Université de Montréal

The roles of the somatosensory cortices in the perception of noxious and innocuous stimuli

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Thèse présentée à la Faculté des études supérieures

en vue de l'obtention du grade de Ph.D.

en Sciences Neurologiques

September 13, 2010

Université de Montréal

Faculté des études supérieures

Cette thèse intitulée :

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Résumé

Les premières études électrophysiologiques et anatomiques ont établi le rôle crucial du cortex somatosensoriel primaire et secondaire (SI et SII) dans le traitement de l'information somatosensorielle. Toutefois, les récentes avancées en techniques d'imagerie cérébrale ont mis en question leur rôle dans la perception somatosensorielle. La réorganisation du cortex somatosensoriel est un phénomène qui a été proposé comme cause de la douleur du membre fantôme chez les individus amputés. Comme la plupart des études se sont concentrées sur le rôle du SI, une étude plus approfondie est nécessaire. La présente série d'expériences implique une exploration du rôle des régions somatosensorielles dans la perception des stimuli douloureux et non-douloureux chez des volontaires sains et patients avec des douleurs de membre fantôme.

La première étude expérimentale présentée dans le chapitre 3 est une méta-analyse des études de neuro-imagerie employant des stimuli nociceptifs chez des volontaires sains. En comparaison aux précédentes, la présente étude permet la génération de cartes quantitatives probabilistes permettant la localisation des régions activées en réponse à des stimuli nociceptifs.

Le rôle du cortex somatosensoriel dans la perception consciente de stimuli chauds a été étudié dans le chapitre 4 grâce à une étude d'imagerie par résonance magnétique fonctionnelle, dans laquelle des stimuli thermiques douloureux et non-douloureux ont été administrés de manière contrebalancée. Grâce à cette procédure, la perception de la chaleur fut atténuée par les stimuli douloureux, ce qui permit la comparaison des stimuli consciemment perçus avec ceux qui ne le furent pas. Les résultats ont montrés que les stimulations chaudes perçues ont engendré l'activation de l'aire SI controlatérale, ainsi que de la région SII.

Grâce à l'évaluation clinique de patients amputés présentant une altération de leurs perceptions somatosensorielles, il est également possible de dessiner un aperçu des régions corticales qui sous-tendent ces

modifications perceptuelles. Dans le chapitre 5 nous avons émis l'hypothèse proposant que les sensations du membre fantôme représentent un corrélat perceptuel de la réorganisation somatotopique des représentations sensorielles corticales. En effet, la réorganisation des sensations peut donner des indices sur les régions impliquées dans la genèse des sensations référées. Ainsi, un protocole d'évaluation sensoriel a été administré à un groupe de patients affligés de douleur au niveau du membre fantôme. Les résultats ont montré que, contrairement aux études précédentes, les sensations diffèrent grandement selon le type et l'intensité des stimuli tactiles, sans évidence de la présence d'un modèle spatialement localisé. Toutefois, les résultats actuels suggèrent que les régions corticales à champs récepteurs bilatéraux présentent également des modifications en réponse à une déafférentation.

Ces études présentent une nouvelle image des régions corticales impliquées dans la perception des stimuli somatosensoriels, lesquelles comprennent les aires SI et SII, ainsi que l'insula. Les résultats sont pertinents à notre compréhension des corrélats neurologiques de la perception somatosensorielle consciente.

Mots clés : La douleur, la chaleur, le toucher, d'imagerie cérébrale fonctionnelle, de l'homme

Abstract

Early anatomical and single-unit recording studies established a crucial role for the primary and secondary somatosensory cortices (SI & SII) in processing somatosensory information. However, recent advances in brain imaging and analysis techniques have called into question their role in somatosensation. Findings from this recent research are relevant to the study of the reorganizational changes occurring in the somatosensory cortices that have been causally linked to the genesis of pain in amputee patients. These patients continue to perceive and experience pain in the absent limb, which is usually referred to as phantom-limb pain; but little research on this phenomenon has focused on other regions outside SI, and further study is needed. The present series of experiments involve an exploration of the roles of the somatosensory cortices in the perception of noxious and innocuous tactile stimuli in healthy volunteers and patients with phantom-limb pain.

The first experimental study in Chapter 3 is a meta-analytic review of neuroimaging studies examining noxious stimuli evoked activation in healthy volunteers. In comparison to previous reviews that have merely reported the prevalence of pain-related activation, the present study yields quantitative probabilistic maps that permit localization of the likelihood of obtaining activation in response to noxious stimuli within any brain region.

The role of the somatosensory cortices in the conscious perception of brief warm stimuli was explored in Chapter 4 using functional magnetic resonance imaging, where noxious and innocuous thermal stimuli were counterbalanced within the experimental protocol. This procedure allowed a gating of the somatosensory system in which the perception of warm stimuli was attenuated by painful stimuli, thus permitting the comparison of detected with undetected stimuli. Results showed that detected warm stimuli significantly activated SI and SII.

It is also possible to draw insight regarding which cortical regions subserve somatosensory processing and its organization by clinical assessment of amputee patients, who demonstrate altered somatosensation. To date, few studies have explored the relationship between referred sensations to the phantom and cortical reorganization. In Chapter 5 we hypothesized that referred sensations to phantom limbs are a perceptual correlates of a somatotopic reorganization of sensory representations. Derangements in referred sensations can give clues to the regions involved in referred sensations genesis. Thus, a quantitative sensory testing protocol was administered to a group of phantom-limb pain patients. Results showed that, contrary to previous reports, referred sensations to the phantom differed greatly based on the type and intensity of the tactile stimuli applied to the body, with no evidence of a spatially localized pattern. Previous reports of referred sensations have solely focused on plastic changes in SI. However, the present results suggest that other cortical regions with bilateral receptive fields also undergo reorganizational changes in response to deafferentation.

These studies present an emerging picture of the cortical regions involved in the perception of somatosensory stimuli, which include SI and SII, as well as the insula. Findings are relevant to our understanding of the neural correlates of conscious perception of somatosensation and the formation of the mental representation of stimuli applied to the body.

Key words: Pain, warm, touch, neuroimaging, human

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List of abbreviations

ACC	Anterior cingulate cortex
aIC	Anterior insula cortex
ALE	Activation Likelihood Estimate
ANOVA	Analysis of Variance
CB	Cerebellum
CTA	Cortical thickness analysis
fMRI	Functional magnetic resonance imaging
FWHM	Full width half maximum
LN	Lentiform nucleus
LEP	Laser evoked potentials
MFG	Middle frontal gyrus
m/DLPFC	Medial/dorsolateral prefrontal cortex
MI	Primary motor cortex
NC	Nucleus accumbens
P	Probability
PCL	Paracentral lobule
PET	Positron emission tomography
PFC	Prefrontal cortex
ROI	Region-of-interest
SMA	Supplementary motor area
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
STG	Superior temporal gyrus
VBM	Voxel based morphometry
VP	Ventral posterior nucleus
VPL	Ventral posterior lateral nucleus
VPM	Ventral posterior medial nucleus

Dedication

I dedicate this thesis to my mother who constantly inspired and encouraged me to go after my dreams and achieve my full potential

Acknowledgements

I would first like to thank my supervisor, Gary Duncan, who has been my scientific mentor over the last seven years during which time he was a member of my Master's thesis committee and my PhD supervisor. Gary has patiently taught me how to design experiments, think critically, and has done his best to try to improve my scientific writing. During these last years he has been an exceptional person to work with and I only hope that I will be able to continue to work with him in the future and to emulate his qualities with colleagues. On a personal note, he has opened his home that he shares with his amazing family (Alice, Stella and Coco) to many lab outings in Parc de la Maurice and taught us to appreciate recreational activities outside the lab.

I would also like to thank Pierre Rainville, who has served as my committee member during my doctorate degree. He has contributed to the majority of the work within this thesis and has helped me on an innumerable amount of occasions to discuss statistics and scientific ideas. Pierre's abilities as a scientist have continually inspired my work. I consider myself very fortunate to have had the honour to work with him and Gary during my doctoral studies.

I am indebted in gratitude to my other committee member, Elaine Chapman, for her advice and encouragement during my doctoral degree. Elaine has an extensive background in studying somatosensory processing and has helped me to improve my understanding of the neurophysiological basis of tactile processing. Additionally, she has mentored me in my professional pursuits to pursue a postdoctoral degree.

I send a huge thank you to Marie-Claire Albanese, who has been my great friend and my colleague during these last few years. I meet Marie-Claire in 2002 during my Master's degree. She imparted all of her knowledge to me on how to conduct a neuroimaging experiment and analyze data. During the last seven years we formed an excellent working

relationship where we could tackle projects as a team. I feel fortunate to have known her and she has contributed immeasurably to my scientific way of thinking. I know that I can rely on her for any scientific or personal discussion and she has supported me in every decision that I have made. She is also a great driver and travel partner as was exemplified during our trips to Toronto, London, New York, Israel and Italy (and that one afternoon in Zurich).

I would also like to thank my lab mates who were always reliable when I needed a subject for the scanner or to discuss projects. In no particular order are Karin Pietruska, Danièle Laverdure-Dupont, Joshua Grant, Mathieu Roy, Mathieu Piché, Jen-I Chen, Audrey-Anne Dubé, Marianne Arseneault, Etienne Vachon-Presseu, Stéphanie Cormier, Mina Khosh Nejad, and Guoming Xie.

I am indebted in gratitude to the patients who participated in the study presented in Chapter 5. Their kindness and willingness to participate in research despite the terrible pain and suffering they have experienced taught me the importance of living life to its fullest and to be optimistic about the future.

Most of all I thank Jason, my mother, Terry, and my sister for their love and endless support during the years I have spent working on this thesis.

Contribution of Authors

Chapter 1: The introduction and background chapter is separated into two chapters. In Chapter 1 the rationale and objectives of the research are presented followed by a brief introduction to mechnoreception and thermoreception. This background information is relevant to the subsequent experimental articles on warmth perception and the use of tactile stimuli for a quantitative sensory testing protocol.

Chapter 2: The second half of the background section of this thesis contains a book chapter entitled “fMRI of Pain” that was published in fMRI Techniques and Protocols. This chapter provides an overview of the neuroanatomical substrates of nociceptive processing and its functional representation in the brain as revealed using brain imaging techniques. This chapter contains relevant information for the first experimental chapter on the representation of pain-evoked activation in the brain. I wrote the manuscript and Dr. Gary Duncan made revisions and comments that contributed to the final form of the manuscript.

Chapter 3: This chapter contains a manuscript in preparation entitled “Localization of pain-evoked activation in the brain: A meta-analysis of functional neuroimaging data”. I generated the concept behind conducting the meta-analysis. I subsequently recruited and trained Joyce Fu to review articles and enter the data to create the probabilistic maps. I reviewed the studies and data points in the meta-analysis. I subsequently performed the analysis, wrote the manuscript, prepared the figures and tables. Drs Gary Duncan and Pierre Rainville provided comments and revisions on the manuscript.

Chapter 4: This chapter contains a manuscript in preparation entitled "Neural correlates of the conscious perception of warmth". I participated in the experimental design, programming of the protocol, tested all of the subjects, analyzed all of the data, wrote the manuscript and prepared all of the figures and tables. Drs. Gary Duncan, Pierre Rainville and Marie-Claire Albanese aided with the experimental design, protocol development, and analysis of the data and provided comments on the manuscript.

Chapter 5: This chapter is a manuscript in preparation entitled "Referred sensations in phantom-limb pain patients provide clues to cortical reorganization". I recruited and interviewed the patients included in the study, developed the quantitative sensory testing protocol, performed the testing, did the analysis, wrote the manuscript, and prepared the tables and figures. Dr. Gary Duncan aided in the development of the testing protocol and provided revisions on the manuscript.

Appendix I: This section contains the agreements of the co-authors and publishers of the book chapter/manuscripts presented in Chapters 2, 3, 4 and 5.

Appendix II: The appendix also contains one published manuscript entitled "Practice makes cortex" that appeared in the *Journal of Neuroscience*. This is a short review of an article that examined grey matter density and functional brain activation changes in response to a two week procedural learning task (Ilg et al. *J Neurosci*. 2008 Apr 16;28(16):4210-5). I included this manuscript as it is related to the general theme of brain plasticity discussed within this thesis. In addition, within the manuscript we used the analytic techniques discussed in Chapter 3, whereby I conducted a meta-analysis of voxel-based morphometry studies demonstrating increased grey matter

density in response to learning induced changes. In collaboration with Danièle Laverdure-Dupont, I co-wrote the manuscript, performed the meta-analysis and prepared the figures.

Introduction

Rationale

Controversy concerning the roles of the primary and secondary somatosensory cortices (SI & SII) in the perception of various types of cutaneous stimuli, including pain, temperature, and vibration, began with the first published experiments in the early 20th century and continues to the present day. Modern advances in brain imaging techniques have made it possible to view the entire human brain *in vivo,* this new information has implicated other cortical regions such as the insula to be involved in processing somatosensory information. The series of experiments described in this thesis were designed to study the involvement of SI, SII, and the insula in the perception of somatosensation. A clearer understanding of the brain regions involved in processing cutaneous stimuli could potentially improve diagnosis of chronic pain and treatment of somatosensory deficits in stroke patients.

In the early 20th century, research in somatosensation relied heavily on the clinical examination of patients who had lesions associated with brain pathology. Reports of patients with lesions to SI have produced conflicting results concerning the perception of touch and temperature information. One study found that patients with damage to SI were unable to identify objects touching their affected hand, but they retained the ability to localize painful pin-prick stimuli (Stewart 1908). Similarly, Head and Holmes reported that patients with lesions to SI had abnormal mechanoreception but intact thermoreception (Head and Holmes, 1911). In contrast to these findings, a more recent study found that a patient with a lesion to SI (and a portion of SII) was unable to perceive temperature and light touch but unimpaired vibration sensation (Ploner, Freund et al. 1999).

During the 1970s, a host of neurophysiological studies with non-human primates demonstrated that SI is involved in the perception of pain (Kenshalo, Jr. and Isensee 1983;Willis, Jr. 1985a;Willis, Jr. 1985b;Chudler,

Anton et al. 1990;Kenshalo, Iwata et al. 2000). This region contains a detailed somatotopic organization of nociceptive neurons, indicating that SI is involved the localization of noxious stimuli (Kenshalo, Iwata, Sholas, and Thomas 2000). Another study implicated SI in the intensity processing of painful stimuli as some of its neurons respond in an intensity-related manner (Kenshalo, Jr., Chudler et al. 1988).

While the 1990s saw advances in non-invasive brain imaging techniques, which facilitated the study of the brain *in vivo*, some initial studies reported inconsistent findings on activation in SI in response to noxious stimuli. Several of these modern imaging studies reported activation in SI in response to pain (Talbot, Marrett et al. 1991;Coghill, Talbot et al. 1994;Coghill, Sang et al. 1999;Chen, Ha et al. 2002), although other studies failed to find any activation in SI (Jones, Brown et al. 1991;Disbrow, Buonocore et al. 1998). In addition to these conflicting results a series of recent studies have postulated that the pain-related activation that has been documented throughout the brain (including SI) is actually involved in the general process of magnitude estimation, rather than pain perception *per se* (Baliki, Geha et al. 2009).

Similarly, studies researching of the role of SII in the processing of somatosensory information have been plagued by contradictory results. For example, single-unit recording studies in animals demonstrated neuronal responses to tactile and thermal stimuli in SII (Robinson and Burton 1980a;Dong, Salonen et al. 1989). Additionally, SII has been shown to have a crude somatotopic organization indicating that this region processes spatial discrimination information (Robinson and Burton 1980b). Corroborating these findings are clinical studies of patients with damage to SII, which demonstrate patients' intact cutaneous and proprioceptive abilities including the ability to process temperature (Caselli 1993;Reed, Caselli et al. 1996). However, a recent conflicting report describes patients with SII lesions who exhibit intact tactile processing, with deficits only in pain and temperature perception (Kim, Greenspan et al. 2007). However, it is

important to note that some of these patients' lesions included the insula, making it difficult to dissociate the processes of either structure in cutaneous processing. Further contradictory evidence regarding SII in somatosensation comes from a single-unit recording study in nonhuman primates, which showed response *suppression* during attention to vibrotactile stimuli (Burton, Sinclair et al. 1997). Therefore, the role of SII in processing temperature and tactile information needs further clarification.

In addition to SI and SII, the insula is increasingly becoming the focus of somatosensory research. It receives information from and projects to parietal (including somatosensory cortices), prefrontal, and temporal cortices, making it well fitted for multisensory integration (Mesulam and Mufson 1982;Friedman, Murray et al. 1986;Preuss and Goldman-Rakic 1989). In terms of the insula's involvement in the processing of somatosensation, it has been implicated mainly in nociceptive processes (Apkarian, Bushnell et al. 2005;Brooks and Tracey 2007). Electrophysiological studies in patients with epilepsy have reported painful and innocuous somesthetic responses in several regions of the insula, which follow a rough topographic organization (Penfield and Faulk 1955;Ostrowsky, Magnin et al. 2002). However, more recent reports from brain imaging studies have implicated the insula in vibration processing (Soros, Marmurek et al. 2007;Albanese, Duerden et al. 2009). Based on the known somatosensory input to the insula and the results of recent studies, in addition to SI and SII, a major focus of the current thesis is the involvement of the insula in processing cutaneous stimuli.

Chapters 1 and 2 of this thesis provide background information on the neurophysiological basis of processing touch and temperature information. Chapter 1 offers a review of the receptors, spinal pathways and cortical regions involved in processing tactile and temperature information. Chapter 2 focuses on both the neuroanatomy of pain processing and neuroimaging methods used to localize pain-evoked activation.

The manuscript presented in Chapter 3 describes a meta-analysis that makes a detailed survey of the pain-imaging literature over the past 20 years; using these data, four separate analyses are performed to address questions that have been difficult to resolve in isolated studies. The initial search of the pain imaging literature revealed 130 studies that satisfied the search criteria of pain, nociception, fMRI, and PET. All three-dimensional (3D) brain imaging coordinates in response to noxious stimuli were compiled and analyzed to create probability maps that can be overlaid on a standard magnetic resonance (MR) image. Each voxel in the MR image was assigned a likelihood value denoting the absence or presence of activation in response to noxious stimuli from the individual studies. The first analysis of these data describes the creation of a general, "quantitative pain matrix" -- a 3-D interactive probability map illustrating the location and extent of the brain activation that is common across the various studies using experimental noxious stimulation. The second analysis examines a subset of these data, searching for regions that process different types of noxious stimulation (e.g. stimuli that evoke the perception of cold pain or heat pain), irrespective of the different experimental paradigms used in the individual studies. Along similar lines, the third analysis assesses the implications of different control conditions in revealing activation related to noxious stimulation; specifically, the location and extent of the apparent "pain-evoked" brain activation is compared for subsets of studies that have employed either a resting baseline or an innocuous warm stimulus condition as a control for activation associated with noxious heat stimulation. This issue is important since warm stimuli are frequently used as a control condition for pain neuroimaging experiments; however, it remains unclear whether warm and noxious heat belong to the same sensory modality. Therefore, in some instances warm stimuli may not be an appropriate comparison for noxious heat. The fourth and final analysis tests for a possible hemispheric dominance for processing noxious stimuli by comparing the location and extent of brain activation across subsets of

studies that presented this type of stimuli either to the left or to right sides of the body. While targeting specific brain regions responsible for pain perception could lead to improved diagnosis of chronic pain, it is rarely studied within the context of a single study.

The manuscript presented in Chapter 4 addresses issues that arose from the third part of the meta-analysis on the use of warm stimuli as an apprpriate control condition for painful stimuli. The third meta-analysis examined stimulus conditions used to examine “pain-related” activation, but did not focus on the brain regions associated with the perception of warmth. We were able to answer this question by using an fMRI data set in which subjects were given painful and warm stimuli presented in a counter-balanced fashion. After repeated presentations of painful stimuli, the perception of some of the warm stimuli became attenuated. In the functional neuroimaging analysis the detected and undetected warm stimuli were entered as separate time periods that permitted the identification of brain activation in response to either condition.

Chapter 5 describes our exploration of abnormal somatosensory processing in a group of amputee patients who experience phantom-limb pain. While the underlying mechanisms remain unknown, research has shown that rapid cortical reorganization of somatotopic maps of the body in SI occurs in response to an amputation, and this has been causally associated with phantom-limb pain intensity (Florence, Garraghty et al. 1994;Flor, Elbert et al. 1995). However, the results from the main meta-analysis from Chapter 3 would indicate that many other regions are involved in pain processing and possess a nociceptive somatotopic organization. Therefore, other regions such as SII and the insula are also likely to undergo reorganizational changes. Early case reports of patients with upper-limb amputations inferred that the perceptual correlates of this cortical reorganization in SI manifest themselves in sensations referred to the phantom by the touching of a patient’s face or arm (Ramachandran, Stewart et al. 1992). However, more recent reports questioned the reorganizational

changes that may occur in SI in response to deafferentaion, implying that other cortical regions that process pain also may be involved in referred sensation genesis (Grusser, Winter et al. 2001a; Sathien, 2001). Moreover, only one report has described referred sensations in lower-limb amputees that described highly localized and detailed remapping of the amputated phantom feet onto the upper thighs in these patients (Aglioti et al., 2005). Findings indicate that referred sensations are generated from body parts that lie adjacent to one another on the somatotopic map in SI. Unfortunately, only a few patients were examined, which makes it difficult to know if similar somatotopic reorganizational changes are generalisable to this population as a whole.

In the research described in Chapter 5, referred sensations were explored in a group of upper and lower-limb amputees using a variety of somatosensory stimuli to target both superficial and deep fibres in the skin. The overall goal in developing this quantitative somatosensory testing protocol was to determine if the referred sensations exhibit a somatotopic organization in order to provide clues as to which cortical regions are involved in the perception of referred sensations.

Objectives

The overall objective of the current thesis was to examine the perception of noxious and innocuous stimuli in healthy subjects using meta-analytic and brain imaging techniques, and also by the development of a quantitative sensory testing protocol for use in patients with phantom-limb pain.

The objective of the meta-analysis presented in Chapter 3 was to investigate the common cortical regions involved in processing nociceptive information. The motivation behind performing this analysis was three-fold. Firstly, meta-analysis overcomes some of the limitations associated with conducting a single brain-imaging study, such as image artefacts or low power resulting from too few subjects. Meta-analysis can also localize

common brain regions associated with a particular task or cognitive function across studies (e.g. working memory). Secondly, the meta-analysis permitted the exploration of a number of questions that have arisen in the pain brain-imaging community. Thirdly, the meta-analysis allowed for the creation of region-of-interest (ROI) maps, which may be used for future analysis of brain imaging data (fMRI or cortical thickness) to localize pain-processing regions in the brain.

Our objective in the manuscript presented in Chapter 4 was to identify the brain regions involved in the cogniscent awareness of innocuous warm stimuli. We explored warm-evoked activation in the brain by using data from an fMRI study we conducted in which noxious and innocuous heat stimuli were presented in a counter-balanced manner within the scanning runs. Throughout the course of the experiment, repeated presentation of painful stimuli caused peripheral fatigue of receptors on fibres transmitting pain and warmth information to the brain, and this resulted in some of the warm stimuli to be undetected by the subjects. In the functional neuroimaging analysis, we identified the time periods when subjects detected or did not detect the warm stimuli. This permitted the localization of brain areas that were activated by consciously detected or undetected stimuli.

Our objective in Chapter 5 was to document the pattern and intensity of referred sensations in phantom-limb pain patients. Such exploration could provide a greater understanding of the cortical regions affected by loss of somatosensory input. Furthermore, the results could later provide a rationale for future studies targeting the remapping of the somatotopic organization of somatosensory cortices.

1 Chapter 1: Background

1.1 Somatosensation

Somatosensation refers to the ability to perceive our body, which includes the perception of anything that comes into contact with our skin and the position of our limbs. It allows us to identify change in our pockets, warns us against touching a hot radiator, and to perform a smooth and coordinated tennis swing. It is a sense that is largely taken for granted, as it rarely becomes noticeably impaired throughout aging, and the detrimental effects of a loss of somatosensation only become apparent after specific brain damage.

Somatosensation can be classed along two lines of perception: somatic sensations including mechanoreception (discriminative touch, vibration, light touch, movements across the skin), thermoception (cool, cold, warm), nociception (pain), and also proprioception (position and movement of the limbs). The latter is further subdivided into two subcategories of joint position sense and kinesthesia, or knowledge of the movement of our limbs.

While each of these senses is processed by different receptors or nerve endings in the skin, and then via separate spinal pathways to the brain, they all converge outside the spinal cord in the dorsal root ganglia cells. The dorsal root ganglia cells have neurites that extend out to the periphery, to the skin or muscle and another process that enters the central nervous system (Davies and Lumsden 1990). Sensory afferent fibres enter the dorsal horn of the spinal cord and terminate either on spinal motor neurons to aid in motor reflexes, and others that ascend to the brain stem and thalamus via several fibre pathways. These pathways serve different types of somatosensation and project to the brain stem, thalamus, and cortex (Willis, 2007). An important note is that these afferents maintain a detailed spatial map of the body surface at all levels of the nervous system. The final termination point of cutaneous input is sent to SI, SII, and the superior parietal lobule (Willis, 2007). These cortical regions interact with

frontal and temporal regions to combine somatosensory information and to compare it with previous experiences (Friedman et al. 1986;Preuss and Goldman-Rakic 1989). These receptors, spinal pathways, and cortical regions provide the underpinnings of the conscious perception of somatosensation.

This thesis is primarily concerned with the study of somatic senses that include mechanoreception, thermoception, and nociception. Proprioception is beyond the scope of the current work. The next section focuses on the receptors, spinal pathways, and cortical regions responsible for somatic sensation. The chapter concludes with an overview of brain imaging techniques with a primary focus on functional MRI of pain.

1.2 Mechanoreception

1.2.1 Cutaneous and Subcutaneous Mechanoreceptors

A variety of specialized receptors innervate the skin, viscera, muscle, joints, and bones to convey somatosensory information to the cortex (Boulais and Misery 2008). Each receptor is classified based on the stimulus that produces the most optimal response. An important characteristic of sensory receptors is their firing pattern in response to their preferred stimuli. Receptors referred to as quickly adapting respond immediately and their firing pattern dissipates after several seconds, while slow adapting receptors maintain their initial response to a sensory stimulus, but do not have a rapid onset (Goodwin and Wheat 2004). Receptors can be further classified along three lines: those with encapsulated endings, free nerve endings, and expanded tip endings.

Encapsulated endings are rapidly adapting receptors found in the dermis that perceive tactile information such as deep pressure, discriminative touch, and vibration. They are aptly referred to as encapsulated because the nerve ending is wrapped in concentric layers of

tissue separated by encapsulated fluid. Pacinian corpuscles and Meissener receptors are considered encapsulated receptors (Vega et al. 2009).

Free nerve endings are responsible for primarily relaying pain and temperature information, although these receptor types also can mediate some tactile information (Fromy et al., 2008). These receptors have fine-grained, gossamer-like projections into the epidermal layer of the skin. Free nerve endings are dispersed all over the body and viscera, and for the most part have non-adapting firing patterns.

Examples of expanded tip endings are Merkel cells and Ruffini endings (Moll et al. 2005;Macefield 2005;Boulais and Misery 2007). These receptors are located in the epidermis, which suits them to be moderately adapting in their firing pattern. The receptors will fire after the application of the stimulus and will not attenuate during its presentation. The structure of the receptors is that of flattened ball-like shapes that transmit touch, pressure, and temperature information.

1.2.2 Neuroanatomy of Tactile Processing

Primary afferent fibres innervating tactile and joint receptors are located in the dorsal root ganglia (Fromy et al. 2008). These fibres are classified as either A-alpha or A-beta and are myelinated, which permits the rapid transmission of information (Provitera et al. 2007). This is true in all instances except for stretch-sensitive free nerve endings that transmit information about excessive force through A-delta fibres, which are responsible for transmitting painful information to the brain. Fibres that innervate the Pacinian corpuscles, Merkel disks, Ruffini endings, and Meissener's and muscle spindles will then synapse on neurons in the dorsal horn of the spinal cord (Macefield 2005). These neurons then project via the dorsolateral funiculus (DLF) and end in the lateral cervical nucleus (LCN) located in the upper cervical spinal cord segments (C1 & C2). LCN fibres then cross the midline and travel to the lower brain stem, where they join fibres in the medial lemniscus located in the medulla (Willis, 2007).

In addition to these pathways, some fibres innervating tactile afferents will traverse through the dorsal columns to the medulla (Rustinioni et al. 1979). Fibres do not cross the midline and ascend through the spinal cord on the side ipsilateral to the site of entry. Afferent fibres from the lower limbs ascend through fasciculus gracilis and fibres from the upper part of the body travel through fasciculus cuneatus. These respective pathways terminate in nucleus gracilis and nucleus cuneatus located at the base of the fourth ventricle on the dorsal surface of the medulla. Axons from the neurons in the nuclei cross the midline and traverse to the thalamus by way of the medial lemniscus. In the thalamus, the fibres terminate in the ventral posterior (VP) nucleus, with the fibres from the lower limbs being laterally located while the upper body are more medial (Herrero et al. 2002).

1.2.3 Subcortical and Cortical Processing of Tactile Input

Thalamocortical projections from the VP terminate in SI. Lesion studies in higher primates have demonstrated that innocuous tactile sensory information is passed in a serial fashion from SI to SII (Pons et al. 1987;Garraghty et al. 1990). Axonal tracer studies have shown that this information is then relayed to the prefrontal cortex (Preuss and Goldman-Rakic 1989) and the mid/posterior insula and then to temporal lobe structures (Friedman et al., 1986;Mesulam and Mufson, 1982).

It has been questioned whether direct projections from VP to SII also exist, which would support parallel processing of tactile information. This has been called into question by experiments demonstrating that surgical ablation of SI renders SII unresponsive to tactile input in macaque and marmoset monkeys (Pons et al. 1987;Garraghty et al. 1990;Burton and Sinclair 1990;Murray et al. 1992). However, parallel tactile processing in SI and SII has been demonstrated in other species such as the cat (Burton and Robinson 1987) and rabbit (Murray et al. 1992). It has been postulated that differences in higher primates may be due to neurons arising from the ventral posterior inferior (VPI) nucleus projecting to SII, while those from the

VP nucleus project to SI (Garraghty et al., 1990;Krubitzer and Kaas, 1992;Friedman and Murray, 1986). Other authors have reported evidence for parallel tactile processing in SI and SII in the marmoset monkey (Zhang et al. 1996). However, these results were based on temporary inactivation by local cooling, rather than lesions of SI, and so it cannot be ruled out that SI may not have been entirely deactivated by the cooling procedure.

1.3 Thermoreception

1.3.1 Thermal Receptors

Separate receptors on the ends of sensory afferent fibres (A-delta & C-fibres) and on the cell bodies of dorsal root ganglia exist for the perception of coolness, warmth, heat, and cold. Six different thermal transient receptor potential (TRP) channels have been identified that mediate from noxious cold to burning heat (Patapoutian et al. 2003;Bandell et al. 2007). TRP channels are activated by changes in temperature, ligands (menthol, capsaicin, anandamide, protons), and two channels have been identified that are dependent on the cell's membrane potential (voltage gated channels). The mechanisms by which these processes occur remain largely unknown. However, it has been suggested that temperature fluctuation could induce ligand production and subsequent binding to TRP channels. An additional possibility may be that TRP channel proteins may undergo structural alterations in response to a change in temperature, causing the opening of the channel. A final alternative may be that TRP channels may be sensitive to alterations in membrane tension.

The cell bodies of sensory neurons are predominantly found in the dorsal root ganglia and they send neurites to the skin and muscle to transmit information about temperature. Incoming information is carried by thinly myelinated A-delta fibres and C-fibres that then terminate in the dorsal horn of the spinal cord. Other fibres that respond to temperature are A-delta fibres, which respond optimally to cool temperatures (Darian-Smith et al.

1973;Dubner et al. 1975;Dykes 1975;Kenshalo and Duclaux 1977), and a class of unmyelinated C-fibres that responds solely to stimuli in the warm temperature range (28-45^{o}C) (Hensel and Kenshalo, 1969;Darian-Smith et al., 1979). Additionally, a separate group of A-delta and C cutaneous afferents respond to noxious cold but not innocuous cool (Georgopoulos 1976;LaMotte and Thalhammer 1982;Simone and Kajander 1996;Simone and Kajander 1997). However, axotomized dorsal root ganglion cells can transmit temperature information indicating that the cell bodies also contain TRP channels. It is of note that the study of TRP channels is a rapidly advancing field of study and while all of the receptors have been identified, there remain many questions concerning their role in temperature perception.

1.3.2 Thermosensory Spinal Pathways

Primary afferents that carry temperature information synapse on dorsal horn neurons. Some second-order neurons that transmit information to the brain have been found to be selectively activated by cold (Craig and Kniffki 1985) and somewhat more rarely by warm stimuli (Dostrovsky and Hellon 1978).

It was a generally held belief that these cold and warm specific second order neurons projected to the cortex through the general pain and temperature pathway, the spinothalamic tract (Craig and Dostrovsky 2001). However, this has recently come into question by a laser-evoked potentials (LEP) study that provides evidence for a warm specific spinal pathway (Iannetti et al. 2003). Short radiant heat pulses by way of a CO^2 laser stimulator were used to generate laser stimuli. These heat pulses selectively activate free nerve endings in the skin and A-delta and C-fibres in the absence of A-beta fibre activation (Bromm and Treede 1984). In the LEP study, selective activation of C-fibres was achieved by using temperatures below those capable of being perceived by the A-delta fibres that transmit information about pain. Results showed significantly different latencies for

warm compared to painful stimuli, which the authors believed provided evidence for a warm specific pathway.

1.3.3 Subcortical and Cortical Temperature Processing

The representation of temperature processing in the thalamus and cortex has remained under dispute during the last several decades. An overwhelming amount of evidence provides proof that temperature information is mediated by the primary sensory thalamus, the VP nucleus, and the somatosensory cortices (for review see (Willis, 2007). However, other evidence exists that the spinothalamic tract terminates in a temperature specific nucleus, termed the ventral medial posterior nucleus (VMpo), which is located posterior to the VP nucleus. VMpo was found to contain somatotopically organized neurons that were specific to pain and temperature (Craig et al. 1994). In addition to antereograde staining studies in animals, the basis of this hypothesis was made largely from patients with lesions to VMpo who exhibited thermal sensory deficits and centrally mediated pain. However, there is a contradictory published report in which a separate patient had exactly the same symptoms as the other group of patients, but whose lesion was localized to the VP nucleus (Montes et al. 2005).

The VMpo was found not to project to SI, but rather to the insula. These findings have been corroborated by imaging studies that found activation in the dorsal posterior insula in response to innocuous cool, but not in SI (Hua et al. 2005;Oshiro et al. 2007). However, current limitations associated with fMRI may not be able to sufficiently identify signals in the thalamus and SI, and further research in this area is needed. This is not the case for cold or warm temperatures, which, in a number of brain imaging studies have been shown to activate the somatosensory cortices, and also the prefrontal cortex and the anterior cingulate cortex (ACC) (Casey et al. 1996;Sawamoto et al. 2000;Iannetti et al. 2003;Olausson et al. 2005;Sung et al. 2007;Rolls et al. 2008).

2 Chapter 2: fMRI of Pain

Preface

The following chapter is a continuation of the background chapter. It is the full text of a book chapter that I co-wrote with my supervisor Gary Duncan called “fMRI of Pain”. It has been published in “fMRI Techniques and Protocols” edited by Fillipo Massimi and published by Humana Press in 2009. I have included it as a background chapter as it reviews extensive information on pain processing. Additionally, it covers meta-analytic techniques and the principles of fMRI, both of which are used in Chapters 3 and 4 of this thesis.

fMRI of Pain

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2.0 Abstract

The field of pain research has progressed immensely due to the advancement of brain imaging techniques. The initial goal of this research was to expand our understanding of the cerebral mechanisms underlying the perception of pain; more recently the research objectives have shifted towards chronic pain – understanding its origins, developing methods for its diagnosis, and exploring potential avenues for its treatment. While several different neuroimaging approaches have certain advantages for the study of pain, fMRI has ultimately become the most widely utilized imaging technique over the past decade because of its non-invasive nature, high-temporal and spatial resolution, and general availability; thus, the following chapter will focus on fMRI and the special aspects of this technique that are particular to pain research. Section 1 begins with a brief review on the spinal pathways and neuroanatomical regions involved in pain processing, and highlights the novel information that has been gained about these structures and their function through the use of fMRI and other neuroimaging techniques. Section 2 reviews a few of the aspects associated with the blood-oxygen-level-dependent (BOLD) signal commonly used in fMRI, as they apply to the particular challenges of pain research. Likewise, Section 3 summarizes some of the special considerations of experimental design and statistical analysis that are encountered in pain research and their applications to fMRI studies. Section 4 reviews special applications of fMRI for the study of higher cognitive processes implicated in pain processing, including pain empathy and cognitive reappraisal of one's own pain perception. The chapter concludes with Section 5, exploring some of the future prospects of fMRI techniques and new applications related to pain research.

Key words: Pain, human, functional neuroimaging, brain, perception

2.1 INTRODUCTION

The history of pain imaging is relatively short, although it has advanced immensely within the last decade due to improvements in imaging techniques, statistical analysis, and specialized equipment for the delivery of painful stimuli. Initially, brain imaging studies sought simply to examine the brain areas that are involved in pain processing, to make comparisons with the long established neurophysiological studies reported in this field. Many of these initial imaging studies were prompted by electrophysiological data from patients undergoing brain surgery in the early part of the 20th century (1), which had questioned the role of the cortex in nociceptive processing. It was initially believed that the thalamus was primarily responsible for nociceptive processing as suggested by deficits in pain perception observed in patients with thalamic lesions (2).

In the early 1990s, activation in the human brain evoked by experimental pain stimuli was studied using positron emission tomography (PET) (3;4) and single photon emission tomography (SPECT) (5). Then in 1995 the first fMRI studies examining the cortical representation of pain (6) were conducted largely to confirm the findings of previous PET studies and to examine whether the cortical nociceptive signal could be detected using fMRI. In more recent years, the field of pain imaging has expanded immensely, allowing researchers to answer complex questions concerning pain processing, such as how cortical regions are connected and modified during the perception of pain and, most importantly, how the cortex responds during the modulation of pain. These experimental studies were conducted in healthy humans in order to answer broad questions regarding pain processing, with the eventual goal of applying this knowledge to a better understanding and alleviation of pain and suffering associated with chronic pain syndromes. The use of fMRI and other imaging techniques has revealed a number of cortical and subcortical changes that may occur as a

result of exposure to chronic pain (7). Indeed, with the advent of high-speed image acquisition and computational processing, not only has the technology of fMRI revealed areas of cortical plasticity associated with chronic pain, it is now possible to use fMRI in real-time to furnish feedback to subjects (and patients) to teach them how to modulate their cortical activation in response to chronic pain (8).

This chapter reviews and discusses the various advances in our knowledge of cerebral pain processing that have been achieved using fMRI, the response properties of cortical nociceptive neurons in relation to both imaging techniques and stimuli used to evoke pain, the applications of this research to treat clinical pain in patients, and the future of pain research using fMRI.

2.2 BACKGROUND

2.2.1 Neuroanatomy of pain processing

Before describing how fMRI measures the cortical and spinal nociceptive signal, it is important to understand how this signal is transferred to the cortex. In the periphery, a painful stimulus applied to the body is transmitted to the central nervous system (CNS) through nociceptors (9). Myelinated A-delta fibers transmit sharp pricking pain (10), while unmyelinated C-fibers transmit slow burning pain, often referred to as second pain (11). The cell bodies of A-delta and C-fibers are located in the dorsal root ganglia, receiving afferent input from the periphery and then sending the information into the spinal cord to terminate in the dorsal horn (12;13). Axons from the second-order dorsal horn neurons rise through several ascending pathways that transmit nociceptive information to the thalamus, reticular formation, and cortex (14-19). Pain and temperature information applied to the face is relayed through cranial nerves to the spinal nucleus V terminating in the thalamus via the trigeminothalamic tract which is then relayed to the cortex. A number of spinal and cortical neurons

respond to noxious stimuli including nociceptive specific (NS) and wide dynamic range (WDR) projection neurons, the latter of which respond to both noxious and innocuous stimuli. Additionally, the dorsal horns and cortical somatosensory regions contain neurons responsive solely to innocuous stimuli called low threshold mechanical (LTM) neurons and thermoreceptive neurons responsive to temperatures in the warm and cold range. This range of responses is an important consideration when interpreting results from fMRI studies of pain in terms of exactly what the activation pattern is reflecting.

Typically, pain-evoked brain activation is achieved by applying contact thermodes to the skin. This technique involves an increase in temperature at the rate of 1°-10°C per second. Depending on the baseline temperature it can take several seconds to reach perceived pain threshold. In addition to activating NS neurons inherently, LTM neurons respond to stimulation of the skin, and, as the temperature rises, thermoreceptors respond to the heating of the skin. Therefore, to examine pain-specific cortical activations, it is necessary to compare pain-related activations to those associated with the presentation of innocuous warm stimuli.

In addition to conductive heating of the skin using contact thermodes, nociceptive afferents can be activated using thermal radiation administered through infrared laser stimulators (20;21). Lasers can deliver heat stimuli without the need for a contact probe, thus selectively stimulating C-fibers and A-delta fibers without contaminant activation of A-beta fibers that transmit touch information. Additionally, laser stimuli can activate nociceptive nerve endings at rapid rates for short durations (1 ms) (22;23) and are therefore well suited for rapid event-related fMRI studies. However, an important consideration associated with the use of laser stimuli is the difficulty of measuring and controlling skin temperature, which is the primary factor triggering the cascade of neural responses that culminate in the processing of heat-related nociceptive information in the brain and likewise, the assessment of pain by the subjects (24). Laser and contact heat stimuli

have been shown to produce similar patterns of blood-oxygen-level-dependent (BOLD) activation in the secondary somatosensory cortex (SII), anterior cingulate cortex (ACC), insula, the primary motor cortex, prefrontal cortex (PFC), parahippocampal gyrus, thalamus, basal ganglia, peri-aquaductal gray (PAG), and cerebellum. However, stronger activation in response to contact heat stimuli was noted in SII, posterior insula, posterior ACC, and regions in parietal and frontal cortices (25). Thus, these two modes of delivering noxious heat stimulation cannot be considered identical in terms of the evoked pain-related BOLD activations, and the advantages and disadvantages of each should be weighed in relation to the research questions and appropriate stimulation paradigms.

2.2.2 Supraspinal processing of nociceptive stimuli

A recent review of 68 pain neuroimaging studies using healthy subjects revealed a homogeneity of reported activations across cortical regions, thus implicating a cerebral network for pain processing (26). Regions most frequently activated by painful stimuli include primary somatosensory cortex (SI), SII, ACC, the insula, the PFC, and the thalamus.

Regions responsible for pain processing are categorized along two functional lines – the first being the sensory-discriminative (lateral pain system) component involved in the perception of temporal, intensity and localization aspects of pain processing, and the second, the affective-motivational (medial) component associated with the emotional aspects of pain (27). Dissociations between the two systems are made through subjective reports on pain scales. After exposure to noxious stimuli, subjects are asked to quantify separately how intense and how unpleasant is the perceived pain. Subjects' scores are recorded typically using numerical or visual analog scales (VAS) (28). Regions implicated in the lateral pain system include SI, SII, posterior insula and lateral thalamus, while the medial pain system consists of the medial thalamic nuclei, the ACC, and the

PFC. Much of what is known regarding the two components in pain processing was initially explored through single-unit recordings in non-human primates and lesion studies in humans. However, the more recent ability to study these functional components non-invasively in humans using fMRI and other brain mapping techniques has allowed pain researchers to advance rapidly in their understanding of the role of these cortical regions in pain processing and how they interact.

2.2.3 Primary somatosensory cortex

SI is located in the post-central gyrus, is composed of four areas (areas 3a, 3b, 1, and 2) (29), and is involved in the processing of both tactile and noxious stimuli (30). SI is the first relay from the principle sensory nuclei in the thalamus (31-35) and receives input from nociceptive neurons. While this information, gathered from non-human primates, established a role for SI in nociceptive processing, it was long debated whether SI was necessary to perceive pain. Early studies of patients with brain lesions suggested that deficits in nociceptive processing were rather common following lesions to the thalamus, but were very rare when damage was restricted to the area believed to incorporate SI (2). Likewise, later studies, using electrical stimulation of the human cortex during awake brain surgery, reported that direct stimulation of SI rarely evoked any perception of pain in patients (1).

The advent of imaging technology allowed a more global exploration of the role of SI and other cortical regions involved in pain processing, and these studies could be conducted in healthy volunteers, rather than in patients with brain injuries that might alter normal function. The first of these studies involved PET and demonstrated that noxious stimuli applied to the hands was associated with robust activation in SI (3). Several other early studies failed to detect SI activation (4;5), and subsequent reports, using either PET or fMRI, have resulted in contradictory findings (for SI activation, see for example: [36-39]; absence of SI activation, see [40, 41]).

The inconsistency of SI activation reported across imaging studies could be due to several factors. Wide variations in the location of the central sulcus across subjects may lead to a wash out in signal across averaged group data. In addition, a reduction in SI activity below statistically significant levels could be caused in some paradigms by inhibitory effects induced by noxious stimuli on tactile inputs. This effect has been reported at the cortical level using optical imaging (42) and SPECT (5) as well as in thalamus (43). In a review discussing the issue of pain-related activation of SI, Bushnell and colleagues conclude that the BOLD signal in SI largely depends on task design which is likely to influence the attentional state of the subject (44). Results from subsequent studies have likewise indicated that pain-related BOLD activation of SI is increased when subjects attend to pain and decreased when they are distracted (45;46).

On the contrary, attention may also show a deleterious effect on SI activation as noted by Oshiro et al. (47) in their fMRI study examining the neural correlates involved in processing spatial localization of pain. The authors failed to find activation in SI in response to painful stimulation of the calf. However, the authors noted that this lack of activation may have been a result of the response properties of the cortical nociceptive neurons. Nociceptive input to SI is somatotopically organized (48-51), and the small receptive fields of SI (52) suggest that this region is well suited to make fine spatial discriminations of noxious stimuli applied to the body. Oshiro et al. (47) required subjects to focus on stimulation applied to their calves, and this increased attention on the leg area may have caused a reduction in the receptive field sizes of nociceptive neurons, which would enhance spatial acuity needed to perform the task – but cause deterioration in resulting brain activation. In another study using a discrimination task, Albanese et al. (53) explored short-term memory for the spatial location and intensity of painful thermal stimuli applied to the palms. In contrast to the study by Oshiro et al. (47), Albanese and colleagues (53) reported robust pain-related activation in SI/posterior parietal cortex, which was sustained during the memory

component, suggesting that this region has a role in the encoding and retention of noxious stimuli. Differences between the two studies may be due to the larger somatotopic organization of the hand representation of SI. Additionally, subjects in the Albanese study were required to detect the end of each stimulus, a strategy that may have heightened attention towards the stimuli and contributed to a temporal summation of the BOLD signal in SI.

2.2.4 Secondary somatosensory cortex (SII)

SII is also considered to be an important region for processing the sensory discriminative component of pain. SII is located in the parietal operculum in the dorsal bank of the lateral sulcus. Like SI, this region receives projections from the ventroposterior lateral nucleus (VPL) (54), but its major nociceptive input comes directly from the ventroposterior inferior (VPI) nucleus (55). Although few nociceptive neurons have been recorded in SII in non-human primates (56-58), this area is nevertheless commonly activated by noxious stimuli in human imaging studies (26). Likewise, studies of patients with lesions that include SII have demonstrated deficits in the perception of pain intensity (59;60); however, lesions comprised additional cortical regions that may work in concert with SII to process this piece of information. In addition to these clinical findings, converging evidence from a number studies (61-63), now supports the notion that SII possesses a functional capacity to discriminate between different intensities of noxious stimuli presented to the contralateral side of the body. Furthermore, evidence from PET provides a role for this region in intensity processing in that subjects' ratings of pain intensity in response to thermal heat pain have been shown to be highly correlated with activation of SII (64). Additionally, an fMRI study by Maihofner et al. (65) found increased activation in SII in response to painful mechanical stimuli compared to thermal heat pain. In turn, ratings of subjective intensity were correlated with the intensity of mechanical pain. However, dissociative processing was noted in this region as ratings of unpleasantness were not found to correlate

with SII activation. Contrary to these findings, evidence from fMRI suggests this region may be involved in some emotional aspects of pain processing. For example, Gracely et al. (66) found that fibromyalgia patients who scored higher on a pain catastrophizing questionnaire showed increased activation in both the ACC and SII in response to noxious stimuli. Catastrophizing (and in turn anxiety about painful stimuli) is inherently linked with pain perception, where the individual's emotional state augments neural processing of these stimuli. In line with these findings are data that show increased activity in SII during the anticipation of painful stimuli, indicative of an enhanced emotional response (67).

2.2.5 Insular cortex

The insula receives inputs from both SI and SII, and also from thalamic nuclei (VPI, the centromedian-parafasicular, the medial dorsal [MD], and the ventral medial posterior [Vmpo] nuclei); in turn, these nuclei receive nociceptive input via the spinothalamic tract (31;54;68;69). Early clinical reports (70), as well as more recent quantitative studies (71), have indicated that patients with lesions encompassing the insula do not exhibit normal withdrawal or emotional responses to noxious stimuli, indicating an altered or deficient perception of pain affect. Accordingly, fMRI activity in this region in response to noxious stimuli is correlated with subjective ratings of pain unpleasantness (72).

The insula has also been found to process sensory-discriminative features of nociceptive information, making it a likely area of convergence of the two pain systems. Evidence for the role of the insula in sensory-discriminative processing comes from direct electrical stimulation to the region during awake brain surgery, demonstrating evoked painful sensations in the body (73). Furthermore, several other lines of evidence indicate that this region may be involved in the localization of painful stimuli, as it contains a somatotopic map of the body. The dorsal posterior insula receives pain and temperature information from a somatotopically organized

region of the thalamus – the VMpo (74), which in turn receives projections from thermoreceptive and nociceptive neurons residing in lamina I of the spinal cord (75).

Neuroimaging studies of pain perception frequently report insular activation, making it difficult to dissociate it from activation seen in adjacent regions of SII (76). Resolving the precise somatotopic organization of the insula using fMRI has only recently become feasible with the availability of high-field strength magnets. As of late, two fMRI studies at 3T have revealed a nociceptive somatotopic organization in the dorsal posterior insula in response to both cutaneous and muscle pain (77, 78). Henderson et al. (77) also reported a distinct somatotopic organization in the right anterior insula ipsilateral to the muscle pain stimuli, and found that activation of this area was greater in comparison to cutaneous stimuli. The authors attributed the increase as a reflection of the enhanced unpleasantness associated with muscular pain.

2.2.6 Anterior cingulate cortex (ACC)

The ACC plays a prominent role in pain processing. This region receives thalamocortical input from nociceptive neurons in the thalamus (79;80) and contains nociceptive-specific neurons responsive to noxious stimuli (81). Additionally, the ACC is implicated in mediating antinociceptive responses as it contains high numbers of opiate receptors (82;83).

Historically, the ACC was considered key to affective processing, as it was classified along with the retrosplenial cortex, the hippocampus, the amygdala, and several basal forebrain structures as part of the limbic lobe, which was considered central in mediating emotion (84, 85). Likewise, the ACC was targeted for surgical lesions to alleviate the suffering of chronic pain (86-88); patients reported that they still experienced the pain they felt prior to surgery, but its emotional unpleasantness was dampened (89;90).

The ACC is subdivided cytoarchitectonically into several Brodmann

areas (BA), namely 24 and 32 (91), with two further subdivisions BA33 located in the perigenual region, and BA25 located in the subcallosal region. The ACC is functionally divided, rather independent of the cytoarchitectonic borders, into a caudal cognitive division involved in attention (BA24 and BA32) and a rostral affective division, which is more involved in emotional processes (BA24, 25, 33) (92). Dissociation between the cognitive division and pain-related processing region was elegantly demonstrated using fMRI by Davis and colleagues (93), who compared BOLD activation evoked by noxious stimuli to that seen during a demanding cognitive task. Activation associated with the noxious stimuli was found to be inferior and caudal to that produced by the cognitive task.

