Acute Experimental Muscle Pain;

Spatiotemporal Expression of

Local and

Referred Pain in Human Subjects
Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.
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This thesis is assembled from four publications submitted during the course of my PhD. As at the date of submission, three of these papers have been accepted.

**PUBLICATIONS**

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**SUBMITTED:**

ABSTRACT

Each and every scientific observation is precisely defined by the state of the variables affecting the observed system. In this respect the choice and validation of a given experimental model is as important as the as the observations recorded. This thesis presents a series of studies utilising and developing the model of hypertonic saline induced human muscle pain to further our understanding of the peripheral and central spatiotemporal characteristics of local and referred pain. In the first three studies an adaptation of the classical (Kellgren 1938) model of hypertonic induced muscle pain utilises increased sample frequency to quantify the percept of local and referred pain; in the absence of local pain; over the course of multiple stimuli within the same subjects and between different limbs with differing stimuli intensities. In the last study we utilise functional magnetic resonance imaging to characterise somatotopy of pain from this model within the human insula.

The first study examines the effects of local pain blockade on the expression of local and referred pain. This chapter is based on a publication in Journal of Pain titled “Effects of Intramuscular Anaesthesia on the Expression of Primary and Referred Pain Induced by Intramuscular Injection of Hypertonic Saline” (Rubin, Gandevia et al. 2009). Intramuscular injection of hypertonic saline produces pain in the belly of the injected muscle (primary pain) and, pain that projects distally (referred pain). While it is known that referred pain can be induced during complete sensory block of the distal site, there is little evidence as to whether the perception of referred pain depends on ongoing input from the primary stimulus. We assessed whether blocking the noxious input following the induction of pain blocks the primary but not the referred pain. In all subjects, the area and intensity of primary pain rapidly disappeared within 7.5 minutes of intramuscular anaesthesia. With the exception of 2 subjects, in whom the referred pain continued in the absence of primary pain, the referred pain declined in parallel with local pain: the mean total pain intensity declined by 74% in both regions. We conclude that the maintenance of referred muscle pain usually depends on ongoing noxious inputs from the site of primary muscle pain. Referred pain is a significant clinical problem, and commonly occurs with pain originating in muscle but not from skin. It is important to know the primary source of the pain so that treatment can be directed to this site rather to the site of referral.

What happens to the intensity of pain induced by repeated noxious stimuli over time? Does it stay the same, increase or decrease? In the second study we tested the hypothesis that the intensity and area of pain in the local and referred regions exhibits
plasticity when an identical noxious stimulus is delivered to the same site over sequential trials. In this repeated measures study over 4 weeks we demonstrated a progressive reduction in the area and intensity of local pain and a reciprocal increase in the expression of referred pain. We conclude that the decrease in perceived local pain and increase in perceived referred pain reflects plastic processes occurring centrally. We show that weekly injections of hypertonic saline into tibialis anterior cause decreases in local but increases in referred pain, suggesting central changes in processing noxious inputs.

While current data suggests that all referred pain derives from common mechanisms of central sensitisation, there is a paucity of data directly comparing referral in different limbs. Does a common mechanism result in similar precepts of referral from identical stimuli in different limbs? We tested the hypothesis that the incidence, intensity and spatiotemporal expression of referred pain are identical during the muscle pain induced by bolus intramuscular injection of hypertonic saline into flexor carpi radialis (FCR) and tibialis anterior (TA). We also tested the hypothesis that an increase in stimulus intensity causes a parallel increase in the incidence and intensity of local and referred pain, by comparing the responses to 5% and 10% hypertonic saline in two groups of subjects. 36 subjects mapped areas of local and referred pain, rating intensities on a visual analogue scale, every 30s until the cessation of pain. Local pain from injections of the 10% solution into either muscle was significantly higher than that produced by injection of the 5% solution. However, while the mean intensity of referred pain was consistently greater after the 10% injections, the increase was greater for FCR than for TA. Our observation that increasing concentrations can have dramatically different effects on referred pain suggest that local pain may be related to a saturation in the perception of local pain rather than a saturation of afferent barrage. Furthermore, the relationship between the perceptual saturation of local pain and the expression of referred pain may vary from muscle group to muscle group.

It is well established that the insula cortex processes noxious information. We have previously shown that noxious inputs from the arm and leg are coarsely organized somatotopically within the dorsal posterior insula. The same has been shown for inputs from C tactile afferents, which mediate affective touch, and it has been suggested that the insula may be responsible for the localization of some somatosensory stimuli. Knowing the degree of spatial detail may have significant implications for the potential role of the dorsal posterior insula in the processing of noxious stimuli. Using high-resolution functional magnetic resonance imaging (fMRI) we compared insula activation patterns during muscle pain induced by injection of hypertonic saline into
three muscles within the same limb: shoulder (deltoid), forearm (flexor carpi radialis) and hand (first dorsal interosseous). Mapping the maximally activated voxels within the contralateral dorsal posterior insula in each individual subject during each pain stimulus revealed a clear somatotopy of activation within the contralateral dorsal posterior insula. Shoulder pain was represented anterior to forearm pain and medial to hand pain. This fine somatotopic organization may be crucial for pain localization or other aspects of the pain experience that differ depending on stimulation site.
ABBREVIATIONS

ACC  anterior cingulate cortex
AMH  A mechano heat sensitive
ANOVA analysis of variance
ATP  adenosine triphosphate
BG   basal ganglia
BOLD blood oxygen-level dependent
CB   cerebellum
CMH  C mechano heat sensitive
CNS  central nervous system
CT   C-tactile
D1   definition 1
D2   definition 2
DOMS delayed onset muscle soreness
dpi  dots per inch
EEG  electroencephalographic recording
FCR  flexor carpi radialis
FDI  first dorsal first dorsal interosseous
fMRI functional magnetic resonance imaging
HPC  heat-pinched cold
HS   hypertonic saline
IASP International Association for the Study of Pain
IC   insular cortex
LTP  long-term potentiation
M1   primary motor cortex
MDvc medial dorsal nucleus
MEG  magnetoencephalographic recording
MIA  mechano-insensitive afferent
MN   Montreal Neurological Institute
MSA  mechanically sensitive afferent
NCF  nucleus cuneiformis
NGC  nucleus reticularis gigantocellularis
NMDA N-methyl-d-aspartate
NS   nociceptor-specific
PAG  periaqueductal grey
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tbody>
<tr>
<td>PCC</td>
<td>posterior cingulate</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PPC</td>
<td>posterior parietal cortex</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SI</td>
<td>primary somatosensory cortex</td>
</tr>
<tr>
<td>SII</td>
<td>secondary somatosensory cortex</td>
</tr>
<tr>
<td>SMA</td>
<td>supplementary motor cortex</td>
</tr>
<tr>
<td>SPECT</td>
<td>single photon emission computed tomography</td>
</tr>
<tr>
<td>STT</td>
<td>spinothalamic tract</td>
</tr>
<tr>
<td>TA</td>
<td>tibialis anterior</td>
</tr>
<tr>
<td>Th</td>
<td>thalamus</td>
</tr>
<tr>
<td>TRPV1</td>
<td>transient receptor potential cation channel, subfamily</td>
</tr>
<tr>
<td>VAS</td>
<td>visual analogue scale</td>
</tr>
<tr>
<td>VMb</td>
<td>basal ventral medial nucleus</td>
</tr>
<tr>
<td>VMpo</td>
<td>ventral medial nucleus</td>
</tr>
<tr>
<td>VPI</td>
<td>ventral posterior inferior nucleus</td>
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<tr>
<td>VPL</td>
<td>ventral posterior lateral thalamus</td>
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<tr>
<td>WDR</td>
<td>wide dynamic range</td>
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ACKNOWLEDGEMENTS

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Great science only comes about through the collaboration of great people.
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CHAPTER 1

GENERAL INTRODUCTION
1.1 THE SENSATION OF PAIN

The International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merksey, Albe-Fessard et al. 1979). While commonly thought of as a sensation, pain is best considered as a percept - a painful experience may arise without any nociceptive afferent input at all (Eisenberger, Lieberman et al. 2003; Derbyshire, Whalley et al. 2004; Singer, Seymour et al. 2004). Pain is a unique sensory experience in that it includes both a physical and an emotional component. Indeed, it was the 17th century philosopher Benedict Spinoza who described pain as “a localised form of sorrow”. An important component of the IASP definition encompasses pain that may derive from “potential tissue damage.” Indeed, while the sensation of pain is usually initiated by a peripheral noxious stimulus, the perception of pain can be generated in the absence of such a stimulus by spontaneous activity in second-order or high-order neurones anywhere along the spinothalamic (or trigeminothalamic) tracts, such as within the thalamus. In addition to the sensory-discriminative aspects of pain emotional processes are responsible for the affective aspects of pain perception. While certain pain pathologies may be maintained entirely by central mechanisms in the absence of peripheral input (Ploner, Freund et al. 1999), most pain sensations are likely to be a complex interplay between peripheral nociception and central modulation.

1.1.1 The subjective nature of pain

Pain is a subjective experience, the quality and magnitude of which by its nature is impossible to precisely convey. This subjective emotional component is of course unique to the individual and dependent on numerous factors including, but not limited to, past experiences, past and present state of depression, current state of anxiety or arousal, levels of expectation and mood state. Some individuals will consider a mild noxious stimulus in terms of the worst possible outcomes, exaggerating their percept of pain (catastrophising), even though the sensation per se may not be severe.

While the peripheral apparatus of pain produces a far less varied sensory input, it is extremely convoluted relative to other sensory systems. Instead of specialized nerve endings on unique afferents as in other sensory systems, pain is largely mediated by free nerve endings utilising multiple specialized receptors on differing classes of afferent. These fibres often have sensitivity to multiple modalities of noxious stimuli. Spinal interconnectivity (Hoheisel, Mense et al. 1993; Andrew 2010) between nociceptive and nonnociceptive afferents together with convergence tactile and nociceptive inputs. Indeed, nociceptive and tactile inputs are well known to interact,
with tactile sensations able to modulate the percept of pain and vice versa. This is most prominent in cases of underlying pathology. If this wasn’t yet a reductionist’s worst nightmare, input from primary nociceptive afferents is almost immediately the subject of a powerful descending modulatory system as it enters the spinal cord. Couple this with a large degree of independent plasticity at almost every step of neural processing and we see one of the best examples of evolution driving the genesis of a system on the basis of function rather than form.

1.1.2 The Measure of Pain

Pain can be distinguished by its time course – acute, tonic or chronic (pain lasting >3 months) – or its spatial extent. Pain can also be delineated on the basis of language, with the use of words describing sensory qualities, affective qualities and evaluative qualities (Melzack and Torgerson 1971). The McGill Pain Questionnaire - utilising these three classes of terms to form a pain rating index - together with a Present Pain Intensity index has been reliably validated (Melzack and Katz 1994) and is frequently used in its complete or short (Melzack 1987) form.

The quality of pain depends on the tissue from which the pain originates. For instance, the deep, dull ache of muscle pain is consistently different from the sharp or burning pain generated in the skin (Melzack 1987). Most pain originating from connective tissues such as fascia, ligaments and tendon shares the deep quality of muscle pain (Graven-Nielsen 2006).

The intensity of pain is frequently measured with a visual analogue scale (VAS). In this assessment subjects are asked to mark a position along a continuous 10 cm line between two points representing “no pain”, generally on the left, and “worst pain” on the right (Graven-Nielsen 2006). This scale is often adapted to produce a continuous readout by incorporating a linear or radial potentiometer that is calibrated to the VAS. These psychometric measures are representative of the cumulative affective and nociceptive components of pain - in this sense, the pain percept. As described below, the nociceptive input is subject to modulation from the first synapse within the dorsal horn.

1.2 THE NEURAL SUBSTRATES OF PAIN

The neurophysiology that underlies pain has been heavily researched, particularly with respect to the peripheral machinery that encodes noxious stimuli. Psychophysical
approaches have provided information on the interindividual differences in the perceptions of pain. Electrical recordings from structures within the spinal cord and brain, and more recently non-invasive brain imaging techniques, have provided more information on the central neural machinery that is responsible for our perceptions of pain.

1.2.1 Afferent sources of pain

As noted above, a perception of pain can be elaborated in the absence of a nociceptive input. In this section, I deal only with nociceptor induced pain.

Table 1.1: Correlations between axonal diameter, conduction velocity and function in peripheral nerves. This shows the lower conduction velocity of A fibres as their diameter decreases, and their function and equivalent classification based on axonal diameter. The Erlanger and Gasser’s classification is also compared for the larger B and smaller C fibres. *This A-C classification was introduced by Joseph Erlanger and Herbert Gasser, who shared the 1944 Nobel Prize in Medicine or Physiology for describing the relationship among axon diameter, conduction velocity, and function in a complex peripheral nerve (Erlanger 1924). Adapted from (Boron and Boulpaep 2003).

1.2.1.1 Classification of afferents

Nerve fibres can be classified according to either conduction velocity or axonal diameter. Both features are related – the larger the diameter of an axon the greater the conduction velocity. Axons were initially differentiated on the temporal dispersion of voltage peaks from in vitro recordings of compound nerve action potentials (Erlanger 1924); this was later resolved for afferent fibres and then efferent fibres (Eccles and Sherrington 1930; Bessou 1963; Bessou, Emonet-Dénand et al. 1965) (Table 1.1, adapted from (Boron and Boulpaep 2003). Aα and Aβ fibres are thicker myelinated nerve fibres with faster conduction velocities, while C fibres are unmyelinated and
conduct slowly. An alternative classification (for sensory fibres) based on axonal diameters was provided by Lloyd (Lloyd 1943): type I–fibres with diameters from 13 to 20 μm found only in afferent muscle are effectively equivalent to the Aα fibres; type II–fibres with diameters from 6 to 12 μm, predominantly cutaneous nerves and infrequently observed in muscle nerves are equivalent to the Aβ fibres; type III–fibres with diameters between 3 and 4 μm, and type IV unmyelinated fibres equivalent to the C fibres of the previous classification. Further complicating matters, the previous delineations of A fibres are dependent on the species from which they are measured (Djouhri and Lawson 2004).

### 1.2.1.2 Cutaneous nociceptors

Given that the skin forms a fundamental barrier between the internal and external environment, nociceptors in the skin have evolved to respond to the full complement of stimulus energies (thermal, mechanical and chemical) to which they are exposed, but with differing sensitivities. Many fibres are polymodal. Cutaneous nociceptors are defined by their sensitivity to different stimuli and their fibre class; in this manner, C fibre mechano-heat sensitive fibres are termed CMHs, or AMHs for Aδ fibres. Similarly, Aδ fibres responsive to mechanical pressure are termed mechanically sensitive afferents (MSA). Though both C (Torebjork, LaMotte et al. 1984) and A (Davis, Meyer et al. 1993) afferents have differing components of chemoreceptive fibres this modality is often unspecified or included in the term, polymodal. Activation of cutaneous CMHs is typically correlated with burning sensations (Schmidt, Schmelz et al. 1997) from stimuli in excess of 39-41 °C (Tillman, Treede et al. 1995). Mechano-insensitive afferents (MIAs) display heterologous responses to chemical and heat stimuli though are in general more responsive to histamine and capsaicin than CMH's; they have high mechanical threshold and a responsiveness that parallels the perception of burning and itching pain (Schmelz, Schmidt et al. 1997; Schmidt, Schmelz et al. 2000). Notably, there is little correlation between the heat thresholds and the sensitivity to mechanical sensibility (Davis, Meyer et al. 1993).

