pain literature. Rainville et al. (1999) found that the precuneus showed decreased activation when painful stimuli were rendered less intense as a result of hypnotic suggestion. The precuneus is a supplementary part of the sensory processing area and has been shown to activate in conjunction with painful somatic hallucinations (Bar et al. (Bar et al., 2002). Tracey et al. (Tracey et al., 2000)also found activation in the parietal cortical area in response to stimulation with painful thermal stimuli. Iadarola

et al (Iadarola et al., 1998) found that when skin was hypersensitised using application of capsaicin, brushing the skin resulted in activation in the precuneus (hyperalgesia was created using the capsaicin). All of these results suggest that further experiments would be necessary to separate the potential involvement of the precuneus in this study either as a result of the visual rating scales or due to the unpleasantness of the itch. Time course data could shed some light on this matter.

The orbitofrontal activation found in this study as a result of a pruritic stimulus was also found in a study of itch processing carried out by Darsow *et al* and has been found to activate in paradigms involving noxious stimuli (Lotze et al., 2001). This region was found to be active only in the contralateral hemisphere to stimulation. Prefrontal cortical activation was found here and also found to correlate in other studies with ratings of unpleasantness of itch (Drzezga et al., 2001), and this may be its role in this context, the analysis of the emotional or unpleasant aspect of itch.

In terms of similarity to pain processing regions, the prefrontal and orbitofrontal cortices are often found to be activated in such studies {Bantick, 2002 #37}. The other area that overlaps to an extent with a region found to show activation in response to noxious thermal stimuli is the bilateral thalamic activation found in the histamine study. This area is involved in pain processing (Coghill et al., 1994), although other itch imaging studies have not shown activation in this area. The literature that exists to date on the central processing of itch is not abundant, and the mix of methodologies is fairly heterogeneous. This makes it difficult to make comparisons between studies. Itch and pain are compared and contrasted in the next chapter of this Thesis (Chapter 8).

7.6 Conclusion

This method with which to investigate the central processing of histamine-induced itch in humans provides reproducible results in the MRI environment and furnishes us with invaluable information as to how the brain processes pruritic stimuli.

Armed with this information it is now possible to approach the development of drugs to combat itch with more insight and a more specific knowledge base. Allergen-induced itch produces a more intense and protracted pruritic response than histamine, but both areas share some commonality in terms of the central areas

involved in processing the sensation. However, allergen-induced itch possesses a more intense and distinct quality, and the fMRI study carried out here showed that this is reflected by activation in some brain areas involved with the processing of the aversive element of sensations (the posterior cingulate cortex). This indicates that there is a distinction between the two stimuli for producing itch.

The field of itch research is in its infancy, in terms of teasing apart the matrix of regions involved in processing pruritic stimuli. This is exciting and makes the prospect of new discovery and continued research an enthralling one. This study gives some preliminary findings as to which areas are involved and makes some comparisons with the regions involved in processing noxious stimuli, but there is much still to learn. The results reported here are internally consistent which is encouraging and suggests that the paradigms used in these investigations are robust.

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Overall conclusions

This Thesis describes research carried out to better characterise the central processing of two forms of unpleasant cutaneous sensation that produce the subjective experience of pain and itch.

Functional Magnetic Resonance Imaging (fMRI) was used because of its applicability for studying activation relevant to subjective perceptions such as itch and pain within the human brain.

An experimental acute noxious stimulus that produces reliable pain sensations and robust brain activations had already been developed within the pain group. It was immediately possible to adapt these paradigms for an investigation of how behavioural paradigms modulate pain perception in normal healthy subjects. Two large studies were done, both requiring considerable "off-line" paradigm development prior to fMRI experimentation. In the first study the Counting Stroop paradigm was used as a distracter during noxious stimulation. An area in the orbitofrontal and perigenual cingulate cortex showed increased activity (more than additive) during the interaction of pain processing and cognitive distraction. In contrast, several of the key pain processing regions showed decreased activity (less than additive) during the interaction of pain processing and cognitive distraction. This latter result suggests a neuronal basis for the decreased pain perception felt by these subjects.

Further work confirmed this cognitive modulation was specific to pain, as different patterns of brain activation induced by non-noxious warm stimuli were not modulated by the same cognitive task.

To determine whether such behavioural modulation of pain processing can be increased with respect to producing larger changes in pain perception with increasing cognitive load, a parametric working memory task, the n-back task, was successfully developed and tested off-line for the application to the fMRI experiments. Some relationship was observed between the modulation in subjects' pain perception (as seen by parameter estimate changes) and task difficulty in the contralateral SI region. Although the task did reduce subjects' reported pain intensity, the present design did not show that this occurred in a parametric manner. The experimental design used in this Thesis could be developed further to ascertain whether a greater range of n-back task levels would add additional information.

The way in which different response profiles relate to the changing perception of pain from a wider perspective rather than mere intensity rating changes is an interesting issue and future work within the group will aim to address these issues. Cognitive modulation of the pain sensation is increasingly gaining credence as a useful tool in the field of pain management in the clinical arena. Knowledge derived from fMRI and other neuroimaging methods will aid our understanding of the central mechanisms by which these interventions work.

The development and characterisation of a suitable itch stimulus to investigate the central processing of itch sensations was a challenge. Considerable effort was required to develop a paradigm that could produce reliable itch stimuli that could be readily transferred to the scanner environment. A series of off-line experiments were carried out to finalise the stimulation procedure to be used for the itch fMRI protocol. Difficulties in analysing the fMRI data from the itch imaging studies were addressed during the pilot data phase, as the itch stimulus increases relatively slowly in intensity and has a long temporal duration, making fMRI data analysis more difficult compared to conventional imaging studies.

