FUNCTIONAL MAGNETIC RESONANCE IMAGING OF PAIN IN THE SPINAL CORD AND BRAINSTEM

by

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Abstract

Functional magnetic resonance imaging (fMRI) studies performed to date have focused on brain structures rostral to the thalamus, although the first level of sensory information and pain transmission occurs at the spinal cord (SC). The primary goal of this project is to map activity using fMRI, from the entire cervical SC and brainstem following innocuous and noxious stimuli before and after peripheral sensitization in normal human volunteers. This study is unique in that it determines functional activity throughout the lower neural axis in response to mechanical stimuli that are perceived as painful only after sensitization.

Functional MRI studies of the SC were carried out in 18 healthy individuals in a 3T Siemens Magnetom Trio. Innocuous touch and brush (n=8), and noxious touch (n=10) stimuli were applied before and after peripheral sensitization. Peripheral sensitization was induced by topical application of capsaicin. Functional image data spanned from the C7/T1 disc to the superior edge of the thalamus and analyzed using a general linear model to discriminate signal intensity changes from physiological motion. Normalized results were combined to demonstrate the number of volunteers showing activity at each location on a voxel-by-voxel basis. Areas of activity were superimposed onto anatomical transverse drawings and identified visually with comparison to several stereotaxic atlases.

The results from this study confirm previous reports that a non-noxious stimulus translates into a pain response after peripheral sensitization. The brush stimulus, before sensitization activated areas in the ipsilateral dorsal horn (DH), gracile and cuneate nuclei in the medulla and areas surrounding the dorsal column medial lemniscal pathway. Peripheral sensitization produced activity in the contralateral ventral horn (VH), typical of a pain response. The innocuous von Frey stimulus produced activity in typical sensory centres in the DH and brainstem before sensitization, and areas more consistent with a noxious response after sensitization. When examining equi-nociceptive stimuli in a control versus sensitized state, the noxious touch stimuli showed similar activation patterns even though the force of the filaments were different. In all experiments there was indication of descending modulation as activity was observed in the periaqueductal gray, midbrain red nuclei and pontine reticular formation. This study demonstrates how non-painful and pain information is transmitted from the dorsal spinal horn to the brain in healthy individuals and how peripheral sensitization induces changes in non-noxious stimuli that correlate with pain sensory transmission.

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List of Abbreviations

ACC	anterior cingulate cortex
ALF	anterior lateral funiculus
BOLD	Blood Oxygenation Level Dependent
CNS	central nervous system
CSF	cerebrospinal fluid
DC	dorsal column
DH	dorsal horn
DLF	dorsolateral funiculus
DLPT	dorsolateral pontine tegmentum
DTI	diffusion tensor imaging
EEG	electroencephalography
FLASH	fast-low-angle single shot
fMRI	functional magnetic resonance imaging
GM	gray matter
dGM	dorsal gray matter
vGM	ventral gray matter
HASTE	half-Fourier single-shot fast spin-echo
FLAIR-HASTE	fast fluid-attenuated inversion-recovery -HASTE
IASP	International Association for the Study of Pain
LC	locus coeruleus
LT	lissauer's tract
MEG	magnetoencephalography
ML	medial lemniscus
MR	magnetic resonance
MRI	magnetic resonance imaging
NCF	nucleus cuneiformis
NRM	nucleus raphe magnus
PAG	periaqueductal gray

PBN	parabrachial nucleus
PET	positron emission tomography
PFC	prefrontal cortex
PNS	peripheral nervous system
RF	reticular formation
MRF	medullary reticular formation
PRF	pontine reticular formation
RVM	rostral ventromedial medulla
SC	spinal cord
SEEP	Signal Enhancement by Extravascular Protons
SI	primary somatosensory cortex
SII	secondary somatosensory cortex
SPECT	single photon emission computed tomography
STT	spinothalamic tract
T	tesla
TE	echo time
TR	repetition time
TRPV1	transient receptor potential vanilloid 1
VH	ventral horn

Chapter 1
Introduction

Humans depend on sensory cues from the environment for their existence. One such sensory cue is pain. Pain is vital for humans as it provides a means of protection from harm. Indeed, individuals born without pain fibres often die at a young age due to inability to detect trauma (McGrath and Unruh 2006). In contrast, pain can sometimes become chronic, at which point it no longer serves a useful purpose and becomes debilitating and vastly decreases quality of life. Perhaps most disturbing is the fact that most sufferers are disabled for months due to the lack of specialized treatment facilities as well as the poor responses to existing pharmacotherapies.

Persistent, non-purposeful pain can result from lesions to the nervous system, which cause abnormal sensitivity at the site of damage and surrounding areas, spontaneous-evoked pain, and changes within the pain transmission networks of the central nervous system (CNS) so that innocuous input is perceived as pain (allodynia) and painful stimuli are perceived as exaggerated (hyperalgesia). This type of pain is collectively referred to as neuropathic pain. These pain syndromes offer no biological advantage and cause discomfort, distress and suffering. There are numerous mechanisms proposed to underlie the development and maintenance of neuropathic pain states, but no optimal treatment strategy has yet been developed.

Much of what we know about sensory transmission has come from neurophysiological studies in normal healthy individuals and those who have experienced changes in such systems due to disease or trauma (Willis and Coggeshall 1991b).

Complementing and advancing our knowledge of sensory transmission is functional magnetic resonance imaging (fMRI). This technique has proven to be a very powerful tool that is used to study how sensory information is transmitted and processed in the brain, in both healthy individuals and in clinical populations (Blatow *et al.* 2007; Creac'h *et al.* 2000; Hansson and Brismar 1999). Functional MRI has demonstrated differences in neural processing between acute and chronic pain; chronic pain patients show more frontal lobe activation suggesting that chronic pain involves different cognitive processing (Apkarian *et al.* 2005) than acute pain. Functional MRI has also reliably shown the psychological and affective components of pain (Price 2000) and what is commonly known in the pain literature as the 'pain matrix'. That is, activity in the anterior cingulate cortex (ACC), insula cortex (IC), somatosensory cortices and thalamus (Tracey 2005). However, the majority of studies to date have focused on rostral cortical areas of nociceptive processing with relatively little to no literature on brainstem and spinal cord (SC) areas. These are areas where all sensory transmission is integrated and modulated to some extent prior to transmission to higher brain structures.

Much of our understanding of how sensory information is relayed and processed from the SC to the brainstem relies on tracing studies and pre-clinical functional studies. Functional studies in humans are essential for understanding normal transmission of sensory information which is correlated to perception of such information, and will prove invaluable in determining how such processes are altered in disease states such as chronic pain. Investigating healthy and clinical pain populations will provide an opportunity to evaluate the plasticity changes associated with chronic pain states, and how sensitization

in normal healthy subjects may modify sensory transmission. In order to understand how these changes occur, we must first understand how sensory (including noxious) information is transmitted from the first synapse in the SC, to the processing centres in the brainstem and midbrain regions.

1.1 Neuropathic Pain

> "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." (IASP 1994).

Pain is an adaptation and a protective mechanism to warn us of potential or actual tissue damage and elicit a reflex and behavioural response to prevent this damage (Woolf and Mannion 1999). If the damage is unavoidable, neuronal excitability in the periphery and CNS causes hypersensitivity at the site of tissue damage and surrounding area. The human nervous system responds to pain in an evolutionarily beneficial way to increase survival by resting the body while it is damaged, and to accelerate healing (Acerbi and Parisi 2007).

In contrast to purposeful pain (as described above), trauma or disease affecting nerves in the peripheral nervous system (PNS) and CNS often cause a chronic, intractable pain state known clinically as 'neuropathic pain'. Neuropathic pain is associated with many pathological states including diabetic neuropathy, herpes zoster infection, complications from AIDS, physical injuries such as nerve traction or compression, and radiation therapy for different forms of cancer (Ossipov *et al.* 2006). An individual with neuropathic pain may experience sensations described as burning, stabbing, prickling,

lancinating, shooting, tingling or shock-like (Bouhassira *et al.* 2004; Woolf and Mannion 1999). These sensations can be stimulus-evoked or spontaneously generated (Woolf and Mannion 1999; Zambreanu *et al.* 2005). Various changes in both noxious and innocuous sensory transmission occur in neuropathic pain states. One such change is a synaptic reorganization of sensory pathways causing ectopic neuronal firing that is thought to underlie spontaneous, paroxysmal pain (Besson 1999; Ikeda *et al.* 2003; Woolf and Mannion 1999). It is clear that both the PNS and CNS play a role in the development of neuropathic pain states.

Neuropathic pain is thought to affect 2-3% of the North American population (Gilron *et al.* 2006) and creates a tremendous burden on health care costs. There is no clear diagnosis for this condition as it is a general term for multiple conditions and no adequate treatment plan exists for patients with neuropathic pain, although various algorithms have been published to help guide physicians (Gilron *et al.* 2006). This debilitating condition is particularly difficult to treat as it is often refractory to opioid analgesics such as morphine, and non-responsive to non-steroidal anti-inflammatory drugs. Non-traditional analgesics such as tricyclic anti-depressants and anti-convulsants have become first line therapy, but such treatments can be expensive, associated with intolerable side effects and only have modest efficacy in many patients. In the United States alone, an estimate of $150 billion is spent on health care, disability and other related costs to chronic pain; of this expenditure, $40 billion is spent on patients suffering from neuropathic pain (Turk 2002).

1.2 Sensory Pathways

Cell bodies of soma to sensory neurons of the PNS are found in the dorsal root ganglion, trigeminal ganglion and nodose ganglion. These neurons sense and encode internal and external environments and project information via primary afferent neurons from peripheral tissue to the dorsal horn (DH) of the SC, the spinal nucleus of the trigeminal tract, and to the gracile and cuneate nuclei in the brainstem medulla.

Primary afferents that respond to noxious stimuli are known as nociceptors. Nociceptors can either be myelinated or unmyelinated. Small unmyelinated afferents (C-fibres) signal a dull burning pain and are often polymodal being activated by chemical, thermal and mechanical noxious stimuli. Myelinated afferents (Aδ fibres) signal sharp well localized pain from both mechanical and thermal stimuli. Large myelinated afferents (Aβ fibres) signal most non-noxious sensory information, including proprioception and light touch (Meyer et al. 2006).

Non-noxious tactile sensory information is transmitted from the periphery via Aβ fibres that ascend in the ipsilateral dorsal columns to the brainstem where they terminate in the gracile (legs) or cuneate (arms) nuclei. Aβ primary afferent neurons also send collaterals into lamina III of the DH of the SC (Figure 1.1A), where they are thought to contribute to the Gate Control Theory of Pain (Fields et al. 2006).

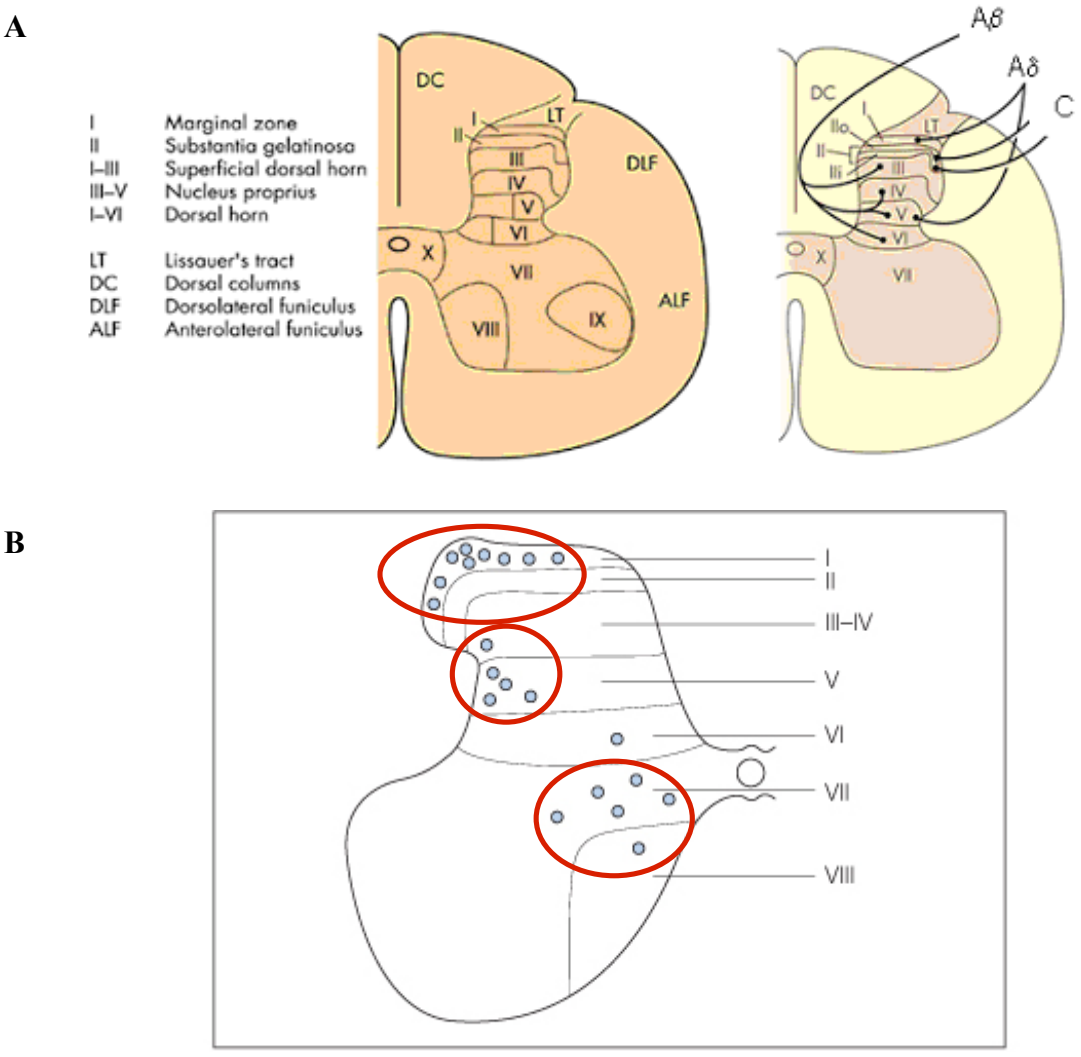

Figure 1.1 Rexed Laminae

A) Rexed laminae distribution of the SC. Sensory primary afferents project to different laminae within the dorsal SC. Aβ fibres terminate in the medulla but send collaterals into the deeper DH (laminae III-V). C-fibres terminate predominantly in laminae I and II, whereas Aδ nociceptors terminate in laminae I and V (Willis and Coggeshall 1991a). B) A schematic diagram showing the concentration of spinothalamic tract (STT) neurons in the SC. The red circles depict the three main areas including lamina I (the marginal zone), laminae IV-V (neck of the DH) and laminae VII-VIII (intermediate zone and ventral horn). Adapted from Dostrovsky and Craig (2006).

Second order neurons in the gracile and cuneate nuclei send axons across the medulla midline where they form the medial leminscus (ML) pathway and synapse with nuclei in the brainstem reticular formation (RF), the periaqueductal gray (PAG), and in the contralateral ventral posterior lateral nucleus of the thalamus. The thalamic neurons (third order) then send projections to the primary somatosensory cortex (SI) (Figure 1.2).

Noxious sensory information is transmitted from the periphery via C and Aδ fibres that terminate in specific regions of the dorsal SC. C-fibres terminate predominantly in lamina II of the DH of the SC (Figure 1.1A) onto inhibitory interneurons that in turn project to SC laminae I and V. Aδ fibres terminate primarily in lamina I and can synapse directly on second order neurons of the anterolateral pathway. These second order neurons decussate at the SC midline to the contralateral SC and project in the white matter columns; consisting of three pathways: spinothalamic, spinoreticular, and spinomesencephalic, with termination points in the thalamus and other midbrain structures. Third order neurons in turn project to the SI, dorsal anterior IC and ACC. The spinothalamic tract (STT) cells are primarily found in the cervical and lumbosacral enlargements (Figure 1.1B). Based on anterograde tracing experiments in non-human primates and silver stained degeneration subsequent to cordotomies in humans, we know that the STT terminates in six areas of the thalamus (Craig 2003). Lamina I STT neurons project to the ventral posterior nucleus, posterior part of the ventral medial nucleus, the ventral posterior inferior nucleus, and the ventral caudal division of the medial dorsal nucleus. Lamina V STT axons (predominantly wide range neurons) project to the ventral posterior nucleus, ventral posterior inferior nucleus, ventral lateral nucleus and

intralaminar nuclei (Tracey 2005). The area with the densest termination points is the posterior part of the ventral medial nucleus. Lamina I projection neurons receiving input from Aδ and C-fibres seem to play a role in detecting homeostatic afferent activity related to pain, temperature and itch sensations (Braz *et al.* 2005; Craig 2003). Lamina I neurons are also a termination point for descending modulatory pathways (Figure 1.2).

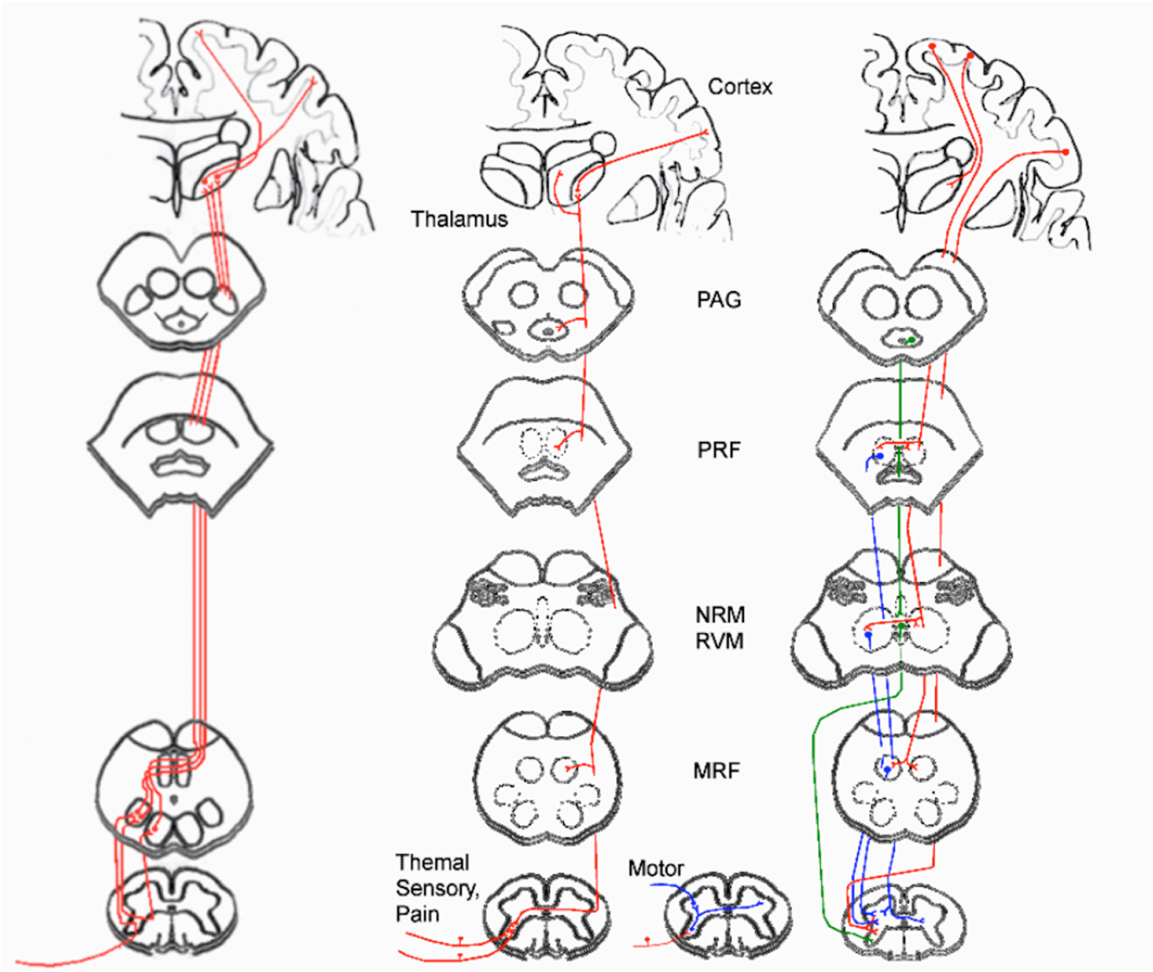

Figure 1.2 Ascending and Descending Pathways

Schematic diagram showing the pathways involved with touch and pain sensations applied to the right hand. The SC and brainstem cross-sectional drawings at the various levels are in radiological orientation to correspond with the orientation of the subsequent fMRI results. The diagram on the left shows ascending touch pathways originating in the DH of the SC. The diagram in the middle is a depiction of a combination of multiple pathways in the anterolateral sensory information system. The schematic at the bottom centre indicates the withdrawal reflex pathways. The diagram on the far right shows the descending pathways involved in pain modulation. The red highlights descending pathways from the cortex, blue from the RF and from the raphe nuclei. Pathways are drawn according to multiple references (Carpenter 1991; Kiernan 1998; Willis and Coggeshall 1991a; Willis and Westlund 1997).