The first direct evidence for the role of ACC in processing affective components of pain came from a PET study, in which subjects under hypnosis were instructed to modulate the perceived unpleasantness of a painful stimulus while maintaining perceived pain intensity (94). Results showed that activation of the ACC was highly correlated with the subjects' ratings of pain unpleasantness, while activation of the SI was unaltered by emotional processes. Nevertheless, these imaging and lesion data should not be interpreted too rigidly, since the ACC has been shown to have some sensory-discriminative characteristics, such as a crude nociceptive somatotopic organization (95). Furthermore, reductions in both pain intensity and unpleasantness have been described following a neurosurgical capsulotomy – interruption of fiber tracts to the ACC (96).

2.2.7 Prefrontal cortex (PFC)

Regions of the PFC have been implicated in both pain processing and pain modulation. PFC activation seen in brain imaging studies of pain is believed to reflect attention towards the stimuli (64;97), but it has also been shown to be directly involved in modulating responses to painful stimuli. Using fMRI, Wager and colleagues have recently demonstrated increased PFC activity during the anticipation of pain, which was interpreted as a pre-

emptive anticipatory response triggering a descending modulation of the pending nociceptive signals via activation of midbrain structures (98).

2.2.8 Amygdala

The amygdala, buried beneath the uncus and located at the tail of the caudate nucleus, is a key limbic structure involved in the processing of emotional stimuli. The amygdala is suited for such processing as it is the sole subcortical structure to receive projections from every sensory area. Interestingly, projections to the amygdala from visual and auditory areas are greater in primates than in other species (for review see [99]).

Functional neuroimaging studies utilizing various types of aversive stimuli including pain, habitually report amygdala activation (100). Studies using fMRI have demonstrated that amygdala activation is associated with extremely unpleasant noxious stimuli, suggesting an involvement of this region in processing the affective component of pain (101). Other evidence from fMRI has implicated the amygdala in processing uncertainty associated with painful stimuli (102).

2.2.9 Brainstem

In addition to cortical regions, a host of midbrain structures are also involved in processing pain affect including the PAG, the superior colliculus, the red nucleus, nucleus cuneiformis, the Edinger-Westphal nucleus, nucleus of Darkschewitsch, pretectal nuclei, the interstitial nucleus, and intercolliculus nucleus. Several of these structures are involved in pain modulation – the best characterized being the PAG. The PAG surrounds the cerebral aqueduct in the midbrain. Inhibitory enkephalin containing neurons in the PAG disinhibit local interneurons and in turn excite neurons in the rostral ventral medulla (RVM) and/or the locus coeruleous (LC). The aminergic projection from the RVM and LC then projects to the spinal cord and dampen pain transmission in dorsal horn neurons through several different mechanisms (103;104).

Presently, the sensitivity and in-plane resolution of 1.5 T and 3.0 T

MRI scanners are limited in their ability to resolve fine spatial localization of many brainstem structures. Brainstem functional imaging also is limited by image distortion and is susceptible to local magnetic field inhomogeneities and pulsation artifacts (105-107).

2.2.10 Motor cortices

A number of other cortical and subcortical regions are commonly activated during fMRI studies of pain including many regions involved in motor processing. Motor regions include the primary motor cortex, the premotor cortex, the supplementary motor area, the cerebellum, and basal ganglia; frequently, these regions are concomitantly activated along with those involved with affective and sensory aspects of pain processing.

The study of pain-motor interactions is just developing in neuroimaging (108), and our current understanding of this complex interaction is still incomplete. The perception of a painful stimulus involves an orienting response and subsequent retraction of the body part being targeted. Activation of motor areas during functional neuroimaging studies is believed to reflect motor preparatory responses. However, several of these areas, such as the nuclei associated with the basal ganglia, are directly responsive to noxious stimuli (109). Using fMRI, a reliable somatotopic organization has been shown in the putamen (110) in response to noxious stimuli, which indicates this region may be involved in sensory discriminative processing of pain.

2.3 USE OF fMRI TO STUDY NOCICEPTIVE PROCESSING

Compared to other brain mapping techniques currently used to study pain experimentally in humans, such as PET, EEG, magnetoencephalography (MEG), or optical imaging, fMRI is the tool of choice, given its high spatial resolution, noninvasiveness, and reasonable temporal resolution, which allows the study of rapid dynamic processes involved in pain processing. A number of methodological issues are

reviewed below concerning the use of the BOLD signal in research involving cortical, and more recently, spinal mechanisms of pain perception.

2.3.1 Nociceptive BOLD signal

Functional MRI measures local blood flow changes in response to brain activity. Increased neuronal activity causes an increase in oxygen consumption resulting in an increase in local blood flow and volume (111). This occurs after a delay of ~2s with the hemodynamic response function (HRF) peaking after ~6-9s after stimulus onset (112). However, for cortical nociceptive processing related to cutaneous heat stimuli, the HRF peaks slightly later and lasts longer in comparison to innocuous stimuli. Chen et al. (113) performed a direct comparison of the temporal properties of the HRF in response to noxious thermal heat pain and innocuous brushing stimuli in SI and SII. While both stimuli were of the same duration, the time course for innocuous stimuli peaked ~10s after the onset of the stimulus and dissipated quickly after its removal. However, noxious thermal heat stimuli produced a time course peaking at ~15s after the onset of the stimulus and the response was sustained for several seconds (Fig. 1). Similar results have been reported in response to painful electrical stimuli (114); identical trains of noxious and innocuous stimuli produced differential time courses, with the HRF for painful stimulation lasting twice as long as that produced by non-painful stimuli.

Time course information on the BOLD response to noxious stimuli is crucial for interpreting data analyzed using the standard canonical HRFs available in the majority of fMRI analysis software, which approximate this time period at ~6 seconds. Ideally, to establish a more representative model of painful stimuli, a canonical HRF should be created based on data from independent studies employing similar noxious stimulation. The BOLD signal can then be regressed against this canonical HRF based on the BOLD nociceptive signal.

A related issue in analyzing data recorded during experimental pain studies is the critical importance of considering the rise time of thermal stimuli when establishing time periods in the event design matrix. As the temperature of the thermode gradually increases, warm and pain fibers will become increasingly activated. In order to maximize sensitivity for detection the pain-related BOLD signal, it is important to enter into the design matrix solely the period of time during which the thermode has exceeded the subjects' pain threshold – not the initial rise-time of the stimulus period, which would be associated with the innocuous warm sensations perceived before the actual onset of pain.

2.3.2 BOLD fMRI of spinal nociceptive signals

A newly developing field in pain fMRI is spinal cord imaging, which is crucial for a better understanding of CNS pain processing. The spinal cord and brainstem receive input from the periphery before relaying this information on to the cortex. These subcortical regions are involved in the modulation of nociceptive input and the potentially abnormal processing of that input that may lead to chronic pain syndromes. Therefore, knowledge concerning the peripheral mechanisms of nociceptive processing is crucial to understanding a number of pathological pain conditions resulting from nerve injury or inflammation. These factors contribute to the generation and maintenance of two key components of chronic pain, namely hyperalgesia and allodynia. Hyperalgesia is the phenomenon where an exaggerated response occurs after exposure to a noxious stimulus. Allodynia is an exaggerated response towards non-painful mechanical stimuli. Both occur when nociceptive fibers become sensitized, after exposure to a noxious stimulus, causing the release of 'painful' substances in the periphery. Peripheral sensitization can occur due to inflammation of peripheral tissues as a result of a burn or cut. Because of this barrage of input, peripheral nociceptors can become hyperexcitable. This peripheral sensitization can also occur due to ectopic firing of peripheral nerves resulting from an

amputation or injury. Central sensitization can occur in the dorsal horns of the spinal cord, when peripheral nerves that were once insensitive to nociceptive input switch their firing patterns and begin to transmit nociceptive information, causing the area of affected skin to become painful to the slightest touch. Much research in this area is focused at the periphery, although these processes have been shown to have supraspinal effects resulting in aberrant cortical activity and the reorganization of body maps in somatosensory cortices.

To fulfill this need to study spinal mechanisms of nociceptive, experimental models directed towards spinal fMRI have begun in humans (115-117). fMRI of the spinal cord is challenging because of several factors. Most importantly, the small size of the spinal cord makes it difficult to achieve high spatial resolution without loss of signal-to-noise ratio (SNR). High SNR is important for good image quality and will increase in relation to voxel size, the number of image acquisitions, phase encodings, or the number of scans. The spinal cord is at its largest in the cervical segment, measuring ~16mm x 10 mm. To achieve high spatial resolution of such a small structure, slice thickness, field of view (FOV), and matrix size can be reduced; however, these strategies reduce the SNR. For example, reducing FOV from 340 to 250 mm causes a reduction in signal of ~50%. On the other hand, thinner slices improve image quality since they are less susceptible to partial volume effects, which are inherent in imaging the spinal cord (due to the small size of the spinal cord, different tissue types and pulsating cerebrospinal fluid [CSF] make it difficult to dissociate one from the other). Spinal fMRI is very sensitive to a number of artifacts, such as magnetic field inhomogeneities; differences in the magnetic susceptibility and field gradients of each component of the spinal cord (bone, discs, cartilage, tissue) result in a loss of signal. Other factors causing increased noise in spinal fMRI signal include physiological motion such as CSF pulsation, respiration and cardiac rhythms. One potential analysis strategy involves

recording these physiological parameters during image acquisition, identifying them during post-processing, and subsequently removing their contribution to the BOLD signal using independent components analysis (116).

To date, only a few reports have assessed the feasibility of studying nociception using fMRI of the spinal cord. One study by Brooks and colleagues (116) examined the spinal nociceptive signal at 1.5 T in response to noxious heat pain stimuli. Using a tailored, high-resolution scanning protocol and postprocessing techniques for controlling for physiological noise, they demonstrated reliable pain-related activation in the ipsilateral dorsal horn.

Applications of spinal fMRI to the study of chronic pain could have vast clinical applications. Use of a non-invasive functional imaging modality could shed light on the spinal mechanisms involved in the generation of neuropathic pain, such as dysesthetic pain in patients with spinal cord injury or syringomyelia. In addition to understanding the effects of chronic pain on neuroplasticity of the spinal cord, spinal fMRI could provide insight into the potential mechanisms of medications and their efficacy at treating chronic pain.

2.4 METHODS FOR fMRI PAIN EXPERIMENTS

2.4.1 Pain Assessment

A key issue in functional imaging of the cortical nociceptive signal is to ensure that the stimuli delivered to the subjects are perceived as noxious. Pain thresholds are commonly determined during a separate session prior to the scan. This procedure also serves to familiarize participants with the stimuli and reduce anxiety, thereby minimizing anxiety-related fluctuations in cardiovascular activity (118). Stimuli utilized for the scanning session are frequently tailored to each individual's pain threshold; otherwise, all subjects can be administered the same level of noxious stimulation, which has been

determined to evoke the perception of pain in all subjects. A corollary to the appropriate choice of noxious stimuli is the confirmation that predetermined levels of stimulation are actually perceived as painful, within the scanning environment. A number of contextual factors can alter the perception of stimuli that were originally considered painful during a pre-scanning test, including the temperature of the scanning suite, the position of the body in the scanner, and distractions of noise, possible feelings of claustrophobia, and other conditions specific to the scanning paradigm.

It is also important to note that the perception of pain can change during the course of a scanning session, due either to habituation (119), sensitization or the potential changes in attention during a long scanning experiment. To address this issue, pain assessment ratings can be obtained *during* the fMRI scanning session through subjective reports from participants using a variety of methods. Subjects can rate their perception after each stimulus, continuously during the stimuli, or at the end of the scanning run by giving an average rating of all the stimuli. Ratings can be obtained using electronic VAS scales, verbal or simple manual reports.

In fMRI experiments, ratings can be obtained during or immediately after the presentation of each stimulus. Conversely, due to methodological issues, with PET studies pain ratings can be taken only at the end of a scanning session several minutes after stimulus presentation. Increased time between stimulus presentation and assessment can cause inaccuracies in subject responses (120). This is a special consideration in studies examining mechanisms of analgesic relief since retrospective ratings can be inflated with increase time after stimulus presentation (121;122).

In addition to ensuring that the noxious stimuli are actually painful, pain assessment ratings (and other behavioural measures) can be used as regressors in the fMRI design matrix to aid in identifying cortical regions involved in various aspects of pain processing. Behavioural data can be incorporated into the fMRI design matrix as a weighting factor

applied to the canonical HRF. Alternatively, continuous pain ratings (recorded during the stimulus presentations) can be modeled in the design matrix (for example, see [123]). The resulting contrasts produce activation sites that are more closely based on the degree to which a region's activity correlates with the perceived intensity of the stimuli rather than with the physical intensity of the stimulus – in other words a "percept-related" activation as opposed to a "stimulus-related" activation (124). This is an important consideration as it has been demonstrated that subjects' continuous ratings of brief (~35 s) thermal heat pain stimuli correlate well with the nociceptive BOLD signal (123).

This experimental approach may have important implications for studying the dissociation that sometimes occurs between the intensity of peripheral stimulation and the perception of pain. For example, presentation of noxious mechanical stimuli over longer durations (~2 min) has been shown to result in an inverse relationship between the firing frequency of nociceptive afferents and the perceived intensity of pain evoked by the stimuli (125;126). This paradoxical relationship may be explained by the process of temporal summation – a disproportionate increase in the firing rate of dorsal horn neurons over time, whereby their response threshold to sensory input is substantially lowered. Additionally, repeated exposure to short duration heat pain stimuli can cause habituation to both the perceived intensity and unpleasantness of the stimuli (127). Therefore, subjective pain ratings can play a key role in the interpretation of nociceptive processing in the cortex, as opposed to utilizing simply the duration or intensity of the noxious stimuli that may not aptly reflect the resulting activations. Only a few studies, however, have explored the possible cerebral mechanisms underlying habituation or sensitization to painful stimuli (119;128;129), and these gave conflicting results concerning any specific association between cerebral activity and ratings of pain intensity. On the whole, however, these ambiguities in the correspondence between stimulus delivery, evoked nociceptive signal, and subjective reports of pain intensity, underscore the

importance of accessing the level of perceived pain during scanning sessions, rather than assuming a fixed relationship between stimulation and percept.

A number of advantages and potential disadvantages are associated with obtaining continuous pain ratings of stimuli during fMRI experiments. Clearly, participants' perceptual evaluations will rely less on memory and will tend to be more accurate, compared with evaluations made after the scanning run. In turn, the resulting brain activation will be less reflective of mnemonic or error detection processes. Additionally, continuous ratings can be used to deduce the time lag between the application of the stimulus and the onset of pain perceived by the subject, and to provide further details about the time course of pain perception and the underlying neural activity.

While continuous ratings provide real-time information about a subject's perception of the stimuli, a clear disadvantage to their use is that the motor activity and motor-related activation can produce a confound that complicates interpretation of sensory-related activity. However, this can be accounted for by including the movements as covariates in the fMRI design matrix. This technique was utilized in a recent fMRI study that explored the impact of continuous rating on brain activity by presenting subjects with painful mechanical stimuli to one hand and requiring that they rate the intensity of every second stimulus using the opposite hand (130); as a control, identical scans were performed utilizing innocuous mechanical stimuli. Interestingly, the BOLD signal in somatosensory regions was found to be heavily dependent on the rating of the stimuli, with t-values more than doubled for rated stimuli compared to unrated stimuli during both the noxious and innocuous scans. The authors note that the enhanced activity was likely dependent on the greater attention paid to the rated stimuli. On the other hand, virtually no differences were seen between the levels of activity evoked by the two intensities of mechanical stimulation – noxious and innocuous, a finding that is contrary to those of previous neuroimaging studies, which had shown intensity-dependent activation in response to

noxious and innocuous stimuli (37;102;131;146). Furthermore, the majority of brain regions activated during the pain task were correlated with the movements associated with the continuous ratings, which the authors attributed to motor planning and attentional effects.

2.4.2 Statistical techniques

2.4.2.1 Conjunction analysis

Conjunction analysis permits the identification of brain regions commonly activated during separate trial epochs (132). For example, to determine whether brain regions responsible for the perception of pain were activated during an anticipation phase, a conjunction analysis was performed on these two time periods (133). Resulting activations from the conjunction analysis, observed in the PAG, ACC, thalamus, and premotor cortex, suggest the importance of these areas in the anticipation of noxious stimuli and their potential role in the subsequent modulation of pain-related activations within these same areas.

Conjunction analysis has also been applied to understand how pain modulates the cognitive processing of concurrent sensory stimuli. Outside of a controlled experimental setting, acute or chronic pain is frequently experienced in the presence of competing stimuli. Bingel et al. (134) addressed this question by presenting painful stimuli during a visual working memory task and an object visibility task. Activation common to these different tasks was reported in the bilateral lateral occipital cortex, a region previously shown to be modulated by the amount of information processed in working memory (135). Results of this conjunction analysis provide insight into the mechanisms responsible for modulating visual input in the presence of an aversive stimulus.

2.4.2.2 Connectivity Analysis

In addition to identifying brain activation associated with the different

stages involved in the processing of pain-related information, it is also important to understand how these different brain regions interact. Advances in multivariate analysis techniques now allow for a non-invasive examination of relationships between coactivated brain regions to understand how these networks covary during the processing of painful stimuli (136).

Analysis of functional connectivity examines patterns of co-activating brain regions but makes no assumptions about their inter-related anatomical connectivity. The basis of this analysis assumes that brain regions with similar or co-varying time courses are likely to interact and are therefore functionally connected during a particular task. Valet et al. (137) used a functional connectivity analysis to examine the relationship among regions involved in modulating pain perception during a distracting cognitive task. Results showed that both pain ratings and pain-related activations in medial pain processing regions were lower during the distracter task. However, the distraction period was associated with increased activations of prefrontal, PAG, and thalamic areas. The time course of the significantly activated voxels in the perigenual ACC and orbitofrontal cortex during pain, with and without distraction, were extracted and included as regressors in the design matrix. Applying this time course in the general linear model (GLM) identified other brain regions showing similar patterns of brain activations (136, 138). Activations of the PAG and thalamus were found to significantly covary with that in the cingolo-frontal cortex during pain accompanied by distraction. These results suggest that distraction may reduce pain through activation of prefrontal regions, which trigger descending inhibitory controls via the PAG and thalamus.

One study also applied an analysis of functional connectivity using a partial least square computation (139) to assess how pain perception modulates a network of cortical regions involved in a cognitively demanding task (140). Acute phasic pain was found to significantly enhance brain activity in several cortical regions, which are involved in processes related to

the cognitive task. Such findings provide insight into the possible mechanisms underlying the detrimental effects of chronic pain on cognitive tasks that demand a high level of attention (141;142). The protective function of nociceptive processes may require focused attention on pain perception, thus engaging the network of interacting cortical regions involved in general attention. The increased activity of this network during chronic pain states may supersede attentional demands of cognition resulting in apparent deficits in the performance of cognitive tasks.

Another approach that compliments the study of functional connectivity is that of "effective connectivity," which describes the causal relationships that one region exerts on another (143). Additionally, psychological or psychophysical data can be modeled into an analysis of effective connectivity (thus, referred to as a psycho-physiological interaction analysis) in order to measure the influence of one cortical region on another based on the experimental context or the behavioural state of the subject (138;144). Such an analysis was applied by Bingel et al. (134) – as an extension of their findings in the lateral occipital cortex – to examine which brain regions were involved in modulating the activity in the lateral occipital cortex during an object-visibility task. A seed region was placed in the ACC, as this region showed increased activation on high pain intensity compared with low pain intensity trial periods. The time course of the activation in the ACC was extracted and used as the physiological variable, while the degree of visibility of the objects was used as the psychological variable. These variables were then implemented as regressors in the fMRI design matrix. Results showed that modulatory activity in the lateral occipital cortex by pain was driven by the ACC – a finding that is consistent with known anatomical connectivity.

Connectivity analyses of pain processing can be complemented through the use of *in vivo* mapping of white matter fiber pathways using diffusion tensor (DT) MRI. A recent DT MRI study examined the role of cortical connectivity in the modulation of pain by the PAG and nucleus

cuneiformis (145), areas that previous neuroimaging studies have implicated in pain processing (105). Focused attention on a noxious stimulus has been shown to increase brain activity in PFC, ACC and thalamus (146). However, during distraction, activations in insular cortex, ACC and thalamus were found to decrease while increased activity was reported in PAG (147;148). Furthermore, results from fMRI indicate significant interactions between the PFC and brainstem structures during pain modulation (98;137). Results from DT MRI showed separate pathways for the PAG and nucleus cuneiformis connecting with the PFC, amygdala, thalamus, hypothalamus and the RVM. Interestingly, no correlation was found between the PAG and the ACC, in spite of previous results from fMRI studies indicating a strong correlation between the activities of the two regions during pain modulation (137). These findings highlight the importance of combining emerging noninvasive imaging techniques to deepen our understanding of pain processing and its modulation.

2.5 fMRI AND THE STUDY OF HIGHER COGNITIVE PAIN PROCESSING

2.5.1 Pain modulation

fMRI is a useful tool for examining cerebral mechanisms of pain modulation, whereby subjects experience either analgesia or hyperalgesia – a decrease or increase in perceived pain, respectively. Pain modulation can occur through both endogenous mechanisms and as a result of exogenously administered agents. One final common pathway for analgesic mechanisms is believed to be through the release of endogeneous opioids (149) acting on sites in the brainstem and midbrain that block the nociceptive signal through their descending pathways; the final effects of this descending modulation are exerted either on the spinal cord and/or at the site of peripheral nerves that transmit the nociceptive stimuli.

Additionally, recent research has implicated endocannibinoids in pain modulation, which may act on similar descending pathways (150). fMRI has been applied to study the initial factors triggering these modulatory processes either through endogenous mechanisms utilizing cognitive strategies, such as attention (147; 151), hypnosis (152-154), and placebo analgesia (155), or through exogenous agents, such as pharmacological interventions (156;157).

While several experimental protocols have been applied using radio-ligands and PET to study neurochemical mechanisms involved in pain modulation – such as in studies of placebo analgesia (158;159) many of the characteristics of fMRI contribute towards its potential to address questions in pain modulation, as has been suggested in several sections above. First, fMRI offers greater spatial and temporal resolution than PET (160). Thus, fMRI is more suited to accurately localize small brain regions involved in pain modulation, such as the RVM or PAG (148;161), and is better able to assess the time-course of activations of those regions. fMRI is also well suited to study procedures that evoke changes in pain perception since it accommodates the use of parametric data, whereby experimental parameters such as pain ratings (intensity, expectation, unpleasantness) can be correlated with brain activations and thus used to characterize cortical structures according to their response profile to various experimental parameters. fMRI also has the advantage of allowing a larger number of scans within a single session (112) and a larger number of experimental conditions during a single experimental paradigm, as opposed to PET studies, which limit the number of measurements that can be taken in order to minimize exposure to radiation. As a corollary of increased temporal resolution, a major advantage of using fMRI to study pain modulation is the possibility of utilizing event-related designs whereby the time course of brain activations over different phases of the modulation period can be studied – the anticipation of the noxious stimulus, the onset of pain perception,

changes in pain perception over time, and post-stimulus ratings. Anticipation of the painful stimulus is a crucial phase of the pain modulation process, since at this time point neural mechanisms act on descending modulatory systems to diminish or enhance the response to the stimulus (162). Rapid event-related fMRI designs also permit a short stimulus-delivery phase (on the order of seconds for thermal stimuli or milliseconds for laser and electrical stimuli), which can have several advantages for a number of different experimental designs. Namely, short-duration stimuli avoid or minimize sensitization of the skin that may occur with the much longer stimulus presentations that are required in PET studies. Additionally, short-duration stimuli minimize the potential attenuation of the BOLD response to noxious stimuli (163) or the reduction in pain sensitivity and activation of antinociceptive responses (149;164) that may be evoked by long-duration tonic stimulation.

2.5.2 Pain empathy

Inherent to processing the emotional component of pain is the ability to understand the emotional reactions of other people who are experiencing pain – i.e., pain empathy (165). This rapidly growing field of empathy research is directed towards studying the mental representation of pain – both that which is perceived to be experienced by others, as well as that which is perceived as one's own. Several different types of experimental stimuli implicating other people in pain have been used in these fMRI paradigms, including photographic images (166-170), or short animations (171) of body parts in potentially tissue-damaging situations, viewing the faces of actors evoking facial expressions of pain (172;173), or subjects actually receiving painful stimuli (174), or those of chronic pain patients (175), or being cued that a loved one in the room was receiving painful stimuli (176). A common finding from these studies is that the processing of pain in others recruits brain regions involved in affective processing – namely the ACC and insula.

In a recent meta-analysis, Jackson et al. (177) compiled brain activation coordinates from 10 studies examining neural correlates of viewing pain in others and compared them with data from 10 studies in which pain was evoked in healthy volunteers. Distinct activation was noted in BA24 in response to pain of the self; however, viewing the pain of others primarily produced activity more anteriorly in the perigenual (BA24/33) and subcallosal (BA32/25) regions. A similar pattern was observed in the left and right insula whereby pain of the self was associated with activation in the mid to posterior, dorsal insula, while processing pain in others was more anterior.

While the majority of imaging studies have not reported modulation of sensorydiscriminative regions associated with pain empathy, emerging evidence, from the use of transcranial magnetic stimulation (TMS), suggests a somatotopic specificity in the perceived pain of others. In two separate TMS studies, Avenanti and colleagues (178;179) reported reduced motor-evoked potentials – a reflection of corticospinal activity – in muscles that were homologous to those of other subjects being targeted by painful stimuli, as seen by the viewer. Furthermore, these reductions were correlated with the viewer's subjective ratings of the pain intensity implied by the noxious stimulation, but not with ratings of pain unpleasantness. Contrary to these findings are those of an fMRI study, which used a similar experimental protocol; Morrison and colleagues (180) administered painful pinprick stimuli to the fingertips of subjects and then later showed images of others receiving the same stimuli. The authors did not report any changes in somatosensory or motor cortices. The lack of concordance between TMS and fMRI studies may reflect subtle differences in the types of tasks, or changes occurring in the sensorimotor system which are below statistical significance thresholds in the fMRI analyses (181).

fMRI has provided considerable insight into the neural mechanisms of processing pain in others, and suggests a number of interesting clinical implications. Since pain is a sensory and emotional phenomenon that is

primarily experienced by the patient – as opposed to an easily measured sign of illness, such as fever or weight loss, for example – health-care professionals who are confronted with patients in pain must be able to infer their discomfort accurately and treat them accordingly. Further understanding of the neural mechanisms underlying how we interpret pain in others is an initial step towards how these neural circuits can change – depending on the clinical context or after years of repeated exposure to those in pain.

2.6 FUTURE OF PAIN IMAGING

2.6.1 Increased sensitivity

The last decade has seen a considerable improvement in the sensitivity of fMRI in both the spatial and temporal localization of regions of activation, and this trend is expected to continue. The shift to higher field strengths of 4 T and 7 T scanners has been shown to significantly enhance the SNR, compared to that observed with the 1.5 T – 3 T scanners (182), which have been used in most pain studies. Several imaging centers have begun human fMRI studies at 7 T; however none to date have applied it to studying pain processing. Pain imaging is poised to benefit from these advances more than other disciplines, because – unlike visual or motor tasks, for example, which produce changes in cerebral blood flow (CBF) on the order of ~40% (183-186) -- BOLD responses to nociceptive stimuli produce signal changes only in the range of ~5% (36;38;40). Improved spatial localization of fMRI pain protocols would provide better information regarding the specificity of somatosensory regions involved in noxious processing and their somatotopic organization; likewise, improved spatial localization and SNR will aid greatly to investigations of small brainstem structures that have been implicated in modulating pain processing at both spinal and supra-spinal levels.

Another burgeoning field in pain imaging is that of arterial spin

labeling (ASL) perfusion MRI, which was first described more than a decade ago (187). ASL directly measures CBF by magnetically labeling water molecules in inflowing arteries. Recent application of ASL to study experimental pain in healthy subjects (188) has shown that this technique offers several advantages. ASL gives a precise localization of neuronal structures and has demonstrated great inter-individual reliability of activation. Additionally, compared with BOLD fMRI, ASL is well suited for pain imaging studies, since it is less susceptible to signal loss and image distortions (189) due to magnetic field inhomogeneities at the air-tissue interface around frontal, medial and inferior temporal lobes (190-192). Although several methods are available to reduce these susceptibility artifacts in the BOLD signal, ASL is nevertheless an attractive alternative for pain studies that target the limbic system where signal loss from susceptibility artifacts are troublesome for such regions as the orbitofrontal cortex and amygdala. ASL also has the additional advantage of permitting longer acquisition times and is thus well suited for studying neuronal processing that may take longer to develop, such as pain modulation through hypnotic induction; fMRI, on the other hand, is limited in terms of its length of acquisition due to drifts in the baseline. However, ASL is limited in that it cannot detect changes occurring faster than 30s and is therefore not suited for event-related designs. Additionally, the technique is limited by its temporal resolution and slice coverage in which whole brain imaging is not possible using current methods. These issues should be resolved with advances made in fast echo planar imaging sequences.

2.6.2 Meta-analysis of functional neuroimaging data

Recent advances in computational techniques have led to more sophisticated tools that can be used for performing meta-analyses on existing brain imaging data. Interpreting the results from individual fMRI studies is limited by factors such as head motion artifacts, small sample size, inter-subject variability, low SNR, and reporting false positives.

Methods to perform meta-analyses of functional neuroimaging data include region- or labeled-based models (193), the spatial density method (194), and the generation of activation likelihood estimate (ALE) maps (195). The ALE method is proving to be especially useful in that it is automated, it allows for a more precise measurement of both the localization and concordance of peak activation sites across studies; it also permits the generation of significance thresholds based on permutation analysis of randomly generated coordinates (196). Using the ALE method to assess possible differences in imaging techniques for this review chapter, we performed a meta-analysis on 30 fMRI and 30 PET studies of noxious stimuli applied to both arms (Fig. 2). Results showed that the probability maps generated for the fMRI and PET studies indicated considerable overlap in a number of cortical regions including SI, SII, ACC, insula, PFC, thalamus, midbrain and cerebellum. However, fMRI studies appear to have a wider distribution of probabilistic values in cortical regions, namely SI, suggesting an enhanced sensitivity of the technique, compared with that of PET. Findings may be due to the fact that fMRI allows for longer scans and a greater repetition of scans within the same session compared to PET, which both contribute to an increased statistical power. However, at a subcortical level, probabilistic values for fMRI data were localized within the territory of the sensorimotor thalamus, while PET probability values (although similar in magnitude to those of fMRI) were more widely distributed across the thalamus. These findings indicate that the likelihood of detecting pain-related activation in the thalamus using either of the two brain imaging modalities is similar, but that the increased spatial resolution of fMRI may allow a better localization of small nuclei within the thalamus. In summary, the meta-analysis of imaging data collected across many studies provides insights and information that may not be obtained from individual studies, no matter how carefully they were designed and executed.

2.6.3 Combining fMRI with morphometry

Recent advances in the analysis of anatomical images derived from MR scanners have led to new research strategies that are expanding the concept of “functional” MRI. Whereas the BOLD signal of an fMRI study is a reliable marker of the current short term function of an activated region, measures of anatomical variability may hold clues to particular aspects of the long-term function of that region. Our growing understanding of processes like learning and memory, and their influences on neuronal plasticity, leads to a measurable corollary of long-term function – specifically, changes in the anatomical features of specific areas of the brain. Just as the power of fMRI lies in the correlation of the BOLD signal with stimulus, motor tasks, or cognitive events, likewise the potential utility of quantifying macro-anatomical changes in the brain – a morphometric analysis – lies in the correlation of these changes in anatomical structure with the subjects’ history of stimulus exposure, practice with motor tasks, and characteristics of their personality that may be associated with certain cognitive traits.

Examples of morphometric analyses include voxel-based morphometry (VBM) (197) and cortical thickness analysis (198). VBM examines changes in gray or white matter density across the entire brain while cortical thickness analysis measures the surface gray matter. It is generally assumed that at least in disease states decreases in gray matter density and cortical thinning are related to neuronal loss (199-201).

For the study of nociceptive processing and pain perception, MRI-based morphometric analyses can be used to examine neuroanatomical changes that are correlated with particular chronic pain states or to examine differences in the anatomy of specific brain regions that may underlie the variability in pain perception that is observed within a population in healthy volunteers.

A number of recent studies have reported changes in cortical and subcortical brain regions in individuals with chronic pain (202-205). In a

recent study examining structural changes in patients with irritable bowel syndrome (IBS), VBM analysis revealed decreases in gray matter density in the anterior medial thalamus (203). This analysis was complemented by cortical thickness analysis and demonstrated cortical thinning in right ACC and bilateral insula. The same group showed in an fMRI study reduced activation in the ACC and anterior insula in response to rectal pain in IBS patients (206). These comparative findings provide a neuroanatomical basis for reduced cortical activity strengthening the importance of relating anatomical structure to physiological function.

To date, a few studies have combined morphometric and functional neuroimaging analysis. However the future of pain fMRI lies in the development of examining complimentary brain imaging analysis techniques to improve our understanding of pain processing.

2.6.4 fMRI as a therapy for chronic pain

Recent improvements in the speed of analysis of fMRI data has led to the possibility that “real-time” fMRI (rt-fMRI) can be developed as a potential “therapy” for chronic pain patients. In principle, if patients can be given feedback regarding the level of activity in specific areas of the brain that are associated with the perception of pain or its unpleasantness, then learning to (self)-regulate this activity can allow them to control their own chronic pain – in much the same way as neurosurgeons attempt to control a patient’s pain by stimulating a specific area of the brain or by placing a lesion in a targeted area. Self-regulation training with EEG has provided the basis for much of the neurofeedback research; however, due to several methodological limitations EEG offers relatively poor spatial specificity within the brain (207;208). By contrast, fMRI offers superior spatial resolution, especially for deeper brain regions, and is more suitable for targeting activity in a small, localized brain region (209;210).

Neurofeedback, using real-time analysis of fMRI data, was initially developed by Cox et al. (211), and several groups have used this

technology to study learned control over brain activity during a number of tasks (212-216). Recently, the use of rt-fMRI has been applied to several clinical conditions whose etiology or symptoms might be linked to abnormal activity in known areas of the brain. In one study testing the feasibility of rt-fMRI as a neuroimaging therapy for chronic pain patients (8), normal subjects receiving experimental noxious stimuli were trained to control activity in a targeted region within the ACC – an area previously shown to be strongly associated with the perception of pain unpleasantness (94). Results demonstrated that these subjects were able to use the feedback provided by rt-fMRI to either increase or decrease, on command, ACC activity, and that the level of this activity correlated with their estimates of pain evoked by the experimental stimuli. Likewise, a small cohort of chronic pain patients, following a similar rt-fMRI training paradigm, reported a significant reduction in their level of chronic pain in comparison to that of a control patient group, which received feedback training based only on autonomic measures. Furthermore, the patients in the rt-fMRI group demonstrated a direct correlation between their ability to control ACC activation and their degree of pain reduction. In the future, rt-fMRI could also be applied to modify cortical hyperactivity that has been described for a number of other pain syndromes (217-219).

2.7 CONCLUSION

The experience of pain is complex: both sensory and cognitive components depend on a network of neural processing spread throughout many cortical and subcortical regions of the CNS. The advent of noninvasive imaging techniques has allowed us to gain a deep understanding of this multifaceted phenomenon in humans – the experimental preparation that is most relevant to our ultimate goal of understanding, managing, and alleviating pain in patients. Pain is a characteristic common to many diseases and injuries, a consequence of many medical and dental procedures, and chronic pain is essentially a

syndrome in its own right – an insufferable sensation that many times has no obvious stimulus. Pain has an enormous impact on society: it costs billions of US dollars annually due to losses in productivity, and strains the health care systems across the world. fMRI in human subjects is helping us to understand the cerebral mechanisms of pain processing and the modulation of pain by both endogenous and exogenous factors. The results of these studies are making substantial contributions to the development of efficacious interventions for treating and alleviating pain.

Figure 1. Time course of the BOLD nociceptive signal

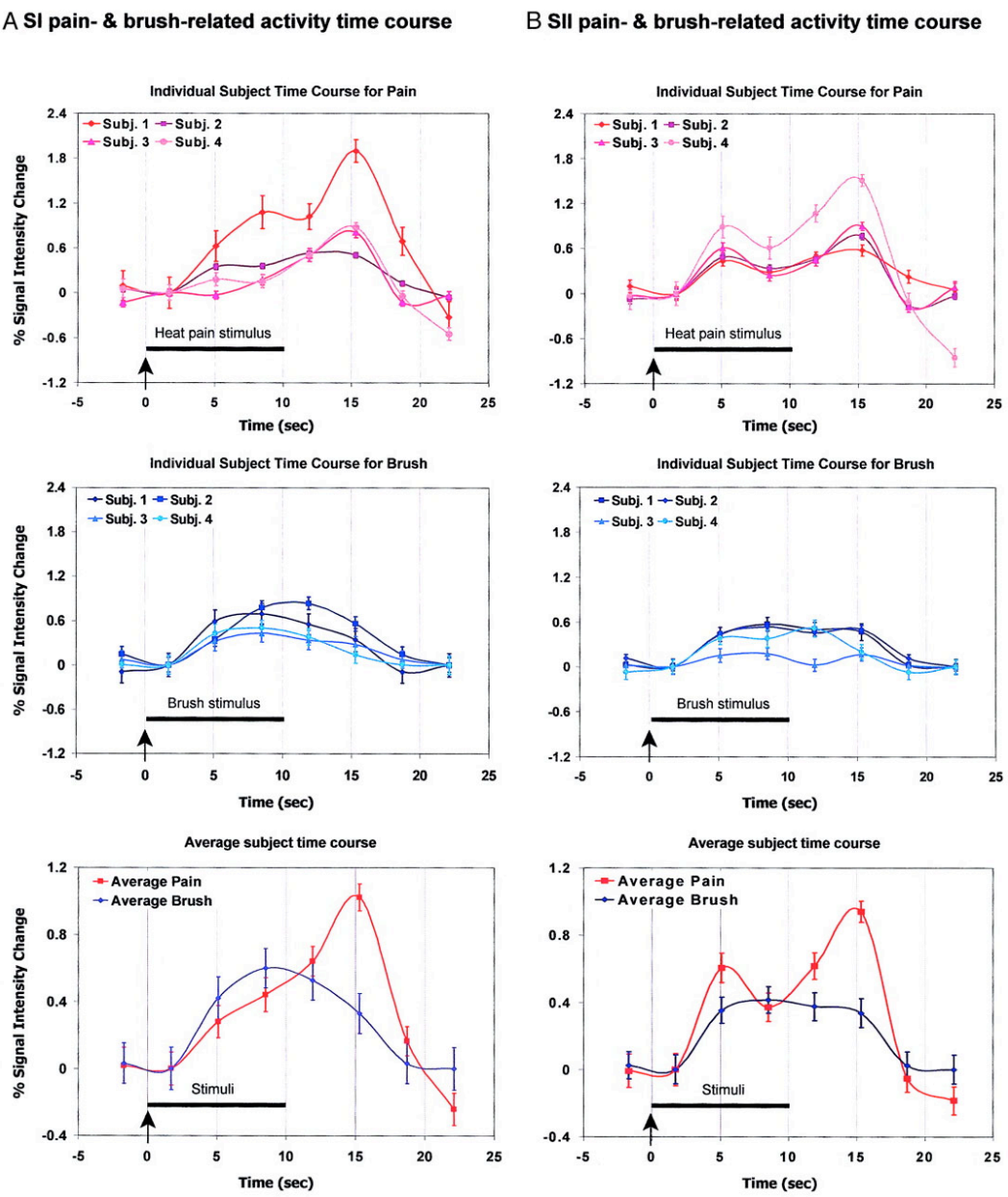

Figure 1. Time course of the BOLD nociceptive signal. (*Top graphs*) Individual subject data showing percent signal change (±SE) in primary somatosensory cortex (SI) (*left*) and secondary somatosensory cortex (SII) (*right*) in response to heat pain applied to the left inner calf. (*Middle graphs*) Time course information obtained in identical cortical regions and from body part locations in response to mechanical stimuli. (*Bottom graphs*) Averaged time course across subjects for heat pain (*red line*) and mechanical stimuli (*blue line*). The response to the thermal stimuli shows a slow gradual increase that peaks on average 15 s after the onset of the stimuli. In comparison, mechanical stimuli (*blue line*) demonstrate a faster rise time with a peak response occurring on average 5–8 s following stimulus onset and was sustained for ~ 10 s. Reprinted with permission from (Chen, Ha, Bushnell, Pike, and Duncan 2002).

Figure 2. Comparison of the sensitivity and resolution of fMRI and PET

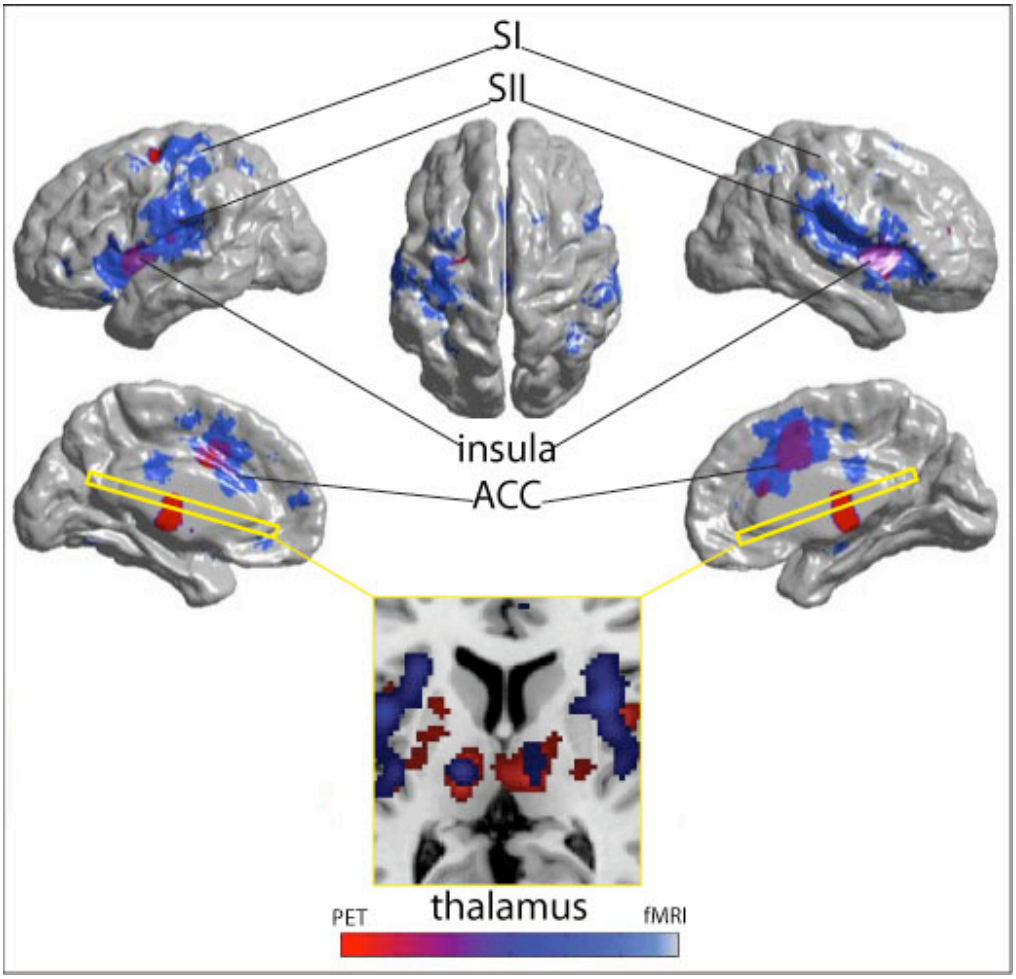

Figure 2. Comparison of the sensitivity and resolution of fMRI and PET. Shown are activation likelihood estimation (ALE) maps resulting from a meta-analysis from fifteen fMRI studies compared to an equal number of PET studies whereby noxious stimuli was applied to the arms. Journal articles were selected through an initial Medline search using the key words “fMRI”, “PET”, “pain”, and “experimental”. Experiments were then screened to ensure that the noxious stimuli were applied to left or right arms in healthy volunteers. Of the resulting studies, comparable types of stimuli and locations across studies were selected for the final selection. Stimuli included thermal (radiant, contact heat and cold), electric shock, pressure, impact, injection of capsaicin and infusion of a phosphate buffer. Relevant information related to the studies including imaging modality, size of the blurring kernel, year, subject number, pain stimulus, stimulus location and activation coordinates. All coordinates points were recorded and converted to a standardized stereotactic space (Collins et al., 1994). This yielded 590 foci for fMRI and 554 foci for PET studies. Using the application GingerALE (www.brainmap.org) the data were subjected to a quantitative voxel-level meta-analysis that produced ALE maps for the fMRI and PET activation coordinates (Turkeltaub et al., 2002). Coordinates were smoothed by 8 mm and then thresholded based on a permutation test (N=1000) and a false discovery rate (FDR) correction of q=0.05. The resulting maps are displayed on an average cortical surface from healthy volunteers registered in MNI space using SurfStat (http://www.math.mcgill.ca/keith/surfstat/). fMRI studies (shown in blue) yielded highest probabilistic values in bilateral SII (right: p=0.85; left: p=0.069), anterior cingulate gyri (right: p= 0.064; left: 0.075), insula (right: p=0.065; left = 0.063), SI (right: p= 0.014; left: p= 0.043), thalamus (right: 0.038; left: p= 0.044), prefrontal cortices (right: p= 0.035; left: p=0.025) left MI (p=0.039), right midbrain (p=0.021) and cerebellum (p=0.022). PET studies (shown in red) showed high probabilistic values in similar regions with the largest values in bilateral anterior cingulate gyri (right: 0.059; left: p=0.046), insula (right: p=0.056; left: p= 0.043), SII

(right: p=0.049; left: p=0.046), thalamus (right: p=0.046; left: p=0.043), prefrontal cortices (right: p=0.031; left: p=0.059), SI (Right: p=0.019; left: p=0.02), and the right cerebellum (p=0.052), right MI (p=0.028), and left periaqueductal gray (p=0.02).

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3 Chapter 3: Localization of pain-related brain activation: A meta-analysis of neuroimaging data

Preface

This chapter explores the neural representation of brain regions involved in processing noxious stimuli. A meta-analysis of brain activation in response to noxious stimuli was created, based on functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies using healthy subjects. A review of the literature produced a total of 130 studies in which various types of noxious stimuli were applied to the skin, muscle or viscera. The brain imaging coordinates were tabulated and converted into a standardized three-dimensional (3D) magnetic resonance imaging space (MRI). Probabilistic maps were created by calculating Activation Likelihood Estimate (ALE) values for each voxel in a template MRI. Additionally, the breadth of data included in the analysis permitted the exploration of several questions that are still contentious in the pain imaging literature including: does cold pain evoke activation within the same brain regions as those involved in processing heat pain?; what are the ramifications on the spatial specificity of brain activation when using either a resting baseline or an innocuous warm stimulus condition as a control for activation associated with noxious heat stimulation; and do specific brain regions process pain regardless of the side of stimulation?

Title: Localization of pain-related brain activation: A meta-analysis of neuroimaging data

Abbreviated title: Meta-analysis of noxious stimuli

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3.1 Abstract

A meta-analysis of 130 neuroimaging studies was performed using the Activation Likelihood Estimate (ALE) method to explore the location and extent of activation in the brain in response to noxious stimuli in healthy volunteers. The first analysis involved the creation of a general “quantitative pain matrix” - a 3D likelihood map illustrating brain activation common across studies using all types of experimental noxious stimuli. Results confirmed the significant overlap between activation sites across studies in brain regions associated with sensory and affective pain processing, with the highest cortical likelihood estimate values located in the anterior insula and the anterior cingulate cortex (ACC). The second analysis contrasted noxious cold with noxious heat stimulation and revealed higher likelihood of activation to noxious cold in the subgenual ACC and the amygdala. The third analysis assessed the implications of using either a warm stimulus or a resting baseline as the control condition for revealing activation attributed to noxious heat. Comparing noxious heat to warm stimulation led to peak ALE values that were more restricted to cortical regions with known nociceptive input, consistent with the increased specificity provided by the warm control. The fourth analysis tested for a hemispheric dominance in pain processing and confirmed the relative importance of the right hemisphere, with the strongest ALE peaks and clusters found in the right insula and ACC, regardless of the side stimulated. These results support the existence of a distributed brain network partly lateralized to the right hemisphere, which responds more strongly to noxious than innocuous stimuli and displays localized sensitivity differences depending on the type of noxious stimulus.

3.2 Introduction

Advances in brain imaging techniques, including functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), have permitted a more detailed view of the nociceptive processing in the human brain. Reviews of neuroimaging studies examining “pain-evoked” activation in the brain have reported an extensive network of cortical regions involved in nociceptive processing, including the primary (SI) and secondary somatosensory cortices (SII), the anterior cingulate cortex (ACC), the insula, the prefrontal cortex, and the thalamus (Iadarola and Coghill, 1999; Peyron et al., 1999; Apkarian et al., 2005). While these reviews have been important for collating information, they only report common *regions* of “pain-evoked” activation. For example, Apkarian et al. (2005) in their review of the pain neuroimaging literature, reported that the most commonly activated region in response to noxious stimuli was the anterior insular cortex. While this information is useful in the general sense, such qualitative approaches do not permit a quantitative appreciation of the probabilistic spatial extent of “pain-related” activation, nor do they allow a more detailed assessment of the relative influence of experimental variables on the likelihood of observing this activation within the broad network of regions implicated in pain processing. Recent advances in meta-analytic methods of assessing brain activation allow some of these limitations to be addressed. Meta-analysis is a statistical technique whereby data is collected, analyzed and compared from multiple independent studies to examine a particular research question. This approach is especially relevant to the study of the cortical and subcortical responses to noxious stimuli. By its nature, pain is a multidimensional sensory experience that leads to numerous candidate areas of brain activation; meta-analysis can be a tool to help decipher the functionality of these varied regions of activation. The quantitative approach of this method yields a brain volume in which the probability of observing activation in response to noxious stimuli is computed at each voxel based on a large number of neuroimaging studies.

The current review applies meta-analytic techniques to examine journal articles published between 1991 and 2008, which report peak activation coordinates in response to noxious stimuli (Study 1). Additionally, as pain can be evoked by different types of peripheral stimuli (eg. heat, cold, impact, capsaicin injection) and under different experimental conditions, the large number of studies included in this review facilitated the exploration of three additional fundamental questions related to the study of brain activation in response to noxious stimuli. The second analysis, presented in Study 2, addresses the specificity of activation across different stimulus modalities by comparing activation sites associated with noxious cold stimulation with those evoked by noxious heat. The third analysis (Study 3) examines the influence of one particular aspect of experimental design (the use of a resting baseline or an innocuous warm stimulus condition) on the apparent activation evoked by noxious heat stimuli. Finally, the fourth analysis (Study 4) tests for possible evidence of hemispheric dominance for activation in response to noxious stimuli.

3.3 Study 1: Meta-analysis of activation in response to all types of noxious stimuli

3.3.1 Methods

3.3.1.1 Study selection

The database was created from a compilation of journal articles retrieved from several sources and using noxious stimuli applied to the skin, muscle, or viscera. Articles reporting brain activation coordinates in response to noxious stimuli were retrieved initially using reference lists from the more recent reviews of "pain-evoked" activation brain imaging studies (Apkarian et al., 2005; Farrell et al., 2005). A subsequent Medline search was initiated using the keywords: pain, noxious, PET, fMRI, experimental, and healthy. Articles were also retrieved from the references in the original research articles collected. The database variables included the year of publication, size of the blurring kernel and the stimulus modality. Further information regarding the study selection, database variables, and inclusion criteria is detailed in the *Supplementary Information*. In total, the activation coordinates from 130 studies drawn from 122 original publications were included in the meta-analysis. A summary of the studies is listed in the *Supplementary Information* (Table S1).

3.3.1.2 Quantitative analysis

To create a probabilistic map of activation evoked by noxious stimuli, we employed the Activation Likelihood Estimate (ALE) analytic strategy as described by Laird et al. (Laird et al., 2005). Details regarding the calculation of the ALE statistic are provided in the *Supplementary Information*. Briefly, the ALE statistic is calculated for each voxel in the template MRI signifying the likelihood of evoking activation in response to noxious stimuli. Reported coordinates were recorded in their original space and then transformed into Talairach space (Talairach and Tournoux, 1988) using the software GingerALE (v.1.0) (Lancaster et al., 2007). The ALE maps were created by

blurring the activation foci using a full-width half maximum (FWHM) of 8mm, which was the average size of the blurring kernel among the studies. This latter step ensures that the data are a realistic reflection of the peak activation sites since all data included in the analysis were smoothed by an average blurring kernel of this size. The statistical significance of the ALE maps was determined by performing a permutation test (N=5000) and the data were thresholded using a false discovery rate (FDR) correction of q=0.05 (Genovese et al., 2002). The ALE method calculates the likelihood that one peak (out of the total number of peaks) actually occurred within a given voxel in the template MRI and tests this against the null hypothesis that the points are randomly distributed across the brain. The resulting ALE values indicate the likelihoods in percentages that any single peak of the total peaks actually occurred in a single voxel located in the template MRI.

