NEURAL CORRELATES OF INTER-INIVIDUAL DIFFERENCES IN PAIN PROCESSING INVESTIGATED BY FUNCTIONAL MAGNETIC RESONANCE IMAGING OF THE ENTIRE CENTRAL NERVOUS SYSTEM

By

Hamza Sami Khan

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Queen’s University
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Abstract

The experience of pain is a highly complex and personal experience, characterized by tremendous inter-individual variability. Pain perception can differ substantially across individuals due to many factors such as age, gender, genetics, cognition and emotionality etc. Some individuals are very sensitive to pain whereas others tolerate pain well. Athletes can play competitive sports even with significant injuries while other people feel tremendous pain while getting a flu shot. This phenomenon of inter-individual variability in pain responses has challenged scientists and clinicians alike. It is difficult to determine whether subjective reports of pain reflect true individual experiences of pain. However, the development of neuroimaging techniques has dramatically progressed our understanding of pain processing. This project investigated the neural correlates of inter-individual differences in pain responses in healthy individuals, by means of functional magnetic resonance imaging (fMRI) of the entire central nervous system. Twenty-healthy participants were asked to rate their pain following a noxious thermal stimulus, while undergoing functional MRI, and considerable inter-individual variability was observed. Results from this project demonstrated central mechanisms in the brain, brainstem and spinal cord that contribute to this variability. Participants that reported higher pain to the noxious stimulus showed greater fMRI responses in some brain, brainstem and spinal cord structures involved in processing the emotional, cognitive and motivational aspects of pain. This showed that the subjective reports of pain are a reliable indicator, and inter-individual differences in pain responses truly reflect variability in pain experience. It is expected that this knowledge will contribute to a better understanding of the neuronal processes, as well as substantial inter-individual variability observed in chronic neuropathic pain populations such as fibromyalgia, patients with spinal cord injuries etc.
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Abbreviations

ACC  Anterior cingulate cortex
ANOVA  Analysis of variance
B₀  Magnetic field
BA  Brodmann area
BCd  Body of caudate nucleus
BG  Basal ganglia
BOLD  Blood oxygenation-level dependent
C₆  6th cervical segment
CL  Claustrum
CNS  Central nervous system
CSF  Cerebrospinal fluid
DH  Dorsal horn
DLPFC  Dorsolateral prefrontal cortex
DLPT  Dorsolateral pontine tegmentum
fMRI  Functional magnetic resonance imaging
FOV  Field-of-view
GE  Gradient echo
GLM  General linear model
HASTE  Half-Fourier single-shot fast-spin echo
HC  Hippocampus
INS  Insula
LGP  Lateral globus pallidus
M₁  Primary motor cortex
MRI  Magnetic resonance imaging
MFG  Medial frontal gyrus
MTG  Medial temporal gyrus
PAG  Periaqueductal gray matter
PFC  Prefrontal cortex
RESPITE  Retrospective spinal cord motion time-course estimates
| **RF**    | Radiofrequency                        |
| **RVM**   | Rostral ventromedial medulla          |
| **S1**    | Primary somatosensory cortex          |
| **SE**    | Spin echo                              |
| **SEEP**  | Signal Enhancement by Extracellular Protons |
| **SFG**   | Superior frontal gyrus                |
| **SMA**   | Supplementary motor area              |
| **SMT**   | spinomesencephalic tract              |
| **SNR**   | Signal-to-noise                       |
| **SPM**   | Statistical parametric mapping        |
| **SRT**   | spinoreticular tract                  |
| **STT**   | Spinothalamic tract                   |
| **SubN**  | Subthalamic nucleus                   |
| **THAL**  | Thalamus                              |
Chapter 1

Introduction

Pain is a complex, multi-dimensional, and highly subjective experience that is crucial for the survival of the organism. It is described by The International Association for the Study of Pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. Therefore, one’s pain experience involves a complex blend of a sensory-discriminative component as well as a cognitive-emotional component that results in an experience that is unique to every individual. It is situation dependent with abundant research over the past 15 years showing variability in pain experience across individuals based on sex (Fillingim, 2000), gender (Greenspan et al., 2007), age (Fillingim, 2007), ethnicity (Riley et al., 2014) as well as multitude of psychosocial factors such as affective factors (Price, 2000), cognitive processes (Pincus & Morley, 2001), personality (Haythornthwaite, 2007) etc. It is this individual nature of pain that underlies the rationale and hypotheses of the research described herein.

Individual differences in pain sensitivity present a challenging problem for physicians. It is a perplexing clinical problem when a sensory/painful experience in one individual is vastly different from another patient, even when presented with the same sensory input. Pain is a fundamental component of many disease and injury states. For example, heterogeneity in pain experiences is especially prevalent in chronic neuropathic pain populations such as patients with spinal cord injury (SCI). Depending on the exact location (cord level) and nature of injury (complete or incomplete), pain experiences can differ widely among patients, with some having reduced sensitivity to pain while others develop increased sensitivity in affected areas. It is important for clinicians to know whether a patient reporting high pain or low pain is simply
based on his/her personality or do they really have much more/less pain than other patients. Therefore, using subjective ratings of pain in patients is of profound importance in the diagnosis and treatment of pain. However, it is also difficult to determine whether such subjective reports of pain reflect true experiential differences. Individual differences in psychophysical ratings are often viewed as artifacts of scale usage (Coghill 2010).

The development of neuroimaging techniques, such as functional magnetic resonance imaging (fMRI) has dramatically enhanced our understanding of the structures important in processing acute and chronic pain. Advancement in the understanding of individual differences and the mechanisms that support them has progressed as psychophysical findings are combined with measures of brain activation provided by functional brain imaging techniques. Emerging brain functional magnetic resonance imaging studies indicate that inter-individual differences in subjective reports of pain intensity are closely related to the degree of activation in several brain regions important in the processing of pain. Coghill and colleagues addressed the issue that some individuals claim to be ‘sensitive’ to pain, whereas others claim they tolerate pain well. In the trial, using psychophysical ratings to define pain sensitivity and functional magnetic resonance imaging to assess brain activity they found that highly sensitive individuals (i.e. individuals who rated the pain highest) exhibited more frequent and more robust pain-induced activation of the primary somatosensory cortex (SI), anterior cingulate cortex (ACC), and prefrontal cortex (PFC), than did insensitive individuals (i.e. individuals that rated pain lowest) (Coghill, McHaffle & Yen, 2003). Taken together, these results confirm that inter-individual differences in subjective pain intensity ratings do indeed reflect inter-individual differences in the pain experience. However, we know that this does not give us the complete picture. Even though higher brain centres represent an important site in the pain pathway, the spinal cord (SC) and brainstem also
include synaptic sites through which the pain experience is processed before going up to higher cortical areas. Some of the important structures in the SC and brainstem, known to be part of the pain matrix include the dorsal horn of the spinal cord, reticular formation, periaqueductal gray (PAG) and rostral ventral medulla (RVM) (D’Mello & Dickinson, 2008). No study to date has performed fMRI of the spinal cord and brainstem to identify the neural basis of an individual’s subjective experience of pain and characterized them in relation to those of other individuals receiving exactly the same stimulus.

This purpose of this thesis is to investigate these inter-individual differences spanning the entire central nervous system (Purpose, Rationale, Hypothesis and Objectives are discussed in detail in section 1.9). It reports the results of a functional MRI study spanning the brain, brainstem and cervical spinal cord, to investigate the individual differences in pain processing in a healthy human population. We hypothesize consistent patterns of activity as well as significant inter-individual differences in fMRI results in the brain, brainstem and SC, in response to painful stimuli. We expect the neural activity to be correlated with subjective ratings of pain intensity, in regions associated with the pain pathway such as the ACC, PFC, periaqueductal grey (PAG), rostral ventromedial medulla (RVM), the dorsal horn of the spinal cord etc.

Twenty healthy volunteers were recruited to participate in a combined psychophysical and functional MRI (fMRI) study of inter-individual differences in pain sensitivity. Thus, the current study first attempts to support prior psychophysical findings by confirming the existence of individual differences from behavioral data. Furthermore, we investigate the key structures in the brain, brainstem and spinal cord that correspond to this variability in pain perception when participants are inflicted by a noxious thermal stimulus. Because noxious stimulation will result in variable responses in psychophysical ratings across individuals, it is expected that patterns of
neural activity will also be similarly variably. Thus, many regions within the brain, brainstem and spinal cord, known to contribute to painful experiences will show differential activation when the stimulation is delivered.

This thesis will start by providing a general overview of pain pathways as well as fMRI. It will then discuss the intersection of pain and fMRI research. Finally, it will delve into the specifics of the research study in terms of the rationale, purpose, methods, results and discussion.

1.1 Pain Transmission

1.1.1 Nociceptors

Nociceptors are specialized sensory neurons that alert us to potentially damaging stimuli. They are widespread and present in every tissue of the body, with the exception of the brain. Three distinct classes of nociceptors are activated by noxious stimuli. Thermal nociceptors are activated by noxious heat (>~45˚C) or cold stimuli (<~15˚C) (Craig & Andrew, 2001) whereas mechanical receptors respond to excess pressure or mechanical deformation. Lastly, polymodal receptors respond to a wide range of noxious stimuli such as heat or cold and pain producing chemicals.

Nociceptors can either be myelinated or unmyelinated. Small diameter, unmyelinated nerve afferents, termed C fibres, respond polymodally to chemical, thermal and mechanical noxious stimuli. Myelinated afferents, termed Aδ fibres, respond to both mechanical and thermal stimuli. Large myelinated afferents, termed Aβ fibres, transmit non-noxious sensory information such as proprioception and light touch (Meyer et al. 2006). The ratio of myelinated to unmyelinated fibres in cutaneous nerves is about 1:4. Approximately 10% of cutaneous myelinated fibres and 90% of unmyelinated fibres are nociceptive.
Sensation of pain can be described in two categories: first pain or second pain. First pain is caused by the activation of faster-conducting cutaneous Aδ fibres and is perceived as a transient and sharp, pricking type of pain. It is responsible for the sensory discriminative component of pain, and, through various imaging studies, has been shown to activate the primary and secondary somatosensory cortex (Ploner et al., 2002). In contrast, second pain is caused by the activation of slower-conducting cutaneous C fibres, and is perceived as a dull, long lasting, poorly localized and non-discriminative burning type of pain. It is mostly responsible for the affective component of pain, and activates a number of regions throughout the brain including the anterior cingulate cortex (ACC) and secondary somatosensory cortex (Ploner et al., 2002).

Activation of nociceptors by a noxious stimulus initiates the pain response. The noxious stimulus is transduced into an action potential by the nociceptors. The intensity of the stimulus determines the frequency of action potentials. These action potentials are then transmitted to the CNS by first propagating towards the dorsal horn of the spinal cord.

1.1.2 Spinal Cord

The spinal cord is a bundle of nervous tissue and support cells that extends from the brain, and makes up the central nervous system. It is the main pathway for information connecting the brain and the peripheral nervous system. The spinal cord is located in the vertebral foramen and is made up of 31 segments: 8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 1 coccygeal. A pair of spinal nerves leaves each segment of the spinal cord and innervates specific areas on each side of the body.