1.3 Descending Systems

The brainstem RF is heavily involved in the inhibition and facilitation of noxious information. Inhibitory inputs can be segmental (restricted to within the SC) or descend from the brainstem where they reduce activity in DH neurons and act as a spinal "gate" for incoming sensory information (Woolf and Mannion 1999). Mayer and Price (1976), in a classic early experiment, showed that electrical stimulation of the PAG (in the midbrain) of alert animals produced analgesia at the level of the SC. Sectioning the dorsal columns of the SC blocked the effect. This could be because the analgesia produced by stimulating the PAG indirectly stimulated other brainstem nuclei that sent descending projections to the dorsal SC. From animal studies and tracing experiments, we now know the specific areas involved in modulation of pain include the midbrain PAG, locus coeruleus in the pontine reticular formation (PRF), dorsolateral pontine tegmentum (DLPT) in the medulla, adjacent nucleus cuneiformis (NCF), brainstem raphe nuclei and rostral ventral medulla (RVM) (Suzuki *et al.* 2002; Urban and Gebhart 1999; Williams and Beitz 1993). The PAG receives ascending projections from the spinomesencephalic tract and descending projections from the amygdala, cortex and hypothalamus. The PAG can modulate pain transmission through the RVM and DLPT. The NCF receives descending projections from cortical areas (Keltner *et al.* 2006) and ascending afferent input from lamina I neurons in the DH (McMahon and Wall 1985; Wiberg *et al.* 1987; Yezierski 1988). The NCF projects to the RVM and bilaterally controls DH nociceptive neurons (Fields *et al.* 2006; Zambreanu *et al.* 2005). The NCF is also activated with repeated noxious stimuli in healthy humans with experimentally

induced sensitization (Zambreanu *et al.* 2005). Activation in the NCF can be attributed to an expectancy state that develops because of repeated noxious stimulation (Keltner *et al.* 2006). Thus, the NCF is able to exert its modulatory effects through the RVM as well as through cortical receiving areas to enhance or inhibit pain transmission. The PAG and NCF both project to the RVM (Basbaum and Fields 1984; Behbehani and Zemlan 1986) and the RVM contains both serotonergic and non-serotonergic projections. Serotonin is released from nucleus raphe magnus (NRM) neurons while the DLPT provides noradrenergic projections; releasing norepinephrine to the dorsal SC. Pain transmission in second order neurons can therefore be inhibited via the PAG-RVM and PAG-DLPT descending systems (Monhemius *et al.* 2001; Pertovaara and Wei 2003; Sillery *et al.* 2005; Wall *et al.* 2006; Waters and Lumb 2007) and the cortical-NCF-RVM circuitry (Keltner *et al.* 2006; Zambreanu *et al.* 2005) (Figure 1.2).

1.4 Sensitization

Sensitization of nociceptors causes an increase in neuronal activity, expanded receptive fields, increased responsiveness to noxious C-fibre mediated stimuli and induction of low threshold (innocuous) response from Aβ fibres (Millan 1999) which initiate action potential discharge (Woolf and Mannion 1999). These changes create hypersensitivity at the site of injury. Additionally, prolonged activity of C-fibres induces changes in synaptic transmission in the CNS (Flor *et al.* 1995; Ochoa and Yarnitsky 1993).

1.5 Allodynia/Hyperalgesia

Stimulus evoked pain, common in neuropathic pain states, can result from peripheral nerve injuries and are manifested as allodynia or hyperalgesia (Woolf and Mannion 1999). Allodynia is perception of pain in response to a non-painful stimulus such as a very soft touch, i.e. cotton brushed against the skin. Allodynia is generally thought to involve altered CNS modulation. Hyperalgesia is an increased sensitivity and exaggerated response to a normally painful stimulus, i.e. heat applied to an area that has a sun-burn. Hyperalgesia can be further subdivided into primary and secondary hyperalgesia. Primary hyperalgesia is thought to be caused by altered nociceptors, but may involve central changes as well; whereas secondary hyperalgesia and allodynia are centrally mediated (Lindblom and Hansson 1991; Ochoa and Yarnitsky 1993).

Mechanical stimuli such as von Frey filaments (static) and brushes (dynamic) are often used experimentally to evoke pain in neuropathic pain patients. Von Frey filaments are thread-like mechanical devices that when applied perpendicular to the skin to create a semi-circle, apply a calibrated amount of force. Various mechanisms have been proposed to account for pain to such stimuli including abnormal processing of nociceptor input (Woolf and Mannion 1999), spontaneous activity of low threshold touch-pressure mechanoreceptors with large afferents (Magerl *et al.* 1998), increased sensitivity of Aδ and C-fibre tracts activation (Meyer *et al.* 2006) or abnormal CNS modulation (Woolf and Mannion 1999). There can also be spontaneous activation of sensitized primary nociceptors, which do not normally respond to low intensity mechanical stimuli (Ochoa and Yarnitsky 1993). Two types of mechanical allodynia exist in neuropathic pain

patients: dynamic and static (Ochoa and Yarnitsky 1993) according to the type of stimulus that leads to their induction. Dynamic allodynia is pain in response to a brush stimulus and static allodynia is pain in response to a light touch or pressure stimulus (LoPinto *et al.* 2006). It has been suggested that static and dynamic allodynia result from different mechanisms and are transmitted via different sensory neurons of the PNS. Dynamic allodynia is generally thought to be independent of C-fibre activation and mediated by Aβ fibre activation; since selective blockage of Aβ fibres by compression-ischemia abolishes dynamic, but not static allodynia (Ochoa and Yarnitsky 1993). Static allodynia is proposed to be signaled by nociceptive Aδ fibres and mediated by central sensitization, but may also involve C-fibre activity (Field *et al.* 1999).

1.6 Capsaicin

Capsaicin is a chemical compound used as a model to induce peripheral sensitization. Capsaicin is the active ingredient in chili peppers that selectively excites the transient receptor potential vanilloid 1 (TRPV1) receptor present on primary afferent nociceptors. Activation of TRPV1 by capsaicin causes neuronal excitation leading to the perception of pain and release of local inflammatory mediators (Caterina *et al.* 1997). The TRPV1 receptor can also be activated by other stimuli, including high temperatures (above 43 °C) (Caterina *et al.* 2000) and acidic solutions (pH <5.5) giving it polymodal characteristics that match nociceptive neurons (Caterina *et al.* 1997; Rang *et al.* 2003). In addition to localization on nociceptors, there is a dense TRPV1 receptor population in the locus coeruleus, medial basal hypothalamus and preoptic areas of the hypothalamus;

as measured by immunohistochemistry. Many of these areas are thought to play a major role in sensory and autonomic function, including pain processing (Svensson 1987).

Topical application of capsaicin causes a burning pain sensation by sensitizing primary afferent C and Aδ neurons and induces an area of primary and secondary hyperalgesia (Dray 1992). In adjacent non-irritated areas, activation of C-fibres induces an axon reflex (flare reaction) to punctate stimuli (von Frey) or light touch (cotton or brush stimuli) (Kilo *et al.* 1994). An axon reflex reaction occurs because C-fibres cause over-excitation of axons, causing activation of inflammatory mediates and thus dilated blood vessels in the vicinity. Repeated capsaicin exposure can desensitize nociceptor terminals to capsaicin and other noxious stimuli (Szolscanyi 1993), which is the principle underlying its use as an analgesic and treatment of neuropathic pain. Eventually, long term exposure to capsaicin causes death of nociceptors or destruction of the peripheral terminals (Campbell 1993).

One mechanism underlying sensitization is changes is modulation of the TRPV1 receptor (Woolf and Salter 2000). Studies have shown that partial nerve injury produces TRPV1 down regulation in damaged afferents, whereas this cation channel is up-regulated in adjacent, uninjured C-fibres and A fibres (Hudson *et al.* 2001). This supports the idea that these changes might contribute to the development of C-fibre sensitization and the consequent hyperalgesia. Extracellular inflammatory mediators released from immune cells and neurogenic sources following tissue injury will also trigger peripheral sensitization. These chemicals (inflammatory 'soup') decrease the

threshold of activation of TRPV1 so that normally non-painful stimuli are capable of activating TRPV1, thus generating a pain response.

Painful sensory abnormalities that occur after application of capsaicin have been used to investigate the peripheral and spinal components contributing to allodynia and hyperalgesia, and form the basis of an acute, reversible human model for neuropathic pain (Park *et al.* 1995). With this model, large areas of hypersensitivity occur in skin surrounding the capsaicin injection. Capsaicin is an effective nociceptive agent in that it is an experimental pain stimulus, yet circumvents the potential for tissue damage inherent with painful thermal stimuli (LaMotte *et al.* 1991; Simone *et al.* 1991). Moreover, capsaicin provides a pain stimulus with minimal contributions from other somatosensory modalities and allows comparisons between nociceptive and non-nociceptive stimuli.

1.7 Capsaicin produces activation of brain nuclei

Capsaicin is an effective model to study CNS activity related to sensitization (Petersen and Rowbotham 1999). Intradermal injections of capsaicin in humans are dose-dependent for the duration of pain, the area and duration of mechanical hyperalgesia, and the area of flare (Simone *et al.* 1989). Kilo *et al.* (1994) characterized the different hyperalgesia patterns and types of inflammatory changes caused by topical capsaicin and after freezing, using different sensory tests involving punctate, mechanical, pressure, impact, and thermal stimuli. Brush stroking and punctate stimuli after capsaicin produced hyperalgesia at the application site but this response was absent after freezing. The authors suggest that this is most likely due to central plasticity rather than nociceptor

sensitivity because in the freezing state, the nociceptors on the peripheral end are sensitized yet there was no hyperalgesia observed. Brush stroking stimuli are sustained by persistent peripheral nociceptor activity, whereas punctate stimuli are not dependent on further peripheral nociceptor input (Kilo *et al.* 1994). Capsaicin also increases the skin temperature in the treated arm by 2-3°C compared with the contralateral side. Using PET, Iadarola *et al.* (1998) were one of the first to identify activation in brain areas specific to capsaicin. Experimental allodynia with light brushing showed prominent activity in the secondary somatosensory cortex (SII), but was not activated by a noxious stimulus. Allodynia on its own bilaterally activated the inferior prefrontal cortex (PFC) suggesting that the response in the PFC is context dependent (Iadarola *et al.* 1998) and there are differences seen between the two allodynic conditions.

1.8 Functional Magnetic Resonance Imaging

Functional MRI is the only means of indirectly mapping neuronal activity with high spatial resolution in humans non-invasively. Other imaging techniques like positron emission tomography (PET) require injections of radioactive isotopes, while electroencephalography (EEG) can be invasive and magnetoencephalography (MEG) is unable to detect activity in subcortical structures. Functional MRI provides better spatial resolution with adequate temporal resolution as compared to PET, EGG and MEG (Table 1.1).

Table 1.1 Temporal and spatial resolution for different neuroimaging techniques		
	Temporal (s)	**Spatial (mm)**
FMRI (Logothetis 2008)	1-4	1-2
EEG (Dale and Halgren 2001; Gevins *et al.* 1991)	0.001	25
MEG (Lounasmaa *et al.* 1996)	0.001	5
PET (Cherry and Phelps 2002)	60-120	4-5

Temporal resolution refers to how closely the measured activity corresponds to the timing of the actual neural activity and is displayed in seconds. Spatial resolution refers to how accurately the measured activity is localized to a specific region and is displayed in millimeters (Kimberley and Lewis 2007).

1.9 MRI Basics

Magnetic resonance imaging detects the magnetization produced by the nuclei of hydrogen atoms within lipids and water in the body. With the use of spatially-dependent magnetic fields, we can determine where the signal originates and with enough of this information we can create an image. To create images, a MRI scanner uses changing magnetic field gradients and oscillating electromagnetic fields to adjust the properties of the hydrogen nuclei (Buxton 2002). Different tissue types are represented by the density and environment of the hydrogen nuclei and this helps differentiate gray and white matter.

The hydrogen nucleus has one proton that spins creating its own magnetic signal. In a living organism, the spins from all the hydrogen nuclei are randomly pointing in different directions in at any given time. When a volunteer enters the magnet, the magnetic field of the MRI scanner aligns the individual spins of each hydrogen nucleus, creating a net internal magnetic field. During a MR session, a radio-frequency pulse is

applied that causes the protons to wobble around their axis. This wobbling generates an electric current that is picked up by receivers in the MR bore. This signal has both horizontal and vertical components that create an image as the proton spins relax back to their original resting state.

Magnetic resonance imaging is focused on measuring two processes of relaxation characterized by time constants T_1 and T_2. A T_1-weighted scan measures the 'tipping' of the proton as it re-aligns with the original magnetic field. The rate of this relaxation is influenced by the surrounding environment and is used for anatomical images; water molecules shows up as dark areas and lipids or white matter are lighter on the MR scan. A T_2- weighted scan measures the dephasing of the synchrony of the rotating protons. The rate of this relaxation is fairly quick and depends on the loss of energy from nearby spinning nuclei. This type of scan is used to differentiate healthy tissue from diseased tissue where water now shows up lighter and lipids are darker. A T_2^*- weighted scan accounts for the T_2-weighted factors, but also takes into effect the inhomogeneities of the magnetic field, and this is the basis of fMRI (Ugurbil K *et al.* 1999).

1.10 FMRI contrast mechanisms

Most fMRI studies rely on the blood oxygenation-level dependent (BOLD) contrast method. Functional MRI using the BOLD contrast mechanism detects neuronal activity indirectly based on image intensity changes arising from related hemodynamic effects (Ogawa *et al.* 1990; Ogawa *et al.* 1993b; Ogawa *et al.* 1993a). The basis of the BOLD signal is as follows: upon an increase in spiking rate, neuronal cell bodies require more energy and take up more oxygen from their surroundings. Simultaneously,

vasoactive substances are released and the local supply of oxygenated blood exceeds that of the demand, increasing the concentration of oxy-hemoglobin (Dreier *et al.* 1995; Lee 2000; Paulson and Newman 1987). The oxygenated hemoglobin is diamagnetic and does not affect the MR (magnetic resonance) signal. However, the deoxy-hemoglobin is paramagnetic and affects local relaxation times. The subtle change in MR contrast is detected by T_2^*-weighted imaging and has reliably demonstrated areas of neuronal activity in the brain (Logothetis *et al.* 2001) by comparing images during rest and stimulation periods. T_2^*-weighted imaging shows areas with water content as bright and is very effective in revealing anatomical detail and provides excellent tissue contrast. This is also an effective method of imaging pain in the brain since multiple studies have shown an increase in regional cerebral blood flow in normal subjects in the parietal, insular and anterior cingulate cortices during evoked pain sensations (Iadarola *et al.* 1998; Rainville *et al.* 1997).

In the vast majority of published fMRI studies, the imaging field-of-view has been limited to higher brain structures because the BOLD signal is difficult to detect in the brainstem and virtually absent in the SC. A small number of studies have attempted to detect the BOLD effect in the brainstem (Dunckley *et al.* 2005; Fairhurst *et al.* 2007) and SC (Govers *et al.* 2007; Komisaruk *et al.* 2002; Madi *et al.* 2001; Stracke *et al.* 2005; Yoshizawa *et al.* 1996), but all these studies state inadequate spatial resolution as the number one limitation. There are also additional substantial problems in imaging the SC (Giove *et al.* 2004; Stroman 2005). The SC's small cross sectional dimensions (max 12-14 mm) (Elliott 1945), limits adequate spatial resolution. There are also different

magnetic field inhomogeneities caused by the SC's vicinity to body tissues having different magnetic field susceptibilities i.e. bone/tissue interfaces, chest cavity, tissue/air interfaces. Chest cavity expansion causes changes in the magnetic field while contractions and the heart beat causes pulsations in cerebral spinal fluid flow and blood flow which move the SC with each beat (Figley and Stroman 2007); movement of the tissue confounds imaging.

It is for these reasons that the majority of published fMRI studies in the SC (spinal fMRI) have employed primarily proton-density weighted spin-echo imaging (Stroman 2005). This contrast mechanism has been termed "Signal Enhancement by Extravascular water Protons" or "SEEP" (Stroman *et al.* 2001; Stroman *et al.* 2004; Stroman *et al.* 2005b; Stroman 2005) with neuronal activity detection based on changes in tissue water content. Neuronal activity causes local changes in fluid balance producing extracellular fluid, which is taken up by glial cells causing cell swelling. Upon neuronal activation, the concentration of potassium ions in the extracellular space increases because discharging neurons release K+. The K+ is taken up by K+ channels on astrocytes and other transport mechanisms while water follows intracellularly from the extracellular space causing the astrocytes to swell (Andrew *et al.* 2007; Andrew and MacVicar 1994; Fujita *et al.* 1997; Ohta *et al.* 1996; Stroman *et al.* 2008c; Svoboda and Sykova 1991). That water originates from the vascular space so the elevated SEEP signal tells us water has moved from there into the extravascular space. Proton-density-weighted spin-echo imaging methods are necessary in the SC and brainstem because they provide significantly better image quality and higher signal-to-noise ratio than BOLD-

based methods. With fast spin-echo imaging methods, it is also possible to obtain higher image resolution to accommodate the small cross-sectional dimensions of the SC, and to obtain images in sagittal slices to provide large volume coverage, without incurring image distortion (Stroman *et al.* 2005a). This contrast method then allows for fMRI in the SC and brainstem with both high quality images and sensitivity to changes in neuronal activity (Agosta *et al.* 2007; Stroman *et al.* 2005b; Stroman 2006b).

A study done by Stroman *et al.* (2005b) compared the BOLD and SEEP effect in duplicated experiments in healthy volunteers. The results from this study showed that the areas of activity from the two contrast mechanisms are localized primarily to gray matter. As well, areas of SEEP activity are immediately adjacent to areas of BOLD activity with very little overlap. The response functions for the two contrast mechanisms were also found to be distinct. The peak SEEP response lagged the BOLD response by approximately 1 s and the decay for the SEEP signal was slower. This study showed how these two contrast mechanisms are distinct with different properties.

1.11 Using fMRI to study pain

Numerous studies which have shown that the magnitude of the fMRI signal reflects perceived pain intensity (Bornhovd *et al.* 2002; Porro *et al.* 2002; Schweinhardt *et al.* 2006; Wiech *et al.* 2005) thus making it an effective technique for the study of pain. Functional MRI studies have identified a 'pain matrix' in the brain. Activation in the ACC, IC, SII and thalamus commonly occurring when testing the perception of pain (Tracey 2005). However, there is also an affective component to pain and other studies

have found activation in the: SI, PFC, PAG, cerebellum, striatum, nucleus accumbens, amygdala and the hypothalamus, that support this hypothesis (Bushnell and Apkarian 2006). Thus, it is reasonable to assume that functional MRI is a very powerful technique in neurological studies of pain. By using fMRI and the capsaicin model to induce hyperalgesia and allodynia, we can also understand the neural activity involved in sensitization. The current literature suggests that it is possible to use fMRI techniques to study sensitization; but these studies have largely focused on the brain. Functional MRI studies have detected tactile stimuli activity in the cervical and lumbar SC and using capsaicin to model sensitization for neuropathic pain. Functional MRI studies have detected activity in the brain and brainstem to different mechanical stimuli (the exact details of these studies will be described in the subsequent sections). However, no group to our knowledge has been able to study how sensitization affects transmission pathways in the CNS, at lower or more caudal sites, including the SC.