Each subject's subjective itch intensity profile obtained outside the scanner environment (averaged over 3 sessions) was correlated with the objective fMRI data. This allowed the regions involved in the processing of itch sensation to be isolated effectively using statistical analyses and modelling of the fMRI data.

On-line rating methods were subsequently developed and applied to accurately monitor subjects' perception of itch intensity using a VAS scale. The same on-line rating methodology was employed in the n-back experiment. Measuring the intensity of sensations such as itch and pain online avoids any reliance on subjects' recall of their intensity subsequent to the experiment and also provides direct behavioural data acquired during the scanning experiment. This makes the on-line paradigm a potentially useful contribution to research in the area of itch.

A robust, reliable and intense itch stimulus was developed using the prick test method with histamine, and, in later studies, allergens to induce a strong itch sensation in atopic subjects. The itch imaging results showed convincing differences to the saline control data, and complemented those previously reported in the itch literature.

The aim of this Thesis was to compare the unpleasant sensations of itch and pain in terms of the central processing involved. Suitable paradigms for inducing both

sensations were utilised and translated to the fMRI imaging environment in order to investigate the two sensations in more detail.

The hypothesis was that the processing of both sensations by the brain would involve many common central regions. To an extent, this was supported by the results of this Thesis. When the central brain regions activated in response to noxious stimuli are compared with those activated by pruritic stimuli, areas common to both sensations can be identified that perhaps relate to the unpleasant perception generated by both stimuli. Regions commonly activated by both sensations include the ACC and OFC regions, as well as the posterior cingulate, primary somatosensory cortex (SI) and thalamus. The pilot experiment found activation in the insula, although this was not replicated when using the on-line rating method. The insula is one of the most frequently activated regions in pain imaging studies and the lack of activation seen here in histamine-induced itch is a key difference between the two sensations that warrants further investigation. However, again, it is possible that studies conducted using a larger cohort of subjects might replicate the pilot experimental results and show activation in this region.

The posterior ACC was activated in the allergen itch paradigm. This region is involved in the affective processing of unpleasant sensations, and which is found to activate in response to noxious thermal stimulation. Allergic itch is an extremely aversive sensation and is reported by subjects challenged with their own allergen to be more intense than their response to histamine. There is also a difference in terms of motor activation. The itch paradigm produced pronounced activation in premotor regions as well as in the cerebellum. During the itch experiments, subjects were not permitted to scratch, though itch sensations produce an ever increasing urge to scratch. Such regions are likely to represent the intention to scratch and the inhibition of this movement.

It is difficult to directly compare the sensations of itch and pain since they possess distinctly unique time courses. The pain stimulus used in the experiments described in this Thesis are acute or "short, sharp" sensations. The motor response to pain, withdrawal, is controlled for in our experiments, as the pain intensity used to stimulate subjects is intense, but not sufficiently so for them to flinch. Such movement would produce undesirable movement artefact in the fMRI data obtained and confounding activations in brain regions corresponding to motor withdrawal responses.

In addition, the itch sensation takes a longer time to develop and is accompanied by a gradually increasing desire to scratch to relieve the itch. The motor drive to scratch the itch is inhibited as subjects were asked not to scratch in the experiments described in this Thesis. Scratching would again produce undesirable motion artefact in the data and contaminate the fMRI data. However, preparation for scratching with the contralateral limb and intention to scratch may be reflected in the premotor and cerebellar activations found in this Thesis and by other authors in the field of itch research.

These time courses of itch and pain within the experimental arena are so different that further research is necessary to draw direct comparisons between them. Future studies could involve delivering itch and pain stimuli to the same subject group during the same session. A larger subject cohort would minimize inter-subject variation and increase the validity of statistical analyses of the fMRI data. Paradigms could be developed where subjects were allowed to scratch in a controlled way and the motor activation could be modelled out to investigate the regions involved in the desire to scratch and the relief that is obtained.

The work contained in this Thesis provides a firm basis for further work which could extend these observations and unravel which aspects of pain and itch perception have a common subjective basis and corresponding neural correlates. The final piece of research described here extrapolated the itch paradigm to a clinically relevant group of subjects: atopics. Behavioural, physiological and fMRI measures were taken and data compared between allergen-induced itch and histamine-induced itch. Common brain regions were active in both types of itch experiments, but with stronger activation in the more itch-inducing allergen atopic group compared to histamine. The histamine-induced itch control group had a wider spectrum of brain regions involved, perhaps indicative of the perceptual differences in the quality of itch.

In summary, several robust paradigms have been developed for the investigation of the central components of pain and itch processing using fMRI. These studies have shown that it is possible to apply behavioural paradigms as an alternative non-pharmacological method of modulating painful sensations. This body of work forms the basis for many possible future studies that can extend these observations, using the paradigms and methods developed in this Thesis, to relevant patient groups. It is hoped that these studies will pave the way for a better understanding of

unpleasant cutaneous sensations and how they are modulated using alternative cognitive therapies within the clinical arena.

Publications arising from my research

Bantick SJ, Wise RG, Ploghaus A, Clare S, Smith SM, Tracey I. Imaging how attention modulates pain in humans using functional MRI. Brain 2002; 125: 310-9.

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Wise R, Rogers R, Painter D, Bantick S, Ploghaus A, Williams P, et al. Combining fMRI with a Pharmacokinetic Model to Determine Which Brain Areas Activated by Painful Stimulation Are Specifically Modulated by Remifentanil. Neuroimage 2002; 16: 999.

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