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

By

Jordan Kelly Leitch

A thesis submitted to the Centre for Neuroscience Studies
in conformity with the requirement for
the degree of Master of Science

Queen’s University
Kingston, Ontario, Canada
(July 2010)

Copyright © Jordan Kelly Leitch, 2010
Abstract

To date, most studies investigating the neural signature of pain in humans have focused on the brain, and those studies concerned with more caudal areas (such as the spinal cord (SC) or brainstem) have used only experimental models of pain. The objectives of this study were 1) to determine the neural activity in the human brainstem and SC that is caused by a noxious mechanical stimulus and 2) to compare the neural response to noxious stimuli in healthy controls and a patient population diagnosed with peripheral neuropathic pain. The SC and brainstem contain important synaptic points in several major pain pathways, and comparing the neural response between a control and patient population in these areas provides a more complete picture of healthy and pathological pain processing.

Functional MRI studies of the SC and brainstem were carried out in healthy control subjects and patients diagnosed with carpal tunnel syndrome (CTS) in a 3T Siemens Magnetom Trio. Subjects reported the point at which the pressure (in mmHg, applied to the wrist at the location of the median nerve) corresponded to a pain level of 2, 4, and 6 on a numerical 11 point pain scale. Sagittal image slices were selected to span from the C7/T1 interface to the superior edge of the thalamus and were analyzed using a general linear model to improve the discrimination between physiological motion and signal intensity changes arising from neural activity. Spatially normalized group results superimposed on anatomical templates in the axial orientation were visually identified using several stereotaxic atlases.

We observed consistent signal intensity change in areas implicated in the transmission and modulation of pain in both control and CTS groups. Both groups showed a similar decrease in signal change with increasing pain, as results at pain level 2 are predominantly positive signal change and at pain level 6 are typically negative. This may indicate a reduction in the tonic inhibition of painful sensations. Differences between groups were readily visible in regions anatomically consistent with the dorsal horn (DH) of the cervical SC, rostral ventromedial medulla (RVM), dorsolateral pontine tegmentum (DLPT), and midbrain periaqueductal gray (PAG). The anatomical variation in signal change between groups may represent, for the first time, a visualization of the functional difference between healthy and pathological pain processing in the SC and brainstem using spinal fMRI.
Acknowledgments

“Flatter me, and I may not believe you. Criticize me, and I may not like you. Ignore me, and I may not forgive you. Encourage me, and I may not forget you.” (William Arthur Ward)

Research is not for the faint of heart. You have to ready yourself for inexplicable results, international travel, nerve-wracking presentations, and more than a few all-nighters. Your confidence and drive will inevitably wane, mine surely did, and you should count yourself lucky to have someone there to support you when they do. Thank goodness I get to use page three of my thesis to thank everyone who listened to me, drank with me, and supported and encouraged me over the course of my graduate work.

I would not have accomplished any of what I have so far had it not been for the unwavering and unconditional support of my mom and dad, Terry and Andy. You are the first people I call when I am euphorically happy, completely miserable, or impossibly stumped, and you always know what to say to make me feel better or whip me into shape (whichever I need). Maybe even more than that, you are the reason I am who I am in every aspect of my life. I only hope I can impart upon my children the confidence, ambition and work ethic you have both instilled in me. Thank you for driving all night to rescue me, moving me (and all my stuff) four times, and always being there. You are just the best parents a girl could ask for, and I am so lucky to have you and I love you!

Alex, my little sister, I am so proud of you and all that you have accomplished. You are going to make a fabulous nurse, no doubt about it. Your humour and lightheartedness combined with a strong work ethic will make you a favourite among staff and patients alike. Good luck in your new position at the hospital, you’re going to be great. Don’t forget our great times as roommates, the late night chats, the road-trips home, the poutine, or the big blue couch. I know I won’t.

One of the many great things about research is being surrounded by brilliant, passionate people on a daily basis in the lab. Chase Figley, Rachael Bosma, Chris Kidd, Celina Nahanni, Natalie Kozyrev, and Randi Beazer, thank you for all the helpful input and good
times. You have all helped make the Stroman Lab my home for the last two years. Chase, what can I say? I feel like I really lucked out ending up in a lab you were a part of, because I’m sure my grad school experience would be incredibly different (in a bad way, no doubt) if we had never crossed paths. Thanks for all the talks, movies, and memories. Good luck at Johns Hopkins next year, and stay in touch!

I have met some really amazing people throughout my time at Queen’s that I am now fortunate to call my friends. My herdmates from my undergrad heyday, Jessie Gill, Lauren (Yukon) Trimble, and Hilary Dugan: you are all exceptional women. I cannot imagine my Queen’s experience without you and frankly I don’t want to. Thank you for too many fabulous memories to count! During grad school, I met some pretty exceptional people as well. Christine Cotie, Ryley Beddoe, Diala Habib, Tomek Banasikowski, Emily Hawkin, Apostolia Petropoulou and Evelyne Gentilcore-Saulnier, I am so glad to have met you and formed what I hope are lifelong friendships. You have all already accomplished so much and, knowing each of you, that is just the tip of the iceberg.

Thanks to the members of my advisory/defense committee, Dr. Michael Kawaja, Dr. Linda McLean, Dr. Angela Garcia, Dr. Khem Jhamandas and Dr. Steve Scott as well as Sharon David for all your helpful input and advice on my project. I’m also fortunate to be supervised by not one but two excellent researchers, Dr. Cathy Cahill and Dr. Patrick Stroman. Cathy, you were my initial contact, and I have you to thank for starting me on this path that has been so fruitful and enjoyable. Thank you for sharing your experience with me and helping me through important presentations, deadlines, and decisions, and for sending me to Stockholm! Also, thank you for introducing me to Pat, who, with his primary appointment in a field in which I am rather ill-equipped (physics) I would probably never have met. Pat, you have been a truly great supervisor, and I have learned so much from you (Self reliance is the key to a vigorous life!). Despite your favourite quote, you have always been there to help me, and I can’t tell you how much I appreciate every opportunity you have given me. From being first author on a review article to three international conferences (Honolulu, Las Vegas, and Stockholm), I have so much to thank you for. Since I will be calling Queen’s home for a few more years, I hope we can continue to collaborate on projects and show the world (or at the very least the vehement BOLD supporters) how valuable spinal fMRI can really be.
Table of Contents

Abstract................................................................................................................................. ii
Acknowledgements .................................................................................................................. iii
Table of Contents....................................................................................................................... v
List of Figures .......................................................................................................................... vii
List of Tables ........................................................................................................................... viii
List of Abbreviations................................................................................................................ ix

Chapter 1: Introduction .......................................................................................................... 1
  1.1 Pain Transmission ........................................................................................................... 3
    1.1.1 Nociceptors ............................................................................................................. 3
    1.1.2 Dorsal Horn ............................................................................................................ 4
    1.1.3 Ascending Pain Pathways ....................................................................................... 7
  1.2 Descending Modulation of Pain ...................................................................................... 11
  1.3 Neuropathic Pain .......................................................................................................... 15
    1.3.1 Allodynia .............................................................................................................. 16
    1.3.2 Hyperalgesia ......................................................................................................... 17
  1.4 Carpal Tunnel Syndrome ............................................................................................... 17
  1.5 Principles of MRI ........................................................................................................... 19
  1.6 FMRI Contrast Mechanisms .......................................................................................... 22
    1.6.1 Blood Oxygenation-Level Dependent (BOLD) .................................................... 23
    1.6.2 Signal Enhancement by Extravascular Water Protons (SEEP) .............................. 24
  1.7 Spinal fMRI .................................................................................................................. 25
    1.7.1 Methodological Developments ............................................................................. 26
    1.7.2 Applications in Clinical Populations ..................................................................... 30
  1.8 Using fMRI to Investigate Acute and Chronic Pain in Humans ................................... 35
    1.8.1 Brain ..................................................................................................................... 35
    1.8.2 Brainstem & Spinal Cord ....................................................................................... 37
  1.9 Proposed Research ........................................................................................................ 39
    1.9.1 Purpose ................................................................................................................ 39
    1.9.2 Rationale .............................................................................................................. 40
    1.9.3 Hypothesis ............................................................................................................ 40
    1.9.4 Objectives ............................................................................................................ 40

Chapter 2: Methods ............................................................................................................ 41
  2.1 Volunteer Recruitment .................................................................................................. 41
  2.2 Confirmation of Carpal Tunnel Syndrome and Neuropathic Pain ............................... 42
  2.3 Part I – Psychophysical Testing ..................................................................................... 45
  2.4 Part II – Imaging .......................................................................................................... 46
  2.5 FMRI Data Acquisition ............................................................................................... 47
  2.6 Data Analysis ................................................................................................................. 48
    2.6.2 Group Analysis ...................................................................................................... 49
    2.6.3 Correlation Analysis ............................................................................................. 51
  2.7 Statistical Analysis ....................................................................................................... 51
Chapter 3: Results ......................................................................................................................... 52
  3.1 Psychophysical Data .............................................................................................................. 52
  3.2 Group Results ....................................................................................................................... 54
  3.3 Regional Comparison of Signal Intensity Change ............................................................... 62

Chapter 4: Discussion ................................................................................................................... 70
  4.1 Principle Findings .................................................................................................................. 71
  4.2 In Light of the Literature ...................................................................................................... 71
    4.2.1 Transmission and Modulation of Pain: A Brief Refresher ............................................. 71
    4.2.2 Comparing SC and Brainstem Activation between Groups ........................................... 72
    4.2.3 A Conundrum ................................................................................................................. 83
  4.3 Interpretations ...................................................................................................................... 84
    4.3.1 The Neuromatrix: A Pattern-Generating Mechanism .................................................. 85
    4.3.2 Experimental versus Clinical Pain .................................................................................. 86
  4.4 Limitations ........................................................................................................................... 88
  4.5 Significance and Future Directions ...................................................................................... 91

Chapter 5: Summary ..................................................................................................................... 93

References ................................................................................................................................... 95

Appendix A: Recruitment Poster – Control ............................................................................ 113
Appendix B: Recruitment Poster – Patient .............................................................................. 114
Appendix C: Newspaper Advertisement .................................................................................... 115
Appendix D: MRI Safety Checklist ............................................................................................. 116
Appendix E: Volunteer Consent Form ......................................................................................... 117
Appendix F: Volunteer Details ..................................................................................................... 124
Appendix G: CTS Patient Information ......................................................................................... 125
Appendix H: Short Form McGill Pain Questionnaire ................................................................. 126
Appendix I: Pain Questionnaire Administration ........................................................................ 127
Appendix J: Psychophysical Administration & Results Table .................................................... 128
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Axial view of spinal cord segment</td>
<td>6</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Ascending pain pathways</td>
<td>10</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Descending modulation of nociception</td>
<td>14</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Positions for spatial normalization of the spinal cord</td>
<td>30</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Stimulation device</td>
<td>46</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Block Paradigm</td>
<td>47</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Results display of individual analysis</td>
<td>49</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Normalization for spinal cord and brainstem</td>
<td>50</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Overview of group results</td>
<td>56</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Group results for regions of interest</td>
<td>57</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>Group results in the cervical spinal cord</td>
<td>58</td>
</tr>
<tr>
<td>Figure 3.4</td>
<td>Group results in the rostral medulla</td>
<td>59</td>
</tr>
<tr>
<td>Figure 3.5</td>
<td>Group results in the pons</td>
<td>60</td>
</tr>
<tr>
<td>Figure 3.6</td>
<td>Group results in the midbrain</td>
<td>61</td>
</tr>
<tr>
<td>Figure 3.7</td>
<td>Comparison of mean signal intensity change in regions of interest</td>
<td>64</td>
</tr>
<tr>
<td>Figure 3.8</td>
<td>Signal intensity change in the ipsilateral dorsal horn</td>
<td>65</td>
</tr>
<tr>
<td>Figure 3.9</td>
<td>Signal intensity change in the contralateral dorsal horn</td>
<td>66</td>
</tr>
<tr>
<td>Figure 3.10</td>
<td>Signal intensity change in the RVM</td>
<td>67</td>
</tr>
<tr>
<td>Figure 3.11</td>
<td>Signal intensity change in the DLPT</td>
<td>68</td>
</tr>
<tr>
<td>Figure 3.12</td>
<td>Signal intensity change in the PAG</td>
<td>69</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Comparison of group results following equi-intense stimulation</td>
<td>81</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>The neuromatrix</td>
<td>86</td>
</tr>
</tbody>
</table>
List of Tables

Table 2.1 Confirmation of CTS and relevant background information ........................................ 43
Table 2.2 Patient results from pain questionnaires ................................................................. 45
Table 3.1 Individual results from psychophysical assessments ................................................. 53
Table 3.2 Group results from psychophysical assessments ...................................................... 54
Table 3.3 Summary of group results ....................................................................................... 62
Table 3.4 Summary of mean signal change in regions of interest ............................................ 63
Table 4.1 Mean pressure used to elicit pain response in subjects ............................................ 80
List of Abbreviations

5-HT  serotonin
ACC  anterior cingulated cortex
ASIA  American Spinal Injury Association
B0  uniform magnetic field (of magnet)
Bi  transverse magnetic field
BK  bradykinin
BOLD  Blood Oxygengation Level Dependent
CBP  chronic back pain
CGRP  calcitonin gene-related peptide
CL  central lateral
CN  cuneate nucleus
CNS  central nervous system
CSF  cerebrospinal fluid
CTS  carpal tunnel syndrome
DH  dorsal horn
DLPT  dorsolateral pontine tegmentum
fMRI  functional magnetic resonance imaging
GABA  [gamma]-aminobutyric acid
GE  gradient echo
GLM  general linear model
GM  gray matter
dGM  dorsal gray matter
vGM  ventral gray matter
GN  gracile nucleus
HASTE  half-Fourier single-shot fast spin-echo
IASP  International Association for the Study of Pain
IBS  irritable bowel syndrome
IC  insular cortex
LC  locus coeruleus
M0  net magnetization
MPQ  McGill Pain Questionnaire
MRI  magnetic resonance imaging
MS  multiple sclerosis
NCF  nucleus cuneiformis
NCS  nerve conduction study
NGc  nucleus gigantocellularis
NP  neuropathic pain
NPS  neuropathic pain scale
NRM  nucleus raphe magnus
ON  olivary nucleus
PAG  periaqueductal gray
PaIN  pain imaging group
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBN</td>
<td>parabrachial nucleus</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PNS</td>
<td>peripheral nervous system</td>
</tr>
<tr>
<td>PQAS</td>
<td>pain quality assessment scale</td>
</tr>
<tr>
<td>RESPITE</td>
<td>retrospective spinal cord motion time-course estimates</td>
</tr>
<tr>
<td>RF</td>
<td>reticular formation</td>
</tr>
<tr>
<td>RR</td>
<td>relapsing-remitting (MS)</td>
</tr>
<tr>
<td>RVM</td>
<td>rostral ventromedial medulla</td>
</tr>
<tr>
<td>S1</td>
<td>primary somatosensory cortex</td>
</tr>
<tr>
<td>S2</td>
<td>secondary somatosensory cortex</td>
</tr>
<tr>
<td>SC</td>
<td>spinal cord</td>
</tr>
<tr>
<td>SCI</td>
<td>spinal cord injury</td>
</tr>
<tr>
<td>SE</td>
<td>spin echo</td>
</tr>
<tr>
<td>SEEP</td>
<td>Signal Enhancement by Extravascular Protons</td>
</tr>
<tr>
<td>SF-MPQ</td>
<td>Short Form McGill Pain Questionnaire</td>
</tr>
<tr>
<td>SMT</td>
<td>spinomesencephalic tract</td>
</tr>
<tr>
<td>SP</td>
<td>substance p</td>
</tr>
<tr>
<td>SRT</td>
<td>spinoreticular tract</td>
</tr>
<tr>
<td>STT</td>
<td>spinothalamic tract</td>
</tr>
<tr>
<td>T</td>
<td>tesla</td>
</tr>
<tr>
<td>T1</td>
<td>longitudinal relaxation time</td>
</tr>
<tr>
<td>T2</td>
<td>transverse relaxation time</td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>TRPVI</td>
<td>transient receptor potential vanilloid I</td>
</tr>
<tr>
<td>VAS</td>
<td>visual analog scale</td>
</tr>
<tr>
<td>VH</td>
<td>ventral horn</td>
</tr>
<tr>
<td>VPI</td>
<td>ventral posterior inferior</td>
</tr>
<tr>
<td>VPLc</td>
<td>caudal ventral posterior lateral nucleus</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
<tr>
<td>WM</td>
<td>white matter</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

Pain is a complex, subjective experience, comprised of both sensory-discriminative and motivational-affective components which combine to produce a sensation that is unique among individuals (Kenshalo and Willis 1991; Millan 1999). Broadly, pain can be classified as either acute or chronic. Acute pain is adaptive, generally of short duration and identifiable cause, and is focal to the site of injury (Gupta and Raja 1996; IASP Subcommittee on Taxonomy 1979; Millan 1999). Acute pain functions as a symptom: it is self-limited, generally responds to treatment, and has a good to excellent prognosis (Gupta and Raja 1996). Conversely, chronic pain is maladaptive, debilitating, unrelenting, and often spreads beyond the original site of injury (Gupta and Raja 1996; IASP Subcommittee on Taxonomy 1979; Millan 1999). Chronic pain has the characteristics of a disease state: it can produce psychological disturbances, requires complex treatment strategies, and typically has a poor prognosis (Gupta and Raja 1996). Neuropathic pain (NP) is a subtype of chronic pain, caused by trauma or lesion to the nervous system (Kenshalo and Willis 1991). NP is characterized by paresthesia, as well both spontaneous and stimulus-evoked pain (such as allodynia or hyperalgesia) (Costigan et al. 2009).

Functional magnetic resonance imaging (fMRI) has enabled the non-invasive study of many neural mechanisms in humans, and has yielded insight into the differences between acute and chronic pain. For instance, fMRI was used to show that chronic pain results in more frontal lobe activity than does acute pain (Apkarian et al. 2005). Price and colleagues used fMRI to demonstrate the brain areas that are involved in the affective component of
pain, the so-called “pain matrix” (Price 2000), such as the anterior cingulate cortex (ACC), insular cortex (IC), somatosensory cortices, and thalamus (Tracey 2005). While the brain is undoubtedly an important integrative site in the pain pathway, the spinal cord (SC) and brainstem are comprised of synaptic sites through which nociceptive information is processed prior to higher cortical involvement. Despite this, few fMRI studies have investigated pain transmission caudal to the cortex, and almost no studies have utilized a clinical chronic pain population. A recent study by Ghazni and colleagues investigated the SC and brainstem activity in response to a painful stimulus in healthy control subjects following capsaicin sensitization, and found that the fMRI response to pain was more localized following sensitization, even when the self-reported pain intensity was equal (Ghazni et al. 2009).