1.12 Spinal fMRI is a useful technique

The SC, contained within the vertebral column, can only be investigated by physical examination or invasive measures; it is inaccessible otherwise. Using fMRI to investigate SC activity is valuable, especially since the original point of sensory transmission occurs in the SC. The neural basis of sensation and motor control in humans has been extensively investigated at the cerebral level using fMRI. However, this approach has rarely been used in the SC although the SC is as involved in sensory transmission to higher CNS structures. Many studies have looked at eliciting a response in the SC with a sensory and/or motor stimulus and detecting a reliable signal using

BOLD fMRI. Yoshizawa *et al.* (1996) was the first study to test the BOLD response in the cervical SC. Using a fast-low-angle-single-shot (FLASH) sequence on 1.5T magnet, volunteers performed a hand opening/closing task. The motor task caused a bigger fMRI signal change (as compared to rest conditions) in the intermediate and ventral zones of the lower cervical SC gray matter ipsilateral to the hand performing the task; changes in the contralateral SC were not significant. A significant limitation of this study was the prolonged temporal window, which could not resolve the rise and decay times of the neuronal hemodynamic response (5–9 seconds). As well, the authors used 1 cm thick slices, and together, these effects limited spatial precision in the SC. However, this study sparked interest in this field of research and researchers sought out to acquire spinal images with neuronal activity in the cervical SC related to a sensory or motor task.

Madi *et al.* (2001) also wanted to detect a reliable BOLD signal in the cervical SC with a simple motor task, but correlated the signal to the force applied. Volunteers performed tasks to test three different muscle groups (elbow flexor, wrist extensor and small finger abductor) to activate three different segments of the SC. Another group of volunteers was imaged while performing isometric exercises (variable weight holding) to study the relationship between the BOLD signal and the applied force. In three of four subjects, the authors found increased fMRI signals during the task at cervical levels C5/C6, primarily in the site for spinal motoneurons and segmental interneurons controlling the biceps muscle. There were also multiple segments of the SC activated, however activity was concentrated in the level of the SC expected of the activated myotome. In addition, there was a linear relationship between the force applied by the

muscles during the isometric task and fMRI signal. Invasive electrophysiological studies have reported a linear relation between neural activity in the SC and contractile force (Maier *et al.* 1998). This study along with the results from Madi *et al.* (2001), supports the existence of a linear association between neural activity and fMRI signal in the SC, which has already been demonstrated in the brain by Logothetis *et al.* (2001). Madi *et al.* (2001) study showed that a mechanical task linearly relates to the detect fMRI signal change and that fMRI can be used to reliably study the activity induced by mechanical stimuli in the cervical SC.

In 2005, Stracke *et al.* (2005) used BOLD fMRI to examine activity in cervical SC with a somatosensory stimulus generator. This study was unique because stimuli were applied to the first, third and fifth finger tip of the right hand to activate the respective C6, C7, and C8 dermatomes. By doing this sequential order of stimuli application, the authors claimed that they found spinal activation in the corresponding segments and activity in the brainstem in three different areas. This is an interesting finding because it reflects the accuracy of spinal fMRI by identifying the respective levels of the SC for the different activated dermatomes. Moreover, it shows that the different stimulated areas on the hand mapped onto different areas of the brainstem. Although the results are encouraging, the authors do note that their functional echo-planar imaging images showed major distortions in addition to significant susceptibility artifacts leading to incomplete delineation of the SC.

Govers *et al.* (2007) wanted to examine whether a reliable fMRI signal can be elicited in the cervical SC during a complex motor activity, finger tapping with a 1.5T

system. At this point, there had been previous studies (Stroman *et al.* 2002b; Stroman *et al.* 2002c; Stroman and Ryner 2001) that had demonstrated sensory activity in the SC using thermal, pain or small tactile stimuli. The purpose of this study (Govers *et al.* 2007) was to know whether the BOLD signal could be detected in SC segments C5 to T1. The authors found predominant activity around C8; which corresponded to the anatomical location of the neurons that activate the muscles in use for finger tapping. However, this study failed to demonstrate the expected ipsilateral and contralateral activity with a motor task and failed to identify the location of nerve tracts above or between the DH and VH (ventral horn). The authors state their methodology (detecting BOLD signal) resulted in poor spatial resolution. Maieron *et al.* (2007) attempted to overcome the methodological hurdles specific to spinal BOLD fMRI and investigate the spatial activity patterns in the cervical SC with a motor task. Volunteers performed a simple finger to thumb opposition task either at a fixed frequency, alternating between right and left hands, or at two different frequencies with the right (dominant) hand. The functional data showed caudal areas of the cervical SC activated; this is where the cervical roots from the median nerve, which control finger-thumb opposition movements, enter the SC. Activation was detected on both sides of the SC with either hand, yet there was more activation on the side ipsilateral to the performing hand. When comparing the two frequencies on the right hand, the spatial extent of BOLD activation remained constant yet the intensity of activation was higher on the ipsilateral side of the SC, as the frequency increased. This study was able to demonstrate the lateral activity of a motor response in the SC and show modulation of the spinal neuronal activity as a function of

different movement characteristics. With the results of these studies, it was evident that reliable activity could be detected in the SC with different sensory tasks and that laterality could even be determined using spinal fMRI although there were still some methodological hurdles in terms of anatomical specificity and spatial precision.

An innovative 2002 study (Komisaruk *et al.* 2002) published a functional map of the lower human brainstem nuclei using fMRI of specific sensory and motor tasks. The authors wanted to visualize the location of the cranial nerve, pontine, bulbar and cervical SC nuclei using BOLD. Using different tests, they were able to activate certain brainstem regions to visualize the respective nuclei involved. For example, swallowing activates the nucleus tractus solitarius while pushing the tongue on the hard palate activates the nucleus cuneatus. Using BOLD fMRI, Komisaruk *et al.* (2002) were able to localize the cranial nerve nuclei of the pons and medulla and other nuclei of the lower brainstem and cervical SC in humans. This study incorporated head, cardiac and respiratory motion artifact compensation and post processing analysis to increase localization precision. Although the prospect of localizing specific nuclei in the SC and brainstem sounds very promising, the authors do mention that their findings provide relative approximate localizations for nuclei and not exact anatomic locations; which should always be taken into consideration with spinal fMRI studies.

The discovery of SEEP (Stroman *et al.* 2001; Stroman *et al.* 2002a), allowed the possibility of performing fMRI studies in the SC with more precise spatial localization. In sensory system research, it became logical then to use this method to examine the activity of different sensory modalities in the SC. In 2002, Stroman *et al.* (2002c) applied

a graded thermal stimulus to the calf to stimulate the L4 dermatome and examined the relationship betweens signal change and neural activity in the lumbar SC. Activity was detected in the DH at L4 and in components of the motor reflex circuitry. The authors also noticed a larger signal change as the thermal stimulus became more noxious (falling below 15ºC) and the activity shifted more to the DH. This study not only showed that thermal stimuli applied to the limb map onto a specific dermatome but also showed the graded response of thermal stimuli in the lumbar SC.

Subsequent studies using a proton-density weighted signal change proved the reliability and effectiveness of using spinal fMRI to image the cervical SC. These studies also overcame the motion artifact, CSF flow, magnetic field inhomogeneities and spatial precision issues arising from of using BOLD contrast mechanism in the SC. Li *et al.* (2005) used SEEP to look at the neuronal activity of acupuncture. Acupoint stimulation at LI4 and LI1[1] caused consistent signal changes C6/C7 and the authors noted a common bilateral activation amongst the volunteers. Eight out of 11 participants showed positive activation (72%) in gray matter regions with peak activity at C7. There was also widespread activity from C5 to T1. The multiple levels of activity in the SC are common to sensory transmission because of Lissauer tracts. Moffit *et al.* (2005) used a proton density signal change in the lumbar SC with a modified version of the HASTE (half-Fourier single-shot fast spin-echo) sequence (which is commonly used to detect SEEP),

[1] LI4 is located at the midpoint of the line bisecting the angle between the first and the second finger when the thumb is fully extended. LI11 is located at the end of the lateral transverse elbow crease when the forearm is flexed at a right angle to the upper arm (Stux and Pomeranz 1998).

called fast-fluid-attenuated-inversion-recovery HASTE (FLAIR-HASTE) to reduce artifact signal in the SC due to CSF flow. Although they were successful in removing the CSF signal, they were unable to observe a consistent fMRI signal in response to a thermal stimulus to the hindlimb. However, the authors used transverse slices, each 1 cm thick, which might obscure the activity by creating partial volume effects. Spinal fMRI commonly used 2-4 mm thick transverse slices in the SC (Agosta *et al.* 2007; Li *et al.* 2005; Stroman *et al.* 2005b). However, there is now a transition into using sagittal slices because it is possible to acquire image data faster and cover larger areas. This is especially useful when imaging the cervical SC and brainstem simultaneously. Moffit *et al.* (2005) justify their unusual large slice thickness under the assumption that groups of associated spinal neurons have elongated shapes in the rostral-caudal direction. However, the large slice thickness also increases the noise and this might account for the lack of consistent activity seen in the lumbar SC. Ng *et al.* (2006) used proton density fMRI in a low field (0.2T) to detect activation in the cervical SC with the hand grip task. Eleven out of 14 subjects showed consistent activity at C6/C7 spinal levels with distinct activity in the DH and VH. The low magnetic field and the short echo time used in this study (24 ms) diminished the BOLD effect and they showed that the observed signal change during neuronal activity was attributed to proton density changes.

Using spinal fMRI, Agosta *et al.* (2007) examined the activity in the cervical SC (C5-C8) with tactile stimulation of the right palm. The authors reported fMRI activity in the cervical SC ipsilateral to the point of stimulation, i.e. right, and a higher frequency of fMRI activity at C6 and C7. This level of the SC corresponds with the respective

dermatomes of the palm. This study (Agosta *et al.* 2007) confirmed the findings from previous studies (Li *et al.* 2005; Ng *et al.* 2006; Stroman *et al.* 2004; Stroman *et al.* 2005a) and also showed consistent cervical SC activity related to a sensory task, using SEEP. More recently, Lawrence *et al.* (2007) used fMRI to detect different dermatome activation with a vibration stimulation. The authors stimulated six different dermatomes at 50 Hz and examined the activity in the cervical SC. The segmental distribution and rostrocaudal distribution of the activity corresponded with the dermatomes activated. Activity was primarily localized in dorsal areas but also spread to ventral and intermediate areas of the gray matter. These recent studies show how the SEEP signal change is the most practical method to detect sensory stimuli activity in the SC.

1.13 FMRI is useful to study sensitization

There are many potential clinical applications of fMRI, especially in the field of pain transmission. Using fMRI, we can determine the areas of neuronal activity related to specific pain sensations (i.e. prickling, shooting, burning, dull etc.) and develop treatment strategies based on the areas affected in the brain. Neuropathic pain is a commonly studied area because there are effective experimental models of pain and because the implications of the findings have potential for future treatment strategies; which currently do not exist for chronic pain patients.

Brain fMRI using capsaicin is a common method to understand the brain areas involved in response to experimentally-induced allodynia and hyperalgesia. In 1998, Iadarola *et al.* used the capsaicin pain model to induce secondary hyperalgesia and

imaged the brain using PET. This study was the first to identify the areas in the brain activated by capsaicin. Iadarola *et al.* (1998) were interested in the pure dynamic component of secondary hyperalgesia and induced dynamic hyperalgesia by light brushing the skin and compared the brain activity patterns before and after injection of capsaicin. The results of the experiment identified the pain component of dynamic hyperalgesia in areas of the PFC (inferior and superior frontal gyri), some activity in SI and SII and none in the ACC. Using the findings in the Iadarola *et al.* (1998) study, Baron *et al.* (1999) examined the pain component of capsaicin-induced secondary mechanical hyperalgesia in the brain using fMRI. Baron *et al.* (1999) indicated that fMRI rather than PET was used, to obtain better spatial and temporal resolution. In this latter study, the forearms of nine individuals were mechanically stimulated and capsaicin was injected adjacent to the stimulation site. The same mechanical stimulus was then perceived as painful after the capsaicin injection. After comparing the two activity patterns, the authors were able to extract the pain specific component of mechanical hyperalgesia and it was comparable to the results in the Iadarola *et al.* (1998) study. For example, mechanical stimuli activated areas in SI and SII. Activity in SII has been identified with non-painful touch stimuli and this area is implicated in a variety of tasks including integration of tactile inputs, pain perception, tactile identification, and attention (Schnitzler and Ploner 2000). Mechanical hyperalgesia showed additional activity in the contralateral PFC. The PFC is commonly activated in studies of secondary hyperalgesia when painful experiences occur in the context of tissue alterations from capsaicin. The PFC is also linked to cognitive processing and experimental pain and clinical pain often

causes changes in this area (Apkarian *et al.* 2001; Derbyshire *et al.* 1997; Hsieh *et al.* 1995). It has been proposed that the PFC exerts a top-down influence on the midbrain, via the thalamus and ACC to control pain perception (Lorenz *et al.* 2003; Valet *et al.* 2004).

In a later study, Lorenz *et al.* (2002) examined thermal allodynia using intradermal capsaicin injections and showed that heat allodynia was functionally and neuroanatomically distinct from normal heat pain. Slow heating preferentially excites capsaicin-sensitive C-fibres (Yeomans and Proudfit 1996) and this form of heat allodynia is perceived differently than just heat pain. In this study, (Lorenz *et al.* 2002) showed specific activation of the medial thalamic pathway to the frontal lobe during heat allodynia, which was not present during normal heat pain. This could be due to the unpleasantness of heat allodynia, but can also be explained by the specific activation of peripheral afferent and brain mechanisms mediating responses to pain caused by inflammation or tissue damage. Maihofner *et al.* (2004) examined the brain activity arising from dynamic mechanical allodynia using fMRI. Following topical application of capsaicin, brush evoked allodynia was induced followed by heating at 45ºC to combine physical and chemical sensitization. Brushing the untreated arm activated areas common to a touch response: contralateral SI, contralateral parietal cortex, bilateral SII and contralateral IC. Brushing the affected arm in addition activated areas in the PFC: contralateral inferior and ipsilateral IC. These areas form a network involved in dynamic mechanical allodynia.

Maihofner and Handwerker (2005) sought to distinguish the differences between primary and secondary hyperalgesia using fMRI. They induced thermal and pin-prick hyperalgesia on the forearms of 12 healthy individuals using topical capsaicin. The activity patterns before and after capsaicin showed that pin-prick hyperalgesia produced activity in the areas of SI, SII, and PFC (insular, superior and inferior frontal cortices). Thermal hyperalgesia-induced activity in parts of the medial pain system, which are linked to different parts of the middle frontal and inferior frontal cortices. In this latter study, the authors were able to show a clear difference in brain activity patterns between thermal and mechanical hyperalgesia.

From these imaging studies, researchers have been able to distinguish clinical pain from experimentally-induced pain. Clinical pain is felt by patients in chronic pain conditions and experimentally-induced pain is inflicted onto healthy individuals with the use of different mechanical stimuli and sensitization agents, i.e. capsaicin. This distinction helped develop a 'pain network' of areas in the brain activated in response to a noxious stimulus and identify areas in the brain activated by a mechanical stimulus in allodynia and hyperalgesic states.

Few groups have examined the role of central sensitization (with the capsaicin model) in brain structures caudal to the thalamus. Zambreanu *et al.* (2005) were one of the first groups to examine the supraspinal activity within the brainstem following sensitization using fMRI. They recorded brain responses to mechanical hyperalgesia and thermal hyperalgesia on the right lower legs of 12 volunteers. Brain areas activated during hyperalgesia included the cerebellum, thalamus, SI, SII, and areas of the PFC.

Interestingly though, areas in the brainstem activated included two distinct regions of the midbrain RF that are consistent with the location of the NCF and rostral superior colliculi/PAG. These areas are dominant sources of output to the RVM and main areas involved in the descending pain modulatory system. This study demonstrated a potential facilitatory role in the development of central sensitization and suggested that areas involved in descending modulation may also be involved in central sensitization. Dunckley *et al.* (2005) also found that the PAG and NCF were activated during visceral and somatic pain. In this study, subjects were scanned while they received electrical stimulation to the midline lower abdomen or rectum. The authors found activation in regions consistent with the PAG, NCF, ventral tegmental area/substantia nigra, parabrachial nucleus (PBN)/nucleus coeruleus and red nucleus bilaterally to both stimuli. Furthermore, the NCF and RVM activations were correlated with the NCF receiving primarily ascending input. Hadjipavlou *et al.* (2006) were able to show the tract paths involved in this descending modulatory circuitry with diffusion tensor imaging (DTI). They used DTI to look at the white matter connections originating in the PAG and NCF, and were able to find connections from the PAG to the PFC, amygdala, thalamus, hypothalamus and RVM bilaterally. The same connections were found originating from the NCF. The descending pain modulatory system drives both anti and pro-nociceptive pathways to elicit a change in pain perception; as identified by others (Gebhart 2004; Porreca *et al.* 2002). The PAG and NCF are part of the brainstem anti-nociception network that control ascending information from the spinal level and connect to the

dorso-lateral PFC via the thalamus to create a top-down descending system which influences pain processing via brainstem structures in humans.

Mainero *et al.* (2007) mapped primary and secondary dynamic mechanical allodynia in the human trigeminal system. They used the heat/capsaicin model and mapped changes in the spinal trigeminal nucleus and brainstem nuclei. The authors found increased activity in the spinal trigeminal nucleus during primary and secondary mechanical allodynia and the ventrolateral PAG showed decreased activity, although pain ratings increased during primary allodynia. Areas of descending modulatory systems (pons, RVM, dorsolateral PAG) were all active during primary allodynia while the caudal MRF was more active during secondary allodynia. This study also confirmed the involvement of descending modulatory systems in central sensitization. Moulton *et al.* (2007) also looked at activity in the human trigeminal system. The difference with this study was the ability to distinguish nociceptive information from capsaicin-induced hyperalgesia. Topical capsaicin was applied to the maxillary skin and when the pain subsided, brush and two levels of painful heat were applied to the same site. Thermal hyperalgesia evoked greater activity in the trigeminal nuclei, thalamus and SI. Comparing capsaicin to untreated skin, there were significant changes in the bilateral dorsolateral PFC and amygdala indicating a cognitive and emotional component to the pain response. There are over 100 fMRI studies that have examined the affective component of pain in humans. Such studies have even shown that the PAG activation might be due to the anticipatory phases of processing noxious stimuli (Fairhurst *et al.* 2007). It has even been suggested that the anticipatory response to pain may modulate

the perception and maintenance of chronic pain (Koyama *et al.* 2005; Porro *et al.* 2002); but those studies go beyond the scope of this thesis. These studies all show observed changes that reflect a reorganization of descending processes and ascending information in the trigemino-thalamo-cortical nociceptive pathways.

1.14 Preliminary study on using spinal fMRI in the SC and brainstem

Using the established spinal fMRI method (Stroman *et al.* 2002c; Stroman *et al.* 2005a; Stroman 2006a), we (Foad Ghazni *et al.* 2007) mapped the areas of neuronal activity in the brainstem and cervical SC that are involved with touch and brush sensations in healthy volunteers. This was the first study to focus on determining the functional activity induced by sensory stimulation within the SC and lower supraspinal structures simultaneously in human subjects who at the same time, could rate their sensory experience. This study (Foad Ghazni *et al.* 2007) systematically examined innocuous mechanical stimulus-induced activity along the entire cervical SC and brainstem simultaneously in healthy humans.