Polymodal chemoreceptive C and Aδ fibres play a significant role in the detection of tissue damage. The ensuing inflammatory response involves the release of numerous endogenous chemical mediators from non-neuronal cells and the afferent fibres themselves. These chemicals include bradykinin (Dray and Perkins 1993), prostaglandins (Schmelz, Schmidt et al. 2003), leukotrienes (Martin, Basbaum et al. 1987), serotonin (Taiwo and Levine 1992), histamine (Handwerker, Forster et al. 1991), substance P (Dubner and Ruda 1992), thromboxanes, platelet-activating factor, adenosine and ATP (Aley, Green et al. 1995), protons, cytokines (Sommer and Kress...
and free radicals. Some of these agents act on inflammatory cells - such as macrophages and mast cells that subsequently release algogenic substances - while others bind directly to nociceptive ligands. Release of these mediators often results in the sensitisation of peripheral afferents, underlying the cellular basis of primary hyperalgesia (increased responsiveness to noxious stimulus).

In the skin, the activation of C fibre nociceptors are generally associated with with burning pain, this is termed slow pain or second pain. Conversely, sharp pricking pain in the skin – the so-called first pain – is mediated by A fibre nociceptors, which provide more spatially discrete sensations (Ploner, Gross et al. 2002). Classification of these thicker, myelinated Aβ and Aδ fibres is similarly based on their responsiveness to differing stimuli, though two distinct groups have become apparent (Treede, Meyer et al. 1998)(Table 1.2, adapted from Textbook of pain 5th edition). Type I and type II Aδ fibre nociceptors are delineated primarily on their response properties to heat and their mechanical sensitivity. Typically responsive to chemical, mechanical and intense heat stimuli, type 1 fibres are often called high-threshold mechanoreceptors, AMH’s or polymodal receptors. They readily sensitise to heat injury, displaying increasing responsiveness to intense heat stimuli. Conversely type II Aδ fibres are mechanically insensitive, and frequently adapt to heat stimuli.

The responsiveness of C fibres is strongly influenced by stimulus history. Microneurographic studies in human subjects have shown that repetitive electrical stimulation of C fibres gradually decreases conduction velocity in an activity-dependent manner. During low-frequency electrical stimulation of the receptor terminals, changes in latency of the recorded action potentials can be used to identify different C fibres, each of which has a different conduction velocity; activity-dependent slowing is used to document activation of C-fibres during mechanical, thermal or chemical stimulation of the receptor. These decreases are more significant in C MIA’s than C MSA’s (Weidner, Schmelz et al. 1999). Fatigue in CMH’s can be observed after heat stimuli (LaMotte and Campbell 1978) or cross-modally through mechanical stimuli (Peng, Ringkamp et al. 2003) in a manner dependent on the interstimulus interval. Sensitisation of C fibres, has been used to explain the hyperalgesia frequently observed following tissue injury (Torebjork, LaMotte et al. 1984).

Although cutaneous C fibres have classically been thought to transmit exclusively nociceptive and thermal (warm) information, there is another class of afferent C fibre – the C tactile (CT) afferents. These afferents, present only in hairy skin, are particularly sensitive to light stroking of the skin (Vallbo, Olausson et al. 1999; Wessberg,
It has been suggested that C-tactile fibres subserve an affective component of touch, engaging areas of the brain involved in the processing of emotion rather than those comprising the discriminative somatosensory system. Indeed, studies in two patients lacking large-fibre sensory axons yet preserved small-fibre function reveal that their capacity to feel this light touch remains. Moreover, the stroking stimuli feel “pleasant”, and as described by Olausson and colleagues (Olausson, Cole et al. 2008), functional magnetic resonance imaging (fMRI) studies have shown that light stroking of the hairy skin in these patients activates the posterior insular cortex but not the primary somatosensory cortex. The contribution of C-tactile fibres to affective touch has been further explored by (Loken, Wessberg et al. 2009).

Table 1.2; Differentiation of Type I and Type II A fibres based on their heat threshold, adaptation in response to heat stimuli, latency in response to heat, mechanical threshold, conduction velocity, sensitization and location.

1.2.2 Muscle nociceptors

Deep penetrating injuries will typically activate nociceptors in muscle and other deep tissues. Like nociceptors in skin, those in muscle are defined according to their sensitivity to mechanical, thermal and chemical stimuli. Accordingly, similar nomenclature is used to describe the nociceptive structures responsible for muscle pain, such that squeezing (Mense and Meyer 1985) or pressure (Marchettini, Simone et al. 1996) mediated pain would be deemed to derive largely from high-threshold mechanosensitive (HTM) muscle receptors. As observed in cutaneous CMHs and AMHs, muscle nociceptors responsible for mechanically induced pain are frequently polymodal to chemical stimuli or heat stimuli (Kumazawa and Mizumura 1976; Caterina and Julius 2001; Hoheisel, Reinöhl et al. 2004).

Muscle and other deep structures have free nerve endings that are primarily believed to originate in the walls of arterioles (Stacey 1969; Graven-Nielsen and Mense 2001). Electrophysiological recordings have shown that some of these muscle afferents, i.e.

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33% of Group III and 43% of Group IV, are nociceptive (Graven-Nielsen, Arendt-Nielsen et al. 2002). The distal sensory ending of group III and IV nerve fibres consists only of the sensory axon and associated Schwann cells but lacks a myelin sheath and is not surrounded by perineurium. The nature of the model systems utilised to elucidate these fine nerve structures makes it difficult to determine their specific receptor modality. Electron microscopy of the sensory axon reveals significant arborisation at the nerve terminals with branches in the order of 200um for group III fibres and 300um for group VI fibres (Heppelmann, Messlinger et al. 1990). The branches traverse independently or in parallel within small Remak bundles along vessel walls and also extend into dense connective tissue. The number of C fibre axons in each Remak bundle varies with location with animal studies revealing smaller numbers of C fibres in distal nerves relative to those terminating in more proximal regions (Murinson and Griffin 2004).

1.2.3 Spinal processing of noxious stimuli
Almost all nociceptive primary afferents enter the dorsal horn of the spinal cord and arborise to form rostro-caudal projections (Noback, Strominger et al. 2005). Patterns of termination in the dorsal horn are primarily related to axonal diameter and receptive field modality. The dorsal root ganglion contains the cell bodies of primary nociceptive neurones, which send axons that synapse primarily within the superficial laminae of the dorsal horn. This region of the spinal cord contains intrinsic or interneurones that synapse locally, projection neurones that pass into the white matter with rostrally extending axons, and descending neurones from higher brain centres that are believed to be involved in top-down modulation of spinal processing. The feline dorsal horn was initially divided into 6 parallel laminae on the basis of local cytoarchitecture, known as the laminae of Rexed (Rexed 1952). Laminae I and II, the latter of which can be subdivided into two parts, lamina II inner (IIi) and lamina II outer (Iio), are generally referred to as the superficial dorsal horn. While the initial afferent termination patterns (Cervero and Iggo 1980) described by electrophysiological studies have given way to a more convoluted picture, there are, however, a few general principles of organization (Cervero and Jensen 2006). Lamina I, also known as the marginal zone and laminae II, the substantia gelatinosa, primarily receive input from small diameter (group III and group IV) neurones. Large diameter Aβ fibers, such as low-threshold cutaneous mechanoreceptors, terminate in laminas III, IV, V and the dorsal part of lamina VI (Brown 1981). Nociceptive afferents from muscle and joints in animal models have been observed to terminate in laminae I, V–VII but not laminae II, III or IV (Mense 1986). By contrast, fine afferents from the viscera project to lamina I and bilaterally to laminae V and X (Sugiura, Terui et al. 1989; Mizumura, Sugiura et al. 1993), though
these findings are likely to vary widely dependent on the species studied. In general, large diameter axons enter medially while small diameter fibres enter more dorsally via the lateral funiculus. Two main projection systems are known to convey noxious information to higher centres: the spinothalamic and spinobulbar projections of the anterolateral tract. Classes of neurones defined by these tracts differ physiologically, morphologically, and biochemically; furthermore, their activity corresponds with distinct sensations, albeit after integration in the forebrain (Craig, Krout et al. 2001; Craig 2003).

1.2.3.1 Spinothalamic projections
The spinothalamic (and trigeminothalamic) pathways of the anterolateral system have been implicated in pain for over 100 years. Lesions at sites as caudal as the segmental innervation to medullary, mesencephalic or thalamic levels manifest as ipsilateral analgesia and therm anaesthesia (Craig and Burton 1981). Mammalian retrograde staining (Craig 2003) and human post-mortem studies (Kuru and Takase 1947) demonstrate three distinct origins of spinothalamac tract (STT) cells. Though marginal interspecies differences exist, 50% of primate STT cells synapse in laminae I, 25% originate in laminae IV–V and about 25% in laminae VII and VIII. As demonstrated by pathological conditions 85-90% of STT cell axons decussate in the commissure of the grey matter and ascend on the contralateral side before converging at the medulla and ascending to higher centres (Boivie 1971).

1.2.3.2 Spinobulbar projections
Spinal projections to the lower brainstem form essential linkages between nociceptive afferent activity and homeostatic processes. Integration in these primordial nuclei precedes subsequent interaction with forebrain centres and subsequent descending modulation. Retrograde staining in the monkey, cat and rat reveals simular constituents of collaterals from laminae I, V and VII to that seen in STT projections (Wiberg, Westman et al. 1987). These projections, while ascending in the same region of the STT, are believed to terminate bilaterally in the ventrolateral medulla (A1-), parabrachial nucleus, the periaqueductal grey (PAG) and the reticular formation (Basbaum, Clanton et al. 1978; Fields and Basbaum 1978). While laminae I projections innervate all but the later nuclei, laminae V and VII cells primarily project to the reticular formation (Andrew, Krout et al. 2003).

There are three functional classes of second order nociceptive neurones in the dorsal horn: the nociceptor-specific (NS), wide dynamic range (WDR) neurones (Simone, Sorkin et al. 1991) and the polymodal nociceptive heat-pinsh-cold (HPC) neurones.
HPC cells (usually multipolar cells) display little ongoing discharge after stimuli whereas NS (fusiform) cells display no ongoing discharge. These cells receive predominantly C and Aδ fibre inputs, respectively (Craig 2003). Static muscle contraction activates spinobulbar projections of lamina I neurones that either respond selectively to muscle afferents or have cutaneous HPC responses with convergent input from muscle (Wilson, Andrew et al. 2002). Consistent with the properties of muscle Aδ and C fibres, some of these cells respond during and some after the contraction. While some of these projections are selective to mechanoreceptive input (from muscle contraction) others respond to lactic acid and other metabolites (i.e. they were chemoreceptive). It is proposed that these cells integrate tissue metabolic needs, and dynamically drive a variety of regional and whole-body homeostatic adjustments to muscular work, including the exercise pressor reflex (Wilson and Hand 1997; Kaufman and Hayes 2002; Craig 2003) and somato-autonomic reflex responses (Sato and Schmidt 1973).

As implied by their name, WDR neurones in the spinal cord act as convergent centres from multiple modalities of touch and pain (nociceptive, tactile, itch and temperature) generally with greater responses to noxious stimuli. More recently WDR neurones, also known as Class 2 cells, have become synonymous with multi receptor cells and convergent neurones with receptive fields in multiple tissue types such as skin + viscera or deep muscle. Laminae V is primarily populated by WDR neurones integrating large-diameter input from cutaneous and deep tissues together with polysynaptic C and Aδ fibre input. They integrate sensory input from all afferent modalities, including tactile, mechanoreceptive and proprioceptive input (Surmeier, Honda et al. 1988). The dendrites of these large cells often extend across much of the dorsal horn and are likely to account for their equally large receptive fields. In contrast to laminae I neurones, the WDR cells of laminae V are not somatotopically organised but are instead regarded as musculotopically organised (Nyberg and Blomqvist 1984; Schouenborg, Weng et al. 1995). It has been postulated that the WDR neurones of lamina V are likely to account for the increased responsiveness (wind-up) observed in hyperalgesia and allodynia (Maixner, Dubner et al. 1989), particularly notable after repetitive C fibre stimulation.

Independently, neurones projecting to the various ascending tracts may be classified on the basis of their gross response to innoxious or noxious stimuli - low threshold and high threshold respectively. Further diversifying the action of WDR neurones is the presence of inhibitory receptive fields, whereby afferent input from one region or
modality inhibits the effective output of another. Indeed, neuronal inhibition in the spinal cord, as in the brain, plays as significant a role as neuronal excitation.

The WDR neurone became widely popularized in the convergent view of pain, and indeed lay at the heart of the “gate control theory” by Melzack and Wall in 1965 (Melzack and Wall 1995). This theory postulated that large diameter (group II) fibres inhibited the small fibre (group III and IV) activation of WDR cells thereby closing the gate. The ability of a distant noxious stimulus to inhibit a local sensation - “counter-irritation” of pain - is said to result from inhibitory WDR neurones with wide large receptive fields. (Zahn, Pogatzki et al. 2002; Brennan, Carr et al.; Jang, Clark et al. 2010). The gate control theory in many ways marked a shift in our understanding of the dorsal horn from a simple relay nucleus to a fundamental modulatory centre of pain processing. Though the subject of much controversy (Nathan 1976), and ultimately an oversimplification (Craig 2003), the concept of gating is now fundamental to our appreciation of afferent integration within the spinal cord.

1.3 SUPRASPINAL PROCESSING OF NOXIOUS STIMULI

While our initial neuroanatomical understanding of pain processing derived from animal and human lesion studies, the development of non-invasive neuroimaging techniques has enabled great advances in our knowledge of the neural correlates of the sensory processing of noxious information and the perception of pain. These techniques - including positron emission tomography (PET), functional magnetic resonance imaging (fMRI), magnetoencephalographic recording (MEG), single photon emission computed tomography (SPECT) and electroencephalographic recording (EEG) - each have their strengths and weaknesses, based on sensitivity and differing spatio-temporal acuity. Elements of this thesis dealing with neuroimaging exclusively employ the imaging technique of fMRI.

1.3.1 Functional magnetic resonance imaging

The most common type of fMRI, blood oxygen-level dependent (BOLD) MRI, is so named because it differentiates between diamagnetic (oxygenated) and paramagnetic (deoxygenated) haemoglobin. Within the constraints of signal and noise at a given spatial resolution, this technique is thus able to measure deoxygenation of
haemoglobin. This molecular reaction is known to result from aerobic glycolysis and thus approximate regional synaptic activity (Siesjö 1978; Schwartz, Smith et al. 1979; Casey, Minoshima et al. 1995). With the assumption that neurones have minimal energy stores for anaerobic metabolism, the so-called hemodynamic response increases neuronal blood flow to energetically active regions within a timeframe of 1-4 seconds. Increases and decreases are measured relative to a baseline. In this respect the explicit controlled nature of the baseline can be as fundamentally important to the outcome of an imaging study as the stimuli itself (Gusnard and Raichle 2001).