3.3.2 Results

An ALE analysis was performed on 2699 coordinate points associated with activation in response to noxious stimuli. The greatest likelihood of evoking activation in the cortex in response to all types of noxious stimuli was in the right anterior insula (ALE = 23%) and ACC (BA 24, ALE = 22.8%; Fig. 1). The resulting ALE values reflect the likelihood of activation in a single voxel, which is a very small region of gray matter within the insula and ACC. The likelihood of activation occurring in the full brain regions is of course much larger. Note that these ALE values are large compared with the likelihood (0.3%) of the highest value in the background noise being interpreted as an activated voxel during the permutation testing. By calculating the conditional probability of the ALE values, we can infer that these results represent 76% and 75% likelihoods that the values in the single voxels in the insula and ACC are not due to noise or artifacts.

Additional cortical regions with significant likelihoods of activation were observed in left insula (ALE = 21.3%), bilateral SII (right: ALE = 17.8%; left: ALE = 16.8%), the prefrontal cortex (right BA 44, ALE = 12.9%; left BA 10, ALE = 5.2%), and SI/PPC (right: ALE = 6.4%; left: ALE = 6.5%). A complete list of brain regions with significant likelihoods of being activated is detailed in Table S2.

3.4 Study 2: Differential brain activation in response to noxious cold and heat stimuli

The second section of this review examines differences in brain regions that process experimental noxious cold stimuli in comparison to those that process noxious heat stimuli. Three previous studies have suggested that cold pain evokes a similar pattern of brain activation as that seen in response to heat pain (Casey et al., 1996; Craig et al., 1996; Tracey et al., 2000); however, cold pain is typically induced using the cold-pressor task, which is considered a massive autonomic stressor with a high degree of unpleasantness. Kwan and colleagues reported large inter-individual differences in brain activation evoked by cold-pain stimulation (Kwan et al., 2000), which could be explained by the potential cultural and situational influences on pain affect. However, it is difficult to draw conclusions based on the results of all these previous studies as they used relatively small numbers of subjects (N=6-13) and did not perform any direct subtractions on the data to determine which brain areas were preferentially associated with processing noxious cold or noxious heat stimuli.

3.4.1 Methods

3.4.1.1 Study selection

Study 2 consists of two meta-analyses conducted on reports selected from the database described in Study 1. The first meta-analysis was performed on data obtained from the 9 studies that applied noxious cold stimuli to the upper limbs (Table S3). For purposes of comparison, the second meta-analysis was conducted on data from 9 studies employing noxious contact heat stimuli applied to the upper limbs (Table S4). Additional details regarding the study selection and inclusion criteria are listed in the *Supplementary Information*.

3.4.1.2 Quantitative analysis

To determine the quantitative extent of activation likelihood associated with the processing of noxious cold or heat stimuli, we calculated two separate ALE maps according to the methods described in the *Supplementary Information* for Study 1. Subsequently, to test for regions preferentially associated with the processing of noxious cold or heat stimuli, we performed a voxel-by-voxel subtraction of the two ALE maps. The analysis involved the subtraction of the ALE values in condition 2 from the ALE values in condition 1 at each voxel (step 1). Two sets of random peak coordinates are then generated using the same number of peaks observed in conditions 2 and 1 and the random ALE maps undergo a pair-wise subtraction (step 2). Subsequently, this method of random peak generation and subtraction is repeated 5000 times (step 3). This process results in a single statistical map representing a null distribution of activation peaks (step 4). At each voxel, the observed ALE statistic in the original subtraction map (step 1) is compared to the random ALE statistic subtraction map (step 4) and a p value is generated to denote the statistical significance of the test. The ALE map is then thresholded at $p<0.05$ using the FDR method.

3.4.2 Results

3.4.2.1 Noxious cold meta-analysis

An ALE analysis was performed on 112 coordinate sites compiled from the nine studies that used noxious cold stimuli applied to the upper limbs. For the noxious cold stimuli meta-analysis, the likelihood of activation was significant in several brain regions involved in affective pain processing such as bilateral insula/claustrum (right: ALE = 3%; left: ALE = 2.8%), right subgenual ACC (ALE = 2.3%) and the amygdala (ALE = 1.2%; Table 1). A complete description of brain areas showing significant likelihood of activation in response to noxious cold stimuli is given in the *Supplementary Information* (Table S5).

3.4.2.2 Noxious heat meta-analysis

The ALE analysis was conducted on 122 coordinates that were published in the 9 selected studies that used noxious heat stimuli. Areas with the most significant likelihood of activation associated with noxious heat stimulation were observed in bilateral insula/claustrum (right: ALE = 3.3%; left: ALE = 2.5%), the left ACC (ALE = 2.4%), the right thalamus (ALE = 2.9%), and SII (ALE = 2.1%); see Table S6 for a complete list.

3.4.2.3 Comparison of noxious cold vs. noxious heat stimuli

Remarkable overlap in the extent of activation sites occurred in a number of brain regions in response to noxious cold and heat stimuli, including the prefrontal cortex, the ACC (Brodmann Area (BA) 24), and insula (Figure S1-A). Statistical subtractions of the noxious cold and noxious heat maps revealed that the likelihood of noxious cold-related activation was significantly greater in the amygdala and the subgenual ACC (BA 25/47; Table S7; Figure S1-B) while the likelihood of noxious heat-related activation was significantly greater in bilateral SII (Table S8; Figure S1-B).

3.5 Study 3: Control conditions for noxious heat

During a pain-imaging experiment, noxious heat stimuli are commonly generated using contact thermodes. The probe is placed on the skin and kept at a baseline temperature (30-32°C) between stimulus presentations. During the stimulation period the temperature is increased to reach a level that is rated as painful by the subject. The gradual rise in temperature will inherently activate fibres that transmit warmth information (Raja et al., 1999) and may trigger orienting responses towards the stimulus. Therefore, when using a resting baseline as a control condition for noxious heat stimuli, the resulting statistical maps may reflect a contamination of the "pain-related" brain activation with that associated with the warming of the skin and orienting responses that preceded the perception of pain.

Only a few imaging studies have specifically examined brain activation in response to warmth. Two of these studies reported that warm stimuli activate brain areas similar to those that process pain, with somewhat less robust activation (Craig et al., 1996; Becerra et al., 1999); however, one group reported both similar regions and similar activation levels in the brain in response to noxious heat and innocuous warm stimuli (Moulton et al., 2005). If innocuous warm- and noxious heat-responsive cortical neurons are distinct and co-exist within spatially defined regions of the brain, then warm stimuli may be an inappropriate control for a noxious-heat condition, since a statistical comparison between the two may result in an underestimation of activation associated with noxious stimuli.

On the other hand, another potential confound may result if warm stimuli evoke activation in brain regions that do *not* process pain. For example, Sung and colleagues (2007) reported activation in several regions outside of the commonly described "pain matrix" (as well as in regions frequently associated with pain perception) evoked by warm stimuli that were perceived as pleasant and comfortable. Although Sung et al (2007) did not present a noxious heat condition, their results underscore the

potential problems that would arise in a statistical comparison for “pain-evoked” responses across regions that are more activated during a warm “control” condition – i.e. apparent inhibition by noxious heat, which may or may not be an appropriate interpretation. Furthermore, as indicated by the perceptual ratings of the warm stimuli used by Sung et al (2007), statistical contrasts between innocuous and noxious heat stimuli may not be appropriate, as the perception of warmth is not merely a lower intensity of thermal pain or unpleasantness, but may be considered a separate sensory modality with distinctly different (positive) affective qualities. In turn, this may render the subsequent subtractions difficult to interpret.

To date, no study has compared the effects of using either a resting baseline or innocuous warm stimuli on the apparent activation in the brain in response to noxious heat stimuli. We examined the costs and benefits of the two subtraction strategies by performing a meta-analysis on a similar number of studies that used either contrast.

3.5.1 Methods

The database created for the general meta-analysis of Study 1 was searched for studies that used either innocuous warm stimuli or a resting baseline as a control condition for evaluating brain activation associated with noxious heat stimuli (applied to any part of the body). Only 9 of the 130 studies described in the Study 1 database matched the inclusion criterion for examining noxious heat in comparison to a warm control condition (Table S9). Seventeen studies from Study 1 met our inclusion criterion of comparing noxious heat stimuli with a resting baseline. Of these 17, nine were chosen for the comparison meta-analysis (Table S10). Further information regarding the selection of studies is given in the *Supplementary Information*.

3.5.1.1 Quantitative analysis

ALE statistical maps were calculated separately for the two contrasts (noxious heat vs. baseline and noxious heat vs. warm) using the same methods described in Study 1. The two maps were subtracted from one another as described in Study 2.

3.5.2 Results

3.5.2.1 Noxious heat minus warm

An initial ALE analysis was conducted on 131 coordinate sites compiled from the 9 studies that used a warm-stimulus control in comparison to noxious heat stimulation. Results of this ALE analysis yielded a total of 31 regions with a significant likelihood (ranging from 1.3% to 4.8%; $p< 0.05$ FDR corrected, cluster volume = $100mm^3$) of showing "pain-related" brain activation. The greatest likelihood that activation will be evoked in the cortex in response to noxious heat stimuli in comparison to warm was in the anterior and posterior cingulate gyrus (BA 24, ALE = 4.8% and BA 23, ALE = 2.9%), the insula (ALE = 2.8%), followed by SI and SII (both ALEs = 1.4%; Table S11). Additionally, the likelihood of evoking activation in response to noxious heat stimuli was significant within the cerebellum, thalamus and basal ganglia.

3.5.2.2 Noxious heat vs. resting baseline

An ALE analysis was applied to the 149 coordinate sites obtained from the 9 studies that used a resting baseline in comparison to noxious heat stimulation. As expected, the noxious heat vs. baseline condition yielded a substantially greater number of activation loci with ALE values above our statistical threshold ($p< 0.05$ FDR corrected, cluster volume = $100mm^3$) than had been observed in the more restrictive comparison of noxious heat to warm stimulation (40 versus 31 regions). Brain regions of interest that had a significant likelihood of exhibiting stimulus-related activation in comparison to a resting baseline were observed throughout the cortex and included the ACC (BA32, 4.2%), the inferior frontal gyrus (BA 44, 2.6%), the insula (2.4%), SI (1.9%), SII (left and right: 1.4%), and the superior frontal gyrus BA 6, 2.1%); see Table S12 for a complete list.

3.5.2.3 Statistical comparison of noxious heat vs. baseline and noxious heat vs. warm

The two ALE maps (noxious heat vs. warm and noxious heat vs. resting baseline) were overlaid on the template MRI. It was evident that for both types of contrasts, the likelihood of activation was significant within the ACC (BA 24), supplementary motor area (SMA), insula, SII, and thalamus (Figure S2).

We performed a direct subtraction of the two maps to assess significant differences in the patterns of activation that resulted from the two analysis strategies. Studies using a no-stimulation baseline control as a comparison for noxious heat stimuli were more likely to reveal stimulus-related activation in the anterior portion of the ACC (BA 32, ALE =3.9%, Table S13), and SI/PPC (ALE = 1.9%); while those using a warm-control condition as a comparison were significantly more likely to observe noxious-heat-related activation in the middle regions of the ACC (BA 24, ALE = 4.8%), and the posterior cingulate cortex (ALE = 2.9%;Table S14).

3.6 Study 4: Hemispheric dominance for activation in response to noxious stimuli

It is generally believed that somatosensory stimuli are processed primarily or preferentially by the hemisphere that is contralateral to the point of stimulation. However, evidence from clinical studies in patients with brain lesions and from brain imaging studies of normal pain processing has called this theory into question.

Results suggesting the possibility of a bilateral pain-processing network come from psychophysical data obtained from patients. For example, hemispherectomized patients can perceive painful stimuli that are either contralateral *or* ipsilateral to their only functioning hemisphere, albeit with poor localization (Olausson et al., 2001). Additionally, recent evidence from an fMRI study with callosotomized patients demonstrated that ipsilateral brain regions responsible for processing pain (SI, SII, insula, cingulate cortex) could be activated in response to noxious heat stimuli (Duquette et al., 2008).

Neuroimaging studies examining the BOLD nociceptive signal associated with stimuli applied exclusively to one side of the body have often reported bilateral activation in a number of brain regions involved in sensory-discriminative and affective-motivational pain processing. Common regions of bilateral activation include ACC, prefrontal cortex, SII, insula, thalamus, inferior parietal lobule (for example see (Bornhovd et al., 2002; Buchel et al., 2002; Bingel et al., 2004b; Bingel et al., 2007a; Bingel et al., 2007b; Boly et al., 2007), and in some instances, SI (for example see (Bingel et al., 2004b; Staud et al., 2007; Cole et al., 2008; Straube et al., 2008). A previous ALE meta-analysis examined concordant brain activation sites evoked by noxious stimuli from 22 original studies that applied stimuli to the upper arms (Farrell et al., 2005). The authors of that meta-analytic study reported that the likelihood of activation was generally bilateral, except in left prefrontal cortex and right SI. However, the finding of a significant likelihood of activation in right SI, instead of in bilateral SI (as would be

predicted given that the stimuli were applied to both sides of the body) was likely due to the inclusion of a greater number of foci from studies that had presented stimuli to the left arms (Left: 249 vs. Right: 140). For this reason, it is difficult to draw conclusions about lateralization of nociceptive processing from this previous meta-analysis as they did not perform their comparisons on a comparable number of activation sites.

Additional evidence that is inconsistent with a strictly contralateral processing of nociceptive information comes from psychophysical studies on healthy subjects suggesting a possible right-hemisphere dominance for pain processing. For example, individuals exhibit lower pain thresholds and rate pain as more intense when noxious stimuli are applied to the left side of the body (contralateral to the right hemisphere) (Haslam, 1970; Jensen et al., 1992; Pauli et al., 1999b; Lugo et al., 2002; Sarlani et al., 2003). In a study of chronic pain patients, Hsieh et al. (1995) found activation lateralized to the right ACC regardless of the limb in which pain was experienced. However, other regions, such as the anterior insula, posterior parietal, lateral inferior prefrontal, and posterior cingulate cortices, were activated bilaterally.

Two imaging studies, which specifically tested for hemispheric differences in pain processing in healthy subjects, have provided additional evidence that some brain regions in the right hemisphere preferentially process pain. For instance, Coghill et al. (2001) reported right-lateralized activation in thalamus, inferior parietal lobule, dorsolateral and dorsal prefrontal cortex in response to noxious and innocuous heat stimuli applied to either forearm. More recently, Symonds et al. (2006) described an fMRI study in which noxious electrical stimuli applied to the right and left fingertips evoked a predominant right hemispheric activation of the ACC (BA 32), the middle frontal gyrus (BA 9/46/10), the medial and superior frontal gyri (BA 6/8), ventrolateral prefrontal cortex, and the inferior parietal lobule. Both studies, however, used relatively small samples (N=9), making it rather uncertain if the results can be generalized to the population as a whole.

To better distinguish brain regions that may participate in a lateralized dominance of pain processing, we conducted a meta-analysis on a similar number of imaging studies that applied noxious stimuli to the left or to the right sides of the body.

3.6.1 Methods

3.6.1.1 Study selection

The studies selected for this meta-analysis were restricted to those found within the database of Study 1 that had used noxious heat or cold applied exclusively to one side of the body. Thirty-seven studies using left-sided noxious stimuli (Table S15) were matched with a comparable number of studies that utilized right-sided noxious stimuli (Table S16). Further details regarding the inclusion and exclusion criteria are given in the *Supplementary Information*.

A Mann-Whitney U test was performed to assess the mean and the distribution of coordinates reported in the studies included in the two comparison groups indicated that no single study unduly influenced the calculations of the meta-analyses ($p=0.190$).

3.6.1.2 Quantitative analysis

ALE statistics were calculated separately for right- and left-sided stimuli as described in Study 1. Analyses were complemented by calculating subtraction ALE statistics (left vs. right and right vs. left) to identify regions that may be preferentially activated in response to noxious stimuli applied to one side of the body or the other, according to the methods described in Study 2.

Results

3.6.1.3 Left-sided stimuli

ALE statistical maps were calculated using the 694 coordinates extracted from the publications included in the left-sided meta-analysis. According to predictions, analysis of studies using left-sided stimulation showed a substantially larger number of sites with significant activation-likelihood values in the contralateral right hemisphere, compared to those observed in the left hemisphere (31 vs. 18). The most statistically significant ALE sites were located in the right insula (ALE = 10.8%) and right ACC (ALE = 9.5%). High ALE values were also found in bilateral thalamus (right: ALE = 8.9%; left: ALE = 8.2%; Table 2). Other brain regions that also had a significant likelihood of being activated are listed in Table S17.

3.6.1.4 Right-sided stimuli

The ALE analysis was calculated on 699 coordinates that were extracted from the studies that applied noxious heat to the right side of the body. Surprisingly, the number of statistically significant activation sites were equivalent in both hemispheres (24 vs. 24), rather than being concentrated in the left hemisphere, as suggested by the traditional view of preferential cutaneous processing through the contralateral sensory pathways. The highest likelihood of evoking activation in response to noxious stimuli applied to the right side of the body was found in right anterior insula (ALE = 10.5%). Other regions showing high likelihood values were the left insula (ALE = 10%), bilateral SII (right = 8.4%; left = 9.1%), left thalamus (ALE = 8.2%) and the right ACC (BA 24, ALE = 8.1%; Table 3). These results provide strong support for a right hemispheric dominance for pain processing. A complete list of the ALE values for right-sided noxious stimulation is in Table S18.

3.6.1.5 Comparison of noxious stimuli applied to the right or left sides of the body

The greatest likelihood of evoking activation in the cortex in response to noxious stimuli presented to either side of the body was in the right anterior insula. Additionally, in both meta-analyses large clusters of likelihood estimate values were significant within the right ACC. The ALE maps for noxious stimuli applied to the right and left sides of the body can be viewed in the *Supplementary Information* in Fig. S3.

Given the strong evidence for lateralization of nociceptive processing in the right insula and ACC, we wished to assess whether other brain areas may be preferentially activated when stimuli are presented to one side of the body or the other. To explore this possibility we directly compared the two ALE maps by subtracting them from one another.

Upon performing the subtractions, (left-sided stimuli minus right-sided stimuli) the results showed preferential likelihood values that were significant within contralateral (right) SI, MI, PPC, and the superior frontal gyrus, and the ipsilateral (left) midbrain. The likelihood of activation evoked by right-sided stimuli was significant (exclusively) within contralateral (left) SI, ACC (BA32), MI, inferior parietal lobule, and the medial frontal gyrus. However, some regions in the right hemisphere were also found to have distinctive activation likelihood values in response to right-sided stimuli such as ACC (BA 32), the inferior parietal lobule, and the middle frontal gyrus.

3.7 General Discussion

3.7.1 Study 1: Meta-analysis of activation evoked by all types of noxious stimuli

We explored common brain regions activated by noxious stimuli by performing a meta-analysis on the activation sites reported by 130 fMRI and PET studies published between 1991-2008. In contrast to previous reviews, our approach provides a quantitative assessment of activation in the brain in response to noxious stimuli through the creation of likelihood estimate maps, which permit precise localization of cortical regions involved in processing pain. The maps can be particularly useful for targeting subregions of a brain area, such as SII, which has no anatomically distinct boundaries to delineate the extent and location of where to predict activation evoked by noxious stimuli.

Our results are consistent with previous qualitative reviews of the literature that have described a “pain network” comprised of SI, SII, ACC, insula, prefrontal cortex, and the thalamus (Iadarola and Coghill, 1999; Peyron et al., 1999; Apkarian et al., 2005). Additionally, our results are consistent with one of these previous recent reviews (Apkarian et al., 2005) that found the insula to be the most commonly reported activation site evoked by noxious stimuli. Our quantitative results expand upon these previous reviews by pointing to the inclusion of the posterior cingulate cortex and the basal ganglia in the “pain network”.

An important finding of the meta-analysis is that the anterior insula was the most likely cortical area to be activated by noxious stimuli. This result is in agreement with a previous qualitative review of the pain neuroimaging literature (Apkarian et al., 2005). These findings may be explained by the insula’s role in processing pain affect. For instance, patients with insular cortex damage were found to show abnormal emotional responses to painful stimuli (Berthier et al., 1988; Schon et al., 2008). However, the anterior insula receives input from peripheral autonomic

receptors, and therefore it may become activated during affective tasks or during the perception of pain due to increases in heart rate, changes in blood pressure, etc. (Cechetto and Saper, 1987; Yasui et al., 1991; Zhang et al., 1999). A number of neuroimaging studies have reported activation in the insula during tasks that involve heightened autonomic activity (Critchley et al., 2000; Cameron and Minoshima, 2002; Gianaros et al., 2007). Furthermore, the right anterior insula also has a key role in interoception, or monitoring the internal state of the body (Critchley et al., 2004). In turn, a high likelihood of obtaining activation in response to noxious stimuli in the insula may reflect an increased awareness of physiological functions during exposure to noxious stimuli.

While the anterior insula is a major site for emotional processing, it also processes sensory-discriminative aspects of pain perception. For example, direct electrophysiological stimulation of the anterior insula produces painful and non-painful somesthetic responses (Penfield and Faulk, 1955; Ostrowsky et al., 2002). Furthermore, a crude somatotopic organization was reported in the insula based on electrophysiological stimulation and functional neuroimaging of this region (Ostrowsky et al., 2000; Henderson et al., 2007).

In sum, the significant likelihood of evoking activation in the insula in response to noxious stimuli is consistent with its role in processing multidimensional aspects of pain perception and pain-related responses, including affective and autonomic processing, self-monitoring, as well as sensory-discriminative functions including stimulus localization.

Surprisingly, the likelihood of activation in SI was significant even though the values reported in this region are from the global analysis and included studies that stimulated different parts of the body. As this region has a detailed somatotopic organization, the activation peaks were in different locations of the postcentral gyrus. Another important factor is that large individual differences in the location of the central sulcus may reduce the ability to detect spatially restricted activation in SI based on multiple-

subject averaging (Geyer et al., 2000). Therefore, the probabilistic values in SI produced by this meta-analysis may not reflect accurately the likelihood of activation in this region in individual studies.

The meta-analysis has also identified cortical regions that are not typically associated with nociceptive processing, such as the posterior cingulate gyrus. Activation in the posterior cingulate cortex is often reported in pain neuroimaging studies as a finding being unrelated to processing noxious stimuli, as its role in pain processing has not been thoroughly explored. However, studies in animals have indicated that this region receives a direct projection from the main pain and temperature transmitting pathway in the spinal cord, the spinothalamic tract (Apkarian and Shi, 1998) and contains nociceptive neurons (Sikes et al., 2008) thus suggesting it processes sensory-discriminative aspects of pain. Additionally, the meta-analysis identified motor regions that invariably become activated during a pain imaging experiment, but are not typically associated with processing noxious stimuli. Activation of motor areas during pain imaging studies has been attributed to preparatory motor responses. However, several motor areas, such as the nuclei in the basal ganglia, are directly responsive to noxious stimuli (Chudler and Dong, 1995) with some regions showing a nociceptive somatotopic organization (Bingel et al., 2004a) consistent with an involvement in stimulus localization.

To conclude, this meta-analysis represents a comprehensive quantitative review identifying the specific location and spatial extent of activation evoked by noxious stimuli in the brain. Given the all-inclusive nature of the types of stimuli included in the analysis, the specific role of these structures in processing noxious stimuli cannot be addressed within the limits of the current study. More detailed information can be obtained by contrasting activation likelihood estimates associated with distinct noxious stimuli as discussed in the following sections.

3.7.2 Study 2: Noxious cold compared with noxious heat

This is the first meta-analysis of brain imaging data to directly compare noxious cold with noxious heat. The most important finding from the noxious cold meta-analysis was that these stimuli were associated with the activation of a number of sensory and affective pain processing cortical regions, including bilateral insular cortices, the right ACC, subcallosal gyrus, SII, and the right amygdala. In comparison, the highest likelihood of obtaining activation in response to noxious heat was localized in bilateral insula and thalamus. Based on the subtraction analysis (noxious heat minus noxious cold), noxious-heat related activation was more likely to occur in somatosensory cortices, which perhaps reflects the substantially lesser autonomic reaction and unpleasantness associated with these stimuli (Rainville et al., 1992).

To date, very few imaging studies have explored the neural representations of noxious cold and noxious heat pain within the same experimental protocol (Casey et al., 1996; Craig et al., 1996; Tracey et al., 2000). In one study, Tracey et al (2000) reported that cold and heat pain activated similar brain areas. However, these authors applied cold stimuli using relatively short (30s) stimuli delivered via a computer-controlled thermode that were potentially not as aversive as the stimuli used in the other cold-pain studies included in the meta-analysis.

Some studies in the meta-analysis administered noxious cold stimuli using the cold pressor task, which involves the immersion of a limb into freezing water for several minutes. In general, subjects report cold-pain sensations to be “aching” and “deep”, in comparison to heat pain, which has been described as “stinging” and “superficial” (Davis et al., 1998). Additionally, subjects rate cold pain as more unpleasant than heat pain (Rainville et al., 1992; Greenspan et al., 2003). In turn, the findings from the noxious cold meta-analysis are in line with results showing high probabilistic values in regions associated with emotional processing and negative affect

such as the amygdala, insula, and the ACC (Mayberg et al., 1999; Neugebauer et al., 2004; Wiech and Tracey, 2009).

3.7.3 Study 3: Localizing activation in response to noxious heat stimuli

In this systematic study, we examined the effects of using either innocuous warm stimuli or a resting baseline as the control condition on the apparent brain activation evoked by noxious heat stimuli. As expected, our findings indicate that contrasts with a resting baseline suggest a more widespread network of brain regions activated by the noxious test stimuli. This was demonstrated by the greater number of ALE peaks, the larger clusters of significant ALE values, and the detection of activation peaks outside of the classical spino-thalamo-cortical system (e.g. in the superior frontal gyrus). Of particular interest, the contrast with a resting baseline has the advantage of increasing the likelihood of detecting stimulus-evoked activation in SI, an area that is often missed because of a variety of factors difficult to control in brain imaging studies, as discussed above (see section 1.1.11).

A major finding from the meta-analysis in which innocuous warm was used as a control condition for noxious heat was the localized peak ALE values in BA 24 of the ACC. This important result suggests that the pain vs. warm contrast may not simply reveal a subset of activation peaks detected in pain vs. baseline. Electrophysiological studies have recorded neurons in the ACC responding to noxious stimuli, with or without attentional modulation, or solely during attentive tasks (Hutchison et al., 1999; Davis et al., 2000). An fMRI study examined BOLD activity either during the presentation of a painful stimulus or an attention-demanding task (Davis et al., 1997). Activation evoked by pain was reported in BA 24, while the attention demanding task activated BA 32. In the present results, the significant probabilistic value in BA 32 for the pain vs. baseline condition might reflect attentional resources directed towards the stimuli. Notably, this

cluster largely overlapped with another cluster that had a significant likelihood of being activated in the pain vs. warm contrast, consistent with increased attention-related responses to pain. However, the more ventral peaks found in the pain vs. warm contrast are consistent with a spino-thalamo-cortical input to BA 24 (Sikes and Vogt, 1992) which might be more closely related to the processing of noxious signals and to the experience of pain. Thus, an important incentive for using warmth as a control for pain is that it may help to discriminate activation associated with nociceptive processes and pain experiences from cognitive processes involved in the registration and attention to both noxious and innocuous stimuli.

3.7.4 Study 4: Hemispheric lateralization of nociceptive processing

This fourth meta-analysis examined the hemispheric lateralization of nociceptive processing by comparing two groups of independent studies that reported brain activation coordinates evoked by noxious stimuli applied either to the left or the right sides of the body. Regardless of whether the left or the right sides of the body received noxious stimulation, the meta-analysis revealed that the most significant probabilistic values were in the right insular cortex. Additionally, the other region to show large clusters and high ALE values for both analyses was the right ACC (BA 24).

The likelihood of activation in the contralateral hemisphere was significant within right SI, MI, PPC, and the superior frontal gyrus, for the left-sided stimuli. For the right-sided meta-analysis, the likelihood of contralateral activation was significant within left SI, ACC (BA32), MI, inferior parietal lobule, and the medial frontal gyrus.

In the ipsilateral hemisphere, the likelihood of activation was significant within the midbrain, for the left-sided stimuli. The likelihood of activation in the ipsilateral hemisphere for right-sided stimuli was significant within the ACC (BA 32), inferior parietal lobule, and the middle frontal gyrus.

Findings from this meta-analysis provide credence to the previously proposed right hemispheric dominance for pain processing (Craig, 2005). This is likely due to the role of the right hemisphere in mediating affective processing, which has been seen across a number of sensory modalities (Borod et al., 1998; Killgore and Yurgelun-Todd, 2007; Coen et al., 2009). Pain in itself is recognized as an emotional state, and in turn is highly modifiable by emotions and mood (Meagher et al., 2001; Villemure and Bushnell, 2002), an effect recently shown to involve the right anterior insula (Craig, 2005; Roy et al., 2009). An additional consideration is that unlike sensory aspects of pain, emotional responses to pain do not depend on localization, and therefore may not rely on precise spatial topographically organized maps. This is consistent with our findings of significant activation likelihood within contralateral SI.

It should be noted that the majority of studies included in the meta-analysis tested only right-handed individuals, and therefore the results may not be applicable to the population as a whole. In turn, the results may reflect differential pain processing by right-handed people. For example, the pain is more tolerable when presented to the dominant (right) side of the body (Pauli et al., 1999a). In contrast, pain sensitivity measures in left-handed people are essentially equivalent for stimuli presented to either side of the body (Pauli et al., 1999b). Therefore, left-handed individuals may process pain either in additional brain regions or in a more distributed fashion in comparison to right-handed people.

3.8 Study Limitations

While the ALE method is an exceptional research tool, one limitation associated with its use is that it does not take into account the magnitude (i.e. statistical significance) or reliability (i.e. variance, number of subjects) of the activation peaks (Sergerie et al., 2008). However, given the large number of studies and activation sites included in the meta-analyses, the data are unlikely to be weighted by the results of a single study. This latter

point is exemplified by our comparison of the number of foci included in the two meta-analyses used in Study 4. This comparison showed no significant differences between two meta-analysis distributions of foci across the studies. This result suggests that neither of the meta-analyses were likely to have been biased by a single study.

3.9 Conclusions and future work

Substantial information from functional brain imaging research can be gained through our ability to combine results across multiple studies that used a large variety of experimental conditions. Meta-analytic techniques permit the extraction of common patterns of brain responses thought to reflect the processes that are common across studies. This meta-analysis provides a detailed assessment of brain responses to different types of noxious stimuli. This technique allowed for an objective, quantitative determination of findings across imaging studies, and produced a spatial likelihood map of activation evoked by noxious stimuli. In addition to providing very strong confirmatory evidence for the activation of brain areas typically associated with pain and supporting a right-hemisphere dominance in the processing of noxious stimuli, the detailed analyses further demonstrated significant differences associated with the type of noxious stimulus employed, as well as the control condition used to reveal noxious-related responses.

Future research lies in comparing data from the current work with brain activation associated with spontaneously induced pain in chronic pain patients. Few studies have directly compared brain activation evoked by chronic and acute pain; however, a review article indicated that patients were more likely to have activation in the prefrontal cortex (Apkarian et al. 2005). A whole brain meta-analysis would offer a more expansive comparison with patient data to explore additional areas of the brain demonstrating differential activation in response to chronic versus acute pain.

3.10 Acknowledgements

The authors would like to thank Jen-I Chen for aiding in data entry. The funding for this research was from a grant provided to the authors by the Canadian Institutes for Health Research (CIHR).

Figure 1. Study #1 ALE map of all noxious stimuli evoked activation in the brain

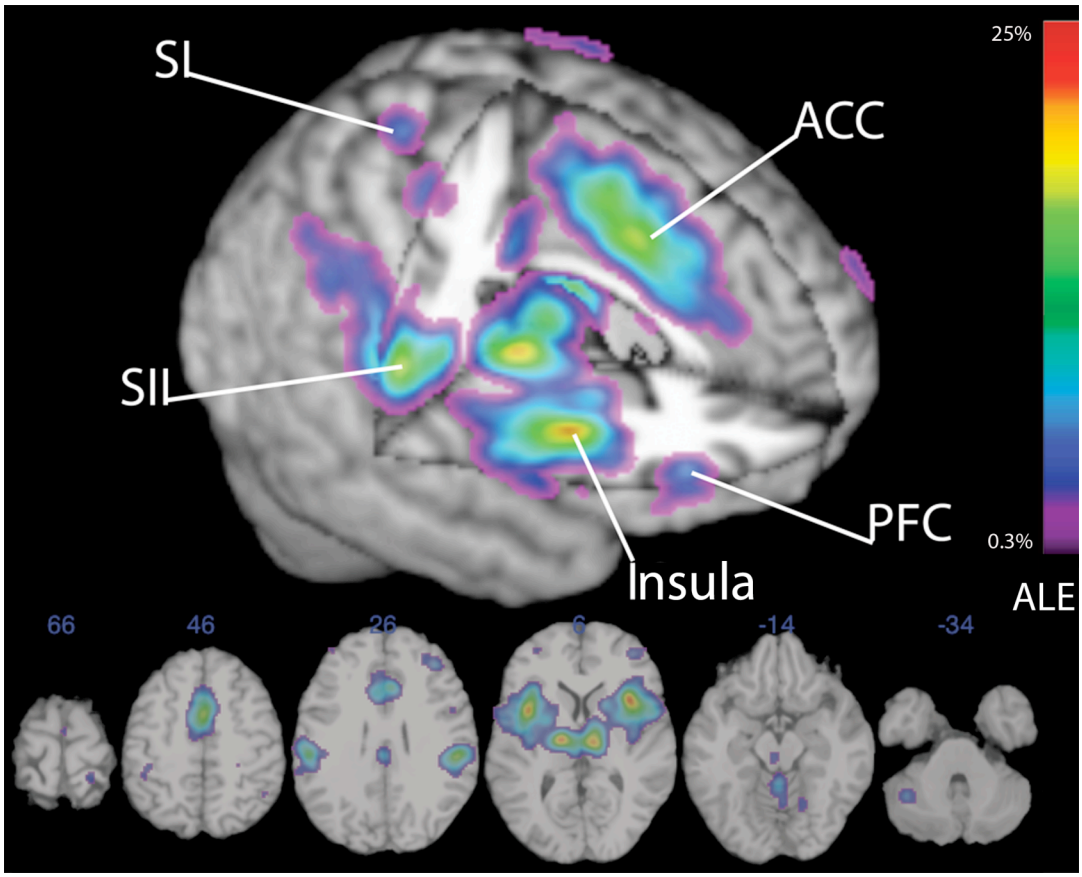

Figure 1. Study #1: ALE map of all noxious stimuli evoked activation in the brain. Likelihood values were found in the thalamus, insula, secondary somatosensory cortex (SII), anterior cingulate cortex (ACC), the prefrontal cortex (PFC) and the primary somatosensory cortex (SI).

Table 1. ALE values (percentages) for Study #2 (noxious cold)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Insula/Claustrum		28	6	12	3.0%	1	1656
Left	Insula		-38	6	4	2.8%	2	1520
Right	Subgenual ACC	47/25	18	18	-10	2.3%	10	496
Right	Amygdala		24	-8	-22	1.2%		

Table 1. ALE values (percentages) for Study #2. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm^3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; ACC = anterior cingulate cortex; SI = primary somatosensory cortex; PPC = posterior parietal cortex; SII = secondary somatosensory cortex; MI = primary motor cortex.

Table 2. ALE values (percentages) for Study #4 (left sided noxious stimuli)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Thalamus		10	-20	6	8.9%	1	18008
Left	Thalamus		-12	-16	10	8.2%		
Right	Insula		36	-20	18	10.8%	2	17912
Right	ACC	24	2	4	38	9.5%	3	12552

Table 2. ALE values (percentages) for Study #4. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm^3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior. ACC = anterior cingulate cortex.

Table 3. ALE values (percentages) for Study #4 (right sided noxious stimuli)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Insula		34	12	8	10.5%	1	41464
Left	Insula		-38	4	4	10.0%		
Left	SII		-54	-26	22	9.1%		
Right	SII		56	-22	20	8.4%		
Left	Thalamus		-16	-16	10	8.2%		
Right	ACC	24	4	8	36	8.1%	2	14016

Table 3. ALE values (percentages) for Study #4. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; SII = secondary somatosensory cortex; ACC = anterior cingulate cortex.

3.11 SUPPLEMENTARY INFORMATION

3.11.1 Study 1: Meta-analysis of activation evoked by all types of noxious stimuli

3.11.1.1 Methods

3.11.1.1.1 Study Selection

We initially conducted a search of the neuroimaging literature published between1980-2008 to retrieve articles that used noxious stimuli. Articles selected for inclusion in the database satisfied the following criteria: a) data were acquired in healthy subjects; b) the activation sites were the result of a contrast that compared a noxious stimulus condition to a resting baseline, or to a control condition, or to a noxious stimulus condition that was rated by participants as less painful, or to a no-stimulus condition conducted in a control group of participants. Likewise, articles were included in which the activation sites were determined by correlating brain activity with participants' perceptual levels of pain intensity or unpleasantness. Excluded from the analysis were studies that reported coordinates that combined painful and non-painful stimuli.

In total 130 studies were included in the analysis, 8 of which were based on further analysis of data from previous publications, leading to a total of 122 original articles (Table S1). The majority of studies (98) utilized cutaneously administered stimuli (contact thermodes, laser, impact, pressure, electric shock, pin prick, or topical capsaicin). However, some of these studies used more than one type of noxious stimulus within the same experimental protocol. Eleven studies used painful visceral stimuli (oesophageal, rectal, stomach, vascular distension), 4 used intracutaneous stimuli (ethanol injection, capsaicin injection, electric shock, or infusion of a phosphate buffer), 7 used transcutaneous stimuli (electric shock), 5 used subcutaneous injections (ascorbic acid, capsaicin, hypertonic saline), 7 were intramuscular (electric shock, hypertonic saline injection, infusion of a phosphate buffer), 1 used intranasal gaseous CO^2, and 1 applied noxious stimuli to the tooth pulp. In most instances, stimuli were applied to the upper

limbs (97 studies). Of the remaining studies, 20 utilized noxious stimuli applied to the lower limbs, 8 to the face, and 3 to the trunk.

3.11.1.1.2 Database Variables

Articles were searched to compile the following information:
(1) Author names
(2) Year of publication
(3) Blurring kernel size
(4) Number of subjects
(5) Stimulus modality (laser, electrical, impact etc.)
(6) System targeted by noxious stimuli (cutaneous, muscle, visceral etc.)
(7) Side of the body
(8) Body part
(9) Type of standardized space
(10) Brain activation coordinates

3.11.1.1.3 Quantitative Analysis

The Activation Likelihood Estimate (ALE) analytic strategy was employed to explore the location and extent of activation in the brain that could be evoked by noxious stimuli (Turkeltaub et al. 2002). The ALE value denotes the likelihood (or probability) that an activation coordinate will fall within a voxel in the template magnetic resonance image (MRI). To create the ALE map, the coordinates were recorded in their original space and then transformed into Talairach space (Talairach and Tournoux 1988) using the software GingerALE (Lancaster et al. 2007; www.brainmap.org).

The coordinates were smoothed by the size of the average blurring kernel used in the studies included in the meta-analysis (8mm). The ALE statistic was calculated for each voxel in the template MRI using the following formula from Laird et al., (2005):

$$\Pr(X_i) = \frac{\exp(-d_i^2/2\sigma^2)}{(2\pi)^{3/2}\sigma^3} \cdot \Delta V$$

Where Xi is the probability that a focus of activation is going to occur within a voxel. The value of *d* is calculated as the Euclidean (3D) distance between the centre of mass of the voxel and the focus of activation reported from a journal article, as indicated by its coordinates. The value for σ refers to the standard deviation of the size of the blurring kernel (SD=3.4mm). The value for ΔV is equal to $8mm^3$ and corresponds to the voxel dimensions of the 3D template MRI (2 x 2 x 2).

A non-parametric permutation test was performed to test the null hypothesis that the ALE values were distributed evenly throughout the template MRI (Good 1994). This process involved the random generation of 5000 sets of 2699 coordinates (the same number of coordinates contained within the meta-analysis) and the calculation of the ALE statistic for each random focus. At each voxel, the random ALE statistic was compared to the observed ALE statistic and a p value was generated to denote the statistical significance of the tests.

The ALE map was then thresholded by applying the false discovery rate (FDR) correction (Benjamini and Hochberg 1995). The FDR correction ensures, when setting the alpha value at 0.05, that on average no more than 5% of the voxels will be false positives. Although not as strict as a Bonferroni correction, this method is reasonably conservative and makes no assumptions about the distribution of the data. The value for critical threshold was calculated using the following formula:

$$P_{(i)} \leq \frac{i}{V}\frac{q}{c(V)}$$

where *i* = the index of a ranked ALE value (from lowest to highest), V = total number of voxels, q = 0.05, and c(V) =

$$\Sigma_{i=1}^{V} 1/i$$

The value for critical threshold is equal to a corrected p value of 0.05. In the present meta-analysis, the critical value for threshold was determined to be p=0.003. All of the voxels in the ALE map with corresponding p values above threshold were considered statistically significant.

3.11.2 Study 2: Differential brain activation evoked by noxious cold and heat stimuli

3.11.2.1 Methods

3.11.2.1.1 Study Selection

To determine the activation likelihood associated with the processing of noxious cold or noxious heat stimuli, we initially searched the database created for Study 1 to select two different sets of studies that used one or the other of these stimulus modalities, respectively. For the noxious cold meta-analysis, stimulus conditions included water baths, contact thermodes, and ice packs. To simplify the comparison to the noxious heat meta-analysis, we only included the 9 studies from the Study 1 database that applied noxious cold stimuli to the upper limbs (Table S3). Studies for the noxious heat meta-analysis were selected if they employed stimuli that were similar to those included in the noxious cold analysis in terms of stimulation site, imaging modality, and year of publication (Table S4). The GingerALE method does not take into consideration the number of studies, but rather the number of coordinates. Therefore, studies were also selected based on the number of reported coordinates.

Study 3: Control conditions for noxious heat

3.11.2.2 Methods

3.11.2.2.1 Study Selection

To assess the implications of using either a resting baseline or a warm stimulus as the control condition for revealing activation attributed to noxious heat, two different sets of studies were selected from the Study 1 database that used one or the other type of control condition, respectively. To obtain a just comparison between the two experimental strategies, only studies that applied stimuli to the upper limbs were included in the analyses. Nine of the 122 studies described in the Study 1 database that examined noxious heat in comparison to a warm control condition were selected for inclusion in the first meta-analysis (Table S9). Seventeen studies from Study 1 met our inclusion criterion of comparing noxious heat stimuli with a resting baseline. Of these 17, nine were chosen for comparison to the first meta-analysis (Table S10). These 9 studies were matched to those included in the first analysis according to the following criteria: imaging modality, number and extent of activation sites, year of publication, and site of stimulation.

3.11.3 Study 4: Hemispheric dominance for activation evoked by noxious stimuli

3.11.3.1 Methods

3.11.3.1.1 Study Selection

To examine a possible hemispheric dominance for processing noxious stimuli, the database for Study 1 was searched to select different sets of studies that applied noxious stimuli either exclusively to the left side or to the right side of the body. For both meta-analyses, studies were selected if they applied stimuli to the arms, legs, or sides of the face. However, to simplify the comparison, the meta-analysis included studies that used stimuli generated using contact thermodes or laser stimuli, since other modalities of noxious stimulation may evoke activation that is unequally weighted in terms of their intensity or emotional valence, which might lead to a non-uniform comparison among studies and brain activation coordinates. The data from the studies included in both meta-analyses were from contrasts that resulted from a noxious stimulus (heat or cold) compared to either a resting baseline or a control condition (innocuous warm or cool). Coordinates that were reported based on correlations of pain ratings with percent blood-oxygen-level-dependent (BOLD) signal change were also included in the analyses. Studies were excluded if they applied stimuli to the midline (back or chest), simultaneously to both sides of the body, or if they reported data combined from scans in which stimuli were applied to either side of the body. The left sided meta-analysis included 43 studies and a total of 694 coordinates (Table S15). Studies chosen for the right-sided meta-analysis were matched to those included in the left sided analysis based on the year of publication, the imaging modality, and the site of stimulation. Additionally, to have an equal number of coordinates to compare across the two sets of studies, we selected 40 studies for the right-sided meta-analysis.

Figure S1. Study #2: ALE maps of noxious cold (blue) versus noxious heat (red)

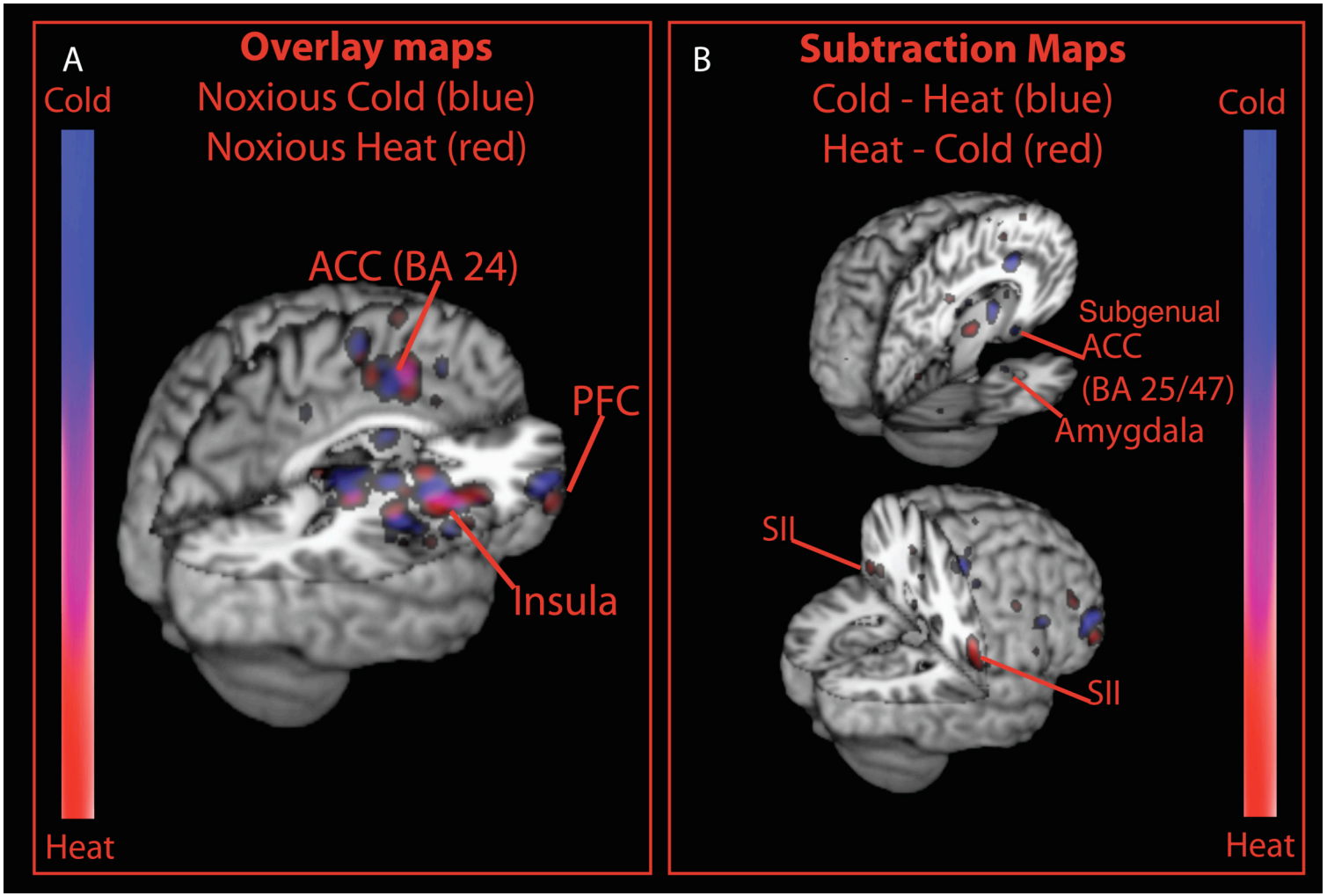

Figure S1. Study #2: (A) Overlay of the noxious cold (blue) and heat (red) ALE maps. The results show overlap of activation sites in the anterior cingulate cortex (ACC) Brodmann Area (BA) 24, the prefrontal cortex (PFC), and the insula. (B) Overlay of the subtraction ALE maps for noxious cold vs. heat (blue) and noxious heat vs. cold (red). These maps demonstrate the regions that exhibited ALE values evoked by noxious cold (blue) or noxius heat (red). For noxious cold stimuli, preferential ALE values were found in the subgenual ACC and the amygdala. For noxious heat stimuli, two large clusters of ALE values were located in the secondary somatosensory cortices (SII).

Figure S2. Study #3: ALE maps for noxious heat using either a resting baseline (yellow) or warm (blue) as a comparison

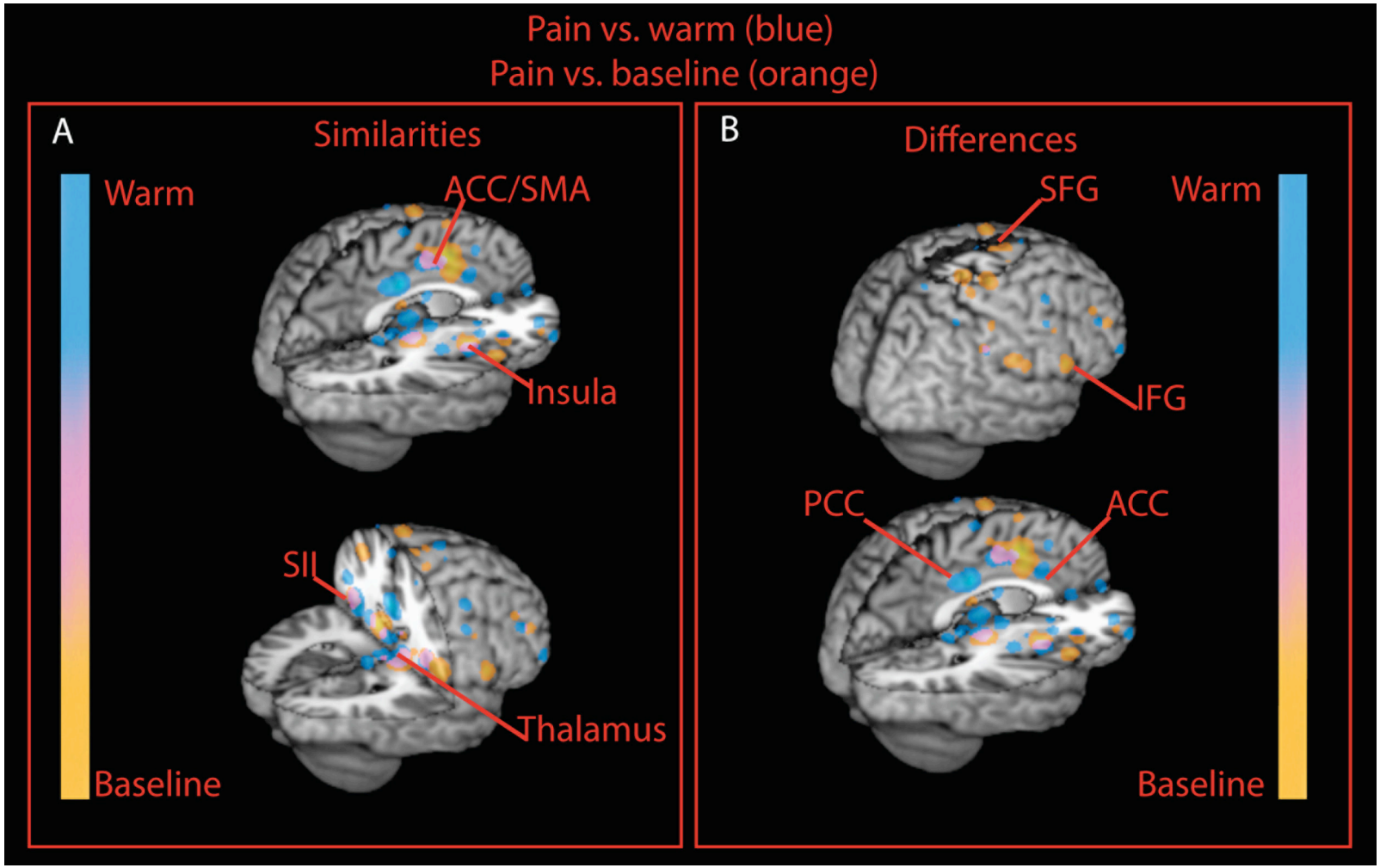

Figure S2. Study #3: ALE maps for noxious heat using either a resting baseline (yellow) or warm (blue) as a control condition. (A) Regions showing overlap of activation sites (shown in pink) were in the anterior cingulate gyrus (ACC)/supplementary motor area (SMA), the insula, the secondary somatosensory cortex (SII) and the thalamus. (B) Top: Preferential ALE values for the noxious heat vs. resting baseline contrast were found in the superior (SFG) and inferior frontal gyri (IFG; yellow). Bottom: Preferential values for the noxious heat vs. warm contrast were seen in the anterior cingulate cortex (ACC) and the posterior cingulate cortex (PCC).