Functional MRI (using SEEP) of the SC of eight healthy volunteers was carried out in a 3T Siemens Magnetom Trio. Light touch (2 g and 15 g von Frey filaments) and brush stimuli were applied manually to the dorsal surface of the hand. Spinal fMRI data were acquired from a volume spanning from the thalamus to the C7/T1 vertebral disc. The results were very consistent with the known neuroanatomy of sensory transmission pathways. The 2 g von Frey filament showed predominant activity in the medulla around the ipsilateral dorsal gracile and cuneate nuclei. There was also more medullary activity

with the 2 g filament than 15 g filament. The 15 g filament elicited significant activity in the ipsilateral dorsal and contralateral ventral gray matter areas of the SC, areas around the olivary nuclei, PRF, PAG, and raphe nuclei in the rostral pons and midbrain. The brush stimuli elicited far less activity in the SC, as compared to the von Frey filaments, but activity was detected around the ipsilateral cuneate and gracile nuclei in the medulla (Figure 1.3).

The findings from our study identified activity in all of the expected SC areas and brain structures related to sensation and pain, such as the dorsal gray matter (dGM) areas of the SC, gracile and cuneate nuclei in the medulla, and PAG (Maihofner *et al.* 2003; Peyron *et al.* 2004; Schweinhardt *et al.* 2006; Witting *et al.* 2001; Witting *et al.* 2006). Our study also demonstrated changes in activity consistent with descending modulation and facilitation, and is consistent with results from previous studies (Mainero *et al.* 2007; Zambreanu *et al.* 2005).

Although many studies to date have employed fMRI to study activity induced by tactile stimuli, primarily in the cortex, none have yet employed spinal fMRI as a tool to study sensory transmission in the SC and brainstem simultaneously. This information is valuable because of its application in SC injured patients or clinical populations with neuropathic pain. Spinal fMRI can be used to detect neuronal activity caudal to the injury site (Kornelsen and Stroman 2007; Stroman *et al.* 2004) or in chronic pain patients, regardless if the patient is able to feel the stimulus. Stroman *et al.* (2004) used spinal fMRI to detect activity in spinal cord injured patients in the entire lumbar cord and 11^{th} and 12^{th} thoracic segments and proved to be successful. The ability to detect

neuronal activity in the brainstem and SC is of considerable value for studies that aim to understand abnormal sensory responses due to injury or disease, plan treatment strategies, and monitor recovery of function during and after treatment.

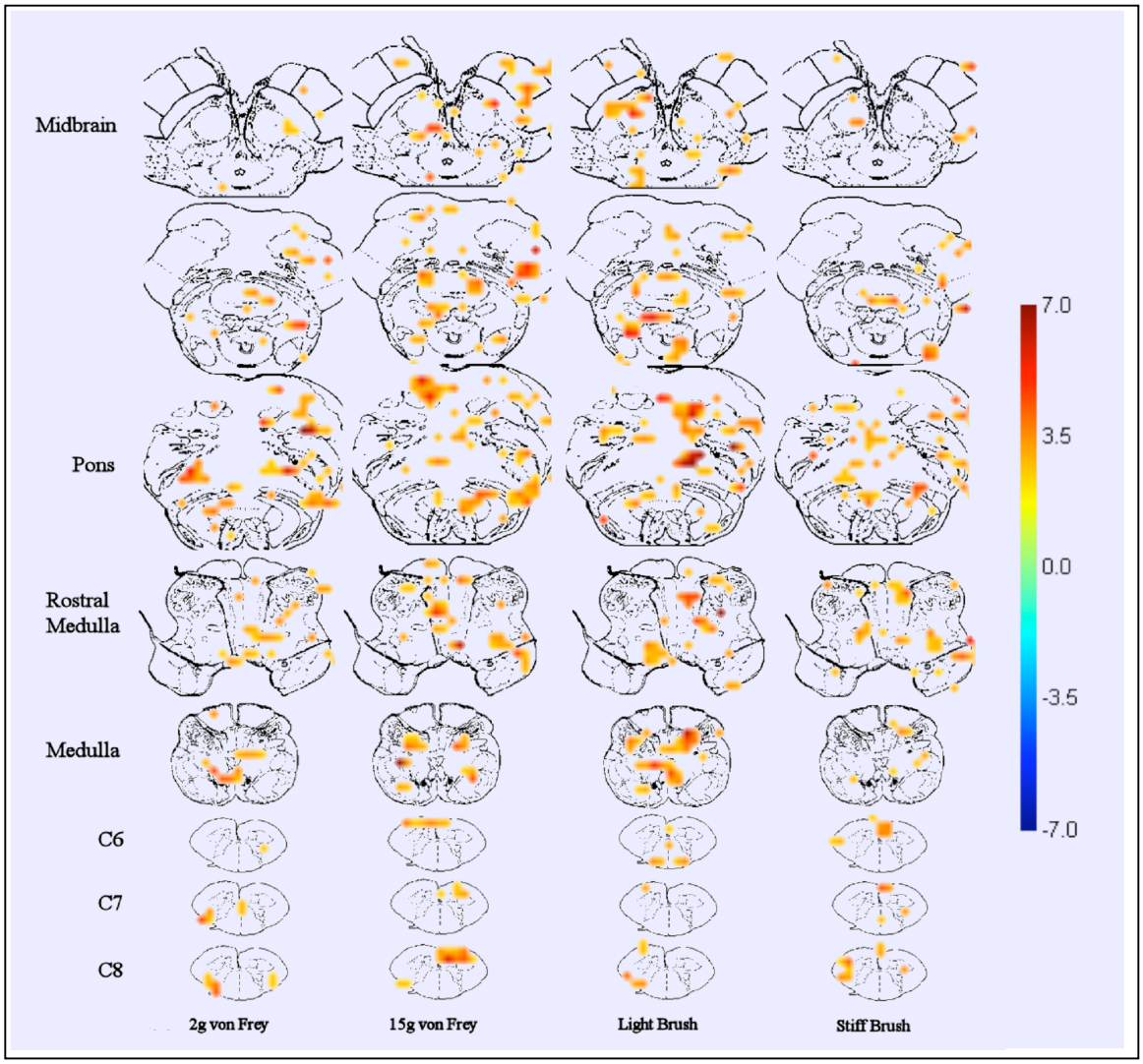

Figure 1.3. Innocuous Touch and Brush Activity Maps

Combined data showing location of neuronal activity in touch (2 g and 15 g filament) and brush stimuli from eight healthy volunteers superimposed onto transverse anatomical drawings. The stimuli were applied to the right thenar eminence. The figure shows significant areas of activity (T>2.5) across the group. The T-value correlation map on the right indicates the corresponding colour for each T-value.

1.15 Proposed Research

1.15.1 Purpose

The purpose of the proposed research is to determine, by means of fMRI, the neuronal activity that occurs in the human brainstem and SC with abnormal pain responses after peripheral sensitization of the skin in healthy volunteers.

1.15.2 Rationale

It is important to understand the exact neural mechanisms for experimentally-induced allodynia and hyperalgesia to obtain a better appreciation for why patients with neuropathic pain experience such phenomena (Ochoa and Verdugo 2001; Ochoa and Yarnitsky 1993; Verdugo *et al.* 2004). Hence, this valuable information can be used to assess patients with neuropathic pain, plan effective treatment strategies and monitor function and behaviour with the treatment approach. By doing so, we can understand the mechanisms underlying neuropathic pain and how different regions and nuclei of the CNS – from the nociceptors to transmission in the spinal columns to higher brain processing centres – contribute to this debilitating condition.

1.15.3 Hypothesis

We hypothesize that there will be significant differences in the anatomical regions that are activated and the magnitude of MR signal intensity changes in response to a non-painful stimulus, and a painful one, before and after peripheral sensitization.

1.15.4 Objectives

The specific objectives of this study are:

1. To determine the activity elicited by mechanical stimulation before and after peripheral sensitization caused by the application of capsaicin (Chapter 2).
2. To determine the activity elicited by equi-nociceptive mechanical stimulation in normal and sensitized skin (Chapter 3).

Chapter 2

Altered spinal cord and brainstem activation in response to peripheral sensitization to sensory stimuli in healthy humans: a spinal fMRI study

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2.1 Introduction

Neuroimaging methods have substantially increased our understanding of sensory processes in the central nervous system (CNS). Functional magnetic resonance imaging (fMRI) studies have identified multiple brain structures involved in the pain experience yet most of these studies have focused on structures rostral to the thalamus. However, since the first level of which sensory transmission can be modulated occurs at the level of the spinal cord (SC), functional studies in this region could provide valuable information on how pain transmission is processed. Currently there is only one (to our knowledge) human high resolution functional study that has correlated perception of sensory information with neuronal activity within the SC and brainstem simultaneously (Foad Ghazni *et al.* 2007). More studies of this nature are needed to fully understand how pain is transmitted and modulated and additionally may provide fundamental basics for

developing diagnostic criteria for various chronic pain conditions and idealistically lead to the implementation of tailored treatments to alleviate such pain.

In this study, we used fMRI techniques to examine the activity within the SC, brainstem, and midbrain of healthy volunteers to two types of innocuous stimuli: von Frey filaments and brush stimuli that produce static and dynamic mechanical responses, respectively. Using tactile stimulation for fMRI experiments is practical because we can potentially use it in patients who cannot detect active sensory paradigms i.e. paraplegics. The two stimuli were applied before and after peripheral sensitization, induced by topical application of capsaicin. Capsaicin is a chemical compound used extensively to study CNS activity related to sensitization (Petersen and Rowbotham 1999). Topical application of capsaicin causes a burning painful sensation by sensitizing primary afferent C and Aδ fibres (Dray 1992). This creates an area of primary and secondary hyperalgesia in response to punctate stimuli (von Frey) or light touch (brush) (Kilo *et al.* 1994) and can therefore serve as a model of mechanical allodynia. By using capsaicin, a state of altered sensitivity was induced, allowing a comparison of the activity responses of innocuous touch and brush before and after peripheral sensitization. Accordingly, it provided an opportunity to examine abnormal pain processing in normal subjects. The objective of the study was to determine whether differences in neuronal activity produced by a specific stimulus type and force could be detected following peripheral sensitization and to identify whether areas of activation consistent with pain transmission was induced by the non-painful stimuli following sensitization. This study is unique in that it can assess the various components of dynamic and static stimuli before peripheral

sensitization and the pain experiences after peripheral sensitization. By detecting the differences in activity between normal nociceptive pain and abnormal pain responses, we can understand altered sensory conditions such as in neuropathic pain.

2.2 Materials and Methods

2.2.1 Volunteer Recruitment

Recruitment of healthy volunteers was performed by means of poster advertisements placed around Queen's University (see Appendix A). Respondents (ages 18-40) were interviewed by phone and then asked to answer a questionnaire to exclude anyone with neurological disorders, previous injury to the brain or SC, any peripheral injury that affects the sensitivity of their hands to touch, or with any MRI safety risks (implants, pacemaker, etc) (see Appendix B for the MRI Safety Checklist). All protocols were approved by Queen's University Human Research Ethics Board and informed consent (Appendix C) was obtained for all subjects meeting the inclusion/exclusion criteria (Appendix D). Twelve healthy individuals participated in the study, median age 23 (range 20-35 years). No individual was excluded because of age, race or previous participation in fMRI studies. Four of the twelve participants were not used in the analysis because:

1) volunteers were unable to lie still (n = 2)
2) request to withdraw from the study (n = 1) and
3) the pain thresholds did not fall within the study's criteria (n = 1); the volunteer did not report a pain score for the greatest gram force von Frey filament.

Due to the elimination of the individuals noted above, a total of eight volunteers (2 males and 6 females) were included in the data analysis. All data were treated confidentially with each set of data assigned a unique identifying number that was only accessible by the experimenter.

2.2.2 Experiment Protocol

Volunteers were asked to participate on two consecutive days. On day 1, psychophysical testing was performed to determine pain thresholds, using different mechanical stimuli in a sham MRI system, which replicates the environment in the actual MRI system. On day 2, volunteers were imaged in the 3T MRI system. Various mechanical stimuli were applied to the volar surface of the participant's forearm prior to and followed by capsaicin on each day, although opposite arms were used on the two days of testing.

2.2.3 Day 1 – Psychophysical Testing

Psychophysical testing was performed by applying a 2 cm wide artist brush and von Frey filaments to the left volar forearm. The von Frey filaments are calibrated to apply a certain force when they are applied perpendicular to the skin and form a semi-circle. Prior to experimentation, subjects were read a description of how to rate pain intensity and unpleasantness (Appendix E). After each stimulus, subjects were instructed to report their pain intensity using an 11 point numerical scale; where 0 = no pain at all and 10 = worst possible pain imaginable. In addition, subjects were asked to rate the sensation in the context of unpleasantness where 0 = not unpleasant, 10 = excruciatingly

uncomfortable and intolerable (Rainville *et al.* 1992). A 3 cm x 3 cm box was drawn on the volar surface of the participant's left forearm (approximately 2 inches above where the wrist bends). The capsaicin was applied to the skin within this box. A mark, with a non-permanent marker, was made on the skin to ensure that the von Frey hairs were applied to the same location each time. Each volunteer was subjected to both types of mechanical stimulation. The two types of touch stimuli were chosen to represent a static (von Frey) or dynamic (brush) response since these two types of stimuli are known to activate different pathways (Field *et al.* 1999).

The brush stimulus was applied to the forearm only once in a back and forth motion. Von Frey filaments, calibrated to produce the correct force when applied perpendicular to the skin until the filament bent, were applied three times for each filament. Application of filaments began at 1 g force and continued to 60 g, or until the stimulus was described as painful. The von Frey filament stimulus producing a pain rating of one (intensity scale) on the eleven point numerical scale was noted. The sequence of whether the brush or von Frey filament was applied first was randomized between volunteers but consistent within an individual. Hence, if brush preceded von Frey stimulation then brush always preceded the von Frey applications prior to and following capsaicin on both days 1 and 2.

Following application of the brush and von Frey filaments, capsaicin (0.075% Zostrix HP®) was applied with a cotton swab to the entire 3 cm square area. Capsaicin was left on the arm for 30 minutes after which it was gently removed with a cotton swab. Subjects were then exposed to the same mechanical stimuli as was done prior to the

application of capsaicin. Subjects were asked to rate their pain in the context of intensity and unpleasantness after each mechanical stimulus.

2.2.4 Day 2 - Imaging

A 3 cm x 3 cm box was drawn on the participant's right arm, contralateral to the arm that the psychophysical testing was performed on the previous day. Stimuli were applied in a block paradigm consisting of three stimulation periods of 56 seconds duration, interleaved with baseline periods of 140 seconds in which no stimuli were applied, and an initial baseline of 84 seconds for a total of 11 minutes 12 seconds for each experiment (Figure 2.1).

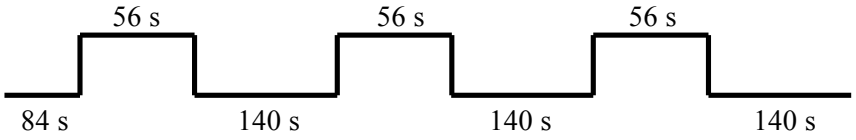

Figure 2.1. Block Paradigm for Each Stimulus

Each stimulus was applied three times interleaved with baseline periods. The total scan time for each experiment was 11 minutes 12 seconds.

All stimuli were applied at a frequency of 1 Hz so that it was possible to apply manually while providing a nearly continuous stimulus related to the speed of the fMRI signal change response. The stimuli were applied by the experimenter who was in the scanner room throughout the duration of the experiment. The pace of stimulation was maintained by a visual prompt on a digital projector that was only visible to the experimenter. Both the brush and von Frey stimuli were used on all the volunteers and the order of the stimuli were randomized across the volunteers to avoid order effects across repeated experiments. This sequence variation was implemented to minimize

extra-pyramidal factors such as anxiety and interest, over time. Subjects were instructed to focus on the stimulus throughout each scanning paradigm and then immediately report a rating for pain intensity and unpleasantness after each experiment.

After application of both brush and the von Frey filament (pain intensity = 1 determined on day 1 during psychophysical testing), topical capsaicin cream was then applied to the right forearm over the 3 cm square surface, to induce peripheral sensitization. After a wane period of 30 minutes (when the initial burning sensation has subsided), the same mechanical stimuli were applied in the same order (Figure 2.2). During the capsaicin wane period, an anatomical scan was performed.

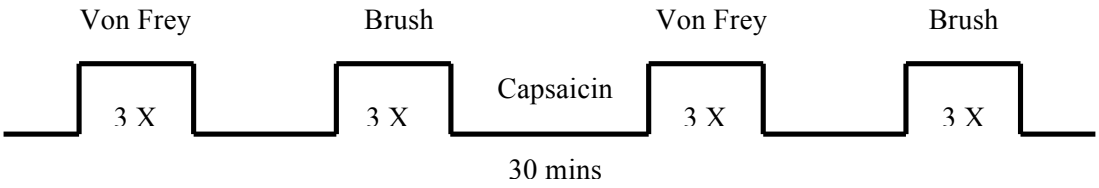

Figure 2.2. Experimental Design for Innocuous Brush and von Frey Filaments

The brush and same von Frey filament were both applied before and after capsaicin in the same order. The order of stimuli was randomized from one subject to the next. Each stimulus was applied three times (3X).

2.2.5 FMRI Data Acquisition

Functional MRI of the SC was carried out in a 3 T Siemens Magnetom Trio using a phased-array spine receiver coil with subjects lying supine. Localizer images were first acquired in 3 planes as a reference for slice positioning for subsequent fMRI studies. Functional MRI data were acquired for each study with a half-Fourier single-shot fast spin-echo sequence (HASTE) with an echo time (TE) of 38 msec, and repetition time

(TR) of 1 second per slice. Signal intensity changes observed upon a change in neuronal activity were the result of the SEEP effect, as described previously (Stroman *et al.* 2002c; Stroman *et al.* 2005a; Stroman 2006a) with a contribution from BOLD. Sagittal image slices were selected to span from the C7/T1 disc to the superior edge of the thalamus, with a 20 cm x 10 cm FOV, a 192 x 96 matrix, in 14 contiguous sagittal slices, each 2 mm thick. The resulting voxel size was 1 mm x 1 mm x 2 mm. Spatial suppression pulses were employed to eliminate signal anterior to the spine to eliminate motion artifacts from the heart etc, and flow-compensation gradients were applied in the rostral-caudal direction to reduce artifacts from flowing cerebrospinal fluid. The peripheral pulse was recorded throughout each study for use in subsequent data analysis.

2.2.6 Data Analysis

The resulting three-dimensional functional image data were analyzed with custom-made software written in MatLab®. In brief, sagittal-slice data were analyzed as described previously (Stroman *et al.* 2008b) by first drawing a reference line along the anterior edge of the SC in a mid-line slice and extending along the entire brainstem up to the anterior edge of the thalamus. The reformatted SC and brainstem were normalized to a standard coordinate space for all studies to facilitate group comparisons of results (Stroman *et al.* 2008b) (Figure 2.3). The accuracy of the spatial normalization has been shown to be within 2 mm (Stroman *et al.* 2008b). Smoothing was applied only parallel to the long axis of the SC and brainstem. The data were then analyzed using a General Linear Model, using the peripheral pulse trace sampled at the time of acquisition of each slice to account for confounding effects arising from cardiac motion, as described

previously (Figley and Stroman 2007; Stroman 2006a). Group results were determined by means of a fixed effects analysis (Friston *et al.* 2007; McGonigle *et al.* 2000). Areas outside the defined boundaries of the SC and brainstem were not masked. Areas of activity were identified visually with comparison to a stereotaxic atlas (DeArmond *et al.* 1974; Tamraz and Comair 2006).

2.2.7 Statistical Analysis

The psychophysical data were analyzed with a two-tailed, paired, Student's t-test ($p < 0.05$). Mean pain intensity and unpleasantness ratings were compared before and after capsaicin with results displayed as mean ± standard deviation.