1.3.2 The neuromatrix of pain
After hundreds of imaging studies examining the cortical and subcortical processing of pain in nonclinical subjects, a somewhat consistent anatomical pattern of altered neuronal activity has emerged. Initially referred to as the neuromatrix (Melzack 1999), and more recently the “Pain Matrix” (Singer, Seymour et al. 2004), this network includes the primary and secondary somatosensory cortices (SI & SII), anterior cingulate cortex (ACC), insular cortex (IC), prefrontal cortex (PFC), thalamus (Th) and cerebellum (CB). Other areas less consistently demonstrating pain-related increases in activation include the prefrontal cortex (Wager, Rilling et al. 2004; Jackson, Brunet et al. 2006), the basal ganglia (Chudler 1998), cerebellum (Helmchen, Mohr et al. 2003; Saab and Willis 2003), amygdala (Becerra, Breiter et al. 2001), hippocampus, and areas within the parietal and temporal cortices. It must be reiterated that pain associated changes in sensory, “limbic”, associative and motor areas are representative of a multidimensional experience that in many respects is unique to each individual. This is particularly pertinent in patients experiencing chronic pain, ongoing or spontaneous pain (Baliki, Chialvo et al. 2006).
Figure 1.1; Brain regions commonly activated in pain studies Adapted from (Price 2000; Apkarian, Bushnell et al. 2005). Cortical and sub-cortical regions involved in pain perception, their inter-connectivity and ascending pathways. Locations of brain regions involved in pain perception are colour-coded in A, a schematic drawing; and B; their location plotted over sagittal MRIs. (A) Schematic shows the regions, their inter-connectivity and afferent pathways. The schematic is modified from (Price 2000) to include additional brain areas and connections. (B), The areas corresponding to those shown in the schematic are shown in an anatomical MRI, on a coronal slice and three sagittal slices (1,2 and 3) as indicated on the coronal slice (Apkarian, Bushnell et al. 2005).

Regions indicated included the thalamus (Yellow), Primary and secondary somatosensory cortices (S1, SII, red and orange), anterior cingulate (ACC, green), insula (light blue), and prefrontal cortex (PF, purple). Other regions indicated include: primary and supplementary motor cortices (M1 dark blue and SMA), , pink), hypothalamus (HT), amygdala (AMYG), parabrachial nuclei (PB), and periaqueductal gray (PAG).

1.3.3 Thalamus
The thalamus is a fundamental relay structure for somatosensory information travelling to the cortex. The nociceptive processing and relaying properties of the thalamus are defined by its spinal inputs, the majority of which originate in the lateral laminae of the dorsal horn. The ventral posterior lateral thalamus (VPL) in particular is known to receive both noxious and innocuous stimuli from cutaneous, muscular and visceral origin (Almeida, Roizenblatt et al. 2004). Direct lamina I spinothalamic tract (STT) projections are observed in numerous nuclei of the thalamus, including the ventral posterior nucleus (VP), the posterior part of the ventral medial nucleus (VMpo) (Craig, Bushnell et al. 1994), ventral posterior inferior nucleus (VPI) and the ventral caudal division of the medial dorsal nucleus (MDvc). STT axons from lamina V are seen to terminate in the VP, VPI, ventral lateral nucleus and the intralaminar nuclei, which project to the basal ganglia and to motor and parietal cortices (Craig 2003; Pralong, Pollo et al. 2004; Montes, Magnin et al. 2005; Seghier, Lazeyras et al. 2005).

Involvement of the thalamus in nociceptive processing has been substantiated by numerous studies of healthy and pathological conditions in humans and animals. Microstimulation of the VMpo within awake humans induces discrete, well-localized pain, cooling or visceral sensations (Hassler and Riechert 1959; Davis, Lozano et al. 1999). In healthy subjects, stimulation of the VP elicits localised paraesthetic sensations and has been used effectively to treat chronic pain (Craig 2003), while patients with
neuropathic pain report an increase in pain during electrical stimulation of the VP (Bittar, Kar-Purkayastha et al. 2005). In addition, PET studies have demonstrated hypoperfusion (indicative of reduced neuronal activity) in the contralateral thalamic in patients with cancer pain (Di Piero, Jones et al. 1991).

1.3.4 Thalamic projections
Anterograde tracing studies demonstrate VMpo and VMb projections to the fundus of the superior limiting sulcus at the dorsal margin of insular cortex (Frot and Mauguiere 2003) and area 3a at the fundus of the central sulcus (Craig 2003). The MDvc projects to Brodmans area 24c in the fundus of the anterior cingulate sulcus (Craig, Bushnell et al. 1994; Vogt 2005). The spatial discriminative aspects of the somatosensory cortices are thought to be mediated by connections from the ventral posterior nuclei, particularly VPL, to SI and SII (Friedman and Murray 1986; Shi and Apkarian 1995). The VPM is understood to have a similar sensory discriminative role as the VPL, though the convergence of fibres originating from the parabrachial region and the paratrigeminal nucleus, its projections to the prefrontal cortex, together with the interconnections with the amygdala, hypothalamus and PAG, suggests an involvement in the emotional aspects and autonomic responses of pain (Li, Kaneko et al. 1997; Gauriau and Bernard 2002; Monconduit, Bourgeais et al. 2002; Almeida, Roizenblatt et al. 2004).

1.3.5 Lateral and medial components of the pain neuromatrix
Current conceptual models of the pain neuromatrix suggest lateral (sensory-discriminatory) and medial (affective-cognitive-evaluative) neuroanatomical components (Albe-Fessard 1985; Tracey and Mantyh 2007). In this respect the somatosensory cortices are primarily thought to encode the intensity and spatiotemporal qualities of pain (Kenshalo and Isensee 1983; Chudler, Anton et al. 1990), together with SII and the posterior insula, while the so-called limbic and paralimbic regions - such as the PFC and ACC - play a more prominent role in the emotional and motivational aspects of pain. Supporting this view, lesion studies demonstrate a sensory discriminatory role of SI and SII (Greenspan, Lee et al. 1999; Ploner, Freund et al. 1999) though in the latter, MRI suggests that the lesion also included regions of the dorsal posterior insular cortex. PET studies (Rainville, Duncan et al. 1997; Kulkarni, Bentley et al. 2005) have provided evidence for distinctive medial affective components.

1.3.5.1 Primary somatosensory cortex
Numerous imaging studies of both deep and cutaneous pain demonstrate activity within the human SI, as reviewed in (Apkarian, Bushnell et al. 2005). Furthermore,
single nociceptive neurones to superficial thermal mechanical stimuli have been identified in SI of the monkey (Kenshalo and Isensee 1983; Chudler, Anton et al. 1990). However, direct electrical stimulation in awake humans of SI (and SII) elicits frequent reports of tactile paraesthesia but not pain (Craig 2003). In contrast, electrical stimulation of the dorsal posterior insular cortex can elicit localized pain sensations in humans (Ostrowsky, Magnin et al. 2002). This further supports the idea that the somatosensory cortices play a more fundamental role in the sensory classification of pain rather than its innate quality. MEG studies of laser-induced cutaneous pain demonstrate initial contralateral SI activation prior to bilateral ACC activity, corresponding respectively with perceptual and functional differences of high acuity fast Aδ and subsequent slow C fibre activity (Ploner, Gross et al. 2002). In this study bilateral SII activation was equally activated during first and second pain. While the degree of activation in SI is seen to correlate with the reported intensity of pain, supporting a role in discriminative perception, activation of the suprasylvian opercular cortex (SII) occurred only at levels well above pain threshold, suggesting that the latter was more related to recognizing the noxious quality of pain (Timmermann, Ploner et al. 2001).

SI is, however, not a homogeneous area but consists of four cytoarchitectonically distinct interconnected (Burton and Fabri 1995; Burton, Fabri et al. 1995) fields in the rostral bank of the postcentral gyrus. These are arranged rostrocaudally and known respectively as areas 3a, 3b, 1, and 2 (Brodmann 1909; Vogt and Vogt 1919). Areas 3b and 1 are predominantly involved in the processing of tactile cutaneous information while areas 3a and 2 mainly receive information from deep body structures (Hyvärinen and Poranen 1978; Iwamura, Tanaka et al. 1993). Physical and anatomical studies propose a somewhat hierarchical organisation of these regions, with area 3b representing the first cortical component (Iwamura 1998). Stimulation of tactile afferents induces two sequentially peaking (30 ms) sources within SI whereas nociceptive stimuli evoked a single response centred at the same region of the second tactile response about 10mm more medially than the first (Ploner, Schmitz et al. 2000). Indeed, the authors of this study propose that the first response was located in area 3b while the second was centered in area 1. While there is some debate as to their exclusively nociceptive activation, laser evoked potentials (Kanda, Shindo et al. 1999) and C fibre microstimulation (Ochoa and Torebjork 1989) are able to be well localised. These finding have fuelled suggestions that the nociceptive system encodes spatial discrimination and localisation independent from tactile inputs. While this may be evolutionarily counterintuitive based on the frequent co-activation of nociceptive and tactile afferents, it is seen in other systems such as proprioception.
1.3.5.2 Secondary somatosensory cortex

SII, or the interoceptive cortex as it has been described by Bud Craig (Craig 2003; Craig 2003), lies in the dorsal posterior margin of the lateral operculum. This region demonstrates increased activity to noxious cold or hot stimuli (Derbyshire and Jones 1998; Hofbauer, Rainville et al. 2001; Brooks, Nurmikko et al. 2002). In addition, it displays increased activity in chronic pain patients (Kupers, Gybels et al. 2000) and neuropathic pain patients during allodynia (Petrovic, Ingvar et al. 1999; Peyron, Garcia-Larrea et al. 2000). Data from animal (Burton and Carlson 1986) and human (Disbrow, Roberts et al. 2000; Ruben, Schwiemann et al. 2001) studies show somatotopy within the SII in relation to tactile inputs. After initial studies demonstrating basic lateral activation (Andersson, Lilja et al. 1997; Xu, Fukuyama et al. 1997), Bingel demonstrated differential activation of the foot and hand in the contralateral SII (Bingel, Lorenz et al. 2004).

1.3.5.3 Anterior cingulate cortex

The ACC is well known to be involved in error detection, problem solving, (Procyk, Tanaka et al. 2000), anticipation (Ploghaus, Tracey et al. 1999; Clark, Brown et al. 2008), motivation and modulation of emotional responses (Bush, Vogt et al. 2002). Cingulotomies, resulting in attenuated emotional responses to pain (Foltz and White Jr 1962; Foltz and White 1968), support the postulate that the ACC plays a prominent role in the affective emotional dimension of pain. Furthermore, hypnosis has been demonstrated to modify the affective component of heat pain in a manner involving the IC and ACC with (Hofbauer, Rainville et al. 2001) and without (Rainville, Duncan et al. 1997) demonstrable changes in the somatosensory cortex. As summarised in (Vogt 2005) numerous studies have investigated the distributed activity within the cingulate cortex in response to both deep (Thunberg, Lyskov et al. 2005; Henderson, Bandler et al. 2006; Macefield, Gandevia et al. 2007) and superficial (Vogt, Derbyshire et al. 1996; Lenz, Rios et al. 1998; Hutchison, Davis et al. 1999; Henderson, Bandler et al. 2006; Frot, Mauguiere et al. 2008) stimuli.

The cingulate cortex, or for that matter any cortical structure, may be divided into distinct cortical regions comprised of areas sharing similar underlying cytoarchitectural motifs, common circuitry and proposed functions. These regions and their subdivisions in the ACC include the (s, subgenual; p, pregenual), the midcingulate cortex (anterior & posterior), the posterior cingulate cortex (dorsal & ventral), and the retrosplenial cortex ventral bank of the posterior cingulate gyrus (Vogt, Berger et al. 2003; Vogt 2005).
The ACC is classically divided anatomically into pregenual (cognitive) and subgenual (emotional) components (Bush, Luu et al. 2000). The pACC is heavily connected with the prefrontal and parietal cortices, while sACC regions receive input from the nucleus accumbens, amygdala, anterior insula, and hypothalamus. More recently, functional studies have convoluted this functional distinction, suggesting the sACC is associated with negatively valanced memories while the more positively events were observed to activate the pACC (Vogt, Berger et al. 2003).

Recent evidence for a role of the pACC in unpleasantness derives from laser evoked potentials though this study did not show significant activation of the sACC (Kulkarni, Bentley et al. 2005). In general, visceral stimuli produce greater differential responses in the pACC while cutaneous stimuli induce foci that are distributed throughout the midcingulate cortex though there is no discernable somatotopy in activation.

1.3.5.4 Insular cortex

The insula has long been thought to be responsible for the perception of the body's internal state (Reiman, Lane et al. 1997; Damasio, Grabowski et al. 2000; Craig 2003), in this respect it is not at all surprising that it is explicitly involved in pain perception (Coghill, Sang et al. 1999; Craig, Chen et al. 2000). As noted above, stimulation of the dorsal posterior insula in awake humans causes well-localized pain (Ostrowsky, Magnin et al. 2002; Mazzola, Isnard et al. 2009). Furthermore, lesions of the dorsal posterior insula are seen to significantly reduce pain and temperature sensibility (Schmahmann and Leifer 1992; Greenspan, Lee et al. 1999). While blurring the line of a purely discriminatory function, large lesions within the insular have been correlated with pain asymbolia - a failure of appropriate high level motor responses to pain (Berthier, Starkstein et al. 1988). This is not inconsistent with evidence suggesting activation of the insula in states of anger, fear (Phan, Wager et al. 2004), frustration (Abler, Walter et al. 2005) and disgust (Phillips, Young et al. 1997), together with evidence correlating activity in the insula with the unpleasantness of visceral or muscle pain (Dunckley, Wise et al. 2005). It has been proposed that the somatotopy of the STT is preserved in its connections with the VMpo, which then projects to the contralateral insula (Craig, Bushnell et al. 1994; Craig 2003; Craig 2009). Indeed, direct electrical stimulation of the human insular cortex has demonstrated somatotopic organization of pain responses (Mazzola, Isnard et al. 2009). In support of this finding, functional imaging studies of innocuous C fibre mediated tactile (Björnsdotter, Löken et al. 2009), superficial thermal pain (Brooks, Zambreanu et al. 2005), innocuous cooling (Hua, Strigo et al. 2005) and muscle pain induced by intramuscular injection of hypertonic
saline (Henderson, Gandevia et al. 2007) all reveal somatotopy within the posterior insula.

In a recent study, innocuous stroking (a means of activating C tactile afferents) of the right forearm or thigh resulted in differential activation of the contralateral insula. All individual clusters of activated voxels from stroking the forearm were located anterior to those of the thigh, such that the mean distance between the centroids was significantly different in the anteroposterior axis (Björnsdotter, Löken et al. 2009). Similarly, trains of innocuous cooling to the thenar aspect of the hand or lateral aspect of the neck resulted in two similarly distinct areas of activation within the posterior insula, with the focus of neck activation being anterior to that of the hand (Brooks, Zambreanu et al. 2005). These activation differences have also been observed with C-fibre mediated heat pain and C/Aδ-fibre mediated muscle pain induced by hypertonic saline, consistent with observations that this somatotopy is maintained in a patient without any myelinated fibres (Björnsdotter, Löken et al. 2009). In addition, heat pain to the face, hand and foot resulted in distinctly separate activation clusters within the posterior insula (Brooks, Zambreanu et al. 2005). Finally, intramuscular and cutaneous injection of hypertonic saline was found to reflect a similar somatotopographic organisation, with the representation of both cutaneous and muscle forearm pain being located lateral and anterior to the leg representation within the posterior insula (Henderson, Gandevia et al. 2007). Interestingly, this study also revealed a reversed posteroranterior gradient in the ipsilateral posterior insula, and the representation of muscle pain in the ipsilateral insula was located anterior to that of cutaneous pain.