Figure S3. Study #4: ALE maps of noxious stimuli applied the right (blue) and left (red) side of the body.

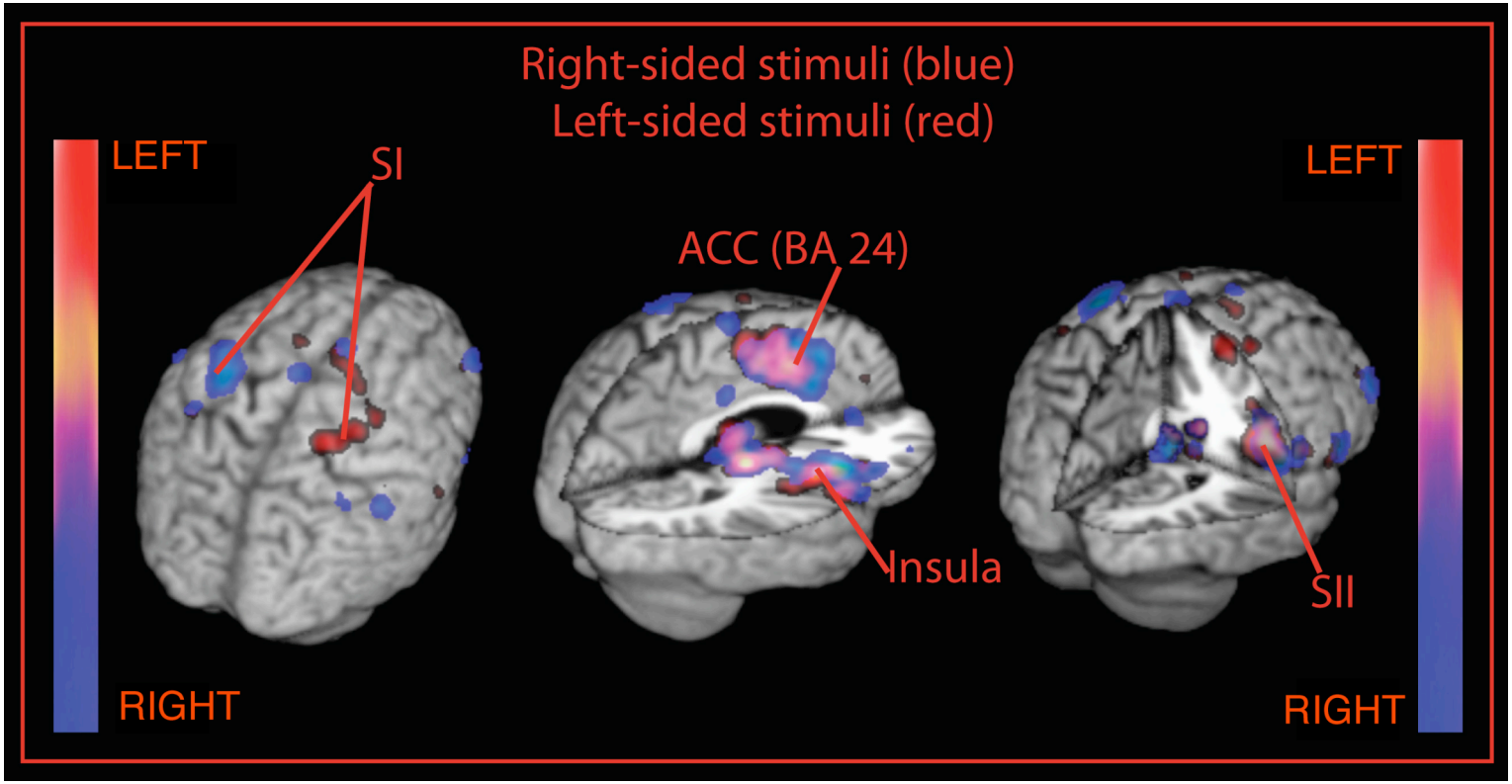

Figure S3. Study #4: ALE maps of noxious stimuli applied to the right (blue) or left (red) side of the body displayed on a template MR. Unique ALE values evoked by right-sided noxious stimuli were found in the left primary somatosensory cortex (SI) and the left-sided stimuli produced unique ALE values in the right SI. Activation sites exhibiting overlap were in the anterior cingulate cortex (ACC) Brodmann Area 24, the insula, and the secondary somatosensory cortex (SII).

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Adler	1997	PET	9	Heat	Thermal	Cutaneous	Left	Forearm	
Aharon	2006	fMRI 1.5	6	Heat	Thermal	Cutaneous	Left	Hand	
Albanese	2007	fMRI 1.5	8	Heat	Thermal	Cutaneous	Right	Hand	
Andersson	1997	PET	6	Capsaicin injection	Chemical	Intracutaneous	Right	Hand, foot	
Apkarian	2000	fMRI 1.5	7	Heat	Thermal	Cutaneous	Right	Fingers	
Aziz	1997	PET	8	Esophageal distention	Mechanical	Visceral	Bilateral	Esophagus	
Becerra	1999	fMRI 1.5	6	Heat	Thermal	Cutaneous	Left	Hand	
Becerra	2001	fMRI 1.5	8	Heat	Thermal	Cutaneous	Left	Hand (dorsum)	
Bingel	2003	fMRI 1.5	14	Laser	Thermal	Cutaneous	Left, right	Hand	
Bingel	2002	fMRI 1.5	14	Laser	Thermal	Cutaneous	Right and left	Hand	Data are shared with Bingel et al., 2003

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Bingel	2006	fMRI 1.5	19	Laser	Thermal	Cutaneous	Right and left	Hand	
Bingel	2004	fMRI 1.5	20	Laser	Thermal	Cutaneous	Left, right	Hand, foot	
Bingel	2004	fMRI 1.5	18	Laser	Thermal	Cutaneous	Left	Hand, foot	
Bingel (Neuron)	2007	fMRI 3	16	Laser	Thermal	Cutaneous	Left	Hand	
Bingel (Pain)	2007	fMRI 3	20	Heat	Thermal	Cutaneous	Left	Forearm	
Binkofski	1998	fMRI 1.5	5	Esophageal distention	Mechanical	Visceral	Bilateral	Esophagus	
Boly	2007	fMRI 3	24	Laser	Thermal	Cutaneous	Left	Hand	
Bornhovd	2002	fMRI 1.5	9	Laser	Thermal	Cutaneous	Left	Hand	Data are shared with Buchel et al., 2002
Borsook	2003	fMRI 1.5	9	Heat	Thermal	Cutaneous	Right	Face	Data are shared with Dasilva et al., 2002

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Botvinick	2005	fMRI 1.5	12	Heat	Thermal	Cutaneous	Left	Thenar Eminence	
Brooks	2005	fMRI 3	14	Heat	Thermal	Cutaneous	Right	Face, hand, foot	
Buchel	2002	fMRI 1.5	9	Laser	Thermal	Cutaneous	Left	Hand	
Carlsson	2006	fMRI 1.5	9	Electric shock	Electrical	Cutaneous	Right	Wrist	
Casey	1994	PET	18	Heat	Thermal	Cutaneous	Left	Arm	
Casey	1996	PET	27	Cold	Thermal	Cutaneous	Left	Hand	
Casey	2000	PET	11	Cold	Thermal	Cutaneous	Left	Hand	
Casey	2001	PET	14	Heat	Thermal	Cutaneous	Left	Forearm	
Chen	2002	fMRI 1.5	4	Heat	Thermal	Cutaneous	Left	Inner calf	
Christmann	2006	fMRI 1.5	6	Electric Shock	Electrical	Transcutaneous	Right	Thumb	
Coen	2007	fMRI 1.5	7	Esophageal distention	Mechanical	Visceral	Bilateral	Esophagus	

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Coghill	1994	PET	9	Heat	Thermal	Cutaneous	Left	Forearm	
Coghill	1999	PET	16	Heat	Thermal	Cutaneous	Right	Upper arm	
Coghill	2001	PET	9	Heat	Thermal	Cutaneous	Left, right	Forearm	
Coghill	2003	fMRI 1.5	17	Heat	Thermal	Cutaneous	Right	Leg	
Cole	2008	fMRI 1.5	30	Pressure	Mechanical	Cutaneous	Right	Thumb	
Craig	1996	PET	11	Cold/heat	Thermal	Cutaneous	Right	Hand	
Davis	2002	fMRI 1.5	NR	Cold prickle	Thermal/ mechanical	Cutaneous	Right	Thernar eminence	
De Leeuw	2006	fMRI 1.5	9	Heat	Thermal	Cutaneous	Left	Masseter muscle	
Derbyshire	1998	PET	7	Heat	Thermal	Cutaneous	Left	Hand	Data are shared with Vogt et al., 1996
Derbyshire	1997	PET	12	Laser	Thermal	Cutaneous	Right	Hand	

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Derbyshire	1998	PET	12	Heat	thermal	Cutaneous	Right	Hand	
Derbyshire	2002	PET	21	Laser	Thermal	Cutaneous	Right	Hand	
Derbyshire	2002	PET	16	Heat	thermal	Cutaneous	Right	Hand	
Derbyshire	2004	fMRI 3	8	Heat	Thermal	Cutaneous	Right	Hand	
Downar	2003	fMRI 1.5	10	Electric Shock	Electrical	Transcutaneous	Right	Median nerve	
Dunckley	2005	fMRI 3	10	Heat, rectal distention	Thermal, mechanical	Cutaneous, visceral	Bilateral, left	Back, rectum, foot	
Fairhurst	2006	fMRI 3	12	Heat	Thermal	Cutaneous	Left	Hand	
Farrell	2006	PET	10	Pressure	Mechanical	Cutaneous	Left	Thumb	
Ferretti	2003	fMRI 1.5	8	Electric Shock	Electrical	Cutaneous	Right	Median nerve	
Frankenstein	2001	fMRI 1.5	12	Cold	Thermal	Cutaneous	Right	Foot	
Gelnar	1999	fMRI 1.5	9	Heat	Thermal	Cutaneous	Right	Finger	

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Gyulai	1997	PET	5	Heat	Thermal	Cutaneous	Left	Forearm	
Helmchen	2003	fMRI 1.5	18	Heat	Thermal	Cutaneous	Right	Hand	
Helmchen	2006	fMRI 1.5	18	Heat	Thermal	Cutaneous	Right	Hand	Data are shared with Helmchen et al., 2003
Henderson	2007	fMRI 3	23	Hypertonic saline	Mechanical	Intramuscular, subcutaneous	Right	Leg, forearm	
Hofbauer	2001	PET	10	Heat	Thermal	Cutaneous	Left	Hand	
Hofbauer	2004	PET	15	Heat	Thermal	Cutaneous	Left	Forearm	
Hsieh	1996	PET	4	Ethanol injection	Chemical	Intracutaneous	Right	Upper arm	
Iadarola	1998	PET	13	Capsaicin injection	Chemical	Subcutaneous	Left	Forearm	
Iannilli	2008	fMRI 1.5	18	Electric shock, gaseous CO2	Electrical, chemical	Cutaneous, intranasal	Right	Forehead, trigeminal branch	

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Ibinson	2004	fMRI 1.5	6	Electric shock	Electrical	Cutaneous	Right	Median nerve	
Jantsch	2005	fMRI 1.5	8	Electric shock	Electrical	Cutaneous	Left	1st upper incisor	
Keltner	2006	fMRI 4	16	Heat	Thermal	Cutaneous	Left	Hand	
Kong	2006	fMRI 3	16	Heat	Thermal	Cutaneous	Right	Forearm	
Korotkov	2002	PET	16	Hypertonic saline	Mechanical	Intramuscular	Left	Tricep	
Koyama	2003	fMRI 1.5	9	Heat	Thermal	Cutaneous	Right	Leg	
Koyama	2005	fMRI 1.5	10	Heat	Thermal	Cutaneous	Right	Leg	
Mohr	2005	fMRI 1.5	18	Heat	Thermal	Cutaneous	Right	Hand	Data are shared with Helmchen et al., 2003
Mohr	2008	fMRI 1.5	17	Heat	Thermal	Cutaneous	Right	Thigh	
Nemoto	2003	PET	12	Laser	Thermal	Cutaneous	Right	Forearm	

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Niddam	2002	fMRI 3	10	Electric shock	Electrical	Intramuscular	Left	Hand	
Ochsner	2006	fMRI 3	13	Heat	Thermal	Cutaneous	Right	Forearm	
Oshiro	2007	fMRI 1.5	12	Heat	Thermal	Cutaneous	Left	Leg	
Owen	2007	fMRI 3	14	Heat	Thermal	Cutaneous	Left	Hand	
Paulson	1998	PET	20	Heat	Thermal	Cutaneous	Left	Forearm	
Petrovic	2002	PET	7	Cold	Thermal	Cutaneous	Left	Hand	
Petrovic	2004	PET	10	Cold	Thermal	Cutaneous	Left	Hand	
Petrovic	2004	PET	7	Cold	Thermal	Cutaneous	Left	Hand	Data are shared with Petrovic et al., 2002
Peyron	1999	PET	7	Heat	Thermal	Cutaneous	Right and left	Hand	
Porro	1998	fMRI 1.5	24	Ascorbic acid	Chemical	Subcutaneous	Right and left	Foot	

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Porro	2002	fMRI 1.5	26	Ascorbic Acid	Chemical	subcutaneous	Right and left	Foot	
Qiu	2006	fMRI 3	13	Laser	Thermal	Cutaneous	Right	Hand	
Raij	2005	fMRI 3	14	Laser	Thermal	Cutaneous	Left	Hand	
Rainville	1997	PET	8	Heat	Thermal	Cutaneous	Left	Hand	
Remy	2003	fMRI 3	12	Heat	Thermal	Cutaneous	Left	Hand	
Rolls	2003	fMRI 3	8	Pressure	Mechanical	Cutaneous	Left	Hand	
Ruehle	2006	fMRI 1.5	11	Electrical shock	Electrical	Transcutaneous/ intracutaneous	Right	Foot	
Sawamoto	2000	fMRI 1.5	10	Laser	Thermal	Cutaneous	Right	Hand	
Schneider	2001	fMRI 1.5	6	Vascular Distention	Mechanical	Vascular	Left	Foot	
Schoedel	2007	fMRI 1.5	11	Impact	Mechanical	Cutaneous	Left	Middle finger	
Schreckenberger	2005	PET	10	Infusion of phosphate	Mechanical	Intracutaneous/ intramuscular	Left	Hand	

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Seifert	2007	fMRI 1.5	12	Cold	Thermal	Cutaneous	Right	Forearm	
Seminowicz	2004	fMRI 1.5	16	Electrical Shock	Electrical	Transcutaneous	Left	Median nerve	
Seminowicz	2006	fMRI 1.5	22	Electrical Shock	Electrical	Transcutaneous	Left	Median nerve	
Seminowicz	2007	fMRI 1.5	23	Electrical Shock	Electrical	Transcutaneous	Left	Median nerve	
Song	2006	fMRI 3	12	Cold/rectal distention	Thermal and mechanical	Cutaneous and visceral	Left/ bilateral	Foot/rectum	
Sprenger	2006	PET	8	Heat	Thermal	Cutaneous	Right	Forearm	
Stammler	2008	fMRI 1.5	12	Pin prick	Mechanical hyperalgesia	Cutaneous	Right	Forearm	
Staud	2007	fMRI 3	11	Heat	Thermal	Cutaneous	Right	Foot	
Straube	2008	fMRI 1.5	24	Electrical Shock	Electrical	Cutaneous	Left	Finger	
Strigo	2003	fMRI 1.5	7	Esophageal Distention	Mechanical	Visceral and cutaneous	Bilateral	Esophagus and chest	

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Strigo	2005	fMRI 1.5	7	Esophageal Distention	Mechanical	Visceral and cutaneous	Bilateral	Esophagus and chest	Data are shared with Strigo et al., 2003
Svensson	1997	PET	10	Electrical Shock and	Electrical and thermal	Intramuscular and cutaneous	Left	Forearm	
Svensson	1998	PET	10	Heat	Thermal	Cutaneous	Right	Forearm	
Symonds	2006	fMRI 3	9	Electrical shock	Electrical	Transcutaneous	Left and right	Index finger	
Talbot	1991	PET	8	Heat	Thermal	Cutaneous	Right	Forearm	
Terekhin	2006	fMRI 1.5	14	Impact	Mechanical	Cutaneous	Right	Index Finger	
Thunberg	2005	PET	19	Hypertonic Saline	Mechanical	Intramuscular	Right	Erector Spinae	
Tolle	1999	PET	12	Heat	Thermal	Cutaneous	Right	Forearm	
Tracey	2000	fMRI 1.5	6	Cold and heat	Thermal	Cutaneous	Left	Hand	
Vandenbergh	2005	PET	11	Gastric Distention	Mechanical	Visceral	Bilateral	Stomach	

Table S1. List of studies included in Study #1 (all noxious stimuli)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Vogt	1996	PET	7	Heat	Thermal	Cutaneous	Left	Hand	
Wagner	2007	PET	7	Heat	Thermal	Cutaneous	Right	Forearm	
Xu	1997	PET	6	Laser	Thermal	Cutaneous	Left	Hand, foot	

Table S1. Study # 1 (all noxious stimuli): List of studies reporting brain activation coordinates evoked by externally and internally applied noxious stimuli. fMRI = functional magnetic resonance imaging; PET = position emission tomography; NR = not reported.

Table S2. ALE values (percentages) for Study #1 (all noxious stimuli)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Left	Thalamus		-14	-16	8	24.8%	1	87592
Right	Insula		34	12	8	23.0%		
Right	Thalamus		10	-18	6	21.5%		
Left	Insula		-36	4	6	21.3%		
Left	Insula		-40	-20	16	19.0%		
Right	SII		52	-26	22	17.8%		
Left	SII		-52	-24	20	16.8%		
Right	Insula		36	-20	16	16.6%		
Right	Inferior frontal gyrus	44	50	2	10	12.9%		
Left	Putamen		-24	-2	6	8.8%		
Right	Putamen		20	8	4	8.5%		
Left	Insula/clastrum		-38	-18	-4	7.2%		
Left	Putamen		-24	2	-2	6.6%		
Right	Pallidus		20	-4	0	6.3%		
Right	IPL	40	52	-44	38	5.9%		
Right	IPL	40	46	-54	44	4.6%		
Right	Cingulate gyrus	24	4	6	38	22.8%	2	23424
Right	Cingulate gyrus	32	6	22	28	11.7%		
Left	Cingulate gyrus	32	-2	32	22	5.6%		
Right	Medial frontal gyrus	6	2	-10	64	4.7%		
Right	Middle frontal gyrus	10	34	42	20	9.8%	3	4920
Right	Middle frontal gyrus	10	42	46	14	7.7%		
Right	Superior frontal gyrus	9	28	40	30	6.6%		
Left	Cerebellum		-36	-56	-34	6.9%	4	2552
Left	Cerebellum		-30	-58	-30	6.9%		
Left	Cerebellum		-22	-60	-24	5.5%		
Right	Cerebellum		0	-48	-16	8.5%	5	2208
Right	Cerebellum		4	-62	-16	6.3%		
Right	Cerebellum		24	-60	-22	6.7%	6	1744
Right	Cerebellum		18	-62	-14	5.4%		
Right	Cerebellum		18	-48	-22	4.7%		
Left	SI	2	-32	-36	60	6.5%	7	1368
Left	MI	4	-32	-24	52	6.0%		
Left	MI	4	-38	-26	62	5.7%		
Left	Cingulate gyrus	23	0	-28	28	8.2%	8	1304
Left	Cingulate gyrus	24	0	-20	36	5.1%		
Right	MI	4	32	-28	56	7.0%	9	872
Left	IPL	40	-40	-40	38	6.6%	10	632
Right	SI/PPC	5	20	-44	64	6.4%	11	464
Left	Superior frontal gyrus	10	-34	48	18	5.2%	12	448
Right	Premotor cortex	6	26	-16	52	5.5%	13	152
Right	Inferior frontal gyrus	9	48	6	26	4.5%	14	104
Left	Middle frontal gyrus	10	-30	48	6	4.7%	15	64
Right	Middle frontal gyrus	47	38	38	-6	4.6%	16	48
Right	Cerebellum		16	-38	-22	4.5%	17	40
Right	Paracentral lobule	5	8	-40	60	4.6%	18	24
Left	Middle frontal gyrus	46	-40	46	8	4.2%	19	16

Table S2. ALE values for Study #1. ALE values (percentages) refer to the likelihood of obtaining activation evoked by noxious stimuli in a given voxel of the standard template MRI. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; IPL = inferior parietal lobule; SI = primary somatosensory cortex; PPC = posterior parietal cortex; SII = secondary somatosensory cortex; MI = primary motor cortex.

Table S3. List of studies included in Study #2 (noxious cold)

				Stimuli			
Author	**Year**	**Imaging**	**Subjects**	**Modality**	**System**	**Side**	**Body Part**
Casey	2000	PET	11	Thermal	Cutaneous	Left	Hand
Casey	1996	PET	27	Thermal	Cutaneous	Left	Hand
Craig	1996	PET	11	Thermal	Cutaneous	Right	Hand
Davis	2002	fMRI 1.5	NR	Thermal/ mechanical	Cutaneous	Right	Palm
Mochizuki	2007	fMRI 3	14	Thermal	Cutaneous	Left	Wrist
Petrovic	2002	PET	7	Thermal	Cutaneous	Left	Hand
Petrovic	2004	PET	10	Thermal	Cutaneous	Left	Hand
Seifert	2007	fMRI 1.5	12	Thermal	Cutaneous	Right	Forearm
Tracey	2000	fMRI 1.5	6	Thermal	Cutaneous	Left	Hand

Table S3. Study # 2 (noxious cold): List of studies reporting brain activation coordinates evoked by noxious cold stimuli. fMRI = functional magnetic resonance imaging; PET = position emission tomography; NR = not reported.

Table S4. List of studies included Study #2 (noxious heat)

					Stimuli			
Author	**Year**	**Imaging**	**Subject (N)**	**Type**	**Modality**	**System**	**Side**	**Body Part**
Ariak	2005	fMRI 1.5	12	Heat	Thermal	Cutaneous	Left	Forearm
Brooks	2005	fMRI 3.0	14	Heat	Thermal	Cutaneous	Right	Hand
Casey	2001	PET	14	Heat	Thermal	Cutaneous	Left	Forearm
Coghill	1994	PET	9	Heat	Thermal	Cutaneous	Left	Forearm
Lorenz	2002	PET	14	Heat	Thermal	Cutaneous	Left	Forearm
Maihofner	2006	fMRI 1.5	14	Heat	Thermal	Cutaneous	Right	Forearm
Nemoto	2003	PET	12	Laser	Thermal	Cutaneous	Right	Forearm
Tracey	2000	fMRI 1.5	6	Heat	Thermal	Cutaneous	Left	Hand
Xu	1997	PET	6	Laser	Thermal	Cutaneous	Left	Hand

Table S4. Study # 2 (noxious heat): List of studies reporting brain activation coordinates evoked by noxious cold stimuli. fMRI = functional magnetic resonance imaging; PET = position emission tomography.

Table S5. ALE values (percentages) for Study #2 (noxious cold)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Insula/Claustrum		28	6	12	3.0%	1	1656
Right	Insula		40	8	0	1.3%		
Left	Insula		-38	6	4	2.8%	2	1520
Left	Claustrum		-36	-8	4	1.3%		
Left	Insula		-38	4	14	1.3%		
Left	Cingulate gyrus	32	-10	6	40	2.1%	3	1496
Right	Cingulate gyrus	24	2	2	36	1.9%		
Left	Cingulate gyrus	32	0	10	38	1.7%		
Left	Thalamus		0	-20	6	2.3%	4	1232
Right	Thalamus		6	-22	14	1.4%		
Right	Thalamus		16	-22	12	1.4%		
Right	Thalamus		4	-12	12	1.3%		
Left	Claustrum		-30	10	14	1.5%	5	712
Left	Claustrum		-30	12	10	1.4%		
Left	Putamen		-26	6	12	1.4%		
Left	Putamen		-18	4	8	1.4%		
Left	Caudate		-12	8	10	1.3%		
Right	Cingulate gyrus	24	12	14	30	2.3%	6	656
Right	Thalamus		12	-4	8	2.1%	7	552
Right	Middle frontal gyrus	10	42	46	12	2.0%	8	544
Left	SI/PPC	43	-54	-6	14	1.9%	9	520
Right	Subgenual ACC	47/25	18	18	-10	2.3%	10	496
Right	Medial frontal gyrus	25	10	16	-14	1.3%		
Right	Claustrum		38	-14	8	2.0%	11	400
Left	Putamen		-22	12	-8	2.4%	12	384
Right	Claustrum		36	-4	0	2.0%	13	352
Right	MI	4	32	-26	56	1.7%	14	328
Right	SII		46	-24	16	1.6%	15	256
Left	SII		-40	-46	46	1.6%	16	248
Right	Midbrain		8	-20	-2	1.4%	17	240
Left	Medial frontal gyrus	6	-4	-10	56	1.4%	18	240
Right	Inferior frontal gyrus	9	50	4	24	1.4%	19	232
Right	Inferior frontal gyrus	9	52	10	26	1.4%		
Left	Superior frontal gyrus	10	-26	44	18	1.3%	20	184
Left	Middle frontal gyrus	10	-30	38	14	1.3%		
Right	Insula		50	-40	18	1.4%	21	152
Right	Insula		44	-36	20	1.3%		
Right	Cerebellum		2	-58	-20	1.3%	22	72

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Middle frontal gyrus	9	38	28	34	1.2%		
Right	Lingual gyrus	19	30	-68	-2	1.1%	24	64
Right	Superior frontal gyrus	10	28	54	4	1.2%	25	64
Right	Premotor	6	50	-2	10	1.1%	26	64
Right	Insula		36	-16	20	1.2%	27	64
Right	Paracentral lobule	31	6	-10	46	1.3%	28	64
Left	Cingulate gyrus	24	0	0	46	1.3%	29	64
Right	MI	4	24	-22	50	1.3%	30	64
Right	Cerebellum		26	-64	-22	1.3%	31	56
Right	Thalamus		12	-30	6	1.3%	32	56
Right	Insula		38	18	8	1.3%	33	56
Left	Thalamus		-10	-16	8	1.2%	34	56
Right	Insula		46	-12	12	1.3%	35	56
Left	Cingulate gyrus	32	-10	18	26	1.3%	36	56
Right	Cingulate gyrus	24	6	-10	32	1.3%	37	56
Right	SI	3	44	-24	52	1.3%	38	56
Left	Fusiform gyrus	19	-22	-66	-6	1.3%	39	48
Right	Superior frontal gyrus	6	12	-6	64	1.3%	40	48
Left	Midbrain		-4	-18	-10	1.3%	41	40
Left	Putamen		-20	16	2	1.2%	42	40
Left	Cerebellum		-36	-56	-32	1.2%	43	32
Right	Parahippocampal	35	22	-8	-22	1.2%	44	32
Right	Amygdala		24	-8	-22	1.2%		
Right	Amygdala		22	-8	-20	1.2%		
Right	Amygdala		24	-8	-20	1.2%		

Table S5. ALE values (percentages) for Study #2 (noxious cold). ALE values (percentages) refer to the likelihood of obtaining activation evoked by noxious cold a given voxel of the standard template. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; IPL = inferior parietal lobule; SI = primary somatosensory cortex; PPC = posterior parietal cortex; SII = secondary somatosensory cortex; MI = primary motor cortex.

Table S6. ALE values (percentages) for Study #2 (noxious heat)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Left	Insula		-40	18	6	2.5%	1	4432
Left	Insula		-44	-24	16	2.4%		
Left	Lentiform Nucleus		-22	0	-2	2.4%		
Left	Lentiform Nucleus		-22	-10	8	1.8%		
Left	Insula/claustrum		-34	4	6	1.7%		
Left	Insula/claustrum		-34	10	6	1.7%		
Left	Insula		-30	18	8	1.7%		
Left	Lentiform Nucleus		-24	-4	6	1.6%		
Left	Insula/claustrum		-34	-16	10	1.4%		
Right	Insula/claustrum		34	4	10	3.3%	2	2432
Right	Insula/claustrum		34	12	6	3.0%		
Right	Thalamus		12	-20	4	2.9%	3	1544
Right	Thalamus		10	-10	6	1.3%		
Left	Cingulate Gyrus	32	-4	10	40	2.4%	4	1288
Left	Cingulate Gyrus	24	-4	12	32	1.6%		
Right	SII	40	52	-30	22	2.1%	5	1160
Right	Insula	40	52	-22	14	1.4%		
Right	Lentiform Nucleus		30	-14	8	1.8%	6	688
Right	Insula		32	-10	18	1.8%		
Right	Cingulate Gyrus	24	2	-4	44	2.0%	7	576
Left	Thalamus		-12	-24	12	2.2%	8	552
Right	Inferior Frontal Gyrus		38	46	2	2.1%	9	464
Right	Caudate		16	8	12	2.1%	10	368
Left	MI	4	-32	-22	50	2.0%	11	304
Right	Cingulate Gyrus	32	4	22	26	1.8%	12	256
Left	Insula		-46	6	16	1.3%	13	168
Left	Inferior Frontal Gyrus	44	-48	0	12	1.3%		
Right	Middle Frontal Gyrus	46	42	36	24	1.4%	14	152
Right	Superior Frontal Gyrus	9	40	34	28	1.3%		
Right	Insula		46	6	16	1.4%	15	144
Right	Inferior Frontal Gyrus	44	52	6	12	1.4%		
Left	Insula		-40	-4	10	1.3%	16	80
Left	Superior Temporal	42	-54	-30	14	1.3%	17	80
Right	Thalamus		6	-18	14	1.3%	18	72
Left	Insula		-52	-34	20	1.3%	19	64
Left	Cingulate Gyrus	24	-10	4	30	1.3%	20	64
Right	Lentiform Nucleus		24	4	16	1.4%	21	56
Right	IPL	39	48	-62	38	1.3%	22	56
Left	Medial Frontal Gyrus	6	0	-10	52	1.3%	23	56
Right	Lentiform Nucleus		22	-4	12	1.2%	24	48

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Left	Superior Frontal Gyrus	6	-4	8	60	1.3%	26	48
Right	Medial Frontal Gyrus	6	2	-12	62	1.1%	27	48
Right	Cerebellum		30	-76	-28	1.3%	28	40
Right	Cerebellum		8	-60	-12	1.2%	29	40
Right	Precentral Gyrus	6	50	-4	38	1.2%	30	40
Right	SI		20	-36	52	1.3%	31	40
Right	Middle Frontal Gyrus	6	18	-10	58	1.3%	32	40
Left	Cerebellum		-28	-40	-42	1.2%	33	32
Right	Cerebellum		18	-72	-30	1.2%	34	32
Left	Cerebellum		-20	-60	-20	1.3%	35	32
Right	Cerebellum		0	-52	-16	1.2%	36	32
Right	Inferior Frontal Gyrus	47	42	20	-4	1.3%	37	32
Right	Insula/claustrum		36	-6	0	1.3%	38	32
Right	Lentiform Nucleus		20	10	0	1.2%	39	32
Right	SII	42	56	-12	12	1.3%	40	32
Left	Insula		-48	-20	24	1.2%	41	32
Left	IPL	40	-62	-40	28	1.2%	42	32
Right	Cingulate Gyrus	23	4	-22	28	1.2%	43	32
Right	Medial Frontal Gyrus	8	14	30	38	1.2%	44	32
Left	Paracentral Lobule	5	-10	-34	46	1.2%	45	32

Table S6. ALE values (percentages) for Study #2 (noxious heat). ALE values (percentages) refer to the likelihood of obtaining noxious heat activation in a given voxel of the standard template. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann's Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; SI = primary somatosensory cortex; IPL = inferior parietal lobule; SII = secondary somatosensory cortex; MI = primary motor cortex.

Table S7. ALE values (percentages) for Study #2 (noxious cold minus noxious heat)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Cingulate Gyrus	24	12	14	30	2.3%	1	408
Left	Insula		-40	6	2	2.3%	2	384
Right	Lentiform Nucleus		26	6	12	2.2%	3	360
Right	Middle Frontal Gyrus	10	42	46	12	2.0%	4	360
Right	Subgenual ACC	25/47	18	18	-10	2.3%	5	344
Left	SII	43	-54	-6	14	1.8%	6	296
Right	Thalamus		12	-4	8	1.9%	7	280
Left	Lentiform Nucleus		-22	12	-8	2.2%	8	264
Left	Thalamus		0	-20	6	2.1%	9	248
Left	IPL	40	-40	-46	46	1.6%	10	232
Left	Cingulate Gyrus	32	-10	6	40	1.9%	11	224
Right	MI	4	32	-26	56	1.7%	12	208
Right	Insula/claustrum		38	-14	8	1.6%	13	88
Right	Inferior Frontal Gyrus	9	52	10	26	1.4%	14	88
Left	Cingulate Gyrus	24	0	2	36	1.5%	15	64
Right	Lingual Gyrus	19	32	-68	-2	1.1%	16	48
Right	Insula/claustrum		36	-2	-2	1.3%	17	48
Right	Insula		46	-24	16	1.3%	18	40
Left	Superior Frontal Gyrus	10	-26	44	18	1.3%	19	40
Left	Caudate		-12	8	10	1.3%	20	32
Left	Cerebellum		-36	-54	-32	1.2%	21	24
Right	Parahippocampal Gyrus	35	22	-8	-22	1.2%	22	24
Right	Amygdala		24	-8	-22	1.2%		
Right	Amygdala		24	-8	-20	1.2%		

Table S7. ALE values (percentages) for Study #2. ALE maps of noxious heat were subtracted from noxious cold. ALE values (percentages) refer to the likelihood of obtaining noxious cold in a given voxel of the standard template. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; IPL = Inferior Parietal Lobule; SII = secondary somatosensory cortex; MI = primary motor cortex.

Table S8. ALE values (percentages) for Study #2 (noxious heat minus noxious cold)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Left	Putaman		-22	0	-2	2.4%	1	1072
Right	Insula/claustrum		34	12	6	2.3%	2	960
Right	Insula/claustrum		34	2	10	2.3%		
Right	Insula		34	22	8	1.4%		
Left	Insula		-40	18	6	2.5%	3	616
Left	Insula		-30	20	8	1.4%		
Right	SII/IPL		52	-32	22	2.0%	4	584
Left	Insula		-44	-24	16	2.4%	5	576
Right	Thalamus		14	-20	4	2.1%	6	504
Right	Insula		32	-8	18	1.7%	7	360
Right	Lentiform Nucleus		30	-14	8	1.6%		
Left	Thalamus		-12	-24	12	2.2%	8	360
Right	Inferior Frontal Gyrus		38	46	2	2.0%	9	352
Left	MI	4	-32	-22	50	2.0%	10	296
Right	Cingulate Gyrus	32	4	22	26	1.8%	11	200
Right	Caudate		14	8	12	2.0%	12	168
Left	Cingulate Gyrus	24	-6	12	32	1.4%	13	112
Left	Cingulate Gyrus	24	0	-6	42	1.5%	14	72
Left	Superior Temporal Gyrus	42	-54	-30	14	1.3%	15	64
Right	Insula		46	6	16	1.3%	16	64
Right	Inferior frontal gyrus	44	52	6	12	1.3%		
Right	Middle Frontal Gyrus	46	42	36	24	1.4%	17	40
Right	Cingulate Gyrus	23	2	-22	28	1.2%	18	32
Right	SII/IPL	39	48	-62	38	1.3%	19	32
Left	SI/PPC	5	-10	-34	46	1.2%	20	32
Left	SI/PPC	5	-10	-32	46	1.2%		
Right	SI/PPC	5	30	-42	58	1.2%	21	32
Left	Insula		-52	-34	20	1.2%	21	24
Left	Insula		-48	-20	24	1.2%	21	24

Table S8. ALE values (percentages) for Study #2. ALE maps of noxious cold were subtracted from noxious heat pain. ALE values (percentages) refer to the likelihood of obtaining noxious heat in a given voxel of the standard template. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; IPL = Inferior parietal lobule; SI/PPC = Primary somatosensory cortex/posterior parietal cortex; MI = primary motor cortex.

Table S9. List of studies included in Study #3 (noxious heat vs. warm)

				Stimuli				
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**
Adler	1997	PET	9	Heat	Thermal	Cutaneous	Left	Forearm
Botvinick	2005	fMRI 1.5	12	Heat	Thermal	Cutaneous	Left	Hand
Casey	2001	PET	14	Heat	Thermal	Cutaneous	Left	Forearm
Vogt	1996	PET	7	Heat	Thermal	Cutaneous	Left	Hand
Derbyshire	1997	PET	12	Laser	Thermal	Cutaneous	Right	Hand
Derbyshire	1998	PET	12	Heat	Thermal	Cutaneous	Right	Hand
Ochsner	2006	fMRI 3	13	Heat	Thermal	Cutaneous	Right	Forearm
Svensson	1998	PET	10	Heat	Thermal	Cutaneous	Right	Forearm
Wagner	2007	PET	7	Heat	Thermal	Cutaneous	Right	Forearm

Table S9. Study # 3 (noxious heat vs. warm): List of studies reporting brain activation coordinates evoked by noxious heat stimuli in comparison to a warm control condition. fMRI = functional magnetic resonance imaging; PET = position emission tomography.

Table S10. List of studies included in Study #3 (noxious heat vs. resting baseline)

				Stimuli				
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**
Albanese	2007	fMRI 1.5	8	Heat	Thermal	Cutaneous	Right	Hand
Coghill	1994	PET	9	Heat	Thermal	Cutaneous	Left	Forearm
Coghill	2001	PET	9	Heat	Thermal	Cutaneous	Left	Forearm
Kurata	2005	fMRI 3	6	Heat	Thermal	Cutaneous	Right	Hand
Kurata	2002	fMRI 3	5	Heat	Thermal	Cutaneous	Right	Forearm
Maihofner	2006	fMRI 1.5	14	Heat	Thermal	Cutaneous	Right	Forearm
Nemoto	2003	PET	12	Laser	Thermal	Cutaneous	Right	Forearm
Tracey	2000	fMRI 1.5	6	Heat	Thermal	Cutaneous	Left	Hand
Xu	1997	PET	6	Laser	Thermal	Cutaneous	Left	Hand

Table S10. Study # 3 (noxious heat): List of studies reporting brain activation coordinates evoked by noxious heat stimuli in comparison to a resting baseline. fMRI = functional magnetic resonance imaging; PET = position emission tomography.

Table S11. ALE values (percentages) for Study #3 (noxious heat minus warm)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Left	SII		-50	-4	6	2.7%	1	3432
Left	Insula		-48	6	4	1.5%		
Left	Insula		-44	6	2	1.5%		
Left	Inferior frontal gyrus	44	-46	8	12	1.4%		
Right	Insula		38	8	-4	2.8%	2	1448
Right	Insula		38	0	12	2.0%		
Right	Cingulate gyrus	24	4	2	38	2.7%	3	1440
Left	Cingulate gyrus	24	-6	-4	40	2.2%		
Right	Medial frontal gyrus		2	0	52	1.4%		
Right	Cingulate gyrus	24	6	20	24	4.8%	4	1096
Right	Thalamus		6	-20	0	2.6%	5	824
Right	Thalamus		12	-22	8	1.6%		
Left	Cingulate gyrus	23	-2	-22	32	2.9%	6	800
Right	Cerebellum		16	-58	-12	2.7%	7	752
Right	Putamen		30	-14	8	1.8%	8	712
Right	Insula		36	-12	16	1.5%		
Right	Insula		34	-22	14	1.4%		
Right	Insula		36	-18	20	1.4%		
Left	Insula		-40	-20	16	2.9%	9	648
Left	Thalamus		-8	-16	8	1.7%	10	480
Right	Thalamus		30	44	20	2.6%	11	360
Left	Thalamus		-22	-16	10	1.4%	12	208
Left	Putamen		-26	-20	12	1.4%		
Right	SII		48	-38	30	1.4%	13	184
Right	SII		52	-34	24	1.3%		
Right	Cerebellum		22	-58	-28	1.4%	14	168
Right	Cerebellum		22	-60	-32	1.4%		
Left	SII		-50	-26	28	1.4%	15	152
Left	SI	2	-48	-20	26	1.4%		
Right	Cingulate gyrus	32	4	42	12	1.3%	16	120
Left	Cingulate gyrus	24	0	38	6	1.3%		

Table S11. ALE values (percentages) for Study #3. ALE values (percentages) refer to the likelihood of obtaining noxious heat contrasted with innocuous warm stimuli in a given voxel of the standard template. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; SI = primary somatosensory cortex; PPC = posterior parietal cortex; SII = secondary somatosensory cortex.

Table S12. ALE values (percentages) for Study #3 (noxious heat vs. baseline)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Left	Cingulate	32	-2	10	40	4.2%	1	3768
Left	Cingulate	24	-4	12	30	2.9%		
Left	Cingulate	24	0	-2	44	2.4%		
Left	Cingulate	32	-8	24	30	1.4%		
Left	Medial	6	0	-10	52	1.3%		
Right	SII	43	50	-18	16	2.0%	2	2408
Right	Insula		36	-20	16	2.0%		
Right	IPL	40	48	-34	28	1.8%		
Right	IPL	40	56	-30	24	1.6%		
Right	IPL	40	60	-30	26	1.6%		
Right	SII	40	50	-32	34	1.4%		
Right	SII		56	-12	12	1.4%		
Right	Putaman		30	2	8	3.2%	3	1952
Right	Insula/claustrum		30	4	12	3.1%		
Left	Insula		-40	2	8	2.4%	4	1360
Left	Insula		-46	6	16	1.3%		
Left	Insula		-42	-10	12	1.3%		
Left	Insula/claustrum		-30	4	8	1.3%		
Left	Thalamus		-16	-20	12	3.7%	5	1032
Right	Inferior frontal gyrus	44	52	6	10	2.6%	6	824
Right	Cerebellum		0	-66	-16	2.3%	7	784
Right	Cerebellum		2	-62	-14	2.1%		
Right	Thalamus		12	-20	4	2.7%	8	760
Right	Cerebellum		20	-66	-24	2.0%	9	656
Right	Cerebellum		30	-76	-28	1.4%		
Left	Insula		-44	-24	16	2.4%	10	576
Right	Medial	6	6	-6	62	1.9%	11	408
Left	Putaman		-22	0	-2	2.2%	12	312
Right	PPC	5	22	-42	66	2.1%	13	280
Left	MI	4	-32	-22	50	2.0%	14	264
Right	SI	3	30	-30	62	1.9%	15	264
Left	Putaman		-28	-14	10	1.4%	16	256
Left	Putaman		-22	-10	8	1.4%		
Left	Putaman		-30	-12	2	1.3%		
Right	Insula		32	-10	18	1.7%	17	248
Left	Cerebellum		-22	-54	-28	1.6%	18	240
Right	Premotor cortex	6	46	0	30	1.8%	19	232
Right	Cerebellum		38	-54	-36	1.7%	20	216
Right	Insula		36	18	8	1.5%	21	160
Left	Superior frontal gyrus		-10	-8	72	2.1%	22	152

Table S12. ALE values (percentages) for Study #3. ALE values (percentages) refer to the likelihood of obtaining activation evoked by noxious heat in comparison to resting baseline. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; IPL = inferior parietal lobule; SI = primary somatosensory cortex; PPC = posterior parietal cortex; SII = secondary somatosensory cortex; MI = primary motor cortex.

Table S13. ALE for Study # 3 (noxious heat vs. baseline minus noxious heat vs. warm)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Left	Cingulate gyrus	32	-2	10	40	3.9%	1	2168
Left	Cingulate gyrus	24	-6	14	30	2.9%		
Left	Cingulate gyrus	32	-8	24	30	1.3%		
Right	SII	43	50	-18	16	2.0%	2	1040
Right	SII	40	60	-30	26	1.5%		
Right	SII	42	56	-12	12	1.4%		
Right	Putamen		30	2	6	2.7%	3	944
Left	Thalamus		-16	-20	12	3.5%	4	688
Right	Inferior frontal gyrus	44	54	6	10	2.6%	5	624
Right	Cerebellum		0	-66	-16	2.3%	6	536
Right	Cerebellum		20	-66	-24	2.0%	7	488
Right	Cerebellum		30	-76	-28	1.4%		
Left	Insula		-40	0	8	1.9%	8	448
Left	Insula		-38	12	12	1.4%		
Right	Medial frontal gyrus	6	6	-6	62	1.9%	9	320
Right	SI/PPC		22	-42	66	2.1%	10	240
Left	MI	4	-32	-22	50	2.0%	11	224
Right	SI	3	30	-30	62	1.9%	12	216
Left	Putamen		-22	0	-4	2.1%	13	192
Left	Insula		-44	-26	16	1.8%	14	168
Right	Thalamus		14	-20	4	1.8%	15	160
Right	Inferior frontal gyrus	6	46	0	30	1.7%	16	152
Right	Cerebellum		38	-54	-36	1.6%	17	144
Left	Superior frontal gyrus	6	-10	-8	72	2.1%	18	120
Right	Insula/claustrum		30	-8	18	1.6%	19	112

Table S13. Study #3: ALE values (percentages) refer to the likelihood of obtaining activation evoked by noxious heat in comparison to resting baseline minus ALE values (percentages) obtained for noxious heat in comparison to innocuous warm. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; SI = primary somatosensory cortex; PPC = posterior parietal cortex; SII = secondary somatosensory cortex; MI = primary motor cortex.

Table S14. ALE values (percentages) for Study #3 (noxious heat vs. warm minus noxious heat vs. baseline)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Cingulate Gyrus	24	6	20	24	4.8%	4	976
Left	Cingulate Gyrus	23	-2	-22	32	2.9%	8	600
Right	Insula	13	38	8	-4	2.8%	11	464
Right	Cerebellum		16	-58	-12	2.1%	14	256
Right	Lentiform Nucleus		30	-14	8	1.8%	19	192
Left	Superior Temporal Gyrus	22	-50	-4	6	2.1%	20	184
Right	Cingulate Gyrus	24	6	0	38	1.9%	21	184
Left	Insula	13	-38	-20	14	1.8%	24	152
Right	Insula	13	38	-2	12	1.6%	28	136
Left	Cingulate Gyrus	24	-6	-6	40	1.8%	30	112
Right	Thalamus		4	-20	0	1.7%	31	104
Left	Thalamus		-4	-14	8	1.5%	32	104

Table S14. Study #3: ALE values (percentages) refer to the likelihood of obtaining activation evoked by noxious heat in comparison to warm subtracting ALE values (percentages) obtained for noxious heat minus baseline. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior.

Table S15. List of studies included in Study #4 (noxious stimuli to the left side of the body)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Adler	1997	PET	9	Heat	Thermal	Cutaneous	Left	Forearm	
Aharon	2006	fMRI 1.5	6	Heat	Thermal	Cutaneous	Left	Hand	
Becerra	1999	fMRI 1.5	6	Heat	Thermal	Cutaneous	Left	Hand	
Becerra	2001	fMRI 1.5	8	Heat	Thermal	Cutaneous	Left	Hand (dorsum)	
Bingel	2003	fMRI 1.5	14	Laser	Thermal	Cutaneous	Left	Hand	
Bingel	2004	fMRI 1.5	20	Laser	Thermal	Cutaneous	Left	Hand, foot	
Bingel	2004	fMRI 1.5	18	Laser	Thermal	Cutaneous	Left	Hand	
Bingel	2007	fMRI 3	16	Laser	Thermal	Cutaneous	Left	Hand	
Bingel	2007	fMRI 3	20	Heat	Thermal	Cutaneous	Left	Forearm	
Boly	2007	fMRI 3	24	Laser	Thermal	Cutaneous	Left	Hand	
Bornhovd	2002	fMRI 1.5	9	Laser	Thermal	Cutaneous	Left	Hand	Data are shared with Buchel et al. 2002
Botvinick	2005	fMRI 1.5	12	Heat	Thermal	Cutaneous	Left	Thenar Eminence	
Buchel	2002	fMRI 1.5	9	Laser	Thermal	Cutaneous	Left	Hand	
Casey	1994	PET	18	Heat	Thermal	Cutaneous	Left	Arm	
Casey	1996	PET	27	Cold	Thermal	Cutaneous	Left	Hand	
Casey	2000	PET	11	Cold	Thermal	Cutaneous	Left	Hand	
Casey	2001	PET	14	Heat	Thermal	Cutaneous	Left	Forearm	

Table S15. List of studies included in Study #4 (noxious stimuli to the left side of the body)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Chen	2002	fMRI 1.5	4	Heat	Thermal	Cutaneous	Left	Inner calf	
Coghill	1994	PET	9	Heat	Thermal	Cutaneous	Left	Forearm	
Coghill	2001	PET	9	Heat	Thermal	Cutaneous	Left	Forearm	
De Leeuw	2006	fMRI 1.5	9	Heat	Thermal	Cutaneous	Left	Face	
Derbyshire	1998	PET	7	Heat	Thermal	Cutaneous	Left	Hand	Data are shared with Vogt et al. 1996
Dunckley	2005	fMRI 3	10	Heat	Thermal	Cutaneous	Left	Foot	
Fairhurst	2006	fMRI 3	12	Heat	Thermal	Cutaneous	Left	Hand	
Gyulai	1997	PET	5	Heat	Thermal	Cutaneous	Left	Forearm	
Hofbauer	2001	PET	10	Heat	Thermal	Cutaneous	Left	Hand	
Hofbauer	2004	PET	15	Heat	Thermal	Cutaneous	Left	Forearm	
Keltner	2006	fMRI 4	16	Heat	Thermal	Cutaneous	Left	Hand	
Kurata	2002	fMRI 3	5	Heat	Thermal	Cutaneous	Left	Forearm	
Lorenz	2002	PET	14	Heat	Thermal	Cutaneous	Left	Forearm	
Oshiro	2007	fMRI 1.5	12	Heat	Thermal	Cutaneous	Left	Leg	
Owen	2007	fMRI 3	14	Heat	Thermal	Cutaneous	Left	Hand	
Paulson	1998	PET	20	Heat	Thermal	Cutaneous	Left	Forearm	
Petrovic	2002	PET	7	Cold	Thermal	Cutaneous	Left	Hand	
Petrovic	2004	PET	10	Cold	Thermal	Cutaneous	Left	Hand	

Table S15. List of studies included in Study #4 (noxious stimuli to the left side of the body)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Petrovic	2004	PET	7	Cold	Thermal	Cutaneous	Left	Hand	Data are shared with Petrovic et al. 2002
Raij	2005	fMRI 3	14	Laser	Thermal	Cutaneous	Left	Hand	
Rainville	1997	PET	8	Heat	Thermal	Cutaneous	Left	Hand	
Remy	2003	fMRI 3	12	Heat	Thermal	Cutaneous	Left	Hand	
Svensson	1997	PET	11	Laser	Thermal	Cutaneous	Left	Elbow	
Tracey	2000	fMRI 1.5	6	Heat, cold	Thermal	Cutaneous	Left	Hand	
Vogt	1996	PET	7	Heat	Thermal	Cutaneous	Left	Hand	
Xu	1997	PET	6	Laser	Thermal	Cutaneous	Left	Hand, foot	

Table S15. Study # 4 (noxious heat applied to the left side of the body): List of studies reporting brain activation coordinates evoked by noxious heat stimuli applied to the left side of the body. fMRI = functional magnetic resonance imaging; PET = position emission tomography.