Results from these studies suggest a functional difference in the way in which chronic pain is processed compared to healthy, acute pain. Furthermore, this difference may be a result of a series of changes within the nociceptive system, an expression of the maladaptive plasticity which constitutes the neural disease state (Costigan et al. 2009). While sensitization models in healthy controls are useful tools with which to study nociception, they cannot serve as a surrogate for clinical chronic pain. Using fMRI to investigate a clinical population suffering chronic, NP provides the opportunity to assess the neural plasticity postulated to occur in actual chronic pain states. Moreover, by examining the fMRI response to pain in the human SC and brainstem, we can develop a more complete picture of nociceptive transmission that includes primary synaptic points caudal to the brain.
1.1 Pain Transmission

1.1.1 Nociceptors

Sensory receptors occur throughout the body (with the exception of the brain and AC) and are activated by a wide variety of stimuli: thermoreceptors respond to temperature, chemoreceptors to chemicals, and mechanoreceptors to mechanical stress. However, when the stimulus involves actual or potential tissue damage, nociceptors are activated and the pain response is initiated (Willis and Westlund 1997). Nociceptors have been described in most structures in the body that give rise to a pain response, such as skin, muscle, joints, and viscera (Willis and Westlund 1997), and the quality of pain that results from nociceptor activation depends upon a) the type of nociceptor and fibre (Aδ or C) stimulated and b) the type of tissue innervated (Konietzny et al. 1981; Ochoa and Torebjork 1989). Aδ fibre afferents are small, myelinated axons which transmit the sensation of sharp, localized pain relatively quickly. C fibre afferents are very small, unmyelinated axons which transmit the sensation of dull, diffuse pain more slowly (Meyer et al. 2006). Thus, activation of cutaneous Aδ nociceptors leads to a pricking pain, whereas activation of cutaneous C nociceptors leads to a dull, burning pain (Konietzny et al. 1981; Ochoa and Torebjork 1989).

Not all nociceptors are constitutively active, as many, and possibly most, are “silent” and rather unresponsive under normal circumstances (Schaible and Schmidt 1983a; Schaible and Schmidt 1983b). These silent receptors can be activated via sensitization due to second-messenger systems by the release of inflammatory mediators from surrounding tissues such as bradykinin (BK), prostoglandins, serotonin (5-HT), and histamine (Dray et al. 1988; Schepelmann et al. 1992). After sensitization, nociceptors will spontaneously discharge and
are more sensitive to peripheral stimulation (Schaible and Schmidt 1985; Schaible and Schmidt 1988). Primary hyperalgesia, an exaggerated response to a painful stimulus, is thought to be a consequence of sensitization of nociceptors following inflammation (Meyer and Campbell 1981).

The activity of nociceptors can be controlled not only by mechanical, thermal, or chemical stimuli with specific features and intensity, but also modulated by the activation of pharmacological receptors on the cell surface membrane. Primary afferents typically express several types of receptors, including opiate, \(\text{gamma}\)-aminobutyric acid (GABA), BK, histamine, 5-HT, and capsaicin (TRPV1) receptors, each modulating the nociceptive response upon activation (Dray 1994). Opiate receptor activation results in analgesia, diminishing the sensation of pain (Machelska and Stein 2000). GABA\(_A\) receptors appear to have a bimodal effect. In animal studies, low concentrations of GABA\(_A\) agonists attenuate pain while high concentrations enhance pain-behaviours (Carlton \textit{et al.} 1999). BK (Couture \textit{et al.} 2001), histamine (Simone \textit{et al.} 1991), and 5-HT (Lang \textit{et al.} 1990) receptor activation all potentiate nociception. Similarly, following sensitization by BK, TRPV1 receptors exhibit decreased threshold and contribute to hyperalgesia (Caterina \textit{et al.} 2000).

1.1.2 Dorsal Horn

The dorsal horn (DH) of the spinal cord is the first central synaptic point in all pain pathways, and is comprised of four major components:

- The central terminals of primary afferent axons
- Intrinsic neurons (Interneurons) – axons that remain in the spinal cord and terminate locally or in other segments
• Projection neurons – axons that project rostrally in the white matter to various areas in the brain

• Descending axons – axons that project caudally from various brain regions to the spinal cord and contribute to the modulation of nociceptive sensory information.

Nociceptive primary afferent axons terminate (almost exclusively) in the dorsal horn, forming the first synapse in all ascending pathways which convey sensory information to the brain (Mehler et al. 1960). The dorsal horn is divided into six parallel laminae (of Rexed) based on differences in the size and packing density of neurons (Figure 1). Lamina I and II, jointly referred to as the superficial dorsal horn, comprise the main target for nociceptive primary afferents. Lamina I, the marginal layer, contains 5% projection neurons (the highest in the dorsal horn) and 95% interneurons (Spike et al. 2003). Lamina II is dubbed the substantia gelatinosa because the absence of myelinated fibres gives it a translucent appearance when unstained. Densely packed, small interneurons comprise virtually the entire substantia gelatinosa (Spike et al. 2003). Lamina V is also associated with the transmission of nociception, as some Aδ (Dubner and Bennett 1983) and C (Lorenzo et al. 2008) fibres appear to terminate in this layer.
Figure 1.1. Axial view of spinal cord segment, including the laminae of Rexed. Adapted from Blumenfeld, H. pp 217 Figure 6.3 (Blumenfeld 2002).

Sensitization of neurons in the dorsal horn, and subsequent transmission of nociceptive information, is attributed to the release of excitatory amino acids (ie. Glutamate and aspartate) and peptides (ie. Substance P (SP) and Calcitonin Gene-Related Peptide (CGRP)) from primary afferent terminals (Dougherty et al. 1992; Dougherty et al. 1993; Dougherty et al. 1994; Dougherty et al. 1995). The primary afferent fibres synapse either directly or indirectly (through interneurons) with projection neurons to transmit the nociceptive information rostrally to the brainstem and cortex via ascending tracts (Willis 1985).
1.1.3 Ascending Pain Pathways

Ascending tracts are composed of the axons of projection neurons that transmit nociceptive information rostrally to various supraspinal structures in the brainstem and diencephalon (Willis 1985). Projection neurons synapse with subsequent neurons within the supraspinal target structures of the ascending pathways which transmit the nociceptive signal to cortical (higher cognitive) and limbic (emotional) structures where the signal is interpreted as pain (Millan 1999). There are three major ascending tracts associated with transmitting nociceptive information:

- Spinothalamic Tract
- Spinomesencephalic Tract
- Spinoreticular Tracts

Spinothalamic Tract

The spinothalamic tract (STT) (Figure 2) mediates the sensations of cold, warmth, touch, and pain (Willis 1985; Willis and Westlund 1997). A large portion of STT cells originate in lamina I and V, however, some cells are located in other lamina (IV, VI, and X) and in the ventral horn (VH) (Apkarian and Hodge 1989; Willis et al. 1979). There are two distinct subpopulations of STT cells: one that projects to the lateral thalamus, and one that projects to the medial thalamus (Willis et al. 1979). The lateral projecting STT neurons originate in lamina I and V, cross midline in the ventral gray commissure at a level near the origin cell bodies, ascend in the ventral, then lateral funiculus, and finally pass through the brainstem to synapse in the lateral thalamus. The nuclei of termination include the caudal ventral posterior lateral nucleus (VPLc) and the ventral posterior inferior nucleus (VPI).
Some even project medially to the central lateral (CL) (Willis et al. 1979) nucleus. In contrast, the medial projecting STT neurons originate in the deep DH (lamina VI) and the VH, cross midline in the ventral white commissure and ascend in the ventral and ventrolateral white matter (as above), and finally pass through the brainstem to synapse in the medial thalamus, specifically the CL nucleus (Willis et al. 1979).

**Spinomesencephalic Tract**

The spinomesencephalic tract (SMT) (Figure 2) is comprised of several projection pathways that terminate in different areas of the midbrain. The cells of the SMT originate in lamina I, IV-VI, and X (Trevino 1976; Willis et al. 1979), decussate in the ventral white commissure, and ascend to the midbrain in the lateral funiculus (Trevino 1976; Willis et al. 1979). In the midbrain, the SMT terminates in the periaqueductal gray (PAG), cuneiform nucleus (NCF), intercolliculus nucleus, deep layers of the superior colliculus, nucleus of Darkschewitsch, pretectal nuclei, red nucleus, Edinger-Westphal nucleus, and interstitial nucleus of Cajal (Mehler et al. 1960).

SMT neurons are nociceptive, and respond best to noxious and only somewhat to innocuous stimuli (Willis and Westlund 1997). Due to the diverse nuclei in which the SMT neurons terminate, the different components have many functions. In particular, the projections to the PAG and pretectal nuclei (both of which produce analgesia when stimulated) implicate the SMT as one of several mechanisms which modulate nociception, in addition to the role of transmitting nociceptive information to supraspinal structures (Rees and Roberts 1993).
Spinoreticular Tracts

The spinoreticular tracts (SRTs) (Figure 2) have also been shown to convey nociceptive information (Millan 1999; Willis 1985). SRT neurons originate in lamina VII and VIII (in the ventral horn), the axons decussate in the ventral white commissure, and ascend in the lateral funiculus and brainstem with the STT (Mehler et al. 1960). The projections terminate in the reticular formation of the medulla and pons, in particular in the nucleus gigantocellularis (NGc), the lateral reticular nucleus, and the lateral parabrachial nucleus (PBN) (Cechetto et al. 1985; Hylden et al. 1985). An additional SRT originates in lamina I and V, decussates in the ventral gray commissure, ascends in the lateral funiculus, and projects through the brainstem to terminate in the ventral subcoeruleus, Kölliker-Fuse and dorsal PBN, locus coeruleus (LC) in the pons (Craig 1995).

Both parabrachial and reticular formation nuclei are comprised of cells which contain an assortment of immunoreactive peptides associated with the pain response (ie. SP) (Leah et al. 1988), and reticular cells appear to respond preferentially to noxious stimuli (Besson and Chaouch 1987; Willis 1982).
Figure 1.2. Ascending Pain Pathways. The spinothalamic (STT, top), spinomesencephalic (SMT, bottom left) and spinoreticular (SRT, bottom right) tracts, with relevant nuclei labeled. Axial brainstem sections presented in anatomical orientation. Adapted from Blumenfeld, H. pp 227 Figure 6.11 (Blumenfeld 2002). Abbreviations: Superior colliculus (SC), periaqueductal gray (PAG), nucleus cuneiformis (NCF), locus coeruleus (LC), and nucleus gigantocellularis (NGc).
1.2 Descending Modulation of Pain

The brainstem contains critical components of a network which modulates pain, with major synaptic relay points in the PAG and rostral ventromedial medulla (RVM, comprised of the nucleus raphe magnus (NRM) and the reticular formation in the medulla). This network is bidirectional, using separate pro- and anti-nociceptive outputs from the RVM to control dorsal horn nociceptive transmission. The PAG-RVM system can be reliably recruited to suppress the response to noxious transmission both following pharmacological intervention (as the primary site of action of opioid analgesics) or in circumstances wherein survival depends upon ignorance of pain (commonly experienced by soldiers in battle who have been known to ignore a serious injury until they are out of immediate danger). Conversely, the system can also enhance the nociceptive response in various conditions, such as prolonged noxious stimulation, inflammation, nerve injury, and acute opioid withdrawal, presumably to promote recuperative behaviour and healing (Fields et al. 2006). In animals, higher-order factors such as fear, attention, and expectancy regulate pain-processing via links between the hypothalamus, amygdala, ACC, and interior insula with the PAG-RVM system (Watkins et al. 1998; Watkins and Mayer 1982). Recent functional imaging studies show strong evidence that expectancy (Fairhurst et al. 2007) and attention (Stroman et al. 2009; Tracey et al. 2002) also modulate the pain response in humans.

Mayer and Price (1976) provided the first evidence that the descending system can selectively cause analgesia. They found that electrical stimulation of the PAG in rats eliminated typical behavioural responses to pain, such as orientation, vocalization and escape, but left responses to innocuous environmental stimuli intact (Mayer and Price 1976).
Stimulation-produced analgesia has also been successfully performed in human patients with chronic pain by likewise electrically stimulating the PAG (Baskin et al. 1986), though this procedure is rarely performed in humans at the present time.

PAG stimulation selectively inhibits nociceptive neurons in the DH of the SC, but this effect is indirect. PAG excitatory projections synapse in the RVM and dorsolateral pontine tegmentum (DLPT, comprised of the LC, subcoeruleus, Kölliker-Fuse nuclei) which in turn send inhibitory projections to synapse in the DH (Figure 3) (Fields et al. 1991). Electrostimulation or microinjection of excitatory amino acids (glutamate or aspartate) in the RVM or DLPT inhibit DH neural responses to noxious stimulation and results in analgesia (Beitz 1982). These observations of robust stimulation-produced analgesia from the PAG and RVM led to the view that the system was purely analgesic, but a mounting body of evidence suggests this system also facilitates nociception, contributing to hyperalgesic states associated with inflammation, nerve injury, and opioid dependence. It appears that the PAG-RVM system exerts true bidirectional control of nociceptive processing.

An early example of descending facilitation of nociception showed an enhanced pain response in the rat (tail flick reflex) following low-intensity electrical stimulation of the RVM (Gerhart et al. 1981). Subsequent studies have implicated the PAG-RVM system in inflammatory and neuropathic pain models of hyperalgesia and allodynia (Heinricher and Neubert 2004). The collective observations that the PAG-RVM system can enhance pain in some, but not all, models emphasizes the need to determine the mechanism by which nociception is inhibited or enhanced, and which conditions recruit each distinct function (Fields et al. 2006). Gebhart’s study suggests that there are parallel inhibitory and facilitatory
output pathways from the RVM to the spinal cord (activated by high and low intensity electrical stimulation, respectively), and consequently that these pathways may be characterized by specific subtypes of RVM neurons and associated circuit connections. (Fields et al. 2006; Gerhart et al. 1981)

There are three distinct neuronal populations in the RVM. “On” cells discharge just prior to withdrawal from noxious heat, “off” cells stop discharging just prior to a withdrawal reflex, and “neutral” cells showing no consistent changes in activity when withdrawal reflexes occur (Fields et al. 1991; Fields and Heinricher 1985). “On” and “off” cells both project to lamina I, II, and V of the dorsal horn of the spinal cord and respond to manipulations of the PAG (Fields and Heinricher 1985). “Off” cells exhibit a net inhibitory effect, and activation of this subtype produces behavioural antinociception (Heinricher et al. 1994). In contrast, “on” cells exhibit a net facilitatory effect, and selective activation of this subtype is associated with an enhanced pain response in some models (Bederson et al. 1990) and produces hyperalgesia in rats (Neubert et al. 2004). Interestingly, PAG and DLPT neurons can be divided into the same three classes (Haws et al. 1989), pointing to a common neural mechanism for pain modulation.
Figure 1.3. Descending modulation of nociception. Regions in the frontal lobe and amygdala project to the PAG, which in turn controls spinal nociceptive neurons through relays in the RVM and DLPT. The RVM exerts bidirectional control over nociception, and has been shown to have pro- (green) and anti- (red) nociceptive circuitry. Adapted from Wall and Melzack’s Textbook of Pain (Fields et al. 2006). Abbreviations: Periaqueductal gray (PAG), dorsolateral pontine tegmentum (DLPT), and rostral ventromedial medulla (RVM).
1.3 Neuropathic Pain

Neuropathic pain is a subtype of chronic pain that is caused by trauma or lesion to the nervous system (Merskey and Bogduk 1994). It is especially challenging clinically because it is generally a) severe and resistant to over-the-counter analgesics (Boulton et al. 1983), b) it is often experienced in parts of the body that appear normal, and c) it is further aggravated by allodynia (touch-evoked pain) (Fields et al. 1998).

Neuropathic pain is caused by various central and peripheral nerve disorders. Peripheral neuropathic pain results from lesions to the peripheral nervous system (PNS) caused by mechanical trauma, metabolic disease, neurotoxic chemicals, infection, or tumour invasion, involving pathophysiological changes in both the PNS and the central nervous system (CNS) (Dworkin et al. 2003; Woolf and Mannion 1999). Central neuropathic pain results from lesions to the CNS, most commonly due to spinal cord injury (SCI), stroke, or multiple sclerosis (MS) (Ducreux et al. 2006).

Neuropathic conditions are characterized by nerve dysfunction, resulting in numbness, weakness, loss of deep tendon reflexes, and symptoms of spontaneous and stimulus-evoked pain. Spontaneous pain is typically described as burning, shooting, or shock-like (Masson et al. 1989; Turk 2002), while temporary, intermittent, or long-term varieties of stimulus-evoked pain are typically manifested as allodynia or hyperalgesia (Woolf and Mannion 1999) (described below). The symptoms associated with neuropathic pain are postulated to be a manifestation of maladaptive plasticity that the nociceptive system undergoes following lesion (Costigan et al. 2009).
Neuropathic pain affects up to 5% of the population (Bouhassira et al. 2008). It is extremely costly to the health care system and utterly devastating to the individuals who experience it. Patients describe their pain using descriptors from the McGill Pain Questionnaire (Melzack 1975) such as “punishing-cruel” and “tiring-exhausting” (Masson et al. 1989). Emotional consequences of neuropathic pain parallel those of chronic pain, and of the estimated $150 billion per year spent on costs associated with chronic pain, $40 billion is attributed to the neuropathic variety (Turk 2002).

### 1.3.1 Allodynia

Allodynia is a noxious (painful) response to a normally innocuous (non-painful) stimulus (such as cotton brushed against the skin) that can be thermal or mechanical in variety, the latter of which can be further divided into two subtypes, static or dynamic (Ochoa and Yarnitsky 1993). These subtypes are classified according to the type of innocuous stimulus that elicits the response. Static allodynia results in pain in response to a light touch or pressure stimulus (LoPinto et al. 2006), and is thought to be signaled by Aδ fibres and mediated through central sensitization, but could potentially involve C-fibre activity (Field et al. 1999). Dynamic allodynia yields pain in response to a brush stimulus (LoPinto et al. 2006), and is generally considered to be mediated by Aβ fibre activation, as selective blockage of these fibres eliminates dynamic, but not static allodynia (Ochoa and Yarnitsky 1993).
1.3.2 Hyperalgesia

Hyperalgesia, an exaggerated pain response to a normally noxious stimulus (such as heat applied to a sunburn), can be broadly divided into primary and secondary hyperalgesia. Similar to allodynia, hyperalgesia may be further classified into subgroups on the basis of either mechanical, thermal, or chemical modality (Woolf and Mannion 1999). In general, primary hyperalgesia is thought to be due to altered peripheral nociception, whereas secondary hyperalgesia is centrally mediated (Lindblom and Hansson 1991; Ochoa and Yarnitsky 1993), and various mechanisms have been proposed to account for the heightened painful response. Abnormal processing of nociceptive input (Woolf and Mannion 1999), spontaneous activity of low threshold touch-pressure mechanoreceptors with large afferents (Magerl et al. 1998), increased sensitivity of Aδ and C-fibre tracts (Meyer et al. 2006), abnormal CNS modulation (Woolf and Mannion 1999), as well as spontaneous activation of sensitized primary nociceptors (Ochoa and Yarnitsky 1993) are all plausible mechanisms by which hyperalgesia may occur.