The spinal cord is composed of the gray matter and white matter. A transverse section of the spinal cord will show the gray matter in the form of a butterfly or the capital letter H, surrounded by the white matter. The white matter consists primarily of bundles of longitudinally
running myelinated axons. The gray matter is made up of neuronal cell bodies, dendrites, axons, and glial cells. It can be macroscopically divided into dorsal and ventral horns with an intermediate region between them. As illustrated in Figure 1.1, the spinal gray matter is further divided into 10 regions on the basis of cytoarchitecture such as variation in size and density of the neurons. The most superficial of these is lamina 1, and the dorsal horn extends to lamina VI. The ventral horn comprises laminae VII-IX. Laminae X is a circle that surrounds the central canal. The dorsal horn of the spinal cord is the major site of termination of nearly all nociceptive afferents, irrespective of the peripheral origin. Small myelinated Aδ nociceptors terminate principally in the laminae 1 and laminae V, while the unmyelinated C fibre nociceptors terminate in laminae II (the substantia gelatinosa). The terminations of these primary afferent nociceptors transmit information to the first relay of neurons in the dorsal horn, also known as second-order neurons. The dorsal horn contains a host of peptide (such as substance P and Calcitonin Gene-Related Peptide (CGRP)) and amino acid neurotransmitters (such as Glutamate and aspartate) that assist in the transmission of nociceptive information. The primary afferents synapse either directly or indirectly (through interneurons) with the second order neurons located in the dorsal horn of the spinal cord, and their axons then decussate and ascend to the brainstem and higher brain centres.
Figure 1.1. Diagrammatic axial section of the spinal cord showing the 10 layers of the gray matter which includes the dorsal horn and ventral horn. Adapted from P. Stroman et al. (2002)

1.1.3 ANTEROLATERAL SYSTEM

The ascending spinal projections transmit sensory information by connecting the spinal cord to supraspinal levels. Spinal cord neurons project to the brainstem, cerebellum, midbrain, diencephalon and telencephalon. The anterolateral system, which primarily consists of the spinothalamic, spinoreticular, and spinomesencephalic tract (Figure 1.2), transmits nociceptive, thermal, and non-discriminatory (crude) touch.

1.1.3.1 SPINOThALAMIC TRACT

The spinothalamic tract is regarded as having a critical role in pain perception. It transmits information regarding the location and magnitude of pain to the thalamus and is composed of two anatomically distinct tracts: the lateral pathway and the small anterior pathway.
The lateral pathway transmits pain and temperature while the anterior pathway transmits crude touch and pressure. The neurons of the spinothalamic tract originate predominantly within laminae I and IV-VI of the dorsal horn. There are also some that are found in laminae X and the ventral horn. The primary fibres of the lateral STT ascend or descend 1-2 spinal cord segments before synapsing with secondary fibres. Secondary axons make up the lateral STT travelling in the lateral column of the spinal cord. The collaterals of the secondary fibres project to the reticular formation and stimulate wakefulness and consciousness while the secondary fibres themselves project to the ventral posterolateral (VPL) nucleus of the thalamus. From the thalamus, tertiary fibres terminate in the insula, the postcentral gyrus and other cortical areas.

1.1.3.2 SPINORETICULAR TRACT

The neurons of the spinoreticular tract originate primarily in the deep layers of the dorsal horn and in laminae VII and VIII of the ventral horn. They terminate into several nuclei within the brainstem reticular formation which includes the lateral reticular nucleus, nucleus gigantocellularis, nucleus paragigantocellularis lateralis in the medulla, the pontine nuclei oralis and caudalis, and the parabrachial region. There is no clear somatotopic organization of the spinoreticular tracts. Neurons from the reticular formation then project and terminate in close apposition to the hypothalamus, thalamus, and both directly and indirectly to the limbic forebrain and neocortex. These regions are mostly involved in blood pressure, motor control and the descending inhibition of pain. Therefore, this pathway is involved in the basic motor, sensory, and autonomic functions. It plays a critical role in the endogenous analgesic responses to nociceptive input. It may contribute to the motivational, affective and aversive response aspects of pain.
1.1.3.3 SPINOMESENCEPHALIC TRACT

The neurons of the spinomesencephalic tract are primarily located in laminae I and IV, V and VI in the dorsal horn of the spinal cord. There are also some that are found in lamina X and the ventral horn. These neurons terminate in several nuclei in the midbrain, including the PAG, the nucleus cuneiformis, red nucleus, superior colliculus, pretectal nuclei, and Edinger-Westphal nucleus. As opposed to the spinoreticular tract, the spinomesencephalic tract is somatotopically organized. The projections from caudal body regions terminate in the caudal midbrain while the projections from rostral body regions terminate in more rostral regions of the midbrain. As opposed to the spinothalamic tract, cells in this tract have large and complex receptive fields. The sites of termination of the spinomesencephalic tract suggest that the connections produce affective and aversive behaviours associated with pain and fear. It is also involved in a wide range of organized and integrated motor, autonomic, and antinociceptive responses to noxious input, such as orienting, defence, and confrontation.
Figure 1.2. Ascending Pain Pathways. The spinothalamic (STT, top), spinomesencephalic (SMT, bottom left) and spinoreticular (SRT, bottom right) tracts. Also showing the relevant termination sites of the tracts. Adapted from Blumenfeld (2002). (Jordan, 2010)
1.2 Descending Modulation of Pain

Modulation of pain pathways involves several brain, brainstem and spinal cord regions either directly or indirectly, such as prefrontal, insular cortices, and anterior cingulate; amygdala; periventricular and posterolateral hypothalamus; PAG; dorsolateral pons; and RVM (Tracey & Mantyh, 2007; Basbaum & Fields 1978; Gebhart, 2004). These areas act together to exert their influences on the perception of pain by regulating nociceptive processing through either inhibition or facilitation of the transmission of nociceptive information at the level of the dorsal horn (Basbaum & Fields 1984; Tracey & Mantyh, 2007; Gebhart, 2004; Ren & Duber, 2002). Descending monoaminergic pathways that utilize serotonin (5-HT), norepinephrine, or dopamine largely mediate these modulatory effects (Millan, 2002; Zhao et al., 2007; Pertovaara, 2006). These monoamines bind to different receptor subtypes and modulate the release of neurotransmitters from nociceptive afferents and the excitability of DH neurons.

The PAG and the RVM are two most well-known and important structures involved in endogenous pain control through the inhibitory PAG-RVM-DH descending inhibitory pain pathway (Fields, 2006). Intriguingly, research has also shown that, in addition to inhibition of spinal nociceptive transmission, the RVM has also been known to be involved in the descending facilitation of pain (Fields 2006; Porreca, Ossipov & Gebhart 2002). It achieves this through independent systems that are anatomically, pharmacologically, and physiologically distinct (Urban & Gebhart, 1999)

The PAG receives input from limbic and forebrain structures such as the insular cortex, frontal cortex, amygdala, and hypothalamus. It then projects the majority of its efferent input to the RVM and lower brainstem, which acts as a relay site for both modulatory systems (Mantyh 1983; Beitz 1982; Bingel & Tracey, 2008). The role of PAG in the descending modulation of
pain has been implicated in studies in both animals and humans. PAG has primarily been shown to be involved in the inhibition of nociceptive transmission. Stimulating the PAG has been shown to inhibit responses to noxious stimuli in rats (Mayer et al., 1971; Reynolds 1969). Similarly, in humans, stimulation of the PAG has been shown to cause pain relief by inhibiting responses to noxious stimuli (Baskin, Hosobuchi & Grevel, 1986; Hosobuchi, Adams & Linchitz 1977).

The PAG has little direct projections to the spinal cord, but as stated previously, it plays a key role in the initiation of the descending modulatory system. Therefore, it uses the RVM and dorsolateral pontine tegmentum (DLPT) as intermediaries in pain modulation. The RVM sends bilateral projections directly to the dorsal horn of the spinal cord via the dorsolateral funiculi and ventromedial funiculi and has differential effects on spinal nociception (Porreca, Ossipov & Gebhart 2002; Behbani 1995; Urban & Gebhart, 1999; Fields, Malick & Burstein 1995). The dorsolateral pontine tegmentum also contributes to the descending modulation of pain by sending projections right through the spinal cord and into the neurons in the DH. These projections are primarily excitatory and thus sometimes facilitate nociception.

In addition to the PAG, the RVM also receives neuronal input from the parabrachial nucleus (PBN), nucleus tractus solitaries (NTS), and other supraspinal sites that are involved in receiving and processing nociceptive information (Millan 1999; Fields, Heinricher & Mason 1991). The RVM is a heterogeneous region where it incorporates several nuclei, each of which provides descending pathways to the dorsal horn laminae, mainly in laminae I and II.

There are two populations of neurons, on-cells and off-cells, that have been identified in the RVM as pain modulatory neurons (Basbaum & Fields 1984; Fields, Heinricher & Mason 1991; Mason 1999). It was determined that activation of off-cells by opioids, inhibits ascending
noxious stimuli from the periphery by triggering descending inhibition. On the other hand, activation of on-cells contributes to enhanced sensitivity to noxious stimulation and thus triggers descending facilitation. (Fields, Heinricher & Mason, 1991; Fields & Basbaum, 1999; Gao & Mason, 2000).

In addition to the interplay between brainstem structures described above, structures at higher cortical levels also contribute to modulation of pain. These include the frontal lobe, anterior cingulate cortex (ACC), insula, amygdala, and hypothalamus, as well as motor areas including the basal ganglia, supplementary motor area, and cerebellum (Peyron, Laurent, & Garcia-Larrea, 2000; Tracey & Mantyh, 2007; W. D. Willis & Westlund, 1997). Most of these structures are involved in the limbic system and thus are important in the mediation of the motivational-affective and autonomic responses to painful stimuli. Brainstem reticular systems receive ascending input from the spinoreticular pathway and relay the information regarding the nature of pain to these limbic structures (Willis & Westlund, 1997). Through various research avenues, a number of factors such as arousal level, anxiety, attention and distraction, depression, expectation or anticipation etc. have been demonstrated to modulate pain processing. Therefore, the resulting perception of pain is a complex blend of sensory-discriminatory and cognitive-emotional factors.
1.3 Gate Theory of Pain

In 1965, Melzack and Wall formulated the classic “gate control” theory of pain which was the first to clearly describe the existence of a pain-modulating system (Melzack & Wall, 1965). The theory proposed that pain is not just a result of a linear process which starts from sensory stimulation of skin and ends with the person experiencing pain. Rather, pain impulses are influenced by a number of modulating factors before reaching the brain. As described in the previous section (section 1.2), when nociceptive information from the periphery reaches the
dorsal horn of the spinal cord, neural mechanisms in the spinal cord can either inhibit or facilitate the flow of nociceptive information. This propagation or restriction of nociceptive information is achieved through opening and closing of neural “gates”. These are located in the dorsal horn of the spinal cord and also along the ascending pain pathways.

The noxious information is influenced by a “gating mechanism” which involves the interplay between large diameter fibers and small fibers (Figure 1.4). The substantia gelatinosa in the dorsal horn is hypothesized to be the location of the gate. Small A-delta, large A-beta and C-fibres travel through the substantia gelatinosa and the arrangement of these neurons forms the basis for the modulation of incoming information. Melzack and Wall (1982) proposed that activity in small A-delta and C fibers causes prolonged activity in the spinal cord and “opens” the gate. This promotes sensitivity and thus increases the sensitivity to pain. On the other hand, when the large A-beta fibers are stimulated, the gate is “closed” and transmission of pain is inhibited. The gating is also influenced by nerve impulses that descend from the brain to the dorsal horn of the spinal cord.

The opening and closing of the gate in the spinal cord by descending impulses from the brain are affected by cognitive processes. Thus, the gate control theory of pain was the first to suggest that perception of pain is influenced by psychological factors. Pain not only has a sensory component but motivational and emotional components as well. The theory guided research towards finding cognitive-behavioral approaches to pain management. It provided a framework for understanding how negative emotions such as anxiety, worry, and depression can increase pain and positive emotions and distraction (e.g. music therapy) can decrease pain perception.
1.4 Principles of Magnetic Resonance Imaging

The primary origin of the MR signal used to generate images comes from hydrogen nuclei. A large proportion of the human body is made up of fat and water, both of which contain an abundance of hydrogen. Hydrogen nuclei consist of a single proton which carries a positive electrical charge. The positive charge results from a non-uniform distribution of smaller charges within the proton which is constantly spinning. The movement of an electrical charge is called a current and this electrical current generates a magnetic field. Thus, protons have their own magnetic fields and behave like little bar magnets. Therefore, the two key properties of the hydrogen nucleus that contribute to it producing a signal that can be used for MRI are: 1) it is magnetic with a north and a south pole and 2) it spins on its axis.