Table S16. List of studies included in Study #4 (noxious stimuli applied to the right side of the body)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Albanese	2007	fMRI 1.5	8	Heat	Thermal	Cutaneous	Right	Hand	
Apkarian	2000	fMRI 1.5	7	Heat	Thermal	Cutaneous	Right	Fingers	
Bingel	2003	fMRI 1.5	14	Laser	Thermal	Cutaneous	Right	Hand	
Bingel	2004	fMRI 1.5	20	Laser	Thermal	Cutaneous	Right	Hand, foot	
Borsook	2003	fMRI 1.5	9	Heat	Thermal	Cutaneous	Right	Face	Data are shared with DaSilva et al., (2002)
Brooks	2005	fMRI 3	14	Heat	Thermal	Cutaneous	Right	Face, hand, foot	
Coghill	1999	PET	16	Heat	Thermal	Cutaneous	Right	Upper Arm	
Coghill	2001	PET	9	Heat	Thermal	Cutaneous	Right	Ventral Forearm	
Coghill	2003	fMRI 1.5	17	Heat	Thermal	Cutaneous	Right	Leg	
Craig	1996	PET	11	Cold, heat	Thermal	Cutaneous	Right	Hand	
DaSilva	2002	fMRI 1.5	9	Heat	Thermal	Cutaneous	Right	Face, thumb	
Davis	2002	fMRI 1.5	0	Cold	Thermal/ mechanical	Cutaneous	Right	Thernar eminence	
Derbyshire	1997	PET	12	Laser	Thermal	Cutaneous	Right	Hand	
Derbyshire	1998	PET	12	Heat	Thermal	Cutaneous	Right	Hand	
Derbyshire	2002	PET	21	Laser	Thermal	Cutaneous	Right	Hand	
Derbyshire	2002	PET	16	Heat	Thermal	Cutaneous	Right	Hand	

Table S16. List of studies included in Study #4 (noxious stimuli applied to the right side of the body)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Derbyshire	2004	fMRI 3	8	Heat	Thermal	Cutaneous	Right	Hand	
Frankenstein	2001	fMRI 1.5	12	Cold	Thermal	Cutaneous	Right	Foot	
Gelnar	1999	fMRI 1.5	9	Heat	Thermal	Cutaneous	Right	Finger	
Helmchen	2003	fMRI 1.5	18	Heat	Thermal	Cutaneous	Right	Hand	
Helmchen	2006	fMRI 1.5	18	Heat	Thermal	Cutaneous	Right	Hand	Data are shared with Helmchen et al., 2003
Kong	2006	fMRI 3	16	Heat	Thermal	Cutaneous	Right	Forearm	
Koyama	2003	fMRI 1.5	9	Heat	Thermal	Cutaneous	Right	Leg	
Koyama	2005	fMRI 1.5	10	Heat	Thermal	Cutaneous	Right	Leg	
Kurata	2005	fMRI 3	6	Heat	Thermal	Cutaneous	Right	Forearm	
Maihofner	2006	fMRI 1.5	14	Heat	Thermal	Cutaneous	Right	Forearm	
Mohr	2005	fMRI 1.5	16	Heat	Thermal	Cutaneous	Right	Hand	Data are shared with Helmchen et al., 2003
Mohr	2008	fMRI 1.5	17	Heat	Thermal	Cutaneous	Right	Thigh	
Nemoto	2003	PET	12	Laser	Thermal	Cutaneous	Right	Forearm	
Ochsner	2006	fMRI 3	13	Heat	Thermal	Cutaneous	Right	Forearm	
Qiu	2006	fMRI 3	13	Laser	Thermal	Cutaneous	Right	Hand	
Sawamoto	2000	fMRI 1.5	10	Laser	Thermal	Cutaneous	Right	Hand	
Seifert	2007	fMRI 1.5	12	Cold	Thermal	Cutaneous	Right	Forearm	
Sprenger	2006	PET	8	Heat	Thermal	Cutaneous	Right	Forearm	
Staud	2007	fMRI 3	11	Heat	Thermal	Cutaneous	Right	Foot	
Svensson	1998	PET	10	Heat	Thermal	Cutaneous	Right	Forearm	
Talbot	1991	PET	8	Heat	Thermal	Cutaneous	Right	Forearm	

Table S16. List of studies included in Study #4 (noxious stimuli applied to the right side of the body)

				Stimuli					
Author	**Year**	**Imaging**	**Subjects**	**Type**	**Modality**	**System**	**Side**	**Body Part**	**Notes**
Tolle	1999	PET	12	Heat	Thermal	Cutaneous	Right	Forearm	
Wagner	2007	PET	7	Heat	Thermal	Cutaneous	Right	Forearm	

Table S16. Study # 4 (noxious heat applied to the right side of the body): List of studies reporting brain activation coordinates evoked by noxious heat stimuli applied to the right side of the body. fMRI = functional magnetic resonance imaging; PET = position emission tomography.

Table S17. ALE values (percentages) for Study #4 (noxious stimuli applied to the left side of the body)

Side	Region	BA	X	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Thalamus		10	-20	6	8.9%	1	18008
Left	Thalamus		-12	-16	10	8.2%		
Left	Insula/claustrum		-34	2	8	6.9%		
Right	Thalamus		16	-18	14	5.5%		
Left	Insula		-32	12	10	4.4%		
Right	Thalamus		10	-6	6	4.2%		
Left	Midbrain		-2	-16	-8	3.6%		
Right	Lentiform Nucleus		18	-6	0	3.2%		
Right	Putamen		2	-28	-6	2.9%		
Left	Lentiform Nucleus		-28	-10	4	2.8%		
Left	Insula		-40	6	-4	2.7%		
Right	Insula		36	-20	18	10.8%	2	17912
Right	Insula/claustrum		32	4	12	9.8%		
Right	IPL	40	52	-30	26	7.3%		
Right	Superior Temporal Gyrus	22	52	4	8	5.0%		
Right	IPL	40	46	-34	40	3.1%		
Right	Insula		46	10	0	2.7%		
Right	Insula/claustrum		36	-12	-4	2.2%		
Right	Cingulate Gyrus	24	2	4	38	9.5%	3	12552
Right	Medial Frontal Gyrus	6	2	2	52	7.9%		
Right	Medial Frontal Gyrus	6	10	8	50	4.9%		
Left	Cingulate Gyrus	24	-8	16	28	4.8%		
Left	Medial Frontal Gyrus	6	-2	-10	52	3.7%		
Right	Cingulate Gyrus	24	8	-12	40	3.7%		
Right	Medial Frontal Gyrus	6	8	-10	52	2.7%		
Right	Superior Frontal Gyrus	6	14	-6	62	2.6%		
Left	IPL	40	-52	-32	28	3.2%	4	1384
Left	IPL		-50	-36	22	3.2%		
Left	SII		-56	-22	20	3.0%		
Left	Cerebellum		-34	-56	-30	3.3%	5	1352
Left	Cerebellum		-28	-54	-30	3.1%		
Left	Cerebellum		-24	-56	-18	3.1%		
Right	SI/PPC	5	22	-42	64	4.0%	6	1048
Right	SI	3	30	-30	62	3.3%		
Right	Cerebellum		4	-58	-14	3.2%	7	864
Right	Cerebellum		0	-50	-16	2.6%		
Right	Precentral Gyrus	6	26	-16	54	4.2%	8	784
Right	MI	4	34	-18	58	2.6%		
Right	Middle Frontal Gyrus	10	32	40	22	2.9%	9	720
Right	Middle Frontal Gyrus	10	40	38	22	2.9%		

Side	Region	BA	X	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Superior Frontal Gyrus	9	28	40	30	2.6%		
Left	SII		-40	-24	14	2.9%	11	456
Right	Inferior Frontal Gyrus		38	22	6	2.7%	12	184
Right	Cerebellum		24	-66	-24	2.5%	13	160
Right	Paracentral Lobule	5	8	-40	60	3.0%	14	152
Left	Insula/claustrum		-34	-18	4	2.5%	15	128
Right	Cingulate Gyrus	32	4	22	26	2.7%	16	128
Left	Cingulate Gyrus	32	-6	32	-4	2.8%	17	104

Table S17. ALE values (percentages) for Study #4. ALE values (percentages) refer to the likelihood of obtaining activation evoked by noxious stimuli applied to the left side of the body. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; SI = primary somatosensory cortex; SII = secondary somatosensory cortex; ; MI = primary motor cortex.

Table S18. ALE values (percentages) for Study #4 (noxious heat applied to the right side of the body)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Insula		34	12	8	10.5%	1	41464
Left	Insula		-38	4	4	10.0%		
Left	SII		-54	-26	22	9.1%		
Right	SII		56	-22	20	8.4%		
Left	Insula		-38	-20	14	8.2%		
Left	Thalamus		-16	-16	10	8.2%		
Right	Thalamus		4	-18	4	6.9%		
Right	Thalamus		12	-12	8	5.4%		
Right	Lentiform Nucleus		24	-2	8	4.9%		
Left	Precentral Gyrus	43	-54	-8	12	4.3%		
Right	Precentral Gyrus	44	50	6	12	4.0%		
Right	Insula		36	-2	14	4.0%		
Right	Inferior Frontal Gyrus	47	42	18	-2	3.9%		
Left	Lentiform Nucleus		-20	4	10	3.8%		
Left	Precentral Gyrus	6	-52	-4	6	3.5%		
Left	Thalamus		-4	-26	0	3.5%		
Right	Insula		38	-14	16	3.4%		
Right	Insula		44	-14	16	3.3%		
Right	Inferior Frontal Gyrus		42	26	4	2.6%		
Left	Lentiform Nucleus		-20	12	0	2.5%		
Left	Supramarginal Gyrus	40	-54	-38	32	2.2%		
Right	Cingulate Gyrus	24	4	8	36	8.1%	2	14016
Right	Cingulate Gyrus	32	6	22	26	6.7%		
Left	Cingulate Gyrus	24	-4	-4	42	6.2%		
Left	Cingulate Gyrus	32	-2	24	38	4.6%		
Left	Anterior Cingulate	24	-4	20	24	4.5%		
Left	Medial Frontal Gyrus	8	-10	26	42	2.5%		
Left	SII	3	-32	-34	60	4.9%	3	1664
Left	MI	4	-38	-24	62	3.4%		
Right	Middle Frontal Gyrus	10	30	44	20	3.6%	4	1424
Right	Middle Frontal Gyrus	10	42	46	14	3.0%		
Right	Superior Frontal Gyrus	9	38	36	26	2.8%		
Right	Middle Frontal Gyrus	9	38	30	30	2.4%		
Left	Cerebellum		-34	-54	-36	3.4%	5	968
Left	Cerebellum		-20	-62	-24	3.3%		
Left	Cerebellum		-30	-58	-30	2.9%		
Right	Cerebellum		22	-58	-24	4.0%	6	720
Right	Cerebellum		2	-46	-16	3.4%	7	616
Right	IPL	40	50	-32	34	3.8%	8	480
Left	Supramarginal Gyrus	40	-40	-40	36	4.2%	9	480
Left	Cerebellum		-4	-56	-28	2.8%	10	328
Right	Cerebellum		4	-62	-16	3.4%	11	288
Right	Inferior Parietal Lobule	40	50	-46	38	3.1%	12	280
Left	Middle Frontal Gyrus	10	-30	46	4	3.3%	13	216

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Left	Angular Gyrus	39	-40	-58	34	3.0%	14	160
Left	Medial Frontal Gyrus	6	-4	-20	66	2.7%	15	128
Right	Uncus	36	20	-4	-34	2.5%	16	104
Right	Medial Frontal Gyrus	6	6	2	62	2.5%	17	104

Table S18. Study #4: ALE values (percentages) refer to the likelihood of obtaining activation evoked by noxious heat applied to the right side of the body. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; SI = primary somatosensory cortex; SII = secondary somatosensory cortex; MI = primary motor cortex.

Table S19. List of studies included in Study #4 (left sided stimuli minus Right-sided stimuli)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Right	Insula		36	-20	18	9.3%	1	3024
Right	Insula/claustrum		38	-14	8	4.0%		
Right	Cingulate Gyrus	24	4	2	38	4.7%	2	2576
Right	Medial Frontal Gyrus	6	2	2	54	4.6%		
Right	Medial Frontal Gyrus	6	12	8	50	4.3%		
Right	Cingulate Gyrus	24	8	-12	42	2.9%		
Right	Medial Frontal Gyrus	6	8	-10	52	2.4%		
Right	Insula/claustrum		32	4	12	5.5%	3	1976
Right	Thalamus		16	-18	14	4.9%	4	1488
Right	Thalamus		10	-20	4	4.1%		
Right	SII		52	-30	26	5.8%	5	1272
Right	SI/PPC	5	22	-42	64	3.9%	6	880
Right	SI	3	30	-30	62	3.1%		
Right	Precentral Gyrus	6	26	-16	54	4.1%	7	712
Right	MI	4	34	-18	58	2.5%		
Left	Thalamus		-10	-16	10	3.5%	8	416
Left	Thalamus		-6	-20	16	2.7%		
Left	Midbrain		-2	-16	-10	3.1%	9	304
Left	Cingulate Gyrus	24	-10	16	28	3.5%	10	264
Right	Inferior frontal gyrus	44	52	2	4	3.1%	11	240
Left	Cerebellum		-24	-56	-18	2.9%	12	232
Right	SI/PPC	5	8	-40	60	3.0%	13	168
Right	Superior Frontal Gyrus	6	14	-6	62	2.5%	14	160
Left	Medial Frontal Gyrus	6	-4	-12	56	2.6%	15	152
Right	Lentiform Nucleus		18	-6	0	2.5%	16	144
Right	Thalamus		10	-4	2	2.2%		
Left	Insula		-46	2	14	2.7%	17	136
Left	Cingulate gyrus	32	-6	32	-4	2.8%	18	128
Left	Insula		-50	-36	22	2.7%	19	128

Table S19. ALE values (percentages) for Study #4. ALE values (percentages) for applying noxious stimuli to the left side of the body subtracting the ALE maps for applying stimuli to the right side of the body. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abbreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; SI = primary somatosensory cortex; PPC = posterior parietal cortex; SII = secondary somatosensory cortex; MI = primary motor cortex.

Table S20. ALE values (percentages) for Study #4 (right sided noxious stimuli minus left sided noxious stimuli)

Side	Region	BA	x	y	z	ALE value	Cluster #	Volume (mm^3)
Left	SI		-54	-26	22	6.7%	1	8128
Left	Insula		-38	6	4	6.6%		
Left	Insula		-38	-18	12	6.1%		
Left	SII		-56	-10	12	3.9%		
Left	Precentral Gyrus	6	-52	-4	6	3.0%		
Left	Cingulate Gyrus	32	-2	24	40	4.2%	2	2256
Right	Cingulate Gyrus	32	6	20	26	4.2%		
Right	Cingulate Gyrus	32	2	24	32	4.0%		
Left	Cingulate Gyrus	24	-2	20	24	3.3%		
Left	Medial Frontal Gyrus	8	-10	26	42	2.5%		
Right	Insula		34	14	8	7.9%	3	1648
Left	Cingulate Gyrus	32	-8	8	38	4.1%	4	1568
Left	Superior Frontal Gyrus	6	0	12	48	3.4%		
Right	Cingulate Gyrus	32	8	12	40	3.3%		
Right	Cingulate Gyrus	32	12	14	38	3.3%		
Left	SI	3	-32	-34	60	4.9%	5	1528
Left	MI	4	-38	-24	62	3.3%		
Right	SII	40	56	-22	20	5.1%	6	656
Left	Cingulate Gyrus	24	-6	-4	42	4.9%	7	528
Right	Lentiform Nucleus		24	-4	8	4.0%	8	480
Left	Supramarginal Gyrus	40	-40	-40	36	4.2%	9	432
Right	Cerebellum		22	-56	-26	3.2%	10	296
Left	Cerebellum		-4	-54	-28	2.7%	11	240
Right	IPL	40	50	-46	38	3.1%	12	232
Left	Middle Frontal Gyrus	10	-30	46	4	3.3%	13	224
Left	Lentiform Nucleus		-20	4	10	3.2%	14	224
Right	Inferior Frontal Gyrus	47	42	20	-4	3.2%	15	208
Left	Insula		-32	20	4	2.9%	16	176
Right	Thalamus		2	-16	4	3.2%	17	168
Left	Angular Gyrus	39	-40	-58	34	3.0%	18	160
Right	Middle Frontal Gyrus	46	42	46	16	2.4%	19	144
Left	Thalamus		-4	-26	0	2.9%	20	120
Left	Cerebellum		-34	-52	-38	2.7%	21	104

Table S20. ALE values (percentages) for Study #4. ALE values (percentages) refer to the likelihood of obtaining activation evoked by noxious stimuli applied to the right side of the body subtracting the ALE maps for applying noxious stimuli to the left side of the body. Coordinates are in Talairach space (Talairach and Tournoux 1988). Cluster #: The clusters are ranked according to their size in millimetres cubed (mm3). Abreviations: BA = Brodmann Area; x = medial-lateral; y = anterior posterior; z = superior-inferior; SI = primary somatosensory cortex; SII = secondary somatosensory cortex; MI = primary motor cortex.

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4 Chapter 4: Neural correlates of the conscious perception of warmth

Preface

Using functional magnetic resonance imaging (fMRI), this study explored the roles of the somatosensory cortices, insula, anterior cingulate cortex (ACC), and the thalamus in the conscious and unconscious processing of thermal stimuli in healthy participants. Within the context of a delayed-discrimination and detection task, subjects received painful or warm thermal stimuli that were administered in a counterbalanced order within each run and across six scanning runs. Within the context of this paradigm the perception of the warm stimuli was attenuated, and as a result, some of the stimuli were undetected by the subjects. The detection-task paradigm required subjects to respond to the onset (and offset) of the stimuli, making it possible to identify the trials where the subjects did and did not detect the stimuli. We performed an analysis using the aforementioned regions-of-interest (somatosensory cortices, insula, ACC, thalamus) to examine the activation associated with detected and undetected warm stimuli.

Title: Neural correlates of the conscious perception of warmth

Abbreviated title: Conscious perception of warmth

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4.0 Abstract

The neural correlates of conscious and unconscious processing of somatosensory stimuli remain unclear. Conscious perception of touch, pain, and temperature information is believed to be dependent upon activation of thalamo-cortical projections to the primary and secondary somatosensory cortices (SI and SII), insula, and to the anterior cingulate cortex (ACC). Some evidence has indicated that unconscious processing of somatosensory stimuli is associated with weaker activation of similar brain regions; however, other studies have reported negative blood-oxygen-level-dependent (BOLD) signal changes in somatosensory cortices in response to undetected stimuli. We sought to assess the roles of SI, SII, insula, ACC and thalamus in the conscious processing of warm stimuli using functional magnetic-resonance-imaging (fMRI) data acquired during a task involving the detection and discrimination of noxious heat and innocuous warm stimuli applied to the right forearm. Within scanning runs, noxious and innocuous trials were presented in a counterbalanced order. In a proportion of trials, subjects did not detect some of the warm stimuli, possibly due to interactions between heat pain and warm processes. This allowed for a comparison of brain responses to "detected" and "undetected" warm stimuli. In comparison to a pre-stimulus baseline, significant stimulus-related activation (detected stimuli) was found in SI and SII but only weak non-significant activation was found in the insula. In contrast, undetected warm stimuli presentations were associated with significant negative BOLD-signal change in the insula, SII, ACC, and the thalamus. Direct statistical comparison of detected and undetected stimuli further confirmed the significantly stronger activation to detected stimuli in somatosensory cortices, insula, ACC and thalamus. Our findings of negative BOLD-signal change associated with undetected stimuli may reflect gating of the somatosensory system by the thalamus, or be a result of top-down attentional mechanisms. Furthermore, this suggests that the somatosensory cortices may be involved in the processing of stimulus-related activity that leads to the conscious perception of warmth.

4.1 Introduction

The roles of the somatosensory cortices in the conscious and unconscious processing of somatosensory stimuli are still poorly understood. The conscious perception of pain, warmth, or touch begins with activation of receptors on afferents in the periphery, which send information via the spinal cord to the brainstem, thalamus, and from there to the primary and secondary somatosensory cortices (SI and SII), insula, and the anterior cingulate cortex (ACC) (Rowe et al., 1996; Iwamura, 1998; Schnitzler and Ploner, 2000; Shyu and Vogt, 2009). The processing of stimuli that do not reach conscious awareness is less clear, although electrophysiological and neuroimaging studies have suggested that similar brain regions are involved in processing imperceptible stimuli (Libet et al., 1967; Palva et al., 2005). For example, weak evoked fields can be elicited from SI in response to undetected stimuli without awareness (Preifll et al., 2001; Palva et al., 2005). In another study, neuronal responses were recorded in SI and the medial prefrontal cortex (PFC) during the presentation of threshold stimuli in a discrimination task (de Lafuente and Romo, 1995). Neural activity in SI changed as a function of stimulus amplitude; however perceptual judgments of the stimuli only correlated with neuronal responses recorded in the PFC. These findings indicate that neurons in SI may only contribute to low level processing of a stimulus.

Contrary to these previous findings are reports of decreased blood-oxygen-level-dependent (BOLD) signal or ‘deactivation’ in somatosensory cortices associated with the presentation of undetected somatosensory stimuli. For example, in a functional magnetic resonance imaging (fMRI) study with healthy subjects, deactivation was reported in SI and SII during undetected electrical shocks (Blankenburg et al., 2003). The authors attributed their results to intracortical inhibition of the somatosensory system. Additionally, another group reported negative BOLD-signal change in the somatosensory cortices in individuals with conversion syndrome, which often presents as a loss of sensation in an affected limb (Mailis-Gagnon et al., 2003). Positive BOLD-signal

change was seen in somatosensory regions such as SI, SII, insula and thalamus when stimuli were presented to the patients' unaffected limbs, but unperceived stimuli presented to their hypoesthetic limbs produced negative BOLD-signal change in SI and SII. The physiological and neural mechanisms underlying the loss of somatosensory function in patients with conversion syndrome remain unclear but may result from damage to central pathways that send touch and pain information to the somatosensory cortices. The authors attributed their results to reflect reduced neuronal activity or potentially top-down attentional mechanisms that may have suppressed activity in the somatosensory cortices. However, the authors had no controlled means to assess the patients' detection of the stimuli. Rather, the analysis was based on patients' verbal reports at the end of the scanning run – making it uncertain whether the stimuli were actually detected or undetected. Another fMRI study (Boly et al., 2007) compared randomly presented "unperceived" laser stimuli with those that were "perceived" by subjects. The authors reported negative BOLD signal changes in regions outside the somatosensory cortices in "default network" brain areas (posterior cingulate, precuneus, medial frontal cortices, temporoparietal junctions, inferior temporal cortex, superior frontal and parahippocampal gyri) – or in brain regions that are active during rest. Increased activity occurred in these regions when unperceived stimuli were anticipated to be imperceptible. However, an important consideration is that the subjects were expecting to either perceive or not perceive a stimulus after a variable delay period. This may have influenced the negative BOLD signal, as a previous fMRI study demonstrated enhanced negative BOLD signal during the anticipation of somatosensory stimuli (Drevets et al., 1995). Additionally, another fMRI study provided evidence that predictive activation was seen in other brain regions such as the anterior insula before the onset of an unpredictable thermal stimulus (Ploner et al., 2010). These results would indicate that this region may also play a role in the conscious perception of somatosensory stimuli. Lastly, another fMRI study scanned healthy individuals before and after somatosensory decline was induced using repetitive electrical shocks for 35 minutes. In comparison to the before-stimulation period, the after-

stimulation period (somatosensory decline) was associated with a decrease in the perception of somatosensory stimuli and significant negative BOLD-signal change in somatosensory areas (Stammler et al., 2008).

The neurophysiological mechanisms underlying negative BOLD-signal change in functional neuroimaging data are unclear, and have been a focus of interest in the brain imaging literature (Menon et al., 1995; Shmuel et al., 2002; Shmuel et al., 2006; Kastrup et al., 2008). It has been theorized that negative BOLD-signal change in the somatosensory system may be a result of inhibitory surround receptive fields in SI (Apkarian et al., 2000). Other authors have suggested that negative BOLD-signal change in somatosensory cortices may be a result of thalamocortical projections to inhibitory interneurons in SI (Gibson et al., 1999; Swadlow and Gusev, 2000; Swadlow, 2003).

Our study explored the roles of the somatosensory areas (SI, SII, ACC, insula, and thalamus) in processing detected and undetected warm stimuli using fMRI. Previous neuroimaging studies have shown that these brain regions process warm stimuli (see Table 1 for a list of studies), but in general do not produce robust brain activation (Craig et al., 1996; Becerra et al., 1999; Becerra et al., 2001; Lorenz et al., 2002; Maihofner and Handwerker, 2005). The lack of robust brain activity may reflect fatigue in the warm sensitive peripheral fibres that is sometimes induced by painful stimulation (Peng et al., 2003; Greffrath et al., 2007). For example, Olausson et al., (2005) reported weak activation in somatosensory cortices in response to warm stimuli. However, the warm stimuli were presented very soon after a painful stimulus (10s). This is of importance, as firing rates of warm fibres are dampened after presenting innocuous thermal stimuli with fixed intensities and durations at short inter-stimulus intervals (Darian-Smith et al., 1979). Therefore, fatigue of peripheral afferents may have led to a reduction in cortical activation.

In the current study using a detection and delayed-discrimination task, a group of healthy volunteers were presented with brief innocuous and noxious heat stimuli to the right volar surface of the forearm. Subjects were asked to signal the detection of each stimulus by pressing a response key. In a pre-scan

training session the temperatures of innocuous stimuli were individually determined so that they would be easily perceptible. Additionally, the warm and noxious-heat stimuli were counterbalanced and separated using inter-stimulus intervals so the influence of noxious stimuli on the perception of warm stimuli presented in different trials would be minimized. Despite these precautionary measures, the subjects still did not perceive some of the warm stimuli. This permitted the identification of trials where warm stimuli were either detected or undetected by the subjects, and analysis of stimulus-related brain activation associated with detected vs. undetected warm sensation. We hypothesized that the conscious processing of detected stimuli would result in positive signal changes in SI, SII, insula, the ACC and thalamus. We also wanted to assess the underlying neural correlates associated with the unconscious processing of warm stimuli in these brain areas of interest by examining activation associated with undetected stimuli.

4.2 Methods

4.2.1 Subjects

A total of eight young healthy subjects (four male, four female; mean age = 27.5 years; sd = 4.28) were recruited from the University community. The study was approved by the Research Ethics Board at the Centre de recherche de l'Institut universitaire de gériatrie de Montréal (CRIUGM). All subjects gave written informed consent and were financially compensated for their time.

4.2.2 Stimuli

Thermal stimuli were delivered through two computer-controlled Peltier-contact thermodes (9 cm^2; Medoc Advanced Medical Systems, Ramat Yishai, Israel) applied to the volar surface of the right forearm. Thermal probes were positioned adjacent to one another in the territories of the T1 and C6 dermatomes.

Prior to the imaging study, the temperatures for thermal stimuli were individually determined while the subject lay supine in an MRI simulator. The baseline temperature was 32°C with a ramp rate of 10°C/s. Stimuli were 6 seconds in duration with a 4-second plateau, making them identical to those presented during the actual imaging experiment.

The primary focus of this report is on the trials where innocuous stimuli were delivered to the subjects. However, the subjects also received noxious stimuli that ranged in temperature from 49°C to 51.7°C. All the innocuous stimuli were individually chosen to be within a range of temperatures that were above warm-detection threshold, but below pain threshold. During the experiment, participants received different combinations of pairs of innocuous stimuli that were of low, medium or high intensity. For three participants, the low innocuous temperature was 42°C, and is referred to as “W1”. For the remaining four participants, the low temperature (W1) was 42.5°C. The medium temperature,

referred to as “W2”, was 1.8°C above W1. The high temperature, referred to as “W3”, was 2.5°C above W1.

4.2.3 Experimental Paradigm

The experimental paradigm was a delayed-discrimination task designed to explore the neural correlates associated with the short-term memory of innocuous and noxious thermal stimuli. However, the current report focuses on the activation associated with the warm stimuli.

Each experiment consisted of six scanning runs. Each run contained an equal number of innocuous and noxious trials. In half the scanning runs (3 runs), the first trial involved the presentation of innocuous stimuli followed by a trial with noxious stimuli. In the other half of the scanning runs, the first trial involved the presentation of noxious stimuli followed by a trial with innocuous stimuli. The order of the trials (pain and warm) was always counter-balanced and was not pseudorandomly presented, so that no more than two noxious or innocuous stimuli were presented in succession.

A single scanning run consisted of twelve trials that included 3 separate trial types: four required an intensity discrimination of two sequentially presented stimuli, four required a spatial discrimination of the stimuli, and four were perceptual trials during which the subjects received similar stimuli and performed similar motor responses but did not have to make a discrimination decision about the stimuli. The trial types were pseudorandomly presented within a scanning run: two intensity discrimination, two spatial discrimination and two perceptual trial types for the six innocuous and six noxious trials, for a total of twelve trials. This resulted in a different program (set of trial types) for each of the six scanning runs. To address potential order effects, the different programs (six) were randomly presented to the subjects. The description of the trial types is given below.

Intensity discrimination trials

A third of the warm trials (2 trials/run x 6 runs/subject = 12 trials/per subject) required the participants to make an intensity discrimination between the pair of stimuli presented during that trial. The first stimulus (W1 or W3) was delivered to either dermatome T1 or dermatome C6, followed by the presentation of the second stimulus (W1, W2, or W3) to the dermatome that did not receive the first stimulus. At the end of the trial, the participants were asked either “Was the first stimulus weaker?” or “Was the first stimulus stronger?” and had to signal (yes or no) by tapping a mouse button with their contralateral (left) index or middle finger, respectively.

Spatial discrimination trials

A third of the warm trials (2 trials/run x 6 runs/subject = 12 trials/per subject) required the subjects to make a spatial discrimination concerning the location of the first stimulus. The first stimulus (W1 or W3) was delivered to either dermatome T1 or dermatome C6, followed by the presentation of the second stimulus (W1, W2, or W3) to the dermatome that did not receive the first stimulus. At the end of the trial the participants were asked either “Was the first stimulus on the right?” or “Was the first stimulus on the left?” and they were asked to signal (yes or no) by tapping a mouse button with their contralateral (left) index or middle finger, respectively.

Perceptual trials

A third of the warm trials (2 trials/run x 6 runs/subject = 12 trials/per subject) involved the presentation of the same sequence of stimuli and motor responses but did not require a delayed discrimination. The first stimulus (W1 or W3) was delivered to either dermatome T1 or dermatome C6, followed by the presentation of the second stimulus (W1, W2, or W3) to the dermatome that did not receive the first stimulus. The subjects were instructed to “Tap index finger” or “Tap middle finger” by tapping a mouse button with their contralateral (left) index or middle finger.

Stimulation Protocol

A single trial began with the notification of the trial type (2s; intensity discrimination, spatial discrimination, or perceptual) and was followed by the presentation of an innocuous or noxious stimulus (Figure 1). For all trial types, the first stimulus (W1 or W3) was delivered to either dermatome T1 or dermatome C6. After a short delay (6, 7, or 8-s) a second stimulus (W1, W2, or W3) was delivered to the dermatome that did not receive the first stimulus. After a short interval (3s), subjects were asked to make a decision (4s) about the stimuli they had received during the trials.

Regardless of the trial type (intensity discrimination, spatial discrimination, or perceptual) the subjects were required to detect the onset and the offset of each stimulus by clicking a mouse button with their left index finger (contralateral to the stimulated forearm). At the end of the scanning run, subjects rated the average intensity of the thermal stimuli separately using a computerized horizontal VAS that was part of the E-prime program (0-100). For the innocuous stimuli, the value 0 (located at the left side of the screen) signified no sensation and the value 100 (located at the right side of the screen) was intense heat, but not painful. For the noxious stimuli, the value 0 (located at the left side of the screen) indicated no pain and the value 100 (located at the far right side of the screen) corresponded to extremely intense pain.

The analysis was based on the responses to the innocuous stimuli (12 stimuli/per scanning run x 6 runs/subject). The subjects' detection of the stimuli (onset and offset) was assessed in relation to the intensity ratings.

4.2.4 Functional Brain Imaging Parameters

The fMRI experiment was performed at the Unité de Neuroimagerie Fonctionelle (UNF) at the CRIUGM. Medical images were acquired on a Magnetom 3T Trio Siemens scanner (Siemens Medical Solutions, Erlangan, Germany) using an 8-channel head coil. Subjects were positioned in the scanner by the MRI technician and were given earplugs. To minimize movement during

the scan, subjects' heads were fixed in position using foam pads and they were instructed to minimize their movements.

The scanning session consisted of 6 functional scanning runs. The functional scans were acquired using a high-resolution blood-oxygen-level-dependent (BOLD) protocol with a T2*-weighted gradient echo-planar imaging (EPI) sequence (TR=2.0 s, TE=30ms, flip angle=90°, 128x128 matrix, 253 volume acquisitions) using 22 coronal slices (voxel size: 2 x 2 x 3mm). The slices did not cover the whole brain, but rather were positioned over SI, SII, PPC, ACC, and insula. Subjects were given several minutes of rest between the scans, and an 8-minute break halfway between the 6 functional scanning runs, during which the subjects had a T1-weighted high-resolution anatomical scan (TR=13 ms, TE=4.92 ms, flip angle=25°, FOV=256 mm, 1-mm isotropic sampling).

4.2.5 Data Analysis

4.2.5.1 Behavioural Data

Subjects' data (detection and intensity ratings of thermal stimuli) were collected via E-prime and were imported into SPSS for statistical analysis (SPSS Inc., Chicago, Il).

4.2.6 Functional Brain Imaging Data

4.2.6.1 Warm Region-of-Interest Analysis

In addition to a global search of the brain, we performed a ROI analysis of the functional imaging data to localize activity in cortical regions related to thermosensory processing. The ROI were determined by a thorough review of the existing neuroimaging literature that described studies using warm stimuli. We selected studies that applied innocuous warm stimuli in the absence of motor responses. A total of 9 studies fit our inclusion criteria (see Table 1 for details). Based on their results, *a priori* ROI were manually drawn on contralateral SI, bilateral SII, ACC, thalamus and insula by one of the authors (EGD) on each

subject's anatomical MRI. For the thalamus, the ROI was drawn to encompass the VPL and VMpo. These regions were determined in respect to the location of the internal capsule, the pulvinar, the posterior commissure, and the third ventricle. The ROI drawn for each subject were then combined to make a single mask file that included all voxels for each subject. The resulting mask file was a combination of all voxels drawn on each subject's anatomical image. This means that the voxels which did not overlap between each subject's anatomical area were included in the warm ROI.

4.2.6.2 Pre-processing and General Linear Model (GLM)

Imaging data were analyzed using BrainVoyager QX (Brain Innovation, Maastricht, the Netherlands; www.brainvoyager.com). Functional scans underwent pre-processing (motion correction, slice-time correction, high-pass filtering at 3 cycles per run, and smoothing using a 6mm FWHM blurring kernel) before being registered to the anatomical scan. Both the functional and anatomical MRIs were then transformed into a standardized stereotactic space (Talairach and Tournoux, 1988).

A random-effects GLM was applied to the data. To minimize the influence of motor-related activity (associated with the detection of the stimuli) on stimulus-related activity, the stimulus-event period modeled in the design matrix was limited to the 4s plateau of stimulation. Regressors modeled into the design matrix included: the notification of the trial type, the plateau of detected warm stimuli – STIM1 (4s), the undetected warm stimuli-STIM1 (4s), warm-STIM2 (4s) pain stimuli-STIM1 (4s), pain-STIM2 (4s), delays 1 (6,7,8s) and 2 (3s), response (4s), and baseline (6s) for each of the three different trial types (Figure 1). To assess whether the time course associated with the detected stimuli was influenced by the motor response associated with the detection (onset/offset detection) of the stimulus-one presentations, we modeled in the design matrix the 1 second time periods preceding and following the 4s plateau of the stimulus-one presentations.

In the present analysis, we focused on the stimulus-one presentations (detected and undetected) rather than analyzing stimulus-one and –two presentations together. This was because the task demands were different for stimulus-one presentations where the subjects were encoding the stimuli to compare to the stimulus-two presentations. Some of the stimulus-two presentations did not involve encoding. During trials where the subjects detected both stimuli, during the stimulus-two presentations the subjects may have been formulating a decision for the upcoming motor response period.

The threshold for significance was set using a two-tailed test with df = 6 (number of subjects -1) and a p-corrected = 0.05 (t=4.21), adjusted using the Bonferroni correction based on a directed-search volume defined by the nine ROIs (volume of 9 resels; p=0.05/9=p-uncorrected = 0.0056). For the global search outside the warm ROI, the p values were corrected for multiple comparisons based on the brain volume scanned using an alpha level set at p=0.05 (t=4.67) using stat-threshold (www.math.mcgill.ca/keith/fmristat).

4.3 Results

4.3.1 Psychophysical data

The data from one subject were removed due to equipment failure; thus, data from 7 subjects were included in the final analysis. The psychophysical analysis is based on 2 stimuli/trial x 6 trials/run x 6 runs/subject x 7 subjects = 504 total innocuous stimuli in this analysis.

The warm intensity ratings were tested for normality using a Shapiro-Wilks Test for small samples. Some of the ratings (runs 3-5) were non-normally distributed ($p<0.05$), and consequently all data were analyzed non-parametric statistical tests. The overall intensity rating of the warm stimuli was 20.43 (SE=7.96). Based on a repeated measures analysis of variance (ANOVA), subjects' intensity ratings were significantly decreased across the successive scans (average rating: run 1=31; run 2=24; run 3=21; run 4=21; run 5=17; run 6=9; Friedman test: chi-square=11.3, p=0.046; Figure 2).

We subsequently assessed the subjects' detection responses. The subjects failed to detect 36% (91/252) of the stimulus-one presentations. Of these undetected stimuli, subjects did not detect some of both temperatures (W1 and W3) for stimulus 1. Additionally, subjects did not detect 15% (38/252) of the stimulus-two presentations (see Table 3 for group results and Table 4 for individual subject results). Of these undetected stimuli, subjects did not detect some of all three temperatures for stimulus 2 (W1, W2, W3).

We examined the relationship between the subjects' detection responses with respect to the perceptual ratings of the intensity of the stimuli. The data for the detection responses for stimulus 1 and 2 were subjected to tests for normality. The detection responses from the stimulus-one presentations for some of the runs (1, 2, 5) were not normally distributed ($p<0.05$). The majority of the detection responses for the stimulus-two presentations were not normally distributed (runs 1-5, $p<0.05$). The data were subsequently analyzed using non-parametric statistics. The detection responses for the stimulus-one presentations showed a significant decrease across the scanning runs (Friedman Test: chi-square: 12.4, $p=0.03$). This would indicate that the decline in the number of detection responses of the stimulus-one presentations was not random and was likely due to perceptual decline over the scanning runs. The results demonstrating that the stimulus-one detection performance followed a similar pattern to the subjects' perceptual ratings are potentially unrelated to the order of scans (order effects). This occurrence was unlikely as the subjects received a different order of trial types and therefore stimuli across the scanning session. For the stimulus-two presentations, a trend towards perceptual decline was seen across the scanning runs; however, this was not significant (Friedman test: chi-square: 8.28; $p=0.14$). To further investigate if the detection responses for stimulus 2 significantly decreased across the scanning runs, the detection responses for the first and last runs were compared. While the data displayed a small decline, this was not significant (Wilcoxon's signed ranks test: $Z=-1.84$, $p=0.065$).

The perception of stimulus 2 may have been under similar sensory

detection constraints as stimulus 1. When examining the data from all subjects, the percentage of trials in which both the first and second stimuli were undetected (31/91=34%; Table 3 for group results; Table 4 for individual results) was similar to that of the percentage of undetected stimulus-one presentations (91/252=36%).

In the resulting fMRI analysis, only the events associated with the detection or missed detection of the stimulus-one presentations were included in the analysis. This was based on the fact that the task demands were different for stimulus 1 (encoding) vs. stimulus 2 (retrieval and possible preparatory motor responses). However, some of the stimulus-two presentations that the subjects detected in the absence of detecting stimulus-one presentations would have also involved encoding processes. These stimuli (stimulus-two presentations following undetected stimulus-one presentations) were more similar to the stimulus-one presentations. Potentially the addition of these stimuli (combining both stimulus-one and –two) in the fMRI analysis would lead to greater percent signal changes in our predefined areas of interest. To explore this possibility, we analyzed the detected stimulus-two presented in which the subjects failed to detect the stimulus-one presentations combined with the stimulus-one presentations (reported in Supplementary Information - Table S1). Additionally, the undetected stimulus-two presentations that followed undetected stimulus-one presentations were combined with the undetected stimulus-one presentations (See Supplementary Information).

4.3.2 Functional Brain Imaging Data

4.3.2.1 BOLD responses associated with detected stimulus-one presentations

4.3.2.1.1 Warmth-related brain activation

Directed search in Warm ROI

Consistent with our predictions, detected warm stimuli evoked significant brain activation in contralateral SI (t=4.77, p=0.003) and ipsilateral SII (t=4.75, p=0.003; Figure 3; Table 6). Weak activation was found in the contralateral (left) SII (t=3.14, p=0.02) and bilateral insula (left: t=3.23, p= 0.02; right: t=3.87, p=0.08), but was below the cut-off level for statistical significance within the warm ROI (t=4.21). The ACC (left: t=0.52, p=0.6; right: t=0.98, p=0.36) and the thalamus (left: t=0.52, p=0.12; right: t=-1.64, p=0.12) did not demonstrate a trend towards a response associated with the detected stimuli. No significant negative peaks were associated with the detected-stimuli time periods within the warm ROI.

Search outside the warm ROI

Detected stimuli positively activated the ipsilateral (right) inferior frontal gyrus (t=7.57, p< 0.0001), the superior parietal lobule (t=7.76, p<0.0001), and the posterior parietal cortex (t=6.96, p<0.001). Additionally, significant positive activation was seen in motor regions including the premotor cortex (t=6.98, p<0.001), and the superior frontal gyrus in the supplementary motor area (t=5.33, p=0.003), which likely reflects activity related to the subjects' motor response indicating the onset and/or offset of the stimuli. No significant activation was seen in the primary motor cortex (MI). Outside the warm ROI, detected stimuli were found to produce significant negative BOLD-signal change in the contralateral (left) amygdala (Table 6). Additional negative peaks were seen in midline structures such as the precuneus and the posterior cingulate cortex.

4.3.2.1.2 BOLD responses to undetected innocuous stimulation

Directed search in Warm ROI

Within the search region, no voxel contained a significant positive t-value above the threshold for significance that was associated with the undetected warm stimuli. However, a significant BOLD decrease was seen in bilateral SII (left: t=-6.62, p=0.0006: right: t=-7.29, p=0.0003), ACC (left: t=-6.01, p=0.0009; right: t=-8.78, p<0.0001), thalamus (left: t= -6.22, p<0.0001; right: t=-8.01,

p=0.0002), and the contralateral (left) insula (t=-8.48, p<0.0001; Table 5). No significant negative BOLD-signal change was seen in contralateral (left) SI or in the ipsilateral (right) insula.

Search outside the warm ROI

A global search outside the warm ROI revealed significant positive activation associated with the undetected stimuli in the ipsilateral (right) superior parietal lobule (t=5.38, p=0.002) and the inferior parietal cortex (t=9.52, p<0.0001; Table 6). Additionally, weak non-significant activation was seen in the inferior frontal gyrus (t=4.62, p=0.06) based on a global search of the slice coverage (threshold: t=4.67). Negative peaks were found in the contralateral (left) temporal lobe structures and the posterior cingulate cortex.

4.3.2.1.3 Time course for detected and undetected stimuli

We extracted the time courses from the peak positive or negative voxels associated with the detected or undetected stimuli within the warm ROI to assess brain activity in response to these stimuli. As the subjects clicked the mouse button with the contralateral (left) hand during the ramp up and ramp down periods of the stimuli, we wanted to assess whether activity in the warm ROI was influenced by this slight motor activity. To this end, we extracted the time course of the peak voxel (t=4.99, p=0.01) in MI, ipsilateral (right) to the warm stimuli, which was associated with the detection of the onset and the offset of the stimuli. We overlaid this time course from MI on top of those derived from the detected and undetected stimuli obtained from contralateral SI. The BOLD percent signal change associated with the clicking of the mouse button was weak and at its maximum it produced a percent signal change equal to 0.04%. The time course of the motor detection response peaked at 6s after the start of the stimulus before the start of the temperature plateau (ramp up period) during the time period when the subjects detected the onset of the stimuli (mean detection time = 733.07msecs; Figure 4). The percent signal change associated with the response dissipated during the stimulus plateau and weakly peaked again 13s

after the onset of the stimulus. Positive signal increases for detected stimuli and negative signal changes in response to the undetected stimuli peaked on average 6s after onset of the stimulus plateau (equivalent to 7s after the start of the stimulus and 1s after the detection response). These findings suggest that the time courses associated with the stimulus plateau period may not have been heavily influenced by the motor detection response generated by signaling the onset or the offset of the stimulus-one presentations.

We subsequently wanted to confirm our findings through the use of an alternative technique to select the time course information. We extracted the peak (positive) voxel associated with the contrast for detected vs. undetected stimuli. We found that the time courses were comparable to those extracted from the peak positive or negative voxels in SI, SII, and insula that were produced by detected or undetected stimuli alone (Figure S1 in Supplementary Information).

4.3.2.2 Detected versus Undetected Trials

Within the warm ROI, the direct contrast between detected vs. undetected warm stimuli produced significant positive activation in contralateral (left) SI ($t=5.82$; $p<0.001$), bilateral SII (left: $t=4.33$, $p=0.005$; right: $t=4.64$, $p = 0.003$), ACC (left: $t=5.45$, $p = 0.002$; right: $t=4.83$, $p=0.003$), insular cortices (left: $t=4.61$, $p=0.003$; right: $t=6.14$, $p=0.0009$) and the thalamus (left: $t=5.49$, $p = 0.002$; right: $t=4.44$, $p=0.004$; Table 5). No negative peaks were associated with this comparison within the warm ROI.

Outside the warm ROI, positive activation for this contrast was seen in the contralateral (left) inferior frontal gyrus ($t=7.69$, $p<0.0001$) and putamen ($t=6.8$, $p<0.0001$, Table 6). Additionally, positive activation was found in the ipsilateral (right) precentral gyrus (BA6; $t=6.71$, $p<0.0001$), the premotor cortex ($t=6.94$, $p<0.0001$), the posterior parietal cortex ($t=5.47$, $p=0.002$), the basal ganglia ($t=8.09$, $p<0.0001$) and the thalamus ($t=7.71$, $p<0.0001$).

4.4 Discussion

The current study explored the roles of SI, SII, insula, ACC and the thalamus in the processing of detected and undetected warm stimuli. During the imaging experiment, pairs of warm stimuli were counterbalanced with pairs of heat pain stimuli across twelve trials that were performed in six runs. This method of stimulus presentation led to an attenuation of the perception of some of the warm stimuli, so that they were undetected by the subjects. This permitted the examination of brain activation associated with the processing of detected and undetected warm stimuli. The current report focuses primarily on detected and undetected stimulus-one presentations. For purposes of comparison the detected and undetected stimulus-two presentations (following undetected stimulus-one presentations) were combined and analyzed with the detected and undetected stimulus-one presentations (See Supplementary Information).

Our results showed significant positive BOLD-signal changes in response to the detected stimulus-one presentations in contralateral SI and ipsilateral SII, but only weak, non-significant positive activation in our other predicted areas, which included contralateral SII and bilateral insula. Conversely, significant negative BOLD-signal change or deactivation was associated with undetected warm stimuli in bilateral SII, ACC and thalamus, as well as in contralateral insula. Weak, but non-significant negative BOLD-signal change was also seen in contralateral SI and the ipsilateral insula. The implications of these findings are discussed below.

4.4.1 Brain activation associated with detected stimulus-one presentations

The findings from this study provide support for a role of the primary and secondary somatosensory cortices in thermoception. Findings are consistent with electrophysiological studies, which have shown that cortical neurons in SI produce graded responses to temperatures in the innocuous range (Kenshalo and Isensee, 1983). Additionally, responses to innocuous thermal stimuli have

been directly recorded in thalamic neurons that project to SII (Gauriau and Bernard, 2004).

The results are also in agreement with previous brain imaging studies that have demonstrated activation in SI and/or SII in response to warmth (Craig et al., 1996; Becerra et al., 1999; Maihofner and Handwerker, 2005; Sung et al., 2007). However, some brain imaging studies have failed to find activation in SI and/or SII in response to warm stimuli (Casey et al., 1996; Becerra et al., 2001; Bornhovd et al., 2002; Olausson et al., 2005). As these studies often presented warm stimuli as a control for painful stimuli, the absence of significant activation may reflect attenuation effects leading to reduced cortical activation. In none of these previous studies were the subjects required to detect their perception of the stimuli. Therefore, the findings from the current report may have important methodological implications for future neuroimaging studies using innocuous and noxious thermal stimuli, in that conscious detection of stimuli is associated with higher levels of brain activity while undetected stimuli can produce significant negative BOLD percent signal change.

Previous neuroimaging studies that have examined the neural correlates of detected and undetected somatosensory stimuli have also reported activation in the primary and secondary somatosensory cortices (Blankenburg et al., 2003; Mailis-Gagnon et al., 2003). However, these previous studies utilized different somatosensory stimuli (electric shocks, brushing, noxious thermal stimuli), different paradigms, or they tested individuals with somatosensory dysfunction. The current report is the first, to the authors' knowledge, to offer evidence for the roles of the primary and secondary somatosensory cortices in the conscious processing of innocuous thermal stimuli by analyzing functional brain imaging data associated with detected or undetected warm stimuli.

It is of note that the BOLD response in contralateral (left) SII did not meet our threshold for significance. The significant activation in right SII is consistent with a previous finding showing responses in this region during the perception of ipsilaterally administered stimuli (Tommerdahl et al., 2005). Additionally, SII contains bilateral receptive fields and potentially transcallosal connections (Petit

et al., 1990; Disbrow et al., 2003). The greater activity on the right side may reflect the role of the right hemisphere in awareness of stimuli presented to the body (Heilman and Van Den Abell, 1980; Fierro et al., 2000; Coghill et al., 2001). Consistent with this finding is a neuroimaging study demonstrating greater activity in the ipsilateral (right) posterior parietal cortex during a task where attention was paid to a thermal stimulus (Peyron et al., 1999).

Activation in bilateral insula was associated, albeit non-significantly, with detected warm stimuli. This was inferred from the examination of the time course curves extracted from the voxels in bilateral insula, which peaked in response to the plateau of the stimulus, thus indicating that these regions were involved in the processing of the detected stimuli. The insula has been implicated in thermoception as it receives input from the main pain and temperature pathway in the spinal cord (Craig et al., 2000). Additionally, clinical studies have reported thermal sensory deficits in patients with damage to this region (Bowsher et al., 2004). Furthermore, direct electrophysiological stimulation of this region in humans can evoke sensations of warmth (Penfield and Faulk, 1955; Ostrowsky et al., 2002). Lastly, activation in the anterior insula has been associated with the subjective perception of thermal stimuli (Ploner et al., 2010). The neural activity before painful and non-painful stimuli was examined in an fMRI study. Activation preceding the stimuli was found in the anterior insula. Additionally, functional connectivity analysis revealed activation in the anterior insula was predictive of an upcoming stimulus – indicating that it may be involved in the cognitive evaluation of the stimuli. Potentially, the lack of robust activity in the insula during the time periods when the stimuli were detected may reflect a decrease in the body's autonomic reaction within the context of a task involving the presentation of noxious and innocuous stimuli. This is an important consideration as the insula is involved in autonomic regulation (Craig, 2002), and is activated during stressful tasks (Stein et al., 2007; Rosenberger et al., 2009). As the warm stimuli were presented within the context of a stimulation protocol that also used painful stimuli, this lack of robust activity in the insula may reflect a reduction in the stress experienced by the subjects during trials where warm stimuli were present.

Very weak positive activation in the contralateral thalamus and bilateral ACC was associated with detected stimuli. These findings are consistent with previous single-unit recording studies that have described few warm-specific neurons in these regions (Poulos and Benjamin, 1968; Martin and Manning, 1971; Davis et al., 1998; Hayama and Ogawa, 2003; Kuo and Yen, 2005; Lee et al., 2005). Therefore the warm-specific neuronal activity may not have been robust enough to be detected as a BOLD response. Another consideration is that weak, non-significant negative activation was seen in the ipsilateral thalamus. This may indicate that some suppression of the BOLD response occurred even during the time periods when the stimuli were detected. The mechanisms of suppression are discussed in the section below on undetected stimuli. Another possibility may be that the spatial resolution of fMRI is not precise enough to separate positive and negative responses that occur within the confines of closely positioned nuclei in this midline structure.