Summarised in figure 2, these data clearly demonstrate an anteroposterior gradient to rostrocaudally presented stimuli in posterior contralateral insula. In this thesis I build on this work by exploring anteriorposterior relationships of muscle pain within a single limb.
Figure 1.2: Activation centres (in MNI coordinates) within the posterior insular cortex resulting from application of stimuli known to activate small-diameter afferents (soft brushing, innocuous cooling, and pain) on various body parts in human subjects. Data have been reproduced from [1] (Bjornsdotter, Loken et al. 2009), [2] (Hua, Strigo et al. 2005), [3] (Henderson, Gandevia et al. 2007) and [4] (Brooks, Zambreanu et al. 2005). White markers indicate an upper body stimulus location and black a lower body location. Gentle tactile stimulation (brushing) fits the somatotopic pattern in the posterior contralateral insular cortex well, with upper body stimulations projecting anterior (and slightly lateral) to lower body stimulations.

1.3.6 Neural correlates of deep pain

While many studies have focussed on the neural correlates of superficial pain, only a handful have investigated deep muscle or visceral pain. This is, in a sense, surprising as the vast majority of debilitating chronic pain conditions arise from deep tissues.

In an fMRI study comparing superficial thermal pain with the visceral pain of rectal distension, only visceral stimuli induced bilateral deactivation of the perigenual cingulate. While the latter stimuli showed greater activation of the contralateral insula, somatic (cutaneous) pain induced greater signal changes in the left (ipsilateral) dorso-lateral prefrontal cortex and bilateral inferior parietal cortex (Dunckley, Wise et al. 2005). This may suggest a greater encoding of affect during visceral pain and a greater encoding of spatial orientation and perceptual valence of the stimulus in response to superficial pain. Comparison of deep and superficial pain evoked by intramuscular or subcutaneous injection of hypertonic saline revealed similar levels of BOLD activation in the mid-cingulate and insula but greater deactivations of the anterior cingulate cortex from deep pain, together with a larger area of SI activation (Henderson, Bandler et al. 2006). This is consistent with the poor locognosia of muscle pain relative to cutaneous pain. In an earlier PET study of jaw pain, induced by injection of hypertonic saline into...
the masseter muscle, there were widespread increases in regional cerebral blood flow in the bilateral posterior insula, ACC and prefrontal cortex, right posterior, parietal cortex, brainstem, cavernous sinus and cerebellum. Interestingly noxious stimulation induced no detectible changes in SI or SII, contrasting significant increases in blood flow during tactile stimulation of the same area (Kupers, Svensson et al. 2004). In another PET study, comparison of laser-evoked cutaneous pain with intramuscular electrical stimulation demonstrated similar levels of blood flow in the contralateral anterior insular cortex, contralateral SII and inferior parietal lobule. Cutaneous stimulation resulted in significant increases in the contralateral lateral PFC and ipsilateral premotor cortex, while intramuscular injection resulted in increased blood flow to the contralateral ACC and contralateral primary sensorimotor cortex (Svensson, Minoshima et al. 1997).

Deep pain induced by intramuscular injection of hypertonic saline into the leg and arm has been shown to increase BOLD signal intensity in mid-cingulate, bilateral insula and SII (Macefield, Gandevia et al. 2007). In instances where this deep pain was referred to the hand or foot, respectively, signal differences were observed in the perigenual ACC, SI, ipsilateral insula and the cerebellar cortices. SI changes corresponded with the appropriate region depending on the extent of pain; moreover, referred pain displayed increased activation in the area of SI that corresponded with activation from tactile stimulation in the referred site. Interestingly, regional signal increases in the prefrontal cortex, SI and cerebellar cortex of referrers corresponded to signal decreases from the baseline in non-referrers (Macefield, Gandevia et al. 2007).

1.3.7 “Limbic” components
The subjective emotional component of the pain percept cannot be understated. This is exemplified by the accounts of pain experienced without any nociceptive input (sometimes referred to as psychogenic pain), using hypnosis and empathy manipulations (Derbyshire, Whalley et al. 2004; Singer, Seymour et al. 2004; Raji, Numminen et al. 2005). These individual non-nociceptive components of the painful experience broadly include attention and context, as well as emotional state.

1.3.7.1 Descending modulatory system
This system was first revealed by Sherrington in a study that demonstrated enhanced nociceptive reflexes after spinal transaction (Sherrington 1906). An early elegant demonstration of selective modulation of pain utilised electrical stimulation of the midbrain PAG to produce analgesia, inhibiting simple noxious stimulus-evoked reflexes
(such as tail flick or paw withdrawal) in awake alert rats (Reynolds 1969; Mayer and Price 1976).

This work was extended in the seminal work of Basbaum & Fields 1978 detailing a three-tiered pain control system. Here, the analgesic action of opiates was postulated to result from excitatory connections between the PAG and the nucleus raphe magnus which in turn project caudally to the dorsal lateral funiculus of the spinal cord. (Basbaum, Clanton et al. 1978; Fields and Basbaum 1978). This model proposed at least two descending control systems, one originating in the midline raphe/ nucleus raphe magnus, the second in the adjacent reticularis paragigantocellulararis lateralis (Rpg) of the rostral medulla. A more general revised model was published incorporating pharmacologically distinct bulbospinal control systems deriving from the Rpgl and parallel descending connections from the nucleus reticularis magnocellularis. The latter projecting independently of the dorsolateral funiculus (Basbaum and Fields 1984).

These circuits were initially thought to serve as an endogenous mechanisms for antinociception in states of stress (Terman, Shavit et al. 1984), though it has subsequently become clear that certain regions - such as the medullary nucleus reticularis gigantocellularis (NGC) - are able to mediate both inhibitory and facilitatory influences on nociception (Zhuo and Gebhart 1992). Indeed, some descending circuits are tonically active and able to dynamically effect their net analgesic or pronociceptive effect as a result of environmental, pathogenic or emotional states.

From an evolutionary perspective it is assumed that this modulation facilitates a protective behavioural response to threatening or stressful stimuli, providing suppression of nociception and associated counter-productive motor responses, thus enabling escape and survival. In this scenario a reduction or inhibition of reflex arcs and valuable tactile information would be counterproductive, thus placing a greater onus on a selective inhibition of only nociception. Indeed, the differential effect observed on A and C fibres (Waters and Lumb 2008) has been a recent focus of many laboratories (Leith, Wilson et al. 2007; Simpson, Headley et al. 2008), confirming a predominantly C-fibre directed response. Descending modulatory pathways terminate heavily, though not exclusively, within the superficial dorsal horn (Basbaum, Clanton et al. 1978), corresponding to the termination of the majority of C-fibres.

1.3.7.2 Attention

The extent to which a subject attends to a painful experience is known to play a significant role in the magnitude and quality of pain he or she experiences. Typically
the perceived pain intensity is more intense when a subject attends to the stimuli relative to a state of distraction (Bushnell, Duncan et al. 1999). These effects are however dependent on a multitude of other factors, not least the stimuli itself. Since severe pain demands attention and this in itself is difficult to control it can be difficult to construct a consistently demanding distraction task (Miron, Duncan et al. 1989). While there is some debate as to the neural substrates of attentional modulation of pain, regions such as the PFC, VLM, PAG (Tracey, Ploghaus et al. 2002), and RVM are likely components (Villemure and Bushnell 2002) are likely components of an opiate sensitive pathway (Fields 1999). Studies showing increased nociceptive dorsal horn activity in primates attending to stimuli relative to those partaking in distracting tasks (Hoffman, Dubner et al. 1981) have been suggested to imply involvement of descending inhibitory systems. Other primate studies recording single from single neurons in the medullary dorsal horn (Bushnell, Duncan et al. 1984) and the medial thalamus (Bushnell and Duncan 1989) have demonstrated distraction related reduction in neuronal activity. Put together, these and many modern imaging studies (Petrovic, Petersson et al. 2000; Bantick, Wise et al. 2002; Legrain, Guérit et al. 2002; Ohara, Crone et al. 2004; Ohara, Crone et al. 2004; Valet, Sprenger et al. 2004) paint a clear picture that the degree of conscious awareness results in numerous parallel changes in the perception of pain.

1.3.7.3 Context

The beliefs and expectations held by a given person at the time they experience pain have been demonstrated to have a substantial impact on their percept of pain. One of the easiest ways to study context is through the use of differing placebo paradigms. Indeed, the placebo effect and resultant analgesia has been extensively studied (Benedetti, Mayberg et al. 2005; Zubieta and Stohler 2009). In a seminal fMRI study Wager and colleagues demonstrated decreased BOLD signal intensity in regions that included the thalamus, insula, and ACC in response to placebo analgesia, and anticipation-related increases in the PFC, a region known to be involved in internal representations of expectation (Wager, Rilling et al. 2004). Positive correlations were observed between the level of anticipation and perceived pain. These effects are mediated, at least in part, by activity within the ventral tegmentum, the entorhinal cortex, and the PAG (Fairhurst, Wiech et al. 2007).

Other psychological states such as depression can have a large impact on the transient percept of pain, though there is a great deal of debate as to the direction of causality, whether one condition leads to the other or if underlying predispositions are involved. While the neuronal plasticity associated with any long term condition can
confound imaging studies, increased activation of the amygdala is seen to differentiate fibromyalgia patients with and without significant depression (Giesecke, Gracely et al. 2005). The construct of exacerbating pain by catastrophizing incorporates rumination about pain, feelings of helplessness, and pessimism about pain-related outcomes (Sullivan, Thorn et al. 2001; Edwards, Bingham III et al. 2006). Comparison of fibromyalgia cohorts with and without this collection of behaviours found a significant association with increased activity in the ipsilateral claustrum, cerebellum, dorsolateral prefrontal cortex, parietal cortex, and in the contralateral dorsal anterior cingulate gyrus, dorsolateral prefrontal cortex, medial frontal cortex and lentiform nuclei (Gracely, Geisser et al. 2004). Indeed, there is mounting evidence to suggesting that these modulatory effects share some similar mechanisms (Valet, Sprenger et al. 2004) with other forms of analgesia such as opioid- or placebo-induced analgesia (Petrovic, Kalso et al. 2002; Price, Craggs et al. 2009).

1.4 STUDIES OF CUTANEOUS PAIN

As exemplified by the aforementioned studies, the majority of research on pain has focused on pain originating in skin, which affords easier access than muscle. We have a very good capacity to localise cutaneous pain: we can correctly identify the location of a pin prick, for instance. Indeed, locognosis of cutaneous pain is comparable to our capacity to localise a tactile stimulus (Koltzenburg, Handwerker et al. 1993).

1.4.1 Experimental models of cutaneous pain

The most common form of experimental cutaneous pain is thermal pain, induced by either heating elements or by focal laser-induced heating (Arendt-Nielsen and Bjerring 1988); alternatively, thermal pain can be induced by a Peltier element inducing cold pain (Davis, Kwan et al. 1998). Other stimuli aim to model other modalities of pain such as topical application of mustard oil (Urban, Jiang et al. 1996), capsaicin (Schmelz, Schmid et al. 2000) to activate chemoreceptors or pinching to activate mechanoreceptors. Our laboratory has also used subcutaneous injection of hypertonic saline (Henderson, Bandler et al. 2006; Henderson, Gandevia et al. 2007; Macefield, Gandevia et al. 2007; Nash, Macefield et al. 2009).

1.5 STUDIES OF MUSCLE PAIN
1.5.1 Basic epidemiology
Muscle pain has featured less in scientific investigations of pain, yet given that chronic pain often develops from a primary noxious stimulus involving deep tissues (such as muscle), there is no doubt that musculoskeletal pain is an important area of study. Indeed, estimates for the prevalence of chronic widespread muscle pain in the population range from 4.2% (Lindell, Bergman et al. 2000) to 11.4% (Bergman, Herrstrom et al. 2001). It is estimated that some 44 million Americans have myofascial pain problems (Wheeler 2004). The vast majority of these complaints are idiopathic, with varied and debated aetiology that would presently be classified myofascial trigger-point pain syndromes and fibromyalgia. As the work presented in this thesis deals exclusively with muscle pain, the study of muscle pain will first be considered in an historical perspective.

1.5.2 Historical perspective
The term rheumatism was first used by Guillaume de Baillou in the late 16th century to describe a number of musculoskeletal conditions, including those resulting from infectious agents such as rheumatic fever (Ruhmann 1940). Over the next 500 years the nosological disarray of rheumatism was perpetuated by Thomas Sydenham’s exclusive use of the term to describe acute rheumatic fever (Sydenham 1676), and William Wells association with myocardial carditis (Baldry 1971) in 1810. Subsequent debate arose between British physicians and a German-Scandinavian collaboration as to whether the pain associated with rheumatism derived from inflammation of the fibrous connective tissue associated with muscles, or inflammation of the muscles themselves (Balfour 1815; Scudamore 1827). It was not until 1840 that Jean Bouillard formally distinguished muscular rheumatism from rheumatic fever (Reynolds 1983). On the back of debated microscopy findings (Stockman 1904) reporting hyperplasia of fibrous tissue, Sir William Gowers proposed rheumatism be replaced by the term “fibrositis” (Gowers 1904). The very next year this term was hijacked to describe a variety of conditions including fibromyalgia and gout (Llewellyn and Jones 1915). In attempting to ascribe a single term to what were surely a myriad of separate conditions, aetiological confusion subsequently spiralled with numerous terms including myofascitis (Albee 1927), myofibrositis (Murray and Durh 1929), idiopathic myalgia (Gutstein-Good 1940), rheumatic myalgia (Good 1941) and myodysneurea (Gutstein 1940).

Amidst this diversity of associations arose a common albeit infrequent set of physical observations. In 1816 William Balfour described the presence of physically defined nodules in patients suffering from what he called muscular rheumatism after using a
form of massage therapy to relieve their pain (Balfour 1815). These findings were later reported in 1876 by the Swedish physician Uno Helleday, in what he called chronic rheumatic myitis Helleday, and further elaborated upon by Strauss (1898) who also described the presence of palpable bands (Strauss). The French physician Valleix, however, was the first to propose that the hyperactivity of nerve endings was responsible for the shooting pain that resulted from palpation of these tender points (Valleix 1841). Unfortunately, this insight was clouded by confusion between neuralgia and rheumatism, together with statements suggesting the referred pain experienced from stimulation of these nodes resulted from the activation of neighbouring nerves. Indeed, mechanisms governing the somatic distribution of muscle pain outside the local stimuli “referred pain” continue to be the subject of great contention to the extent of justifying this publication. In 1904 Stokman suggested the distribution of pain derived from the nodule compressing neighbouring nerves or surrounding nerves that may run through it (Stockman 1904). Though some thought pain resulted from pressure on the nerve as the muscle went into spasm, the general consensus well into the 1900’s involved rheumatic inflammation of the connective tissue and the surrounding nerves (Gowers 1904; Llewellyn and Jones 1915). This belief ran in stark contrast to the remarks of Thomas Inman, who noted as early as 1858 that radiation of muscle pain “is independent entirely of the course of nerves” (Inman 1858). This sentiment was shared by Cornelius, who went further in exclaiming “this radiation... absolutely does not keep to the individual nerve trunks” (Cornelius 1903).