1.4 Carpal Tunnel Syndrome

Carpal tunnel syndrome (CTS) is the most commonly diagnosed entrapment neuropathy, with an incidence of approximately 10-20%, depending on the population studied (Atrosi et al. 1999; Mondelli et al. 2002). CTS is caused by the idiopathic compression of the median nerve in the carpal tunnel, which is formed by the carpal bones on the dorsal, medial, and lateral sides and by the deep transverse carpal ligaments on the ventral side of the wrist (Kuhlman and Hennessey 1997). Though the etiology is often
unknown, predisposing conditions such as diabetes mellitus, hypothyroidism, trauma, or collagen vascular diseases are present in 15-20% of patients, and may contribute to the condition (Stevens et al. 1999; Tay et al. 2006). CTS is characterized by pain and paraesthesia (sensations of tingling, prickling, and numbness) in the first three digits (thumb, index and middle fingers) of the affected (and typically the dominant) hand, often exacerbated by use of the hand or at night during sleep (Bland 2001; Kuhlman and Hennessey 1997).

The diagnostic signs of CTS include sensory loss along the lateral side of the hand, motor weakness, and wasting of the abductor pollicis brevis (APB) muscle, as well as Tinel’s and Phalen’s sign. Tinel’s sign is a tingling sensation which radiates into the first three digits following light tapping of the median nerve at the wrist, while a positive Phalen’s (wrist-flexion) test results in paraesthesia in the first three digits following 30 to 60 seconds of complete wrist flexion. Wrist flexion causes the median nerve to become more compressed in the carpal tunnel, thus exaggerating the symptoms of CTS (Phalen 1970). Nerve conduction study (NCS) is used to distinguish between mild, moderate, and severe CTS based on median nerve motor and sensory latency (Bland 2001). Mild and moderate CTS are typically managed conservatively with wrist splinting (to minimize wrist flexion, particularly at night) (American Academy of Neurology 2006), physical and occupational therapy (Gard 2005), and over-the-counter anti-inflammatory medications such as aspirin and ibuprofen (Sato et al. 2005).

When symptoms become constant, severe CTS can be treated using carpal tunnel release surgery, in which the goal is to sever the transverse carpal ligament to relieve the
pressure in the carpal tunnel (Hui et al. 2004). Surgery is highly effective in patients with properly diagnosed CTS, with 90% able to return to their jobs following the procedure (Kouyoumdjian et al. 2003). CTS can result in neuropathic pain, as diagnosed by The Neuropathic Pain Scale (NPS) (Galer and Jensen 1997), supplemented by the Pain Quality Assessment Scale (PQAS) (Jensen et al. 2006) and the McGill Pain Questionnaire (MPQ) (Melzack 1975). In particular, paresthesia is implicated as a critical component of neuropathic pain (Bouhassira et al. 2004; Bouhassira et al. 2005) as well as a principle symptom of CTS.

1.5 Principles of MRI

Magnetic resonance imaging (MRI) detects the magnetization produced by hydrogen nuclei within lipids and water in the body when in a strong magnetic field. Additional magnetic fields are applied to vary the strength of the magnetic field with respect to position, thus providing a means from which to select specific areas of the body to excite and measure signal. MRI scanners employ changing magnetic field gradients and oscillating electromagnetic fields to adjust the properties of hydrogen nuclei, thus differentiating various tissue types based on the density and environment of hydrogen nuclei of the tissues in question (Huettel et al. 2004).

The keystone of every MRI method is the single proton comprising the nucleus of the hydrogen atom, the source of the magnetic resonance signal. The hydrogen atom is found in great abundance in the body, primarily in water and lipids (both key components of neural tissue). Hydrogen nuclei also have magnetic properties. In particular, the hydrogen nucleus
has two properties that are critical to MR imaging methods: 1) it is magnetic, with a north and south pole, and 2) it spins on its axis. In the normal physical state, hydrogen nuclei are oriented randomly in such a way that the individual magnetic moments sum to cancel each other out. However, when a collection of individual hydrogen nuclei are placed inside a strong magnetic field ($B_0$), such as when a volunteer is placed inside the MRI system, the nuclei will align with the magnetic field. This produces a net magnetization which is parallel to the magnetic field of the MRI system, and designated $M_0$ (Stroman 2010b).

To detect an MR signal from the tissue in question, a radiofrequency (RF) pulse is applied to tip $M_0$ away from equilibrium. An RF pulse is the brief application of a small magnetic field ($B_1$), applied perpendicular to $B_0$. The angle to which the magnetization is tipped is called the “flip angle”, so while the rotating magnetization induces the electrical signal in a receiver coil, the magnitude of the “flip angle” determines the strength of the MR signal that can be obtained (in addition to the magnitude of the magnetization) (Stroman 2010b).

After the magnetization has been disturbed from resting state, it must return to equilibrium. This process is called “relaxation”, and occurs as a result of magnetic interaction amongst hydrogen nuclei which causes them to lose energy. Importantly, different tissue types relax at different rates, providing the basis for tissue contrast in MRI. There are two components of relaxation: 1) Longitudinal, the recovery of the magnetization parallel to $B_0$, characterized by time $T_1$ and 2) Transverse, the decay of the transverse component of the magnetization to zero, characterized by time $T_2$. In a $T_1$-weighted scan, the free water in cerebrospinal fluid (CSF) relaxes more slowly and results in a long $T_1$, thus appears dark in the
images. Lipids, a main constituent of white matter (WM), relax quickly and result in a short $T_1$, appearing light in the images. Gray matter (GM) has an intermediate duration of relaxation, and appears darker than WM, but lighter than CSF. In a $T_2$-weighted scan, which measures the decay of the transverse component of magnetization due to accumulated phase differences caused by spin-spin interactions, CSF (free water) appears bright and WM (lipids) appears darker (Stroman 2010b). A $T_2^*$-weighted scan measures the transverse magnetization decay due to both accumulated phase differences and local magnetic field inhomogeneities, and is the basis for BOLD-fMRI (Huettel et al. 2004; Stroman 2010a). Perhaps the simplest form of MR contrast is proton-density imaging, as it provides contrast based on the sheer number of protons within a voxel, which varies with tissue type (Huettel et al. 2004).

Finally, there are two fundamental imaging methods used in MRI; the “spin-echo” (SE) and the “gradient-echo” (GE). The imaging method selected (SE or GE) will determine the overall image appearance and how well anatomical features or neural functions are contrasted from their surroundings. Immediately after the RF pulse, the magnetizations of all nuclei are oriented in the same direction, producing the greatest MR signal. This signal decays exponentially due to transverse relaxation and dephasing caused by spatial variations in the static magnetic field and magnetic field gradients. The signal “echo” is the return of the MR signal after it has decayed, produced by the temporary reversal of the dephasing, and providing more time to measure the MR signal before it decays to zero. A SE is produced by inverting the initial 90° RF with a 180° pulse to generate the MR signal “echo”. Incidentally, this cancels out the dephasing caused by static spatial field variations, and SE is $T_2$-weighted as a result. A GE is produced by applying a magnetic field gradient after the RF pulse then
reversing it, causing the two gradients to cancel out and generating an “echo”. Because the
dephasing by the static spatial field variations is not cancelled along with the gradient, GE is
T₁*-weighted (Stroman 2010c).

1.6 **FMRI Contrast Mechanisms**

Functional MRI (fMRI) is a particular type of MRI method which noninvasively
detects neural function. By imaging the brain, brainstem, or spinal cord at various states of
neural function, we can infer that any regions of the CNS that changed are likely to be
involved with the function that was varied between states. In a typical study, MR images are
repetitively acquired over a time series during which the subject performs a motor, sensory,
or cognitive task which, theoretically, systematically varies their neural function and reliably
 corresponds to the different images acquired over the course of the study. Differences
between healthy and injured or diseased neural states can also be detected using fMRI in
patient populations, such as Parkinson’s Disease (Cameron et al. 2009), Multiple Sclerosis
(MS) (Agosta et al. 2008b; Agosta et al. 2008a), spinal cord injury (SCI) (Stroman et al.
2002b), or neuropathic pain (Moisset and Bouhassira 2007), to name only a few. Perhaps the
most significant challenge for fMRI is separating the measured changes due to neural function
from those caused by random noise, physiological motion, or subject movement. Another
important consideration is that fMRI does not measure neural function directly, but rather
detects indirect physiological correlates of neural activity. Blood oxygenation-level
dependent (BOLD) fMRI is the dominant contrast mechanism, especially in studies
investigating neural function in the brain. Several years following the advent of BOLD-fMRI,
signal enhancement by extravascular water protons (SEEP) fMRI contrast was found to be particularly useful when measuring neural activity in the brainstem and spinal cord (SC) (Figley et al. 2010).

1.6.1 Blood Oxygenation-Level Dependent (BOLD)

BOLD contrast occurs from a combination of three effects. One, the magnetic field around individual red blood cells and in blood vessels (in particular the capillaries) can be manipulated depending on the amount of oxygen in the blood. Oxy-hemoglobin is diamagnetic (very weak magnetic effect), whereas deoxy-hemoglobin is paramagnetic (stronger magnetic effect), and the latter affects local relaxation times. Two, the magnetic field distortion caused by a magnetic field gradient between the inside and the outside of the blood vessels alters relaxation times, especially $T_2$ and $T_2^*$. Three, when the neuronal firing rate increases, the rate of oxygen consumption likewise increases due to additional energy demands, resulting in a counter-intuitive increase in local blood-oxygenation. Since the increase in oxygen consumption occurs simultaneously to the increase in local blood flow, the increase in oxygen delivery exceeds the increase in oxygen demand, resulting in a net change of increased blood oxygen-level at sites of increased neural activity. Therefore, the signal intensity detected in $T_2$- or $T_2^*$-weighted MR images is increased in regions with increased metabolic demand, which is an indirect indication of neural activity. It has been shown that changes in energy requirements and oxygen consumption is closely linked to pre-synaptic input, both excitatory and inhibitory, which should be considered when interpreting signal intensity change in a given location (Stroman 2010a).
1.6.2 **Signal Enhancement by Extravascular Water Protons (SEEP)**

SEEP is an alternative contrast mechanism to BOLD which has proven particularly useful in fMRI of the spinal cord (spinal fMRI) (Figley et al. 2010). The SEEP contrast mechanism was discovered during several studies which demonstrated that while the BOLD effect does occur in the SC, an additional process contributes to the MR signal change that could not be attributed to a change in relaxation times. SEEP contrast is the result of activity-dependent changes in tissue water content due to swelling of neurons and glial cells. Upon neuronal activation, the concentration of potassium ions in the extracellular space increases as a result of discharging neurons releasing potassium. The potassium ions are subsequently taken up by astrocytes via potassium channels, with water following (Andrew et al. 2007; Andrew and MacVicar 1994; Fujita et al. 1997; Ohta et al. 1996; Stroman et al. 2008b). The extracellular/intracellular volume ratio of astrocytes changes significantly during neural activity, such that the net result of water movement out of the blood and into the astrocytes is a local increase in tissue water content, or proton density. This tissue swelling can be measured with proton-density-weighted MRI to demonstrate sites of neural activity. Studies which have attempted to utilize BOLD-fMRI to investigate neural activity in the brainstem (Dunckley et al. 2007; Fairhurst et al. 2007) and SC (Govers et al. 2007; Komisaruk et al. 2002; Madi et al. 2001; Yoshizawa et al. 1996) have yielded results all limited by inadequate spatial resolution. Because SEEP does not rely on relaxation times, it can be detected using proton-density weighted imaging, which has the distinct advantage of producing images with the highest possible signal-to-noise ratio (SNR). Another advantage of
SEEP-fMRI is that data can be acquired using spin-echo methods, resulting in high SNR, lower spatial distortion, and less sensitivity to image artifacts. Spin-echo methods also facilitate the acquisition of data in areas of poor magnetic field homogeneity near air/tissue and bone/tissue interfaces, of which there are many in the SC. While SEEP offers many advantages over BOLD, particularly in the SC, it is not without its limitations. SEEP is slower than BOLD, taking approximately 7 seconds to reach peak response and longer to return to baseline following a stimulus. This results in a longer acquisition time which in turn results in longer study times which can be more difficult for subjects, and patients in particular. The SEEP response is also more localized than the BOLD response, which is both an advantage and a limitation. On the positive side, this reflects greater precision and specificity. On the negative side, however, it makes the areas of activity more difficult to detect, more affected by motion, and more difficult to align when comparing results from different subjects (compared to the relatively broad areas of activity demonstrated by BOLD contrast) (Stroman 2010a).

1.7 Spinal fMRI

NB: The contents of section 1.7 have been reproduced in accordance with the terms of the copyright transfer agreement Magnetic Resonance Imaging (In Press), 2010. © 2010 Elsevier Inc. For citation purposes, please reference the original publication:


Functional magnetic resonance imaging of the spinal cord (spinal fMRI) has facilitated the noninvasive visualization of neural activity in the spinal cord and brainstem of both animals and humans. This technique has yet to gain the wide-spread usage of brain fMRI,
due in part to the intrinsic technical challenges spinal fMRI presents and to the narrower scope of applications it fulfills. Nonetheless, methodological progress has been considerable and rapid. To date, spinal fMRI studies have investigated SC function during sensory or motor task paradigms in healthy controls as well as spinal cord injury (SCI) and multiple sclerosis (MS) patient populations, all of which have yielded consistent and sensitive results (Leitch et al. 2010).

1.7.1 Methodological Developments

Key technical challenges for spinal fMRI include motion of the spinal cord within the spinal canal, and variability of the results across repeated experiments. Additional challenges include the lack of a standardized coordinate system, such as that defined by Talairach and Tournoux (Talairach and Tournoux 1988) for the brain, in order to enable objective comparisons of results between different people and group analyses. In the past four years, significant developments have addressed these issues, and are described below.

Reduction of Variability in Results

Two sources of variability in spinal fMRI results have been identified (Cohen-Adad et al. 2009; Stroman 2009b), One, Type I and Type II errors, arising from physiological motion and random noise, and two, actual variation in the neuronal activity between repeated experiments. The same problem of physiological motion for brain fMRI has been addressed by modeling the motion and retrospectively accounting for the consequent MR signal changes in a general linear model (GLM) analysis (Glover et al. 2000), and the method was termed “RETROICOR”. This method allows reliable detection of neuronal-activity related
signal changes in spite of the confounding motion. A similar approach has been investigated for spinal cord fMRI data acquired with neuronal activity detected with SEEP contrast (Stroman 2006). This study demonstrated that GLM analysis produces reliable results for spinal fMRI data, and that inclusion of recordings of cardiac, but not respiratory, movement during the fMRI time-series improves the reliability of the results. An analysis by Valsasina et al (Valsasina et al. 2008) similarly demonstrated that the GLM is effective for spinal fMRI data acquired with SEEP contrast, and also that the sensitivity may be improved by also including terms obtained by independent component analysis (ICA) in the GLM basis set. Brooks et al (Brooks et al. 2008) then used the RETROICOR approach for spinal cord fMRI data acquired with BOLD contrast. Their study showed that by including cardiac and respiratory motion in the GLM basis functions, rates of detection of false-positive activity were reduced. In two separate studies, Figley et al (Figley et al. 2008; Figley and Stroman 2007) measured the spinal cord motion within the spinal canal as a function of the cardiac cycle, and developed a model of the motion at each level of the cord. This model was then used in a GLM with a method analogous to RETROICOR, termed “retrospective spinal cord motion time-course estimates (RESPITE)” (Figley and Stroman 2009). In this study, 100 spinal fMRI data sets acquired with SEEP contrast were analyzed and the results showed the addition of the RESPITE terms to the GLM improved the sensitivity by 15%-20%, and the specificity by 5%-6%. Overall, these studies demonstrate that the GLM approach for analysis of spinal fMRI data is highly effective, and that physiological motion terms can significantly improve the quality of the results. We propose that the disagreement between these studies as to whether or not respiratory terms need to be included in the GLM can be explained by
BOLD fMRI likely being more sensitive to magnetic field fluctuations arising from respiratory movement than SEEP fMRI.

**True Physiological Variation**

A key source of variation in fMRI results are the true differences in neuronal activity that can occur between repeated studies. While this variability cannot be considered an error, *per se*, as its detection demonstrates the reliability and sensitivity of the fMRI method, it can present a challenge for repeated or group studies.

It is well known (Gebhart 2004; Hoffman et al. 2005) that activity in the SC in both ventral and dorsal regions is modulated by descending input from the brainstem and cortex, and depends on factors of awareness, alertness, and attention as well as control of motor reflex responses. Studies of emotional and cognitive influences on activity in the spinal cord have been carried out by systematically varying participants’ attention focus, whether toward a thermal sensation on the hand, toward a movie, or toward mentally challenging tasks (Stroman et al. 2009). The results showed that activity in the cord, in response to a thermal sensory stimulus, did indeed depend on the participant’s attention focus in each study. A separate study demonstrated that the activity in the spinal cord, in response to thermal stimuli applied to the hand, depends on both the stimulus temperature, and on the order of experiments (Stroman 2009b). This result again implicates factors of emotion and attention. The overall conclusions from these studies are that emotional and attention factors need to be controlled, as much as possible, in spinal fMRI studies of any specific function. Even changes in anxiety or alertness over time, as participants become accustomed to being inside the MRI system, and potentially become bored with the study, were seen to affect spinal fMRI results.
These observations demonstrate that spinal fMRI results are sensitive to subtle response variations which may be features of true neuronal activity.

**Spatial Normalization**

A means of spatially normalizing spinal fMRI results acquired in sagittal slices has been developed (Stroman *et al.* 2008a) by first defining a three-dimensional coordinate system, with the primary axis parallel to the long axis of the spinal cord at every position along the rostral-caudal direction, and orthogonal axes in the right-left and anterior-posterior directions. For data spanning the cervical spinal cord and brainstem in sagittal slices, rostral-caudal position reference points were selected to be at the caudal edge of the pons (the pontomedullary junction), and at the C5/T1 disc, and are spaced 140 mm apart in the normalized space (Stroman *et al.* 2008a). For images of the lumbar spinal cord, reference points were chosen to be the T8/T9 disc and the tip of the conus, with a normalized spacing of 157 mm (Kozyrev *et al.* 2008). In order to complete the entire span of the spinal cord, the thoracic region is spanned with reference points at the C7/T1 disc and the T8/T9 disc spaced 176 mm apart (unpublished results). The three reference volumes therefore overlap and span the entire spinal cord and brainstem, as shown in Figure 4, with a total normalized span of 448 mm from the top of the C1 vertebra to the bottom of the conus, consistent with the average expected spinal cord length of 45 cm in adults. The definition of this normalized space, analogous to the Talairach space for the brain, has been shown to enable voxel-by-voxel group analyses in the spinal cord and brainstem, and allows side-by-side comparisons of results from patients after trauma and reference results from healthy subjects. (Stroman 2009a)
Figure 1.4. Positions for spatial normalization of the spinal cord. The positions are fixed based on the pontomedullary junction (PMJ) being placed at 65 mm along the primary axis (parallel to the long axis of the spinal cord at all points along the cord), and fixed spans of 140 mm between the PMJ and C₇/T₁ intervertebral disc, 176 mm between the C₇/T₁ disc and the T₈/T₉ disc, and 157 mm between the T₈/T₉ disc and the caudal tip of the conus (approximately in line with the L₁/L₂ disc). The positions of each intervertebral disc have been selected based on multiple sagittal MR images spanning different ranges of the spinal column in healthy volunteers, and the positions were scaled and aligned to be consistent across each overlapping span. Data from each volunteer are shown with a different symbol and color.