Outside the warm ROI, significant positive activation was seen in the right PFC and the posterior parietal cortices. These regions are involved in internal and external awareness, monitoring, maintenance, and performance control during tasks that involve relevant and irrelevant stimuli (Berns et al., 1997; Blakemore et al., 1998; Mesulam, 1998; Frith et al., 2000; Bunge et al., 2001; Kircher et al., 2002; Schott et al., 2005; Uddin et al., 2005; Jardri et al., 2007; Scheuerecker et al., 2007; Esslen et al., 2008; Kozasa et al., 2008; Mimura, 2008; Voss et al., 2008; Zahn et al., 2008; Lafargue and Franck, 2009). The increased activation in these regions during the time periods when the subjects detected the stimuli most likely reflects enhanced monitoring and attention to the body during the detection of the stimuli. An additional explanation for the current findings may be that the activation in the PFC reflects the subjective evaluation of somatosensory stimuli. This was a conclusion of a single-unit recording study where non-human primates were trained to detect the presence or absence of near-threshold somatosensory stimuli (de Lafuentes and Romo, 2005). Neuronal responses in SI were correlated with stimulus amplitude; however, only neuronal

activation originating from the PFC was associated with perceptual judgments about the stimuli.

Additional areas outside the warm ROI showing significant positive activation included several association motor regions such as the ipsilateral (right) premotor and supplementary motor cortices. As these regions are involved in motor planning, these results likely reflect preparation for the upcoming detection of the offset of the stimulus (Winstein et al., 1997; Simon et al., 2002; van den Heuvel et al., 2003; Bischoff-Grethe et al., 2004; Huang et al., 2004; Cavina-Pratesi et al., 2006; Cunnington et al., 2006). Below threshold activation was found in right MI (contralateral to the stimulus) in response to the stimulus plateau. Significant activation was seen only during the time period associated with the detection response (start of the stimulus to the plateau). Additionally non-significant activation was seen in left MI near sensory regions contralateral to the stimulation site. Thus findings indicate that the positive BOLD-signal associated with the somatosensory perception of the stimulus plateau may not have been heavily influenced by the motor response made during the onset/offset detection.

4.4.2 Brain activation associated with undetected stimulus-one presentations

Undetected warm stimuli were associated with significant negative BOLD-signal change in SI, SII, ACC, insula and the thalamus. While the mechanism of negative BOLD seen in brain regions during an fMRI experiment remain largely unknown, it is believed that it reflects a focal decrease in blood flow and oxygen consumption, and has been associated with either the activation of inhibitory neurons (Shmuel et al., 2002) or a decrease in neuronal firing (Hamzei et al., 2002; Shmuel et al., 2006). A previous fMRI study examined neural activity during the presentation of imperceptible stimuli and reported negative BOLD-signal change in somatosensory cortices during time periods when weak electric shock stimuli were undetected by subjects (Blankenburg et al., 2003). These authors interpreted the negative BOLD-signal change to be a result of activity of

SI inhibitory interneurons that receive feed-forward inhibition from excitatory thalamocortical cells. However, the authors did not report any activation in the thalamus, which makes it arduous to assess their interpretation. In relation to the current findings, we found negative BOLD-signal change in the thalamus while no significant positive or negative BOLD-signal change was found in SI. This finding may reflect a decrease in the inhibitory feed-forward thalamocortical cycle during the time periods when the stimuli were undetected, but expected by the subjects, as a means of preparation for the perception of a stimulus.

The negative BOLD signal seen in our data set associated with the undetected stimuli may also be explained by suppression of neuronal firing. This was a conclusion by authors of a previous fMRI study that found negative BOLD-signal change in the somatosensory cortices when individuals with conversion disorder did not perceive a stimulus that was presented to a limb with reduced sensory perception (Mailis-Gagnon et al., 2003). Other authors who have reported negative BOLD-signal change in somatosensory regions ipsilateral to stimulation have attributed their results to suppression of neural activity (Drevets et al., 1995; Hamzei et al., 2002; Staines et al., 2002; Hlushchuk and Hari, 2006). In relation to the current findings, brain regions that mediate intrapersonal awareness and monitoring, such as the posterior parietal cortex could have suppressed activation of the somatosensory cortices. Additionally, the right PFC showed activation just below the threshold for significance during the time periods when the stimuli were undetected. Suppression of neural activity in the somatosensory cortices has been attributed to an excitatory projection from the PFC to the reticular nucleus in the thalamus that sends inhibitory projections to the sensory relay nuclei (Guillery et al., 1998). Therefore, the negative BOLD-signal change that we see in the present study may reflect suppression of the somatosensory cortices via a frontothalamic pathway.

A last possible explanation for the negative BOLD signal seen in the warm ROI is that it reflects surround inhibition in cells in the somatosensory regions. A previous fMRI study using somatosensory stimuli, reported focal increases in SI that were accompanied by focal decreases in activation in nearby voxels

(Apkarian et al., 2000). This result may reflect a mechanism by which the somatosensory system is able to enhance the relative activity in cells that directly receive stimulation by inhibiting those in surround. In the present study, a global type of inhibition in the somatosensory areas may have occurred due to enhanced attention during the time periods when the subjects expected to receive a somatosensory stimulus.

4.4.3 Brain activation associated with the detected vs. undetected stimulus-one presentations

Examination of the detected vs. undetected stimuli produced significant robust positive responses in all areas of interest. This contrast produced a more localized and direct comparison between the voxels that were activated positively for the detected stimuli and negatively for the undetected stimuli. The response produced by this contrast was most likely driven by the greater negative response seen during the undetected trials.

An earlier fMRI study examined the effects of baseline fluctuations in the BOLD signal in brain regions that preceded the presentation of thermal stimuli to determine if it contributed to the cognitive evaluation of the stimuli (Boly et al., 2007). Similar to our findings of increased frontoparietal activation for detected vs. undetected stimuli, in the Boly et al. (2007) study consciously perceived stimuli in comparison to intensity-matched unperceived stimuli produced increased BOLD signal change in bilateral dorsolateral prefrontal, posterior parietal cortex. However, Boly and colleagues found negative BOLD signal change in regions involved in “default mode” processing (bilateral posterior cingulate precuneas, medial frontal cortices, temporoparietal junctions, right inferior temporal, superior frontal gyri) - or brain activation associated with task-unrelated processing. Activation in these regions was associated with the prediction of an unperceived stimulus, which might enhance the subjects’ ability to perceive a subthreshold stimulus. In the current study these aforementioned brain regions were outside the slice coverage that was designed to improve

spatial resolution of the somatosensory cortices and subcortical structures. The role of default mode processing on the perception of subthreshold stimuli is still unknown and future work in this area is warranted.

4.4.4 Brain activation associated with the stimulus-one and -two presentations

The examination of the brain activation associated with both the detected stimulus-one presentations and the detected stimulus-two presentations (where subjects failed to detect stimulus-one presentations) increased the t-values in contralateral SI, bilateral SII and insula (see Supplementary Information Table S1) to a level that was above our significance threshold in comparison to analyzing the detected stimulus-one presentations alone. This provides further support for the role of the somatosensory cortices and the insula in the conscious perception of thermal stimuli. This finding indicates that the low statistical values seen in contralateral SII, and bilateral insula associated with the undetected stimulus-one presentations were likely due to the fewer number of stimuli included in the contrast. However, consistent with the findings for the detected stimulus-one presentations, non-significant activation was seen in bilateral ACC and thalamus. The values were slightly more positive than seen for the stimulus-one presentations alone. Potentially (as mentioned previously) the low percent BOLD signal change may reflect the fewer number of warmth-responsive neurons in this region and the low spatial resolution associated with fMRI.

Examination of the undetected stimulus-one presentations combined with the undetected stimulus-two presentations (that followed undetected stimulus-one presentations) also revealed significantly negative activation in bilateral ACC, thalamus and ipsilateral SII. Consistent with the findings for the undetected stimulus-one presentations, no significant negative activation was found in contralateral SI. However, unlike the previous findings, no significant negative BOLD signal change was found in contralateral SII or bilateral insula. This would indicate that the BOLD signal change was less negative (or more positive) in

these regions in comparison to the undetected stimulus-one presentations. Potentially, the activation in these regions may have been influenced by the longer delay period. Variable delay periods before a thermal stimulus have been shown to modulate activation in the somatosensory cortices (Porro et al., 2002). The activation in these regions may have received less neuronal suppression from frontothalamic pathways during the stimulus-two presentations due to this longer delay period. A previous fMRI study noted BOLD signal decreases during the anticipation of an impending somatosensory stimulus (Drevets et al., 1995). In relation to the current experiment, it is possible that the initial decreases in BOLD signal dissipated because the subjects knew that they were more likely to receive the second stimulus upon missing the detection of the first stimulus.

4.4.5 Limitations of the interpretation

A limitation of the current study is the relatively few number of subjects included in the sample. Small sample sizes in fMRI studies are generally not recommended, as this is largely dependent on the type of analysis strategy applied to the data. The statistical analysis of fMRI data involves several steps where the effects can be modeled using either a fixed or random approach. In a fixed-effects analysis all images for all subjects are included in the analysis and the variance and degrees of freedom over all of these data points are calculated. This will produce a large number of degrees of freedom and possibly lead to highly significant effects; however, in a fixed-effects analysis the variance is computed over scanning runs, the subjects are treated a fixed effect and inference is thus limited to the specific set of subjects included in the analysis. More sophisticated fMRI analysis techniques treat subjects a random effect thus allowing the inference to be extended to the population from where the subjects were sampled.

In the current experiment, the data were analyzed using random-effects, meaning that the data were not driven solely by the contribution of a few subjects. The results demonstrate significant positive and negative activation in several of our regions of predefined interest. Although a random-effects analysis

is typically not performed with so few subjects (Penny et al., 2003; Penny and Holmes, 2006; Holmes and Friston, 1998; Strange et al., 2003), the decision to analyze data using fixed- or random-effects should be based on the task design in terms of the type of experimental protocol and the stimuli used to examine the effect on brain activation (Friston et al., 1999). Had the current experiment involved a more complex cognitive task design this may have produced more variability in the data; however, as the goal of the current analysis focused on brain regions known to process somatosensory stimuli – the effects were significant in a few brain areas, which suggests that the variance was lesser for this type of task. In support of this conclusion, in our previous experiments using either thermal or vibrotactile stimuli we had included data from 8 or 9 subjects in a random-effects analysis and also obtained significant activation in somatosensory cortices (Albanese et al., 2007; Albanese et al., 2009). However, in the current report the conclusions drawn concerning the activation of the insula may have been spurious given that the inclusion of additional stimuli (stimulus-two presentations following an undetected stimulus-one presentation) increased the significance of the t-values in bilateral insula.

Another possible limitation of the current design is potentially the inclusion of the stimulus-onset and –offset motor detection response. A potential improvement to the current design would have required subjects to indicate the detection of the stimuli several seconds after their offset. The addition of the motor detection response may have contributed stimulus-unrelated variance to the data thus augmenting the percent signal changes in the somatosensory cortices. An additional consideration is that should several events in the fMRI design matrix be collinear with one another, this may have detrimental effects on the stimulus-related BOLD signal changes in the somatosensory cortices as several events so close in time may be difficult to disambiguate. Colinearity issues are difficult to address after the data in an fMRI experiment have been collected. The effect of the stimulus-onset and –offset motor responses was assessed by examining the associated time-course information extracted from right MI (ipsilateral to the stimulus-evoked activation). This was then compared to

the time-course extracted from the somatosensory cortices (SI & SII) and bilateral insula. The time-course associated with the motor detection response increased during the stimulus ramp up period (start of the stimulus to the stimulus plateau), but then dissipated afterward. In contrast, the time-course extracted from the somatosensory cortices continued to peak just after the time-course of the motor response decreased. This result provides an indication that the results obtained in the somatosensory cortices may not have been heavily affected by the motor detection responses.

A last potential limitation of the study is that different temperatures were used for the stimulus-one presentations (W1=42.0/42.5^{o}C and W3=44.5/45^{o}C). All stimulus-one presentations (detected and undetected) were not coded separately in the design matrix and were combined. This method did not allow us to examine whether differential brain activation would produce variations in BOLD signal in our predefined regions of interest. Previous brain imaging studies have examined brain activation in response to varying levels of thermal stimuli and found corresponding increased activation in a number of regions known to process somatosensory stimuli (Casey et al., 1994; Coghill et al., 1999); however, none have compared brain activation using temperature differences as small as in the current experiment. The effects remain largely unknown and could be addressed in future experiments.

4.5 Conclusions

This study examined the roles of the somatosensory cortices associated with the processing of detected and undetected warm stimuli. The results of this study confirm that SI and SII are involved in the conscious processing of warm stimuli. Conversely, undetected stimuli produced significant negative BOLD-signal change in SII, ACC, insula and the thalamus. While the mechanisms behind negative BOLD-signal change remain uncertain, our findings could be explained by gating of the somatosensory system, top-down attentional mechanisms, inhibitory thalamocortical projections, or surround inhibition. Future studies are needed to understand how these factors influence negative BOLD-signal change and could be aided by complementary electrophysiological or optical imaging techniques.

4.6 Acknowledgements

The authors would like to thank Jen-I Chen for aiding in subject testing. The funding for this research was provided from a grant provided to the authors by the Canadian Institutes for Health Research (CIHR).

Figure 1. Stimulation Protocol

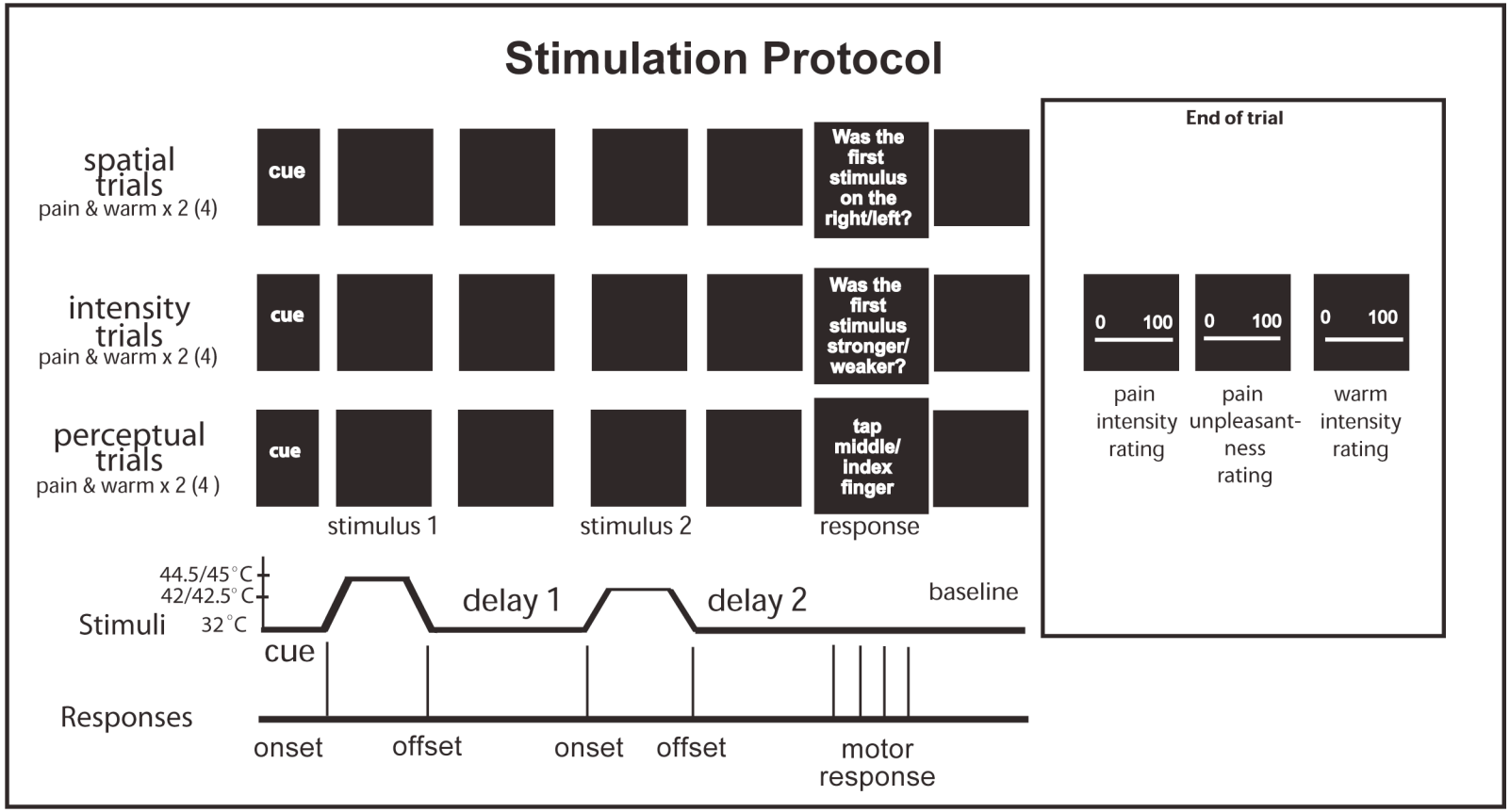

Figure 1. Stimulation protocol - A single trial began with the notification of the trial type (2s; intensity discrimination, spatial discrimination, or control) and was followed by the presentation of an innocuous or noxious stimulus. For all trial types, the first stimulus was delivered to either dermatome T1 or dermatome C6. After a short delay (6, 7, or 8-s) a second stimulus was delivered to the dermatome that did not receive the first stimulus. After a short interval (3s), subjects were asked to make a decision (4s) about the stimuli they had received during the trials. For the intensity discrimination trials, the subjects were asked either “Was the first stimulus weaker?” or “Was the first stimulus stronger?” For the spatial discrimination trials, the subjects were asked either “Was the first stimulus on the right?” or “Was the first stimulus on the left?” For both of these trial types, the subjects had to answer (“Yes” or “No”) by clicking a mouse button. For the control trials, the subjects were instructed to “Tap index finger” or “Tap middle finger” by pressing a mouse button. This was followed by a post-response period (3s) and a resting baseline period (3s). Half of the trials involved the presentation of innocuous (warm) stimuli and the other half presented noxious (painful) stimuli. The trial types were counter-balanced across the trials with varying intensities of thermal stimuli (warm and pain).

Figure 2. Averaged warm ratings across the six scanning runs

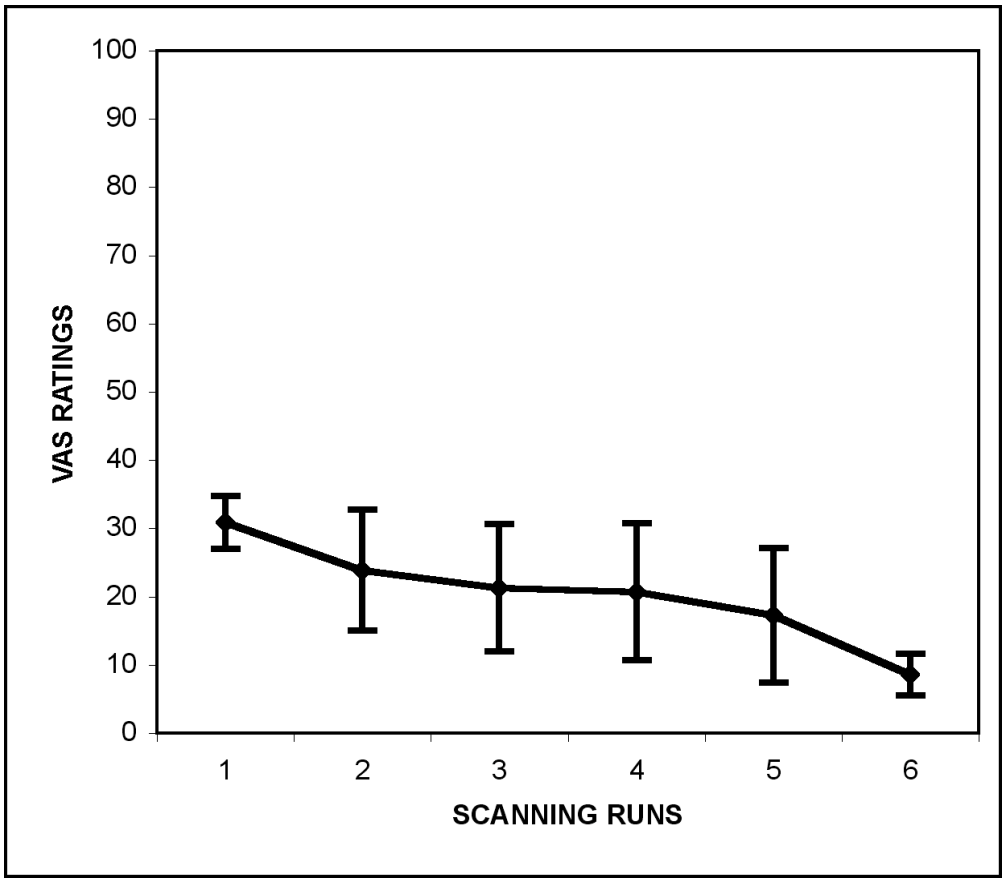

Figure 2. Averaged warm ratings across the six scanning runs. Subjects rated the stimuli on a scale ranging from 0 = no sensation to 100 = very hot but not painful. The average intensity was 20.43 (SEM 7.96). Significant attenuation to the stimuli was seen across the scanning session (runs 1 through 6) based on the non-parametric version of a within-subjects ANOVA (Friedman test: chi-square=11.3, p=0.046).

Figure 3. Statistical parametric maps associated with the time periods for detected (left) or undetected (middle) stimuli and the comparison between detected vs. undetected stimuli (right)

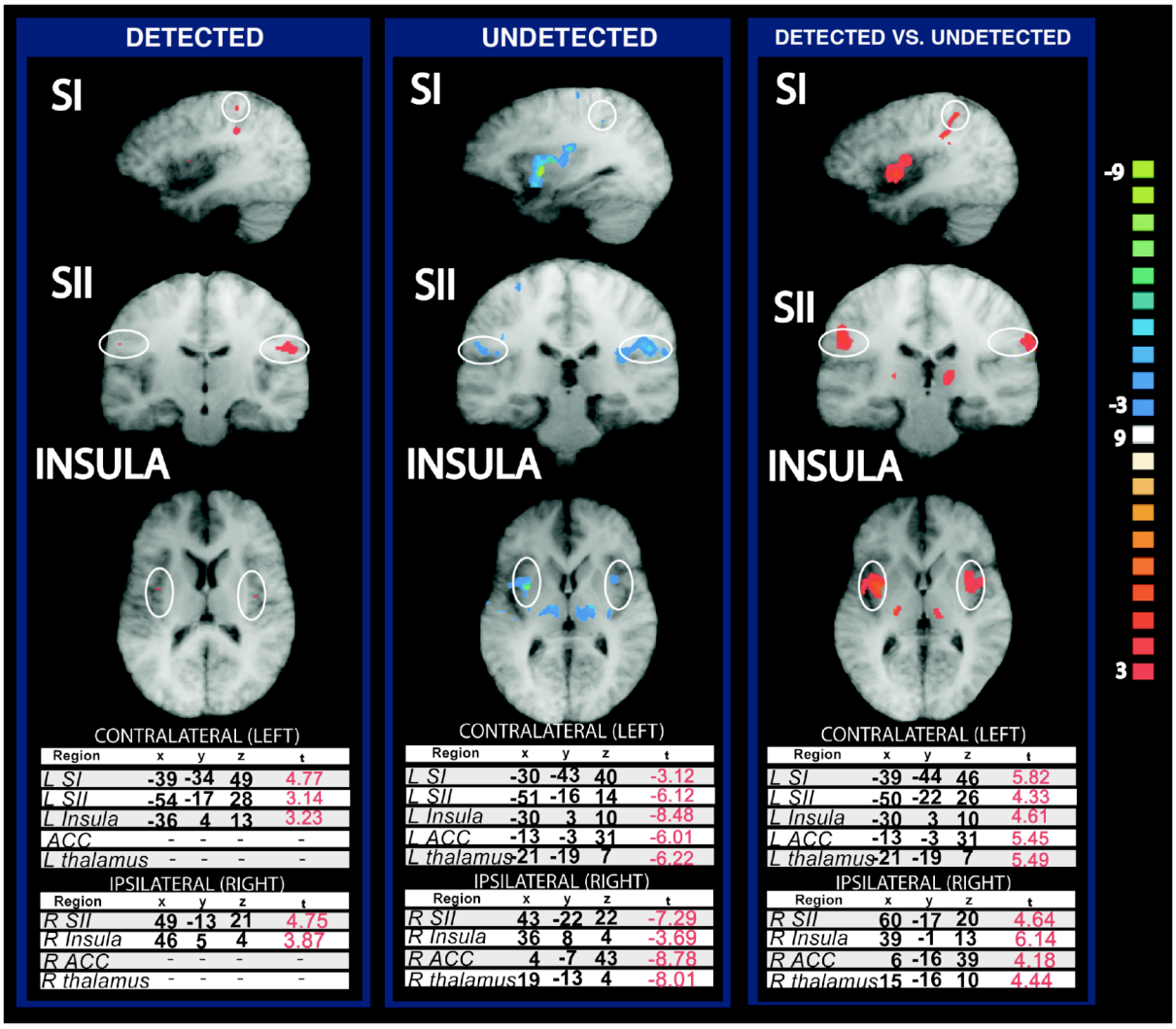

Figure 3. Statistical parametric maps associated with the time periods when stimuli were detected (left) or undetected (middle) and the comparison between detected and undetected stimuli. The values for the positive and negative activation coordinates were taken from the peak voxels with the warm ROI that contained t-values above 3.0. Coordinates are given in Talairach space (Talairach & Tournoux, 1988). Medial-lateral (X), anterior-posterior (Y), and superior-inferior (Z) stereotaxic coordinates (mm) are relative to midline, anterior commissure, and commissural line, respectively (positive values are right, anterior and superior). L: left; R: right; ACC: Anterior cingulate cortex; SI: primary somatosensory cortex; SII: secondary somatosensory cortex.

Figure 4. Time courses extracted from the peak positive or negative voxels in the warm ROI associated with the time periods when the stimuli were detected (blue line) or undetected (red line)

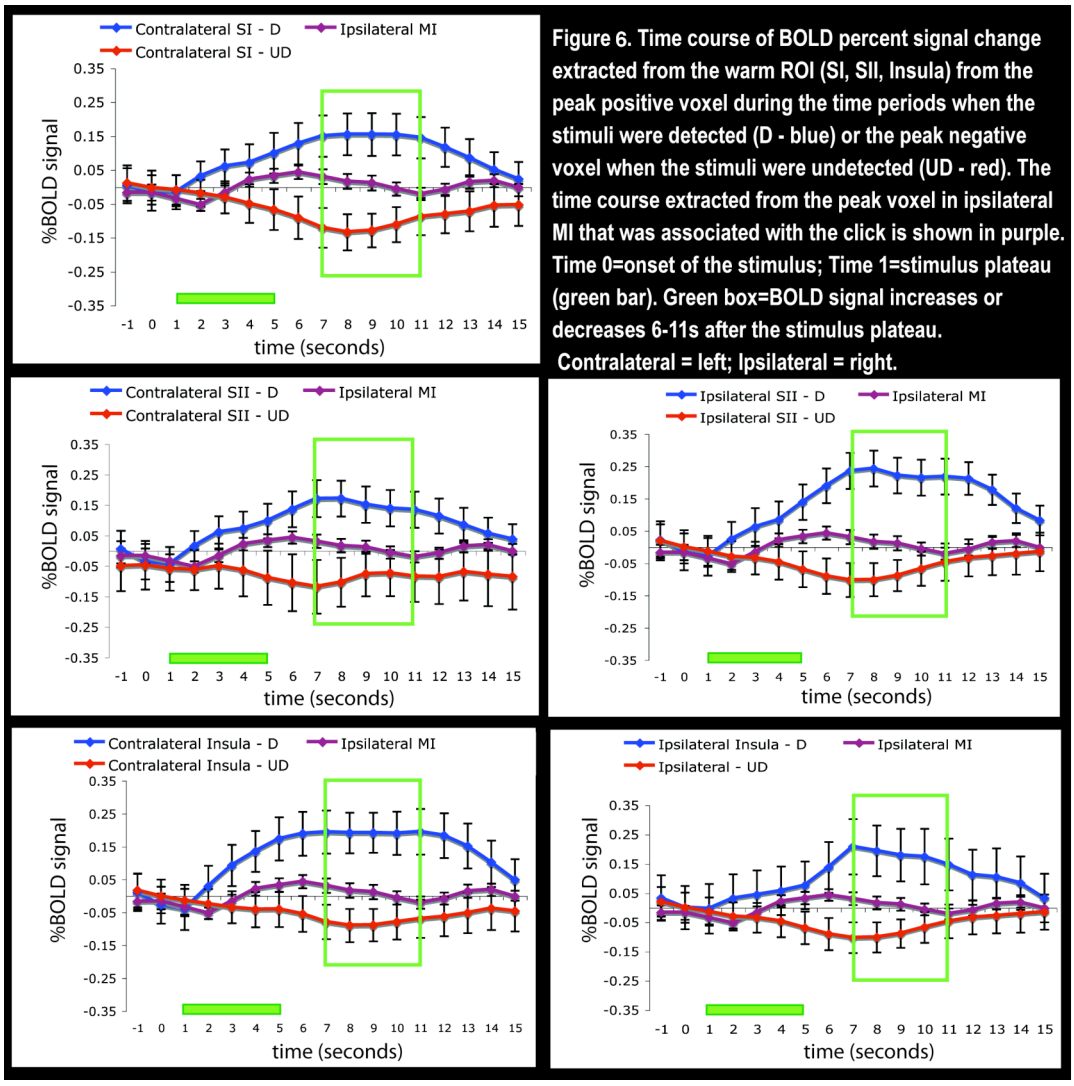

Figure 6. Time course of BOLD percent signal change extracted from the warm ROI (SI, SII, Insula) from the peak positive voxel during the time periods when the stimuli were detected (D - blue) or the peak negative voxel when the stimuli were undetected (UD - red). The time course extracted from the peak voxel in ipsilateral MI that was associated with the click is shown in purple. Time 0=onset of the stimulus; Time 1=stimulus plateau (green bar). Green box=BOLD signal increases or decreases 6-11s after the stimulus plateau. Contralateral = left; Ipsilateral = right.

Table 1. List of studies utilizing warm stimuli in the absence of motor responses

			Stimuli			
Author	**Year**	**Imaging**	**Type**	**Side**	**Body Part**	**Regions**
Becerra	1999	fMRI 1,5	Contact Heat	Left	Hand	IC, SII, PPC, MFG, SI, MI, ACC, STG, thalamus, CB
Becerra	2001	fMRI 1,5	Contact Heat	Left	Hand	ACC, mPFC, DLPFC, STG, NC, thalamus
Bornhovd	2002	fMRI 1,5	Laser	Left	Hand	PPC, DLPFC
Casey	1996	PET	Contact Heat	Left	Forearm	mPFC, LN, CB, thalamus
Craig	1996	PET	Contact Heat	Right	Hand	IC, SII, SI
Lorenz	2002	PET	Contact Heat	Left	Forearm	IC, SII, PPC, ACC, SII, DLPFC, LN, thalamus
Maihofner	2005	fMRI 1,5	Contact Heat	Left	Forearm	IC, SII, SI, MI, MPFC, IFC
Olausson	2005	fMRI 1,5	Contact Heat	Left	Leg	IC, ACC
Sawamoto	2000	fMRI 1,5	Laser	Right	Hand	SII, ACC
Sung	2007	fMRI 3,0	Contact Heat	Right	Lower leg	IC, SI, PCL, MFG, IFG, LN, CB

Table 1. List of brain imaging studies reporting warm-evoked activation. Articles were retrieved via a literature review search on Medline (1980-2009) using the terms warm AND (fMRI or PET). This produced a total of 103 studies. Studies were searched as to whether the reported brain-imaging coordinates reflected somatossensory responses to warm stimuli in the absence of any motor activity (i.e. Intensity ratings of the stimuli), and data were reported in healthy subjects. The references of the studies that met these inclusion criteria were also searched for suitable articles to be included in the literature review. Abbreviations: ACC: Anterior cingulate cortex; CB: cerebellum; IC: insular cortex; MFG: Middle frontal gyrus; m/DLPFC: Medial/dorsolateral prefrontal cortex; MI: Primary motor cortex; PCL: Paracentral lobule; PPC: Posterior parietal cortex; SI: primary somatosensory cortex; SII: secondary somatosensory cortex; STG: Superior temporal gyrus; LN: lentiform nucleus: NC: nucleus accumbens.

Table 2. Temperature Tasks and Delta T calculation

TRIAL	STIMULUS 1		STIMULUS 2		Delta-T (relative to stimulation on C6)						
	Stimulus Location				T1<C6				T1>C6		
	T1	C6	T1	C6							
1	**W1**			**W1**				**0**			
2		W1	W2							1.8	
3	**W3**			**W2**					**0.7**		
4		W3	W2				-0.7				
5	**W1**			**W3**	**-2.5**						
6		**W3**	**W1**		**-2.5**						
7	**W1**			**W2**		**-1.8**					
8		**W1**	**W3**								**2.5**
9	**W3**			**W1**							**2.5**
10	**W3**			**W3**				**0**			

Table 2. Temperature Tasks and Delta-T calculation between the stimuli presented to dermatomes T1 and C6. During the experiment, participants received different combinations of pairs of innocuous stimuli that were of low, medium or high intensity. For three participants, the low innocuous temperature was 42°C, and is referred to as “W1”. For the remaining four participants, the low temperature (W1) was 42.5°C. The medium temperature, referred to as “W2”, was 1.8°C above W1. The high temperature, referred to as “W3”, was 2.5°C above W1. For the intensity discrimination trials and the perceptual trials, the pairs of stimuli presented to dermatome T1 or dermatome C6 varied by seven temperature differences ranging from positive 2.5°C to negative 2.5°C degrees and were presented in ten different combinations. For the spatial discrimination trials the participants received five out of the seven temperature combinations (in BOLD font in the list) in eight different combinations.

Table 3. Number of detected and undetected stimuli across warm trials

Raw data (sum across all subjects)

	stimulus 2 detected	stimulus 2 undetected	Total
stimulus 1 detected	154	7	**161**
stimulus 1 undetected	60	31	**91**
	214	**38**	**252**

Percentages

	stimulus 2 detected	stimulus 2 undetected	Total
stimulus 1 detected	**61%**	**3%**	**64%**
stimulus 1 undetected	**24%**	**12%**	**36%**
	85%	**15%**	**100%**

Means

	stimulus 2 detected	stimulus 2 undetected	Total
stimulus 1 detected	22	1	**23**
stimulus 1 undetected	9	4	**13**
	31	**5**	**36**

SD

	stimulus 2 detected	stimulus 2 undetected
stimulus 1 detected	**9**	**1**
stimulus 1 undetected	**5**	**6**

Range

	stimulus 2 detected	stimulus 2 undetected
stimulus 1 detected	**10-33**	**0-3**
stimulus 1 undetected	**3-15**	**0-16**

Table 3. Number of detected and undetected stimuli across all 252 warm trials. The percentages were derived by dividing each number in each of the cells by the total number of warm trials (N=252). The means and standard deviations (SD) were calculated based on the sum of the detected and undetected stimuli across the number of warm trials (N=36) for each subject. The range is the minimum and maximum number of warm trials where the stimuli were detected or undetected.

Table 4. Number of detected and undetected stimuli: individual subjects

Raw Values

S1

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	29	0	29
stim 1 undetected	5	2	7
	34	2	36

S2

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	20	1	21
stim 1 undetected	13	2	15
	33	3	36

S3

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	33	0	33
stim 1 undetected	3	0	3
	36	0	36

S4

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	20	1	21
stim 1 undetected	13	2	15
	33	3	36

Percentages (divided by trials N=36)

S1

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	81%	0%	81%
stim 1 undetected	14%	6%	19%
	94%	6%	100%

S2

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	56%	3%	58%
stim 1 undetected	36%	6%	42%
	92%	8%	100%

S3

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	92%	0%	92%
stim 1 undetected	8%	0%	8%
	100%	0%	100%

S4

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	56%	3%	58%
stim 1 undetected	36%	6%	42%
	92%	8%	100%

S5

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	11	2	13
stim 1 undetected	7	16	23
	18	18	36

S5

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	31%	6%	36%
stim 1 undetected	19%	44%	64%
	50%	50%	100%

S6

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	10	3	13
stim 1 undetected	15	8	23
	25	11	36

S6

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	28%	8%	36%
stim 1 undetected	42%	22%	64%
	69%	31%	100%

S7

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	31	0	31
stim 1 undetected	4	1	5
	35	1	36

S7

	stim 2 detected	stim 2 undetected	Total
stim 1 detected	86%	0%	86%
stim 1 undetected	11%	3%	14%
	97%	3%	100%

Table 4. Numbers of stimuli that were detected or undetected by individual subjects. The percentages were derived by dividing each number in each of the cells by the total number of warm trials for each subject (N=36).

Table 5. Peak coordinates associated with detected and undetected stimuli, and the contrast of detected vs. undetected stimuli within the warm ROI

	Detected					Undetected					Euclidean Distance D - UD (cm)	Detected vs. Undetected				
Contralateral	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**		**x**	**y**	**z**	**t**	**p**
SI	-39	-34	49	**4.77**	**0.003**	-30	-43	40	-3.13	0.02	1.6	**-39**	**-44**	**46**	**5.82**	**0.001**
SII	-54	-17	28	3.14	0.02	-51	-16	14	**-6.62**	**0.0006**	1.4	**-50**	**-22**	**26**	**4.33**	**0.005**
IC	-36	4	13	3.23	0.02	-34	3	4	**-8.48**	**0.0001**	0.9	**-30**	**3**	**10**	**4.61**	**0.003**
ACC	-9	-10	31	0.52	0.6	-3	-16	34	**-6.01**	**0.0009**	0.9	**-6**	**-13**	**31**	**5.45**	**0.002**
Thalamus	-21	-23	7	0.52	0.6	-12	-14	10	**-6.22**	**0.0008**	1.3	**-21**	**-19**	**7**	**5.49**	**0.002**
Ipsilateral	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**		**x**	**y**	**z**	**t**	**p**
SII	49	-13	21	**4.75**	**0.003**	43	-22	22	**-7.29**	**0.0003**	1.1	**60**	**-17**	**20**	**4.64**	**0.004**
IC	46	5	4	3.87	0.008	36	8	4	-3.69	0.01	1.0	**39**	**-1**	**13**	**6.14**	**0.001**
ACC	6	-13	27	0.98	0.36	4	-7	43	**-8.78**	**0.0001**	1.7	**6**	**-16**	**39**	**4.83**	**0.003**
Thalamus	21	-22	7	-1.64	0.12	19	-13	4	**-8.01**	**0.0002**	1.0	**15**	**-16**	**10**	**4.44**	**0.004**

Table 5. Cortical areas showing postive or negative BOLD-signal change associated with detected (left) or undetected (middle) stimuli and for the comparison between detected and undetected stimuli (right) within the warm ROI. The Euclidean distance (3D) between the peak positive and negative coordinates for the Detected (P) and Undetected (UP) stimuli are given in centimeters (cm). The average distance between the sites was 1.2 cm. Coordinates are given in Talairach space (Talairach & Tournoux, 1988). Medial-lateral (X), anterior-posterior (Y), and superior-inferior (Z) stereotaxic coordinates (mm) are relative to midline, anterior commissure, and commissural line, respectively (positive values are right, anterior and superior). Contralateral = left; Ipsilateral= right; ACC: Anterior cingulate cortex; SI: primary somatosensory cortex; SII: secondary somatosensory cortex; IC: insular cortex; the threshold for significance was based on a two tailed test with 6 degrees of freedom for the directed search within the 9 areas of interest (equal to one resel each) corresponding to an uncorrected p=0.0056 (t=4.21). Values that met the criterion for threshold are in **bold** in the table.

Table 6. Brain areas showing significant postive or negative BOLD-signal change outside the warm ROI that was associated with the detected or undetected stimuli, and the comparison between detected vs. undetected stimuli

	Detected					Undetected					Detected vs. Undetected				
Contralateral															
Positive peaks	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**
Inferior frontal gyrus (BA 9)	-	-	-	-	-	-	-	-	-	-	**-47**	**4**	**31**	**6.38**	**< 0.0001**
Inferior frontal gyrus (BA 9)	-	-	-	-	-	-	-	-	-	-	**-44**	**2**	**9**	**7.69**	**< 0.0001**
Putamen	-	-	-	-	-	-	-	-	-	-	**18**	**-10**	**18**	**6.8**	**< 0.001**
Negative peaks															
Amygdala	-20	-1	-17	**-4.71**	**0.04**	-18	-7	-11	**-5.53**	**0.001**	-	-	-	-	-
Superior temporal gyrus	-	-	-	-	-	-51	-10	-8	**-7.45**	**< 0.0001**	-	-	-	-	-
Posterior cingulate cortex	-	-	-	-	-	-3	-28	28	**-9.12**	**< 0.0001**	-	-	-	-	-
Midline															
Negative peaks	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**
Precuneus (BA 7)	0	-58	46	**-7.16**	**< 0.0001**	-	-	-	-	-	-	-	-	-	-
Posterior cingulate cortex	0	-44	22	**-14.96**	**< 0.0001**	-	-	-	-	-	-	-	-	-	-
Ipsilateral															
Positive peaks	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**
Superior frontal gyrus (BA 6)	3	-10	6	**5.33**	**0.003**	-	-	-	-	-	-	-	-	-	-
Inferior frontal gyrus (BA 9)	51	2	31	**7.57**	**< 0.0001**	50	9	28	4.62	0.06	-	-	-	-	-
Precentral gyrus (BA6)	-	-	-	-	-	-	-	-	-	-	**52**	**-4**	**49**	**6.71**	**< 0.001**
Superior parietal lobule	30	-37	61	**7.76**	**< 0.0001**	**36**	**-56**	**43**	**5.38**	**0.002**	-	-	-	-	-
Posterior parietal cortex	55	-34	49	**6.96**	**< 0.001**	**36**	**-37**	**34**	**9.52**	**< 0.0001**	**42**	**-58**	**48**	**5.47**	**0.002**
Premotor cortex (BA 6)	48	-7	46	**6.98**	**< 0.001**	-	-	-	-	-	**0**	**10**	**58**	**6.94**	**< 0.001**
Basal ganglia	-	-	-	-	-	-	-	-	-	-	**21**	**5**	**15**	**8.09**	**< 0.0001**
Thalamus	-	-	-	-	-	-	-	-	-	-	**13**	**-15**	**13**	**7.71**	**< 0.0001**
Negative peaks	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**
Superior temporal gyrus	**-**	**-**	**-**	**-**	**-**	**57**	**-12**	**1**	**-8.15**	**< 0.0001**	-	-	-	-	-
Middle temporal gyrus	**54**	**8**	**-16**	**-10.47**	**< 0.0001**	-	-	-	-	-	-	-	-	-	-
Inferior temporal gyrus	**57**	**-10**	**-14**	**-11.65**	**< 0.0001**	-	-	-	-	-	-	-	-	-	-
Putamen	-	-	-	-	-	**21**	**8**	**4**	**-5.44**	**0.001**	-	-	-	-	-

Table 6. Cortical areas outside the warm ROI showing activation during the time periods when the stimulus-one presentations were detected (left) or undetected (middle) by the subjects, and the comparison between detected vs. undetected stimuli (left). Coordinates are given in Talairach space (Talairach & Tournoux, 1988). Medial-lateral (X), anterior-posterior (Y), and superior-inferior (Z) stereotaxic coordinates (mm) are relative to midline, anterior commissure, and commissural line, respectively (positive values are right, anterior and superior). Contralateral = left; Ipsilateral= right; BA=Brodman Area. The threshold for significance was set at p=0.05 correcting for multiple comparison based on global search of the slices covering the brain (t=4.67). Values that met the criterion for threshold are in **bold** in the table.

Supplementary Information

Figure S1. Time courses of the peak voxel associated with the comparison between detected (blue line) and undetected (red line) stimuli

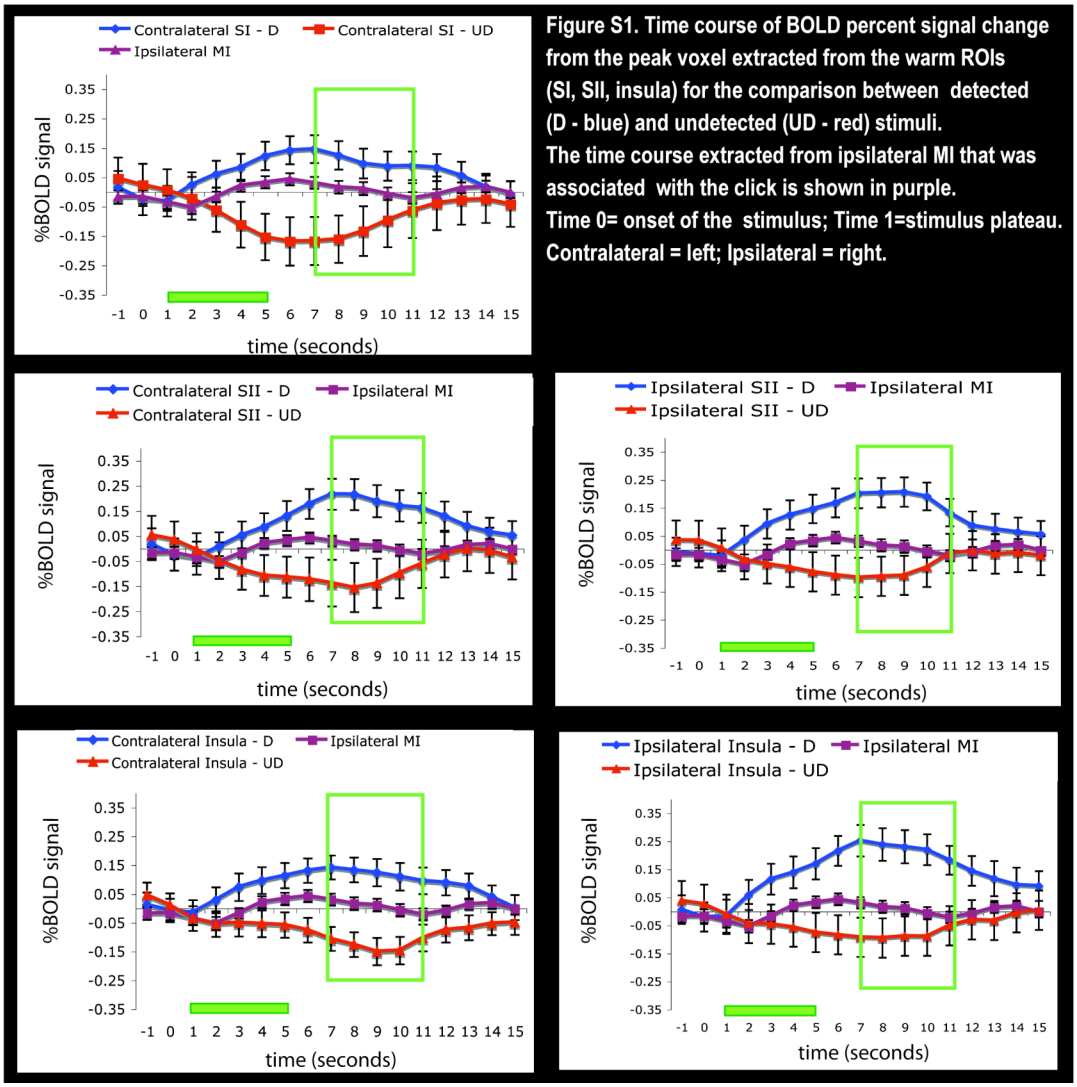

Figure S1. Time course of BOLD percent signal change from the peak voxel extracted from the warm ROIs (SI, SII, insula) for the comparison between detected (D - blue) and undetected (UD - red) stimuli.
The time course extracted from ipsilateral MI that was associated with the click is shown in purple.
Time 0= onset of the stimulus; Time 1=stimulus plateau.
Contralateral = left; Ipsilateral = right.

Table S1. Brain activation associated with dectected and undetected stimulus-one and -two presentations within the warm ROI

	Detected					Undetected					Detected vs. Undetected				
Contralateral	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**
SI	**-41**	**-31**	**41**	**5.91**	**0.001**	-51	-35	35	-1.78	0.13	**-48**	**-34**	**36**	**7.85**	**0.0002**
SII	**-51**	**-22**	**25**	**6.29**	**0.001**	-45	-31	25	-2.22	0.07	-45	30	27	2.42	0.05
IC	**-29**	**4**	**10**	**4.41**	**0.004**	-27	5	9	-2.7	0.04	-29	0	10	3.76	0.009
ACC	-6	-13	34	2.65	0.07	**-1**	**1**	**44**	**-4.65**	**0.004**	-6	-16	40	2.8	0.03
Thalamus	-18	12	10	2.22	0.08	**-8**	**-15**	**4**	**-4.26**	**0.006**	**-18**	**16**	**10**	**4.59**	**0.004**
Ipsilateral	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**	**x**	**y**	**z**	**t**	**p**
SII	**48**	**-13**	**24**	**5.98**	**0.001**	**30**	**-28**	**19**	**-5.1**	**0.002**	46	-36	28	3.41	0.02
IC	**36**	**4**	**10**	**4.7**	**0.003**	23	16	1	-2.07	0.08	43	1	10	2.88	0.03
ACC	9	-16	37	3.7	0.01	**6**	**10**	**41**	**-4.93**	**0.003**	6	-16	34	2.41	0.05
Thalamus	14	-16	10	0.41	0.7	**-6**	**-16**	**6**	**-5.4**	**0.002**	15	-19	10	2.46	0.05

Table S1. To examine brain activation associated with detected stimulus-one and –two presentations we focused on the stimulus-two presentations that would not have been influenced by the delay-discrimination task. The time periods associated with the stimulus-two presentations were first divided into four categories: (1) detected stimulus-two presentations following detected stimulus-one presentations; (2) detected stimulus-two presentations following undetected stimulus-one presentations; (3) undetected stimulus-two presentations following detected stimulus-one presentations; (4) undetected stimulus-two presentations following undetected stimulus-one presentations. The results for “Detected” (left) reflect the contrast of detected stimulus-one presentations plus detected stimulus-two presentations (that followed an undetected stimulus-one presentation). The results for “Undetected” (middle) reflect the contrast of undetected stimulus-one and –two presentations (which followed an undetected stimulus-one presentation). The results for “Detected vs. Undetected” (right) stimuli reflect the contrast of detected stimulus-one and –two presentations (which followed and undetected stimulus-one presentation) minus undetected stimulus-one and –two (which followed and undetected stimulus-one presentation) presentations. Coordinates are given in Talairach space (Talairach & Tournoux, 1988). Medial-lateral (X), anterior-posterior (Y), and superior-inferior (Z) stereotaxic coordinates (mm) are relative to midline, anterior commissure, and commissural line, respectively (positive values are right, anterior and superior). Contralateral = left; Ipsilateral= right; ACC: Anterior cingulate cortex; SI: primary somatosensory cortex; SII: secondary somatosensory cortex; IC: insular cortex; the threshold for significance was based on a two tailed test with 6 degrees of freedom for the directed search within the 9 areas of interest (equal to one resel each) corresponding to an uncorrected p=0.0056 (t=4.21). Values that met the criterion for threshold are in **bold** in the table.

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5 Chapter 5: Referred sensations in phantom-limb pain patients provide clues to cortical reorganization

Preface

This chapter describes the exploration of somatosensory processing in a group of upper and lower-limb amputee patients who continue to perceive and experience pain in the missing limb – the phenomenon also known as phantom-limb pain. Previous studies have shown that amputees exhibit altered somatotopic organization in the primary somatosensory cortex (SI), and in upper-limb amputees the adjacent representation of the stump and remote representation of the face “invade” the cortical territory once occupied by the amputated limb. It has been inferred that the cortical reorganization that occurs in the cortex may manifest itself in the perception of referred sensations to the phantom limb when other parts of the body are touched. Previous studies with upper and lower-limb amputees have shown precise one-to-one mapping of referred sensations to the phantom in response to applying stimuli to body parts adjacent to the stump and to other body parts more remote from the stump, such as the face or leg.