The magnetic field for each proton is known as a magnetic moment. Magnetic moments are normally randomly orientated. However, they align either parallel or antiparallel with the external field when an external magnetic field ($B_0$) is applied. The preferred state of alignment is parallel to $B_0$ because it is the lower energy state.
When put in an external static magnetic field, protons precess due to a combination of their spin giving them angular momentum, and the magnetic field providing a force to change its direction (towards alignment with the field). Precession can be thought of as the movement of a spinning top. When spun, the top wobbles but does not fall over and the axes of the top circles form a cone shape.

When an individual is put in the magnet of the MRI system, protons precessing parallel to $B_0$ cancel each other out in all directions, except, the direction of the z-axis, along $B_0$. This results in a total net magnetic field, often given the symbol $M$, with the value $M_0$. As this magnetization parallels the external magnetic field it is also referred to as longitudinal magnetization. The individual essentially becomes a magnet with a magnetic vector aligned with $B_0$.

Because the magnetic force of the patient cannot be measured as it is in the same direction as the external field, a magnetization is required that lies at an angle to $B_0$. Therefore, RF pulses are switched on and off. The purpose of the RF pulse is to disturb the protons so that they are pushed out of alignment with $B_0$. This occurs through the transference of energy from the RF pulse to the protons. However, this can only occur if the RF pulse has the same frequency as the precessional frequency of the protons. The activation of an RF pulse has two main effects on the protons. First, some protons gain energy and move to the higher energy state of being antiparallel to $B_0$. Consequently, opposing 'little bar magnets' (those parallel and antiparallel to $B_0$) once again cancel each other out, resulting in a reduction in overall longitudinal magnetization. Second, the RF pulse causes the protons to move in phase (ie, in the same direction, at the same time) with each other rather than in random directions. The result is
transverse magnetization in which a new magnetization vector is created in the x–y plane and moves in line with the precessing protons.

The transverse magnetization vector is a moving magnetic field and if a conductive receiver coil is placed in proximity, an alternating voltage will be induced across it. This generates an electrical current, which can be recorded and is the MR signal. As soon as the RF pulse is switched off the protons start to fall out of phase with each other, due to interactions between their individual magnetic fields, and also return to a lower energy state, that is, the protons relax. Relaxation occurs in two different ways. Transverse magnetization begins to decay back to equilibrium value of zero, a process called transverse (or T2) relaxation and the longitudinal magnetization starts to return to its original value, a process termed longitudinal (or T1) relaxation. For human tissue transverse relaxation is typically a much faster process than longitudinal relaxation. Different tissues relax at different rates, thus providing the basis for tissue contrast in MRI.

Two fundamental imaging methods used in MRI are: the “spin echo” (SE) and the “gradient” echo. The overall image appearance, determined by how well anatomical features or neural functions are contrasted from their surroundings, depends on the imaging method selected. When the RF pulse tips the magnetizations of all nuclei that are initially oriented in the same direction, the greatest MR signal is produced. However, this signal slowly decays exponentially to zero due to transverse relaxation as well as dephasing caused by spatial variations in the static magnetic field and magnetic field gradients. It takes milliseconds for the MR signal to decay to zero. Thus a signal echo, which is the return of the MR signal after it has decayed, produced by temporarily reversing the dephasing, is needed in order to have more time to measure the MR signal before it decays to zero again.
A SE is produced by applying a second RF pulse of 180° after the initial 90° RF pulse to create the MR signal “echo”. This cancels out the dephasing caused by static spatial field variations. However, since applying spin echo does not recover the entire MR signal and does not cancel out the effect of tranverse relaxation, SE is T2-weighted as a result. In contrast, a GE is produced by first using a single RF pulse and applying a magnetic field gradient and then reversing it. This causes the two gradients to cancel each other out and generating an “echo”. Since the dephasing caused by the static magnetic field variations are not cancelled out, the signal decays exponentially, and is T2*-weighted (Stroman 2011).

1.5 fMRI Contrast Mechanisms

1.5.1 BOLD

The most common contrast mechanism in fMRI is known as blood oxygenation level dependent (BOLD) contrast. Ogawa et al. (1990) first discovered the BOLD contrast in rat brains (Ogawa et al., 1990). In these studies, they observed that the intensity of the signal was depended on the blood oxygen levels. Signal intensity decreased when blood was deoxygenated, and increased when the flow of the freshly oxygenated blood increased. Later on, the BOLD contrast was shown to be able to detect functional activation in humans (Kwong et al., 1992; Ogawa et al., 1992). The BOLD contrast is based on the concentration of deoxyhemoglobin in the blood. Deoxyhemoglobin is paramagnetic and acts as a contrasting agent. When deoxyhemoglobin concentration increases, the relaxation time (T2*) decreases. Therefore, when there increase in oxygen supply to the active tissue and the oxygen supplied is more than the oxygen that is used by the neurons, there is a relative increase in the oxyhemoglobin concentration and a decrease in
Deoxyhemoglobin concentration. This increases T2* and leads to a signal increase in the GE images (Kwong et al., 1992; Ogawa et al., 1992) It also increases T2 and leads to signal increase in SE images. Therefore, the key effects that work together to produce a BOLD effect are: 1) changes in neural activity coupled with 2) changes in blood oxygenation which then results in an increase or decrease in 3) relaxation times. These three effects combine to influence the MR signal. (Stroman, 2011)

1.6 Spinal fMRI

Functional MRI (fMRI), a technique for measuring brain activity, is a recent addition to the world of diagnostic imaging. The advantages of fMRI have made it a commonly used tool in research fields for imaging brain activity since first being introduced successfully by Seiji Ogawa and Ken Kwong. An addition to the field of fMRI is the extension of this imaging technique to detecting neuronal function in the spinal cord. Functional MRI of the spinal cord (spinal fMRI) is another non-invasive tool used to investigate neuronal activity and reveal important insights with regards to spinal cord function. This technique is currently only being used for research purposes but, like brain fMRI, significant potential exists for spinal fMRI to become an important tool for clinical purposes. Even though the application of fMRI to the spinal cord requires certain specific modification to the conventional brain fMRI methodology, the underlying theory of conventional brain fMRI still applies. Constant work is being undertaken to make spinal fMRI methods as optimal as possible with goal of eventually using them in clinical settings. Spinal fMRI was first shown to be feasible one decade ago, and great advances have been shown with regards to its use in clinical populations.
1.6.1 Applications in Clinical Populations

As far as clinical populations are concerned, spinal fMRI has been used in the study of individuals with spinal cord injuries. Studies that have been carried out to date have demonstrated that spinal fMRI can detect important changes that occur as a result of traumatic injury in both the cervical and lumbar segments of the spinal cord (Goldfarb et al., 2011; Kornelsen and Stroman, 2007; Stroman et al., 2004). Stroman et al. 2004 imaged the lumbar spinal cord during noxious thermal stimulation of the L4 dermatome (leg) of complete and incomplete spinal cord injured volunteers and compared the activity with healthy volunteers (Stroman et al., 2004). Results showed significant activity in all participants, including SCI patients, in the lumbar segments of the spinal cord, below the level of injury. In addition to this, participants with SCI, who were able to feel or have some sensation of the stimulus, had similar spatial patterns of activity to that observed in healthy participants. However, the areas of activity in gray matter of the SCI patients were altered. Another more recent study, conducted by Saranathan et al., investigated responses in the cervical spinal cord and brainstem to warm thermal stimuli in regions both above and below the level of injury (Saranathan et al., 2012). Results were dependent on the level and extent of injury. Responses above the level of injury were mostly similar to those seen in healthy participants, whereas responses below the level of injury were shown to be altered depending on how severe the injury was. Differences were also observed in the right- and left-side of the spinal cord, as well as corresponding activity in the brainstem. This shows that spinal fMRI was able to detect neuronal response in the spinal cord even in subjects who could not feel the noxious stimulus. In addition to neural activity elicited as a result of thermal stimuli, spinal fMRI has also been used to detect neuronal activity elicited by passive and active lower limb movement tasks in regions caudal to the injury site in patients with
spinal cord injury (Kornelson & Stroman, 2007). Results showed activity in all volunteers regardless of the extent of injury. During both active and passive lower limb movement tasks, activity was seen caudal to the injury site. However, the number of active voxels detected with passive movement was less than with the active movement task. This shows that spinal fMRI was able to detect a neuronal response in the spinal cord caudal to the injury site during both lower limb movement tasks. Based on the two studies described above, spinal fMRI has proven to be useful for revealing activity in areas that have been impaired as well as areas that were preserved as a result of spinal cord injury. This shows that spinal fMRI methods are sensitive enough to detect changes in activity as a result of injury or trauma and can thus be used as a research tool for studying the effects of injury. However, the eventual goal is to be able to demonstrate that the results obtained in each individual SCI patient are reliable enough that they can be suitable for clinical purposes e.g. for diagnosis or monitoring treatment outcomes.

In addition to patients with SCI, spinal fMRI has also been used to investigate nociceptive processing in the brainstem and spinal cord to better understand pain. Spinal fMRI has been used to study the structural and functional correlates of pain in order to advance our understanding of mechanisms of nociceptive processing (Kornelsen & Mackey, 2007).

In relation to pain research, spinal fMRI may aid in identifying the pathogenesis of many chronic pain conditions. Spinal fMRI could be used to explore the neuronal abnormalities underlying several pain conditions such as irritable bowel syndrome or chronic lower back pain (Kornelsen & Mackey, 2007). Present ongoing research in Stroman Lab is looking at pain processing networks in patients with fibromyalgia with a goal of eventually developing a biomarker of the disease. Great potential exists for spinal fMRI to reveal the underlying initiation or maintenance of central sensitization, which is involved in many chronic pain conditions.
A disease that has benefitted from advanced spinal cord imaging techniques such as fMRI is Multiple sclerosis (MS). MS results in spinal cord lesions that are frequently observed in the cervical region of the cord and so the use of spinal fMRI might be able to aid in tracking disease progression and prognosis. Spinal fMRI has been used to investigate and assess the functional differences in the SC gray matter in patients with relapsing-remitting MS and healthy controls. Following tactile stimulation of the palm of the right hand, neural activity in the cervical spinal cord was detected and found in regions C5 to C8 in all participants, both patients and controls (Agosta et al. 2008a). However, MS patients demonstrated almost 20% greater signal intensity changes than controls. Neural activity was distributed throughout the dorsal, central, and ventral cord, which may be due to the interneuronal systems of the spinal cord. MS patients also tend to show a reduced functional lateralization in the SC and gray matter reorganization. MS patients show bilateral dorsal horn activity, whereas healthy controls show predominantly ipsilateral dorsal activity. It is unclear what the purpose of this gray matter reorganization is. Spinal fMRI could prove be the tool that may be needed to examine changes in the functional activity of gray matter throughout the evolution of multiple sclerosis, and may provide further insight into the disease. (Stroman et al., 2014)
1.7 Using fMRI to study Pain in Humans

1.7.1 Brain

In recent years, non-invasive functional neuroimaging techniques such as functional MRI have provided us with valuable insight into the brain systems engaged in the experience of pain (Tracey & Mantyh, 2007). Studies began by first using experimental pain in healthy volunteers and then have now, more recently, transitioned into patient populations with real pain conditions. FMRI techniques have helped us to investigate various components of the pain experience and to understand how the CNS changes, both in the short term and the long term as a result of injury. FMRI is also developing as a tool to investigate the mechanisms of action of pharmacological and non-pharmacological agents used for the treatment of pain. This may prove to be important in the drug development processes (Wise & Preston, 2010).