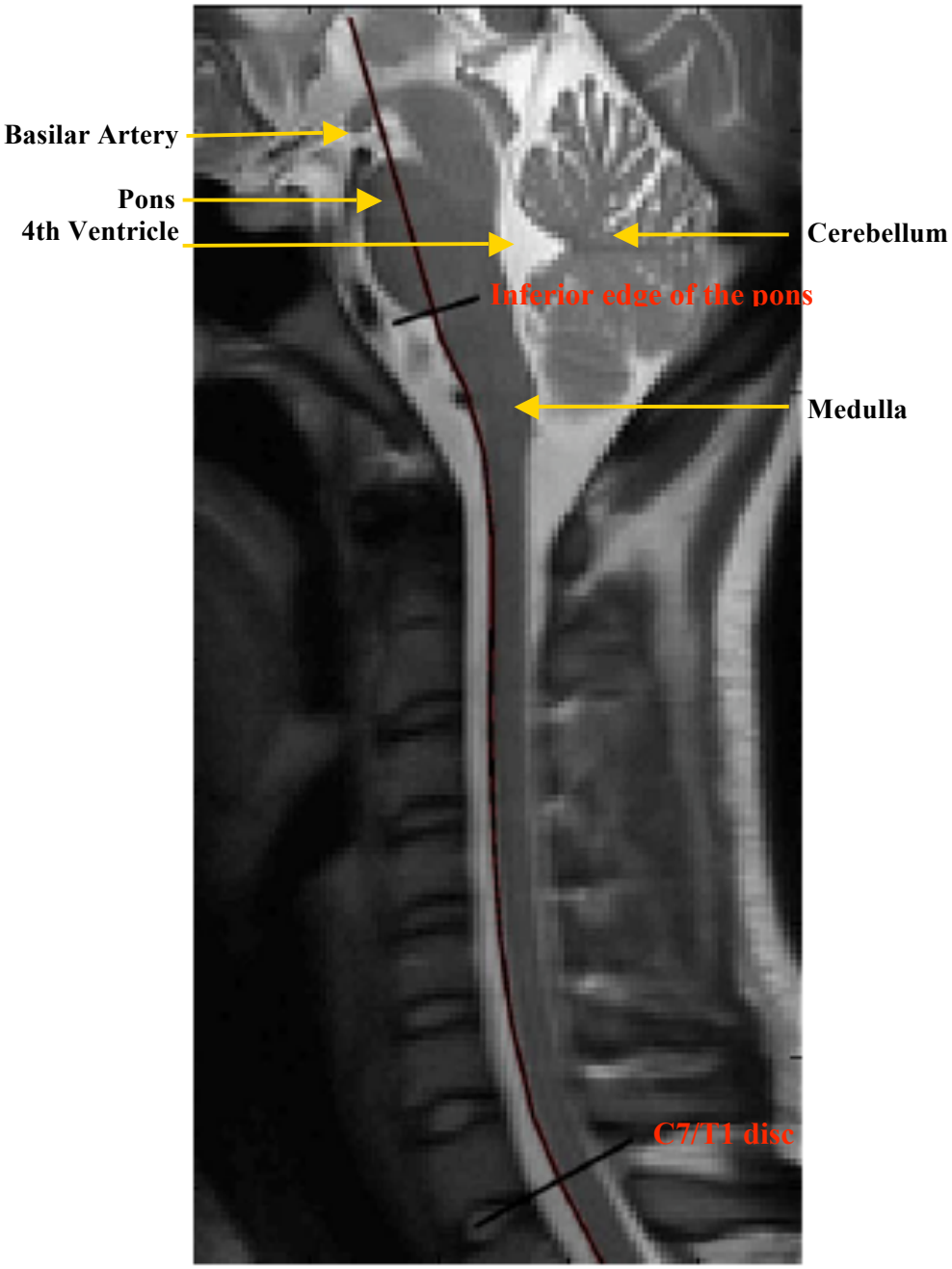

Figure 2.3. Normalized spatial space for the SC and brainstem

A reference line was drawn along the anterior edge of the SC along the brainstem up to the anterior edge of the thalamus. Sagittal images spanned from the C7/T1 disc (bottom red line) to the superior edge of the thalamus (top red line). Modified from Stroman *et al.* (2008b).

2.3 Results

2.3.1 Psychophysical Data

Psychophysical testing was done prior to scanning to determine each volunteer's pain threshold (Table 2.1, Table 2.3). We used a topical application of capsaicin to produce sensitization, to avoid the invasiveness of injections, as used in other studies (Baron *et al.* 1999; Iadarola *et al.* 1998; Lorenz *et al.* 2002). Following capsaicin treatment, subjects reported higher pain intensity and unpleasantness ratings to light brushing and the non-painful von Frey filament; the same filament used prior to capsaicin.

Before capsaicin, all volunteers rated the brush stimulus as not painful prior to capsaicin on day 1 (mean intensity = 0, mean unpleasantness = 0.3 ± 0.5) (Table 2.1) and on day 2 (mean intensity = 0, mean unpleasantness = 0.1 ± 0.3) (Table 2.2). Following the application of capsaicin on day 1, mean pain intensity and unpleasantness ratings to the brush stimulus increased, but these were not found to be significant (mean intensity = 0.4 ± 0.4, mean unpleasantness = 0.6 ± 1.1) (Table 2.1). After capsaicin treatment, on day 2, mean unpleasantness ratings to the brush stimulus were significantly increased (1.3 ± 1.3, $p<0.05$), but there was no difference in the ratings of pain intensity prior to (0) and following capsaicin (0.4 ± 0.7) (Table 2.2).

Table 2.1. Brush Stimuli Psychophysical Data – *Day 1*		
	Before	**After**
Intensity	0	0.4 ± 0.4
Unpleasantness	0.3 ± 0.5	0.6 ± 1.1

Table 2.2. Brush Stimuli Psychophysical Data – *Day 2*		
	Before	**After**
Intensity	0	0.4 ± 0.7
Unpleasantness	0.1 ± 0.3	1.3 ± 1.3*

Psychophysical data showing pain intensity and unpleasantness scores across 8 healthy volunteers with brush stimuli before and after capsaicin. Table 2.1 shows psychophysical data from day 1 while Table 2.2 shows psychophysical data from day 2. Scores are displayed as mean ± standard deviation. Significance (*) was determined using a two-tailed, paired, Student t-test ($p<0.05$).

The von Frey filaments selected for psychophysical testing ranged from 2 g – 26 g of force on day 1 (Table 2.3) and the filament that produced a pain intensity rating of 1 before capsaicin was chosen for fMRI studies on day 2. On day 1, the mean pain intensity of the selected von Frey filament slightly increased following the application of capsaicin (1.9 ± 1.4), but was not significant. However, the mean unpleasantness ratings significantly increased from 1.4 ± 0.7 to 2.5 ± 1.5 (Table 2.3) following the application of capsaicin. On day 2, the pain intensity ratings for the pre-determined von Frey filament significantly increased following application of capsaicin where ratings ranged from 0-2 (mean = 0.7 ± 0.9) before and 0-5 (mean = 1.4 ± 1.5) after capsaicin (Table 2.4). Similarly, pain unpleasantness was reported significantly higher following capsaicin.

Hence, unpleasantness ratings ranged from 0-3 before (mean = 1.2 ± 1.0) and 0-6 after (mean = 2.8 ± 2.3) capsaicin (Table 2.4). The psychophysical data demonstrate that the volunteer's pain threshold decreased after the skin was sensitized with capsaicin, and thus they reported higher pain intensity and unpleasantness ratings for both stimuli. The von Frey filament that produced a pain intensity of 1, as determined on day 1 for psychophysical testing (Table 2.3) was used prior to and following capsaicin for fMRI studies.

Table 2.3. Psychophysical Data with Innocuous Touch (von Frey) Stimuli – *Day 1*

	Before	**After**
Intensity	1.0	1.9 ± 1.4
Unpleasantness	1.4 ± 0.7	2.5 ± 1.5*

Table 2.4. Psychophysical Data with Innocuous Touch (von Frey) Stimuli – *Day 2*

	Before	**After**
Intensity	0.7 ± 0.9	1.4 ± 1.5*
Unpleasantness	1.2 ± 1.0	2.8 ± 2.3*

Psychophysical data showing intensity and unpleasantness scores across 8 healthy volunteers with von Frey stimuli before and after capsaicin. Volunteers reported a pain rating of one before capsaicin. Table 2.3 shows psychophysical data from day 1 while Table 2.4 shows psychophysical data from day 2. Scores are displayed as mean ± standard deviation. Significance (*) was determined using a two-tailed, paired, Student t-test ($p<0.05$).

2.3.2 Group Results

Group fMRI results across the eight volunteers, as determined by fixed effects analysis, for the ***brush*** stimulus are shown in Figure 2.4 with areas of consistent activity colour-coded, according to the legend described in the figure caption. Areas of significant signal changes ($p<0.001$) are inferred to reflect changes in neuronal activity when the stimulus was applied. In the cervical SC, activity was noted in the ipsilateral DH and contralateral DH *prior to* capsaicin. There appeared to be more activity in the medulla relative to in the SC, in anatomical regions consistent with the location of the gracile and cuneate nuclei. The majority of the activity appeared to be located in the pons and midbrain regions. Almost all of the volunteers had activity in the posterior medial pons where the ML pathway is found. There was also activity in the PRF and in the anterior pons where sensory nuclei are found. In the midbrain, there was predominant activity in anatomical regions consistent with the location of the ipsilateral red motor nuclei, PAG, and areas where the STT and ML pathways are found.

After capsaicin, the ***brush*** stimulus produced activity in the ipsilateral DH and contralateral VH. There was activity in the area of the ML pathway in the medial medulla. There was also PRF activation, activity in the sensory nuclei in the anterior pons, the ipsilateral red motor nuclei, and at the border between the thalamus and midbrain, consistent with activation of descending pathways originating in the thalamus. There was no significant activity across subjects in regions consistent with the location of the PAG with the brush stimulus after sensitization. The results for the brush stimulus are summarized in Table 2.5.

Table 2.5. Main Areas of Activity – Brush		
	Before	**After**
Spinal Cord	▪ Ipsilateral DH ▪ Contralateral DH	▪ Ipsilateral DH ▪ Contralateral VH
Brainstem	▪ More medullary than SC activity ▪ Vicinity of the gracile and cuneate nuclei ▪ Posterior medial pons ▪ PRF ▪ Sensory nuclei in the anterior pons ▪ Ipsilateral red motor nuclei ▪ STT and ML synaptic areas ▪ PAG	▪ Medial medulla in the area of the ML pathway ▪ PRF ▪ Sensory nuclei in the anterior pons ▪ Ipsilateral red motor nuclei ▪ Thalamic-midbrain border ▪ No PAG

The main areas of activity in the SC and brainstem for brush stimuli before and after application of capsaicin.

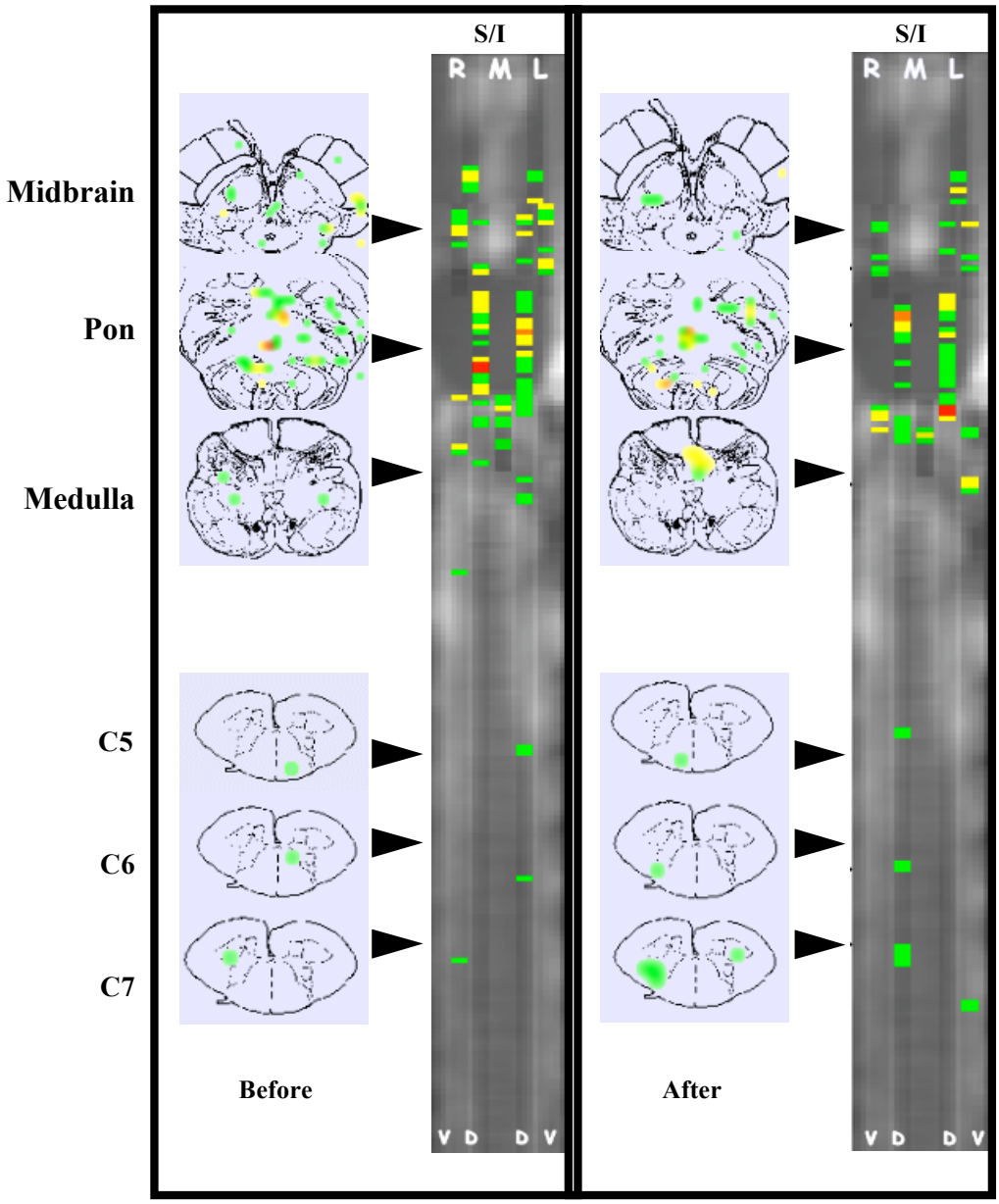

Figure 2.4. Innocuous Brush Stimuli Before and After Peripheral Sensitization

Combined group results showing areas of activity with brush stimuli in 8 healthy volunteers before and after capsaicin. The right hand of the frame shows the rostral-caudal distribution of activity. Activity in areas of significant signal changes are overlaid onto a spatially normalized mid-line coronal slice. The rostral-caudal activity is then superimposed onto transverse anatomical images of the respective segments (left) in radiological orientation. The colours represent activity in each voxel or an immediate neighbour where green represents activity in 4 out of 8 subjects; yellow-5; orange-6; and red-7 out of 8 subjects. D: dorsal; V: ventral; R: right; M: midline; L: left.

Application of the ***von Frey filament*** produced cervical SC activity in the ipsilateral and contralateral VH and around the central canal *prior to* capsaicin (

Figure 2.5). In the medulla, there was activity in the area where the STT synapses with secondary neurons and activity was significant in the regions consistent with the location of the olivary nuclei. In higher brainstem structures, there was activity in the anterior pons where sensory nuclei are found, PRF, PAG, contralateral red motor nuclei and activity in the area where descending tracts from the thalamus synapse in the midbrain.

After sensitization with capsaicin, application of the **von Frey filament** produced minimal SC activity (contralateral DH at C7) as compared to prior capsaicin. In the medulla, pons, and midbrain regions, the activity pattern was similar to that seen prior to capsaicin. The only notable increase in activity following capsaicin was in the midbrain where there appeared to be more activity at the thalamic-midbrain border; an area where descending pathways from the thalamus pass through to the midbrain and other supraspinal structures. The important areas identified as active before and after capsaicin are displayed in Table 2.6.

Table 2.6. Main Areas of Activity – Innocuous von Frey		
	Before	**After**
Spinal Cord	▪ Ipsilateral VH ▪ Contralateral VH ▪ Central canal	▪ Contralateral DH
Brainstem	▪ STT synaptic areas in the medulla ▪ Olivary nuclei ▪ Sensory nuclei in the anterior pons ▪ PRF ▪ PAG ▪ Contralateral red motor nuclei ▪ Thalamic-midbrain border	▪ Medial medulla in the area of the ML pathway ▪ Sensory nuclei in the anterior pons ▪ PRF ▪ Contralateral red motor nuclei ▪ More activity at the thalamic-midbrain border

The main areas of activity in the SC and brainstem for innocuous von Frey touch stimuli (pain intensity =1) before and after application of capsaicin.

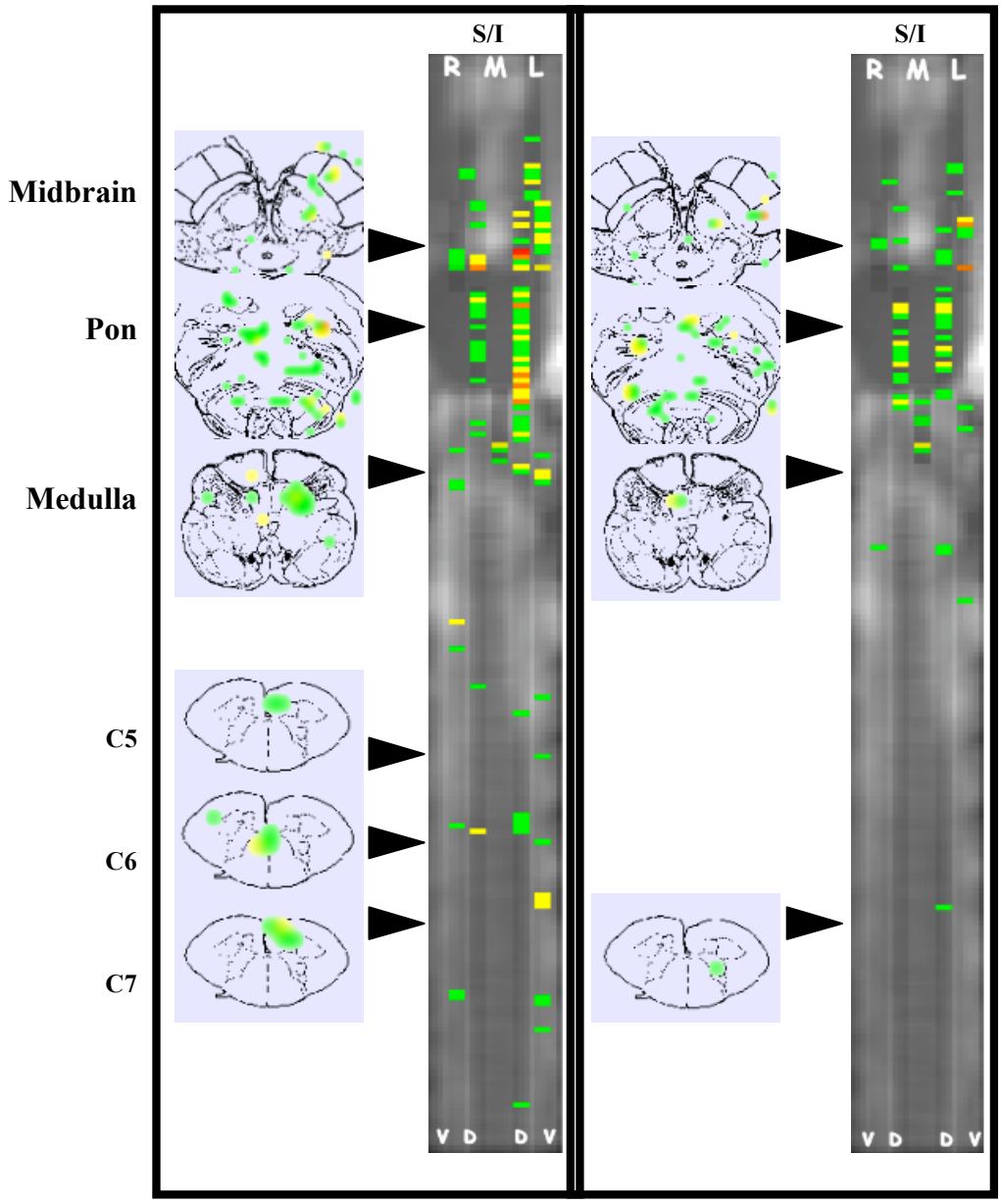

Figure 2.5. Innocuous Touch Before and After Peripheral Sensitization

Combined group results showing areas of activity with von Frey stimuli in 8 healthy volunteers. Von Frey touch (pain rating = 1) stimuli were applied before (left) and after (right) capsaicin. The right hand of the frame shows rostral-caudal distribution of activity. Significant signal changes reflecting neuronal activity are overlaid onto a spatially normalized mid-line coronal slice. The rostral-caudal activity is then superimposed onto transverse anatomical images of the respective segments (left frame) in radiological orientation. The colours represent activity in each voxel or an immediate neighbour where green represents activity in 4 out of 8 subjects; yellow-5; orange-6; and red-7 out of 8 subjects. D: dorsal; V: ventral; R: right; M: midline; L: left.