For example, in an upper-limb amputee, application of a stimulus to a specific location on the cheek will produce a unique sensation in a localized point on the phantom digit. Contrary to these reports are other studies that have reported non-specific referred sensations in the phantom in response to applying tactile stimuli to any part of the body. In the research reported in this chapter, as a prelude to a future neuroimaging study where we intended to map the somatotopic organization of body part representations in upper and lower-limb amputees, we explored the pattern of referred sensations to the phantom that would be evoked by applying stimuli to body parts adjacent to the amputated limb and to more remote body parts such as the face or lower leg. This allowed us to determine if referred sensations follow an organized pattern and to infer the brain regions that may be involved in the generation of these percepts.

Title: Referred sensations in phantom-limb pain patients provide clues to cortical reorganization

Abbreviated title: Referred sensations in phantom-limb pain

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5.1 Abstract

Amputation of a limb leads to changes in the representations of body parts in the primary somatosensory cortex (SI). It has been presumed that the physiological correlates of this reorganization are manifested in sensations referred to the amputated or “phantom” limb, and can be evoked by touching body parts near the site of the amputation or “reference zones” on the skin. Some controversy exists concerning the organization of referred sensations to the phantom limb evoked by tactile stimuli applied to the reference zones. Early reports indicated that referred sensations in upper and lower-limb amputees followed a highly detailed organization with a one-to-one mapping of the phantom that were evoked by stimuli applied to single points on adjacent or neighbouring body part representations ipsilateral to the amputation. However, some of these previous studies tested amputees who had premorbid conditions and/or had a mean amputation duration over 50 years. Moreover, some studies did not explore other factors that might influence referred sensations such as the perception of shortening (telescoping) of the phantom limb or prosthesis usage. The other more numerous studies with upper-limb amputees who recently lost their limbs found that referred sensations could be evoked in a non-specific location on the phantom in response to applying stimuli to any part of the body. We explored referred sensations in recent upper and lower-limb amputees by applying tactile stimuli to body parts adjacent to the amputation and to other body parts more remote to the stump such as the face and leg. We found no topographic organization of referred sensations to the phantom limb in either upper or lower-limb amputee patients, and the sensations were poorly localized, and occasionally were evoked by stimuli applied to both sides of the body. Lastly, we found no significant relationship between the perception of referred sensations to the phantom limb and the sensation that it is telescoping into the stump. Nor was there an association between the perception of referred sensations to the phantom limb and the use of a prosthetic limb.

5.2 Introduction

In human upper-limb amputees, the cortical territory of the amputated arm in the primary somatosensory cortex (SI) becomes invaded by neighbouring body part representations (Flor et al., 1995). Clinical observations and anecdotal evidence have presumed that the physiological correlate of the somatotopic reorganization that occurs in the brain manifests itself in sensations referred to the phantom limb when touching reference zones on the skin involved in the cortical reorganization. These reference zones can be either closely adjacent body parts (e.g. the residual stump) or neighbouring cortical representations (the face in the case of upper limb amputation) in SI (Katz and Melzack, 1987; Ramachandran and Hirstein, 1998).

In upper-limb amputees, referred sensations to the phantom hand have been evoked by stimuli applied to the reference zones on the face and the residual portion of the amputated limb in a near perfect topologic map – in which the entire somatotopic representation of the phantom hand appears to be transposed on the ipsilateral cheek or stump (Ramachandran et al., 1992; Halligan et al., 1993). For example, if a stimulus were to be moved down the cheek in a near straight line it would be perceived by the patient as though the phantom index finger was being touched in exactly the same way. Moving the stimulus sideways on the cheek would feel as though the middle finger was being touched and so on. These findings suggest that the perception of referred sensations in the phantom limb are caused by the reorganization of body part representations in a region such as SI that has small receptive fields, which occurs when adjacent representations “invade” the territory once subserved by the amputated limb.

Contrary to these findings are those from more recent studies that have rarely evoked referred sensations to the phantom limb (Hunter et al., 2005;Knecht et al., 1996; Grusser et al., 2001; Grusser et al., 2004). For example, one study was only able to evoke referred sensations to the phantom limb when the experimenter applied stimuli to the stumps of half of

the patients tested (Hunter et al., 2005). Yet this study did not specifically target localized points on the skin as the investigators utilized a light-brushing stimulus (Hunter et al., 2005). However, some other more recent studies that used localized stimuli reported that referred sensations could be evoked in a non-spatially localized manner in that they could occur in any part of the phantom limb in response to stimuli applied to both sides of the face or different parts of the body (Knecht et al., 1996; Grusser et al., 2001; Grusser et al., 2004). These studies found no evidence that the somatotopic representation of the referred sensations to the phantom hand would be maintained in the cortex, which indicates that cortical regions with bilateral or large receptive fields may be involved in their generation. Also of note is that the use of different types of somatosensory stimuli (heat, pin-prick, vibration) evoked entirely different sensations in the phantom limb (Grusser et al., 2001; Grusser et al., 2004). The differences between the older and more recent studies could be due to factors such as age, the length of time since the amputation (Grusser et al., 2001), or the inclusion of patients with pre-existing somatosensory dysfunctions prior to the amputation, such as chronic pain or cancer (Halligan et al., 1993; Grusser et al., 2004).

Most of the existing literature has been performed with upper-limb amputees and only one report has been published on data from three lower-limb amputees (Aglioti et al., 1994). In the Aglioti et al., (2004) study with lower-limb amputees, tactile stimuli applied to the remaining lower leg portion above the stump caused referred sensations to be evoked in the phantom limb in all patients. However, only one patient demonstrated a highly topographic organization of referred sensations to the phantom limb. This patient exhibited a preserved somatotopic map of the phantom foot on the residual leg, so that by touching a single point on the skin, a highly localized referred sensation would be evoked in the first phantom toe and moving the stimulus over would also generate a localized sensation in the second phantom toe and so on. However, a potential confound of this study is that they included in their sample patients with and without progressive

arteriopathy that may have caused reduced sensation in their limbs prior to the amputation. Additionally, an important measure not taken in these patients was an assessment of the amount of phantom limb telescoping, which is the perception that the phantom is retracting into the stump. This sensation may be a result of the expansion of the receptive fields of the adjacent representations into the region of SI once subserved by the phantom limb, so that the remote portion of the limb (fingers or toes) is perceived as shrunken into the stump. As this neurophysiological reorganization is believed to underlie the generation of referred sensations, we hypothesized that there would be an association between telescoping and referred sensations. This line of reasoning was based on results from a neuroimaging experiment with upper-limb amputees that found separate significant correlations between telescoping and cortical reorganization, and between cortical reorganization and referred sensations (Grusser et al., 2001). In the Aglioti et al., (2004) study, no information was given on prosthesis usage; an important consideration given that in upper-limb amputees the use of a prosthetic limb can reduce cortical reorganization and is inversely related to limb telescoping (Lotze et al., 1999).

In the current study, we explored referred sensations to the phantom limb in upper and lower phantom-limb pain patients to understand their pattern of evocation as we intended in the future to study the somatotopic organization of body part representations using functional neuroimaging. We were specifically interested in studying patients who had recent upper or lower amputations, with no history of premorbid conditions (e.g. persistent chronic pain prior to the amputation or sensory neuropathy). We wanted to determine in this group of recent traumatic amputees, if they would exhibit the same pattern of referred sensations that had previously been reported in patients with longer amputation durations – or were a result of peripheral sensory neuropathy (i.e. damage to the peripheral nerves). Secondly, as it remains unclear in the literature whether referred sensations differ based on the type of somatosensory stimuli used for their evocation, we applied one

to three different types of tactile stimuli to the face and stump in upper-limb amputees, and to the stump and leg in lower-limb amputees. Thirdly, we wanted to evaluate whether referred sensations would be localized to specific points on the phantom limb and if they would follow a topographic one-to-one organization evoked by stimuli applied to body parts adjacent to the amputated limb on the somatotopic map in SI. Lastly, we wanted to explore the relationship between phantom-limb telescoping and several variables such as referred sensations, use of a prosthesis, and the duration of the amputation.

5.3 Methods

5.3.1 Patients

Patients were mainly recruited from the Institut de réadaptation de Gringras-Lindsay de Montréal (IRM), or through advertisements, but some patients contacted the researchers directly as the study was publicized in the media. The research was approved by the Research Ethics Board of the Institut universitaire de gériatrie de Montréal (IUGM), centre de recherche interdisciplinaire en réadaptation (CRIR) du Montréal métropolitain, and the IRM. All patients gave written informed consent before participating in the study and were compensated for their time.

Patients were included in the study if they had either an upper or lower limb amputation and experienced phantom-limb pain. Patients with multiple amputations, pre-existing somatosensory dysfunction, or co-existing medical conditions (eg. diabetes, cancer, neurological disease, sensory neuropathies) that might also induce changes in somatosensory processing were excluded from the study.

The same investigator (EGD) interviewed all patients. Patients were prescreened by telephone to determine their medical history. They were also verbally asked to rate their level of pain in the phantom limb and the stump on a scale of 0 to 10. A value of 0 corresponded to no pain and a value of 10 indicated extremely intense pain. A total of 7 relatively recent traumatic amputees were recruited for the study (Table 1). One patient, P2 did not undergo referred sensation mapping due to time constraints. This patient was suffering from extreme emotional distress and was unable to complete the interview; only data for his pain ratings and telescoping of the phantom limb were recorded. Four of the patients had an amputation of the upper limb and three of the lower limb. Two of the patients were female and both had upper limb amputations. The five remaining patients were males, of whom 2 had upper limb amputations and 3 had lower limb amputations. The average age of the participants was 43.2 years (SEM=6.17; range 21-

58 years). The average length of time since the amputation was 36.6 months (SEM = 15.2 months). Five of the seven patients used a prosthetic limb. All of the lower-limb amputees (P2, P3, P7) and two of the upper limb (P5, P6) amputees used a prosthetic limb, while the two other upper-limb amputees (P1, P4) did not.

5.3.2 Endogenous pain ratings

At the beginning of the testing session, patients were asked to describe and rate their pain at the time of testing, specifically the quality and sensation of the pain that they experienced in the phantom limb and stump. Patients then rated their pain (intensity and unpleasantness) in the phantom limb and stump using a visual analog scale (VAS) scale (0-10). A rating of zero corresponded to no pain/not unpleasant; increasing to 10, which was defined as extremely intense pain/extremely unpleasant.

A repeated measures Analysis of Variance (ANOVA) was applied to the patients' ratings of pain intensity and unpleasantness in the phantom limb and stump. This was done to assess the main effects of pain ratings (unpleasantness and intensity), pain location (phantom and stump), and possible interactions between these two factors. The level of statistical significance was set at $p < 0.05$.

5.3.3 Quantitative sensory testing

All patients but one were tested at the IUGM. The exception was P4 who was tested at home. Patient testing involved mapping of referred sensations and measurements of the degree of telescoping of the amputated limb. Details of these procedures are listed below.

5.3.3.1 Referred sensations

The referred sensations to the phantom limb were assessed in a clinical testing session that was conducted prior to a future neuroimaging experiment. The purpose of the planned neuroimaging study was to map the somatotopic organization of body part representations in SI in upper and

lower-limb amputees. The body part representations to be mapped included those that would be either adjacent to the stump or more remote sites from the amputation (i.e. upper leg, face) – referred to in this study as “reference zones”. Therefore, we only tested these sites of interest during the clinical testing session and chose not to apply stimuli all over the body.

The stimuli consisted of cotton buds, non-painful electric shocks, or vibration (see below for details on these types of stimuli). Two of the seven patients (P6 and P7) received all three types of stimuli in the order described above. Three patients (P1, P3, P5) received only the cotton buds and electrical shocks (in that order). Patient 4 only received only the cotton buds and P2 was not presented any stimuli. At the beginning of the testing session, patients were familiarized with the stimuli and the stimulation procedure. For each stimulus, patients were asked to describe where they perceived the stimulus on their body (including the phantom limb) and what type of sensation it evoked. Following a similar method to previous publications that explored referred sensations in amputees (Knecht et al., 1998; Grusser et al., 2001; Hunter et al., 2005), the patients were not specifically questioned as to whether they had previously experienced referred percepts to the phantom limb. This was done to maintain neutrality across the sessions with different patients and also to avoid possible response biases during the assessment for referred sensations. Therefore, after the procedure was described the interview was left open for the patient to make any remarks concerning the testing or if the patients had ever previously experienced a referred sensation to the phantom limb.

For upper-limb amputees, sites around the stump (proximal to the amputation), residual arm (adjacent to the amputation), and the face (remote to the amputation) were tested for referred sensations using at least one of the different stimuli. Regions were tested at least twice to ensure accuracy of responses. All sites were assessed by systematically applying the stimuli manually across the skin in a grid-like pattern. For the face, stimuli were applied across the area of skin that extended from the forehead

to the mandible (approximately 30 sites). For the stump, the stimuli were applied to the skin on and around the site of the amputation (approximately 20 sites).

For lower-limb amputees, the stimuli were applied to dorsal and ventral sites around the stump (proximal to the amputation) and then progressively upwards to approximately 12 cm above the knee joint (approximately 60 sites). Patients were subsequently asked if they had ever experienced, outside the laboratory, sensations referred to the phantom elicited by stimulation of body parts that were not stimulated during the testing session (for example the viscera), which would be impossible to examine through the application of topical stimuli.

The cotton bud stimulus consisted of two pieces of cotton wrapped around either end of a small rod. The tip of the cotton bud was approximately 5 mm in diameter. The tip of the cotton bud was lightly applied to each site on the skin at distances approximately 0.5-1 cm apart for 1 second and then removed. In upper-limb amputees, the stimuli were manually applied in a grid-like manner across the entire face and stump at sites apart. In lower-limb amputees the stimuli were applied to the stump and area around the knee joint.

The electrical stimuli were bipolar square wave pulses generated by Grass stimulators (S88x, S48). The stimuli were delivered using a flat rectangular bipolar stimulating electrode that was manually placed on the skin. The interelectrode distance was approximately 1 cm. The site of stimulation was cleaned using alcohol swabs before and after testing. A small amount of saline electrode gel (Signa Gel; Parker Laboratories, Orange, NJ) was applied to the electrode tips before they were placed on the skin. During the repeated application of the electrodes it was sometimes necessary to reapply the electrode gel. Stimuli were 10ms in duration and were administered by the experimenter by manually triggering the stimulator. The voltage was individually adjusted to be perceptible but non-painful. The stimuli were increased by 0.5V increments until the patients

reported that they could readily perceive the stimuli. Single-pulse stimuli were manually applied to a site and then removed at a rate of ~1-3 Hz. The stimuli were applied approximately 1cm apart to the sites on face, arm, or leg.

Vibrotactile stimuli (125Hz, 1s duration) were generated using software-constructed .wav files that were played through an amplifier connected to the sound card of a laptop computer. The stimuli were delivered through a small piece of balsa wood (8mm by 16mm) situated on the end of the custom-built piezoelectric stimulators that were 2cm in width. The stimulators were moved from left-to-right across the skin with the sites being approximately 1cm apart.

5.3.4 Telescoping

Telescoping is the sensation that the phantom limb is progressively retracting inwards towards the residual stump. Telescoping was assessed in all participants using the methods of Montoya et al. (1997). The lengths of the intact and amputated limb were first determined. A retractable tape measure was then placed on the end of the amputated limb and extended until the patient perceived that the tips of the fingers/toes of the phantom had been reached. The distance from the phantom fingertips/toes to the residual limb was added to the length of the residual limb (stump + phantom), and this value was then subtracted from the length of the intact body part (Intact limb – (stump + phantom)). The percentage of telescoping was determined using the following calculation (Intact limb – (stump + phantom))/ Intact) x 100.

The association between telescoping and referred sensations was assessed using a Fisher's exact test following a similar method to that of Hunter et al. (2005).

To determine whether telescoping was related to prosthesis usage, a Fisher's exact test was performed on these dichotomous variables. This procedure was utilized to test the null hypothesis that there would be no

difference between the number of patients who did or did not experience telescoping and the number of patients who did or did not use a prosthetic limb.

Lastly, the relationship between the amount of telescoping and amputation duration was explored by performing a Pearson's correlation test. For all tests the level of statistical significance was set at $p < 0.05$.

5.4 Results

5.4.1 Endogenous pain ratings

Patients were asked to describe the overall daily sensations of pain they experienced in the phantom limb and stump. Patients' qualitative descriptions of the pain in their phantom were for the most part unique to each individual (Table 2). Some patients reported primarily somatosensory qualities, including grating, burning, and throbbing pain, while others reported descriptors associated with muscle pain, particularly frozen or cramped muscles. In one of the upper-limb amputees (P1) and all the lower-limb amputees (patients 2, 3, and 7) the painful sensations were reported in the remote extremities such as the fingers and toes.

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A repeated measures ANOVA revealed a significant main effect for the pain ratings obtained for the phantom limb and the stump ($F=6.28$, $p=0.004$; Figure 1). Pair-wise comparisons revealed that ratings of unpleasantness associated with the phantom limb were significantly greater than ratings of pain intensity in the phantom ($p = 0.01$), pain intensity in the stump ($p=0.001$), and pain unpleasantness in the stump ($p=0.027$). No significant differences were seen between ratings of pain intensity for the phantom limb and the stump, nor were there any differences in ratings of pain intensity and unpleasantness associated with the stump. The findings of significance were similar, even after removing subject P2, who

was not tested for referred sensations. In the subsequent analyses on referred sensations, this patient was removed from the calculations.

5.4.2 Referred sensations

Referred sensations were assessed in all patients (except P2) using one or more types of innocuous stimuli in the following order: cotton bud, electric shocks, and vibration. All patients were tested for referred sensations using the cotton bud stimulus. All patients, except P4, were tested using the electrical stimuli; however, for the vibration stimuli, only P6 and P7 were tested.

Patient 1 was the only participant to have the contralateral intact hand tested for referred sensations, as she remarked at the beginning of the testing session that she had perceived referred sensations to her phantom hand when stimuli were applied to that part of her body. Since we were not interested in examining the representation of the contralateral intact hand in other patients in the future neuroimaging experiment, we only examined the referred sensations in the contralateral intact hand in this one patient. For P1, the stimuli were applied across the dorsal surface of the hand starting at the wrist and going towards the fingers. This process was repeated on the palm of the hand and fingers. Electrical stimuli were applied to all patients except P4, and were only applied to the residual stump, chest, and shoulder blade in P5. Patient 5 did not have electrical stimuli applied to his face due to time constraints. This patient had an extensive upper arm amputation and wore a myoelectric prosthesis that was controlled by the muscles in his chest and also covered his shoulder blade, and these regions also were tested for referred sensations. Starting around the site of the amputation, the stimuli were applied across the skin progressively covering the skin over the pectoral muscle. Subsequently, the stimuli were then applied to cover the entire shoulder blade.

Vibrotactile stimuli were only applied to P6 and P7. The details concerning the administration of stimuli and the referred sensations' qualia are given in Tables 3 (upper-limb amputees) and 4 (lower-limb amputees).

Perception of Referred Sensations

In line with previous reports, all patients tested in this study exhibited non-painful and painful referred sensations in the phantom limb in response to at least one of the forms of innocuous cutaneous tactile stimuli (Ramachandran et al., 1992; Halligan et al., 1993). At the beginning of the testing session, most of the patients reported they had never previoiusly experienced the sensation that the phantom was being touched by an external stimulus, but they then later experienced referred sensations during the testing session.

Referred Sensations Differ Based on the Type of Stimulus Applied to the Skin

In agreement with some previous studies (Grusser et al., 2001; 2004), different types of cutaneous tactile stimuli applied to the same locations on the "reference zones" on the skin produced different sensations referred to the phantom limb in all patients who received more than one type of stimulus (P1, P3, P5, P6, P7). For example, P6 perceived no sensations in the phantom in response to light cotton bud stimuli applied to the face. However, when applying electrical stimuli to the same locations on the face, the patient felt a non-specific sensation in the phantom elbow and fingers. On applying the vibrotactile stimulators to the face, the patient experienced intense contraction of the phantom forearm, and applying the vibration stimuli to the stump produced the sensation of clenching the phantom hand and a parallel sensation of vibration in the phantom forearm.

Referred Sensations are Perceived as Different in Quality in the Reference Zone and in the Phantom Limb

In most instances the sensations felt in the phantom limb were entirely unlike the type of stimulus applied to the reference zones on the face or stump. However, this varied across the different stimulus types. For example, when the experimenter applied the cotton bud stimulus to the reference zone on the intact hand of P1, she experienced the cotton bud to be touching her phantom limb. However, when electrical stimuli were applied to the same location on the reference zone in the intact hand, a very different sensation of pain was evoked in the phantom limb (Figure 2). In this same patient, when the cotton bud stimulus was applied to the reference zone on her face, she experienced the sensation that her phantom hand was contracting. But when electrical stimuli were applied to the same location on the reference zone on the face, she experienced mild pain in the phantom. Most patients were more likely to report a referred sensation in the phantom limb that was different from the type of stimulus applied to the skin.

Localization of Referred Sensations

Consistent with the results of Ramachandran et al., (1992) and Halligan et al., (1993) 4 out of 6 patients reported referred sensations that were localized to one specific point on the phantom, at least, with some types of stimuli. However, when using another type of stimulus, an equal number of the patients reported non-localized referred sensations in the phantom limb (see Tables 3 and 4 for specific details). For example, a cotton bud applied to the thigh of P7 produced a non-localized radiating sensation in the phantom leg, but applying electrical stimuli to the same site produced a sensation of an electrical shock in the dorsum of the phantom foot.

Somatotopic Organization of Referred Sensations

In contrast to previous published reports (Ramachandran et al., 1992; Halligan et al., 1993; Aglioti et al., 1994), no patient exhibited referred

sensations in the phantom limb that were arranged in a one-to-one somatotopic organization in response to tactile stimulation of the skin. For example, Ramachandran et al., (1992) reported that when a tactile stimulus was applied to the residual stump of an upper-limb amputee it produced a localized sensation in the phantom index finger. Moving the stimulus over would produce a sensation in the phantom middle finger and so on until the entire phantom hand could be mapped in a precise topographic organization. The same pattern of referred sensations to the phantom was evoked in response to stimuli applied to the cheek ipsilateral to the amputation. This finding of Ramachandran et al., (1982) seems to be consistent with an fMRI study, which found a correlation that suggested a relationship between referred sensations and reorganization of the of adjacent (e.g. the stump) and remote (e.g. the face) body part representations into the cortical territory once subserved by the amputated limb in SI (Grusser et al., 2001). In the current study, three out of the 4 upper-limb amputees in our study were more likely to report referred sensations when the stimuli were applied to the remote sites on the face compared to stimuli applied to the adjacent site around the stump (Table 3). Moreover, these sites were located on the lower portion of the face in the territory of the maxillary (V2) and mandibular (V3) nerves (Siessere et al., 2009). This is contrary to the classical reports of the somatotopic organization of the face where the forehead representation (ophthalmic branch of the trigeminal nerve) is located adjacent to the digit representation (Penfield and Boldrey, 1937; Sato et al., 2002; Sato et al., 2005; Schwartz et al., 2004). Also of note is that P1 reported referred sensations in response to stimuli applied to both sides of her face. In lower-limb amputees, referred sensations were evoked after applying stimuli to both the adjacent and remote sites on the leg (Table 4). In no patients were the referred sensations evoked in a detailed one-to-one somatotopic organization. For example, P3 reported referred sensations in the same location in the

phantom limb after stimuli were applied to different locations on the adjacent and remote sites.

5.4.3 Telescoping

All of the upper-limb amputees experienced some telescoping of the phantom limb into the stump (Mean = 38.33%; SEM = 14.21%; Table 5). However, only one of the three lower-limb amputees (P7) experienced telescoping of the phantom limb retracting into the amputated limb (Mean = 4.43%; SEM = 4.43%). In these lower-limb amputee patients, while they did not perceive telescoping of the phantom limb, they both experienced an altered perception of their amputed legs. Patient 2 reported that his phantom foot was in the same location as his former foot, but he was only able to perceive the outer half of the muscles in his leg (the peroneus longus and peroneus brevis muscles). He reported having the sensation of being able to consciously contract these muscles. Patient 3 only felt sensation in his phantom foot, but had no conscious feeling of his former leg.

We sought to address whether phantom-limb telescoping was associated with prosthesis usage. Three of the five patients who experienced telescoping of the phantom limb wore a prosthetic limb. The two remaining patients in this study did not experience telescoping, but both wore a prosthesis. Two patients did not experience telescoping and did wear a prosthesis. A Fisher's exact test revealed that there was no association between phantom limb telescoping and prosthesis usage ($p=1.0$)

We also assessed whether telescoping of the phantom limb might be associated with the generation of referred sensations in this group of patients. The majority of the patients (5 out of 6) who experienced telescoping of the phantom limb also reported feeling at least one referred sensation. However, the number of patients who reported telescoping was found to be statistically unrelated to referred sensations in the phantom limb ($p=1.0$).

Lastly, we wanted to address whether phantom-limb telescoping was related to the duration of the amputation. We found no significant correlation between these two variables ($r = 0.59$, $p = 0.17$).

5.5 Discussion

In a group of phantom-limb pain patients, we assessed referred sensations to the phantom by applying tactile stimuli to reference zones on the skin. Our results showed that all patients tested on the protocol reported referred sensations in the phantom in response to at least one type of somatosensory stimuli. In some instances, patients reported that they felt a sensation in the phantom limb that was very similar to what they had felt at the site of stimulation when the stimuli were applied to the reference zones on the skin. However, the more common finding across patients was that very dissimilar patterns and sensations were evoked in the phantom limbs when different types of stimuli were applied to the skin. Referred sensations were often reported by patients to be unlike the type of stimulus applied to the skin, and in some instances the perception of muscle contractions was evoked in the phantom limb. Moreover, it was found that, contrary to previous results, the referred sensations reported by this group of patients did not follow an organized pattern. We also found that phantom-limb telescoping was not associated with referred sensations, prosthesis usage or amputation duration.

5.5.1 Endogenous pain characteristics

Intense phantom pain in the densely innervated remote extremities such as the fingers was reported in 4 patients in this study. This finding is in agreement with a previous report with phantom-limb pain patients (Jensen et al., 1985). This result is important in understanding the mechanisms of phantom-limb pain. It has been proposed that the boundaries of the body-part representations, which define the spatial and functional characteristics of the SI somatotopic map, are maintained by lateral inhibition, in which the region of cortex receiving constant input from larger remote extremities such as the fingers and toes may mask the contributions from adjacent cortical regions (Tremere et al., 2001b). Deafferentation could lead to a decrease in inhibition causing disruption in receptive field properties. For example,

deafferentation in animals leads to a decrease in the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (Garraghty et al., 1991). The loss of inhibitory input might lead to enlargement of the receptive fields of previously silenced body part representations (Salimi et al., 1994; Barbay et al., 1999; Tremere et al., 2001b, a). It seems a reasonable assumption that the cortical territories subsubserving the remote extremities would experience a greater loss of inhibition, which might lead to an increased perception of pain associated with the deafferented limb.

The findings of greater pain unpleasantness ratings in the phantom limb, in comparison to all other ratings of pain perception, highlight the importance of the role of affective pain processing in the maintenance of chronic pain states. This has been demonstrated in animals where the medial prefrontal cortex, a region involved in negative emotional processing, has been implicated in the storing of long-term memory of information associated with aversive stimuli (Millecamps et al., 2007). To date, no brain imaging study has explored the effects of negative emotional associations with a spatially specific mental representation of a body part on the maintenance of phantom-limb pain, and future work in this area is needed.

5.5.2 Referred sensations

Our results demonstrate that referred sensations can be commonly evoked by stimuli applied to the cutaneous reference zones. Most of the patients reported that sensations referred to their phantom limbs were non-painful, although two of the patients occasionally experienced painful sensations that were referred to their phantom limbs.

The painful sensations referred to the phantom limbs were evoked by innocuous electrical and vibratory stimuli, but never by the cotton bud stimuli. One patient (P1) reported mild diffuse electrical pain in her phantom hand and the other patient (P7) reported a sharp stabbing pain in his phantom foot. These results are consistent with those from a previous group who also reported painful sensations referred to a phantom limb evoked by

innocuous tactile stimuli applied to the residual stump of an upper-limb amputee (Grusser et al., 2001).

Painful percepts referred to the phantom limbs also may have been caused by similar mechanisms that underlie “referred pain”, where pain can be perceived in an area away from the primary site of injury (e.g. facial pain caused by a myocardial infarction) (Kreiner and Okeson, 1999; de Oliveira Franco et al., 2005; Myers, 2008). It has been proposed that the sensation of referred pain may be due to nociceptive afferents from different tissues converging on the same neurons in the dorsal horn of the spinal cord (Sessle et al., 1986; Hoheisel and Mense, 1990). Higher brain regions may be unable to discern between the different tissue types causing the location of the pain to be misinterpreted (Ruch, 1961). In relation to the current findings, possibly the myelinated afferents that innervated the skin above and below the amputation converged on the same dorsal horn neurons. Subsequent to the nerve injury, these myelinated afferents from the skin may have innervated the superficial dorsal horn laminae, which normally receive their input solely from nociceptive afferents (Woolf et al., 1992). These morphological changes have been demonstrated in animal models of nerve injury and have been attributed to underlie signs of mechanical allodynia (Woolf et al., 1992; Mannion et al., 1996; Nakamura and Myers, 1999).

In the current report, in no patient were the referred sensations organized in a strict one-to-one topographic map, as had been reported by previous authors (Ramachandran et al., 1992; Halligan et al., 1993). Nor, in most individuals were the referred sensations localized to a specific spatial region in the phantom limb. Furthermore, the referred sensations were evoked in regions that were not adjacent to the amputated limb on the somatotopic map in SI. These findings would argue against the notion that referred sensations are always caused by changes in the somatotopic organization of SI. This is inferred based on the knowledge that area 3b has finely tuned receptive fields and contains a detailed organization of body

part representations (Nelson et al., 1980). Potentially, cortical regions outside SI that have large receptive fields may undergo reorganizational changes in response to deafferentation, and this may underlie the genesis of referred percept. Likely candidates outside SI would be higher order cortical regions involved in bodily awareness and perception, such as the posterior parietal cortex (Berlucchi and Aglioti, 2009), the secondary somatosensory cortex (SII) (Pia et al., 2004), and the insula (Karnath et al., 2005). This assumption is in line with the results of an fMRI study with two phantom-limb pain patients who experienced referrred sensations but in whom no reorganizational changes of body part representations in SI were observed (Grusser et al., 2004). While the authors did not report the results from other regions of the brain, they concluded that referred sensations were likely generated through changes occurring in the thalamus, SII or the posterior parietal cortex.

Previous authors have also reported that referred sensations do not follow a somatotopic organization in patients with spinal cord injury (Moore et al., 2000). The authors interpreted their findings as being organizational changes occurring in subcortical structures, including nucleus cuneatus, because it is unlikely that these changes would occur in SI, as cortical sprouting would have to span distances greater than 2cm (Florence et al., 1982; Jones and Powell, 1969; Manger et al., 1997). The authors note that previous research with squirrel monkeys had shown that the chest and arm representations are quite close to one another in nucleus cuneatus and therefore the sprouting may occur in this brain stem nucleus (Xu and Wall, 1999).

In the current study it was interesting that P1 reported sensations in her phantom hand when stimuli were applied to both sides of her face and also to her intact hand. These results may reflect that referred sensations are associated with reorganization of brain regions with bilateral receptive fields such as SII (Burton et al., 1998) or the occurrence of transcallosal connections in area 2 of SI (Killackey et al., 1983). However, bilateral

receptive fields have been described in SI (Iwamura et al., 2002; Hansson et al., 1999). Similar reports of referred sensations occurring in response to stimuli applied to the contralateral side of the body are rare but do exist in the literature (Sathian, 2000; Grusser et al., 2004). Referred sensations generated from applying stimuli to the intact side of the body may be due to mirrored plasticity in the contralateral hemisphere or unmasking of inputs from inter-hemispheric transcallosal connections as has been proposed by previous authors (Calford and Tweedale, 1990; Schroeder et al., 1995).

Unique to this study was the finding that in all four of the upper-limb amputees, cotton swab, electrical and vibratory stimuli applied to various regions, including the face, chest and shoulder blade prompted the sensation of muscle contraction in the phantom fingers or forearms. Only one other instance of a referred sensation of a muscle contraction in a phantom limb in response to touching a reference zone on the skin has been reported (Grusser et al., 2004). Our findings would potentially indicate that cortical motor regions are involved in the generation of referred sensations. However, muscle afferents also project to area 3a and b in SI (Heath et al., 1976) and therefore the muscle contraction responses may be a result of the receptive fields of these afferents overlapping with purely cutaneous afferents. Additionally, as muscle contraction responses can sometimes be painful, the referred muscle contractions may be related to damage to muscle afferents that project to nociceptive dorsal horn neurons (M⌀rch et al., 2007).

A limitation of the present study is that the tactile stimuli were not applied to the patients in a randomized order. The patients were always tested initially with the cotton bud stimuli, followed by electrical stimuli and then vibratory stimuli. Potentially, the repeated application of the tactile stimuli to the reference zones on the skin may have reduced the threshold for evoking referred sensations. However, the referred-sensation testing procedure took place over 1-2 hours, and the patients were given breaks of 5-10 minutes before the application of a new stimulus. This should have

reduced sensitization of the receptors associated with cutaneous afferents innervating the reference zones on the skin.

It is also important to note that attention to the reference zones on the skin may have potentially altered the threshold for the generation of referred sensations. This is said in light of the fact that top down mechanisms, such as attention, can modulate tactile receptive field sizes (Haggard et al., 2007). An additional top-down influence is the possibility that the instructions for the referred-sensation procedure may have had a powerful suggestive effect prompting the patients to comply with the testing instructions. This is said in light of the fact that the majority of the patients had not experienced referred sensations before the testing session. This highlights the importance of providing non-biased instructions to the patients prior to the testing session.

5.5.3 Telescoping

Telescoping of the phantom limb into the stump is a commonly reported phenomenon among amputees, occurring in 49-63% of cases within several weeks post-amputation (Carlen et al., 1978). Consistent with these previous reports, telescoping of the phantom limb was found in 5 out of 7 patients examined in the current study. Telescoping can increase over time (Katz, 1992); however in the current report no significant correlation was seen between telescoping and amputation duration. This may likely be due to the low number of patients included in the study and their small range of amputation duration.

It has been theorized that limb telescoping may occur as the lower arms or legs have a smaller and therefore weaker cortical representation than the feet or hands (Ramachandran and Hirstein, 1998). The remaining representation of the stump, and larger cortical representation of the face may invade that territory causing the sensation that the arm or leg is shrinking. Or alternatively, an upper-limb amputee may perceive that their

limb is shortened more than in comparison to a lower-limb amputee as the cortical representation of the hand has a large cortical territory in a number of sensorimotor brain regions due to the hand's role in fine manual control (Ramachandran and Hirstein, 1998). This is in agreement with our findings that telescoping of the phantom limb was more common in the upper-limb amputees.

Only one of the lower-limb amputees experienced mild telescoping of the limb while the other two did not perceive any shortening of their phantom legs. Previous research has shown reductions in phantom limb telescoping in patients who habitually wear prosthetic limbs (Mayer et al., 2008). In the current study, no association was found between phantom-limb telescoping and prosthesis usage. However, this lack of a statistically significant result may have been due to the few number of patients in the sample. It is still of interest to note that the telescoping was quite pronounced in two upper-limb amputees (P1 and P4) who did not use a prosthetic limb. One of the upper limb patients only experienced mild telescoping (P5) but he used a myoelectric prosthesis – an electric artificial limb where patients can learn to control its functionality through contracting muscles in the stump (Weiss et al., 1999). Use of this dynamic type of prosthesis has been associated with decreases in cortical reorganization in comparison to patients who use cosmetic prostheses (Lotze et al., 1999). Consistent with this notion is that the one upper-limb amputee patient (P6) who used a cosmetic prosthesis also experienced a shortening of his phantom limb.

5.6 Conclusions

Referred sensations to the phantom limb in patients who experience phantom-limb pain provide some insight into the massive cortical reorganization that occurs after amputation. In this small group of upper and lower-limb amputees, the results indicate that referred sensations can be evoked in a non-localized pattern in response to tactile stimuli applied to ipsilateral body parts near the site of the amputation, as well as on the contralateral side of the body. To a large extent, the referred sensations to the phantom, showed little or no topographic organization in response to tactile stimulation delivered to the reference zones. The reorganization of SI has been the primary focus of research on the genesis of referred sensations. The present research does not discount the possibility that SI may be involved in the perception of referred sensations. However, based on our findings of poor spatial localization of referred sensations, it could be inferred that other cortical regions that have a crude somatotopic organization and bilateral receptive fields may also contribute to the altered body percept and evocation of referred sensations. However, as noted before, SI does contain bilateral receptive fields associated with the distal upper extremities (Iwamura et al., 2002; Hansson et al., 1999). The relationship between referred sensations and cortical reorganization would benefit from further exploration using whole-brain, high-field neuroimaging. Functional activation maps may provide information regarding where referred sensations are represented in the brain, and provide insight into whether these regions play a role in the generation or maintenance of chronic pain in amputee patients.

5.7 Acknowledgements

The authors would like to thank the patients and their families for their time and positive dispositions, despite traveling long distances to participate in the study. Additionally, the authors are indebted to Audrey-Anne Dubé, Jen-I Chen, and Nadine Leblanc for help with patient testing. The funding for this research was from a grant provided to the authors by the Canadian Institutes for Health Research (CIHR).

Figure 1. Pain ratings (intensity and unpleasantness) for the phantom limb and stump

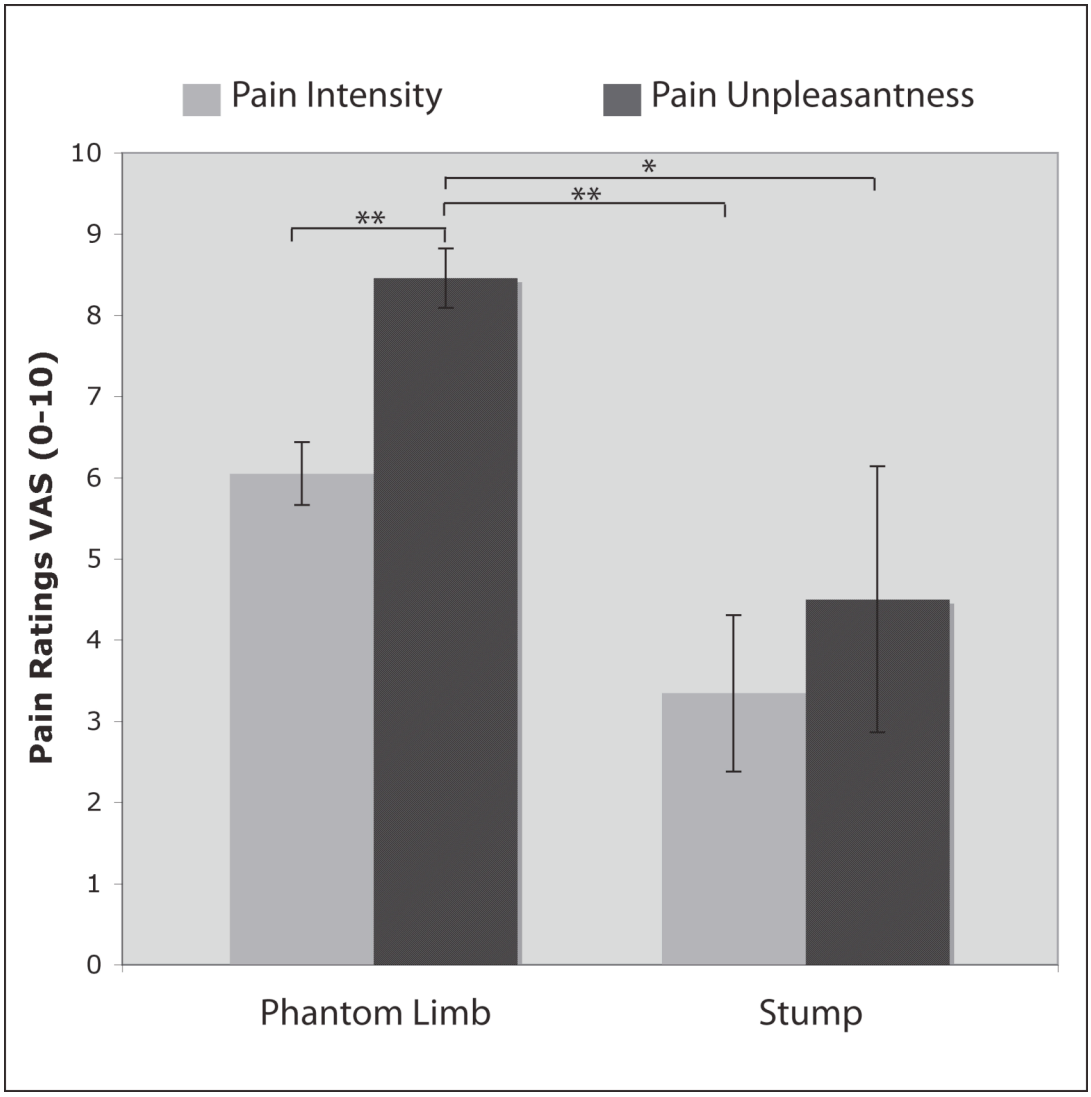

Figure 1. Average pain intensity and unpleasantness ratings in the phantom limb and stump. The ANOVA revealed a significant main effect for the pain ratings in the phantom limb and the stump ($F = 6.28$, $p = 0.004$). Pair-wise comparisons showed a significant difference between unpleasantness ratings in the phantom limb (Mean = 8.46; SEM = 0.37) in comparison to its pain intensity (Mean = 6.05; SEM = 3.03, $p = 0.01$). A significant difference was found between pain unpleasantness ratings in the phantom and stump pain intensity (Mean = 3.34; SEM = 0.96, $p=0.001$) and unpleasantness ratings (Mean = 4.5; SEM = 1.64, $p=0.027$). Abbreviation: Visual Analog Scale (VAS).

Figure 2. Referred sensations to the phantom limb in an upper-limb amputee

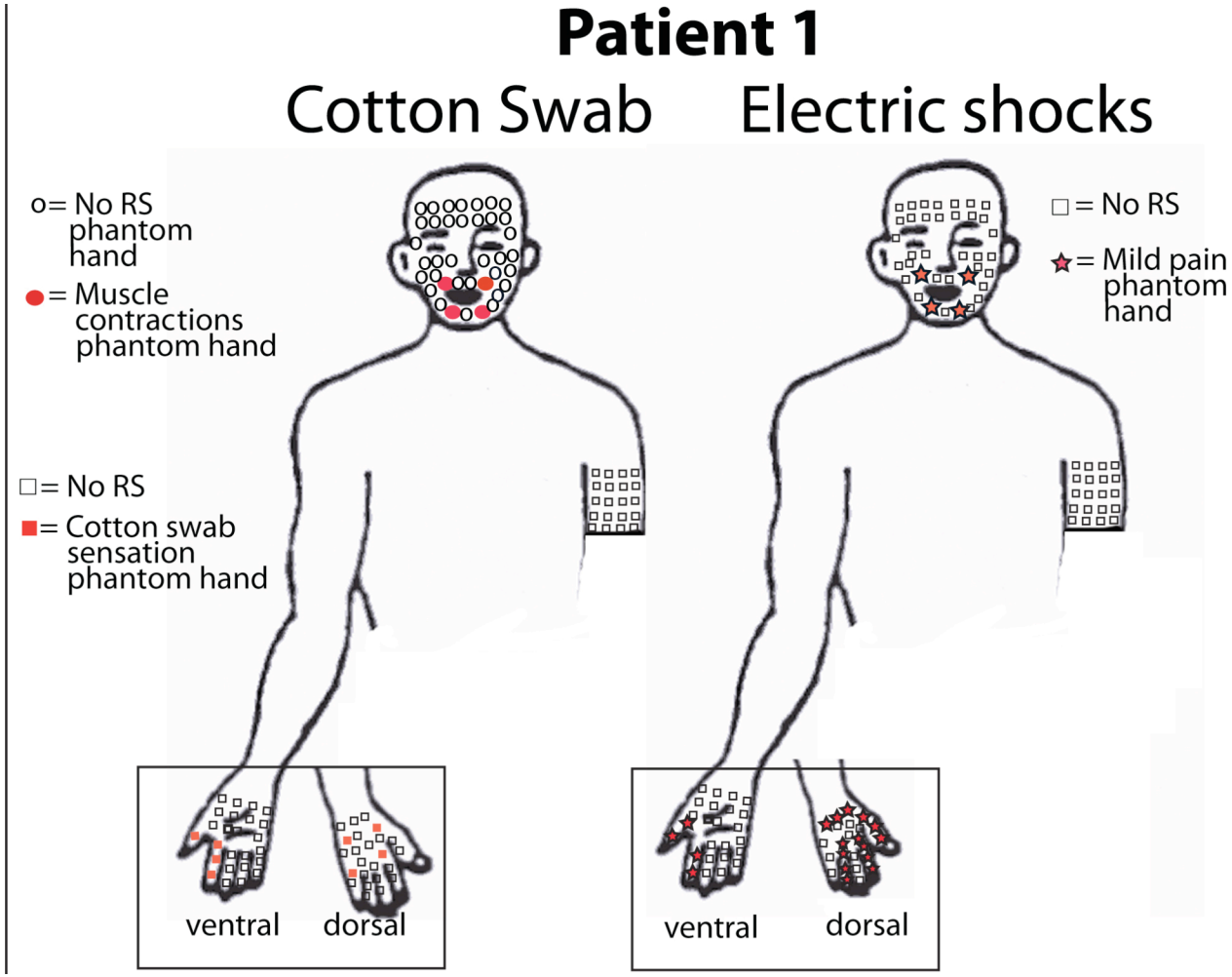

Figure 2. Referred sensations in an upper-limb amputee patient. Cotton bud stimuli applied to the patient's face produced the sensation of muscle contractions in the phantom hand. In response to the same stimulus being applied to the intact hand, the patient experienced an identical sensation of the cotton bud touching the phantom hand. In the same areas, after applying electrical stimuli to the face and the intact hand the patient reported feeling mild pain in the phantom hand. Abbreviation: RS = Referred sensations in the phantom hand.

Table 1. Patient characteristics

Patient	Sex	Handedness	Age	Duration (months)	Amputation location	Prosthesis
Upper-limb amputees						
1	f	R	22.4	12	Left upper humerus	No
4	f	R	58	118	Left upper humerus	No
5	m	R	21.12	13	Left upper humerus	Yes
6	m	L	57.8	16	Right upper humerus	Yes
Lower-limb amputees						
2	m	R	49	60	Left BKA (fibula and tibia)	Yes
3	m	R	37.6	9	Left BKA (fibula and tibia)	Yes
7	m	R	56.6	28	Left BKA (fibula and tibia)	Yes
		Average =	**43. 22**	**36.6**		
		SEM =	**6.17**	**15.2**		

Table 1. Patient characteristics. Abbreviations: m = male; f = female; R= right; L = left; BKA = below knee amputation; SEM = Standard error of the mean.

Table 2. Endogenous pain ratings

Patient	Phantom		Stump (Residual Limb)		Phantom Pain Sensations
P	**Pain Intensity**	**Pain Unpleasantness**	**Pain Intensity**	**Pain Unpleasantness**	
			Upper-limb amputees		
1	6.5	7.2	3	2	Grating skin in fingers
4	5.5	8.5	5.5	3	Arm in a vice
5	5.25	8	0	0	Frozen muscles, numbing pain
6	6.5	8	6	6.5	Frozen muscles, numbing pain
			Lower-limb amputees		
2	5.1	9.5	3.4	10	Cramping in toes
3	8	8	0	0	Metal bar across toes
7	5.5	10	5.5	10	Burning, throbbing, radiating pain in toes
Mean	**6.05**	**8.46**	**3.34**	**4.50**	
SEM =	**0.39**	**0.37**	**0.96**	**1.64**	

Table 2. Patients rated their pain on a scale from 0-10. The lowest value represents no pain with pain becoming incrementally worse till the highest rating of extremely intense pain. Likewise for pain unpleasantness, 0 represents not unpleasant at all and 10 represents an extremely unpleasant sensation. Abbreviation: SEM = standard error of the mean.

Table 3. Referred sensations in upper-limb amputees

P	Site	Cotton bud	Electric Shocks	Vibration
Upper-limb amputees				
1	**Adjacent (stump)**	**Stump:** No referred sensations	**Stump:** No referred sensations	**Stump:** Not tested
	Remote (face)	**Face**: Sensation of clenching the phantom fingers (bilateral).	**Face:** Non-localized sensation of mild pain in phantom (bilateral).	**Face:** Not tested
	Remote (contralateral hand)	**Contralateral hand:** Localized sensation of touching the phantom hand and fingers with the cotton bud.	**Contralateral hand:** Non-localized sensation of mild pain in phantom hand.	**Contralateral hand:** Not tested
4	**Adjacent (stump)**	**Stump:** No referred sensations	**Stump:** Not tested	**Stump:** Not tested
	Remote (face)	**Lower lip:** Sensation of clenching the phantom fingers (ipsilateral to amputation).	**Lower lip:** Not tested	**Lower lip:** Not tested
5	**Adjacent (stump)**	**Chest:** Sensation of scratching in specific locations on the phantom hand. **Shoulder blade:** Sensation of numbness in the phantom triceps.	**Chest:** Sensation of movement in phantom fingers. **Shoulder blade:** Sensation of movement in phantom fingers.	**Chest:** Not tested **Shoulder blade:** Not tested
	Remote (face)	**Face:** Sensation of scratching specific locations on the phantom shoulder and hand (ipsilateral to amputation).	**Face:** Not tested	**Face**: Not tested
6	**Adjacent (stump)**	**Stump:** No referred sensations	**Stump:** No referred sensations	**Stump:** Sensation of clenching the phantom fingers; Sensation of vibration in a specific location on the phantom forearm.
	Remote (face)	**Face:** No referred sensations	**Face:** Non-specific sensation in phantom fingers and elbow (ipsilateral to amputation).	**Face:** Sensation of contraction of the phantom forearm muscles (ipsilateral to amputation).

Table 4. Referred sensations in lower-limb amputees

P	Site	Cotton bud	Electric Shocks	Vibration
Lower-limb amputees				
3	**Adjacent (stump)** **Remote (thigh)**	**Upper shin:** Sensation of the cotton bud applied to a non-specific location on the dorsum of the phantom foot. **Thigh:** Sensation of the cotton bud applied to a non-specific location on the dorsum of the phantom foot.	**Upper shin:** Sensation of electric shocks traveling down the phantom leg to the phantom foot. **Thigh**: Sensation of electric shocks traveling down the phantom leg to the phantom foot.	**Upper shin:** Not tested **Thigh:** Not tested
7	**Adjacent (stump)**	**Knee/shin:** Sensation of contracting the hallux and second phantom toe. **Knee (back):** Sensation of cotton bud applied to a localized location in the phantom shin, tickling bottom of foot.	**Knee/shin:** Stabbing sensation shin, numbness toes. **Knee (back):** Pin-prick sensation in heel, electric shock in heel, and fifth toe.	**Knee (front):** Sensation of pain in the phantom shin; Sensation of vibration in the plantar of the phantom hallux, ball of phantom foot, and heel. **Knee (back)**: Pin-prick sensation in the phantom heel, numbness in plantar aspect of the second through fifth phantom toes.
	Remote (thigh)	**Thigh:** Sensation of radiating heat in a non-specific area of the phantom.	**Thigh:** Electric shock sensation in dorsal surface of phantom foot.	**Thigh:** Numbness in the fourth and fifth toes.

Table 5. Phantom limb telescoping

				Telescoping (cm)		
P	**Duration (months)**	**Amputation location**	**Prosthesis**	**Intact Limb (cm)**	**Phantom +Stump (cm)**	**Percentage**
Upper-limb amputees						
1	12	Left upper humerus	no	74	55	25.7%
4	109	Left upper humerus	no	70	20	71.4%
5	22	Left upper humerus	yes	48	45	6.2%
6	16	Right upper humerus	yes	70	35	50%
					Mean =	**38.33%**
					SEM =	**14.21%**
Lower-limb amputees						
2	60	Left BKA	yes	73	73	0
3	9	Left BKA	yes	76	76	0
7	28	Left BKA	yes	53	46	13.3%
					Mean =	**4.43%**
					SEM =	**4.43%**

Table 5. Phantom limb telescoping into the stump. The amount of telescoping was calculated by first measuring the length of the intact limb (Intact Limb) in centimetres (cm). The researcher then placed a retractable tape measure on the end of the amputated limb and extended it until the patient decided that the tips of the fingers/toes of the phantom had been reached. The distance from the phantom fingertips/toes to the residual limb was added to the length of the residual stump (Phantom + Stump), and this value was then subtracted from the length of the intact body part to yield the magnitude of telescoping (Intact-Phantom). These values are given in percentages. Abbreviation: BKA = below the knee.