Because pain is a complex and a highly subjective experience, a large distributed network is accessed during nociceptive processing. The brain areas that most consistently appear to be involved in pain processing are often referred to as the “pain matrix”. These brain areas include: sensory–discriminatory involving areas such as primary somatosensory cortex (S1), secondary somatosensory cortex (S2), thalamus, and posterior parts of insula and affective–cognitive involving areas such as the anterior parts of insula, anterior cingulate cortex (ACC), and prefrontal cortex (PFC) (Apkarian et al., 2005). It is important to note that the pain matrix is not necessarily a defined static entity because, depending upon the interplay of different factors involved in influencing pain perception (e.g. cognition, mood, injury etc.), different brain regions will play a more or less active role in the pain matrix. The most common regions found active during an acute pain experience have been determined by different imaging studies such as
positron emission tomography (PET) and fMRI (Apkarian et al., 2005). These areas include: S1 and S2, insular, ACC, PFC, and the thalamus. However, subcortical regions such as basal ganglia (caudate & putamen), cerebellum, amygdala, hippocampus, and areas within the parietal and temporal cortices have also been shown to be active dependent upon the particular set of circumstances for that individual (Tracey & Mantyh, 2007).

Through experience we know that attention is very effective in modulating the sensory and affective aspects of pain (Levine et al., 1982; Miron, Duncan & Bushnell, 1989; Villemure & Bushnell, 2002). Functional MRI and neurophysiological studies have demonstrated numerous modulations related to attention- and distraction of pain-evoked activations in many parts of the pain ‘matrix’. (Bantick et al., 2002; Legrain et al., 2002; Ohara et al., 2004; Petrovic et al., 2002; Peyron et al., 1999). Results from these studies showed that regions that appear critical during the attentional modulation of pain include the descending pain modulatory system and key elements of the pain ‘matrix’ (Bantick et al., 2002; Tracey et al., 2002, Valet et al., 2004).

1.7.2 Brainstem and Spinal Cord

Even though fMRI of the spinal cord presents several challenges, technological developments in this field have provided opportunities to examine pain processing in the lower region of the CNS. Studies by The Stroman Lab have been able to accurately demonstrate activity in the spinal cord and brainstem in response to both innocuous and noxious stimuli. Studies were carried out with thermal stimuli at 42°C (warm) and 46°C (hot) of the palm of the hand (Cahill & Stroman, 2011). The results showed distinct differences between the warm and hot stimuli and were in line with the neuroanatomy for sensation vs. pain. Areas of activity were detected in the ipsilateral dorsal gray matter in areas of the spinal cord with both stimuli, but
were noticeably increased in the ventral gray matter regions of the spinal cord and in the rostral ventromedial medulla, reticular formation in the pons, raphe nucleus, pariaqueductal gray matter, locus coeruleus, red nucleus, and in the contralateral thalamus, with the hot stimulus. Another study showed that increased stimulus intensity corresponds to increased activity in the brain stem and spinal cord regions (Ghazni, Cahill & Stroman, 2010). This demonstrated the ability of spinal fMRI to discriminate sensation from pain and to reliably identify specific areas of activity that can especially useful in future studies in patients with neuropathic pain.

Spinal fMRI studies have also been able to demonstrate modulation in pain due to attention/distraction. Behavioral studies have shown that subjective ratings of pain sensations are increased when subjects focus their attention on a painful sensation. These findings also corresponded to fMRI studies of the brain, showing altered activity depending on attention. Therefore, a study on the effects of directing the subjects’ attention to or away from noxious thermal stimulus was carried out to determine whether or not changes in attention can also influence fMRI results in the cervical spinal cord. Results showed the effects of attentional modulation of activity in the cervical spinal cord that resulted from descending input from the brainstem. When the subjects were asked to focus attention away from the stimulus (watching a movie) there was consistently more activity in the periaqueductal gray matter and raphe nuclei in the medulla (Duckley et al., 2005; Fairhurst et al., 2007), as well as increased activity in the sixth cervical spinal cord segment in the ipsilateral dorsal gray matter and ventral gray matter regions. Similarly, recent studies conducted at Stroman Lab have also shown the effect of emotional modulation of pain through listening to music or watching pictures that affect an individuals’ emotional state (both positively and negatively).
With the advancement in pain and spinal fMRI literature, researchers have now begun looking at the entire central nervous system to study the interaction of brain, brainstem and spinal cord and of our ability to endogenously control pain (Stroman et al., 2013). Scientists have now started examining the impact of analgesic compounds on pain-related activity in the human spinal cord as well.

1.8 Individual Differences in Pain Experience

There is a large amount of evidence that normal, healthy, pain responses vary substantially between individuals. In other words, an identical noxious stimulus produces a wide range of experiences of pain in different people. Therefore, such individual differences in pain responses cannot be argued; however, the contributing factors as well as the clinical importance of these individual differences in pain remain an important topic of study.

![Figure 1.5: A model of pain showing that pain experience is influenced by interactions among biologic, psychologic and sociocultural factors. Adapted from Fillingim (2005)](image-url)
1.8.1 Factors/Mechanisms

As illustrated by Figure 1.5, pain is a complex personal experience which is influenced by multiple biopsychosocial factors such as age, sex, ethnicity, etc., and these contribute to tremendous inter-individual differences in responses to pain and its treatment. This has profound implications for the management of chronic pain, and efforts to better understand the many factors contributing to patients’ pain will permit more effective tailoring of treatment producing improved clinical outcomes. While biopsychosocial factors have individual qualities and influences on pain, as discussed below, their influences are inter-related and are not necessarily separable, or distinguishable.

**Sex/Gender differences**

Recent years have seen a substantial increase in research regarding sex differences in pain. A large body of literature clearly suggests differences in responses to pain in men and women, with an increased pain sensitivity and risk for clinical pain commonly observed in women. Several pain-related conditions such as rheumatoid arthritis, systemic lupus erythematosis, and fibromyalgia (Buckwalter & Lappin, 2000) have been shown to be more common among women than men. Arthritis pain and disability has also been reported to be greater among women compared to men (Keefe et al., 2000). Women also respond less to analgesic therapy, including therapy with ibuprofen and morphine, than men. In addition to clinical pain, substantial evidence suggests that relative to men, women exhibit more robust perceptual responses to pain in an experimental setting. More specifically, women display greater sensitivity (lower pain thresholds and tolerances) to multiple pain modalities than men. They also demonstrate greater temporal summation of heat (Fillingim et al., 1998; Robinson et
al., 2004) and mechanical pain (Sarlani & Greenspan, 2002), while men display greater conditioned pain modulation (Popescu et al., 2010). Therefore, sex differences in pain perception have been widely reported across multiple measures and stimulus modalities.

*Ethnic Differences*

Considerable evidence suggests that disparities in pain experience have also been shown to be present when looking at different ethnic groups (Edwards, Fillingim & Keefe, 2001). Most studies have compared Caucasians and African-Americans, with only a few studies examining other ethnic groups. Laboratory research has shown increased experimental pain sensitivity among blacks as compared with whites. Chapman and Jones, documented lower heat pain thresholds and tolerances among blacks compared with white subjects, almost six decades ago (Chapman & Jones, 1944). White subjects have also shown higher tolerance for cold pressor pain when compared with a combined group of blacks and Hispanics (Walsh et al., 1989). Campbell, Edwards & Fillingim 2005, examined responses to multiple experimental pain stimuli in black and white subjects. Results showed that black subjects reported lower pain tolerance and thus are more pain sensitive across three stimulus modalities (heat, cold, and ischemic pain) compared with white participants (Campbell, Edwards & Fillingim, 2005). In addition to experimental pain, these ethnic differences in pain experience and sensitivity have also been seen in clinical pain. For example, arthritis has been shown to be more prevalent among blacks and Hispanics than in whites. This greater prevalence has also been documented in these minority groups when it comes to experiencing greater arthritis-related pain and limitations in daily activity as a result of pain (Dominick & Baker, 2004).
**Age-related differences**

Vast amounts of literature on the epidemiology of pain have indicated age as being a contributing factor for an increased prevalence and impact of pain (Helme & Gibson, 2001). For example, the onset of and persistence of clinical pain has been associated with older age (Gureje, Simon & Von Korff, 2001). It is also reported that older adults appear to expect more pain, report pain at a greater number of body sites than younger adults, and show higher levels of interference in daily activities as a result of pain (Gagliese & Melzack, 1997; Gibson et al., 1994). Apart from these clinical and epidemiologic findings, it has also been shown that pain perception changes with age. The strongest data documenting age-related differences in pain perception, induced by thermal stimuli in a laboratory setting, comes from a review article by Gibson & Helme, 2001. They showed that age related differences in pain perception vary across stimulus modalities and pain measure. They concluded that older adults not only exhibited a decrease in pain threshold but also showed moderate to large reductions in pain tolerance in comparison to younger adults (Gibson & Helme, 2001). Loss of inhibition has also been shown among older adults using conditioned pain modulation (CPM) models. Older adults showed greater temporal summation of heat pain and decreased pain inhibition compared to young adults (Edwards & Fillingim, 2001; Edwards, Fillingim & Ness, 2003). Taking this together, research reports significant differences in pain sensitivity and perception as a result of age.

**Genetic factors**

Increasing evidence suggests that genetic factors contribute significantly to individual differences in pain responses in both a clinical and experimental setting. Multiple genes interact...
among themselves along with a variety of environmental factors to influence pain sensitivity and contribute to differences in pain responses in both normal and chronic pain conditions. Twin studies have been performed to provide insight into the genetic influence on pain. Studies show that genetic factors account for approximately 26-32% of the inter-individual variability in heat pain, 21% in chemical pain and as much as 60% of the inter-individual variability in cold pressor pain (Coghill, 2010). Candidate gene association studies have identified genes that may contribute to clinical and experimental pain. Studies have found significant associations between single nucleotide polymorphisms (SNPs) of specific genes and experimental pain responses. Kim et al. 2004 reported the genetic influence of SNPs on variability in human acute experimental pain sensitivity. They showed that a SNP of the δ- opioid receptor gene (OPRD1) was associated with thermal pain responses in males but not in females. They also found an association between vanilloid receptor subtype 1 (TRPV1) genotype and perception of cold pain (Kim et al., 2004). Other researches have shown an association between SNP of the catechol-O-methyltransferase gene (COMT) to be marginally associated with pain report and muscle pain stimulus (Zubieta et al., 2003); COMT haplotype to be associated with experimental pain sensitivity (Diatchenki et al., 2005); and a SNP of the mu-opioid receptor gene to be associated with mechanical pain sensitivity (Fillingim et al., 2005). Thus research has been able to suggest genetic factors as playing a role in individual differences in pain sensitivity.

**Psychosocial factors**

An extensive amount of literature shows that pain is heavily influenced by a vast diversity of psychosocial factors, including affective factors, cognitive processes, psychosocial history, social learning, personality etc. In both healthy individuals as well as among individuals
with chronic pain, it is widely reported that negative mood such as anxiety, anger, depression etc. is more prevalent and associated with greater levels of clinical pain (Fillingim, 2005). In addition to clinical settings, laboratory settings have also shown that negative mood predicts greater severity of acute pain and is related to greater pain sensitivity. As in the case with pain sensitivity, pain coping strategies also vary across individuals and are related to clinical pain symptoms (Nicassio et al., 1995; Riley, Robinson & Geisser, 1999). In addition to chronic pain, coping has also been related to acute pain. For example, pain catastrophizing has been shown to cause greater severity of postoperative pain (Tripp et al., 2003; Jacobsen & Butler, 1996) and has also been shown to enhance experimental pain sensitivity (France et al., 2002; Geisser, Robinson & Pickren, 1992). Thus, psychosocial factors such as mood states and pain coping strategies are associated with experimental pain sensitivity and thus contribute to inter-individual differences.