2.4 Discussion

This study demonstrated activity in areas of the SC and brainstem elicited by innocuous touch (static) and brush (dynamic) mechanical stimuli before and after capsaicin. To our knowledge there have been no other fMRI studies that have systematically examined innocuous mechanical stimulus-induced activity along the cervical SC and brainstem in the presence of peripheral sensitization.

2.4.1 Comparing Brush Data Before and After Peripheral Sensitization

The key differences between the activity observed before, as compared to after, the application of capsaicin will be discussed and how these differences can help us understand processes involved in sensitization. In the SC, there is a lack of contralateral DH activity after the application of capsaicin and the presence of contralateral VH activity. Activity in the contralateral DH with the brush stimulus is evidence of descending projection efferents of the bulbospinal tract, originating in supraspinal structures and terminating in the SC (Yezierski *et al.* 1982). These descending efferents have been known to modulate incoming sensory responses and release serotonin to produce analgesia at the SC level (Besson 1999). Contralateral DH activity may also arise from descending efferents of the RF (Stroman *et al.* 2008a); indeed there is significant activity in the PRF before capsaicin. The lack of activity in the contralateral DH after sensitization may reflect activation of an alternative descending modulatory pathway, not specifically originating in the PRF or rostral brainstem. Accordingly, activity at the thalamic-midbrain border could indicate descending fibres that originate from the thalamus and terminate in the SC (Stroman *et al.* 2008a; Willis and Coggeshall

1991c). The contralateral VH activity after the application of capsaicin with the brush stimulus is indicative of descending modulatory pathway activation. The activity at the thalamic-midbrain border supports this idea because this area is a key termination site for the STT (Craig 2003).

Before capsaicin, the brush stimulus produced activity in the medulla in the area where the gracile and cuneate nuclei are found. However after capsaicin, the only activity observed was in the medial medulla where the ML pathway is found and no activity in the area of the gracile and cuneate nuclei. The gracile and cuneate nuclei are key regions involved in carrying proprioception and fine touch information. The presence of activity in these regions suggests that the brush stimulus was perceived as non-painful before capsaicin, but the lack of activity in this region and the appearance of activity in the ML pathway subsequent to sensitization indicates that the brush stimulus activated pathways consistent with pain transmission. The psychophysical data also supports this finding with volunteers rating the brush stimuli as more painful and more unpleasant following peripheral sensitization.

After sensitization the brush stimulus failed to elicit PAG activity, which was evident with the same stimulus prior to sensitization. The PAG is a major site for homeostatic control and limbic motor output. Stimulation of different areas of the PAG elicits aversive behaviours, cardiovascular changes and antinociceptive modulation. The PAG is strongly connected to the thalamus which is a key area of convergence of ascending spinal nociceptive information (Bushnell and Apkarian 2006) showing activity during painful states and decreased activity during nociceptive control. The absence of

activity in the PAG could suggest activation of another pathway that involves the PRF and thalamus, but not the PAG. PRF activity at the midbrain-thalamic border was present and may indicate activation of the spinoreticular tract.

The psychophysical data for the brush stimulus indicates that after peripheral sensitization, the volunteers found the stimulus more painful and more unpleasant. The brush stimulus was perceived as not painful before capsaicin by all of the volunteers on both days of testing. The range for unpleasantness (0-4) and the mean unpleasantness rating (1.3 ± 1.3) significantly increasing after capsaicin on day 2 of testing although this result was not seen on day 1. The difference in unpleasantness ratings between the two days is currently unknown but may be an effect of application time and the brush was applied for a longer duration on day 2. More importantly, since pain unpleasantness, but not intensity increased following sensitization, the differences seen in activity cited above may reflect regions controlled by pain affect (emotion). Further studies with connectivity analysis will be necessary to determine whether areas such as the ACC or PFC (regions involved in affective components of pain) influence the spinoreticular tract and ML pathways.

2.4.2 Comparing von Frey Data Before and After Peripheral Sensitization

The main difference in neuronal activity generated by von Frey filaments before and after capsaicin was the lack of activity in the SC after peripheral sensitization. Accordingly, the only SC activity evident after sensitization was found in the contralateral DH. Contralateral DH activity may indicate activation of descending

efferents of the bulbospinal tract and PRF (Stroman *et al.* 2008a; Yezierski *et al.* 1982). Such activation is consistent with the reported increase in pain intensity and unpleasantness ratings after capsaicin. There also appeared to be more activity at the midbrain-thalamic border, as compared to before capsaicin. The activity in the rostral midbrain, along with the significant activity in the PRF, provides evidence of descending modulatory pathway activation.

Another interesting finding was the presence of VH activity before the application of capsaicin. Ipsilateral VH activity could indicate activation of the motor reflex circuitry. It could also be indication of descending projections from the PRF as found in a study by Stroman *et al.* (2008a) who summarized the correlations between different areas of the spinal cord architecture and brainstem structures and found an inhibitory connection between the PRF and ipsilateral vGM. The activity in the contralateral VH could also indicate descending pathway activity.

2.4.3 Comparing von Frey and Brush Stimuli

The von Frey and brush stimuli are both mechanical stimuli that activate Aβ and Aδ fibres to evoke a touch and/or pain response. The pattern of activity produced by both stimuli were comparable in many aspects. The following discussion will examine the similarities and differences between the two tactile stimuli, in the presence and absence of peripheral sensitization.

The brush stimulus was an innocuous sensation that activated Aβ fibres, which sent collaterals to the ipsilateral DH of the SC, but projected predominantly to the

medulla. The von Frey stimulus was a sub-threshold sensation of pain that may activate both Aβ and Aδ fibres (Ochoa and Yarnitsky 1993). The differences in CNS activity patterns generated by the static and dynamic stimuli most likely arise from their differences in activation of primary afferent neurons.

The activity distribution for the von Frey and brush stimuli differ before capsaicin, but both produce neuronal activity consistent with non-painful sensory pathways. Before capsaicin, the brush stimulus activated areas in the DH while the von Frey stimulus activated areas in the VH. The DH processes sensory information while activity in the VH could reflect activation of descending modulatory pathways and motor responses. There appeared to be more activity in the medulla with the von Frey stimulus than the brush stimulus and as expected, more medullary activity than in the SC with both stimuli. In the medulla, the brush stimulus activated areas consistent with the location of the gracile and cuneate nuclei, while the von Frey stimulus evoked activity in areas where the STT synapses onto secondary neurons. This finding may indicate that the brush stimulus is transmitting non-painful sensory information while the von Frey stimulus may also be activating pain sensory information pathways.

The activity patterns for brush and von Frey stimuli after peripheral sensitization are different yet the activity is consistent with both activating pain pathways. The brush stimulus appeared to produce more activity in the SC after capsaicin than the von Frey stimulus. With the brush stimulus, there was very strong indication of STT activity as evident by the ipsilateral DH. Although the von Frey stimulus was perceived as painful after capsaicin, the only activity seen in the SC is in the contralateral DH and that could

be evidence of descending efferents from the brainstem. There appeared to be more activity in the medulla with the brush than the von Frey stimulus. However, the activity for both stimuli was concentrated in the anterior medial medulla where the ML pathway crosses. These two stimuli are transmitted via different sensory neurons based on the different fibre types they activate. The brush stimulus produces a dynamic response while the von Frey filament produces a static response. In the presence of capsaicin, they both produce mechanical allodynia. Static allodynia has been reported to be signaled by nociceptive A fibres and mediated by central sensitization (Field *et al.* 1999), but may also involve C-fibre (nociceptive) neurons. Dynamic allodynia is generally thought to be independent of C-fibre activation and mediated by Aβ fibre activation; because selected blockage of Aβ fibres by compression-ischemia abolishes dynamic, but not static allodynia (Ochoa and Yarnitsky 1993)..

2.4.4 Conclusions

This study demonstrated observable differences between von Frey (static) and brush (dynamic) stimuli. The innocuous stimuli used in this study activated areas of the SC and brainstem involved in non-painful sensory transmission. This study also demonstrated that an innocuous stimulus perceived as painful after peripheral sensitization with capsaicin induced activity consistent with activation of pain pathways. This study also suggests that different descending modulatory pathways are activated in response to an innocuous brush stimulus, an innocuous von Frey stimulus, and in the presence of peripheral sensitization.

Chapter 3

Noxious tactile stimuli in response to peripheral sensitization in healthy humans: a spinal fMRI study

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3.1 Introduction

Neuropathic pain is a debilitating condition that affects 2-3% of the population in North America (Gilron *et al.* 2006). It often leaves individuals either partially or totally disabled for weeks to months, diminishing their quality of life. It is associated with the occurrence of allodynia (a painful response to a normally non-painful stimulus) and hyperalgesia (exaggerated response to a normal painful stimulus). Treatment of neuropathic pain is particularly challenging as this pain is typically refractory to conventional treatment such as opioid analgesics. There are numerous theories that have been proposed to underlie the development and maintenance of neuropathic pain states, but the mechanisms are yet unknown.

Functional MRI has revolutionized our understanding of central pain processes. For example, fMRI has shown differences in signal change responses to specific

modalities such as brush, heat, and cold in normal or unaffected regions in patients with neuropathic pain (Borsook *et al.* 2004). In the present study, we aimed to determine the changes that occur in sensory transmission in human subjects following peripheral sensitization by means of fMRI in the SC. The goal of the project was to better understand abnormal pain processing at caudal areas of sensory integration. Hence, although functional imaging studies have reported differences in cortical regions of activation between healthy and clinical pain patients, such differences may arise from how pain is processed even from the first synaptic integration of sensory information in the SC.

The present study was undertaken to determine if pain generated by two different methods (von Frey filaments without prior peripheral sensitization and von Frey filaments following peripheral sensitization induced by topical capsaicin) would produce similar patterns of activation. Accordingly, the stimuli consisted of application of von Frey filaments and were deemed to be equi-nociceptive based on a 11 point numerical scale, as described previously (Rainville *et al.* 1992).

The objectives of this study were:

1) To determine the activity produced by a tactile stimulus that volunteers report as painful
2) To determine if two equi-nociceptive stimuli, but of different force produced similar or different patterns of neuronal activity.

We hypothesize that stimuli that produce same pain ratings before and after peripheral sensitization will produce similar activity patterns in the brainstem and SC, even though the force of the filament used to evoke the responses is different.

This study is unique in that it proves to demonstrate how pain information is transmitted from the first synapse in the DH to the brain in healthy individuals and how we may be able to replicate this experience after peripheral sensitization. The changes induced by sensitization can then help us understand abnormal pain processing in altered sensory disorders such as neuropathic pain.

3.2 Materials and Methods

Refer to Chapter 3 for Volunteer Recruitment, Experimental Protocol, FMRI Data Acquisition and Data Analysis.

3.2.1 Volunteer Recruitment

Twelve healthy individuals participated in the study with a median age of 24 (range 19-29 years). Two participants were not used in the analysis because:

1) volunteer was unable to lie still (n=1) and

2) request to withdraw from the study (n=1)

A total of 10 volunteers (4 males and 6 females) completed the study and were included in the data analysis.

3.2.2 Mechanical Stimuli

The tactile stimuli used in this study were von Frey filaments that were calibrated to produce a specific known force when applied perpendicular to skin until the filament bends. Tactile stimuli were applied to the skin over a range of forces starting at 1 g, and then in incremental amounts until the stimulus was described as being painful.

3.2.3 Day 1 - Psychophysical Testing

Prior to experimentation, subjects were read specific instructions for how to rate pain intensity and unpleasantness (Appendix E). Von Frey filaments were applied to the volar surface of the subject's left arm (approximately 2 inches above where the wrist bends). The subjects were instructed to report their pain intensity on an 11 point numerical scale; where 0 = no pain at all and 10 = worst possible pain imaginable. In addition, subjects were asked to rate the sensation in the context of unpleasantness where 0 = not unpleasant, 10 = excruciatingly uncomfortable and intolerable (Rainville *et al.* 1992).

A 3 cm x 3 cm box was drawn on the participant's left forearm. A mark, with a non-permanent marker, was made on the skin to ensure that the von Frey filaments were applied to the same location. Von Frey filaments were applied with increasing force until the subject reported a pain intensity rating between 4 and 6. Subsequently, capsaicin was applied to the skin within this box, a wane period of 30 minutes was invoked prior to applications of von Frey filaments starting at the 1 gram force filament. Filaments were applied until a pain intensity rating between 4 and 6 was achieved. The filament that produced pain prior to capsaicin was (in all cases) higher than following capsaicin

treatment. Hence, two different von Frey filaments were used during imaging on the following day; the von Frey filament that produced a pain intensity rating between 4-6 *before* capsaicin and a different (lower force) filament that produced a pain intensity rating between 4-6 *after* capsaicin.

3.2.4 Day 2 - Imaging

A 3 cm x 3 cm box was drawn on the participant's right arm, contralateral to the arm that the psychophysical testing was performed on the previous day. The stimulus was at a frequency of 1 Hz so that it was possible to apply manually while providing a nearly continuous stimulus related to the speed of the fMRI signal change response. The stimuli were applied by the experimenter who was in the scanner room throughout the duration of the experiment. The pace was maintained by a visual prompt on a digital projector that was only visible to the experimenter. Stimuli were applied in a block paradigm, with each experiment lasting 11 minutes 12 seconds.

Capsaicin cream was applied to the right volar forearm over a 3 cm square area and imaging proceeded while the burning sensation subsided (30 minutes). During the capsaicin wane period, an anatomical scan was performed. After the 30 minutes wane period, a different force filament was applied (Figure 3.1). Participants were instructed to focus on the stimulus during the experiment and to rate the pain intensity and unpleasantness for each stimulus after each experiment. From here on in, the filament used before the application of capsaicin will be referred to as the "noxious filament' and the von Frey filament used after capsaicin will be referred to as the "innocuous filament".

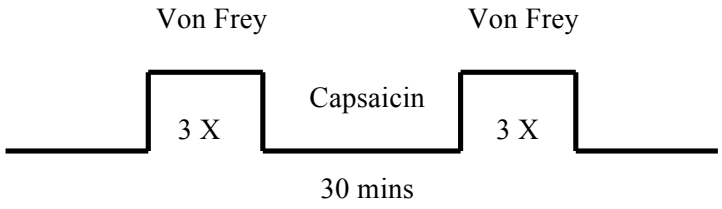

Figure 3.1. Experimental Design for Equi-Nociceptive von Frey Filaments

The noxious von Frey filament was applied three times (3X) without capsaicin. After the capsaicin wane period, a different innocuous von Frey filament was applied. The volunteer reported the same pain intensity for both filaments but they were of different forces.

3.3 Results

3.3.1 Psychophysical Data

On day 1, von Filaments ranged in force from 8 g – 300 g (median = 140 g) before the application of capsaicin and from 2 g – 180 g (median = 26 g) after the application of capsaicin. A paired t-test significantly showed ($p<0.001$) that the force of the filament with capsaicin was always lower. Mean pain and unpleasantness scores were 4.0 with and without application of capsaicin (Table 3.1). It should be noted that some subjects did not report a pain intensity rating of 4 even at the largest gram force von Frey filament in the series, therefore the largest (300 g) von Frey Filament was used for these subjects.

Although psychophysical testing on day 1 was aimed to determine the von Frey filaments that produced a pain intensity between 4-6 with and without sensitization, the subjective pain ratings ranged from 1-4 for pain intensity and 1-6 for unpleasantness before capsaicin on day 2 of imaging. Mean intensity ratings were comparatively similar

before (2.6 ± 1.0) and after (3.0 ± 1.9) capsaicin was applied and ranged between 1-8. Mean unpleasantness ratings before (3.5 ± 2.0) and after (4.2 ± 2.6) were significantly different and also ranged from 1-8, although this difference was not evident on day 1 of psychophysical testing (Table 3.2).

Table 3.1. Pain Intensity and Unpleasantness Ratings with Noxious Touch Stimuli – *Day 1*		
	Before	**After**
Intensity	4.0 ± 0.9	4.0 ± 0.9
Unpleasantness	4.0 ± 1.7	4.0 ± 1.4

Table 3.2. Pain Intensity and Unpleasantness Ratings with Noxious Touch Stimuli – *Day 2*		
	Before	**After**
Intensity	2.6 ± 1.0	3.0 ± 1.9
Unpleasantness	3.5 ± 2.0	4.2 ± 2.6*

Psychophysical data showing mean intensity and unpleasantness scores across 10 healthy volunteers with von Frey stimuli before and after capsaicin. Volunteers reported a pain rating between 4-6 before capsaicin and similar pain scores after capsaicin. Table 3.1 shows ratings from day 1 while Table 3.2 shows ratings from day 2. Scores are displayed as mean ± standard deviation. Significance (*) was determined using a two-tailed, paired, Student t-test ($p<0.05$).

3.3.2 Group Results

Group fMRI results across the ten volunteers, as determined by fixed effects analysis, are shown in Figure 3.2 with areas of consistent activity colour-coded, according to the legend described in the figure caption. Areas of significant signal changes are inferred to reflect changes in neuronal activity when the stimulus is applied. *Before*

capsaicin, there was very little activity in the SC and located in the contralateral DH of C5. Activity in the medulla was also nominal and concentrated in the area where the STT synapses onto secondary neurons in the contralateral medulla. However, in higher brainstem structures, the areas of activity were very localized to the pontine reticular formation (PRF), in the anterior pons where sensory nuclei are found, areas around the PAG and areas where descending modulatory fibres project from the thalamus. *After capsaicin* was applied, a lower force filament was used to induce the same pain response, and the activity pattern was fairly similar but more localized to specific regions. In the SC, there was only ipsilateral VH activity at C7. Activity was also concentrated in the projection sites for STT neurons coming from the SC and in areas where the gracile and cuneate nuclei are found. In higher brainstem regions, there was predominant activity in the pontine nuclei, ipsilateral red motor nuclei and substantia nigra. The activity maps before and after sensitization show similar levels and similar regions within the brainstem structures activated. Table 3.3 highlights the important areas of activity in this experiment.