Nevertheless, by the early 20th century prevailing opinion reflected a view that the pain experienced in conditions such as muscular rheumatism and fibrositis derived from over-activity of nerves rather than pathological processes affecting the muscles or their surrounding tissues (Osler 1909). By the 1930’s a number of reports began to focus on the location of somatic pain relative to defined tender points - termed trigger zones (Hunter 1933; Edeiken and Wolferth 1936). While Pickering (Pickering and Hess 1933) had demonstrated the viability of ischaemic muscle contractions in 1931, the first specific human model of muscle pain, and thus one of the most valuable contributions to the field, was developed by Sir Thomas Lewis and John Kellgren. Lewis initially injected sodium phosphate and sodium chloride solutions of differing tonicity into the dorsal muscles of the forearm, comparing the effects with simultaneously induced pain from ischaemic contractions in the same muscle of the contralateral arm (Lewis 1938). While his findings went little further than the description of a distinct aching pain with distal referral, they precipitated collaboration with Kellgren.
1.5.3 Experimental models of muscle pain

Ultimately the choice of an experimental model must be a compromise. To explore the complexity within one element of a system we must reduce the complexity of another so as to reduce variables. In this sense compromises are often made between assumptions and open exploration or between reduced experimental variables and pathological equivalence. These compromises can, of course, be minimised. Typically, models of perceptual systems aim to deliver a defined input enabling deconstruction of mechanistic information from a measurable output. The output of many sensory models involving humans is often an account of perception, though by its very nature this involves the integration of practically all higher order processing. The input is the afferent drive created by the activation of specific receptors that have evolved to respond to specific modalities. The utility of an experimental model can be measured simply by the degree to which it accurately elucidates the system it is trying to model. Yet without an explicit understanding of its limitations, a model can easily detract from our understanding. These limitations may often be viewed as the assumptions, simplifications and omissions involved in its execution. For example, a model attempting to make inference on the mechanism of chronic pain is practically meaningless unless it embraces the temporal aspects explicit in the term chronic.

1.5.3.1 Intramuscular injection of hypertonic saline

In a seminal paper, John Kellgren utilised the injection of hypertonic saline (NaCl 6%) into muscle belly and surrounding fascia, together with physical trauma of these tissues, to extensively characterise the quality and spatial attributes of pain from these structures (Kellgren 1938). This extensive study involved injections into numerous muscles structures including, but not limited to, the multifidus, rectus abdominis, biceps, gluteus medius, rhomboids, flexor carpi radialis, abductor pollicis longus, third dorsal interosseous, serratus anterior, infraspinatus, latissimus dorsi, sartorius, adductor longus, gastrocnemius, first interosseus, peroneus longus and the sacrospinalis. The diffuse pain from muscular injections was found to radiate with distinct patterns specific to the region of each muscle stimulated, although these patterns were subject to inter-individual differences. Pain was often felt some distance from the noxious stimulus in other structures, such as joints (and even the testes), and frequently referred to distal aspects of limbs. Similar sites of projection have been found following injection into the distal tendon or, especially, the proximal bone-tendon junction of tibialis anterior (Gibson, Arendt-Nielsen et al. 2006). Referred pain has also been reported following injection of hypertonic saline into the infrapatellar fat pad, though this usually projects proximally, towards the groin (Bennell, Hodges et al. 2004).
Although it is possible that muscle spasms could play a role in hypertonic-saline (HS) induced muscle pain no significant electromyographic (EMG) evidence has been observed during and after intramuscular injections (Graven-Nielsen, McArdle et al. 1997; Svensson, Graven-Nielsen et al. 1998); fasciculations are infrequently observed, and only on insertion of the needle. MRI (Graven-Nielsen, McArdle et al. 1997). 3D ultrasound (personal unpublished data) and T1 weighted MRI (Graven-Nielsen 2006) studies suggest that the injectate forms a pool of extracellular fluid that extends proximally or distally, generally along the cleavage planes of the muscle and dissipates up to four hours after the sensation of induced pain. While it is said that this pool of extracellular fluid is not correlated to the actual pain intensity, the “rate of change” of this pool is likely to closely match reported intensity. Such a phenomenon would likely cause interstitial tearing that would be independent of the tonicity of solution, resulting in the rupture of surrounding microvasculature and stimulation of associated nociceptors. Indeed, this would explain the occasional observation of pain from injections of isotonic solutions (Jarvik 1962) and the common observation of bleeding upon withdrawal of the needle. It may be suggested that these mechanisms constitute at least some of the volumetric effects (below) observed from this model. This pool of extracellular fluid has been seen to correlate with a marginal volume-dependent increase in intramuscular pressure (Graven-Nielsen, McArdle et al. 1997) that is slightly higher than that found during normal exercise.

While the precise mechanism of action is unknown, the algesic effects of HS are likely to be mediated by the TRPV1 (Cui, Honore et al. 2006) or a stretch-inactivated variant (Schumacher, Jong et al. 2000) of the TRPV family of ion channels expressed on group III and IV fibres (Caterina, Schumacher et al. 1997). Human compression block experiments confirm that the induced pain is mediated by activation of unmyelinated C fibres and to a lesser extent Aδ fibres (Laursen, Graven-Nielsen et al. 1999). Animal studies have demonstrated robust excitation of group III and IV afferents (Paintal 1960; Kumazawa and Mizumura 1977).

A primary justification for the continuing utilisation of the HS model of muscle pain is the similarity of the quality of induced pain to that of pathological conditions, the safety and absence of any after effects negative or otherwise. In this respect, the quality of HS-induced pain is frequently described as ‘aching’, ‘cramping’, ‘boring’, ‘drilling’, ‘taut’, ‘tight’, ‘spreading’, and ‘radiating’ (Graven-Nielsen, Arendt-Nielsen et al. 1997; Graven-Nielsen, McArdle et al. 1997; Graven-Nielsen, Babenko et al. 1998; Graven-Nielsen, Fenger-Grøn et al. 1998; Graven-Nielsen, Jansson et al. 2003). Furthermore, the induced pain is diffuse and poorly localised (Kellgren 1938).
While the hypertonic saline injections pioneered by Kelgren have become the predominant model of muscle pain in the last 80 years (Graven-Nielsen and Mense 2001) much has been learnt from many other models. Human models of muscle pain are often classified broadly by their stimuli, either endogenous or exogenous. Common techniques in the former category include muscle ischaemia (Moore, Duncan et al. 1979) or heavy repeated exercise involving concentric, isometric and eccentric contractions. Exogenous models of muscle pain aim to specifically stimulate known modalities of pain, these of course include heat, mechanical and chemical stimulation. The modality-specific exception to the exogenous sources of muscle pain involves the use of electrical impulses to directly evoke action potentials in afferents.

1.5.3.2 Ischaemia-induced muscle pain
Repeated muscle contractions under ischaemic conditions are known to induce a deep dull aching sensation of moderate to severe intensity (Rodbard and Pragay 1968; Graven-Nielsen, Jansson et al. 2003). This method is often executed with the application of a tourniquet or a sphygmomanometer cuff proximal to a limb segment prior to eliciting voluntary contractions. Initial accounts of ischaemia-induced pain provoked the suggestion that it was caused by the accumulation of an unknown endogenous substance (Lewis 1932). While numerous studies have investigated the actions of various substances such as potassium, adenosine, lactate (Harpuder and Stein 1943; Saltin, Sjøgaard et al. 1981; Costa, Sulur et al. 1999; Green, Langberg et al. 2000) the precise mechanism of action is still hotly debated. Animal studies suggest that only a minority of muscle nociceptors are activated even though the entire muscle is theoretically under ischemic stress (Mense and Meyer 1985).

1.5.3.3 Exercise-induced muscle pain
While each of these techniques induce pain with a character equivalent to that seen in pathological deep pain, the stimulus is hardly controlled. Each subject may require vastly different amount of exercise to induce a similar level of pain, and it is often hard to induce pain in specific muscles. Concentric exercise reportedly induces short lasting pain (Vecchiet, Marini et al. 1983) resulting from impaired circulation, thus likening it to ischemia-induced muscle pain (O’Connor, Motl et al. 2004). Eccentric exercise is known to cause delayed onset muscle soreness (DOMS) that reaches a peak 24-48 hours after exercise (Howell, Chleboun et al. 1993; Slater, Arendt-Nielsen et al. 2003; Slater, Arendt-Nielsen et al. 2005). The efficacy of non-steroidal anti-inflammatory drugs (NSAID) (Baldwin 2003) in the treatment of this type of pain supports the notion that this pain is largely mediated by the release of inflammatory mediators released in
response to physical damage to the muscle fibres (Howell, Chleboun et al. 1993), though it must be noted that the effects of NSAID's are highly variable.

1.5.3.4 Mechanically-induced muscle pain

Induction of muscle pain through mechanical stimulation is generally achieved through means of a pressure algometer. While utilised in many studies in both humans (Brennum, Kjeldsen et al. 1989; Kosek and Ekholm 1995; Graven-Nielsen, Babenko et al. 1998; Graven-Nielsen, Gibson et al. 2002; Graven-Nielsen, Jansson et al. 2003; Staud, Cannon et al. 2003) and animals (Hoheisel and Mense 1990) though the model suffers from many confounding factors. A primary area of contention with model arises from a lack of reproducibility (Isselee, Laat et al. 1997; Prushansky, Dvir et al. 2004). To combat variability due to the rate at which pressure is applied (List, Helkimo et al. 1991), and the effect of the examiner (Ohrbach, Crow et al. 1998) automated systems have been developed (Staud, Cannon et al. 2003; Graven-Nielsen, Mense et al. 2004) enabling the generation of a predefined peak pressure and rate of onset.

However, the utility of this model is further compromised by the common foe of many muscle pain models. In order to induce noxious stimuli in deep structures and only deep structures, one must avoid the dense nociceptor innervation of the overlying skin. While deep pressure pain experiments do not require physical damage of the skin, it is obvious that cutaneous forces are at least equal to those experienced by deep structures. Many studies have attempted to resolve this issue with topical application of local anaesthetic (EMLA cream) (Kosek and Ekholm 1995; Laursen, Graven-Nielsen et al. 1997; Graven-Nielsen, Babenko et al. 1998; Graven-Nielsen, Mense et al. 2004) and iontophoretic (Fujisawa, Shoji et al. 1999) or subcutaneous injection of local anaesthetics (Arendt-Nielsen and Bjerring 1988; Robinson, Riley 3rd et al. 1998) though a large degree of variance is observed in the efficacy of these treatments with some reporting decreases and others reporting no effect in cutaneous pain. The use of EMLA cream often further confounds studies with the introduction of other experimental variables such as application time, skin thickness and other anatomical differences (Arendt-Neilsen, Bjerring et al. 1990).

1.5.3.5 Intramuscular electrical stimulation of muscle pain

Electrical stimulation of deep muscle has been utilised to explore the properties of temporal summation in healthy (Arendt-Nielsen, Graven-Nielsen et al. 1997) and clinical (Graven-Nielsen, Aspegren Kendall et al. 2000) subjects, stimulus response functions (Graven-Nielsen, Arendt-Nielsen et al. 1997), the spatiotemporal characteristics of referred pain (Laursen, Graven-Nielsen et al. 1997; Laursen, Graven-
Nielsen et al. 1997) and the effects of anaesthetics (Schulte, Segerdahl et al. 2006). Stimulation methodologies are varied though often involve consecutive trains of 200 Hz tetanic stimulation to induce intense, dull, poorly localised aching sensations. While these stimuli allow the on-off induction of pain their nonspecific mode of action is likely to introduce a number of unknown experimental variables.

Intramuscular electrical stimulation of peripheral nerves bypasses receptor transduction, inducing a barrage of non-physiological, synchronous, action potentials. These resultant inputs to the spinal cord may be resistant to normal modulation by interneurone’s and descending modulatory systems. This technique preferentially activates non-nociceptive larger diameter myelinated fibres (both afferents and efferents), further differentiating it from clinical muscle pain. Indeed, differential nerve blocks through compression and local anaesthetic have demonstrated the primary involvement of group III not group IV fibres in electrically induced muscle pain (Laursen, Graven-Nielsen et al. 1999).

1.5.3.6 Thermal induction of muscle pain
To date there has only been one specific model of thermally induced muscle pain involving the injection of heated isotonic saline into the TA (Graven-Nielsen, Arendt-Nielsen et al. 2002). This study showed that intramuscular injections of 1.5 ml isotonic saline at 48°C induced dull pain, while injections at temperatures ranging from 18-38°C did not. However, it should be stressed that the magnitude of the thermally-induced pain (~3/10) was only half of that produced by injection of the same volume of hypertonic saline at 18°C.

1.5.3.7 Chemical induction of muscle pain
Direct chemical stimulation of deep nociceptive afferents allows evaluation of the explicit receptive characteristics. Intramuscular injection of numerous chemicals including capsaicin, (Witting, Svensson et al. 2000), potassium chloride, (Jensen and Norup 1992), and numerous acidic buffers (Jensen and Norup 1992; Hoheisel, Reinöhl et al. 2004; Mense 2009) been demonstrated to induce muscle pain.

Apart from various acids and bases, capsaicin (8-methyl-N-vanillyl-6-nonenamide) is quite possibly the most documented specific nociceptor agonist. Since the molecular cloning of its nonselective cation channel, the transient receptor potential (TRP) cation channel, subfamily V, member 1 (TRPV1) (Caterina, Schumacher et al. 1997) many studies have investigated its ability to activate nociceptive cutaneous C fibres (LaMotte,
1.5.3.8 Neurotransmitters

Muscle pain has also been induced by the intramuscular injection of certain neurotransmitters, including serotonin (Babenko, Graven-Nielsen et al. 1999), bradykinin, ASIC and glutamate (Hoheisel, Mense et al. 1993; Babenko, Graven-Nielsen et al. 1999; Graven-Nielsen and Mense 2001; Mork, Ashina et al. 2003; Schmelz, Schmidt et al. 2003; Wang, Ehnert et al. 2006), 4,5-HT (Babenko, Graven-Nielsen et al. 1999), CGRP (Graven-Nielsen 2006) and substance P (Babenko, Graven-Nielsen et al. 1999; Babenko, Graven-Nielsen et al. 1999; Lawson 2002). In general, comparison studies have demonstrated that bradykinin and serotonin elicit pain of similar magnitude and distribution, greater than that observed from intramuscular administration of serotonin.

While intramuscular injection is an invasive procedure involving penetration of the skin and its densely supplied nociceptors, the sharp pain from needle insertion quickly fades. Indeed, studies have demonstrated no differences in the quality of HS-induced pain with and without anesthetised skin (Arendt-Nielsen, Graven-Nielsen et al. 1996; Svensson, Arendt-Nielsen et al. 1996). This cutaneous pain can be minimised with the use of fine needles and the minimisation of injectate delivery until the needle is well into the muscle belly. Alternatively, intradermal anaesthesia may be applied in the form of subcutaneous injection or topical anaesthetic; while the latter is observed to have varied effects, the former involves exceedingly more painful injections than the intramuscular injections themselves. In the experiments to be reported in this thesis all intramuscular injections were made through an indwelling cannula, and were delivered several minutes following insertion of the cannula; there was no residual pain from the insertion.