1.8.2 Clinical Importance

When it comes to visits to general physicians, pain is the primary reason in nearly 30% of the cases (Hasselstrom et al. 2002). Individual differences in pain sensitivity are of great importance in the treatment of pain. These differences in pain sensitivity also tend to complicate treatment methods and strategies. It is hard to decipher whether a patient who is reporting extremely high pain from a given procedure is simply a personality trait, or does he/she truly have much more pain than the other patients? Similarly, if the pain experience is true, how does one employ a treatment strategy based on that experience? The situation is similar for a patient who is reporting lower pain to the same procedure. Should the patient undergo same standard treatment or should the physician take a more conservative approach? (Coghill, 2010)
An important goal of research when examining individual differences in responses to pain is to eventually be able to develop the ability to accurately predict a person’s response to pain based on assessments of various characteristics of the individuals. For example, with sufficient knowledge we might be able to predict an individuals’ response to pain both before and after surgical procedure, pain therapy etc. based on assessments of his/her characteristics such as age, sex, ethnicity and other factors. Thus, a better understanding of the factors that underlie these individual differences in pain can provide further insights into the treatment in cases of both acute and chronic pain. As evidence continues to accumulate through various research avenues, these insights can then be used to tailor treatment for each individual patient as opposed to having a standard mode of treatment. Thus, complications arising from both under- and over-treatment could be greatly reduced, with the end result of a substantial improvement in pain therapy (Coghill, 2010).

With the information provided previously (section 1.8.1) we see that pain perception is characterized by tremendous inter-individual variability through a multitude of factors. What is particularly interesting is that the discussed demographic, genetic and psychosocial factors have not only been associated with laboratory measures of pain perception but also clinical pain indices as well. Thus, it is reasonable to assume that these individual differences in pain sensitivity have a direct clinical implication as well (Coghill, 2010). As described before, several domains of research support the clinical relevance of assessing experimental pain (Edwards et al., 2004). Various chronic pain populations demonstrate enhanced sensitivity of painful stimuli. Enhanced pain sensitivity has been demonstrated in chronic pain populations such as fibromyalgia, rheumatoid arthritis, and osteoarthritis (Laursen et al., 2005; Williams et al., 2004; Hendiani et al., 2003; Leffler et al., 2002). Experimental pain perception has often been able to
predict the severity of clinical symptoms. It has also been able to be an indicator or a predictor of future pain experiences in a clinical setting. For example, enhanced pain sensitivity before a surgical procedure has been shown to predict greater pain sensitivity after the surgery (Bisgaard et al., 2001; Werner, Duun & Kehlet, 2001; Granot et al., 2003). Similarly, for chronic pain, greater pain sensitivity before treatment with a particular drug has been shown to be a predictor of poorer outcomes following treatment (Edwards et al., 2003; Granot et al., 2004). Thus, through various examples discussed inter-individual differences in pain perception show strong clinical relevance in multiple chronic pain populations.

1.9 Proposed Research

1.9.1 Purpose

The purpose of the proposed research is to use fMRI to characterize the relationship between brain and spinal cord (SC) activity, and the perceived pain intensity in order to investigate variability in neural activity across healthy individuals.

1.9.2 Rationale

An extensive network of brain and spinal cord regions involved in the functional processing of pain has been identified through various electrophysiological and anatomical studies of nociceptive pathways. Interaction of brain regions such as the prefrontal cortex (PFC), anterior cingulate cortex (ACC), insula (INS), periaqueductal gray (PAG), rostral ventral medulla (RVM), and the dorsal horn of the spinal cord (SC) play an important role in processing nociceptive information. To date, functional magnetic resonance imaging (fMRI) studies investigating inter-individual differences in pain experience have focused on brain structures.
The primary goal of this study is to characterize the relationship between brain, brainstem and spinal cord activation with perceived pain intensity in order to understand neurophysiological origins of variability in neural activity among individuals. This study is valuable in providing a better understanding of abnormal sensory responses in neuropathic pain populations such as fibromyalgia, patients with spinal cord injury etc. Detecting neuronal activity and inter-individual differences in the entire CNS can help us understand mechanisms underlying chronic and neuropathic pain and how regions of the CNS, from transmission in the spinal columns to processing in higher brain structures, contribute to this condition. The findings from this study will provide a more complete picture of pain processing in healthy individuals and demonstrate the reliability of fMRI and its clinical potential.

1.9.3 Hypothesis

We hypothesize that consistent patterns of activity as well as significant inter-individual differences in fMRI results in the brain, brainstem and SC, in response to painful stimuli, are correlated with subjective ratings of pain intensity.

1.9.4 Objectives

The specific objectives of this study are:

1) To identify brain, brainstem and SC regions engaged in the processing of pain evoked by a noxious thermal stimulus.

2) To quantitatively characterize the relationship between CNS activity and perceived pain intensity in order to determine variability among different individuals.
Chapter 2

Methods

2.1 Volunteer Recruitment

A total of 20 normal, healthy volunteers (9 males and 11 females) were recruited from the local community. Volunteers were recruited via posted advertisements (Appendix A) placed around Queen’s University campus. All participants’ ages ranged from 19 to 34 years (median age 21). All respondents were provided with the MRI Safety Screening Questionnaire (Appendix B), Subject Consent Form (Appendix C), and Volunteer Details, which were signed and returned prior to enrollment in the study. All volunteers gave written, informed consent acknowledging that they would undergo brain and spinal cord imaging and experience experimental pain stimuli. All methods and procedures were clearly explained, and they were free to withdraw from the experiment at any time. Exclusion criteria for participation in the study included any neurological disorders, previous injury to the brain or spinal cord, any peripheral injury that affects the sensitivity to their hands to touch or thermal sensation, a history of claustrophobia or anxiety, or having any MRI safety risks (e.g., pacemaker, implant, neurostimulator, metallic implants, artificial limbs etc.). All research procedures were reviewed and approved by the Queen’s University Human Research Ethics Board. The research protocol was reviewed and approved by the Queen’s University Human Research Ethics Board. All data were treated confidentially; with each set of subject data images assigned a unique identifying number only accessible by the experimenter.
2.2 Functional imaging

The purpose of this study was to use fMRI to identify regions within the brain, brainstem and spinal cord that have responses to a noxious thermal stimulus that are correlated in intensity with participants’ pain ratings. In order to achieve this, we applied thermal stimulation (49 °C) to the right hand, on the little finger side of the palm corresponding to the 8th cervical dermatome (C8). Thermal stimulus was produced using a Medoc TSA-II thermal sensory analyzer (Medoc Ltd, Haifa, Israel). To ensure that our volunteers were familiar with the pain tasks to be used during the scans, they underwent a training session before scanning. They were trained to recognize various painful stimuli and become familiar with the numerical pain rating scale (Figure. 2.2), before the start of the study (not discussed further). This also allowed the participants to become more familiar with the MRI environment and become accustomed to the heat stimuli, in an effort to reduce anxiety and anticipation. During scanning, participants experienced thermal stimulation consisting of 10 hot, brief, heat pulses, as shown in Figure 2.1. The frequency of administration was 0.33 Hz (pulses every 3 seconds). The thermode was initially held at a warm adaptation temperature for 50 seconds (“rest” period). The spikes in temperature comprised of an 8 °C increase over 1.5 seconds, then a drop back to the warm adaptation temperature within 1.5 seconds. The peak 49 °C temperature and warm adaptation temperature were always 8 °C apart. After the initial 50 seconds of “rest” period, the ten pulses were administered over the next 30 seconds (0.33 Hz) and were followed by again by 75 seconds at the warm adaptation temperature (Figure. 2.1). This procedure stayed constant throughout testing without the knowledge of the participant and so this information did not influence the participants’ pain ratings. Participants were not aware whether the thermal stimulation temperature was the same or whether it was changed between runs. In total, the paradigm was
155 seconds in length with a rest period of 2 minutes between each trial. The trial was repeated six times for spinal fMRI and four times for brain fMRI. This was done in order to avoid sensitization of the skin receptors and allowing them to acclimatize back to room temperature. Therefore, each data collection run lasted 2.5 minutes in duration. Both the initial and final baseline temperatures were the same and were always 8 °C lower than the stimulation temperature.

**Figure 2.1:** Thermal stimulation presented to participants during the imaging session. Heat pulses were presented at a frequency of 0.33Hz. The adaptation temperature (41°C) and peak temperature (49°C) were kept consistent for all participants.

### 2.2.1 Pain ratings

Immediately after thermal stimulation, a standardized numerical pain scale was displayed to participants and they were asked to rate the intensity of the pain produced by the thermal stimulation. The scale ranged from 0 to 100 (participants were allowed to rate in increments of 5), associated with verbal descriptors at intervals of 10: 10, warm; 20, a barely painful sensation; 30, very weak pain; 40, weak pain; 50, moderate pain; 60, slightly strong pain; 70, strong pain; 80, very strong pain; 90, nearly intolerable pain; and 100, intolerable pain. A mirror allowed the
participants to look at the numerical pain scale on a projection screen setup outside the MRI system.

![Pain Rating Scale](image)

**Figure 2.2** Pain rating scale employed by participants

### 2.3 fMRI study setup

Functional MRI studies with thermal stimulation were carried out in a 3.0 Tesla Siemens Magnetom Trio MRI system, using a spine-array coil, anterior neck coil, and anterior head coil, for detection of the MR signal. Participants were made comfortable and positioned supine on the MRI bed. In order to eliminate signal from anterior to the spine to reduce physiological motion or motion artifacts produced by the heart, lungs, throat etc from breathing/swallowing, spatial suppression pulses were applied. Using the physiological monitoring equipment that is incorporated into the Siemens MRI system, each participant’s peripheral pulse was recorded continuously throughout the study. An external trigger generated by a National Instruments data acquisition board (DAQpad- 6020E) controlled by custom software written in MATLAB® (The MathWorks Inc., Natick, MA, USA) triggered the acquisition of each image slice. This allowed for peripheral pulse recordings and acquisition of each imaging slice to be in synchrony. In order
to record peripheral pulse, an optical device was attached to the left index finger and remained there for the duration of the study. Participants were made as comfortable as possible (foam-filled pads, pillows and blankets were used) while they were in the MRI and were encouraged to remain as still as possible throughout the studies. Each imaging session contained six spinal cord imaging runs and four brain imaging runs.

2.4. fMRI Acquisition

Spinal fMRI Acquisition

For slice positioning for subsequent fMRI acquisitions, initial localizer images were acquired in 3 planes to be used as a reference. Nine sagittal slices spanned the spinal cord to include the C7/T1 intervertebral disc to above the superior edge of the thalamus in the brainstem using a half-Fourier single-shot fast spin-echo (HASTE) imaging method for BOLD contrast. This is done to avoid echo planar imaging (EPI) and get the best possible image quality, spatial fidelity and signal-to-noise ratio. The sagittal slice orientation gives the largest coverage of the spinal cord/brainstem in 3D with no gaps and this allows for spatial normalization. The repetition time (TR) was 6.75s, which is the shortest possible with chosen acquisition parameters, and a shorter TR gives more time-points for fMRI analysis and thus leads to higher BOLD sensitivity. The echo time (TE) was 75ms because this provides the optimal BOLD sensitivity. The acquisition matrix is kept as large as possible at 192 x 144 because this gives a higher SNR and thus higher BOLD sensitivity than a smaller matrix. Each slice was 2 mm thick with the resulting voxel size of 1.5mm x 1.5mm x 2mm. This provides a balance between coverage and SNR.
**Brain fMRI Acquisition**

Brain fMRI data were acquired following spinal fMRI acquisition. The acquisition was similar to spinal fMRI protocol except that a 12-channel head coil was used. To ensure optimal BOLD sensitivity, functional images were acquired in 49 transverse slices using a T2*-weighted gradient echo planar imaging (EPI) sequence. The imaging parameters used were as follows: Repetition time/Echo time (TR/TE) = 3 s/30 ms; field of view (FOV) = 192 x 192 mm; matrix = 64 x 64; and the resulting voxel size 3.0 mm x 3.0 mm x 3.0 mm].