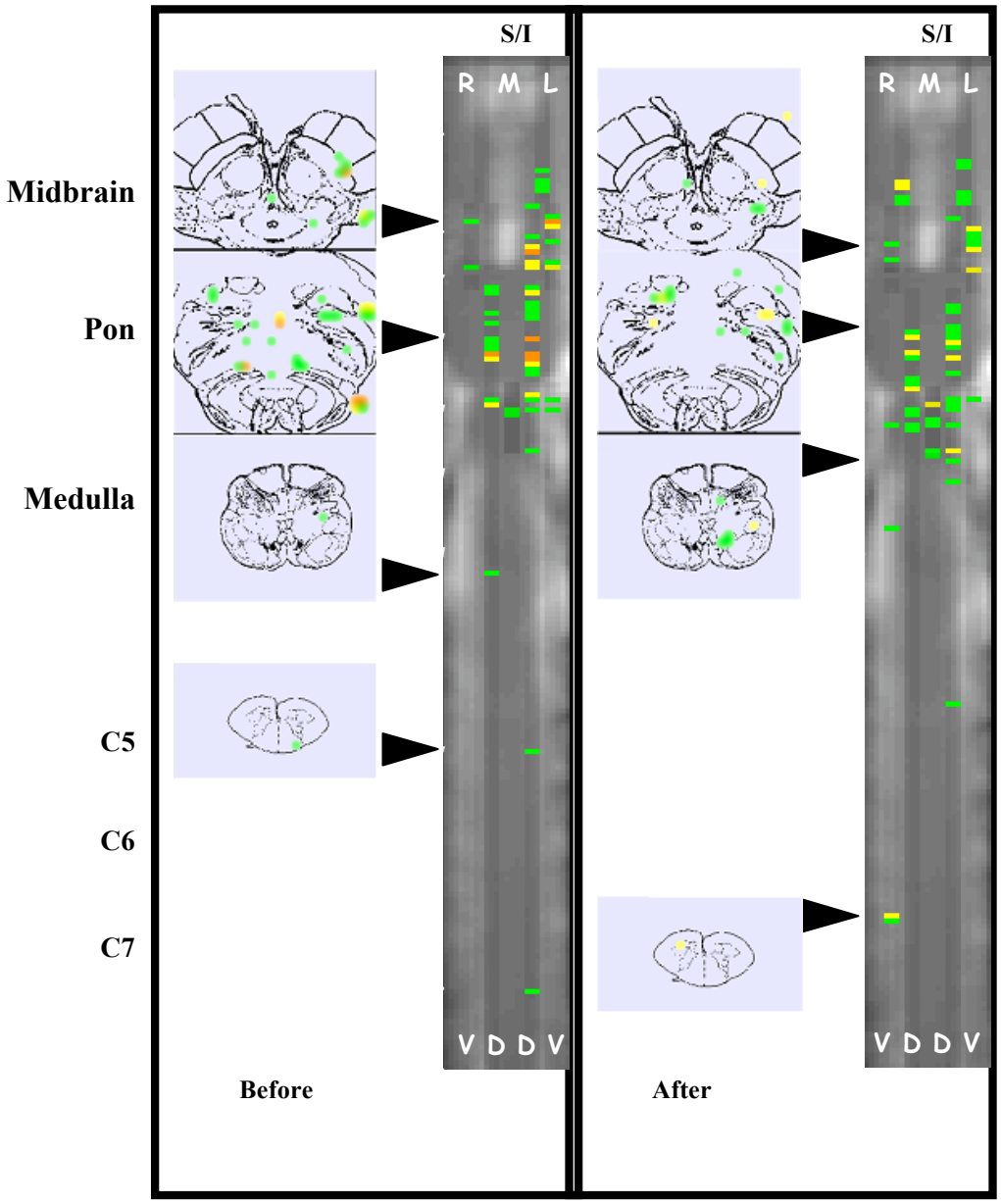

Figure 3.2. Noxious Touch Before and After Peripheral Sensitization

Combined group results showing areas of activity with von Frey stimuli in 10 healthy volunteers. Von Frey touch (pain rating = 4) stimuli were applied before and after capsaicin. The right hand of the frame shows the rostral-caudal distribution of activity. Activity in areas of significant signal changes are overlaid onto a spatially normalized mid-line coronal slice. The rostral-caudal activity is then superimposed onto transverse anatomical images of the respective segments (left frame) in radiological orientation. The colours represent activity in each voxel or an immediate neighbour where green represents activity in 5 out of 10 subjects; yellow-5; orange-6; and red-7 out of 8 subjects. D: dorsal; V: ventral; R: right; M: midline; L: left.

Table 3.3. Main Areas of Activity – Noxious von Frey		
	Before	**After**
Spinal Cord	▪ Contralateral DH at C5	▪ Ipsilateral VH at C7
Brainstem	▪ STT synaptic sites in the medulla ▪ PRF ▪ Sensory nuclei in the anterior pons ▪ PAG ▪ Thalamic-midbrain border	▪ In the vicinity of the gracile and cuneate nuclei ▪ STT synaptic sites in the medulla ▪ Pontine nuclei ▪ Ipsilateral red motor nuclei ▪ Substantia nigra

Main areas of activity in the SC and brainstem for the noxious von Frey stimulus before and after the application of capsaicin.

3.4 Discussion

The purpose of this study was to apply equi-nociceptive stimuli with and without peripheral sensitization induced by capsaicin, to determine if the pattern and intensity of neuronal activation was similar.

3.4.1 Psychophysical Data

Although the filaments identified on day 1 of psychophysical testing did not produce pain intensities between 4 and 6, similar pain intensity scores were achieved and therefore meaningful comparisons could still be made from the imaging data. Nevertheless, the mean pain unpleasantness scores were significantly increased in the sensitized state and therefore should be kept in mind when interpreting the imaging data.

3.4.2 Areas of Significant Signal Changes

A study done by Gracely *et al.* (2002) used fMRI to demonstrate that the amount of pressure stimuli required to cause brain activation in SI and SII was much lower in Fibromyalgia patients than in healthy controls. However, even though comparable subjectively painful conditions were tested, there were similar activation patterns in patients and controls. The authors state that the application of mild pressure produced subjective pain reports and cerebral responses that were qualitatively and quantitatively similar to the effects produced by application of at least twice the pressure in control subjects. This proves the importance of using pain intensity scores to standardize similar pain experiences. This is relevant to this study because we can confidently asses the signal changes related to neuronal activity with and without sensitization because the pain intensity scores for both conditions were similar.

The activity pattern produced by the noxious von Frey filament with sensitization was similar to the activity map seen without sensitization, and both methods activated pain pathways. These results indicate that the similar subjective pain ratings regardless of how pain was induced, also reflect similar activity maps produced by two different force filaments (Gracely *et al.* 2002). The only difference between the activity maps was the distribution of activity: without sensitization, the activity was more disperse whereas with sensitization, the activity was more localized to specific areas. Both painful stimuli produced minimal cervical SC activity. Contralateral DH activity at C5 without sensitization, indicates activation of descending efferents of the bulbospinal tract (Yezierski *et al.* 1982) and from the PRF (Stroman *et al.* 2008a). Ipsilateral VH activity

at C7 seen with sensitization most likely indicates inhibitory connections from the PRF (Stroman *et al.* 2008a).

There was also strong evidence of descending modulation in both the sensitized and non-sensitized pain stimuli. Activity was evident at the thalamic-midbrain border; an area where descending modulatory pathways project from the thalamus. There was also activity in the PAG and pontine nuclei, which are areas that project fibres down to the SC to control incoming pain information.

The activity observed with and without sensitization strongly indicates a pain response with activation from descending tracts to modulate the pain. However, the results possibly suggest that evoked pain after peripheral sensitization (mechanical allodynia) activates very specific regions as compared to pain generated from a non-sensitized state that has a more distributed activity pattern.

3.4.3 Conclusions

The results of this study demonstrated that two equi-nociceptive filaments can produce similar activity patterns, although a lower force filament was used with sensitization to induce similar pain ratings than without sensitization. The activity pattern observed from both stimuli was indicative of a pain response. These findings show that changes in the periphery, causing sensitization, can evoke a pain response from an innocuous stimulus and this is one possible mechanism that could help explain altered pain states.

Chapter 4
General Discussion

4.1 Main Findings

The results from this project strongly indicate that a non-noxious stimulus translates into a pain response after peripheral sensitization and this is strongly linked with the descending modulatory system. A touch response, examined by means of the brush stimuli, before sensitization activated typical areas expected of non-painful sensory transmission. These include the ipsilateral DH, gracile and cuneate nuclei in the medulla and areas surrounding the dorsal column medial lemniscal pathway. Peripheral sensitization produced activation patterns typical of a pain response, such as the contralateral VH, which is also thought to be due to activation of descending pain-modulating systems. The touch stimulus (pain score = 1) produced activity in typical sensory centres in the DH and brainstem before sensitization, but after sensitization, we observed a pain response as demonstrated by the activity in the SC and higher brainstem structures. Interestingly, stimuli that produced the same pain ratings with and without peripheral sensitization (pain score = 4-6) showed similar activation patterns even though different von Frey filament were used to evoke these responses. In all experiments there was indication of descending modulation as activity was observed in and around areas of the PAG, midbrain red nuclei and PRF. This research demonstrates how non-painful and pain sensory information are transmitted from the first synapse in the dorsal spinal horn to the brain in healthy individuals and how peripheral sensitization induces changes in

non-noxious stimuli-induced activation patterns that correlate with pain sensory transmission.

4.2 Interpretations

Pain is a multi-dimensional phenomenon that encompasses many factors. Along with the sensory-discriminative aspect of pain, we were also able to assess the affective component of pain by psychophysical testing. In this project, common themes have emerged that are all involved in pain perception. There are observable differences between experimentally induced chronic pain and clinical pain that patient's experience. The results of this study apply to the clinical setting, but under certain constraints. There is also an affective component of pain that plays an important role in how pain is perceived and how it is processed and interpreted in the brain. These topics contribute to the complexity of chronic pain states and will be discussed in more detail below.

4.2.1 Chronic versus Clinical Pain

It is now widely known that the brain network for pain in chronic clinical conditions is different from the brain activity for acute painful stimuli in normal subjects. This was obvious even from the first pain imaging study which used a hemodynamic response using the radioisotope ^{133}Xe (Lassen *et al.* 1978). This technique provided little spatial resolution but suggested an increase in blood flow to the frontal lobes during pain. The first human brain studies performed using modern technologies used PET (Jones *et al.* 1991; Talbot *et al.* 1991) and SPECT (Apkarian *et al.* 1992) to image pain and also found observable differences in the frontal cortices. One study (Maihofner and

Handwerker 2005) reported different brain activity patterns when comparing pin-prick responses on affected and unaffected limbs of patients with complex regional pain syndrome. They found increased activity in the contralateral S1, bilateral SII, insular cortex, the frontal cortices and parts of the ACC when comparing the affected to the unaffected limb following simulation with von Frey filaments.

A meta-analysis study (Apkarian *et al.* 2005), showed chronic clinical pain conditions more frequently involve the PFC (81% in clinical conditions versus 55% in normal subjects), while in normal subjects, perception of experimental pain more frequently involves the S1, S2, the thalamus and ACC. The preferential activation of PFC in clinical conditions suggests that chronic pain states have stronger, cognitive, emotional, and introspective components than acute pain (Apkarian *et al.* 2005). This suggests that chronic pain conditions may be a reflection of decreased sensory processing and enhanced emotional/cognitive processing. It has been suggested that since the sensory-discriminative, affective-emotional, cognitive-evaluative components of pain are commonly seen in chronic clinical pain conditions, this may be a distinctive feature between chronic and acute pain (Apkarian *et al.* 2005).

Clinical investigations have suggested that tactile allodynia is processed differently than nociceptive pain. The ACC is activated by nociception (Apkarian *et al.* 2005) but not in patients with central pain (pain from injury to the CNS) (Peyron *et al.* 1998; Peyron *et al.* 2000) or tactile allodynia elicited by capsaicin (Baron *et al.* 1999; Iadarola *et al.* 1998). The results of this study also support this finding. The results from Chapter 3 show that tactile allodynia seen prior to capsaicin produces a different

activity pattern than nociceptive pain, produced after sensitization. However, other studies argue this finding showing that allodynia and nociception evoke activity in similar cortical structures including the ACC (Hofbauer *et al.* 2006; Iadarola *et al.* 1998; Lorenz *et al.* 2002; Olausson *et al.* 2001; Petrovic *et al.* 1999; Witting *et al.* 2001). Most investigators will now agree that dynamic mechanical allodynia activates brain regions normally involved in pain processing (Olausson *et al.* 2001), as well as distinct regions not thought to be involved in pain processing such as the ACC.

The aforementioned studies all show that acute and chronic pain states activate different areas in the brain. Previous spinal fMRI studies (Agosta *et al.* 2008; Foad Ghazni *et al.* 2007) have reported similar findings in the SC, but more studies are needed to confirm the exact differences between acute and chronic pain. This project aimed to achieve this and showed differences before and after peripheral sensitization in an experimentally-induced model of pain. However, these results need to be compared with a patient population and by assessing the differences that exist, we can then have a better understanding of the mechanisms involved in neuropathic pain and the role of the SC in altered pain states.

4.2.2 Affective Component of Pain

We know that parallel processing systems, consisting of sensory-discrimination, affective-emotional, and cognitive-evaluative dimensions, contribute to the subjective experience of pain (Brooks and Tracey 2005) and these processes involve different brain regions. The advantage of using neuroimaging methods, and specifically fMRI, to study

pain in the brain (and within in the SC and brainstem as demonstrated in this project) allows us to study pain-associated physiological events in human who can simultaneously report their subjective experiences; hence the utility of psychophysical data. By identifying the areas activated in brain fMRI studies of pain, and by knowing what functions are associated with these areas, we can better understand the affective dimensions of pain.

Various studies have reported how factors such as empathy, arousal, anxiety, depression, attention and expectation influence pain and activity within the CNS (Singer *et al.* 2004; Wager *et al.* 2004). We are aware of the 'pain matrix' that exists, which includes the ACC, IC, SI, SII, and thalamus (Tracey 2005). Several studies have shown activity in these areas and have confirmed the 'pain matrix' in experimental pain studies. SI and SII are commonly activated in heat pain studies (Coghill et al 1999;Peyron et al 1999;Bushnell et al 1999;Chen et al 2002) and it has been suggested that noxious input into these regions may underlie the perception of pain. In PET and fMRI studies of heat pain, the ACC and IC are activated and because these two areas are part of the limbic system, they have been implicated in the affective processing of pain (Fulbright *et al.* 2001; Rainville *et al.* 1997; Tolle *et al.* 1999). The IC is anatomically heterogeneous (Mesulam and Mufson 1982) and activity in its posterior portion may be more related to the sensory aspect of pain. The more anterior IC is anatomically continuous with the PFC and may be more important in the emotional, cognitive, and memory related aspects of pain perception (Apkarian *et al.* 2005). The PFC and parietal association areas, are sometimes activated by heat pain and may be related to cognitive variables, such as

memory or stimulus evaluation (Coghill *et al.* 1999; Strigo *et al.* 2003). Emotional states can also influence pain perception. A study by Phillips *et al.* (2003) showed that negative emotional states can enhance pain-evoked activity in limbic areas, such as the ACC and IC.

There are other components to the pain experience such as distraction, attention or the anticipation of pain. Other regions, besides the 'pain matrix' are activated when subjects are distracted from the pain. These include the PAG, parts of the ACC, and orbitofrontal cortex (within the PFC) (Bantick *et al.* 2002; Brooks *et al.* 2002; Frankenstein *et al.* 2001; Longe *et al.* 2001; Tracey *et al.* 2002). However, these regions could also be involved in the modulatory circuitry related to attention. The anticipation or expectation of pain activate pain-related areas, such as the S1, ACC, PAG, IC, PFC and cerebellum, in the absence of a physical pain stimulus (Beydoun *et al.* 1993; Hsieh *et al.* 1999; Ploghaus *et al.* 1999; Porro *et al.* 2002; Sawamoto *et al.* 2000; Villemure and Bushnell 2002).

The sensation of pain is caused by peripheral stimulation of neurons, but the conscious perception of pain depends heavily on the multi-processing centers involved in the 'pain matrix'. Pain causes an increase in brain arousal due to the many ascending pathways from the SC and brainstem, but the interpretation of pain in the brain heavily depends on the emotional aspect. This is demonstrated by the many areas in the brain activated in response to pain. The results of this project also showed differences in SC areas activated during a more painful state (during peripheral sensitization) with a brush stimulus when the pain intensity ratings did not change but the unpleasantness ratings

did. These studies alone prove how multi-dimensional pain is and how, many factors must be taken into consideration when interpreting pain.

4.3 Limitations

The age of the subject pool (19-35) is not representative of a patient population with an altered pain state such as neuropathic pain. To be able to confidently relate these results with a patient population, further studies are needed to examine older, healthy controls with the same experimental conditions.

There is the possibility of Type I and Type II errors when interpreting the results from fMRI (Stroman 2006a). We may not detect all of the activity in the gray matter, resulting in Type I errors. Type II errors would indicate activity that is not neuronal activity. This would likely occur along the edges of the SC. In this area, there is SC movement from CSF flow and blood flow in larger vessels. These errors might appear random, but true neuronal related activity occurred consistently across repeated experiments and across people, as demonstrated in the two studies. There is also evidence that Type I errors are more common than Type II errors (Stroman 2006a). This means that we are more likely to not detect the activity present in the cervical SC rather than detect activity that does not exist. Even in a single experiment, if all factors are controlled, there are large individual differences that reflect distinct patterns of activity (Davis *et al.* 1998).

The activity maps displaying the patterns of neuronal activity for each stimulus, might at first seem confusing, but there are several reasons for displaying the data in this

form. 1) This is the first time that SC and brainstem data have been spatially normalized so that voxel-by-voxel group analyses are possible. Unlike brain fMRI studies, which can locate certain areas using Talairach coordinates; there is no standard coordinate space yet, to spatially locate areas of the SC and brainstem. This must be done visually by comparing the relative location of the activity with several atlases. 3) These data are unmasked, showing all of the activity that falls within a stringent statistical threshold and thus, all of the activity displayed is significant. Activity that falls outside of the boundaries could be because of motion at the edge of an anatomical feature. The key points to take into consideration are spinal fMRI is an effective tool that can detect neuronal activity in the SC and brainstem and that the activity detected does correspond to a stimulation paradigm and is not random.

The block design used in both experiments consisted of stimulation periods of 56 seconds and baseline periods of 140 seconds. The limitation of this design is that it requires the neuronal activity and the related MR signal changes to be sustained for a period of time and then disappear. However, pain is not necessarily an "on/off" phenomenon but is a continuum with varying degrees from "none" to "worst imaginable". That being said, there is no way to know with certainty that the evoked neuronal activity from the stimulus returns to baseline conditions in time for the next stimulation period, and that there is no residual activity from the previous stimulus overlapping into the next stimulation period. The fMRI method used does span 10 volumes (140 seconds) during the rest periods, which is sufficient to allow for the signal

changes to return to baseline by using this block design. However, what we gain in image quality and spatial precision is traded off with total experimental time.

A study in 2001 (Logothetis *et al.* 2001) suggested that BOLD fMRI reflects neuronal input to a given area rather than its spiking output (Logothetis *et al.* 2001). Although this study examined the effects of neuronal input with BOLD signal changes, previous studies comparing SEEP and BOLD signal changes in the brain have demonstrated related signal changes and areas of activity (Stroman *et al.* 2005b), indicating that we may be able to apply the same interpretation to SEEP signal changes. This is particularly relevant when interpreting results indicating descending modulation. Descending modulation is known to contribute to chronic pain states (Porreca *et al.* 2002) and may also be present in experimental pain. However, we were not able to distinguish whether the activity is excitatory or inhibitory. Therefore, the results allow for more than one interpretation because of this lack of discrimination.

4.4 Directions

The results obtained can be applied to future studies involving clinical populations. These results can serve as control data to compare to a patient population with chronic pain in order to understand the underlying pain mechanisms and to develop objective clinical pain assessments. With this knowledge, we may be able to design treatment strategies for patients suffering from chronic pain.