1.7.2 Applications in Clinical Populations

SENSORY STIMULI

Sensory-related neural activity in the SC has been consistently observed with fMRI in a number of studies involving healthy and clinical subject populations since 2002 (Agosta et al. 2008a; Ghazni et al. 2009; Komisaruk et al. 2002; Li et al. 2005; Ng et al. 2006; Stroman et al. 2002a; Stroman et al. 2002b; Stroman et al. 2004; Stroman 2009b; Wang et al. 2006; Xie et al. 2007; Zambreanu et al. 2005). Early studies of patients with SCI investigated the
functional response in the lumbar SC to thermal stimulation of the 4th lumbar dermatome (Stroman et al. 2002b; Stroman et al. 2004). The thermal probe was placed against the inner skin of the calf, and the temperature was ramped from skin temperature (32°C) to 10°C several times. Neural activity was consistently observed in the lumbar SC caudal to the site of injury, regardless of whether the subject could consciously feel the stimulus or not. Although the percent signal change was similar between healthy controls and SCI patients (2-3% when the temperature ranged from 29°C to 15°C and approximately 8% at 10°C, which subjects reported as a noxious sensation, discussed below), the spatial distribution of activity was notably different. While healthy controls show predominantly ipsilateral dorsal gray matter activity in response to sensory stimuli, absent or diminished dorsal gray matter and enhanced contralateral ventral gray matter activity is observed in subjects with complete SCI (patients who were unable to feel the stimulus). Results from patients with incomplete SCI are essentially divided based on the degree to which subjects were able to perceive the stimulus. For subjects with preserved sensation, the observed activity pattern was similar to that of healthy controls (consistent ipsilateral dorsal gray matter activity, in addition to central and bilateral ventral gray matter activity). Conversely, patients with decreased sensation exhibited diminished ipsilateral dorsal gray matter activity (similar to complete SCI), yet bilateral ventral gray matter activity was similar (in some cases even diminished) compared to healthy controls. The ability of spinal fMRI to distinguish subtle functional differences between well-established classes of SCI lends credence to its capacity to quantitatively assess the function of the SC.
Spinal fMRI has also been used to assess and compare the functional differences in the SC gray matter between healthy controls and patients with relapsing-remitting MS. Neural activity in the cervical spinal cord was investigated following tactile stimulation of the palm of the right hand, and was found from C5 to C8 in all patients and controls (Agosta et al. 2008a), which corresponds to the expected regions of neuronal recruitment. In general, MS patients showed approximately 20% greater signal intensity changes than controls (3.9% compared to 3.2%), with activity dispersed throughout the dorsal, central, and ventral cord, most likely attributable to the interneuronal systems of the SC (Brodal 1981; Kandel et al. 1991; Williams and Warwick 1980). Interestingly, MS patients tend to show an over-recruitment of dorsal gray matter (i.e., show bilateral dorsal activity, whereas healthy controls show predominantly ipsilateral dorsal activity), which is indicative of reduced functional lateralization in the SC. This result appears to support SC gray matter reorganization (previously found in the cortex (Filippi and Rocca 2004)), as well as post-mortem (Bot et al. 2004; Gilmore et al. 2005; Gilmore et al. 2006; Nijeholt et al. 2001) and in vivo MRI studies (Agosta et al. 2007) of the spinal cord, which suggest that gray matter is not spared by MS pathology. The purpose of this gray matter reorganization is not yet clear. However, spinal fMRI could be the tool needed to assess changes in the functional activity of gray matter throughout the evolution of the disease, and may yield insight as to whether these changes are predictive of clinical outcome.

**Motor Tasks**

Neuronal activity in the SC related to various motor tasks has been demonstrated by a number of groups (Agosta et al. 2008b; Backes et al. 2001; Govers et al. 2007; Komisaruk et
al. 2002; Kornelsen and Stroman 2004; Kornelsen and Stroman 2007; Madi et al. 2001; Maieron et al. 2007; Stroman et al. 1999; Wilmink et al. 2003; Yoshizawa et al. 1996). Twelve patients with SCI, classified as ASIA A (American Spinal Injury Association Neurological Standards Committee 2000) (no sensory or motor function preserved, n = 4), ASIA B (sensory but no motor function preserved, n = 3), ASIA C (weak motor function is preserved, n = 3), or ASIA D (motor function preserved in a condition sufficient for near-normal use n = 2) were studied while performing a pedaling motor task (Kornelsen and Stroman 2007). All subjects participated in the passive task (researcher manually moved pedals and subjects’ feet moved in pedaling motion), but only ASIA C and D patients performed the active task (autonomous alternating pedaling). Consistent with results from studies in SCI patients using sensory stimuli, neuronal activity was detected caudal to the site of injury in all subjects, regardless of the extent or level of injury. The number of active voxels in the lumbar SC was greater during active compared to passive participation; however, the overall percent signal change was greater during passive (15.0%) compared to active (13.6%) pedaling. The spatial distribution of neural activity in SCI patients was similar to healthy controls for each task. Active participation resulted in bilateral activity in both dorsal and ventral horns, corresponding to a neural response to motor and sensory stimulation, typical of purposeful movement. Passive participation yielded some ventral horn activity, but most activity was seen in the dorsal horn, typical of a neuronal response to proprioceptive and mechanical information produced by this type of movement. Also, the number of active voxels detected in the SC of each subject population mirrors the severity of the impairment. That is, fewer active voxels were detected in the SC of ASIA C/D SCI
patients than in the healthy control group (Kornelsen and Stroman 2004). Likewise, still fewer active voxels were observed in the SC of ASIA A SCI subjects compared to ASIA C/D SCI subjects. Perhaps most intriguingly, six subjects were only able to use one limb during active participation (unilateral movement generation), as opposed to typical pedaling with both feet (bilateral movement generation). The latter results in neuronal activity distributed across both sides of the cord. In this study, it was found that during unilateral movement generation, neuronal activity appeared to be prominent in the contralateral ventral horn. This corresponds with known physiology (Ferris et al. 2004; Jankowska and Edgley 2006; Kautz et al. 2006; Kawashima et al. 2005; Stroman et al. 2004), and suggests once again that spinal fMRI is able to detect subtle differences in neural function. Although spinal fMRI cannot determine the cause of the observed activity patterns, it may be used to supplement the ASIA diagnosis with functional activity maps, providing additional insight into SC physiology and enhancing the design of rehabilitation programs. Neuronal function could be investigated before, during, and after rehabilitation to provide a quantitative measure of progress in addition to the qualitative measures provided by ASIA and other subjective (outcome-based) tests.

A study similar to the passive participation pedaling task has been carried out in an MS patient population as well, investigating the extent of cervical SC functional activity in healthy controls and patients with relapsing-remitting (RR) or secondary progressive (SP) course MS (Agosta et al. 2008b). Passive and calibrated 45° flexion/extension was repetitively administered (by the researcher) to the relaxed, pronated, right hand of the patient. Activity was observed in the cervical SC from C5 to C8 in all subjects, but several differences between
controls and patients were noted. Approximately 20% greater signal intensity changes were observed in the cervical SC of MS patients (3.4%) compared to controls (2.7%), analogous to results from the study investigating the spinal fMRI effects of tactile stimulation of the palm in MS patients and healthy controls (Agosta et al. 2008a). Also, increased bilateral ventral gray matter activity was observed in MS patients compared to controls. Not only has spinal fMRI been used to detect differences between patient and control populations, but also to investigate functional differences between various classifications of MS severity. One study has shown that patients with less severe MS (RRMS) had a task-related spinal fMRI activity pattern similar to healthy controls, while patients with more severe MS (SPMS) show a pattern of cord activity more similar to SCI patients. If cervical cord functional activity varies over the course of the disease, spinal fMRI may be useful in assessing the nature and evolution of MS within individual patients.

1.8 Using FMRI to Investigate Acute and Chronic Pain in Humans

1.8.1 Brain

Functional MRI has allowed the noninvasive visualization of pain in the human nervous system, yielding intriguing insights into both healthy and pathological pain processing. The literature regarding brain circuitry in acute or experimental pain states is relatively established, and in fact agrees upon a “cerebral signature” for pain. This so-called “pain matrix” can be (somewhat simplistically) described in terms of lateral (primarily corresponding to the sensory-discriminative aspects of pain) and medial (affective-evaluative aspects of pain) neuroanatomical components (Tracey 2008). The lateral component is
composed of the primary and secondary somatosensory cortices (S1 and S2), thalamus, and posterior insula, while the medial component is composed of the anterior cingulate (ACC) and prefrontal (PFC) cortices as well as the anterior insula (Albe-Fessard et al. 1985). These areas are not necessarily the core network of human pain processing, but are consistently detected in some combination in all pain states (Casey et al. 2000; Geha et al. 2007; Rogers et al. 2004). Other areas appear active depending on both the context and the individual, including the basal ganglia, cerebellum, amygdala, hippocampus, and parts of the parietal and temporal cortices (Baliki et al. 2006). Recent fMRI studies have shown that the “cerebral signature” for chronic pain differs from that for acute pain. For example, it appears that the spontaneous pain experienced by individuals suffering chronic back pain (CBP) results in sustained medial PFC and rostral ACC activation compared to the typical “pain matrix” signature observed following experimental thermal pain (Baliki et al. 2006).

Likewise, the “cerebral signature” for neuropathic pain (with allodynia being the primary feature measured) appears distinct, albeit overlapping, with the conventional “pain matrix”. The results between studies are variable, probably due to both the heterogeneity of patients in terms of NP etiology and the utilization of different stimulation procedures, but are intriguing nonetheless (Moisset and Bouhassira 2007). Whereas more generalized chronic pain (such as CBP) results in differences in the medial “pain matrix” components (associated with the affective-evaluative aspect of pain), allodynia appears to affect the lateral component (associated with the sensory-discriminative aspect of pain). Specifically, differences in S1, S2, and lateral thalamus appear correlated with brush-evoked allodynia (Ducreux et al. 2006; Petrovic et al. 1999; Peyron et al. 1998).
“Cerebral signatures” are only a piece of the human pain puzzle. The caudal, more primitive component of the nociceptive system (the brainstem and spinal cord) has rarely been considered in the literature. This is largely due to the technical limitations of investigating these areas in humans using conventional fMRI methods, such as poor spatial resolution, local field inhomogeneity-induced signal losses, image distortion, arterial pulsation, and lack of adequate template images (Bingel and Tracey 2008). However, recent technical and methodological advances in fMRI have facilitated an investigation of the role of the brainstem and spinal cord in pain processing (See Section 1.7 Spinal fMRI).

1.8.2 Brainstem & Spinal Cord

The Pain Imaging Neuroscience (PaIN) group at Oxford University has investigated brainstem activity following a noxious stimulus in a variety of conditions, largely pertaining to the descending modulation of pain. Numerous regions in the brainstem are implicated in this process from previous (mostly animal) work. Tracey and colleagues (2002) found that the fMRI signal increase detected in the PAG following noxious stimulation corresponded with a decrease in the subjective reports of pain when subjects were distracted and not focused on the painful stimulus (Tracey et al. 2002). In another study, subjects were told when a noxious stimulus was about to occur to determine if anticipation of pain resulted in more or less nociceptive sensitivity. A positive correlation between subjective reports of anticipation of pain and the intensity of the pain perceived was observed. Furthermore, activity in the cortical pain matrix during the noxious stimulation was predicted by activity in the entorhinal cortex and ventral tegmental area (VTA) during the anticipatory stage. This
suggests some correlation between the anticipation of pain and the subjective perception of pain, potentially modulated by areas in the brainstem (Fairhurst et al. 2007).

Another group from PaIN electrically stimulated the abdominal muscles and the rectum (experimental somatic versus visceral pain, respectively) to determine the effects of emotion and type of pain on fMRI response in the brainstem. They found that visceral pain was more nocifensive (more emotionally unpleasant) than somatic pain, and in the visceral group higher activity in the PAG was correlated with high self-reports of anxiety. Also, the nucleus cuneiformis (NCF) showed greater signal change in the visceral pain condition than in the somatic pain condition (Dunckley et al. 2007).

Zambreanu and colleagues have investigated central sensitization and secondary hyperalgesia (commonly associated with NP) in the brainstem using fMRI. A 2005 study showed an increase in fMRI signal change in the NCF and PAG when an identical painful stimulus was applied following capsaicin application compared to the control condition (Zambreanu et al. 2005). Capsaicin is a chemical compound commonly used to induce an experimental model of peripheral pain via selective excitation of the transient receptor potential vanilloid 1 (TRPV1) receptor located on primary afferent nociceptors (Caterina et al. 1997). Additionally, a similar study showed a significant increase in the fMRI signal in the mesencephalic pontine reticular formation and anterior thalamus during painful stimulation following central sensitization (Lee et al. 2008).

Despite the methodological challenges described in previous sections, several groups have investigated the fMRI response to pain in the spinal cord (SC) with consistent results. In 2004, Kornelsen and Stroman found that ramping the temperature of a thermode applied to
the lower leg from 32°C to 15°C resulted in a signal intensity change of approximately 3% in the SC. However, when the temperature was decreased just five more degrees from 15°C to 10°C (perceived as painful), the signal increased a significant 7.0% and was more localized to the ipsilateral dorsal horn (DH) (Stroman et al. 2004). Other studies yield similar results using various stimulation paradigms. Ipsilateral DH activity in the cervical SC is observed following 15 g Von Frey application to the volar forearm (Ghazni et al. 2009) as well as noxious heat applied to the hand (Brooks and Tracey 2005; Cahill and Stroman 2010). Contralateral ventral horn (VH) activity is also seen during the painful stimulus, potentially due to a reflexive withdrawal motor response (Cahill and Stroman 2010; Ghazni et al. 2009). A recent study has even shown the descending effects of placebo analgesia in the SC. The expected activity in the ipsilateral DH during noxious stimulation was significantly decreased in the placebo condition, corroborated by the decrease in subjective pain ratings despite identical stimuli (Eippert et al. 2009a; Eippert et al. 2009b). The results of these studies are consistent and promising, and supplement the exponentially growing body of literature regarding pain processing in humans.

1.9 Proposed Research

1.9.1 Purpose

The purpose of the proposed research is to compare the neural response that occurs in the brainstem and spinal cord following noxious stimuli in healthy controls to a patient population diagnosed with neuropathic pain.
1.9.2 Rationale

Acute pain and experimental models of NP are inadequate surrogates for investigating clinical NP (Moisset and Bouhassira 2007). The postulated maladaptive plasticity that causes the pain and paresthesia undoubtedly affects the way pain is processed, effectively altering either the ascending or descending pathways, and possibly both (Costigan et al. 2009). This study is the first to use a clinically diagnosed population suffering from NP to study the fMRI response to pain in the SC and brainstem, an important step in determining the pathophysiology of the NP state in humans. These results may someday lead to clinical methods used to noninvasively assess presence and extent of NP, plan innovative and effective treatment strategies, and monitor treatment efficacy.

1.9.3 Hypothesis

We hypothesize that there will be significant differences in the subjective pain responses, MR signal intensity changes and in the anatomical regions that are activated between the healthy control and the NP patients.

1.9.4 Objectives

The specific objectives of this study are:

1) To determine the neural activity in the human brainstem and spinal cord that is caused by a noxious mechanical stimulus.

2) To compare the neural response to noxious stimuli in both healthy controls and a patient population diagnosed with peripheral neuropathic pain.
Chapter 2

Methods

2.1 Volunteer Recruitment

Healthy volunteers were recruited via poster advertisements (Appendix A) placed around Queen’s University campus and downtown Kingston. Patients diagnosed with carpal tunnel syndrome (CTS) were recruited via poster advertisements (Appendix B) placed in physician and physiotherapy offices as well as a notice (Appendix C) placed in Kingston This Week, a local event newspaper. All respondents were provided with the MRI Safety Screening Questionnaire (Appendix D), Subject Consent Form (Appendix E), and Volunteer Details (Appendix F), each of which was signed and returned prior to enrollment in the study. The MRI Safety Screening Questionnaire is submitted and evaluated by an investigator to exclude individuals with neurological disorders, previous injury to the brain or SC, a history of claustrophobia or anxiety, or any MRI safety risk (implant, pacemaker, artificial limb). Fourteen healthy, right-handed subjects (4 males) participated in this study as controls, with a median age of 21 years (range 19 to 52). Ten subjects who were diagnosed by a physician as suffering from mild, unilateral carpal tunnel syndrome (CTS) of the right hand also participated in this study (2 males), with a median age of 32 years (range 22 to 49). Four subjects diagnosed with mild, unilateral CTS of the left hand participated as well (median age 50, range 39 to 70). The research protocol was reviewed and approved by the Queen’s University Human Research Ethics Board, and each subject provided signed consent before participating. All data were treated confidentially; with each set of subject data images assigned a unique identifying number only accessible by the experimenter.
2.2 Confirmation of Carpal Tunnel Symptoms and Neuropathic Pain

Subjects diagnosed with CTS were used as the clinical population suffering neuropathic pain (NP), and both CTS and NP were confirmed prior to enrollment in the study. Each subject had been previously diagnosed by their physician as having mild, unilateral CTS, but had not undergone any treatment to date. Subjects completed the CTS Patient Information form (Appendix G) regarding onset of symptoms, date of diagnosis, triggers for symptoms, and other relevant information, as well as performed two well-established tests, Phalen’s Test and Tinel’s Sign, to verify the presence of symptoms (Introduction: Section 1.4 for details). To perform Phalen’s Test, the subject was asked to place the backs of his/her hands flush against one another and touch the thumb and middle fingers together for each hand. This position was held for 30 seconds, or until the onset of parasthesia (pins and needles, numbness) in the thumb, index, or middle finger of the affected hand, indicative of a positive test for CTS. To perform Tinel’s Sign, the experimenter lightly tapped the middle of the volar arm 2 cm proximal to the wrist crease in the affected hand for 30 seconds or until the onset of parasthesia, again, indicative of a positive test for CTS. Most subjects recruited to the CTS (right hand) group tested positive in both Phalen’s Test and Tinel’s Sign \( n = 6 \), two only in Phalen’s, and one only in Tinel’s (Table 2.1).
Table 2.1. Confirmation of CTS and relevant background information. Nine subjects diagnosed with mild, unilateral CTS of the right hand were included in the study. Results for Tinel’s Sign and Phalen’s test are denoted by either a ‘+’ (positive diagnostic test) or ‘-’ (negative diagnostic test).