1.5.4 Referred pain

A particular character shared between clinical muscle pain and hypertonic saline induced muscle pain is the induction of both local and referred sensations (Kellgren 1938; Kellgren 1949; Arendt-Nielsen, Graven-Nielsen et al. 1997; Graven-Nielsen, Arendt-Nielsen et al. 1997; Graven-Nielsen 1997; Leffler, Kosek et al. 2000; Schulte, Segerdahl et al. 2006).

Sensory localisation may be thought of as having two distinct components: the ability to distinguish between two concurrent stimuli, referred to as two point discrimination.
(Johnson and Phillips 1981), and the ability to identify any stimuli in the space of the body schema. In tactile and visual perception the former is aided by an extremely high density of receptors and peripheral processes such as surround inhibition (Born and Tootell 1992). Tactile two-point discrimination is known to be heavily dependent on thicker myelinated fibres as demonstrated by compression block experiments (which blocks thicker fibres first).

Referred pain from visceral organs is classically observed in clinical conditions such as angina pectoris (Foreman 1999) and appendicitis (Stawowy, Rossel et al. 2002) as aberrant pain sensations in the left arm and lower right abdomen, respectively. Visceral pathologies are by no means the only manifestations of spatially misrepresented noxious sensations - referred muscle pain is frequently observed in the clinical conditions of myofascial pain (Hong, Kuan et al. 1997; Wheeler 2004; Bennett 2007) and fibromyalgia (Graven-Nielsen, Sörensen et al. 1999; Graven-Nielsen, Aspegren Kendall et al. 2000; Neumann and Buskila 2003; Bennett 2007; Nielsen and Henriksson 2007) though rarely observed from noxious cutaneous stimulation. Indeed the distribution of referral correlates most with spinal segmental input of a given region rather than myotomes and dermatomes. With the use of experimental models, referred pain has been demonstrated to arise from noxious stimulation of numerous musculoskeletal structures including tendon (Gibson, Arendt-Nielsen et al. 2006) - though Kellgren (Kellgren 1938) observed only local pain from these structures – as well as interspinous ligaments (Kellgren 1938; Steinbrocker, Isenberg et al. 1953; Feinstein, Langton et al. 1954), the dorsal rami of spinal nerves (Fukui, Ohseto et al. 1996; Fukui, Ohseto et al. 1997), joint-related structures (Steinbrocker, Isenberg et al. 1953), deep periostium (Kellgren 1949), and muscle (Kellgren 1949; Zhang, Ashton-Miller et al. 1993; Graven-Nielsen, Arendt-Nielsen et al. 1997; Graven-Nielsen, Arndt-Nielsen et al. 1997; Graven-Nielsen, McArdle et al. 1997; Graven-Nielsen 1997; Graven-Nielsen, Aspegren Kendall et al. 2000; Leffler, Kosek et al. 2000; Arndt-Nielsen and Svensson 2001; Hwang, Kang et al. 2005; Svendsen, Edwards et al. 2005; Ge, Madeleine et al. 2006; Gibson, Arendt-Nielsen et al. 2006; Schulte, Segerdahl et al. 2006; Ro, Capra et al. 2007).

The work of this thesis deals explicitly with the local and referred pain originating from intramuscular injections of hypertonic saline into tibialis anterior (TA), flexor carpi radialis (FCR), the first dorsal first dorsal interosseous (FDI) and the deltoid. While the latter muscle does not exhibit referral the FCR, FDI and TA all demonstrate frequent distal referral.
Contrasting the mixed referral from visceral structures to both deep and superficial regions (Ness and Geibhart 1990), referred pain from muscle is always perceived in deep structures (Feinstein, Langton et al. 1954). Referred pain does not always manifest as a result of intramuscular injection, but noxious stimulation can occasionally result in the generation of referred pain but not local pain, suggesting central neural processes. The clear correlation between local and referred pain intensity and area emphasises the direct causal link between local and referred pain (Graven-Nielsen 2006). However, referred pain is extremely variable - far more so than local pain. While some of this variability may be dependent on psychological factors that are difficult to control for, there are many experimental factors such as the volume of infusion, tonicity of the hypertonic saline, and infusion rate that can be manipulated to minimise this variance.

1.5.4.1 Defining referred pain

In order to characterise the mechanisms of referral it is necessary to define the concept of referred pain. While numerous methods have been used to demarcate the local and referred regions of experimental muscle pain, an adaptation of Kellgren’s initial study (Kellgren 1938) has been adopted as the prevailing definition of local and referred muscle pain (Stohler and Lund 1994). This widely used (Graven-Nielsen, Arendt-Nielsen et al. 1997; Graven-Nielsen, Arendt-Nielsen et al. 1997; Graven-Nielsen 1997; Laursen, Graven-Nielsen et al. 1997; Graven-Nielsen, Babenko et al. 1998; Babenko, Graven-Nielsen et al. 1999; Graven-Nielsen, Aspegren Kendall et al. 2000; Leffler, Kosek et al. 2000; Witting, Svensson et al. 2000; Arendt-Nielsen and Svensson 2001; Schulte, Graven-Nielsen et al. 2003; Ge, Madeleine et al. 2006) definition classes pain around the injection site as local pain and referred pain as regions that are isolated, discontinuous and outside the bounds of local pain. By this definition a single contiguous region of pain extending from the injection site to a distant region innervated by a different nerve would be classed as local pain, resulting in underestimation of referred pain. There are, however, other more specific definitions of local and referred pain, such as that used by Schulte and colleagues, whereby local pain from injection into the TA was separated from referred pain by an arbitrary line traversing the line connecting the malleoli at the ankle (Schulte, Segerdahl et al. 2006). However, even this definition may be seen to have weaknesses, in that it would appear to classify pain within the bounds of the upper leg, separated and distinct from the injection site, as local.

As an interesting corollary, Kellgren noted that the distribution of referral was greatly influenced by a subject’s preconception that pain must arise from the source of the
stimulus. This was demonstrated by an increase in the reporting of HS-induced referral from subjects who were blindfolded (Kellgren 1949). These observations are not at odds with our understanding, given the known associations between visual input and proprioception together with the aforementioned contextual modulation of pain.

### 1.5.4.2 Concentration, volume and infusion rate

The magnitude of pain induced is proportional to volume, concentration, and infusion rate (Jarvik 1962; Graven-Nielsen 1997). While hypotonic saline has been shown to induce pain at a similar level to that of hypertonic solutions, the duration of pain is much shorter than that produced by solutions with higher concentrations (Jarvik 1962). Indeed, this variability lead Wolff and Jarvik to conclude that the model of HS-induced muscle pain “appears to be useful only as a measure of pain threshold, but not of supra-threshold pain and pain tolerance” (Wolff BB 1965).

The development of a computer-controlled infusion system (Zhang, Ashton-Miller et al. 1993), which utilized pain intensity feedback to titrate the flow of HS, has allowed the induction of a stable tonic level of muscle pain. This technique is touted as a vast improvement on existing models of HS-induced muscle pain and has subsequently been used in many studies (Zhang, Ashton-Miller et al. 1993; Graven-Nielsen, Arendt-Nielsen et al. 1997; Graven-Nielsen, McArdle et al. 1997; Graven-Nielsen, Babenko et al. 1998; Graven-Nielsen, Jansson et al. 2003), though it assumes a uniform and invariant response from each of the multitude of central processing elements explicit in the percept of pain. Put simply, the nociceptive input is not directly or consistently proportional to the perception of pain as seen by temporal summation of electrical (Arendt-Nielsen, Graven-Nielsen et al. 1997; Graven-Nielsen, Aspegren Kendall et al. 2000), mechanical and HS (Arendt-Nielsen, Graven-Nielsen et al. 1997; Graven-Nielsen, Aspegren Kendall et al. 2000) stimuli.

A latency of approximately 20 seconds has been observed between the onset of local pain and subsequent manifestation of referred pain induced by intramuscular injection of HS (Graven-Nielsen, Arendt-Nielsen et al. 1997). Numerous studies have demonstrated the extent of referral to be directly proportional to the intensity of the painful sensation (Kellgren 1949). Given the nature of the stimulus response curve from HS-induced muscle pain (Gibson, Arendt-Nielsen et al. 2006), it had been proposed appearance referral was entirely dependent on the magnitude of local pain since a rise in local pain is accompanied by a rise in referred pain. Subsequent studies using electrical stimulation (Laursen, Graven-Nielsen et al. 1997) to induce constant tonic pain reiterated this latency, suggesting a time dependent process. Furthermore,
HS-induced referred muscle pain was seen to dissipate before local pain (Graven-Nielsen, Arendt-Nielsen et al. 1997).

1.5.4.3 Spatial properties

In his original 1938 paper Kellgren did not try and define the boundaries between local and referred pain but noted that “the distribution of the diffuse pain from muscle appears to follow a spinal segmental pattern [which] differs from that of the segmental innervation of the skin.” This strictly segmental distribution of pain has been subsequently questioned (Ruch 1947; Steinbrocker, Isenberg et al. 1953; B. Feinstein 1954). Initial responses to Kellgren’s work suggested that the saline spread through the tissue and stimulated nerve trunks (Ruch 1947), resulting in afferent input entering the spinal cord via one or more posterior division(s) of the spinal root. Indeed, Kellgren himself noted that there was a significant degree of variability between coloured muscles from methylene blue injections into cadavers though they were not observed to extend beyond the muscle injected. While this “spread” has subsequently been supported by the aforementioned MRI evidence of injectate spread (Graven-Nielsen, McArdle et al. 1997) it does not account for referral into anatomically distinct compartments, as seen from injections of the FCR referring into the hand, injections into TA that refer into the foot and injections into FDI, in which pain refers into the palm and little finger. Furthermore, some of these distinct compartments are innervated by separate nerve branches. The median nerve, for example - with spinal terminations from C6-T1 - innervates muscles of the anterior forearm compartment, including FCR, palmaris longus and the pronator teres, together with the lumbricals to digits 2 and 3 of the hand and thenar muscles (Moore 2006). As this nerve does not innervate the fourth and fifth digits of the hand, painful sensation in these fingers is difficult to attribute to direct stimulation of the median nerve. Furthermore, FCR is supplied by a separate branch of the median nerve, as are the surrounding muscles: flexor digitorum superficialis, palmaris longus and pronator teres. In this respect, the perception of pain from intramuscular injections of HS into FCR to the greater anatomical regions of the forearm can be argued to result from a central lack of acuity rather than a peripheral activation of multiple regions.

As noted by Feinstein (B. Feinstein 1954), Kellgren’s segmental classification is likely to be academic as “sharply delimited segments of sensory organisation in the spinal cord are not likely to exist, especially not for deep tissues”. Given the aforementioned dorsal root ganglion arborisations in the spinal cord and the apparent lack of evolutionary drive for such acuity from deep inputs, this is by no means an unreasonable statement. However, the consistent observation that referred pain almost
always projects distally in limbs is somewhat mysterious. This phenomenon of unidirectionality is observed in most muscles; for example, pain generated in the TA is seen to refer down the leg and into the foot, while pain generated in the upper forearm will refer down the arm to the hand (Feinstein, Langton et al. 1954). Nonetheless, proximal or bidirectional referral has been observed in certain muscles, such as the extensor digitorum brevis (Graven-Nielsen 2006). It remains to be seen whether the direction of referral has any correlation with the path of the interstitial pool created by the injection, though this is highly unlikely.

1.5.4.4 Possible mechanisms of referral

Many theories have been proposed to explain the inability of higher brain centres to correctly identify the source of painful stimuli. Theories of bifurcating afferents with peripheral receptive fields in both local and referred regions (Sinclair 1948) fail to explain the delayed onset of referred pain (Hockaday and Whitty 1967; Graven-Nielsen, Arendt-Nielsen et al. 1997). Furthermore, they provide no explanation for the observation of unidirectionality. The convergence projection theory (Ruch 1947) describes a convergence of afferents from local and referred regions on the same population of spinal projection neurones, though this too fails to account for unidirectionality and latency. More recent adaptations of Mackenzie’s original convergence-facilitation theory (Mackenzie 1909) propose the unmasking and sensitization of inactive connections within the dorsal horn of the spinal cord (Mense 1994). This theory is based on animal experiments that provide evidence of the development, expansion and persistence of new receptive fields following noxious stimuli (Cook, Woolf et al. 1987; Hoheisel, Mense et al. 1993).

After initial injections of the pro-inflammatory algesic peptide bradykinin, Hoheisel and Mense demonstrated the development of new receptive fields in response to subsequent electrical stimulation and repeated injection of bradykinin (Hoheisel and Mense 1989). These injections into the gastrocnemius-soleus and semitendinosus muscles of cats resulted in an increase in the number of neurones with both deep and cutaneous inputs. In a second series of experiments Hoheisel and colleagues utilised a similar stimulus in rats to show both distal expansion and the development of new receptive fields of group III and IV afferents. Following subcutaneous injection of adjuvant into the hindlimb of the rat, lamina I neurones with cutaneous inputs developed increased receptive field sizes, discontinuous receptive fields, additional responsiveness to deep tissues and increased spontaneous activity when compared to control rats (Hylden, Nahin et al. 1989). Utilising a similar technique, Dubner and
colleagues recorded increases in receptive-field size in NS and WDR (class 2) neurones that were N-methyl-d-aspartate (NMDA) dependent (Ren, Williams et al. 1994). In another rat model, recordings from the dorsal horn demonstrated large increases in receptive field size in wide-dynamic range neurones in response to noxious pinch stimuli (Laird 1989).

In this modern interpretation, convergence of other peripheral afferents continues to play a role, though the precise nature of this role is somewhat convoluted (Sinclair 1948), as anaesthetisation of peripheral inputs from the region of referral has demonstrated varied effects. After a procaine block of the brachial plexus sufficient to induce complete paralysis and anaesthesia of the upper arm, a second interspinous injection of 6% HS (0.5ml) was still able to induce a dull aching deep referred pain in the forearm only slightly less than that seen without anaesthetic (B. Feinstein 1954). Similarly, referred pain from electrical stimulation of the tibialis anterior was reduced but not abolished by compression block and intravenous anaesthesia of the foot (Laursen, Graven-Nielsen et al. 1999). These findings suggest that the myelinated fibres may, in part, mediate this peripheral component of convergence.

1.6 AIMS AND STRUCTURE OF THE THESIS

This thesis aims to further explore the spatiotemporal properties of local and referred muscle pain. I employ a variation of the classic methodology pioneered by Kellgren in the 1930’s, together with fMRI to characterise the local and referred pain produced by intramuscular injection of hypertonic saline.

1.6.1 Methods
In Chapter Two I will concisely described the common methods utilised in the four studies that comprise this thesis. Subsequent methodologies will detail the methods specific to each study

1.6.2 Results
In the four Results chapters I present work from four papers; two accepted and two submitted at the time of printing this thesis. Each chapter contains a brief introduction, a description of methods specific to that chapter and a brief discussion of the findings in the broader context of the literature.
1.6.2.1 Chapter 3:
Effects of intramuscular anaesthesia on the expression of primary and referred pain induced by intramuscular injection of hypertonic saline

Chapter 3 examines the effects of blockade of local pain on the expression of referred pain. This chapter is based on a publication in *Journal of Pain* titled “Effects of Intramuscular Anaesthesia on the Expression of Primary and Referred Pain Induced by Intramuscular Injection of Hypertonic Saline” (Rubin, Gandevia et al. 2009). As noted above, intramuscular injection of hypertonic saline produces pain in the belly of the injected muscle (primary pain) and, often, pain that projects distally (referred pain). While it is known that referred pain can be induced during complete sensory block of the distal site, evidence as to whether the perception of referred pain depends on ongoing input from the primary stimulus derives from the nonspecific intramuscular electrical stimulation (Laursen, Graven-Nielsen et al. 1997). We assessed whether blocking the noxious input following the induction of pain blocks the primary but not the referred pain.