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6 Chapter 6: General Discussion and Final Conclusions

6.1 General Discussion

The studies included in this thesis used a variety of techniques including meta-analysis of existing brain imaging data, functional magnetic resonance imaging (fMRI), and the clinical examination of chronic pain patients to study the roles of the somatosensory cortices in the processing of pain, warmth, and touch.

The meta-analytic review presented in Chapter 3 examined brain regions responsible for processing activation in the brain in response to noxious stimuli in four separate experiments. The main objective of the first experiment in the meta-analysis was to use existing brain imaging data from the pain neuroimaging literature to create likelihood maps that provide detailed, localized information on brain regions responsible for processing nociceptive input. A total of 130 original brain-imaging studies that used noxious stimuli were included in the meta-analysis. We hypothesized that significant likelihood values would occur in the primary and secondary somatosensory cortices (SI and SII), the anterior cingulate cortex (ACC), the insula, the prefrontal cortex (PFC), thalamus, and basal ganglia in response to noxious stimuli. This hypothesis was supported by findings that the activation likelihood was significant all the aforementioned regions. Additionally, a significant likelihood of evoking noxious-stimulus related activation occurred in brain regions outside somatosensory areas, including several motor regions.

The second meta-analysis in Chapter 3 examined the neural representation of noxious cold to determine whether some brain areas would show a preference for its processing in comparison to noxious heat. It was hypothesized that noxious cold and heat would have a similar likelihood of producing activation in common brain regions that process nociception such as SI, SII, the ACC, the insula, the PFC and thalamus. However, it was predicted that the extreme unpleasantness of noxious cold would show

greater likelihood values in regions responsible for processing pain affect, including the ACC and insula (Rainville et al. 1997;Costafreda et al. 2008). We compared 9 studies that used noxious cold as a stimulus and compared the data with those from 9 studies that used noxious heat. Our results showed that noxious cold stimuli did indeed show a likelihood of activation in brain regions known to process nociception. However, unique to the noxious cold meta-analysis were the significant likelihoods of evoking activation in the subgenual ACC and the amygdala.

The third meta-analysis in Chapter 3 assessed the implications of using either a resting baseline or warm stimuli as the control condition for revealing activation attributed to noxious heat. By performing a meta-analysis we compared the data from 9 brain-imaging studies that used innocuous warm as a control condition, in comparison to a comparable number of studies that used a resting baseline as a control condition for noxious heat. The main objective of this analysis was to determine which of these contrasts (warm or baseline) would produce more localized regions of activation sites in response to noxious heat stimuli. It was hypothesized that innocuous warm stimuli would be a better contrast for noxious heat. This was based on the knowledge that warm stimuli, when used as a control condition for noxious heat, will remove activation related to the activation of C warm fibres that is associated with the use of contact thermodes (Raja et al. 1999). This will occur as the thermodes activate warm afferents when heated up to a level that is perceived as painful to the subjects. In keeping with our hypothesis, our results showed that the use of innocuous warm stimuli as a control condition for noxious heat did was associated with localized likelihood values in regions known to process nociception. In comparison, using a resting baseline as a contrast for noxious heat the likelihood of evoking activation was more widespread in additional areas of the brain with no known nociceptive input, such as the superior frontal gyrus. However, one advantage to using a resting baseline as a control

condition for noxious heat is that it was more likely to produce activation in SI.

The fourth meta-analysis in Chapter 3 explored whether nociceptive input is processed preferentially by regions of the brain that are lateralized to one hemisphere, irrespective of the side of the body to which the stimuli are presented. Based on previous reports with patients and brain imaging studies with healthy individuals, it was first hypothesized that the right ACC would show significant likelihood values regardless of the side of the body where the stimuli were applied. Secondly, we hypothesized that activation would be likely to occur in SI in response to contralateral stimulation only, as this region contains few neurons with bilateral receptive fields. In the meta-analysis, we included a total of 40 studies that applied noxious stimuli to the left side of the body, and a comparable number of studies and foci that applied stimuli to the right side of the body. Contrary to our hypothesis, the activation likelihood in the right insula in response to stimuli applied to the right and left sides of the body was highly significant. However, for both meta-analyses, the other region to demonstrate a significant likelihood of stimulus-evoked activation was the right ACC. Other cortical regions involved in processing nociception such as SII, thalamus, and basal ganglia showed a significant likelihood of being activated in response to stimuli applied to either side of the body. Additionally, supporting our hypothesis, regardless of the side of the body the stimuli were presented to - only contralateral SI (not ipsilateral) had a significant likelihood of being activated.

Based on the findings from the third meta-analysis in Chapter 3, which showed that innocuous warm stimuli, when used as a control condition for noxious heat, were associated with a high likelihood of evoking activation in brain regions with known nociceptive input, we explored the neural representation of warmth perception using fMRI, as described in Chapter 4. To date, few neuroimaging studies have examined the brain regions that process warmth perception, although warm stimuli are often

used as a control condition for noxious heat pain. Several authors have reported that the same brain areas that process pain are activated by warm stimuli (Becerra et al. 1999;Lorenz et al. 2002), while other studies have reported either very weak activation (Davis et al. 1998;Olausson et al. 2005) or entirely dissimilar areas of brain activation (Casey et al. 1996). It could therefore be interpreted that warm stimuli are not the most efficacious method to localize pain activation in the brain. Chapter 4 describes the fMRI study we conducted to further understand the roles of the somatosensory and limbic cortices in processing warmth. To determine whether activation would be primarily located in regions that process pain, we performed a region-of-interest (ROI) analysis focusing on SI, SII, ACC, insula, and thalamus. During the experiment, noxious heat and warm stimuli were presented in a counter-balanced manner. The repeated presentation of noxious heat stimuli caused attenuation of the warm fibres, and as a result, some of the warm stimuli were undetected by the subjects. This permitted the identification of trials where the stimuli were either detected or undetected by the participants. Events for detected and undetected stimuli were modeled separately in the functional brain imaging data analysis. In line with our predictions, a direct comparison between the detected and undetected stimuli revealed significant activation in SI and SII, but only weakly in the insula in response to the detected stimuli only. However, contrary to our hypothesis, no activation was seen in the thalamus or the ACC. Additional findings were that in comparison to the rest period the undetected stimuli were associated with significant negative BOLD-signal change in SI and bilateral IC. Somatosensory gating, top-down attentional mechanisms, inhibitory thalamocortical projections or surround inhibition may explain this finding.

The final experiment presented in Chapter 5 was initiated to study the somatosensory system after a loss of sensory input, by exploring the perception of cutaneous stimuli in patients with amputations who experience phantom-limb pain. Several previous studies examining patients with

amputations have mainly focused on the reorganizational changes of body part representations in SI in relation to phantom limb sensations that can be evoked by applying stimuli to body parts near the site of amputation. This was largely based on reports of highly detailed and organized referred sensations in the phantom in response to applying tactile stimuli to "reference zones" on the skin. For example, Ramachandran et al., (1992) reported that the entire somatotopic representation of the phantom hand could be evoked by applying stimuli to the stump and to the ipsilateral cheek. These findings would implicate a cortical region with small receptive fields, such as SI, in their generation. However, as exemplified in Chapter 3 of this thesis, many other regions of the brain process pain. In turn, several other authors have reported non-localized referred sensations in the phantom in response to stimuli applied to widespread regions of the body. However, the results from all of these reports were obtained from small samples of heterogeneous patients, some of whom had amputations for a lengthy amount of time or pre-morbid chronic pain conditions that were likely to have modulatory effects on the cortical representations of body parts. As a lead up to a future neuroimaging experiment where we intended to study the somatotopic organization of body part representations in SI in phantom-limb pain patients with upper and lower limb amputations, we sought to explore the pattern of referred sensations to the phantom by having the patients undergo a quantitative sensory testing protocol. For the neuroimaging experiment, we were interested in exploring the representations of the face and the arm stump in SI in upper-limb amputees, and the leg stump representation in SI in lower-limb amputees. In turn, we chose only to apply stimuli to these regions during the quantitative sensory testing protocol where we mapped referred sensations in the phantom by applying tactile stimuli to the skin. In this group of recent traumatic upper and lower-limb amputee patients, we wanted to determine whether sensations referred to the phantom limb would be evoked in a localized, somatotopic manner in response to tactile stimuli applied to reference zones

on the skin near to the site of the amputation (face and arm stump in upper limb amputees and leg stump in lower limb amputees). In most instances, the referred sensations to the phantom were diffuse and followed no topographic organization in response to tactile stimuli applied to the skin. The referred sensations to the phantom were in most instances entirely unlike the type of stimuli applied to the skin – with some patients reporting referred muscle contraction sensations to the phantom. Additionally, referred sensations to the phantom occurred in response to stimuli applied to either side of the body. These results suggest the involvement of brain regions outside SI in the generation of referred sensations and potentially include SII, the insula, thalamus, and brainstem nuclei.

6.2 Localization of activation in the brain in response to noxious stimuli

6.2.1 Meta-analysis of activation in the brain in response to all types of noxious stimuli

The first meta-analysis described in Chapter 3 was performed by analyzing 130 brain imaging studies that utilized any type of noxious stimuli applied to any part of the body in healthy subjects. For the first time, these results provide the ability to determine the likelihood and spatial extent of evoking activation in response to noxious stimuli in any brain region. The results were in agreement with previous electrophysiological and brain imaging investigations into the processing of nociceptive input to the brain and showed a significant likelihood of evoking brain activation in response to noxious stimuli in SI, SII, ACC, the insula, the PFC, the thalamus, and basal ganglia (Willis 1985a;Willis 1985b;Chudler and Dong 1995;Apkarian et al. 2005).

6.2.1.1 Primary and Secondary Somatosensory Cortices (SI & SII)

One of the most important findings from the primary meta-analysis was that SI had a significant likelihood of being activated. The role of SI in

pain perception was outlined by an early neurophysiological study with non-human primates, which demonstrated stimulus-dependent response curves in this brain region (Kenshalo et al. 1988). This was later complemented by a brain imaging study in humans that showed a stimulus-intensity related response in SI by correlating subjects' pain ratings with brain activation (Coghill et al. 1999).

Despite these results, the role of SI in processing pain is still debated within the brain imaging community. Two recent experiments have argued that activation in SI in response to pain is solely due to estimation of intensity (Baliki et al., 2009; Mouraux and Iannetti, 2009). During an fMRI study, Baliki et al. (2009) presented subjects with either painful or visual stimuli that varied in intensity. During the experiment, subjects were required to constantly rate the intensity of the stimuli. In the analysis, online ratings were correlated with brain activation in their ROIs that included bilateral insula, the premotor cortex, the posterior parietal cortex, the mid-temporal cortex, and the mid ACC and the supplementary motor area. Their results showed that only a "pain-specific" response could be elicited in the insula. All other regions activated by pain were also associated with the rating of the stimuli.

While the results of Baliki et al. (2009) are compelling, an inherent limitation of the study is the use of online ratings to correlate with brain activation. Several of the same cortical regions involved in pain perception would undoubtedly be activated by the use of a manual-rating tool, including the somatosensory cortices. This task is also extremely attention orienting and may have produced negative effects on the signal change in pain-related regions. This is an important consideration given that attention to painful stimuli has been shown to dramatically impact resulting brain activation (Bushnell et al. 1999). For example, when subjects direct attention away from painful stimuli, this causes a reduction in activation in somatosensory areas (Bantick et al. 2002). Therefore, the lack of specific activation in SI associated with nociceptive stimuli may be entirely due to

attentional modulation of the stimuli, in that the subjects were highly engaged in rating of the stimuli, which may have distracted attention from their actual perception.

A recent laser evoked potentials (LEP) study also reported that activation patterns in response to noxious stimuli reflected estimation of the magnitude of the stimuli (Mouraux and Iannetti, 2009). During the experiment, laser stimuli were utilized to activate nociceptive afferent fibres. As this method of stimulation does not involve contact with the skin it permits the selective activation of only A delta and C fibres, which process pain, and avoids activating A beta fibres that process touch. In the experiment the authors presented painful, non-painful, auditory and visual stimuli. At the end of the trials, subjects rated the intensity of the stimuli. The authors found similar activation patterns across all stimulus types, and concluded that no specific regions were responsive for processing pain. However, specific laser evoked potentials were found in response to visual, auditory and tactile stimuli. The authors conceded that the lack of a nociceptive specific signal may be due to the limitations associated with the use of scalp EEG recordings. This technique records large populations of synchronously firing neuronal populations and may not be able to detect the rather sparse distributed firing pattern of nociceptors (Kenshalo et al., 2000; Kenshalo and Isensee, 1983; Robinson and Burton, 1980). Additionally, in the cortex, neurons responding to tactile and nociceptive stimuli are intermixed and therefore it is impossible to detangle their respective evoked responses using this type of analysis (Kenshalo and Isensee, 1983). Additionally, another limitation associated with the analysis is that they did not examine single trials. In LEP data, artifacts have low frequency components that can be averaged out with multiple signal averages. In the single trial data, evoked potentials may have been elicited from SI; however this would have been averaged out if the evoked potentials were not consistent across all trials. Therefore, the results of Mouraux et al., (2009)

should be considered carefully, but this certainly does not discredit the host of experiments that have used LEPs to study pain.

In relation to the findings from the first meta-analysis in Chapter 3, it cannot be entirely ruled out that some regions that process nociceptive input may overlap with those that estimate the magnitude of the stimuli. The studies included in the main meta-analysis either passively presented stimuli to the subjects, or the subjects rated the intensity of the stimuli at the end of the trial. However, given the enormity of the number of studies included in the analysis and the overlap in SI and other somatosensory regions, the results provide a strong role for these regions in processing noxious stimuli.

Bilateral SII was also found to have a significant likelihood of evoking activation in response to noxious stimuli. While the peak values were located in the mid parietal operculum, this region showed widespread likelihood values extending laterally from the inferior aspect of the postcentral gyrus to the dorsal posterior insular cortex. The higher probabilistic values in SII compared with SI may be due to a number of factors. Firstly, this region contains large, bilateral receptive fields (Robinson and Burton 1980). Additionally, SII has been considered a higher order somatosensory processing region involved in the integration of sensorimotor stimuli (Huttunen et al. 1996;Forss and Jousmaki 1998). Therefore, the significant likelihood values in this region may reflect the complex cognitive and physiological processes involved in the perception of noxious stimuli.

6.2.1.2 Insula

A second major finding from the primary meta-analysis in Chapter 3 was that the highest likelihood value associated with evoking activation in response to noxious stimuli in the cortex was found in a voxel located in the anterior insula. The insula has not traditionally been the main concentration of pain research; however, in the last decade it has become a major scientific focus within this field of study. The significant likelihood of evoking activation in the insula in response to noxious stimuli is likely due to several

reasons. Nociceptive neurons have been recorded in the insula in humans (Penfield and Faulk 1955;Ostrowsky et al. 2002). Additionally, this region processes autonomic responses that can occur during the perception of noxious stimuli, which include increases in heart rate, galvanic skin responses, and heightened blood pressure (for review see Craig 2009). Lastly, the insula's involvement in pain perception may stem from this regions' role in monitoring the body and other interoceptive processes (Critchley et al. 2004).

6.2.1.3 Anterior cingulate cortex (ACC)

The ACC had a significant likelihood of being activated, with the highest likelihood of activation being located in Brodmann Area (BA) 24. Based on anatomical studies in animals, lesion studies in patients, and more recent functional neuroimaging studies, the ACC has been implicated in processing the emotional salience or unpleasantness of painful stimuli. The ACC receives direct nociceptive input from dorsal horn neurons by way of the MD and intralaminar thalamic nuclei (Krettek and Price 1977;Goldman-Rakic and Porrino 1985;Giguere and Goldman-Rakic 1988;Wang and Shyu 2004). In humans, cingulotomy for alleviation of chronic pain reduces affective responses without disruption of the ability to appreciate somatosensory aspects of painful stimuli (Foltze and White 1962;Ballantine et al. 1967). Functional neuroimaging studies have also implicated the ACC in affective pain processing. For example, Rainville and colleagues (1997) modulated the affective qualities of a noxious stimulus without changing its perceived intensity using hypnotic suggestion. Changes in activation were noted in the ACC, with no concurrent changes in brain regions involved in sensory-discriminative processing.

While strong evidence exists for a role for the ACC in affective processing of pain, other reports have suggested this region may also subserve sensory-discriminative aspects of pain perception. Namely,

neurons have been recorded in this area in both humans and animals in response to pain whose firing frequencies were correlated with stimulus intensity (Yamamura et al. 1996;Hutchison et al. 1999). An fMRI study reported a crude nociceptive somatotopic organization in this region thus implicating this region in stimulus localization (Henderson et al. 2007). However, this claim has been called into question as there have been a greater number of electrophysiological studies reporting bilateral and large receptive fields in the ACC (Sikes and Vogt 1992;Hutchison et al. 1999;Kuo and Yen 2005).

Based on these previous findings, the significant likelihood of evoking activation in the ACC in response to noxious stimuli may reflect both the processing of the affective component of pain and potentially the localization of stimuli applied to the body.

6.2.1.4 Prefrontal cortices

Several regions in the prefrontal cortex had a significant likelihood of evoking activation in response to noxious stimuli, which were all lateralized to the right hemisphere except in the instances of left BA 10 and 46. These regions comprised those that were involved in mediating executive functions (BAs 9, 10, 44). The dorso- and ventro-lateral prefrontal cortices are implicated in working memory, attentional control, monitoring, temporal coding of stimuli, and multisensory integration (Knight et al. 1995;Funahashi 2006;Stein and Stanford 2008). In relation to pain processing these regions' significant likelihood of being activated could reflect any number of complex cognitive processes being experienced during exposure to noxious stimuli. Incoming information regarding a noxious stimulus is not only perceived, but is also compared to long term memories of previous similar stimuli, and involves subsequent planning of behaviours as to how to react.

Interestingly, unconscious patients receiving noxious stimuli show activation in a number of pain processing regions including SI, but do not show activation in prefrontal cortices (Laureys et al. 2002;Kassubek et al.

2003). Furthermore, Laureys et al., (2002) performed functional connectivity analysis on data obtained from patients in persistent vegetative states receiving painful stimuli. Results showed that SI showed a functional disconnection from the prefrontal cortex. The authors concluded that their findings reflected the importance of the prefrontal cortex in the conscious perception of pain.

6.2.1.5 Motor regions

A number of regions responsible for motor processing had significant likelihoods of being activated by noxious stimuli, such as the primary motor cortex (MI), the supplementary motor area (BA 6), the basal ganglia, and the cerebellum. The role of these motor regions in cortical pain processing remains somewhat unclear. Some areas have been shown to possess nociceptive neurons such as the basal ganglia (Chudler and Dong 1995). Evidence from fMRI has demonstrated a rough somatotopic organization in the putamen, suggesting that this region may be part of a network of areas that could contribute to localization of nociceptive stimuli (Bingel et al. 2004). While the same has not been explored in the cerebellum, Purkinje cells have been shown to respond to nociceptive colorectal distention (Saab and Willis 2001), and this region has been found to contain proton-gated ion channels known to mediate nociception (Alvarez et al. 2003).

The role of MI in nociceptive processing is less clear. However, anterograde tracer studies in animals have shown that the medial thalamus, which is involved in affective pain processing, projects to MI, ACC, prefrontal cortices and the striatum thus suiting this cortical network to be involved in the emotional-motivational aspect of pain processing (Ma et al. 1987;Wang and Shyu 2004). This connection may serve to initiate a withdrawal response after exposure to a noxious stimulus. Additionally, a recent antereograde tracer study demonstrated that one of the main pathways that sends pain and temperature information to the cortex (spinothalamic tract) terminates in SII, insula and motor regions of the

cingulate gyrus (Dum et al., 2009). These regions in the cingulate then send a direct projection to MI. These findings indicate that a somatosensory pathway may influence the motor system.

An important finding regarding the role of MI in nociceptive processing is that electrophysiological stimulation of this region has been used as a method to alleviate chronic pain (Velasco et al. 2002). It was initially believed that the underlying neurophysiological mechanism worked by projections to SI. However, a recent PET study of patients with chronic motor cortex stimulation demonstrated this phenomenon is dependent on the release of endogenous opioids (Maarrawi et al. 2007).

Other motor areas that demonstrated a significant likelihood of being activated in response to noxious stimuli included the supplementary motor area. This finding may reflect direct projections from the ACC to this region that are involved in initiating a response selection (Morecraft and Van Hoesen 1992). The likelihood of evoking activation in this region may reflect the many studies included in the meta-analysis that required subjects to rate the stimulus after its presentation. Alternatively, the likelihood of evoking activation in this region may represent response inhibition (Mostofsky and Simmonds 2008), as subjects may have been given instructions not to move during scanning, but may have had the conflicting urge to retract their limbs during the onset of noxious stimuli.

6.2.1.6 Thalamus

The ventral posterior lateral (VPL) nucleus also exhibited one of the most significant likelihoods of being activated in the main meta-analysis. Findings are consistent with the known anatomical input to this nucleus. This region receives pain and temperature information from the upper and lower limbs via the lateral spinothalamic tract, the primary ascending pathway known to relay pain and temperature information (Willis and Westlund 1997). This nucleus has often been considered to be involved in

purely sensory processing of pain such as spatial localization (Price and Dubner 1977). This conclusion is largely based on the high percentage of WDR neurons found within VPL that are responsive to noxious thermal and mechanical stimulation (Kenshalo et al. 1980;Guilbaud et al. 1980).

Additionally, the mediodorsal (MD) nucleus was significantly likely to be activated by noxious stimuli and is often referred to as the limbic thalamus (Vogt and Pandya 1987;Taber et al. 2004). The MD nucleus receives nociceptive input from the spinothalamic tract (Kerr 1975;Apkarian and Hodge 1989). Its role in pain processing is largely that which mediates emotional responses to noxious stimuli. This conclusion is largely drawn from anatomical studies showing that it receives and projects to a number of brain regions involved in affective processing including orbitofrontal cortex, medial prefrontal cortex, temporal pole, ACC (BA 24), and the amygdala (Russchen et al. 1987;Yeterian and Pandya 1991).

6.2.2 Activation in the brain in response to noxious cold stimuli

The second sub-analysis in Chapter 3 examined the brain regions involved in processing noxious cold stimuli and compared them with those that process noxious heat. Findings revealed that activation in response to noxious cold and heat was likely to occur in several regions such as SII, ACC, insula and thalamus. Additionally, SI was significantly likely to be activated in response to processing both noxious cold and heat. The role of SI in mediating cold perception has been somewhat disputed. To date, few imaging studies have directly compared noxious cold and heat within the same experimental protocol. One study found that noxious heat, but not noxious cold, produced robust activation in SI (Craig et al. 1996). However, by using meta-analytic techniques across a broader range of studies, we have shown that SI has a significant likelihood of being activated in response to noxious cold. This highlights the importance of using meta-analysis to examine brain activation across several studies in that it permits

overcoming the limitations associated with drawing conclusions from a single imaging experiment.

An additional finding from the second sub-analysis in Chapter 3 was that the subgenual ACC and the amygdala were preferentially likely to be activated by noxious cold stimuli. These brain regions are involved in processing aversive stimuli. The greater likelihood of obtaining activation in the brain in response to noxious cold stimuli may reflect the extreme unpleasantness associated with the perception of these stimuli (Rainville et al. 1992).

6.2.3 Control conditions for noxious heat stimuli

The fourth experiment in Chapter 3 examined the implications of using either innocuous warm stimuli or a resting baseline as control conditions for noxious heat. Both types of control conditions showed significant likelihood values in brain regions involved in processing nociceptive stimuli including SI, SII, ACC, the insula, the prefrontal cortex (PFC), and thalamus. However, the noxious heat vs. resting baseline meta-analysis showed that brain regions with no known nociceptive input such as the super frontal gyrus were significantly likely to be activated by noxious stimuli. In contrast, when using innocuous warm stimuli as a control condition for noxious heat, this resulted in fewer nociceptive brain processing regions showing a significant likelihood of being activated.

The findings from the meta-analysis are not surprising given the knowledge that contact thermodes used to generate noxious heat stimuli will invariably activate C warm fibres as it increases in temperature to levels perceived as painful to subjects (Raja et al. 1999). Therefore, when using innocuous warm stimuli as a contrast for noxious heat, activation associated with warmth perception would be subtracted out.

The results from this analysis should not discount the use of a resting baseline as a control condition to examine pain-evoked activation in the brain as it could offer some advantages. Namely, the use of a resting

baseline as a contrast for noxious heat this may result in higher statistically significant activation peaks. The blood-oxygen-level-dependent (BOLD) nociceptive signal is quite weak in comparison to the signal produced by visual, tactile or motor stimuli (Fox et al. 1988;Coghill et al. 1994;Kim 1995;Ramsey et al. 1996;Derbyshire and Jones 1998) and therefore using a time period with no stimulation as a control condition may produce the most robust results. For other experimental protocols, a more ideal contrast to localize activation in the brain in response to noxious stimuli would be to use innocuous warm stimuli. Such a contrast would be ideal for example when examining nociceptive somatotopic organization.

6.2.4 Hemispheric lateralization of noxious stimuli

The fourth and final meta-analysis presented in Chapter 3 examined brain regions that have a hemispheric lateralization for processing noxious stimuli. For both left and right-sided stimuli, the right anterior insula had the highest likelihood of being activated. Additionally, the other region with a similar likelihood of being activated was right ACC. The majority of other brain regions that receive nociceptive input such as SII, thalamus and basal ganglia also had a significant likelihood of being activated in response to stimuli presented to the left or the right side of the body. However, only contralateral SI was found to have a significant likelihood of being activated in response to left or right-sided stimuli.

The likelihood of evoking activation in the right insula and ACC may be attributed to these brain regions' involvement in processing emotion. In a recent review, Craig (2002) attributes activation in the right anterior insula to be associated with the subjective awareness of pain. This conclusion was drawn based on several lines of evidence. Namely the author notes that a previous neuroimaging experiment demonstrated that brain activation in the right anterior insula was correlated with the evaluation of pain (Brooks et al. 2002). Additionally, the right anterior insula is activated during subjective processing of emotional stimuli (for example see (Reiman et al.

1997;Mayberg et al. 1999). The right anterior insula is also activated by the subjective assessment of emotion in others (Winston et al. 2002).

In line with this interpretation is that the likelihood of evoking activation in right ACC may also reflect heightened awareness to one's emotional state during the perception of noxious stimuli. This is due to the highly unpleasant nature of painful stimuli. The right ACC has been associated with processing the perception and expression of emotions (Lane et al. 1998). Lane and colleagues (1998) demonstrated a direct correlation between activation in the right ACC and scores on an emotional awareness scale during an experiment where emotions were induced externally or internally. In turn, the results from the meta-analysis in Chapter 3 may indicate that during the perception of noxious stimuli the right ACC and insula may work in conjunction to enhance attention and awareness to one's affective state.

6.2.4.1 Study limitations

The experiments presented in Chapter 3 were developed using the ALE method (Laird et al., 2005; Turkeltaub et al., 2002). While this remains an exceptional and widely used technique to perform meta-analysis of brain imaging data, limitations are associated with the method. Namely, the analysis technique weights each activation site equally and does not take into account such factors as the statistical value of the reported peaks (e.g. effect size) or the number of subjects (e.g. reliability). However, given the large number of activation sites (<100/per meta-analysis) showing concordance across studies, the magnitude of the effect size for each foci or reliability of the data is less critical for the calculation of the likelihood estimate values.

6.3 Localization of warm-evoked activation in the brain

In Chapter 4, we explored warm-evoked activation in the brain using fMRI. During the experiment, due to the attenuation of warm fibres after

repeated presentation of painful stimuli, some of the warm stimuli were undetected by the subjects. In the functional imaging analysis, the time periods for the detected and undetected stimuli were modeled as separate regressors in the design matrix. This permitted the ability to view activation in the brain in response to the detected or undetected stimuli. The results showed that in response to detected stimuli, activation was seen in SI and SII, but only weakly and non-significantly in the insula.

Contrary to our hypothesis, no activation was seen in the ACC. This result was surprising given that previous fMRI studies exploring warmth perception reported that the ACC was a commonly activated area. However, studies that have recorded directly from the ACC in patients undergoing implantation of deep brain stimulators noted that neuronal responses were absent in this region in response to warm stimuli (Hutchison et al. 1999). Therefore, given the current findings that no activation was produced in the ACC during the detected warm trials this may indicate that the previous fMRI studies may have been capturing attention or orienting responses that have been previously attributed to activation in the ACC (Carter et al. 1999). However, one cannot rule out the possibility that in the current study the lack of a response in the ACC may be due to the method of stimulus presentation, in that warm stimuli were counterbalanced with pain. Consequently, the warm trials may have been detected as a period of relief from the painful stimuli and the subjects potentially attended less during this period.

6.3.1.1 Study limitations

A significant limitation of this study is that a whole brain imaging sequence was not performed. Instead, the acquisition slices were positioned over the somatosensory and limbic cortices, as the intention was to focus on these regions for this particular protocol. This was to determine if warm stimuli would be an appropriate control for noxious heat stimuli. However, a number of other cortical regions such as the orbitofrontal cortex and the

pregenual ACC have also been shown to process warmth. For example, electrophysiological studies have directly linked the pregenual ACC with temperature processing (Kadohisa et al. 2004). Additionally, a recent fMRI study found that activation in the orbitofrontal cortex and the pregenual ACC was correlated with subjects' ratings of pleasantness (Rolls et al. 2008). However, this was not the focus of the current research. In future, further research using high-resolution, whole brain imaging is needed to further elucidate this region's role in the perception of warm stimuli.

6.4 Exploration of referred sensations in patients with phantom-limb pain

The study of patients with amputations offers the unique opportunity to study plasticity of the somatosensory system in the human brain. While the knowledge that the central nervous system undergoes changes in response to amputation was reported as early as 1872 by Mitchell (Mitchell 1872); systematic investigation in animals only began nearly a century later (Merzenich et al. 1984). This early work by neurophysiologists revealed that wide scale cortical reorganization occurred in SI in response to digit amputation. As a result, the representations of the surrounding digits and palmar surface of the hand over took the area of the denervated cortex. Subsequently, clinical investigation utilizing brain imaging techniques reported a similar type of reorganization in patients with phantom-limb pain.

Referred sensations, where patients perceive the phantom in response to stimuli applied to body parts adjacent to the phantom on the somatotopic map in SI were reported in the early 1950s, but only became an area of renewed scientific interest forty years later. In the report by Ramachadran et al. (1992) the authors studied two patients, one with an upper limb amputation and one with a digit amputation. The upper-limb amputee patient reported a one-to-one topographic representation of the phantom hand in response to stimuli applied to the stump and the ipsilateral cheek. For example, one touching the ipsilateral cheek it would be

perceived as though the phantom index finger was being touched by the exact same stimulus, and moving the stimulus to the left would evoke the representation of the middle finger. Given the small receptive fields and detailed somatotopic organization of the receptive fields in SI, it was presumed that this was the primary region that underwent reorganizational changes of the body part representations and was responsible for the generation of referred sensations.

In line with these findings was a report of referred sensations to phantom legs lower-limb amputees (Aglioti et al. 1994). They examined referred sensations to the phantom legs of three patients by applying tactile stimuli to their residual stumps. All patients reported referred sensations to their phantom feet in response to stimuli applied to the residual leg stump. However, one patient reported a highly localized and topographic organization of the referred sensations to the phantom in response to stimulation of the skin. This patient also exhibited a considerable amount of phantom limb telescoping, where the phantom limb is perceived to shrink into the stump representation. This could potentially effect the generation of referred sensations. The underlying mechanisms of phantom limb telescoping remain unknown; however, presumably this would involve the expansion of the neighbouring cortical territory of the stump and the shrinking of receptive field sizes of the phantom arm. Therefore, as the phantom arm receptive fields become overlapped with those of the intact arm, stimuli applied to the latter may result in the perception that the phantom arm is being touched.

Subsequent reports found poor spatial localization of referred sensations in upper-limb amputees (Grusser et al. 2001; Hunter et al. 2005; Sathian 2000). Moreover, the referred sensations in the phantom could be evoked in response to applying stimuli to widespread regions of the body. These findings would indicate that cortical regions with large or even bilateral receptive fields are involved in the generation of referred sensations.

The differences between all of these reports may be due to the heterogeneous samples of patients who had different amputation sites and were tested sometimes more than 50 years after their surgeries. Additionally, some of the patients included in the previous studies had pre-existing chronic pain conditions that has been shown to lead to the generation of referred sensations (McCabe et al. 2003).

The results from Chapter 5 would provide strong support for other cortical regions in addition to SI to be responsible for the generation of referred sensations. SI contains neurons with small receptive fields and a highly detailed somatotopic organization of body part representations. Should referred sensations contain a highly organized pattern, this would indicate that they are originating from changes occurring in SI. However, we report that the referred sensations to phantom limbs in upper and lower-limb amputee patients in most instances followed no topographic organized pattern in response to tactile stimuli applied to the skin. Additionally, some patients also reported muscle contractions referred to the phantom limb. Results would therefore also implicate motor areas in referred sensation genesis.

We also assessed the amount of phantom limb telescoping in relation to referred sensations in the phantom limbs in our group of upper and lower amputee patients. We found that 4 out of the 6 patients tested experienced some degree of telescoping of the phantom limb with the co-occurrence of referred sensations. However, two of the lower-limb amputees also reported referred sensations in their phantom legs, but perceived little or no telescoping. Therefore, as referred sensations are likely to be generated by reorganizational changes outside SI, this may also be the case for the perception of phantom limb telescoping. Likely candidates may be higher order areas of somatosensory perception in the posterior parietal cortex. For example, the superior parietal lobule is essential for the conscious awareness of the body and damage to the right parietal lobe can lead to neglect of the contralateral side of the body (Husain and Nachev, 2007).

The psychophysiological investigation used in the current study was not sufficient to fully explore the relationship between chronic pain and referred sensations, and future research using high-resolution brain imaging would help to further clarify this important issue in phantom-limb pain research.

6.4.1.1 Study limitations

Several limitations are associated with the interpretation of the results from Chapter 5. Namely, due to the rarity of amputees and our strict inclusion criteria, we were only able to test a small number of patients. Additionally, as the entire interview and quantitative sensory testing protocol took sometimes up to 3 hours it was not possible to apply the tactile stimuli to all body parts, or even to some of the sites of interest. However, given that this study was largely exploratory our results still provide new information on referred sensations in phantom-limb pain patients. Another limitation of the current study is that the somatosensory stimuli were always applied in the same order (cotton bud, electrical stimuli, vibrotactile stimuli). Potentially, this may have changed the threshold for the evocation of the referred sensations to the phantom due to the repeated presentation of the stimuli. However, the sensory testing protocol was carried out over a long period of time (1-2 hours) with breaks in between the application of the different types of stimuli. This being said, the order of stimulus presentation should still be considered in future studies with amputees.

Another important consideration is that the perception of referred sensations could have been heightened by top-down mechanisms such as attention, which may have altered the receptive field sizes of tactile neurons (Haggard et al., 2007). Additionally, a number of brain imaging studies have demonstrated increased activity in somatosensory regions during attention to tactile stimuli (see Burton and Sinclair, 2000 for review). In turn, should referred sensations be generated by reorganizational changes in the somatosensory cortices, then increased attention toward them may enhance

their cortical representation that would potentially facilitate their evocation. The current report did not examine the effects of top-down processes on referred sensation perception; however one previous report noted that patients' referred sensations were more salient when they were asked to close their eyes (Hunter et al. 2003). Thus it could be argued that in absence of competing sensory input, this enabled the patients to enhance their attention towards the referred sensations in their phantom limbs.

6.5 Final conclusions

Aristotle wrote in *De Anima* (On the Soul; 384-322 BCE) that if touch is not a single perception, but many instead, then its purposes are also manifold. In this short quote, Aristotle conveys the complexity of perceiving touch, in that a single stimulus applied to the skin signals information to the brain about its location, texture, temperature, weight, and affective value. Through what we have gained by way of intense scientific study of the somatosensory system we now have a greater understanding of how a tactile stimulus is processed in the brain. This process begins with special receptors on afferents in the periphery that signal this information via specialized pathways in the spinal cord up to the brain stem, thalamus, and cortex.

The studies presented in this thesis offer insight into the brain regions involved in cutaneous perception including pain, warmth, and touch. One major contribution this work has made to the field of somatosensory research is that it includes the most comprehensive and detailed review of the current pain imaging literature. The meta-analysis presented in Chapter 3 provides localized information concerning regions in the brain responsible for processing pain and include SI, SII, ACC, insula, PFC, thalamus, and basal ganglia. Additionally, given the wealth of data within the meta-analysis, this permitted the ability to explore several unresolved issues within the pain imaging literature, such as whether cold pain is processed by the same regions as heat pain. Findings indicate that noxious cold stimuli are preferentially processed by certain brain areas, including the subgenual ACC and the amygdala. A third question addressed by the meta-analysis assessed the implications of using a resting baseline or warm stimuli as a control condition for noxious heat. Heat pain versus warm stimuli produced fewer likelihood values in cortical areas involved in processing nociception in comparison to studies that used a resting baseline compared to noxious heat. The last question to be explored by the meta-analysis data was to determine whether the processing of noxious stimuli would be lateralized to

brain regions in one hemisphere. By comparing an equal number of studies that presented noxious stimuli to either side of the body it was shown that regardless of the site of stimulation the right anterior insula had the highest likelihood of being activated.

Based on the results of the third meta-analysis presented in Chapter 3, we were interested in studying the roles of the somatosensory and limbic cortices in the perception of warmth. In Chapter 4, we utilized a unique approach to analyze fMRI data in order to localize regions in the brain that process warm stimuli. During the fMRI experiment warm stimuli were counterbalanced with noxious heat pain stimuli. This resulted in some of the warm stimuli to be perceptually gated by noxious heat pain stimuli - causing some of the warm stimuli to be undetected by the subjects. This permitted the comparison of detected versus undetected stimuli in the resulting analysis. In response to detected stimuli, positive BOLD-signal change was seen in contralateral SI and SII, but non-significantly in the insula. However, significant negative BOLD-signal change was seen in our regions of interest (SI, SII, insula, ACC and thalamus) that was associated with the undetected stimuli. The underlying neural mechanisms responsible for negative BOLD signal remain poorly understood; however, this finding may be attributed to somatosensory gating, top-down attentional mechanisms, inhibitory thalamocortical projections or receptive field surround inhibition.

The last study in Chapter 5 of this thesis explored altered somatosensation in a group of amputee patients who had phantom-limb pain. We conducted a quantitative sensory testing protocol on upper and lower-limb amputees as a prelude to a future neuroimaging experiment where we planned to map the somatotopic organization of body part representations in SI. We mapped sensations referred to the phantom by applying tactile stimuli to the face and arm stump in upper-limb amputees and to the leg stump in lower-limb amputees. This provided insight into the brain regions that might undergo somatotopic reorganizational changes in response to deafferentation. The results indicated that referred sensations

were commonly reported in both upper and lower-limb amputees. However, referred sensations in the phantom were often detected as occurring in non-specific locations on the phantom limb in response to stimuli applied to both sides of the face or even to the contralateral intact hand. Furthermore, the sensations referred to the phantom were more often than not entirely different from the type of stimuli applied to the skin. For example, several patients reported muscle contractions in the phantom limb in response to stimuli applied to the body. Additionally, referred sensations in upper-limb amputees were more likely to be evoked in response to stimulation of the face – that is located more remotely to the hand and arm representation in SI. These findings would indicate that somatosensory regions, perhaps in addition to SI, that have large or even bilateral receptive fields undergo reorganizational changes in response to deafferentation. These brain regions include SII and the insula. Motor responses in the phantom limb could potentially be generated by the expansion of somatosensory cortical neurons in Area 3b into Area 3a in SI that receives muscle afferent input or potentially even the primary motor cortex. Additional plausible subcortical candidates that undergo reorganizational changes include the thalamus, brainstem nuclei such as nucleus cuneatus, nucleus gracilis, and the lateral cervical nucleus.

The studies included in this thesis focused on the roles of the somatosensory cortices in the processing of innocuous and noxious stimuli. The findings from the meta-analyses conducted in Chapter 3 provide insight into the regions that process pain. Furthermore, the results of the third meta-analyses in Chapter 3 showed the advantages of using warm stimuli as a control for noxious heat. When the neural activation associated with the detection of warm stimuli was explored in Chapter 4 the results showed that these stimuli activate similar regions as nociceptive stimuli such as SI, SII, and (albeit weakly) the insula but not the ACC or the thalamus. Additionally the result from the main meta-analysis in Chapter 3 can aid in drawing insight into brain regions that undergo changes in chronic pain states

including phantom-limb pain. As the results of Chapter 5 demonstrated that referred sensations to phantom limbs are likely not generated by solely changes in SI, but also include other regions that process pain. Based on the results from the fourth meta-analysis comparing noxious stimuli applied to either side of the body, potentially patients may exhibit changes in mainly contralateral SI, but bilaterally in SII and insula.

7 References

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à inclure cet article dans ☐ son mémoire de maîtrise ☑ sa thèse de doctorat
qui a pour titre *The roles of the somatosensory cortices in the perception of noxious and innocuous stimuli*

Coauteur	Signature	Date
Gary H. Duncan		18-dec-2009
Coauteur	Signature	Date
Coauteur	Signature	Date
Coauteur	Signature	Date

Imprimer Effacer tout

APPENDIX II

- Published manuscript - Duerden EG, Laverdure-Dupont D. Practice makes cortex. *J Neurosci*. 2008 Aug 27;28(35):8655-7.

Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Practice Makes Cortex

Emma G. Duerden and Danièle Laverdure-Dupont

With the development of high-resolution anatomical magnetic resonance imaging (MRI) and automated statistical analysis techniques, brain morphometry has emerged as a dynamic field in neuroscience. A variety of techniques are now used to assess gray and white matter characteristics, such as thickness and density. Additionally, intrinsic changes in cortical folding can be explored using gyrification and sulcal morphometric analyses. To date, morphometric research has contributed significantly to our understanding of neurodegenerative diseases as well as experience-dependent brain plasticity.

Traditionally, it was believed that the organization of the cortex was static. However, results from the elegant work of Merzenich and colleagues (for review, see Merzenich and Sameshima, 1993) in nonhuman primates has demonstrated the presence of experience-dependent plasticity in the parietal cortex in response to somatosensory training. This extensive remapping was paralleled with improvements in behavioral performance. Findings from animal models have widened neuroscientists' views of the dynamic nature of the brain and have underscored the importance of verifying the presence and functional implications of such processes in humans.

Voxel-based morphometry is a modern, noninvasive computational approach allowing the study of neuroanatomical changes in living human subjects. This technique involves voxelwise measures of local concentrations of gray or white matter (Ashburner and Friston, 2000). In initial steps, MRI data are registered to a standardized template, tissues types are segmented with regards to their intensities, and the resulting images are smoothed. Parametric statistical tests are then used to examine divergences between different cohorts of subjects or longitudinal changes within a single population. Another major advantage of voxel-based morphometry is the possibility of correlating changes in anatomical structure with the modulation of cerebral activation, as measured with a variety of functional imaging techniques.

Several morphometric studies have demonstrated both short- and long-term use-dependent changes in the cortex. Voxel-based morphometry has been used to study experience-related plasticity in response to explicit tasks such as spatial navigation (Maguire et al., 2000) or intensive studying (Draganski et al., 2006) and implicit tasks including language skill learning (Mechelli et al., 2005), musical training (Gaser and Schlaug, 2003), and juggling (Draganski et al., 2004). Although structural changes are commonly found in brain regions known to be functionally involved in the particular skill under study, a meta-analytic review of these studies revealed that additional changes often occurred in associative regions including parietal and temporal cortices (Fig. 1).

The link between functional and anatomical cortical plasticity has remained essentially unexplored. With the advent of computational analysis, it is now possible to spatially correlate loci of functional activation with emerging morphological modifications induced by practice. Ilg et al. (2008) examined this question by combining functional MRI (fMRI) and anatomical MRI to study practice-induced cortical plasticity in response to a procedural learning task. Participants assigned to the experimental group practiced a mirror reading task for 15 min every day over a 2 week period. Subjects' behavioral performance at reading mirrored words was recorded before and after 1 week and again after 2 weeks of training. Practice-related performance increases in mirror-reading were observed as soon as after 1 week of practice, with slight additional improvement in the following week [Ilg et al. (2008), their Fig. 1 (http://www.jneurosci.org/cgi/content/full/28/16/4210/F1)]. Functional and structural imaging was performed only before the initiation of training and after 2 weeks of practice. To examine functional changes, brain activation was compared during the reading of mirrored words before and after training. Significant training-related increases were observed in the blood oxygen level-dependent signal in the right dorsal occipital cortex and left thalamus. Additionally, a significant decrease was noted in the right superior parietal cortex. In turn, voxel-based morphometry analy-

sis was used to explore structural gray matter density changes that occurred before and after training. Although no decreases were detected between training days, a significant increase in gray matter density was detected in the right dorsal occipital cortex, localized to the peak activation increase observed in the fMRI data. In line with previous findings, these results suggest that, after practice, structural changes occurred in areas that were actively recruited during practice at the task. Because behavioral data showed a steep increase after only 1 week of training, a direct comparison of anatomical and functional images collected in the same temporal window might have better clarified the relationship between such changes. Although practice-related changes in fMRI activation and gray matter density were localized in visual areas, the change in the fMRI activation was reported in the right V1 [primary occipital cortex; Brodmann's area (BA)17/18], whereas the peak region of increased gray matter density after training was slightly more lateral and encroached on higher-order visual processing areas (BA18/19) [Ilg et al. (2008), their Fig. 2 (http://www.jneurosci.org/cgi/content/full/28/16/4210/F2)]. In light of the reduced time required to accomplish mirrored reading, one can postulate that, in the early stage of practice, complex visual processing involving mental translation of the mirrored letters was needed to enable comprehensive detection. As the time required to accomplish the task diminished, subjects might have gradually relied on memorized mirrored letters without the need for abstract transformation. Hence, activation of primary visual areas noted after 2 weeks of practice could reflect a stage at which a more automated process based on simple mirrored letter recognition was used. Under those assumptions, whereas early phases of learning could have relied mainly on associative visual areas, later stages of automated detection might have only required the activation of primary visual regions. In this context, the increase in gray matter density of higher-order visual areas could reflect the changes that occurred earlier in the learning process, when the task was still cognitively demanding. However, strategies may have differed among participants, hence the need for correlating functional and structural changes within individuals.

In a voxel-based morphometric analysis, the tissue composition of each voxel is inferred from differences in image intensity, and the average intensity calculated for the gray matter partition is based on a mixture of different cell types, which cannot be differentiated with current techniques. Therefore, morphometric changes in density are somewhat difficult to interpret. Generally, the molecular correlates of gray matter density alterations are believed to be linked to changes in cell size, growth of neurons or glia, synaptogenesis, and even changes in blood flow or interstitial fluids (May, 2008). Central to the findings of Ilg et al. (2008) is that increased functional activity leads to intracortical remodeling. Whether those changes reflect molecular alterations in neurons or glia is hard to ascertain, because capillaries are also found in gray matter, and their density can vary with metabolic demands. In fact, animal studies have suggested that increased synaptic activity can elicit compensatory angiogenesis without necessarily producing new synapses (Black et al., 1990). Potential variations in capillary density are rarely discussed in relation to morphometric brain alterations, although they have been observed during functional activation in living human brains (Kuwabara et al., 1992). Consequently, the use of complementary methods, such as cortical evoked potentials recorded after training, might be a reasonable addition to rule out such causes when an increase in gray matter density is observed (May et al., 2007).

Ilg et al. (2008) showed increases in gray matter density induced after only short daily practice sessions. Additionally, changes corresponded to the location of practice-specific functional activation and not to the global extent of activation associated with the task. In light of these findings, the authors postulate that the gray matter density changes were linked to modifications of the axonal architecture and are unlikely attributable to the production of neurons or glia. Indeed, morphological changes in the brain have been observed over a period as short as 5 d of low-frequency transcranial magnetic stimulation (May et al., 2007), indicating that fast adjusting processes such as synaptic remodeling appear to underlie the

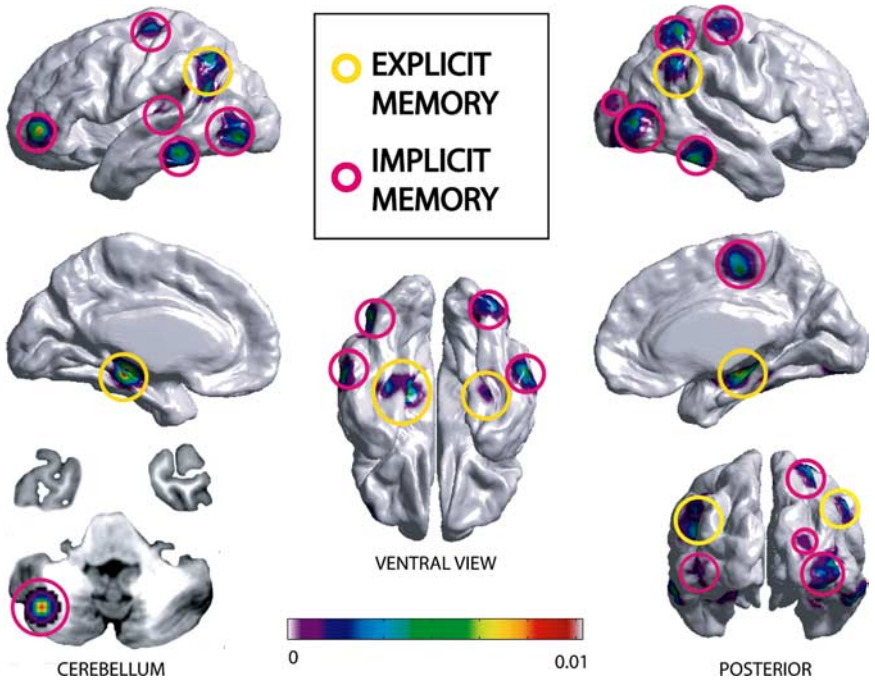

Figure 1. Meta-analysis of voxel-based morphometric studies reporting increased gray matter density after learning in the cortex and cerebellum. Factual knowledge that is recalled by a purposeful effort requires the involvement of the explicit memory system. It is involved in tasks such as spatial navigation (Maguire et al., 2000) and intensive studying (Draganski et al., 2006). Implicit memory refers to intrinsic knowledge about how to perform an action and includes language learning (Mechelli et al., 2005), juggling (Draganski et al., 2004), mirror reading (Ilg et al., 2008), and musical training (Gaser and Schlaug, 2003). The *x*, *y*, and *z* coordinates reported in these studies corresponding to increased density in brain regions were compiled and used to generate an activation likelihood estimate (ALE) map (www.brainmap.org). Coordinates are displayed on an average cortical surface in standard stereotactic space using SurfStat (http://www.math.mcgill.ca/keith/surfstat/). The ALE map reflects the probabilistic likelihood of increased gray matter density occurring within the six studies. Results demonstrate that although structural changes occur in functional areas related to the task, increases also occur in associative areas such as the posterior parietal and temporal cortices. Furthermore, studies examining explicit learning showed an overlap of increased gray matter density in the hippocampal gyrus.

anatomical changes. Neurogenesis as a potential underlying mechanism may not be entirely ruled out because many studies in vertebrates have demonstrated the production of new neurons throughout adulthood, particularly in the hippocampal formation, and show that these newly generated neurons are able to form connections with the CA3 region as soon as 1 or 2 weeks after mitosis (Gould et al., 1999).

In conjunction with these new morphometric techniques, complementary research is needed in animal models to understand the exact molecular events underlying experience-dependent plasticity seen in humans. Indeed, our interpretation of the various findings can hardly be conclusive until learning-dependent morphometric changes observed in animals are coupled with histological and immunological data. Nevertheless, this new exciting field of research undoubtedly highlights the remarkable potential of the adult brain to undergo anatomical changes that have a great impact on its functioning. Improved understanding of experience-dependent changes in cortical plasticity has vast clinical implications for neurorehabilitation programs after stroke, as well as for treatments of chronic pain that focus on use-dependent plasticity to improve mobility and alleviate pain.

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2007-2008	**CRIUGM brain imaging seminar series**
2003	**Medical Imaging Computing and Computed Assisted Intervention**

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