Since CTS does not necessarily result in NP in all patients, we confirmed the presence of NP using several diagnostic questionnaires. Each subject completed the Short Form McGill Pain Questionnaire (SF-MQP, Appendix H), administered by the experimenter using a standardized script to maintain consistency (Appendix I). The SF-MPQ is composed of three parts. Part one is a list of adjectives used to allow the patient to qualitatively describe their pain. Part two is the Visual Analog Scale (VAS) to quantify the intensity and unpleasantness of the individual’s pain over the preceding two weeks. The subject reports this by marking an ‘I’, indicating ‘intensity of pain’, and a ‘U’, indicating ‘unpleasantness of pain’, along a 10 cm line where the left anchor is ‘No Pain’ and the right anchor is ‘Worst Pain Imaginable’ (Appendix H, Part II). The distance from the left anchor to the subject’s ‘I’ and ‘U’ are then measured and reported in millimeters. Part three is a Present Pain Index (PPI) to give an indication of the amount of pain they are experiencing at the onset of the study. NP is characterized by specific descriptors in the SF-MPQ, such as shooting (the “electric shock”
episodic events) and burning, thus subjects included in the study reported at least one of the
two descriptors (in addition to the parasthesia previously described).

Members of the control group did not positively identify any qualifier in the SF-MPQ, and each rated their current pain (both intensity and unpleasantness) as ‘0’ using the
VAS. In contrast, members of the NP patient group positively identified many of the SF-MPQ descriptors used to diagnose neuropathic pain (NP), both sensory (shooting, stabbing, sharp, hot-burning) and affective (tiring, punishing) components. Each subject in the NP
group positively identified at least one of the sensory descriptors, most identified two or more
(n = 8), and most positively identified an affective descriptor as well (n = 7). Using the VAS,
all patients rated their pain for the preceding two weeks as a non-zero value, with a mean
intensity of 43.22 ± 27.4 and mean unpleasantness of 53.11 ± 29.43 (Table 2.2).
Table 2.2. Patient results from pain questionnaires. Each subject diagnosed with right hand, unilateral CTS completed the Short Form McGill Pain Questionnaire (SF-MPQ) and the Visual Analog Scale (VAS) to compare their individual pain experience. Though the SF-MPQ contains fifteen descriptors, only the six associated with neuropathic pain were included in the table. Subjects rated each descriptor as none, mild, moderate or severe with regards to their individual pain experience in terms of each descriptor. To complete the VAS, subjects indicated the intensity and unpleasantness of their pain by marking an ‘I’ and a ‘U’ along a 100 mm line, where the left anchor is “No Pain” and the right anchor is “Worst Pain Imaginable”. The values in the table are measurements from the left anchor to the ‘I’ or ‘U’, in milimetres.

![Table 2.2](image)

2.3 Part I – Psychophysical Testing

A custom-made device (Figure 2.1) fastened around the wrist focused the pressure from a sphygmomanometer onto the median nerve via a rigid sphere (attached to the device) which was placed in the middle of the volar forearm 2 cm proximal to the wrist crease. Pain was induced by slowly and steadily increasing the pressure until subjects reported the pressures that corresponded to a subjective pain intensity of 2, 4 and 6 on and 11-point numerical analog scale from 0 to 10 (where 0 is ‘No pain’ and 10 is ‘Worst pain imaginable’). Both left and right wrists were assessed separately, and these reported pressures were recorded (Appendix J) and used during imaging stimulation blocks.
Figure 2.1. Stimulation device. A regular sphygmomanometer was modified by adding 3 m of clear, plastic tubing (to allow experimenter to stand clear of the magnet, minimizing confounding artifacts) and a rigid sphere to the cuff (to focus the pressure from the device to a more localized region). The cuff was placed around the subject’s wrist and the pressure used was recorded from the gauge.

2.4 Part II – Imaging

Stimuli were applied in a block paradigm consisting of three stimulation periods of 45 seconds each, interleaved with baseline periods of 72 seconds in which no stimuli were applied, and preceded by an initial baseline of 81 seconds, for a total of 7 minutes and 12 seconds (Figure 2.2). The onset of the noxious stimulus was signaled by a 1 second tone, included to keep the subject’s awake and attending to the study. The stimuli were applied continuously during the stimulation blocks by an experimenter who was in the scanner room.
for the duration of the experiment. Series 1 and 2 were performed on the right wrist and series 3 and 4 performed on the left wrist, using the pressures reported during psychophysical testing on each wrist. An intermediate scan was performed between series 2 and 3 for anatomical localization.

![Figure 2.2](image)

**Figure 2.2.** Block paradigm. Timing used during the four series (first two on the right hand, second two on the left hand) to indicate rest and stimulation periods. The experimenter used the same pressures for all four series which corresponded to pain levels 2, 4, and 6 as indicated during prior psychophysical testing.

### 2.5 FMRI Data Acquisition

FMRI studies of the spinal cord and brainstem were carried out in a 3 T Magnetom Trio (Siemens, Erlangen, Germany) using a phased array spine receiver coil. Localizer images were initially acquired (with subjects lying supine) in three orthogonal planes as a reference for section positioning for subsequent fMRI studies. Functional imaging data were acquired with a half-fourier single-shot fast spin-echo (HASTE) sequence with an echo time of 38 msec and a repetition time of 1 sec per slice to obtain primarily proton-density weighted images. The observed signal intensity changes were a result of signal enhancement by extravascular water protons (SEEP) in addition to a contribution from the BOLD effect (Stroman *et al.*).
Sagittal image slices were selected to span from the C7/T1 disc to the superior edge of the thalamus, with a 28 cm x 14 cm field of view (FOV), a 192 x 96 matrix, in nine 2 mm thick contiguous sagittal slices spanning the entire width of the spinal cord and midline brainstem. The resulting voxel size was 1 mm x 1 mm x 2 mm. Spatial suppression pulses were applied to eliminate signal intensity anterior to the spine and motion artifacts from the heart, in addition to flow-compensation gradients applied in the rostral-caudal direction to reduce artifacts from flowing CSF. The peripheral pulse was continuously recorded throughout each study for use in subsequent data analysis. Each image slice was acquired following 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). The signal from the external trigger was recorded simultaneous to the peripheral pulse to provide a time reference for the acquisition of each image relative to the physiological traces.

2.6 Data Analysis

First-level (individual) analysis was performed on all subjects recruited. Individual results are shown in 2 mm thick axial slices spanning from the thalamus to spinal cord segment C8 according to Figure 2.3 (each spinal cord segment is comprised of approximately nine slices). One participant from the control group and one from the right-hand CTS group were excluded from second-level (group) analysis because the peripheral pulse was not properly recorded and the subject was improperly positioned in the magnet which affected the MR signal obtained, respectively.
Figure 2.3. Results display of individual analysis. Slices spanning from the thalamus to the C7/T1 disc are presented sequentially. Each slice is 2 mm thick, and one spinal cord segment corresponds to approximately nine slices.

2.6.1 Group Analysis

The 3D functional imaging data were analyzed with custom-made software written in MatLab®. To perform second-level (group) analysis, a reference line was drawn along the anterior edge of the spinal cord in a midline sagittal section and extended through the brainstem to the anterior edge of the thalamus (Figure 2.4). The reformatted spinal cord and brainstem were normalized to a standard coordinate space for all studies to allow group comparisons of results, and this normalization has been shown to be accurate to within 2 mm (Stroman et al. 2008a). Smoothing was applied only parallel to the long axis of the cord and brainstem. The data were then analyzed using a General Linear Model (GLM), using the peripheral pulse sampled at the time of data acquisition to account for confounding effects due to cardiac motion (Figley and Stroman 2007; Stroman 2006). Group results were determined using a random effects analysis (Friston et al. 2007; McGonigle et al. 2000). Areas of activity outside the defined boundaries of the spinal cord and brainstem were left intact, and areas of activity within the defined boundaries were identified visually with
comparison to a stereotaxic atlas (Blumenfeld 2002; DeArmond et al. 1974; Tamraz and Comair 2006). Data were analyzed with a threshold of p < 0.01, corrected for multiple comparisons by thresholding at a minimum cluster extent as determined with the MatLab function “stat_threshold” written by K. Worsely (Worsley 2005). The extent thresholds were set at 9.05 mm$^3$ for both the control and the CTS patient groups, as they both consisted of the same number of series. The cluster thresholds were determined based on a total volume of the cervical SC and brainstem of 27,400 mm$^3$, determined from a spatially normalized region-of-interest mask.

Figure 2.4. Normalization for spinal cord and brainstem. A reference line was drawn along the anterior edge of the spinal cord, through the brainstem, and along the anterior edge of the thalamus. The inferior border of the pons (top red line) and the C7/T1 disc (bottom red line) are used as additional reference points for spatial normalization. Sagittal images spanned from the superior edge of the thalamus to the C7/T1 interface. Modified from Stroman et al. (Stroman et al. 2008a).
2.6.2 Correlation Analysis

Further second-level analysis was performed on the data. An activity mask was used from the group analysis to present only voxels which showed signal intensity change at all pain levels. A custom-made program written in MatLab® was used to show the change in signal intensity that was observed at each pain level for all individuals in both control and CTS subject groups, in various regions of interest. Regions were chosen based on known synaptic relay points in both ascending and descending pathways involved in pain transmission, including the ipsilateral dorsal horn, rostralventromedial medulla (RVM), dorsolateral pontine tegmentum (DLPT), and periaqueductal grey (PAG). The consistently active voxels across individuals and within each of these regions were averaged together to obtain each group’s mean, and are presented graphically along with individual results.

2.7 Statistical Analysis for Psychophysical Results

Psychophysical data were analyzed with a two-tailed Student’s t-test (p < 0.05). The mean pressures used to elicit subjective pain scores of 2, 4, and 6 out of 10 on a pain scale (a score of ‘0’ indicates ‘No Pain’ and a score of ‘10’ indicates ‘Worst Pain Imaginable’) were compared between control and CTS subjects for both the right and left hand. The results are displayed as mean ± standard deviation.
Chapter 3

Results

3.1 Psychophysical Data

Psychophysical testing was done prior to imaging to determine the pressures required to elicit subjective pain ratings of 2, 4, and 6 out of 10 for each individual. Standardized pain ratings were used (as opposed to standardized pressures) because the pain response is extremely subjective, and varies widely between individuals (Staud et al. 2008). Standardized pressures would have resulted in far too great a range of pain ratings or null results. For example a pressure of 120 mmHg may be perceived as 2 out of 10 on a pain scale for a control subject and as 6 out of 10 for a CTS subject. Likewise, a pressure of 55 mmHg may be perceived as 6 out of 10 for a CTS subject, but fail to elicit any pain response in a control population. All subjects were able to report the point at which the applied pressure corresponded to a subjective pain rating of 2, 4, and 6 (Table 3.1).

The pressures required to elicit ratings were significantly lower for the CTS subject population at all pain levels for the right (affected) hand, but not significantly different for the left (unaffected) hand (Table 3.2). Interestingly, the difference between the affected and unaffected hand within the CTS subject population was also not significantly different.
<table>
<thead>
<tr>
<th></th>
<th><strong>PRESSURE USED (mmHg)</strong></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>PAIN = 2</strong></td>
<td><strong>PAIN = 4</strong></td>
<td><strong>PAIN = 6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>CONTROL</strong></td>
<td><strong>CTS</strong></td>
<td><strong>CONTROL</strong></td>
<td><strong>CTS</strong></td>
<td><strong>CONTROL</strong></td>
<td><strong>CTS</strong></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>70</td>
<td>180</td>
<td>110</td>
<td>210</td>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>75</td>
<td>140</td>
<td>220</td>
<td>190</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>50</td>
<td>190</td>
<td>70</td>
<td>220</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>60</td>
<td>80</td>
<td>95</td>
<td>110</td>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>40</td>
<td>150</td>
<td>75</td>
<td>195</td>
<td>105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>60</td>
<td>200</td>
<td>100</td>
<td>290</td>
<td>130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>40</td>
<td>130</td>
<td>50</td>
<td>160</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>40</td>
<td>130</td>
<td>60</td>
<td>170</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>190</td>
<td></td>
<td></td>
<td>260</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>120</td>
<td></td>
<td></td>
<td>160</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>140</td>
<td></td>
<td></td>
<td>210</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>130</td>
<td></td>
<td></td>
<td>260</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>270</td>
<td></td>
<td></td>
<td>270</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>80</td>
<td></td>
<td></td>
<td>110</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.1.** Individual results from psychophysical assessment. Pressures (mmHg) required to elicit subjective pain scores of 2, 4, and 6 out of 10 on a pain scale for the right (affected) hand (a score of ‘0’ indicates ‘No Pain’ and a score of ‘10’ indicates ‘Worst Pain Imaginable’). The control group is comprised of healthy subjects (n = 13) and the CTS group is comprised of volunteers diagnosed with mild, unilateral CTS of the right hand (n = 9)
Table 3.2. Group results from psychophysical assessment. Pressures (mmHg) required to elicit subjective pain scores of 2, 4, and 6 out of 10 on a pain scale for both the left and right hand (a score of ‘0’ indicates ‘No Pain’ and a score of ‘10’ indicates ‘Worst Pain Imaginable’). The control group is comprised of healthy subjects (n = 13) and the CTS group is comprised of volunteers diagnosed with mild, unilateral CTS of the right hand (n = 9). Scores are displayed as mean ± standard deviation. Significance (*) was determined using a two-tailed Student's t-test (p < 0.05). The only significant difference in pressure was between the control and CTS groups following right hand stimulation.

<table>
<thead>
<tr>
<th>Pain Level</th>
<th>Condition</th>
<th>Control (n = 13)</th>
<th>CTS (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (± SD)</td>
<td>Right Hand</td>
<td>97.5 ± 35.59</td>
<td>53.89 ± 13.18*</td>
</tr>
<tr>
<td></td>
<td>Left Hand</td>
<td>92.5 ± 29.66</td>
<td>70.56 ± 23.91</td>
</tr>
<tr>
<td>4 (± SD)</td>
<td>Right Hand</td>
<td>152.14 ± 50.41</td>
<td>93.33 ± 51.66*</td>
</tr>
<tr>
<td></td>
<td>Left Hand</td>
<td>145 ± 44.85</td>
<td>111.67 ± 53.27</td>
</tr>
<tr>
<td>6 (± SD)</td>
<td>Right Hand</td>
<td>201.07 ± 56.30</td>
<td>115 ± 52.5*</td>
</tr>
<tr>
<td></td>
<td>Left Hand</td>
<td>198.21 ± 61.99</td>
<td>143.33 ± 61.99</td>
</tr>
</tbody>
</table>

3.2 Group Results

An overview of the group fMRI results for control (n = 13) and CTS (n = 9) subjects following noxious stimulation is shown in Figure 3.1, as determined by random effects analysis. Areas of significant signal intensity change are inferred to reflect changes in neural activity caused by the application of the stimulus. Positive signal intensity changes are represented by warm colours (orange and red) and correspond to a regional increase (from baseline) in neural input. Negative signal intensity changes are shown in cool colours (light and dark blue) and correspond to a regional decrease (from baseline) in neural input. Here, results are shown following noxious stimulation of the right (affected) wrist. There are considerably less active voxels at all pain levels in the CTS subject group (Figure 3.2(b) also) compared to the control group (Figure 3.2 (a) also). The difference is most prominent in the thalamus, midbrain, pons, and medulla at pain levels 2 out of 10 and 4 out of 10. Despite the
difference in sheer quantity of activated voxels, those voxels that are active in both the control and CTS group appear to follow the same general trend over the three pain levels. At pain level 2 out of 10, the overall signal intensity change is positive and most robust of all the pain levels. At pain level 4 out of 10, the overall signal intensity change is still positive (with some areas of negative signal intensity change), but less active voxels are observed following this stimulus. However, at pain level 6 out of 10, the overall signal intensity change appears negative, represented by a similar (low) number of voxels as in the pain 4 condition. Signal intensity changes were observed in many areas implicated in the transmission and modulation of pain (See Figure 1.2 and Figure 1.3 in Chapter 1 - Introduction), including synaptic points in the SMT, SRT, and descending modulation pathways.

To evaluate the activity at specific regions of interest with regards to the transmission and modulation of pain, group fMRI results were manually over-laid on templates representing relevant nuclei in the midbrain, pons, medulla, and cervical SC (Figure 3.2). These templates are presented in anatomical orientation for consistency with previous group results. That is, axial slices are rotated 180° clockwise from anatomical orientation (so that the right DH appears at the bottom left of the cervical SC slices). Here, results are shown following noxious stimulation of the right (affected) wrist.
Figure 3.1. Overview of group results. Spinal fMRI group results (T-values; threshold = 2.5) from 13 controls (top) and 9 subjects diagnosed with mild, unilateral CTS of the right hand (bottom). Each subject reported (each) level 2, 4, and 6 out of 10 on the pain scale when various pressures were applied to the right wrist. Each panel shows the axial distribution of activity projected onto anatomical drawings for each level of the brainstem and spinal cord (presented in radiological orientation), and the rostral/caudal (R/C) distribution of activity projected onto left and right sagittal slices.
Figure 3.2. Control (a) and CTS (b) group results for regions of interest for transmission and modulation of pain. The midbrain, pons, medulla, and spinal cord DH are comprised of important synaptic points in both the ascending transmission and descending modulation of pain. Group fMRI results have been enlarged and manually overlaid on simplified templates showing nuclei relevant to pain pathways.
**Cervical Spinal Cord**

At pain 2, the stimulus produced positive signal intensity change in the ipsilateral DH in both control and CTS groups. This positive signal change in the ipsilateral DH persisted at pain 4 for both groups, and persisted still for the CTS group at pain 6. However, in the control group, ipsilateral DH signal intensity change was negative at pain 6. Furthermore, in the CTS group, positive signal change was observed in the central gray matter at pain 6.

![Diagram of pain levels and signal intensity changes in cervical SC at C5 and C8 for control (n = 13) and CTS (n = 9) groups, respectively.](image)

**Figure 3.3.** Group results in the cervical SC at C5 and C8 for control (n = 13) and CTS (n = 9) groups, respectively.

Contralateral DH activity was consistently observed in the control group at all pain levels, predominately positive at pain 2 and pain 4, with both positive and negative signal intensity change observed at pain 6. In addition, positive contralateral and negative ipsilateral VH signal change was seen in controls. In contrast, neither VH nor contralateral DH signal change was observed in the CTS group at any pain level.

**Rostral Medulla**

In the rostral medulla, consistent signal intensity change was observed within both control and CTS subject groups. The most prominent difference between groups was the consistent trend observed in the RVM of the control group compared to the lack of coherent
activity in the RVM of the CTS group (Recall that the RVM is comprised of the NRM and the
adjacent reticular formation (RF)). At pain 2 in controls, we observe positive signal change in
anatomical regions consistent with the NRM, and bilateral positive signal change in the
medullary RF and NGc. This activity becomes more localized ipsilaterally as the pain level
increases, with positive signal change observed in the NRM and ipsilateral RF at pain 4. In
the CTS group at pain 2, negligible activity is observed in the NRM and NGc, however,
bilateral positive signal intensity change occurs in the vicinity of the RF. Interestingly, in the
CTS group at pain 4, there is still a lack of activity in the NRM and NGc, however the
bilateral activity in the RF is notably different. Specifically, there is ipsilateral positive signal
change in the area of the RF, whereas the signal change is negative contralaterally.