While no significant changes were observed in the control element of the crossover design in these experiments, a consistent trend of decreased/attenuated local pain and increased referred pain was observed in the first 90 seconds of subsequent experiments. This and the paucity of published repeated measures data lead us to question the extent of plasticity and variability in this pain model.

1.6.2.2 Chapter 4:
Changes in the spatiotemporal expression of local and referred pain following repeated intramuscular injections of hypertonic saline: a longitudinal study

What happens to the intensity of pain induced by repeated noxious stimuli over time? Does it stay the same, increase or decrease? In Chapter 4 we tested the hypothesis that the intensity and area of pain in the local and referred regions exhibits plasticity when an identical noxious stimulus is delivered to the same site over sequential trials. Based on an publication in the *Journal of Pain* (Xu and Brennan 2010), I show that weekly injections of hypertonic saline into tibialis anterior cause decreases in local but increases in referred pain, suggesting central changes in processing noxious inputs.

The control element of this study made the implicit assumption that hypertonic saline induced muscle pain resulted in a similar perception of local and referred pain in both the arm and the leg. While this is likely to be the case there is, to date no such comparison has been published.
1.6.2.3 Chapter 5:
A comparison of the spatiotemporal expression of local and referred acute muscle pain in the arm and leg

While current data suggests that all referred pain derives from common mechanisms of central sensitisation, there is a paucity of data directly comparing referral in different limbs. Does a common mechanism result in similar precepts of referral from identical stimuli in different limbs? We tested the hypothesis that the incidence, intensity and spatiotemporal expression of referred pain are identical during the muscle pain induced by bolus intramuscular injection of hypertonic saline into flexor carpi radialis (FCR) and tibialis anterior (TA). We also tested the hypothesis that an increase in stimulus intensity causes a parallel increase in the incidence and intensity of local and referred pain, by comparing the responses to 5% and 10% hypertonic saline in two groups of subjects.

In the earlier chapters I reported on psychophysical differences in the expression of primary and referred pain in different muscles. Earlier work suggests that the sensory-discriminative aspects of pain are subserved by the primary somatosensory cortex and, as outlined above, we also know that the insular cortex is involved in the affective aspects of the pain experience. In this Chapter I deal with the potential role of the insula in the sensory-discriminative aspects of pain processing, by extending work from our laboratory showing that the insula cortex is organised in a crude somatotopic fashion.

1.6.2.4 Chapter 6:
Within-limb somatotopic representation of acute muscle pain in the human dorsal posterior insular cortex

It is well established that the insula cortex processes noxious information. We have previously shown that noxious inputs from the arm and leg are coarsely organized somatotopically within the dorsal posterior insula (Henderson, Gandevia et al. 2007). The same has been shown for inputs from C tactile afferents, which mediate affective touch, and it has been suggested that the insula may be responsible for the localization of some somatosensory stimuli. Knowing the degree of spatial detail may have significant implications for the potential role of the dorsal posterior insula in the processing of noxious stimuli. Using high-resolution functional magnetic resonance imaging (fMRI) we compared insula activation patterns during muscle pain induced by injection of hypertonic saline into three muscles within the same limb: shoulder (deltoid), forearm (flexor carpi radialis) and hand (first dorsal interosseous).
1.6.3 General discussion
In this section I will briefly summarize the work described in this thesis and provide a perspective of how this work has contributed to the state of knowledge. I also consider future directions for this research.
CHAPTER 2

GENERAL METHODS
2.1 SUBJECTS

All experiments in this thesis were approved by the Human Research Ethics Committee of both the University of New South Wales and the University of Western and conformed to the Declaration of Helsinki. All experiments were conducted with the understanding and written consent of the subjects, who could stop this study at any time. Participants were informed that they would be likely to feel pain somewhere in the limb that was being injected. Subjects were not taking analgesic drugs and had no knowledge of the experimental hypothesis. Subjects were excluded if they had felt any abnormal persistent pain in the last month. Participants were not included if they had any fractures of the limbs in question within the last year. Short-lasting sporadic symptoms such as minor headaches, 2 weeks prior to, and in-between subsequent experiments were not classified as exclusion criteria. Subjects were only included in the experiments presented in Chapters 4 and 5 if they had not previously participated in any muscle pain experiments. Participants were excluded if they reported muscle pain in normal daily activities.

2.2 PSYCHOPHYSICS

Experiments were conducted with subjects sitting upright with legs supported and outstretched. In order to assess the spatiotemporal characteristics of pain, subjects were asked to draw an enclosed shape around each perceived area of pain on an A4 transparency over the template of the injected limb, with each new transparency being placed over the previous one so that subjects could see the immediate history of the pain profile. Immediately after drawing each separate region of pain, subjects were asked to indicate the intensity of pain in that region on a 10 cm visual analogue scale (VAS) printed on the same transparency with a pen. This was repeated every 30 sec after the hypertonic saline injection until all pain had completely subsided. The VAS was a non-graduated line where 0 cm indicated ‘no pain’ and 10 cm indicated ‘worst pain’. Only subjects with maximal pain ratings equal to or higher than 3 out of 10 were asked to return for subsequent experiments. This served to increase the consistency of a referred response, the accuracy of localisation and decrease the impact of temporally attenuated outliers. This resulted in the exclusion of 4/86 subjects that had pain distribution 3 standard deviations from the mean. Drawings were scanned at 300 dpi, and pixels were tabulated using Adobe Photoshop (Adobe Systems Inc), thus defining the arbitrary unit of area as a pixel. At the end of each experiment subjects indicated the quality of pain with a McGill pain questionnaire that described the overall pain...
experience. Where subjects reported different qualities of pain for individual regions separate questionnaires were utilised

2.3 MUSCLES INJECTED

This thesis deals with injections of hypertonic saline into the muscle belly of the tibialis anterior (in the anterior aspect of the lower leg), the flexor carpi radialis of the forearm (originating on the medial epicondyle of the humerus and inserting on the anterior aspect of the base of the second metacarpal), the first dorsal interosseus of the hand (originating on the radial side of the second metacarpal and the proximal half of the ulnar side of the first metacarpal and inserting in the radial side of the base of the second proximal phalanx) and finally the deltoid muscle of the shoulder. It is assumed that the area of innervation impacted by each injection is contained within the tissue of these muscles, though it remains possible that injections into the first dorsal interosseus resulted in minor spread to the surrounding tissue given its small size in subjects.

2.4 DEFINITION OF LOCAL AND REFERRED PAIN

While local pain is frequently said to incorporate the site of stimulus, definitions for its boundaries are somewhat arbitrary. Furthermore, there is little consensus over the definition of referred pain, except that it must not include the site of stimulus. In Chapters 4 and 5, I employed two definitions of local and referred pain so as to facilitate comparison with other studies and enable a more accurate description of the dynamic spatiotemporal expression of local and referred pain.

Definition 1 (D1) classified local pain as pain appearing in the general area of the lower leg (but not the foot) or the forearm (but not the hand). Specifically, local leg pain was defined as the total painful area in the lower leg from the knee to the line connecting the malleoli (Schulte et al., 2003). Similarly, local forearm pain was defined as all pain within the forearm - between the elbow and the line connecting the distal ends of the radius and ulna. If there were multiple regions within this location, the maximum intensity was taken and their areas were summed. For TA injections, small islands of pain (smaller than the length of the foot) extended from the leg into the foot (traversing the line connecting the malleoli) in some subjects. In these cases such shapes were arbitrarily assumed to lie in the region (local or referred) where greater than 60% of its area was situated. For FCR injections, no islands of pain were observed to cross the
line connecting the head of the ulna and radius. Referred pain was defined as all leg pain distal to the line connecting the malleoli or all hand pain distal to the wrist.

Definition 2 (D2) classified local and referred pain on the basis of spatial separation. Here, only pain shapes overlapping a 2 cm radius (scaled to the subject) of the injection site were classed as local pain. In this respect a single contiguous area of pain extending from the injection site to the foot is counted as local pain. All shapes outside of this area are classed as referred pain.

All statistical analyses were conducted with both definitions of pain. Each subject’s leg (and arm) length was measured. The ratio of the length from the distal edge of the patella to the line connecting the malleoli (and) the length of the elbow to the head of the radius, relative to that of the template, was used for individual scaling factors. This facilitated comparison of pain distribution in the arm with that in the leg, that were scaled differently so maximise spatial representation.

![Figure 2.1; Schematic of pain distribution patterns and classification under different definitions.](image)

Black areas in panels B, C, D and E indicate typically displayed regions classified as local pain irrespective of definitions. The white area in panel B and E represent referred regions irrespective of definition and the gray areas in panel A and F denote typical subject responses that were classified as local pain with definition 1 and referred pain with definition 2. The striped area in the hand of panel C and the foot of panel D demonstrate referred pain in D1 but local in D2. Thus panel C and D displays single continuous regions of pain from the injection site to the hand and foot respectively. The
line connecting the ulna and the radius in the wrist and that bisecting the malleoli in the ankle demarcates the separation of local and referred pain for the purposes of D1, while D2 categorises the entire region as local. Panel B and E demonstrate typical distributions of local and referred pain in either definition.
CHAPTER 3

EFFECTS OF INTRAMUSCULAR ANAESTHESIA
ON THE EXPRESSION OF PRIMARY AND
REFERRED PAIN INDUCED BY INTRAMUSCULAR
INJECTION OF HYPERTONIC SALINE
3.1 INTRODUCTION

Intramuscular injection of hypertonic saline has been used as an experimental model for acute muscle pain in over 130 studies (Graven-Nielsen 2006) since the late 1930s (Kellgren 1938; Lewis 1938). Subcutaneous injection of hypertonic saline induces a sharp, burning sensation limited to a small, well-defined area (Henderson, Bandler et al. 2006). In contrast, intramuscular injection induces pain that is typically described as dull, cramping and aching. This deep pain usually covers a larger local area (Graven-Nielsen, Babenko et al. 1998), (Graven-Nielsen, McArdle et al. 1997) and often refers to distal regions (Gibson, Arendt-Nielsen et al. 2006). Differential nerve blocks produced by compression in human subjects confirm that hypertonic saline activates C-fibers, and to a lesser extent Aδ-fibers (Laursen, Graven-Nielsen et al. 1999).

Two theories have been proposed to explain the inability of higher brain centers to correctly identify the source of painful stimuli. One theory involves a single nociceptive neuron having two (or more) widely separated receptive fields, with a bifurcated axon supplying a receptive field in the local area as well as the referred area (Sinclair 1948). However, this fails to explain the delayed onset of referred pain (Hockaday and Whitty 1967; Graven-Nielsen, Arendt-Nielsen et al. 1997) or the observation that muscle pain frequently refers distally but rarely proximally. The convergence-projection theory (Ruch 1947) requires a convergence of afferents from local and referred regions on the same population of spinal projection neurons, though this too fails to account for semi-directionality and latency. More recent adaptations of Mackenzie’s original convergence facilitation theory (Mackenzie 1909) propose the unmasking and sensitization of inactive connections within the dorsal horn of the spinal cord (Mense 1994). This view is based on animal experiments in which dorsal horn neurons showed expansion and persistence of new receptive fields following noxious stimuli (Hoheisel, Mense et al. 1993). Here activation of latent synaptic connections facilitates both convergent and divergent inputs to projection neurons.

If sensitization plays a part in referred pathologies it is important to establish whether ongoing nociceptive input is required to maintain the sensation of referred pain. Does referred pain persist in the absence of local pain or can it be sustained by central processes? Many studies have used systemic application of anesthetic to abolish hypertonic-saline induced muscle pain (Schulte, Graven-Nielsen et al. 2003; Schulte, Segerdahl et al. 2006) while others have used anesthetics (Laursen, Graven-Nielsen et al. 1997) and nerve blocks (Laursen, Graven-Nielsen et al. 1999; Laursen, Graven-Nielsen et al. 1999) to decrease input from the region of referred pain. While
informative, these experiments have not been designed to determine the effect of abolishing only the local noxious input on the expression of referred pain.

To answer this question we monitored the expression of local and referred pain following injection of hypertonic saline into the tibialis anterior muscle, recording the intensity and area every 30 seconds until the pain had subsided. Intramuscular injection of local anesthetic was used to test the hypothesis that the expression and maintenance of referred pain depends on ongoing sensory activity from the primary site of noxious stimulation.

3.2 METHODS

3.2.1 Experimental protocol
Fifteen healthy subjects (8 males, 7 females) aged 20–45 years subjects attended at least two sessions of a quasi-random cross-over design involving four presentation sequences, with at least one week separating each session. All sequences involved a bolus intramuscular injection of 0.5 ml (over 3 s) of 5% hypertonic saline, followed 90 s later by either a second bolus injection or a bolus injection of 2 ml (over 5 s) lignocaine (1%, no adrenaline) through the same cannula. The lignocaine injection was followed 60 s later either by a second identical injection of hypertonic saline (sequence 1) or a sham injection (sequence 2), as depicted in Fig. 3.1. The two sequential injections of hypertonic saline were followed 60 s later by either a lignocaine injection (sequence 3) or a sham injection (sequence 4). All injections were delivered via an indwelling 23 G cannula inserted into the rostral part of the tibialis anterior muscle connected via a three-way stop-cock to two syringes. Sham injections involved a charade where an empty syringe inserted into the three-way stopcock was touched by the examiner. The identity of the syringes (hypertonic saline or lignocaine) was not known to the subjects, nor was the order of presentation.

Referred pain was defined as being pain isolated and distinct from the local pain caused by the injection and appeared spontaneously, with no stimulation of the referred pain area required (Gibson, Arendt-Nielsen et al. 2006). Local pain within TA is sometimes seen to extend into referred areas, (Graven-Nielsen, Babenko et al. 1998) forming one continuous area. In these instances an arbitrary line was drawn between the malleoli (Schulte, Graven-Nielsen et al. 2003) to define the area of referred pain. Regions distal to this demarcation were classed as referred. All drawings were scanned at 300 dpi, thus defining the arbitrary unit of area as a pixel. Areas are
presented in absolute (pixels) and relative (normalized to the maximal area of pain in a given subject) terms; pain intensity was likewise presented in absolute and relative terms.

Figure 3.1: Flowchart of experimental protocol.

Subjects were asked to attend a minimum of two experimental sessions each separated by at least one week. The quasi-random cross-over design, involved a total of four different experiments each comprised by a unique sequence of injections. The first injection was followed 90 s later by a second that was then followed 60 seconds later by a third injection or a sham. Sequence 1; hypertonic saline (H), lignocaine (L), hypertonic saline. Sequence 2; H, L, Sham (S). Sequence 3; H, H, L, and sequence 4 H, H, S. Data from sequences 3 and 4 (dark tiles) were compared to determine the effects of lignocaine.