Chapter 5
Summary and Conclusion

This project has demonstrated the SC and brainstem activity involved with innocuous and noxious touch, before and after peripheral sensitization. In Chapter 2, we were able to detect differences between different stimuli and understand how this information is processed in the SC and brainstem. There were observable differences between dynamic (brush) and static (von Frey) stimuli that most likely arose because they activate different primary afferent fibre types. Punctate stimuli activate Aβ and Aδ fibres, which carry pain information into the DH and then synapse onto secondary neurons in the VH comprising the anterolateral tract. Brush stimuli signal non-painful information via Aβ fibres to the medulla. There are some projections to the ipsilateral DH but these are few. We were also able to detect obvious differences before and after peripheral sensitization and how different descending pathways might specifically become activated in response to sensitization to produce a pain response. The results from this study strongly indicate that a non-noxious stimulus translates into a pain response after peripheral sensitization. In Chapter 3, we used different force filaments that produced the same pain ratings with and without peripheral sensitization. The activation patterns produced were very similar although the activity produced by a noxious stimulus was more distributed as compared to the activity of an innocuous stimulus with sensitization; which was more localized.

The main conclusions from this project are:

1) Spinal fMRI is a reliable and sensitive technique that can detect changes in neuronal activity related to sensory and pain information transmission in the SC and brainstem.
2) There are observable differences between touch and brush stimuli using spinal fMRI.
3) An innocuous stimulus (brush or von Frey) activates a non-painful sensory pathway, and a noxious stimulus activates a pain pathway.
4) The psychophysical data and activity patterns produced by non-painful stimuli indicate pain pathway activation after sensitization.
5) Different descending pathways might become activated in response to sensitization.
6) We were able to reproduce a noxious activity pattern with an innocuous stimuli following sensitization.
7) The descending pain modulatory system has a very prominent role in sensory and pain transmission.

These studies demonstrate that sensitization induces changes in non-noxious stimuli-induced activation patterns that correlate with pain sensory transmission. The induced changes are replicable and parallel a pain response. With this valuable insight, we now have a better understanding of the alterations that occur during neuropathic pain and can use this information to create treatment strategies for alleviating pain syndromes modulated by peripheral sensitization.

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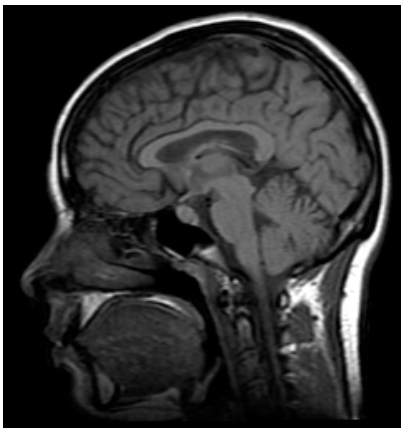
Appendix A

Recruitment Poster

Pain Research using fMRI

Healthy Volunteers are Needed for Studies Using fMRI at Queen's University

(new studies starting January 2007)



Healthy volunteers are needed to study pain pathways in the brainstem and SC after peripheral sensitization of the skin using magnetic resonance imaging (MRI). Participation in the study involves two visits to the Queen's fMRI Facility in the lower level of the Cancer Research Institute, and will last about 3 hours in total. The studies are completely non-invasive. A small honorarium ($20) will be provided to cover your time and expenses (parking etc.)

For more information please contact:

Janet

Recruitment Coordinator

email: **MRI@cogeco.ca**

Appendix B

MRI Safety Checklist

Centre for Neuroscience Studies

fMRI Facility

MAGNETIC RESONANCE (MR) IMAGING SAFETY CHECKLIST FOR RESEARCH SUBJECTS

This MR system has a very strong magnetic field (3 Tesla) that may be hazardous to individuals entering the magnet room if they have certain metallic, electronic, magnetic, or mechanical implants, devices or objects. Therefore, all individuals are required to fill out this form BEFORE entering the magnet room. Be advised, the magnet is ALWAYS ON. This questionnaire must be completed accurately to ensure safety. An answer of "Yes" in a category may not necessarily exclude you from entry into the MRI or its vicinity.

Name:_____ **Age:** _____ **Weight:** _____ **Height:** _____

Please Circle

Have you had prior surgery or an operation of any kind?	Yes No
Have you had an injury to the eye involving a metallic object (e.g. metallic slivers, foreign body)?	Yes No
Have you ever been injured by a metallic object or foreign body (e.g. BB, bullet, shrapnel, etc.)?	Yes No
Are you pregnant or suspect that you are pregnant?	Yes No
Do you have any history of claustrophobia, panic attacks, or seizures?	Yes No
Do you have any history of heart disease (angina, palpitations, heart attack, etc.)?	Yes No

WARNING: Certain implants, devices or objects may be hazardous to you in the MR environment or the magnet room. DO NOT ENTER the MR environment or the magnet room if you have any questions or concern regarding an implant, device object.

Please indicate if you have any of the following:

Yes	No	Aneurysm clip(s)	Yes	No	Neurostimulation system
Yes	No	Cardiac pacemaker	Yes	No	Spinal cord stimulator
Yes	No	Implanted cardioverter defibrillator (ICD)	Yes	No	Cochlear implant or implanted hearing aid
Yes	No	Electronic implant or device	Yes	No	Insulin or infusion pump
Yes	No	Magnetically-activated implant or device	Yes	No	Implanted drug infusion device
Yes	No	Any type of prosthesis or implant	Yes	No	Any external or internal metallic object (e.g. dentures, permanent retainer, IUD, metal sutures)
Yes	No	Artificial or prosthetic limb			
Yes	No	Any metallic fragment or foreign body	Yes	No	Hearing Aid (Remove before entering the magnet room)
Yes	No	Medication patch (Nicotine, Nitroglycerine)			
Yes	No	Tissue expander (e.g. Breast)	Yes	No	Tattoo
Yes	No	Body piercing	Yes	No	Other implant _____

IMPORTANT INSTRUCTIONS: Remove all metallic objects before entering the MR environment or magnet room including hearing aids, beeper, cell phone, keys, hairpins, barrettes, jewelry, watch, safety pins, paperclips, money clips, credit cards, bank cards, magnetic strip cards, coins, pens, pocket knife, nail clipper, steel-toed boots/shoes, and tools. Loose metallic objects are especially prohibited in the magnet room and MR environment.

I attest that the above information is correct to the best of my knowledge. I have read and understand the entire contents of this form and have had the opportunity to ask questions regarding the information on this form.

Person Completing Form:

_____

Print Name Signature Date

Form Reviewed By:

_____

Print Name Signature Date Position

For research study volunteers (to be completed at the end of the study) Total time spent in magnet (minutes) _____

Time entered by (name): _____

Appendix C

Consent Form

INFORMATION/CONSENT FORM

TITLE OF PROJECT: Functional Magnetic Resonance Imaging of SC and Brainstem: Pain Responses after Peripheral Sensitization of the Skin

BACKGROUND INFORMATION: (Overview of study)

You are invited to participate in a research study directed by Dr. Patrick Stroman and Dr. Cathy Cahill. The purpose of this study is to map pain pathways in the brainstem and SC after peripheral sensitization of the skin via magnetic resonance imaging (MRI). The current study is part of a larger one that involves mapping neuronal activity in these areas in patients with abnormal pain responses after peripheral sensitization or in patients with allodynia. You should have been told which part of the study is currently being done when you were given this information package. If you are not sure, please contact the person who gave you this information or Patrick Stroman at (613) 533-3245 or Cathy Cahill (613) 533-6162. Participation in the study involves two visits to the Queen's fMRI Facility in the lower level of the Cancer Research Institute, and will last about 3 hours in total.

DETAILS OF THE STUDY

1. What the aim of the study is:

The current study has three parts, with three different aims.

Part 1: To determine the activity elicited by the application of capsaicin and by mechanical stimulation after peripheral sensitization caused by the application of capsaicin.

Part 2: To determine the neural networks, and magnitude of signal changes, identified with fMRI of the superposition of two painful stimuli in healthy volunteers.

Part 3: To determine the neural networks activated by normally non-noxious thermal or dynamic mechanical stimulation, in patients with carpal tunnel syndrome.

2. Description of visits, dosage, tests to be performed as part of the study:

If you agree to participate, your brainstem and SC will be imaged while you are lying in a 3 Tesla magnetic resonance imaging (MRI) scanner in the Queen's fMRI Facility, and your heart beat and breathing may be monitored using entirely non-invasive methods. The entire session may last up to 3 hours over the course of two visits, including getting ready for the study and positioned in the magnet etc. This study involves two visits to the lower level of the Cancer Research Institute for imaging.

a) You will begin by filling out a checklist and questionnaire to make sure you are eligible. This will be completed first, and will take about 5 minutes. If you are pregnant or are trying to conceive you will not be eligible. If there is any uncertainty regarding whether or not you are pregnant and you want to participate in the study, a pregnancy test must be done prior to the experiment.

b) On your first visit, the mechanical stimuli for eliciting sensations (i.e., von Frey filaments and artist brush) will be shown to you. We will conduct a simulated run of the experiment in the mock scanner. A mock scanner is similar in setup to the actual MRI scanner except there is no magnetic field turned on and we will acquire no images. A trial run in the mock scanner is important so that you can habituate to the actual scanner and its environment. We will first apply each mechanical stimulus to your forearm, after which we will apply capsaicin cream. After a wan period of 30 minutes, to allow for the initial burning sensation to subside, we will re-apply the same mechanical stimuli to your forearm. On the second day, the exact same experiment will be conducted, but we will continuously acquire MR images in the actual scanner, and we will verbally ask you to rate the pain intensity and unpleasantness on a scale of 0-10 (0 = no pain and 10 = worst possible pain imaginable) after each stimulus on a button response system.

c) Please try to wear clothing containing no metal, or bring a change of clothing. Metal in zippers, snaps, and the wire and metal clasps in some bras can interfere with the imaging. Many shoes contain metal as well. You will be asked to remove or change out of any clothes that contain metal that will be near the area being imaged, and you will be asked to remove your shoes. For imaging the brain and upper portion of the SC, the snaps and zippers in jeans or other pants are far enough from the area being imaged that they do not cause a problem.

d) You will be asked to wear earplugs to protect your ears from the noise of the actual scanner on the second day of the experiment. You will still be able to hear the researchers over the two-way communication system with these earplugs in place.

e) You will be asked to lie on your back on the well-padded bed of the scanner. Pillows will be placed under your legs for comfort and a blanket will be placed over your legs if you wish.

f) For studies that require monitoring of your heart-beat and breathing, a small device that uses light to sense your blood flow will be clipped onto your finger. A belt containing a flexible air-filled tube will be placed around the lower portion of your chest for monitoring your breathing. You will be allowed to position this belt yourself, for your comfort.

g) For brain imaging studies, a head coil will be placed over your head. This coil is fitted with a mirror so that you can see out of the magnet towards your head or feet. For SC imaging studies, you will lay on top of a flat spine coil that looks like a part of the bed you are lying on. You and the bed will then slide into a long tube (the magnet).

h) You will need to keep still while the images are taken. To help you, we will make you as comfortable as possible and we will pack soft foam around your head if needed.

i) The MR system has a two-way intercom for communication. During the imaging, you will be asked to provide a rating from 1 to 10 of the sensation you felt. This rating will also be explained to you before the study starts. The different stimuli or tasks are described below. Please tell the researchers if you do not want to volunteer for any particular task or sensation, and remember that you can change your mind about volunteering at any time during the study.

j) All functional MRI studies require periods of rest interleaved with periods of sensation or activity so that we can detect the differences in the brain or SC that show where there was activity. For this study, we will apply an ointment to your skin that contains capsaicin in order to make your skin more sensitive to the mechanical stimulus. Capsaicin is the ingredient in hot peppers that causes a hot sensation. When applied to your skin it can normally cause a warm to hot sensation. Capsaicin will wash off, and its effect will last 20 – 30 minutes. We will inform you about each sensation or task before each experiment begins so that you know what to expect.

k) We will first determine how you rate the sensations of different mechanical stimuli during the mock scan. We will then apply the capsaicin ointment and apply the same mechanical stimuli again to examine whether the sensations change. We will not use any stimuli that you already had perceived as being painful in combination with the capsaicin ointment.

l) At the end of the session, additional images will be taken of the anatomy (or structure) of your brain or SC.

3. An explanation, if special research techniques will be used(e.g. randomization, blinding, placebo control) :

The MRI scanning procedure is very much like other medical imaging used in hospitals, but you will not be exposed to x-rays. This MRI machine uses a strong magnet and radio waves to make images of the interior of your body. You will not feel either. The MRI used in this study is a 3 Tesla MRI that is twice that used for most clinical imaging, although 3 Tesla systems are becoming more common in hospitals. The levels of magnetism and radio waves used in the MRI have not been shown to cause harmful effects. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic objects must be removed from your person before you approach the scanner. If you have a cardiac pacemaker or a metallic clip in your body (e.g., an aneurysm clip in your brain or an I.U.D.) you should not participate in any MRI study. In addition, credit cards and other cards with magnetic strips should also be removed as these will be damaged. (These items will be kept safe for you).

You will be in voice contact with the operator, and the operator will be able to see you via a camera. You may ask the operator to stop the experiment at any time. You should ask to stop the experiment if you feel tired, claustrophobic, or uncomfortable.

4. Alternative Therapies:

Does not apply.

5. Risks/Side-Effects:

There are no known risks involved with magnetic resonance imaging. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic or magnetic objects must be removed from your person before you approach the scanner. The capsaicin ointment that will be used for this study can cause discomfort and increased sensitivity to warm temperatures, and its effect can last for 20 to 30 minutes. It does not cause any damage to the skin though.

6. Benefits

You will not get a personal medical benefit from participating in this study but your participation will help us to better understand pain pathways in the SC and brainstem.

7. Exclusions:

Do to the very high magnetic field you should not be a subject in any MRI experiment if you…:

(any of the following)

a) have a history of head or eye injury involving metal fragments.
b) have ever worked in a metal shop
c) have some type of implanted electrical device (such as a cardiac pacemaker or neurostimulator)
d) have implanted metal objects as a result of surgery such as artificial joints, aneurysm clips, metal staples
e) have severe heart disease (including susceptibility to arrhythmias) or any other serious illness
f) are wearing metal braces on your teeth
g) have non-removable jewelry (body piercing)
h) are, or may be, pregnant

8. Confidentiality

The findings of this study will be reported in scientific journals but your name will remain confidential. Data from your images will be stored on a secure computer system and identified only with the date and a subject code. Only the researchers directly related to this

study will have access to the data files and the subject codes. You will not be identified in any publication or reports.

Although this is not a diagnostic scan and any images obtained are for research purposes only, it is possible that the MR scan may disclose an unknown abnormality. In this event, a medical imaging specialist will be asked to review the images and we would send a report to your physician. The researchers directly involved with this procedure do not have the credentials to diagnose medical conditions.

9. Voluntary nature of study/Freedom to withdraw or participate:

Your participation in this study is voluntary. You may withdraw from this study at any time and your withdrawal will not affect your future medical care, academic standing, or career.

10. Withdrawal of subject by principal investigator:

The study Director may decide to withdraw you from this study if:

1) you do not meet the criteria in the Magnetic Resonance Screening Form.
2) you are unable to perform the tasks requested.

11. Liability:

"In the event that you are injured as a result of taking study medication or of the study procedures, medical care will be provided to you until resolution of the medical problem.

By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities."

12. Payment: Some studies compensate for subject's expenses and inconvenience.

You will receive $20 to cover your costs for parking, transportation to Queen's, etc, for participating in this study.

SUBJECT STATEMENT AND SIGNATURE SECTION:

13. Description of how subject is informed of study (e.g. protocol read with doctor, consent form discussed). List Principal Investigator and Department

Head as contacts, and provide telephone numbers should subjects have questions or problems. The format for this section is standard.

I have read and understand the consent form for this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I chose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I have named Dr. _____ at _____ as the physician to be contacted for follow-up purposes. I am voluntarily signing this form. I will receive a copy of this consent form for my information. If at any time I have further questions, problems or adverse events, I can contact

Dr. Patrick Stroman (P.I.)

Queen's University
Kingston, Ontario
K7L 2V7
Phone: (613) 533-3245
Fax: (613) 533-6840

Dr. Cathy Cahill (P.I.)

Queen's University
Kingston, Ontario
K7L 2V7
Phone: (613) 533-6162
Fax: (613) 533-6412

If I have questions regarding my rights as a research subject I can contact
Dr. Albert Clark, Chair, Research Ethics Board at Queen's University. (613) 533 - 6081

By signing this consent form, I am indicating that I agree to participate in this study.

_____ _____

Signature of Volunteer Date

_____ _____

Signature of Witness Date

STATEMENT OF INVESTIGATOR:

I, or one of my colleagues, have carefully explained to the subject the nature of the above research study. I certify that, to the best of my knowledge, the subject understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

_____ _____

Signature of Principal Investigator Date

Please note:

IF A PARENT, GUARDIAN or PUBLIC TRUSTEE IS REQUIRED TO SIGN A CONSENT FORM, A SEPARATE FORM SHOULD BE DESIGNED FOR THEM SPECIFICALLY.

Participant Consent Form

Project title: Functional Magnetic Resonance Imaging of SC and Brainstem: Pain Responses after Peripheral Sensitization of the Skin

I have read the Letter of Information, have had the nature of the study explained to me, and I agree to participate. All questions have been answered to my satisfaction.

Subject Name (please print): _____

Signature:_____ Date: _____

Individual responsible for
obtaining consent:

Signature:_____ Date: _____

Investigator:

Signature:_____ Date: _____

Appendix D

Volunteer Details

Functional Magnetic Resonance Imaging of SC and Brainstem: Pain Responses after Peripheral Sensitization of the Skin

This study is a small part of the overall project entitled **Functional Magnetic Resonance Imaging of the Brain and SC after Pain**. The details and rationale for this study are as described in the overall project description.

The details of this particular study are as follows:

In this study, we will image the activity in your SC and brainstem. We will apply mechanical stimuli to your forearm and examine the activity before and after peripheral sensitization. We will first apply the mechanical stimuli, then sensitize your skin, then apply the same mechanical stimuli. The mechanical stimuli will consist of von Frey filaments (i.e., nylon threads that vary in width) and a 2 cm wide artist's brush. Most people describe the sensations elicited by the mechanical stimuli as pleasant and soothing. The mechanical stimuli are pressure stimuli and do not cause tissue damage. Peripheral sensitization will be induced using capsaicin cream. Capsaicin is the ingredient in hot peppers that causes a hot sensation. Capsaicin cream elicits a slight burning sensation in most people, and your skin may redden temporarily; however, it will cause no damage to your skin. The design of the study is as follows: 1) While in the scanner, you will initially undergo two scans, one for each mechanical touch stimulus. Each stimulus will be applied for 56 s, three times, with rest periods in between the stimulations; 2) Capsaicin cream will be applied to your forearm; 3) After 30 minutes, the mechanical stimuli will be reapplied while you are in the scanner. Again, you will undergo two scans, one for each stimulus.

Participation in this study involves two separate appointments; ideally, these two appointments would be scheduled on two consecutive days. The procedures for the two days are exactly the same, except that on the first day, we will conduct the described procedure on a mock scanner. A mock scanner is similar in setup to the MRI scanner except there is no magnetic field and we will

acquire no images. On the second day, we will record activation in your SC and brainstem while continuously acquiring MR images.

Both sessions take place at the MRI facility in the basement of the Cancer Institute. Each visit will take approximately 1.5 hours, for a total participation time of approximately 3 hours.

You will receive $20 to cover your costs for parking, transportation to Queen's etc. for participating in this study.

Appendix E

Psychophysical Testing Descriptor

Direct quotation read to the volunteers prior to psychophysical testing:

"There are 2 primary aspects of pain that we are interested in measuring: the intensity, how strong the pain feels, and the unpleasantness, how unpleasant or disturbing the pain is for you. The distinction between these two aspects of pain might be made clearer if you think of listening to music. As the volume of the music increases, I will ask you how loud it is or how unpleasant the music is to hear. The intensity is like the loudness. The pleasantness or unpleasantness of the music depends on how much you like or dislike the music. The pleasantness of pain depends on how much you dislike it.

Rating for mechanical stimulation

1. *"Name" How would you rate the sensation in the context of pain: where '0' is not painful and '10' worst possible pain imaginable*
2. *Now, how would you rate the sensation in terms of unpleasantness where '0' is not unpleasant and '10' is excruciatingly uncomfortable"*