![Figure 3.4](image.png)

**Figure 3.4.** Group results in the rostral medulla for control (n = 13) and CTS (n = 9) groups.

Both the control and CTS groups show overall negative signal intensity change in the
medulla at pain 6, and while the negative signal change is localized to the NRM in the control
group, it is dispersed ipsilaterally in the vicinity of the RF in the CTS group. Again, positive
ipsilateral RF signal change is observed in controls at pain 6, as in pain 2 and pain 4
conditions. Signal change was also seen in anatomical regions not typically associated with
pain transmission, but which are often implicated in other sensory processes. Positive and negative signal change was observed in both groups at pain 2 and pain 4 in regions anatomically consistent with the olivary nucleus (ON), gracile nucleus (GN), and cuneate nucleus (CN); the positive activity was predominantly ipsilateral, while the negative activity appeared to be contralateral in these regions.

**PONS**

The results between the control and CTS groups vary greatly in the pons. As previously noted, the control group exhibited a much greater quantity of active voxels than the CTS group, especially at pain 2. This positive activity is widely dispersed in controls, but more localized contralaterally to the cluster of nuclei collectively known as the DLPT in the CTS group (Recall that the DLPT is comprised of the LC, subcoeruleus, and Kölliker-Fuse nuclei). At pain 4, while the CTS group shows predominantly contralateral positive signal change, the control group exhibits contralateral negative but ipsilateral positive signal change in proximity to the DLPT and parabrachial nuclei (PBN). At pain 6, the CTS group exhibits an almost entirely negative signal intensity change, predominantly in the contralateral DLPT and parabrachial nucleus, while the control group exhibits bilateral, intermingled positive and negative signal change.

**Figure 3.5.** Group results in the pons for control (n = 13) and CTS (n = 9) groups.
Finally, group fMRI results for the midbrain present some interesting differences between the control and CTS groups. At pain 2, the control group shows considerable ipsilateral positive signal change in all the accessory nuclei relevant to the various pain pathways (that is, the interstitial nucleus of Cajal, Darkschewitsch nucleus, NCF, and superior colliculus (SC)) except for the PAG. Conversely, the CTS group shows only slight activation in these various nuclei, yet does show localized positive signal intensity change in the vicinity of the PAG. At pain 4, the control group does show positive signal change in the PAG, yet most activity appears in the proximity of the ipsilateral accessory nuclei. Again, however, the activity in the CTS group at pain 4 is mostly confined to the region of the PAG and is predominantly positive signal change, with adjacent negative signal change observed. At pain 6, very little activity is visible in the control group midbrain while the CTS group presents with an overall negative signal intensity change, largely confined to the PAG. A summary of the signal intensity change observed following noxious stimulation of the right (affected) wrist can be found in Table 3.3.

**Figure 3.6.** Group results in the midbrain for control (n = 13) and CTS (n = 9) groups.
### Table 3.3. Summary of signal intensity change in the cervical spinal cord and brainstem following noxious stimulation to the right wrist. The signal changes at pain 2, pain 4, and pain 6 are compared between control (n = 13) and CTS (n = 9) groups. Groups are compared based on location of signal change within axial slices, whether this activity is ipsi-, contra-, or bilateral, and whether the signal change is positive or negative (see legend below table).

#### 3.3 Regional Comparison of Signal Intensity Change

To perform a comparison of signal change across pain conditions, voxels which were active at each pain level in a specific region of interest were sampled and averaged together. These voxels were measured at pain 2, pain 4, and pain 6 for each individual in both the control and CTS groups. No significant difference was found between the control and CTS groups’ signal change in any region of interest at any pain level (Table 3.4, p > 0.05). There appears to be a consistent trend of decreasing signal intensity change in each region as the...
pain level increases, observed in both groups (Figure 3.7). Thus, though the anatomical areas that appear active differ between groups, those areas that are active show a similar activity profile with increasing pain ratings.

<table>
<thead>
<tr>
<th>Region</th>
<th>Control (n = 13)</th>
<th>CTS (n = 9)</th>
<th>Control (n = 13)</th>
<th>CTS (n = 9)</th>
<th>Control (n = 13)</th>
<th>CTS (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPSILATERAL DH</td>
<td>1.80 ± 2.45</td>
<td>0.40 ± 1.24</td>
<td>1.34 ± 1.28</td>
<td>0.59 ± 2.36</td>
<td>0.19 ± 1.29</td>
<td>-0.39 ± 1.64</td>
</tr>
<tr>
<td>CONTRALATERAL DH</td>
<td>1.51 ± 2.40</td>
<td>1.07 ± 2.18</td>
<td>1.32 ± 0.96</td>
<td>-0.26 ± 0.91</td>
<td>-0.43 ± 1.70</td>
<td>-0.17 ± 1.47</td>
</tr>
<tr>
<td>RVM</td>
<td>0.76 ± 0.56</td>
<td>0.68 ± 0.43</td>
<td>0.86 ± 0.77</td>
<td>0.02 ± 0.83</td>
<td>-0.14 ± 0.88</td>
<td>-0.18 ± 0.63</td>
</tr>
<tr>
<td>DLPT</td>
<td>0.60 ± 0.31</td>
<td>0.30 ± 0.42</td>
<td>0.71 ± 0.27</td>
<td>0.07 ± 0.30</td>
<td>0.00 ± 0.14</td>
<td>-0.02 ± 0.15</td>
</tr>
<tr>
<td>PAG</td>
<td>0.56 ± 0.43</td>
<td>0.21 ± 0.40</td>
<td>0.53 ± 0.39</td>
<td>0.26 ± 0.56</td>
<td>-0.15 ± 0.22</td>
<td>0.04 ± 0.37</td>
</tr>
</tbody>
</table>

Table 3.4. Summary of mean signal change in spinal cord and brainstem regions of interest following noxious stimulation to the right wrist. Control (n = 13) and CTS (n = 9) groups are compared with respect to mean signal change at pain 2, pain 4, and pain 6. There was no significant difference in signal change at any region of interest at any pain level, as determined using a two-tailed Student’s t-test (p < 0.05).
**Figure 3.7.** Comparison of mean signal intensity change in regions of interest following noxious stimulation to the right wrist. Areas of activity consistent across all pain levels within the control (top) and CTS (bottom) groups are compared at each individual pain level. Markers indicate the mean value in each region, and colour-coded lines are used to aid in visualization trends, as these data are not continuous.
Figure 3.8. Signal intensity change in the dorsal horn ipsilateral to noxious stimulation of the right wrist. The percent signal intensity change is shown for pain levels 2, 4, and 6 out of 10 for both control (n = 13) and CTS (n = 9) groups. Each blue line represents the activity profile of one subject, while the red line denotes the mean signal intensity change for the entire group at each pain level.

Despite several outliers, the signal change in the ipsilateral DH appears similar between individuals in both groups at each pain level (Figure 3.8).
Figure 3.9. Signal intensity change in the dorsal horn contralateral to noxious stimulation of the right wrist. The percent signal intensity change is shown for pain levels 2, 4, and 6 out of 10 for both control (n = 13) and CTS (n = 9) groups. Each blue line represents the activity profile of one subject, while the red line denotes the mean signal intensity change for the entire group at each pain level.

In contrast, in the contralateral DH, the control group exhibits considerably more inter-individual variability than does the CTS group, in which the individual results are tightly clustered around a 0% signal change at each pain level (Figure 3.9).
Figure 3.10. Signal intensity change in the region of the RVM following noxious stimulation of the right wrist. The percent signal intensity change is shown for pain levels 2, 4, and 6 out of 10 for both control (n = 13) and CTS (n = 9) groups. Each blue line represents the activity profile of one subject, while the red line denotes the mean signal intensity change for the entire group at each pain level.

With the exception of several outliers, the control group presents with little inter-individual variability in the region corresponding to the RVM, while individuals in the CTS group differ considerably from one another, particularly at pain 4 and pain 6 (Figure 3.10).
Figure 3.11. Signal intensity change in the region of the DLPT following noxious stimulation of the right wrist. The percent signal intensity change is shown for pain levels 2, 4, and 6 out of 10 for both control (n = 13) and CTS (n = 9) groups. Each blue line represents the activity profile of one subject, while the red line denotes the mean signal intensity change for the entire group at each pain level.

The region taken as the DLPT (and the comprising nuclei) was the most consistent between groups and across pain levels (Figure 3.11). Mean signal change in both control and CTS groups appear to be the result of relatively similar inter-individual variability.
Figure 3.12. Signal intensity change in the region of the PAG following noxious stimulation of the right wrist. The percent signal intensity change is shown for pain levels 2, 4, and 6 out of 10 for both control (n = 13) and CTS (n = 9) groups. Each blue line represents the activity profile of one subject, while the red line denotes the mean signal intensity change for the entire group at each pain level.

Finally, the CTS group again shows more inter-individual variability than the control group in the region anatomically consistent with the PAG (Figure 3.12).
Chapter 4

Discussion

In this study, we used fMRI to demonstrate neural activity generated by noxious mechanical stimuli spanning from the lower cervical SC to the thalamus in healthy controls and in patients diagnosed with carpal tunnel syndrome (CTS, shown to result in neuropathic pain (NP)). To our knowledge, no other study has investigated the neural activity induced by peripheral noxious stimulation in the cervical SC and brainstem in a patient population suffering from NP. Early investigators studying pain believed that the cortex played only a minimal role in pain processing, due in part to the observation that soldiers with extensive cerebral damage could still perceive pain (Head and Holmes 1911), thus it was inferred that pain must be primarily processed in subcortical structures. However, over the last two decades the advent of non-invasive neuroimaging has effectively rebutted this archaic belief, and in fact the vast majority of fMRI studies on pain focus exclusively on the cerebral cortex, elucidating a fairly consistent “cerebral signature” for pain. Recent technical and methodological advances in spinal fMRI (Leitch et al. 2010) have facilitated the investigation of the neural signature of pain in the SC and brainstem in a growing number of studies. This discussion will focus mainly on these studies, and other non-fMRI studies, concerning the subcortical substrates of pain transmission, as they are most pertinent to the results from this study.
4.1 Principle Findings

We observed a consistent signal intensity change in response to the noxious stimuli in the SC and brainstem within both the control and CTS groups. The general trend of this activity was similar between groups in almost all areas studied, with a primarily positive signal change at the lowest pain level, transitioning to a negative signal intensity change at the highest pain level. Despite the similarity in the general trend observed, various differences were evident in the anatomical location of the activity, predominantly in areas commonly implicated in both ascending and descending pain systems. Furthermore, we found that individuals in the CTS group required significantly less intense noxious pressure to elicit the same subjective pain rating as control subjects. These results suggest both important similarities and differences between healthy and pathological pain processing.

4.2 In Light of the Literature

4.2.1 Transmission and Modulation of Pain: A Brief Refresher

When noxious insult occurs in the periphery, primary afferents from nociceptors carry this information and synapse in the ipsilateral DH of the SC, forming the first component of the pain transmission pathway. Second-order ascending tracts decussate immediately, transmitting painful stimuli from the DH of the SC to various nuclei in the contralateral medulla and pons (spinoreticular tracts), midbrain (spinomesencephalic tract), and thalamus (spinothalamic tract) (Dostrovesky and Craig 2006). Third-order neurons project from these nuclei to regions in the cortex involved in the integration of the noxious stimuli with the individual's specific emotional and cognitive state. The areas which appear
most consistently to be part of the cerebral “pain matrix” are the primary and secondary somatosensory cortices (SI and S2), insular cortex (IC), ACC, PFC, and the thalamus (Bushnell and Apkarian 2006). The cortex then sends bilateral descending projections to the thalamus, midbrain, pons, and medulla via several important relay points which serve to either enhance or inhibit nociception. Finally, if anti-nociceptive circuitry is recruited, inhibitory projections descend from the brainstem to the DH of the SC to diminish the incoming noxious stimuli (Fields et al. 2006). FMRI does not differentiate between excitatory or inhibitory synapses at a given location. Instead, it is a measure of increasing or decreasing neural input at a synapse (Logothetis et al. 2001), be that excitatory or inhibitory, from baseline. This limits interpretation somewhat, but still yields new and valuable information concerning how both healthy and pathological pain is processed in humans.

4.2.2 Comparing SC and Brainstem Activation between Groups

Spinal Cord

The activity observed at each pain level is compared between groups and within groups, and will be discussed in terms of how the similarities and differences may help us to understand pain processing in humans in both healthy and pathological conditions. The positive signal change observed in both groups in the DH ipsilateral to the noxious stimulus was expected (Figure 3.3), as the primary afferents from peripheral nociceptors synapse here, thereby increasing neural input in the region (Dostrovsky and Craig 2006). Various studies from Stroman and colleagues have shown similar activity in the ipsilateral DH following noxious thermal (Cahill and Stroman 2010; Stroman et al. 2004) and von Frey (Ghazni et al. 2002).
2009) stimulation. However, while the control group shows a negative signal change at the highest pain level, the CTS group shows positive activity, indicative of a functional difference.

This negative signal change may be the result of the endogenous analgesic system recruited to inhibit the increasingly painful sensations generated by the pain pressure. Descending projections from supraspinal locations (Yezierski et al. 1982) have been shown to modulate nociception via serotonin release in the SC (Besson 1999). Though this modulation does represent neural input to the SC, other studies have shown a similar deactivation in the ipsilateral DH in conditions of placebo analgesia in healthy controls (Eippert et al. 2009b).

The negative signal change may also be the result of a signal change response to a painful stimulus that does not return completely to baseline after the stimulation is removed. Recalling that the fMRI signal is merely an increase or a decrease in neural input from baseline, if the neural input did not return to the true baseline during the rest periods of the paradigm, the stimulation measurement could be in reference to this elevated baseline. Given that the temporal resolution of SEEP fMRI is about seven seconds (compared to neuronal firing which is significantly more rapid) this is an extremely likely scenario. So while we did not observe the expected increase in signal change that would result due to increased neural input from the descending inhibition, we observed the resultant decrease in signal change due to said inhibition. Thus it appears that the persistent positive signal change observed in the CTS ipsilateral DH at the highest pain level is atypical, and could represent a lack of descending modulation to the incoming noxious stimulus in the patient group, resulting in persistent pain that is not endogenously modified.
The lack of activity in the contralateral DH in patients compared to controls (at all pain levels) likewise implies a lack of descending sensory modulation, which theoretically descends bilaterally (Fields et al. 2006). The sparse contralateral VH activity at pain 2 in both groups has been similarly observed in sensory stimuli fMRI studies (Ghazni et al. 2009; Stroman et al. 2004; Stroman et al. 2009; Stroman 2009a), and may be the result of an initial withdrawal reflex, particularly following noxious stimuli, or the activation of interneurons in the region. The positive signal change observed in the central GM in the region of lamina X in the CTS group at pain 6 suggests the presence of visceral pain (Westlund et al. 2009), as perhaps the patients can quite literally ‘not stomach the pain’. There may be some link between visceral pain processing and the feelings of ‘unpleasantness’ that NP patients attribute so strongly to their pain. Indeed, NP patients rate their pain as more ‘unpleasant’ than ‘intense’ on the VAS (Table 2.2), and visceral pain is commonly perceived in the same way. In a study comparing somatic and visceral pain, visceral pain was rated significantly more unpleasant than somatic pain (despite the fact the somatic stimulus was more intense) and even resulted in greater activation in various nuclei in the medulla and midbrain associated with the pain response (Dunckley et al. 2007).

**Medulla**

At pain 2, the control group showed robust positive signal change in the RVM (comprised of the NRM and adjacent RF) and the NGc, while the activity in the CTS group medulla was primarily confined to the RF (Figure 3.4). The NGc receives primarily ascending nociceptive information, the RF is a target for both ascending and descending projections (Cechetto et al. 1985), and the NRM receives primarily descending input (projections coming
from the SC are sparse) (Fields et al. 2006). This makes the NRM (along with the contralateral DH) one of two anatomical regions that give us an indication of descending modulation alone, not confounded by ascending sensory or noxious activity. Results for both groups are similar at pain 4, that is, controls showed positive signal change in the NRM and RF while the CTS group showed only RF activity. This result suggests a difference in, and possibly a lack of, descending modulation of pain at the level of the medulla in the CTS group. Though the NRM is only one of two components of the RVM, the main (if not only) relay point for PAG stimulation-induced analgesia (Fields et al. 1991), it is probably the most critical. Studies have shown that when the NRM specifically is lesioned, analgesia via midbrain morphine injection is significantly reduced (Haghparast et al. 2008). So while the medullary RF receives descending projections from the midbrain, they are supplementary to those projections targeting the NRM.

Interestingly, at pain 6 the control group NRM and the CTS group RF both exhibited negative signal intensity change. Decreased neural input at the highest level of pain in a region implicated in the active modulation of nociception is initially counterintuitive, and in fact is not consistent with the results from other fMRI studies of pain in the brainstem which instead support results from pain 2 and pain 4. That is, following painful heat (Cahill and Stroman 2010; Fairhurst et al. 2007), hyperalgesic (Ghazni et al. 2009) or allodynic (Mainero et al. 2007) punctuate mechanical, visceral (Westlund et al. 2009), or noxious intranasal ammonia stimulation (Stankewitz et al. 2009) there is a consistent and robust activation of the entire RVM. All of these studies employed BOLD-fMRI, with the aforementioned issues of spatial resolution, so it is possible that with SEEP-fMRI we are observing a specific result.
other groups are not able to see. A previous study from our laboratory has noted a similar overall decrease in activity as the pain rating to a noxious thermal stimulus increased. This decrease may be a result of a negative feedback loop serving to turn off the modulation of the sensations. In contrast, the subjects could have become accustomed to the stimulus and less anxious about its application, as anxiety has been shown to increase activity in various regions of the brainstem pain systems, including the RVM (Wise et al. 2007).

**PONS**

Activity in the pons varied significantly between the control and CTS group (Figure 3.5), despite being consistently positive at pain 2. The robust bilateral distribution of signal change in the control group appears significantly different from the comparatively sparse distribution of contralateral (left) activity in the CTS group. In fact, this pattern of bilateral-control and contralateral-CTS activity holds at pain 4 and pain 6 as well, in regions consistent with the DLPT, pontine RF, and PBN. Most groups that have found pontine activation in the DLPT (Cahill and Stroman 2010; Dunckley et al. 2007), PBN (Fairhurst et al. 2007; Seifert and Maihofner 2007), and RF (Ghazni et al. 2009) due to noxious stimulation do not report a difference in the laterality of this response, though Weigelt and colleagues did discern predominantly contralateral pontine activity following electrical stimulation of tooth pulp (Weigelt et al. 2010). Importantly, none of these groups used a clinical population suffering NP, at best studying the effects of a painful stimulus following an established model of hyperalgesia or allodynia. However, when a population of patients suffering diabetic neuropathy simply lay in the magnet and were scanned, awake with eyes closed, the left brainstem, including the left pons, was significantly more active (Cauda et al. 2009). It is
possible that the predominantly contralateral (left) pontine activity in the CTS group was caused by this skewed resting state.