**2.5 Data Analysis**

*Spinal fMRI Analysis*

The spinal functional image data were analyzed using custom-made software written in MATLAB® (The Mathworks Inc., Natick, MA, USA), specifically for spinal cord and brainstem fMRI. Data were first converted from DICOM to NifTI format for convenience. Co-registration was applied in order to align each volume in 3D with the first one in the time-series. This corrects any small scale movements. No spatial smoothing or temporal filtering was applied since it has shown no observable advantages rather potential bad effects. Data were combined across all runs (6 in total) which give the advantage of a large number of volumes, without long continuous runs. GLM analysis was done to extract BOLD signal changes in the presence of other sources of signal change. The basis set included a model of the stimulation paradigm convolved with the BOLD hemodynamic response function, the first two principal components as models of the dominant global variance in the data, and a constant function (Stroman, 2013). For group analysis, a random effects analysis (2nd level analysis) was conducted and activity map for the significantly active voxels in all twenty participants was created (p-value cutoff 0.001).
In order for the data to be spatially normalized, regions masks to be generated, and to guide image co-registration, a total of nine reference lines were manually drawn on one volume of each set of time-series image data (Figure 2.3). Lines were drawn on each data set in the midline sagittal plane and coronal plane. Within the midline sagittal plane of each data set, five lines were drawn along the anterior and posterior edges of the cord, around the edge of the pons, along the posterior medulla, as well as the top of the corpus callosum. Within the coronal view four lines were also drawn to mark 1) the bottom of the pons 2) an extension through the middle of the image spanning the corpus callosum to the bottom of the pons and 3) right and left boundaries of the spinal cord. Points were selected and then joined together to create the nine reference lines. The positions of the points were adjusted to fine-tune the lines and maximize the accuracy of normalization.
Figure 2.3: Image showing the normalization procedure for a dataset. Coronal (right) and sagittal (left) view of a midline slice with nine reference lines drawn manually as part of preprocessing step of spinal cord fMRI data.

**Brain fMRI Analysis**

The brain fMRI data were analyzed using the statistical parametric mapping (SPM 8) software package (Wellcome Dept. of Imaging Neuroscience). Image data were first converted to NifTI format and then were realigned, and coregistered with slice timing corrected. Slice time correction was done to correct for differences in image acquisition time between slices. Normalization was done using representative, averaged realigned slices and a standard EPI template available in SPM-8. Additionally, data were smoothed with 5mm full width at half maximum (FWHM) Gaussian kernel.
A random-effects group analysis (a second-level analysis) was carried out on the participants’ activation maps to produce group results. MNI coordinates were converted to talairach coordinates, which were then used to identify cortical regions using the ‘Atlas of the Human Brain’ (Mai, Assheurer, & Paxinos, 1997) and the Talairach Daemon Client (Version 1.1, Research Imaging Center, University of Texas Health Science Center, San Antonio, TX).

**Connectivity Analysis**

A connectivity analysis was carried out on the participant activation maps to produce connectivity maps, characterizing activity in all 20 participants. Analysis was performed using CONN: functional connectivity toolbox. Network theory results were generated from the 2nd level analysis. ROI’s were identified using the Betweenness Centrality measure which is an important concept of graph theory. It is a useful measure of load and importance of node. The exact definition describing betweenness centrality is:

"An important concept related to that of node degree is centrality. Centrality refers to the relative importance of a node or vertex within the network. The betweenness centrality of a particular vertex is the fraction of shortest paths in the network that pass through this vertex which corresponds to the node's relative importance in the network." (Bassett and Gazzaniga, 2011)

The analysis threshold (p-value) was p<0.05 and thus ROIs that were activated in all 20 participants were generated based of their T value (See Results 3.2).
2.6 Statistical/Correlation Analysis

Functional MRI data from the brain or spinal cord were first pre-processed and analyzed using a General Linear Model to identify the magnitude of apparent BOLD response in each voxel that corresponded with the stimulation paradigm. The BOLD magnitudes, represented as a “β” value for each voxel and each participant, were then used in a second-level analysis to determine the correlation with the reported pain ratings for each study. For this analysis, the results of the first-level GLM analysis in spatially normalized format were compiled across participants into a single data set. Pain ratings from each participant were similarly compiled into a single data set, in the same order as the fMRI results. The Pearson’s correlation coefficient between pain ratings and β-values were then determined for each voxel, resulting in a map of correlation coefficients.

The correlation coefficient map was then used to identify regions-of-interest in which the magnitudes of the correlation were highest, and were relatively consistent across a 3D anatomical region.
Chapter 3

Results

3.1 Psychophysical Differences between Individuals

According to subject reports, the individual experience of pain evoked by the 49 °C noxious thermal stimulus, delivered to the right hand at the C8 dermatome, differed substantially across participants. The least sensitive subject rated the 49°C stimulus as 18/100 (warm) whereas the most sensitive individual rated the same 49 °C stimulus as 74/100 (strong pain) (Figure 3.1). The mean rating across all twenty subjects was 43.3 ± 16.9 (mean ± S.D.). A Student’s two-tailed, paired sample t-test was used to determine the significance of the differences in pain ratings between males and females. The test demonstrated that pain intensity ratings did not differ significantly between males and females.

Figure 3.1: Subjective pain responses of 20 participants.
3.2 Spinal Cord and Brainstem Results

To identify the neural correlates underlying these inter-individual experiential differences, the correlation between subject pain ratings and the BOLD percent signal change in various spinal cord and brainstem regions was determined. Spinal cord and brainstem regions important in sensation, attention, and affect showed high correlations between pain ratings and BOLD percent signal changes. Neural activity, in response to thermal stimulation, was detected in all participants within regions associated with the pain pathway; including pons, midbrain, rostral ventromedial medulla (RVM), and the dorsal horn of the 8th cervical segment. The neural activity map for regions consistent in all 20 participants is shown in Figure 3.2.

Significant inter-individual differences were observed and BOLD percent signal changes were highly correlated with pain ratings in the PAG, PBN, and the right dorsal segments of C7, C6, C5, C4, C3 and C1. Individuals that rated the pain as higher exhibited a stronger BOLD percent signal change in these regions as compared to individuals who rated their pain as relatively less painful. The PAG and PBN showed the highest correlation between the pain ratings and BOLD percent signal changes having an $R^2$ value of 0.81 and 0.80 respectively. However, other brainstem regions known to be involved in pain intensity processing such as RVM, LC, and NTS did not show any statistically reliable correlation in BOLD percent signal changes and pain ratings in individuals. The correlation values, $R^2$, between subjective pain ratings provided by each volunteer at the time of the study and signal intensity changes in active voxels, averaged across specific regions of the cervical spinal cord and brain stem, are summarized in Table 3.1. The ratings from each volunteer are plotted against the magnitudes of signal changes in active voxels for PAG, PBN and C7 in Figures 3.3, 3.4 and 3.5 respectively.
Figure 3.2: Group fMRI results showing areas of activity in brainstem/spinal cord that is consistent among all 20 participants. Each square represents slice 1mm thick for a total of 220 contiguous slices spanning the upper thoracic to lower part of the brainstem.
Figure 3.3: Plots of relationship between subjective pain ratings provided by each of the twenty individuals studied, and the magnitude of the BOLD Percent Signal Changes observed in the midbrain – periaqueductal gray. The correlation ($R^2$) value is indicated for a linear regression between the signal changes and pain ratings.

Figure 3.4: Plots of relationship between subjective pain ratings provided by each of the twenty individuals studied, and the magnitude of the BOLD Percent Signal Changes observed in the parabrachial nucleus. The correlation ($R^2$) value is indicated for a linear regression between the signal changes and pain ratings.
Figure 3.5: Plots of relationship between subjective pain ratings provided by each of the twenty individuals studied, and the magnitude of the BOLD Percent Signal Changes observed in the dorsal horn of cervical spinal cord segment (C7). The correlation ($R^2$) value is indicated for a linear regression between the signal changes and pain ratings.

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<td>C1</td>
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Table 3.1: Correlation values, $R^2$, between pain ratings and fMRI BOLD percent signal changes over selected regions of the cervical spinal cord and brainstem
3.3 Brain Results

Even though spinal cord and brainstem include important synaptic sites through which the pain experience is processed, the pain signals eventually end up in higher cortical regions in the brain (pain centres) that play a key role in pain experience. Therefore, like the spinal cord, in order to identify the neural correlates underlying these inter-individual pain experiential differences, correlation between subject pain ratings and BOLD percent signal change was determined in certain regions of the brain. Brain regions important in sensation, attention, and affect showed significantly higher correlations between pain ratings and BOLD percent signal changes. Neural activity in response to thermal stimulation was detected in all participants within regions most commonly associated with the pain pathway; including the anterior cingulate cortex (ACC), the prefrontal cortex (PFC), premotor cortex, insula (INS), thalamus, and the primary somatosensory cortex (S1). In addition to these, regions that are generally not considered and reported in a large majority of human functional imaging studies of pain also generated areas of activity. For example, activity was noted in regions consistent with the location of the parahippocampal cortex, perirhinal cortex as well as the entorhinal cortex. The neural activity map of all 20 participants grouped together is shown in Figure 3.6. Figure 3.7 shows activity taken from one slice of the group map. A connectivity map of brain regions activated in all 20 participants is shown in Figure 3.8. Table 3.2 shows the names of the labels shown in Figure 3.8 as well as the T-values of the regions activated.

Significant inter-individual differences were observed and BOLD percent signal changes were highly correlated with pain ratings in the ACC, PCC, PFC, S1, insular cortex, premotor cortex, thalamus as well as the parahippocampal cortex, and the entorhinal cortex. Individuals that rated the pain as higher exhibited a stronger BOLD percent signal change in these regions as
compared to individuals who rated their pain as relatively less painful. The ACC, PCC, showed the highest correlation between the pain ratings and BOLD percent signal changes having an $R^2$ value of 0.46 and 0.45 respectively. Correlation values between subjective pain ratings provided by each volunteer at the time of the study and signal intensity changes in active voxels, averaged across specific regions of the brain, are summarized in Table 3.3. The ratings from each volunteer are plotted against the magnitudes of signal changes in active voxels for insular cortex, left ventral ACC and left ventral PCC in Figures 3.9, 3.10 and 3.11 respectively.
Figure 3.6: Group fMRI results for 20 participants in brain regions showing areas of activity in response to the stimulus.
Figure 3.7: View of one slice in three orientations from the group fMRI results for 20 participants shown in Figure 3.6.
Figure 3.8: Axial (left) & saggital (right) view of the connectivity map of brain regions showing areas of activity in response to the stimulus in all 20 participants. Table 3.2 shows the names of the labels as well as the T and p values of these regions.

<table>
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**Table 3.2:** Label ROI names, and T-values of the regions activated showing connectivity in all 20 individuals, as shown in Figure 3.8.
INSULAR CORTEX

Figure 3.9: Plots of relationship between subjective pain ratings provided by each of the twenty individuals studied, and the magnitude of the BOLD Percent Signal Changes observed in the insular cortex. The correlation ($R^2$) value is indicated for a linear regression between the signal changes and pain ratings.

$R^2 = 0.37$
Figure 3.10: Plots of relationship between subjective pain ratings provided by each of the twenty individuals studied, and the magnitude of the BOLD Percent Signal Changes observed in the left ventral posterior cingulate cortex. The correlation ($R^2$) value is indicated for a linear regression between the signal changes and pain ratings.

$R^2 = 0.45$

Figure 3.11: Plots of relationship between subjective pain ratings provided by each of the twenty individuals studied, and the magnitude of the BOLD Percent Signal Changes observed in the left ventral anterior cingulate cortex. The correlation ($R^2$) value is indicated for a linear regression between the signal changes and pain ratings.