A lack of descending modulation at the level of the pons could provide another compelling explanation for the unilateral activity observed in the pons. As was previously mentioned, ascending nociceptive tracts cross at midline to reach targets in the medulla, pons, and midbrain contralateral to the stimulus (Dostrovsky and Craig 2006), readily explaining contralateral activity in the brainstem of both groups. In contrast, the bilateral distribution of the descending modulation (Fields et al. 2006) should theoretically produce activity in both the ipsi- and contralateral brainstem. The DLPT, like the RVM, receives excitatory projections from the PAG, in turn sending inhibitory projections to the DH of the SC to modulate nociception (Beitz 1982). The fact that the ipsilateral pons remains relatively inactive in the CTS group implies that the DLPT route is bypassed, providing another piece of evidence suggestive of a deficiency in the descending systems. This is further confirmed by the presence of ipsilateral pontine activity in the control group, which should be an indication of normal ascending and descending function. Intriguingly, the trend of decreasing signal change with increasing pain ratings remains in both groups at the pontine level, and again could represent negative feedback or be the result of an elevated baseline of activity from prior stimuli.

**Midbrain**

The midbrain contains several nuclei that are important targets for the SMT, which projects from the DH of the SC to the PAG, NCF, SC, interstitial nucleus of Cajal, red nucleus and nucleus of Darkschewitsch (Mehler et al. 1960). Additionally, the PAG is the first
synaptic point of the descending modulation system in the brainstem. The PAG integrates inputs from the ACC, amygdala, thalamus, hypothalamus, and other cortical sites with the ascending nociceptive input from the DH (Bandler and Keay 1996). At pain 2, the control group showed positive signal change in the target nuclei of the SMT except the PAG, however we observed positive signal change localized to the PAG in the CTS group (Figure 3.6). As the pain level increases, control group activity in all areas virtually disappears yet the CTS group activity follows the decreasing trend observed in other regions, with the signal change transitioning from positive at pain 2 and pain 4 to negative at pain 6 (discussed previously). Many groups have shown midbrain activation in control subject populations following painful stimulation. Somatic, visceral (Dunckley et al. 2007), thermal (Cahill and Stroman 2010), and punctuate (Ghazni et al. 2009; Mainero et al. 2007; Zambreanu et al. 2005) noxious insult all produce robust activity in the PAG, and (albeit less consistently) in the NCF, SC, and red nucleus. These results coupled with the plethora of non-fMRI research confirming the importance and universality of PAG involvement in ascending ((Dostrovsky and Craig 2006), for a review) and descending ((Fields et al. 2006), for a review) pain systems make the lack of PAG signal change in the control group particularly perplexing.

The manipulation of various cognitive factors (such as attention or anticipation) appears to effect PAG activation during noxious stimulation in controls. If the subject’s attention was distracted from the pain rather than focused on it, a 25% decrease in PAG activation was observed (Tracey et al. 2002). So while the stimulus may have been more salient or intense to subjects in the CTS group, thus holding their attention, the control subjects perhaps became tolerant and in fact bored with the stimulus. They instead may have
attended to the sounds of the MR scanner or simply began thinking about something else, both of which may have served as a suitable distraction to their attention. Conversely, because the onset of the painful stimulus was signaled by a tone, the subject’s anticipation of the stimulus may play a modulatory role in the PAG as well. Berman and colleagues found decreased activity in the dorsal brainstem (including the midbrain PAG) in controls anticipating noxious rectal distension (Berman et al. 2008). The chronic pain population, women diagnosed with irritable bowel syndrome (IBS), exhibited no such decrease. Complications associated with NP may also contribute to the marked difference between control and CTS group PAG activity, as mechanical allodynia appears to result in increased PAG, RVM, and DLPT activity (Mainero et al. 2007). Finally, it must be noted that, in light of the importance and expectation of PAG involvement in normal pain transmission and modulation, the lack of signal change detected in the control group PAG may be the result of a Type II (false negative) error in data analysis (Cohen-Adad et al. 2009; Stroman 2009a).

**Psychophysical Results**

The psychophysical results obtained were expected yet intriguing. Previous groups have standardized the perception of pain between groups using rigorous threshold testing and questionnaire administration (Gracely et al. 2002; Staud et al. 2008), so rather than use the same stimulus they used the same pain ratings. We employed this method as well, for reasons outlined in results (Section 3.1, Table 3.1). Like Gracely and colleagues, we found significant differences in the stimulus intensity needed to achieve pain 2, pain 4, and pain 6 between the control and the patient group (Gracely et al. 2002). In general, both studies showed that the chronic pain (fibromyalgia and CTS) group required a less intense stimulus to produce the
same pain rating as the control group. Furthermore, they found that when pain perception was equalized, similar areas in the brain were activated in both groups. Interestingly, they included a second part to the study which alternatively used an equi-intense stimulus on both patients and controls. In this condition, patients exhibited many more regions of activity than control subjects. We did not include a second part to incorporate an equally intense stimulus in our study design, but upon inspection of our psychophysical results, an interesting pattern appeared which facilitated a similar comparison.

When the mean pressure used to elicit each pain rating was assessed, we determined that almost all comparisons were significantly different (Table 4.1). Specifically, pressures between control and CTS subjects at each pain level and within groups between each pain level. However, the pressure used to elicit a rating of ‘pain 2’ in controls was not significantly different from the pressure required to induce a rating of ‘pain 4’ or ‘pain 6’ in CTS subjects. So while we did not experimentally control for stimulus intensity, we can nonetheless draw comparisons between our results and those from Gracey et al.

<table>
<thead>
<tr>
<th></th>
<th>PAIN = 2</th>
<th>PAIN = 4</th>
<th>PAIN = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
<td>CTS</td>
<td>CONTROL</td>
</tr>
<tr>
<td>PAIN = 2</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>CTS</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>PAIN = 4</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>CTS</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>PAIN = 6</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>CTS</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1. Mean pressure used to elicit pain response in subjects. Using a two-tailed Student’s t-test (p > 0.05), the mean pressures used (on the right wrist) between groups at pain levels 2, 4, and 6 were assessed. Means that were significantly different are indicated (*), and means that were not significantly different are hi-lighted.
We did not observe more activity *per se* in the SC or brainstem regions of interest when the intensity of the stimulus was equalized, but several interesting features appeared (Figure 4.1). The positive signal change in the DH of the SC is virtually the only region consistent between ‘Control pain 2’, ‘CTS pain 4’, and ‘CTS pain 6’, despite the equalized intensity. The negative signal change in the brainstem is relatively consistent between ‘CTS pain 4’ and ‘CTS pain 6’, but markedly different from the positive signal change observed in brainstem regions in ‘Control pain 2’. Moreover, there were a greater number of voxels active in the control group than in the CTS group at either pain level, yet the CTS activity appears rather dispersed across the various brainstem regions, particularly the medulla.

![Figure 4.1](image)

**Figure 4.1.** Comparison of group results following equi-intense stimulation. Group results for the control subjects (n = 13) at pain 2 and CTS subjects (n = 9) at pain 4 and pain 6. Mean pressure used to elicit these ratings are indicated below each group.
General Trends

The major trend observed in our results is the counterintuitive decrease in signal intensity change (from positive to negative) as the pain level increases (Figure 3.7). This decrease occurred in each region of interest, with the notable exception of the DH of the SC in the CTS group. A negative correlation between pain ratings and signal intensity change has been observed before (Cahill and Stroman 2010; Mainero et al. 2007; Stroman 2009a), but because the activity detected with fMRI reflects both excitatory and inhibitory neural input as well as local processing (Logothetis et al. 2001; Stroman et al. 2005a), it is difficult to determine the mechanism which underlies this decrease. Perhaps the most likely explanatory mechanism is a reduction in tonic inhibition of ascending nociceptive information. Tonic inhibition in the CNS occurs via innervations from GABAergic (Halasy and Somogyi 1993; Han et al. 1993) and cholinergic (Proudfit and Hammond 1981; Yaksh 1979) neurons, and has been implicated as a method of spinal analgesia (Zhuo et al. 1993). If this inhibition that produces spinal analgesia is reduced, subjective pain ratings would increase while at the same time neural input to the region decreased, thereby resulting in the observed decrease in signal intensity change. In any case, this trend of decreasing signal change as pain increases is consistent between the control and CTS group in this study, between various studies from this laboratory (Cahill and Stroman 2010; Stroman 2009a), and from studies conducted by other groups (Mainero et al. 2007). If it is a result of reduced tonic inhibition and therefore enhanced pain, it certainly warrants further investigation as a potential mechanism underlying complications associated with NP.
4.2.3 A Conundrum

Perhaps the most intriguing result of this study was the lack of PAG activity in the control group following noxious stimulation. Research, including fMRI studies, suggests that the PAG is a critical relay point in the transmission and (in particular) modulation of pain. In fact, virtually every fMRI study investigating the involvement of the brainstem in pain processing has found robust activation in the PAG (Cahill and Stroman 2010; Dunckley et al. 2007; Eippert et al. 2009a; Fairhurst et al. 2007; Ghazni et al. 2009; Mainero et al. 2007; Tracey et al. 2002; Westlund et al. 2009; Zambreanu et al. 2005). The nature of this lack of activity has been thoroughly discussed (See Section 4.2.2), yet an important question remains.

If the lack of signal change is a true result and not due to an error in data analysis, how is this control group modulating ascending nociceptive transmission? There is evidence of nociceptive modulation in the control group, as the signal change observed in the control NRM and contralateral DH is not attributable to the ascending transmission of pain, yet is consistent with the descending modulation system. The PAG sends very few projections directly to the DH of the SC (Fields et al. 1991), but rather relies on synaptic relay sites in the DLPT and RVM to elicit nociceptive modulation (See Figure 1.3) (Fields et al. 2006). However, there are many reciprocal connections between the DLPT and the RVM, and signal change was observed in both these regions in the control group. Furthermore, electrical stimulation of the LC (part of the DLPT) results in spinal analgesia (Willis and Westlund 1997), which may explain control group modulation of pain despite the lack of activity observed in the PAG.
Regardless of the unexpected lack of control PAG activation, the most important conclusion drawn from this study is the observed activity difference in areas associated *exclusively* with the descending modulation of pain between the control and CTS subject groups. Namely, the NRM and the contralateral DH of the SC, both of which do not receive projections due to ascending nociception, but are targets in the descending modulation system (most of the other regions implicated in pain processing receive both ascending and descending input). The signal change observed in the contralateral DH and the NRM in the control group was not observed in the CTS group. This is a strong indicator of a functional difference between normal and pathological pain processing via the descending modulation system. Equal pain from a weaker stimulus, and potentially the described clinical pain in daily life, may be explained by this lack of descending modulation in the CTS group.

4.3 **Interpretations**

Pain is a conscious experience, influenced by emotional, pathological, genetic, and cognitive factors, and even memories. Pain is therefore not necessarily linearly related to nociceptive input or tissue injury, and in fact, perceived pain has been shown to be independent of physical sensory input altogether (Raij *et al.* 2005). Unfortunately for the 20% of adults that suffer from chronic pain (Breivik *et al.* 2006), these features make pain difficult to assess, investigate, manage and treat (Tracey 2008). The neural signature of pain varies dependent on the neuromatrix (described below), whether the individual is healthy or suffering chronic pain, and whether the stimulus is experimental or a provocation of existing
clinical pain. The results from this study contribute to the growing body of information surrounding clinical, chronic pain.

### 4.3.1 The Neuromatrix: A Pattern-Generating Mechanism

The neuromatrix for pain perception (Figure 4.2) is a theoretical mechanism that combines sensory input and descending modulation with affective and cognitive factors to generate a pain experience unique to each individual (Loeser and Melzack 1999). We have discussed the effects of sensory input, descending modulation, attention, and anticipation on pain at length in previous sections, but mood, genetics and past experience are also important inputs to the neuromatrix. An anxious or depressed mood appears to have the greatest emotional impact on pain perception. Both anxiety (Fairhurst et al. 2007) and depression (Giesecke et al. 2005) result in increased activity in the amygdala and anterior insula, cortical regions associated with pain processing. It is also likely that our genes determine how nociceptive stimuli are processed and in turn how the brain reacts to this stimulation. Coghill and colleagues compared a group of subjects who identified as ‘sensitive’ to pain to a group of subjects who claimed to ‘tolerate pain well’. They found that those individuals who identified as ‘sensitive’ consistently rated pain higher than the ‘tolerant’ group and also exhibited more robust pain-induced activation of S1, ACC and PFC (Coghill et al. 2003). However, the age-old question remains: is it nature or nurture? Thus far, it would appear to be both. Individuals homozygous for the met158 allele showed a reduced opioid system response to a painful stimulus and consistently rated the stimulus as more intense and unpleasant compared to heterozygotes (Zubieta et al. 2003). On the other hand, a one-time substantial nociceptive
insult can permanently alter SC function and lead to chronic pain via the excitatory toxic effects of certain amino acids (Dubner and Ruda 1992). Both our genes and past experience (including non-painful experience) undoubtedly affect the evaluative aspect of pain as our mind attempts to rationalize the experience. An extensive neural network is recruited which incorporates all the inputs discussed to produce a cerebral output unique among individuals.

**Figure 4.2.** The neuromatrix. A schematic of the pattern generating mechanism that combines sensory inputs, as well as affective and cognitive components to yield the unique pain experience of the individual, beyond the relatively simple consequences of tissue injury. Adapted from Loeser and Melzack (Loeser and Melzack 1999).

### 4.3.2 Experimental versus Clinical Pain

It is widely accepted that individuals suffering from chronic pain present with different (albeit overlapping) regions of cortical activation compared to healthy individuals following acute noxious stimulation. A meta-analysis of various studies investigating the difference in acute and chronic pain processing has revealed that chronic, clinical conditions
more frequently involve the PFC (81% of clinical populations versus 55% of normal populations). The enhanced activation of the PFC in chronic pain conditions may suggest stronger cognitive and emotional components, thus increased affective processing of the nociceptive information in patients (Apkarian et al. 2005).

Most studies attempt to differentiate acute and chronic pain by applying the same painful stimulus to both a healthy and clinical population. For instance, painful thermal stimuli applied to the hand of patients suffering from rheumatoid arthritis (Jones and Derbyshire 1997), atypical facial pain (Derbyshire et al. 1994), and patients with post-tooth extraction pain (Derbyshire et al. 1999) resulted in decreased activity in brain regions normally active in control subjects following thermal pain. Unfortunately, these results are mostly un-interpretable because the stimulus was applied to a site remote from where the clinical pain is felt and no psychophysical questionnaires were administered to standardize pain thresholds between groups. Furthermore, this stimulus does not reproduce the clinical pain and therefore does not (necessarily) reflect changes in properties fundamental to the condition.

Derbyshire and colleagues conducted another study, again using a painful thermal stimulus, on a large population of low back pain patients and healthy controls and compared the resulting neural activity. They found no discernible difference in cortical activation between the patient and control groups (Derbyshire et al. 2002). A study by Apkarian and colleagues confirmed and extended upon this result in patients with complex regional pain syndrome (CRPS). Again following painful thermal stimulation to the hand, the activity in the patient group closely matched that of the control group. However, when sympathetic
blocks were used to specifically reduce the ongoing CRPS symptoms but not the activity caused by the thermal pain, significant differences appeared between groups (Apkarian et al. 2001). Thus, there is little compelling evidence that neural activity following experimental painful stimulation predicts the pattern of activity in chronic, clinical pain states. Conversely, provoking clinical pain has provided an alternate means of investigating brain activity associated with pathological pain processing. Our study utilized a mechanical stimulus designed to provoke CTS-like NP symptoms in our patient population. Equalizing the pain perceived between groups and utilizing a stimulus designed to provoke clinical pain has been done previously (Gracely et al. 2002), and resulted in general trends similar to those found in our study. In both cases, equal pain ratings resulted in similar quantities of active voxels, yet equal stimulus intensity resulted in significantly different cortical activity in the patient group.

4.4 Limitations

There are several limitations related to the design of this study specifically and to fMRI in general that may have impacted the observed results. While it is quite simple to recruit subjects aged 18 to 22 years on a university campus, it is not as simple to recruit age-matched controls for a NP patient population (typically 30 to 60 years old). So despite the inclusion of four 40+ year old control subjects, the median age of the control and patient groups are significantly different (control = 21, CTS = 32). Future studies would benefit from age-matching the control population with the patient population to facilitate more useful comparisons and interpretations with regards to healthy and pathological pain processing.
The stimulus itself is a potential source of considerable variability within the study. Though the electromyography guideline was used to locate the median nerve and guide placement of the stimulation device, the anatomical variability that occurs naturally between individuals may have confounded the stimulation paradigm.

The order of painful stimulation in the block paradigm may also have affected the outcome of this study. The stimuli were always applied in the same (increasing) order: ‘pain 2’ then ‘pain 4’ then ‘pain 6’. Though it is standard practice to randomize the stimulation paradigm to eliminate order effects, we always began with the lowest pressure and increased to the highest to allow visualization of the neural response to the least intense stimulus. If the first stimulation block had consisted of the highest pressure, it is possible that the individual’s pain response would have remained elevated for the rest of the study. Therefore, the ramping effect which results from our consistent increase in stimulation pressure is a trade-off to allow assessment of all three pain levels. Finally, the block design used consisted of baseline periods of 72 seconds interleaved with stimulation periods of 45 seconds. This design is limited in that it requires that the neural activity and MR signal changes related to the stimuli appear during stimulation and disappear during rest. Unfortunately, the pain response does not necessarily comply, as the pain may persist despite the removal of the stimulus, and may result in sustained elevated neural activity even during rest periods. Therefore, it is possible that the neural activity does not return to baseline during rest periods, and that this residual activity overlaps with the next stimulation period, potentially confounding the results.
The fMRI method in general presents some unavoidable limitations which impede interpretation of results. There is a possibility of Type I and Type II errors following fMRI data analysis. Type I (false positive) errors indicate activity that is not true neural activity, often occurring along the edge of the SC, as CSF and large-vessel blood flow cause SC movement. Type II (false negative) errors indicate that we did not detect all the neural activity truly present in the gray matter. Evidence also shows that Type II errors are more common than Type I errors, therefore it is more likely to not detect neural activity than it is to detect neural activity that does not exist (Stroman 2006). Even if these errors are minimized, large individual differences reflecting distinct patterns of neural activity are evident within a single experiment despite controlling all factors (Davis et al. 1998).

Unlike fMRI studies utilizing standard Talairach coordinates to investigate the brain, there is no standard coordinate space to spatially locate areas of the brainstem and SC. In fact, it is only recently that SC and brainstem fMRI data have been spatially normalized to facilitate voxel-by-voxel group analysis. Thus, we used various stereotaxic atlases to visually identify the neural activity we measured. The activity shown is unmasked, meaning that all activity that fell within a stringent statistical threshold (and is therefore significant) is shown.

Finally, as has been previously discussed, the interpretation of fMRI results is somewhat limited due to the nature of the signal obtained. In 2001, Logothetis and colleagues helpfully determined that the BOLD fMRI response reflects neural input in a given area as opposed to spiking output (Logothetis et al. 2001). Because previous studies comparing SEEP and BOLD signal changes demonstrate related changes and areas of activity (Stroman et al. 2005b), we infer the SEEP fMRI response to likewise reflects neural input.
Increased neural input to an area can be the result of both excitatory and inhibitory input. We are not able to distinguish the direction of synaptic activity in an area because we are unable to differentiate between increased excitatory and increased inhibitory activity, which elicit opposite physiological effects. Thus, fMRI results can be ambiguous, and allow for more than one interpretation.

### 4.5 Significance and Future Directions

Results from this study are valuable because they represent a clinical population and utilize a stimulus that provokes this clinical pain. Furthermore, they give us insight into the SC and brainstem processing of chronic, NP pain. There are many conditions known to result in NP, and future studies could investigate the neural response to pain in many of these, focusing on the SC and brainstem, both of which have been essentially ignored in most neuroimaging studies.

These future investigations should manipulate and improve upon the stimulus and conditions used to allow for more specific interpretations to be made. If an experimental stimulus is used, it should reliably and quantifiably induce pain. Some groups utilize a YAG (yttrium-aluminum-granate) laser, which induces pain but has no tactile component (Bingel et al. 2002). This could allow the pain system to be assessed without confounding sensory input. Another way to aid interpretation is to manipulate neural responses with various drugs. Several fMRI studies have investigated nociceptive modulation due to analgesic-antagonists (Eippert et al. 2009a), anticonvulsants (Iannetti et al. 2005), and anxiolytics (Wise et al. 2007). Subjects in the drug groups subjectively rate pain differently, and this
difference is manifest in the fMRI response. The knowledge gained from future pain studies which employ varying stimuli, conditions, and subject populations enriches our understanding of pain processing in humans, and will undoubtedly contribute to improving pain management and treatment for those suffering from chronic pain.
Chapter 5

Summary

This study is the first to investigate the neural response to provoked clinical pain in the SC and brainstem of a neuropathic pain patient population. Less noxious pressure was required to elicit equal pain rating in patients compared to controls. When the pain perceived was equalized between groups, both consistent trends and observable differences were apparent in the group fMRI results. Both control and patient groups showed a general trend of decreasing signal intensity change as the pain rating increased. This decreasing signal change may reflect a reduction in tonic inhibition which has the effect of enhancing nociception and causing pain ratings to increase. We observed consistent signal change in both groups in regions anatomically consistent with relay points in ascending nociceptive pathways and descending modulation systems. Controls and patients alike exhibited similar activation in the ipsilateral DH (the primarily synaptic site in every pain pathway) as well as in target nuclei of ascending nociceptive tracts (SMT and SRT). There were few, if any, discernible differences in group results that could be specifically attributed to the transmission of pain. However, there were significant differences between groups in areas anatomically consistent with the NRM and the contralateral DH. The NRM receives few ascending projections from the DH and the contralateral DH is not associated with the transmission of pain whatsoever. Both these regions are specifically associated with the descending modulation of pain. Controls exhibit the expected signal change in both regions, while the patient group does not show activity in either. This result strongly suggests a malfunction in the descending modulation (as opposed to the ascending transmission) of pain.
in the NP population. This may be a manifestation of the maladaptive plasticity postulated to result in the neural disease state and the symptomatic pain. Though there are limits to the conclusions that can be drawn from the results, it is clear that this method of spinal fMRI is a reliable and sensitive technique that is able to detect changes in neural activity related to the transmission and modulation of pain. Furthermore, spinal fMRI has allowed us to not only visualize functional differences between healthy and pathological pain processing, but to specify that the nature of this difference most likely lies in the descending modulation system. Confirmation of this feature of pathological pain processing in humans is an important step in understanding the transmission and modulation of pain. With a better understanding of the changes that occur in the NP state, we may better diagnose, manage and treat chronic pain in the future, for as an ancient Buddhist proverb tells us, “Pain is inevitable, suffering is optional”. The results from this study bring us closer to providing options to those individuals suffering from chronic pain.
References


Dougherty, P.M., Palecek, J., Paleckova, V., Sorkin, L.S., Willis, W.D., 1992. The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal, and electrical stimuli. Journal of Neuroscience 12, 3025-3041.


Li, G., Ng, M.C., Wong, K.K., Luk, K.D., Yang, E.S., 2005. Spinal effects of acupuncture stimulation assessed by proton density-weighted functional magnetic resonance imaging at 0.2 T. Magn Reson.Imaging 23, 995-999.


LoPinto, C., Young, W.B., Ashkenazi, A., 2006. Comparison of dynamic (brush) and static (pressure) mechanical allodynia in migraine. Cephalalgia 26, 852-856.


Ng, M.C., Wong, K.K., Li, G., Lai, S., Yang, E.S., Hu, Y., Luk, K.D., 2006. Proton-density-weighted spinal fMRI with sensorimotor stimulation at 0.2 T. NeuroImage 29, 995-999.


Phalen, G.S., 1970. Reflections on 21 years' experience with the carpal-tunnel syndrome. JAMA 212, 1365-1367.


Stroman, P.W., 2009b. Spinal fMRI investigation of human spinal cord function over a range of innocuous thermal sensory stimuli and study-related emotional influences.


Appendix A: Recruitment Poster - Control

Pain Research using fMRI
Individuals with no history of are needed for Studies at Queen’s University
(New studies starting January 2009)

Individuals (18-60) with diagnosed and untreated carpal tunnel syndrome are needed to study pain pathways in the brainstem and spinal cord using functional magnetic resonance imaging (fMRI). Participation in the study involves a visit to the Queen’s fMRI Facility in the lower level of the Cancer Research Institute, and will last about 2 hours in total. The studies are completely non-invasive and harmless. A $40 honorarium will be provided to cover your time and expenses. We would greatly appreciate your participation!

For more information please contact:
Jordan Leitch
Email: 4jl7@queensu.ca
Appendix B: Recruitment Poster - Patient

Pain Research using fMRI

Individuals with **Carpal Tunnel Syndrome** are Needed for Studies at Queen’s University

(New studies starting January 2009)

Individuals (18-60) with **diagnosed** and **untreated** carpal tunnel syndrome are needed to study pain pathways in the brainstem and spinal cord using functional magnetic resonance imaging (fMRI). Participation in the study involves a visit to the Queen’s fMRI Facility in the lower level of the Cancer Research Institute, and will last about 2 hours in total. The studies are completely non-invasive and harmless. A $40 honorarium will be provided to cover your time and expenses. We would greatly appreciate your participation!

**For more information please contact:**

Jordan Leitch
Email: 4jl7@queensu.ca
Appendix C: Newspaper Advertisement

Carpal Tunnel Syndrome Study

Individuals (18-60 yrs) with diagnosed and untreated CTS are needed to study pain processing in the spinal cord and brainstem using functional magnetic resonance imaging (fMRI). Contact Jordan Leitch by email at 4JL7@queensu.ca or by phone at 613 533 6000 ex. 79981

fMRI is completely non-invasive and harmless

Participation requires one 2 hour visit to the Queen’s University MRI Facility

For your participation, you will receive a $40 honorarium
Appendix D: MRI Safety Checklist

Centre for Neuroscience Studies
fMRI Facility

MAGNETIC RESONANCE (MR) ENVIRONMENT SAFETY CHECKLIST FOR INDIVIDUALS

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.

Please Circle
Have you had prior surgery or an operation (e.g. arthroscopy, endoscopy, etc.) of any kind? Yes No
Have you had an injury to the eye involving a metallic object (e.g. metallic silvers, foreign body)? Yes No
Have you ever been injured by a metallic object or foreign body (e.g. BB, bullet, shrapnel, etc.)? Yes No
Are you pregnant or suspect that you are pregnant? Yes No

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 or object.

Please indicate if you have any of the following:

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

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

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

Person Completing Form:

Print Name
Signature
Date

Form Reviewed By:

Print Name
Signature
Date Position

116
Appendix E: Volunteer Consent Form

TITLE OF PROJECT: Spinal cord and brainstem activation in carpal tunnel syndrome patients in response to noxious stimuli using spinal function magnetic resonance imaging

BACKGROUND INFORMATION: (Overview of study)
You are invited to participate in a research study directed by Dr. Patrick Stroman and Dr. Cathy Cahill. The purpose of this study is to map pain pathways in the brainstem and spinal cord in individuals experiencing chronic, neuropathic pain via functional magnetic resonance imaging (fMRI). Participation in the study involves two visits to the Queen’s fMRI Facility in the lower level of the Cancer Research Institute, and will last about 3 hours in total.

DETAILS OF THE STUDY

1. What the aim of the study is:
The current study has two parts:
Part 1: To determine the activity elicited by a painful stimulus in a population of individuals diagnosed as having carpal tunnel syndrome (CTS).
Part 2: To compare this activity to the activity elicited by the same stimulus is healthy control subjects.

2. Description of visits, dosage, tests to be performed as part of the study:
If you agree to participate, your brainstem and SC will be imaged while you are lying in a 3 Tesla magnetic resonance imaging (MRI) scanner in the Queen’s fMRI Facility, and your heart beat and breathing may be monitored using entirely non-invasive methods. The entire session may last up to 2 hours over the course of the visit, including getting ready for the study and being positioned in the magnet etc. This study involves a visit to the lower level of the Cancer Research Institute for imaging.

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

b) During Part 1 (30 minutes), you will begin by filling out several questionnaires. Then, the pain stimulus will be applied to determine your own personal pain
threshold and to allow you to be comfortable with the procedure. We will conduct a simulated run of the experiment in the mock scanner. A mock scanner is similar in setup to the actual MRI scanner except there is no magnetic field turned on and we will acquire no images. A trial run in the mock scanner is important so that you can habituate to the actual scanner and its environment. We will apply the stimulus to achieve reported pain ratings of 2, 4, and 6. During Part 2 (1.5 hours), the exact same experiment will be conducted, but we will continuously acquire MR images in the actual scanner, and we will ask you to rate the pain intensity and unpleasantness on a scale of 0-10 (0 = no pain and 10 = worst possible pain imaginable) during each stimulus by visually fixating on the number that represents your pain on a pain scale presented to you.

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

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

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

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

g) For brainstem and cervical spinal cord imaging studies, a neck coil will be placed over the lower portion of your face. This coil is fitted with a mirror so that you can see out of the magnet towards a screen. For SC imaging studies, you will lay on top of a flat spine coil that looks like a part of the bed you are lying on. You and the bed will then slide into a long tube (the magnet).

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

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

j) All functional MRI studies require periods of rest interleaved with periods of sensation or activity so that we can detect the differences in the brain or SC that show where there was activity. We will inform you about each sensation or task before each experiment begins so that you know what to expect.

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

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

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

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

4. **Alternative Therapies:**

Does not apply.

5. **Risks/Side-Effects:**

There are no known risks involved with magnetic resonance imaging. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic or magnetic objects must be removed from your person before you approach the scanner.

6. **Benefits**

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

7. **Exclusions:**

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

(any of the following)

a) have a history of head or eye injury involving metal fragments.

b) have ever worked in a metal shop

c) have some type of implanted electrical device (such as a cardiac pacemaker or neurostimulator)

d) have implanted metal objects as a result of surgery such as artificial joints, aneurysm clips, metal staples
e) have severe heart disease (including susceptibility to arrhythmias) or any other serious illness
f) are wearing metal braces on your teeth
g) have non-removable jewelry (body piercing)
h) are, or may be, pregnant

8. Confidentiality

The findings of this study will be reported in scientific journals but your name will remain confidential. Data from your images will be stored on a secure computer system and identified only with the date and a subject code. Only the researchers directly related to this study will have access to the data files and the subject codes. You will not be identified in any publication or reports. Although this is not a diagnostic scan and any images obtained are for research purposes only, it is possible that the MR scan may disclose an unknown abnormality. In this event, a medical imaging specialist will be asked to review the images and we would send a report to your physician. The researchers directly involved with this procedure do not have the credentials to diagnose medical conditions.

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

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

10. Withdrawal of subject by principal investigator:

The study Director may decide to withdraw you from this study if:
1) you do not meet the criteria in the Magnetic Resonance Screening Form.
2) you are unable to perform the tasks requested.

11. Liability:

"In the event that you are injured as a result of taking study medication or of the study procedures, medical care will be provided to you until resolution of the medical problem. By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities."
12. **Payment**: Some studies compensate for subject’s expenses and inconvenience.

You will receive $40 to cover your costs for parking, transportation to Queen’s, etc, for participating in this study.

**SUBJECT STATEMENT AND SIGNATURE SECTION:**

13. **Description of how subject is informed of study** (e.g. protocol read with doctor, consent form discussed). List Principal Investigator and Department Head as contacts, and provide telephone numbers should subjects have questions or problems.

   The format for this section is standard.

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

Dr. Patrick Stroman (P.I.)                           Dr. Cathy Cahill (P.I.)

Queen's University                                 Queen's University
Kingston, Ontario                                   Kingston, Ontario
K7L 2V7                                             K7L 2V7
Phone: (613) 533-3245                                Phone: (613) 533-6162
Fax: (613) 533-6840                                  Fax: (613) 533-6412

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

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

___________________________  ____________________
Signature of Volunteer             Date

___________________________  ____________________
Signature of Witness               Date
STATEMENT OF INVESTIGATOR:

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

____________________________  ______________________
Signature of Principal Investigator                  Date

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

Participant Consent Form

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

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

Subject Name (please print): ________________________________

Signature: ___________________________________ Date: ____________

Individual responsible for obtaining consent:

Signature: ___________________________________ Date: ____________

Investigator:
Signature: ___________________________________ Date: ____________
Appendix F: Volunteer Details

Spinal cord and brainstem activation in carpal tunnel syndrome patients in response to noxious stimuli using spinal function magnetic resonance imaging

My goal is to use spinal functional magnetic resonance imaging (fMRI) to locate the specific pathways through which pain travels in the spinal cord; specifically, in individuals that experience chronic pain, such as carpal tunnel syndrome (CTS). By mapping pain pathways in individuals experiencing chronic pain, I hope to expand our knowledge of pain transmission and contribute to an improved therapeutic strategy to treat all types of chronic pain. To help out with my study, your participation will involve a visit to the MRI Facility located in the Cancer Research Institute.

To find the MRI facility, go to the south end of the Cancer Research Institute (CRI) building, at 15 O'Kill Street. There is a small parking lot between O'Kill street and the CRI building, and from the street the entrance is not clearly visible because it is in small stairwell, but there is a sign on the building. Once you are at the entrance you will see a sign for the MRI Facility on the wall to the left, and below the sign is a call button. Press the call button to have someone come and open the door to let you in. Map: http://www.queensu.ca/neurosci/MRI/MRI_map.jpg

The details of this particular study are as follows:

In this study, we will image the activity in your SC and brainstem. Part 1 (30 minutes) will consist of a brief assessment of your symptoms, two brief questionnaires, and we will establish your personal pain threshold (that is, when you begin to experience pain). For part 2 (1.5 hours maximum), you will fill out a brief MRI safety screening questionnaire and we will proceed to image your spinal cord and brainstem using fMRI. The design of the study is as follows: 1) While in the scanner, you will initially undergo two scans both using the wrist/arm indicated in your CTS. Each stimulus will be applied (with increasing pressure) three times in each run for 45 seconds each. These stimulation blocks will be interleaved with rest blocks of 72 seconds each. 2) Two final runs will be conducted in the same manner but on the opposite wrist not indicated in your CTS. FMRI is completely non-invasive (it does not penetrate the skin or any body cavity), and you are able to withdraw from the study at any time.

You will receive $40 to cover your costs for parking, transportation to Queen’s etc. for participating in this study.
**Appendix G: CTS Patient Information**

<table>
<thead>
<tr>
<th><strong>Volunteer ID</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Personal Information</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td><strong>Handedness</strong></td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>CTS Information</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beginning of Symptoms</strong></td>
</tr>
<tr>
<td><strong>Unilateral(R/L) or Bilateral</strong></td>
</tr>
<tr>
<td><strong>Severity</strong></td>
</tr>
<tr>
<td><strong>Surgery (Y/N)</strong></td>
</tr>
<tr>
<td><strong>Successful?</strong></td>
</tr>
<tr>
<td><strong>Currently in pain (Y/N)</strong></td>
</tr>
<tr>
<td><strong>Triggers for pain</strong></td>
</tr>
<tr>
<td><strong>Tinel’s Sign (tap median nerve firmly)</strong></td>
</tr>
<tr>
<td><strong>Phalen’s Sign (push back of hands together, touch thumb and middle finger)</strong></td>
</tr>
<tr>
<td><strong>Prayer Test (Reverse Phalen’s)</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Medical Information</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Co-morbidities (diabetes/hypertension)</strong></td>
</tr>
<tr>
<td><strong>Medications</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Interested in follow-up</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Email Address</strong></td>
</tr>
<tr>
<td><strong>Phone Number</strong></td>
</tr>
</tbody>
</table>
Appendix H: Short Form McGill Pain Questionnaire

<table>
<thead>
<tr>
<th>Subject Number:</th>
<th>Date:</th>
<th>Pain Rating Index:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NONE (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MILD (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MODERATE (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVERE (3)</td>
</tr>
</tbody>
</table>

- Throbbing
- Shooting
- Stabbing
- Sharp
- Cramping
- Gnawing
- Hot-Burning
- Aching
- Heavy
- Tender
- Splitting
- Tiring
- Sickening
- Fearful
- Punishing

**Visual Analog Scale** (Mark ‘I’ for intensity and ‘U’ for unpleasantness)

<table>
<thead>
<tr>
<th>No Pain</th>
<th>Worst Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imaginable</td>
</tr>
</tbody>
</table>

**Present Pain Intensity** (Circle response)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No Pain</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
</tr>
<tr>
<td>2</td>
<td>Discomforting</td>
</tr>
<tr>
<td>3</td>
<td>Distressing</td>
</tr>
<tr>
<td>4</td>
<td>Horrible</td>
</tr>
<tr>
<td>5</td>
<td>Excruciating</td>
</tr>
</tbody>
</table>
Appendix I: Pain Questionnaire Administration

Short Form McGill Pain Questionnaire

Sensory (Questions 1-11)

“How would you rank your pain with respect to a ____________ sensation; none, mild, moderate, or severe?”

Throbbing; Shooting; Stabbing; Sharp; Cramping; Gnawing; Hot-Burning; Aching; Heavy; Tender; Splitting.

Affective (Questions 12-15)

“How would you rank your pain with respect to a ____________ feeling; none, mild, moderate, or severe?”

Tiring; Sickening; Fearful; Punishing-Cruel.

Visual Analog Scale

“Please mark an ‘I’ on the scale in the location you feel represents your pain in the context of intensity”

Please mark a ‘U’ on the scale in the location you feel represents your pain in the context of unpleasantness”

Present Pain Index

“Please circle the descriptor that reflects the level of pain you are experiencing presently.”
Appendix J: Psychophysical Administration & Results Table

Direct Quotation read to the volunteer prior to psychophysical testing:

“There are 2 main aspects of pain that we are interested in measuring: the intensity, how strong the pain feels, and the unpleasantness, how unpleasant or disturbing the pain is for you. The difference between these two aspects of pain if you compare a painful experience to listening to music. As the volume of the music increases, I will ask you how loud the music is and how much you like the music. The loudness of music is like the intensity of pain, how much you like the music is like the unpleasantness of pain.

Psychophysical Testing of Pain Stimulus

Instructions given to the volunteer prior to determining pressures required to elicit pain levels 2, 4, and 6 out of 10.

I will now increase the pressure until you report that your pain has reached a level 2 out of 10 on the pain scale. (Repeated for levels 4 and 6)

<table>
<thead>
<tr>
<th>Subject Number:</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain Level Target</td>
<td>Pain Level Achieved</td>
</tr>
<tr>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>