$R^2 = 0.46$
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<tr>
<td>Left Perirhinal Cortex</td>
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</tr>
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</table>

Table 3.3: Correlation values, $R^2$, between pain ratings and fMRI BOLD percent signal changes over selected regions of the brain that showed a POSITIVE correlation.

Although the thermal stimulation was applied on the right hand and so, in theory, activity should only be observed in the left hand side of the brain, we observed activity in the right side of the brain as well. This activity was predominantly negative, meaning that individuals who rated the pain as higher exhibited a lower BOLD percent signal change in these regions as compared to individuals who rated their pain as less painful. The regions involved in this negative correlation were PFC, S1, ACC, parahippocampal cortex, PCC and the insular cortex. The right dorsolateral PFC and the right premotor cortex showed the highest correlation between
the pain ratings and BOLD percent signal changes having an $R^2$ value of 0.33 and 0.38 respectively. Again, as observed in regions on the left hand side, other brain regions known to be involved in pain processing such as the secondary somatosensory cortex, amygdala, the supplementary motor area, and the posterior parietal cortex did not show any statistically reliable correlations in BOLD percent signal changes and pain ratings in individuals. Comparisons between subjective pain ratings provided by each volunteer at the time of the study and signal intensity changes in active voxels, averaged across specific regions of the brain, are summarized in Table 3.4. The ratings from each volunteer are plotted against the magnitudes of signal changes in active voxels for the right dorsolateral PFC, right parahippocampal cortex and right anterior PFC in Figures 3.12, 3.13 and 3.14 respectively.

**Figure 3.12:** Plot of relationship between subjective pain ratings provided by each of the twenty individuals studied, and the magnitude of the BOLD Percent Signal Changes observed in the right dorsolateral prefrontal cortex. The correlation ($R^2$) value is indicated for a linear regression between the signal changes and pain ratings.
Figure 3.13: Plot of relationship between subjective pain ratings provided by each of the twenty individuals studied, and the magnitude of the BOLD Percent Signal Changes observed in the right parahippocampal cortex. The correlation ($R^2$) value is indicated for a linear regression between the signal changes and pain ratings.

Figure 3.14: Plot of relationship between subjective pain ratings provided by each of the twenty individuals studied, and the magnitude of the BOLD Percent Signal Changes observed in the right anterior prefrontal cortex. The correlation ($R^2$) value is indicated for a linear regression between the signal changes and pain ratings.
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*Table 3.4:* Correlation values, $R^2$, between pain ratings and fMRI BOLD percent signal changes over selected regions of the brain that showed a NEGATIVE correlation.
Chapter 4

Discussion

4.1 Principle findings

In the present study we demonstrate neural activity generated by a noxious thermal stimulus throughout the entire central nervous system spanning the lower neuroaxis, caudally from the lower cervical spinal cord to all the way up to the cerebral cortical regions, in healthy volunteers. Results also demonstrate that application of the same noxious thermal stimulus (49°C) produced similar regions of activity but different magnitudes of BOLD responses in subjects. Subjective pain ratings were highly positively correlated with the BOLD percent signal changes in most of the active regions in response to the noxious thermal stimuli in brain, brainstem and spinal cord regions. Thus the behavioral data from the current study confirms the existence of inter-individual differences in pain and the imaging data corresponds with the behavioral results by showing these differences in neural activation, giving insight into the structures and mechanisms involved in the differences in pain experience. Brain regions such as ACC, PCC, S1, insular cortex etc. and spinal cord and brainstem regions such as the PAG, PBN, and right dorsal segments of cervical cord exhibited a higher BOLD percent signal change in individuals who were highly sensitive to pain as compared to individuals who were relatively less sensitive to pain. In contrast, brain regions such as S2, amygdala and brainstem regions such as LC, RVM, and NTS etc. which are also known to be critically involved in the afferent transmission and processing of nociceptive information did not show any statistically significant correlation between BOLD percent signal changes and pain ratings.
4.2 Subjective Reports are a Reliable Index of an Individual’s Experience

As shown by means of the correlations of subjective pain ratings to BOLD percent signal intensity changes in active voxels, the variation of neuronal activity across participants in each anatomical region appears to be a consequence of differences in pain perception. This correspondence is especially important because it demonstrates that normal healthy variations in our results are not attributable to errors/uncertainty but instead a significant component of the variance of the results is attributable to physiological differences between individuals. The results can therefore be considered to be sensitive and reliable indicators of neural function in the central nervous system within each individual who was studied. The present finding provides evidence that BOLD signal intensity correlates with psychophysical outcomes (pain ratings). Individuals with similar patterns of activation of CNS regions such as the ACC, PCC, S1, insular cortex, PAG, PBN, and C7 etc. provided similar subjective reports of pain suggests that subjects can accurately capture their conscious experience. Historically, scientists have considered this as errors/nuisance. Our findings suggest that these differential pain responses are also accompanied by differential central processing of the noxious stimuli. This further strengthens and validates the use of subjective reports of pain as being a reliable index of an individual’s actual experience.

4.3 Certain CNS Regions Activated Reflect Inter-individual Differences in Pain Sensitivity

Brain Regions

The brain regions that were activated more robustly in highly sensitive individuals are supported by large amounts of literature as playing an important role in the pain experience.
Functional imaging studies of human subjects have identified a wide array of brain areas that are involved in the processing of pain. Multiple regression analysis revealed correlations between pain ratings and activation of brain regions, including those important in sensation, motor control, affect, and attention. Pain related activation and significant inter-individual differences were observed in the ACC, PCC, PFC, S1, insular cortex, thalamus as well as the parahippocampal cortex, and the entorhinal cortex. Within-subject studies have shown that regions such as S1, ACC and PFC all show increased activation as the intensity of the noxious stimulus increases (Derbyshire et al., 1997; Coghill et al., 1999; Porro et al., 1998). Coghill and colleagues have shown that the ACC, PFC and S1 are activated in processes leading to inter-individual differences in pain sensitivity (Coghill, McHaffie & Yen, 2003). Our study provides further insight into CNS mechanisms that may account for inter-individual differences in pain sensitivity by identifying additional regions of the brain.

Although S1, ACC, PCC, PFC, S1, insular cortex, thalamus etc. are all activated in the processing of pain intensity, each area may make a differential contribution to various aspects of the pain experience. In general, positively correlated activations of these regions were observed contralateral to the stimulation. Negative correlations were observed in sites ipsilateral to the stimulation.

**Primary somatosensory cortex (S1)**

Among the cortical regions that are most often activated by pain is the primary somatosensory cortex (S1). It contains neurons that code spatial, temporal, and intensive aspects of a noxious somatosensory stimuli (Hofbauer et al., 2001), characteristics that subserve the sensory-discriminative aspect of pain processing. The ability to locate pain is essential in
immediate defense and withdrawal behavior (Bingel et al., 2004). The primary somatosensory cortex (SI) shows a clear somatotopic organization, by showing activation in the region contralateral to the stimulation and contributes to early levels of pain localization processing (Kanda et al., 2000). Based on this, it comes as no surprise, that there are inter-individual differences in its activation, since the ability to accurately locate pain differs from person to person.

**Anterior Cingulate Cortex (ACC)**

Unlike SI, where activity is found to be consistent across all subjects in most studies, the ACC has been implicated to show activity in only some individuals. This finding suggests that the ACC does not play an important role in the extraction of information from sensory stimuli, but it may in fact represent individual differences that may occur at a later stage of processing (Arienzo et al., 2006). Various PET studies on somatic or visceral pain have reported activation of the ACC attributed to the emotional response to pain (Hsieh, 1999). Human imaging studies have shown that ratings of pain unpleasantness were correlated with activity with the ACC (Rainville, 2002), which makes it a candidate for the affective-motivational dimension of pain. This may explain the inter-individual differences observed in the activation of both ventral and dorsal subregions of the ACC, both of which are known to be involved in motivational and goal-oriented cognitive processes and processes that provide the negative emotional valence to the experience of pain. (Vogt, 2005; Lavin et al., 2013)
**Posterior Cingulate Cortex**

The posterior cingulate cortex receives afferent axons from the thalamus and sends projections to the anterior cingulate cortex as well as other areas (Maddock et al., 2001). Most pain imaging studies have reported activation associated with the anterior or middle part of the cingulate cortex. Involvement of the posterior part is seldom reported. There is no general textbook consensus about the functions of the PCC. It has been shown to have functional heterogeneity by being associated with a variety of brain functions e.g. evaluative functions (for spatial orientation and memory) (Vogt et al., 1992), memory retrieval (Cabeza and Nyberg, 2000), emotion (Vogt et al., 2001), as well as in eye movements with activations in saccades and pursuit tracking (Berman et al., 1999). While it is hard to exactly say how PCC may be involved in inter-individual differences in pain processing, it does have other functions (such as memory, emotion etc.) that differ from person to person.

**Insular cortex**

The insular cortex is involved in the intensity encoding of painful stimuli (Bantick et al., 2002) and its activations appear in studies involving application of heat (Brooks et al., 2005; Davis et al., 1998). Given its anatomical connections, the insula is viewed as a relay station for sensory information, where it receives input from the spinothalamically activated posterior thalamic nuclei, into the limbic system, where it has links with the amygdala, the temporal pole, the hippocampus, the premotor cortex, the prefrontal cortex and the ACC. It is believed to be involved in consciousness as well as functions linked to emotion. These include perception, motor control, self-awareness and interpersonal experience. A recent study by Isnard et al., 2011
suggested that if the full pain experience involves all the areas of the pain matrix network, the insula plays a leading role in the triggering of this network and the resulting subjective pain experience (Isnard et al., 2011). Based on this, it is conceivable, that an increased activation of this pain area corresponds with higher pain rating and its activation differs from one individual to another when it comes to experiencing pain.

**Thalamus**

The thalamus is the part of the brain which plays a major role in pain perception and where activation would most be expected during an acute pain state (Peyron et al., 1999). Activation of the contralateral thalamus due to pain is known from experimental animals (Zhao et al., 2007) and functional imaging studies in humans (Da Silva & Hadjikhani, 2013). It receives pain signals from the spinal cord and relays these signals to the cerebral cortex and the midbrain. The intensity and speed with which the pain signal is transmitted and then relayed depends on the individuals’ pain experience. Because we see inter-individual differences in the dorsal horn of the spinal cord (discussed later) and thus how pain is transmitted, it makes sense that these differences would also be observed in the thalamus. An increased activation would likely correspond with a higher pain rating since the signals received from the spinal cord might be more in individuals that perceived the pain as more painful compared to those that did not.

**Parahippocampal region: Entorhinal, Parahippocampal and Perirhinal Cortices**

The parahippocampal region which consists of the entorhinal, perirhinal and parahippocampal cortices lies adjacent to the hippocampus. These regions are commonly
implicated in memory and emotion processing. Evidence from human neuroimaging and animal studies suggests a direct role of the hippocampus in the processing of nociceptive information such as pain intensity encoding. Areas within the parahippocampal complex have been more consistently activated in studies where pain perception is modulated by expectation and/or anxiety (Ploghaus et al., 2001). Based on this, it is possible, that a higher pain rating would correspond with individual being more anxious and thus cause an increased activation of this complex.

**Premotor and Prefrontal Cortex**

Thermal stimulation also produced activation of the premotor cortex and the prefrontal cortex. Even though these areas are not widely reported in pain processing studies in humans their role in inter-individual differences is certainly important. The premotor cortex has been known to play an essential role in spatial attentional processing and in encoding the locations of objects in peripersonal space (Coghill et al., 1999). Therefore the activation of premotor cortex in response to pain is not surprising given that increasingly painful stimuli becomes increasingly effective at attracting attention and thus inter-individual differences are also observed.

The role of PFC in pain is still not well-understood; it has been shown to exhibit activation that is positively related to perceived pain intensity in certain stimulation paradigms (Derbyshire et al., 1997). It is also thought to play important roles in working memory, affect, and attention (Coghill, McHaffie & Yen, 2003).
Brainstem and Spinal Cord Regions

A substantial body of evidence indicates that the spinal cord and brainstem regions activated more robustly in the highly sensitive individuals play important roles in the pain experience (Bowes, Stroman & Garcia, 2011; Cahill & Stroman, 2011; Lawrence, Stroman & Malisza, 2008). Cahill and Stroman (2011) showed that in within-subjects, spinal cord and brainstem regions have been shown to exhibit increasing activation as noxious stimulus intensities increase (Cahill & Stroman, 2011). Here, we provide a demonstration that these regions may also be critically important in processes leading to between-individual differences in pain sensitivity, when noxious stimulation remains the same. Although PAG, PBN, and dorsal horn all exhibit responses that are related to pain intensity, each region may make a differential contribution to various aspects of the pain experience.

The PAG and the dorsal horn of the spinal cord receive multiple inputs, which can be either excitatory and/or inhibitory, from the limbic system, the brainstem, and the periphery that influence their individual activity. Areas of activity in the midbrain have been shown to be positively correlated with pain ratings with lower innocuous temperature stimuli, and negatively correlated with ratings with higher noxious temperature (Stroman & Cahill, 2011). This shows that midbrain regions may have roles that are dependent on whether the stimulus is perceived as innocuous or noxious by an individual.

Similarly, brainstem sites, such as the parabrachial nucleus (PBN) appears to be an important site in the transmission of pain related information to midbrain dopaminergic neurons. It is involved in mediating pain relief and using many different neurotransmitters to either facilitate or inhibit activity of the neurons in the dorsal horn (Coizet et al., 2010)
Activity as well as individual differences observed in C6, C5, C4, C3, and C1 are definitely unexpected yet interesting. These areas primarily represent the neck and shoulder area. One explanation for this may be that upon experiencing thermal stimulation, it is not uncommon among participants to flex muscles in these areas depending on how strong or uncomfortable they may feel about the stimulus. This may results in neural activity being detected and thus inter-individual differences being observed.

4.4 Individual Differences in Pain May Result from Factors other than Pain Sensitivity

It is also important to note that a large portion of the variability of inter-individual differences in both the subjective experience of pain and activation of brain, brainstem and spinal cord regions is also likely to be attributed to factors other than differential sensitivity of spinal or peripheral afferent mechanisms. For example, expectations about a stimulus have been shown to have a marked impact on the subjective experience of pain in both brain and spinal fMRI studies (Fields, 1999; Atlas & Wager, 2012). Attention/distraction has also been shown to have a significant effect on the subjective experience of pain corresponded with altered neural activity in both brain and spinal cord (Sprenger et al., 2012; Bantick et al., 2002) Psychological factors, such as emotional manipulation of the experience of pain, have been shown to produce significant changes in the activity of PAG and cervical segments (Sprenger et al., 2012; Terry et al., 2013) as well as cortical regions such as insula and ACC (Rainville et al., 1997; Hofbauer et al., 2001).
4.5 Pain Intensity Information is an Important Component of Pain Experience

As evidenced through this study, there is a widespread distribution of pain intensity–related activation throughout the CNS (brain, brainstem and spinal cord). This suggests that these CNS regions are capable of processing pain intensity–related information, utilizing it and then combining it with other processes associated with cognitive evaluation of the features of a painful stimulus, to form a pain experience (Coghill, 1999). Therefore, it is important to note that pain intensity processing is an integral component of the many processes comprising the pain experience (Figure 4.1). These processes include cognitive evaluation of features of a painful stimulus (feature extraction), affect, motor control and attention. This organization provides the neurophysiological basis for the inter-individual differences in perceived pain intensity observed in this study.

The fact that one aspect of sensory processing (pain intensity) is so widely distributed across a multitude of distinct functional areas is in sharp contrast to other sensory systems e.g. the visual system. In the visual system, afferent information undergoes considerable changes as it is transmitted from one cerebral cortical area to the other. However, this wide distribution of sensory processing of pain intensity ensures that, in the event of critical and extensive CNS damage, the brain can detect tissue injury (Coghill, 1999). This shows that pain is one sensory experience that is absolutely necessary for our survival. Congenital insensitivity to pain is a rare condition in which individuals are born without the ability to perceive pain frequently and thus die from injuries and infections since they lack the ability to feel their pain (Wheeler et al., 2014).
4.6 Limitations

Despite our strong results there are several limitation associated with the study. Firstly, even though we received a wide range of responses from participants we would have liked to see more participants in the strong pain spectrum. The maximum pain rating was 64 which only correspond to “slightly strong pain” on the pain rating scale. More participants, especially in the 70-90, “strong” to “nearly intolerable pain” range would have helped in the correlations and made the results more interesting.

Secondly, even though the total participants were 20, if divided between males and females there were only 9 males and 11 females. This represents a small sample size and it may also be a reason that we did not see any sex/gender differences in the pain ratings in our
participants. Having a large sample size of both males and females separately would have been beneficial and may have shown gender as a possible factor in differences in pain responses.

Thirdly, recruitment for study participation resulted in very narrow types of participants. Participants were mostly Caucasian, white, university students mostly in their early and late twenties. This subset represents a population of very distinct psychosocial and age related differences. Since all of the participants were university students factors that may have played a role in pain responses such as tuition payments, work load from courses, and stress and anxiety levels from other sources are very unique to this population and thus cannot be applied to the general healthy population.

Lastly, and most importantly, our findings are based on pain ratings of experimental stimuli given to healthy individuals. Therefore, one should be cautious in generalizing these results to clinical settings where a variety of very different factors come into play. Just like the psychosocial factors of the university students mentioned previously, clinical cases represent their own distinct set of factors which may influence pain responses for example patients may be having financial problems such as insurance compensation or their eligibility for disability may be at stake etc. (Nielsen, 2005). It will always be challenging exercise to translate experimental findings to the clinical populations, let alone individual patients. However, while this may be true, our results suggest that individuals can use pain rating scales to rate pain and accurately capture their pain experience, if given proper instructions.

4.7 Significance & Future Directions

This study is the first to demonstrate the neural correlates of inter-individual variability in pain sensitivity in the entire CNS. Different pain experiences, measured through participant pain
ratings, are characterized by different patterns of cortical and spinal activation. In this study, we demonstrate that a noxious thermal stimulation of identical intensity can induce variable responses in both subjective reports of pain as well as in signal changes in various anatomical areas known to be involved in pain transmission and processing. While studies have demonstrated such individual differences in higher cortical structures, it is now evident from this study that these differences also exist in nociceptive processing in the lower neuroaxis as low as the spinal cord.

It is expected that this study will aid in understanding how pain-induced activity may be modified in persistent and chronic pain states (e.g. patients with fibromyalgia), since they are also characterized by substantial inter-individual variability. Subjective report of a patient’s experience is a reliable index of the magnitude of pain. As more evidence continues to accumulate such findings may transition into clinical settings and be used to tailor treatment based on each individual patient. This could minimize complications that occur from both over and under treatment and in the end, substantially improve pain therapy.

These findings provide a more complete picture of pain processing in healthy individuals and demonstrate the utility of fMRI and its clinical potential. Since we see these inter-individual differences in the spinal cord as well, this study will help future studies in providing a better understanding of abnormal sensory responses due to spinal cord injury. It would be interesting to know how the transmission of pain differs in patients with injuries at different levels of the spinal cord. Even though it is important to know the factors determining death or recovery from spinal cord injury in the hope that this may lead to better treatment strategies, understanding the neural causes underlying individual differences in pain as a result of injury may also be crucial to its prevention and treatment.
Chapter 5

Summary and Conclusions

This project is the first to investigate and demonstrate neural activity as well as inter-individual differences in CNS activity as a result of noxious thermal stimulation. We were able to detect differences in neural activity in individuals that rated the pain the highest and individuals that rated the pain really low. There were observable differences in neural activity in regions such as ACC, PCC, S1, insular cortex etc. in the brain and the PAG, PBN, C7 etc. in the brainstem and spinal cord. Individuals that rated the pain as high showed a corresponding higher BOLD percent signal changes in areas mentioned, contralateral to the stimulation.

The main conclusions from this project are:

1) Individual differences are of profound importance in the treatment of pain.

2) Subject ratings are essential for diagnosis and treatment of pain.

3) Normal healthy variation in our fMRI results is not attributable to errors/uncertainty.

4) Spinal fMRI is a reliable and sensitive technique that can detect changes in neuronal activity related to pain information transmission in the SC and brainstem.

5) Valuable in providing a better understanding of abnormal sensory responses due to spinal cord injury

6) Provides a more complete picture of pain processing in healthy individuals and demonstrates the reliability of fMRI and its clinical potential.

This study demonstrates that inter-individual differences in pain responses reflect true variability in the experience of pain. With this valuable insight, we may have a better understanding of the
alterations that occur during chronic, neuropathic pain. The eventual goal is to be able to use this information and translate these findings in clinical populations to create better treatment strategies.
REFERENCES


Appendix A: Recruitment Poster

VOLUNTEERS NEEDED!

This study involves Magnetic Resonance Imaging (MRI) to look at pain processing in the brainstem and spinal cord.

MRI uses only magnetic fields (no radiation), and so is not harmful to your body and you cannot feel it.

- We are looking for males and females with:
  - No history of head or eye injury involving metal objects; implanted electrical devices (such as cardiac pacemaker); implanted objects (artificial joints, metal staples).
  - Do not have non-removable jewelry
  - No history of heart disease or any other serious illness.
- 1.5 hour session. Compensation $20.
- Queen’s MRI Facility
- For more information, please contact Hamza: 7hk14@queensu.ca
Appendix B: MRI Safety Checklist

Centre for Neuroscience Studies

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.

Full Name: ___________________________ Date of Birth: ______/____/______
(print) Given / Middle / Family Weight: ______ Height: ______
Family Physician Name: ___________________________ Address or City: ______
(include first name/initial) of Physician

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. BE, bullet, shrapnel, etc.)? Yes No
Have you worked in a metal machine shop for an extended period of time? 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
Are you wearing clothing with silver or gold threading (e.g. liliumon silverescent)? 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 Cardiac pacemaker
- ____________ Yes No Implanted cardioverter defibrillator (ICD)
- ____________ Yes No Electronic implant or device
- ____________ Yes No Magnetically-activated implant or device
- ____________ Yes No Any type of prosthesis or implant
- ____________ Yes No Artificial or prostheto limb
- ____________ Yes No Any metallic fragment or foreign body
- ____________ Yes No Medication patch (Nicotine, Nitroglycerine)
- ____________ Yes No Tissue expander (e.g. Breast)
- ____________ Yes No Body piercing

IMPORTANT INSTRUCTIONS: Remove all metallic objects before entering the MR environment or magnet room including hearing aids, hearing aid, cochlear implants, hearing aids, eyeglasses, hearing aids, eyeglasses, spectacles, jewelry, watches, safety pins, paper clips, money clips, credit cards, bank cards, magnetic strip cards, coins, pins, 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: Volunteer Consent Form

I have read and understand the consent form for this study, entitled “From Research to Clinic: Translation of Functional MRI of the Human Spinal Cord (spinal fMRI)”. 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 understand that I may retain a copy of this consent form for my records.

If at any time I have further questions, problems or adverse events, I can contact

Dr. Patrick Stroman (Principal Investigator)
by e-mail at stromanp@queensu.ca or by phone at 613-533-3245
or
Dr Doug Munoz, Director, Centre for Neuroscience Studies
by e-mail at doug@eyeml.queensu.ca or by phone at 613-533-2111

If I have questions regarding my rights as a research participant I can contact
Dr. Albert Clark, Chair, Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board at 533-6081

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

________________________  _____________
Signature of Participant     Date

STATEMENT OF INVESTIGATOR:

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

________________________  _____________
Signature of Principal Investigator     Date