3.2.3 Analysis

Statistical analysis compared protocols where two subsequent hypertonic saline injections were followed by a lignocaine injection and those where two hypertonic saline injections were followed by a sham. Pain sensations at local and referred regions were compared between these protocols if they were present in both experiments. This resulted in the exclusion of only local pain in one experiment and only referred pain in another. Only subjects (n = 11: 8 males, 3 females) with maximum pain intensities of 3 (moderate pain) or greater were included in the analysis. Area data, both absolute and relative (normalized to each subject’s maximum), together with normalized pain intensity were entered into separate 3-way repeated measures ANOVAs using SPSS for Windows (version 13.0 SPSS Inc. Chicago, IL). This software was also used to determine trend-lines of optimal fit (typically 6th and 7th order polynomials). Separate ANOVAs were performed over the first 2.5 minutes and over the remaining 13.5 minutes, since the lignocaine protocols were identical until the lignocaine injection at 2.5 minutes. The within-subjects factors were Region (Primary vs. Secondary),
Lignocaine (With vs. Without) and Time. Planned contrasts on the Time factor examined the linear and quadratic trends over Time. A significant linear contrast indicates a steady decline over time points, while an exponential trend is indicated by significant linear and quadratic trends. All data are presented as mean ± standard error.

Figure 3.2; Schematic of pain distribution patterns.
(A), local pain primarily in the region of the tibialis anterior muscle; (B), referred pain in the ankle and foot; (C), pain that extended throughout the anterior compartment of the leg and into the ankle and foot; (D), concurrent sensation of both local and referred pain (the line bisecting the malleoli represents the separation of local and referred pain for the purposes of analysis); (E), unusual patterns of pain in three subjects: one subject reported pain internal to the medial and dorsal surfaces of the knee, another reported pain in the medial gastrocnemius, and the third reported pain proximal to the ankle joint.

3.3 RESULTS

Intramuscular injection of hypertonic saline caused a deep, dull ache in the muscle belly (local pain) and often referred pain that extended beyond the area of injection into the ankle or foot. The different distributions of pain are illustrated in Figure 3.2. The most common pattern (n=7) comprised two spatially distinct areas of pain, one local
and one referred distally (Fig. 3.2D). Five subjects experienced only local pain in a total of 6 experiments (Fig. 3.2A), while two experienced only referred pain in three experiments (Fig. 3.2B). In a total of five experiments on three subjects there was no clear distinction between local and referred pain. In these instances, an arbitrary line was drawn between the apices of the malleoli (Fig. 3.2C), this pattern appeared at the onset of pain but then differentiated into two distinct areas. Three other subjects presented the distinct referral patterns illustrated in Fig. 3.2E: one subject reported pain internal to the medial and dorsal surfaces of the knee while another felt pain medial to the tibia; the third localized pain just proximal to the ankle joint, i.e. within the area arbitrarily defined as local.

**Figure 3.3;** 3D projection of raw data from a single experiment in which two sequential intramuscular injections of hypertonic saline were administered (1.5 min apart). Individual local and referred areas, drawn every 30 s, are displayed over the schematic of a leg. The two arrows indicate the times of injection through the indwelling cannula.

Figure 3.3 shows raw data from one subject who reported both local and referred pain. It can be seen that both the areas of pain were spatially distinct but developed
concurrently. Average time-courses of the intensities and areas of pain in the local and referred areas, expressed in absolute and relative terms, are shown in Fig 3.4 (bold lines). Normalized intensity closely paralleled normalized area in experiments with and without anesthetic in both regions. Pain reached a peak within 2.5 minutes and then slowly subsided over 8-15 minutes. While the maximal absolute intensity of the pain was not significantly different between local and referred regions, ($F<1$, $p > 0.05$), the maximum absolute area of pain in the referred region was about a third of that in the local region. Moreover, on average the local pain outlasted the referred pain by up to 8 minutes.
Figure 3.4; Mean pain area and intensity (± SE) in local regions (n=9) and referred regions (n=8) in experiments in which the first injection of hypertonic saline was followed by a second injection at 1.5 minutes (solid vertical lines) and, at 2.5 minutes (dashed vertical line) either a sham injection or an intramuscular injection of local anesthetic. Trend lines are created with 6th and 7th order polynomials. Thick lines refer to experiments in which the third injection was a sham, thin lines to those experiments in which the third injection was local anesthetic. These data demonstrate the consistent effects of lignocaine on the percept of pain within local and referred regions.
3.3.1 Effects of intramuscular anesthesia

Comparison of hypertonic-saline-anesthetic and hypertonic-saline data from the first 2.5 minutes, where both protocols were identical, served as an internal control. This analysis revealed no significant differences in intensity ($F[1.5] = 1.57, p > 0.05$), normalized area ($F[1,6] = 1.60, p > 0.05$) or absolute area ($F[1,5] = 0.001, p > 0.05$) between experiments. As seen in figure 3.3, intramuscular anesthesia caused a reduction in normalized pain intensity ($F[1,5] = 57.22, p < 0.001$, power = 1.00) and normalized area (Linear $F[1,6] = 13.80, p = 0.01$, power = 0.87). The fall in pain intensity after lignocaine injection was greater for the local region than the referred region ($F[1,5] = 7.14, p = 0.044$, power = 0.57). The effect of lignocaine was not significantly different between the normalized local and referred areas ($F[1,6] = 1.93, p > 0.05$). In fact, after the injection of lignocaine the percept of pain at the local region was significantly correlated with that at the referred region, (normalized intensity $R = 0.987, N = 26, p < 0.001$; normalized area $R = 0.965 N = 26, p < 0.001$). After 2.5 minutes the rate of fall in pain intensity was significantly greater following intramuscular anesthesia (Linear $F[1,5]=61.8, p = 1.00$, Quadratic $F[1,5]=9.836, p < 0.05$, power = 0.70; the same was true for the rate of decay of absolute areas of pain (linear $F[1,4]=26.4 p=0.007$, power = 0.96 quadratic $F[1,4]=10.9 p=0.03$, power = 0.70). This anesthetic-induced attenuation was greater in the local region than in the referred region.

The total pain area and intensity, respectively representing integration of the mean area versus time and mean intensity versus time curves were derived for each set of experiments. Total intensity decreased by $25.3 \pm 26.4\%$ and $25.4 \pm 12.9\%$ in the local and referred regions respectively, while total area decreased by $20.6 \pm 15.1\%$ and $10.5 \pm 25.4\%$. The same data of Fig. 3.4 are shown in a different format in Fig. 5 to illustrate the similar time-courses of the reduction of pain following lignocaine.

![Figure 3.5; Mean pain area and intensity (± SE) in experiments in which the first](image-url)
injection of hypertonic saline was followed by a second injection at 1.5 minutes (solid vertical lines) and, at 2.5 minutes (dashed vertical line) either a sham injection or an intramuscular injection of local anesthetic. Trend lines comparing pain percepts in local (black) and referred (grey) regions both with (thin line) and without (thick line) lignocaine. The top graph displays mean normalized pain intensity, the bottom graph normalized mean pain area. The striking similarity between the black and gray thin lines of each graph represents the parity in local and referred pain percepts after the delivery of anesthetic.

Figure 3.6 shows the mean intensity and area profiles from those experiments (n=9) in which the first injection of hypertonic saline was followed by intramuscular injection of local anesthetic at 1.5 minutes and a second injection of hypertonic saline at 2.5 minutes. Intramuscular anesthesia prevented a subsequent injection of hypertonic saline from causing pain.

Figure 3.6; Mean pain area and intensity (± SE) from local and referred regions in experiments in which the first injection of hypertonic saline was followed by intramuscular injection of local anesthetic at 1.5 minutes (dashed vertical line) and a second injection of hypertonic saline at 2.5 minutes (solid vertical line). Thick lines refer to normalized area, thin lines to normalized intensity. The lack of any inflection point in these trend lines at the time of the second hypertonic saline injection indicate the irreversible nature of the lignocaine treatment.

3.4 DISCUSSION

Many studies have used intramuscular injections of hypertonic saline to induce muscle pain. It is known that a small volume of hypertonic saline, such as the 0.5 ml bolus injection used in the present study, can induce pain over a large area of the muscle belly. Such pain can be expressed remote from the site of noxious stimulation. However, it is not known whether this remote - referred - pain, once established by a
primary noxious stimulus, requires an ongoing noxious input to maintain the pain. We have now shown that, once the nociceptors have been activated by hypertonic saline, and both the local and referred pain established, both sites of pain disappear following intramuscular injection of local anesthetic into the site of the primary noxious stimulus. Lignocaine inhibits C fiber and Aδ activity. (Gokin, Philip et al. 2001) (Tanelian and MacIver 1991; Laursen, Graven-Nielsen et al. 1999) Moreover, this anesthesia prevents a subsequent injection of hypertonic saline site from causing pain, indicating that the local anesthetic interferes with the capacity of hypertonic saline to depolarize small-diameter axons.

Areas of referred pain were, on average, one third smaller than the areas of local pain, corroborating earlier studies (Graven-Nielsen 2006). Our incidence of referred pain to an identical stimulus (78%) was high, in the upper end of the range reported in previous studies. (Graven-Nielsen, Arendt-Nielsen et al. 1997; Graven-Nielsen, Babenko et al. 1998; Schulte, Segerdahl et al. 2006). In contrast to other findings of Graven-Nielsen and colleagues (Graven-Nielsen, Arendt-Nielsen et al. 1997), the maximal intensity of pain at the local and referred regions was not significantly different.

While the observed correlations between the area and intensity of pain were expected (Graven-Nielsen, Arendt-Nielsen et al. 1997), the similar rates of attenuation in pain at the local and referred sites were not. Rather, we would have expected that, in most subjects, referred pain would persist following block of the primary noxious input, in support of the central sensitization model of referred pain. Persistence of referred pain was found in two subjects. In contrast to the other 13 subjects, these two subjects had participated in several studies involving injection of hypertonic saline into the tibialis anterior muscle, and it is our impression that, over the course of a few weeks, the relative magnitudes of local and referred pain areas changed: referred pain became more dominant, at the expense of local pain. Indeed, in one of these subjects the intramuscular injection induced referred pain exclusively. This phenomenon is currently being investigated in a longitudinal series of experiments. With the above caveat, our data show that referred pain usually depends on an ongoing nociceptive input from the primary site of noxious stimulation (even though overt pain in this area may not be apparent). While there is a complex time course for describing the decay of pain in the local and referred regions under standard conditions, this may be simplified in the presence of lignocaine. The similar response of these regions after lignocaine may result from a form of “wind-down” at a set of central synapses that convey divergent
information to higher centers representing these separate and distinct regions of the body.

3.4.1 Methodological considerations
Our sample consisted of 8 male and 7 female subjects. While many studies have claimed higher pain thresholds for men, (Giles and Walker 1999) meta-analysis suggests numbers of approximately 40 per group are required to obtain adequate statistical power to draw such conclusions. (Riley, Robinson et al. 1998) We recently documented gender differences in the central processing of experimental muscle pain in an fMRI study involving 12 male and 12 female subjects, despite identical pain ratings between the two groups.(Riley, Robinson et al. 1998) The small number of subjects included in the present study precludes our commenting on gender differences in perceptual responses to pain, but given the robust nature of the lignocaine-induced attenuation of local and referred pain we do not believe that gender differences, and hormonal fluctuations related to the menstrual cycle, would affect our conclusions. Our approach was to inform subjects that they were to receive three injections, but that – apart from the first injection (hypertonic saline) – they would not know the identity (or order) of the subsequent injections. It is known that anticipation of pain can induce an apparently real painful experience (Ploghaus, Tracey et al. 1999), yet lignocaine (but not a dummy “sham” injection) caused the pain to subside. Accordingly, we do not believe expectation played a role in the current findings. And, while subjects could see the intramuscular cannula through which both hypertonic saline and lignocaine were injected via a stopcock, they could not see the experimenter turning the stopcock or injecting the solution. Finally, given that both local muscle pain and referred pain have qualities of depth, our measurement of two dimensional areas, rather than volumes, could be seen as a limitation. However, as the subjective intensity of pain is a dimensionless metric that reflects the volume and/or area of the tissue in which the subject perceives the pain, and that pain intensity is tightly correlated to the spatial area of the pain, it is reasonable to conclude that the two-dimensional measure of area is not a major limitation.

3.4.2 Conclusions
Using intramuscular injection of lignocaine we have shown that the pain induced by a prior intramuscular injection of hypertonic saline falls in the area of referred pain as well as in the area of local pain and conclude that, in general, the maintenance of referred muscle pain depends on ongoing noxious inputs from the site of primary muscle pain. Whether these observations are relevant to chronic pain remains to be seen.
CHAPTER 4

CHANGES IN THE SPATIOTEMPORAL EXPRESSION OF LOCAL AND REFERRED PAIN FOLLOWING REPEATED INTRAMUSCULAR INJECTIONS OF HYPERTONIC SALINE: A LONGITUDINAL STUDY
4.1 INTRODUCTION

Nociceptor activation in deep structures can result in pain perceived at a distance to the site of activation, known as referred pain. This aberrant projected sensation may be observed during activation of visceral nociceptors - such as the pain down the left arm during periods of myocardial ischaemia – as well as nociceptors in skeletal muscle or fascia. For example, it is known that intramuscular injection of hypertonic saline into tibialis anterior induces local pain in the muscle belly surrounding the injection site and, often, referred pain in the anterior aspect of the leg and/or the dorsum of the foot (Kellgren 1938; Feinstein, Langton et al. 1954; Rubin, Gandevia et al. 2009); the same can be induced by injection of hypertonic saline into tendon (Gibson, Arendt-Nielsen et al. 2006). Central mechanisms are likely to be involved as referred pain can be perceived even after complete sensory block in the area of referred pain (Kellgren 1938; Feinstein, Langton et al. 1954). It has been suggested that pain referred from visceral to cutaneous structures results from the convergence of nociceptive afferents in the dorsal horn (Foreman, Blair et al. 1984). Furthermore, electrophysiological studies in animal models reveal changes in the dorsal horn, including expansion of receptive fields, the development of new receptive fields and decreases in nociceptive threshold following peripheral noxious input (Cook, Woolf et al. 1987; Hoheisel and Mense 1989; Hylden, Nahin et al. 1989; Laird 1989; Hoheisel, Mense et al. 1993; Ren, Williams et al. 1994). However, it is also possible that plastic changes could occur supraspinally. Indeed, our recent fMRI studies have shown that intramuscular injection of hypertonic saline activates the region of the primary somatosensory cortex corresponding to the sensory representation of local muscle pain and, in those subjects who experienced referred pain, expansion of this sensory representation to include the area in which referred pain is perceived (Henderson, Bandler et al. 2006; Macefield, Gandevia et al. 2007). Nevertheless, we do not know whether these changes are established spinally or supraspinally.

It is known that referred pain can be induced by mechanical stimulation of specific trigger points in individuals with myofascial pain syndrome (Li, Ge et al. 2009). There is also an increase in pain in response to intramuscular injection of hypertonic saline in such individuals, with the incidence of referred pain being higher the greater the intensity of local pain (Li, Ge et al. 2009). Given that allodynia can also be induced by (non-noxious) stimulation at latent trigger points (Li, Ge et al. 2009), these observations support the idea that referred pain does indeed reflect changes in central neural processing. But how dependent is referred pain on local pain? It has been reported that the incidence of referred pain is positively correlated to the intensity of local pain.
following intramuscular injection of hypertonic saline (Graven-Nielsen 1997), and in myofascial pain syndrome it is known that treating the trigger point with local anaesthesia can abolish both the local and referred pain produced by mechanical stimulation (Li, Ge et al. 2009). This fits with our recent study in healthy subjects, in which we abolished both the local and referred pain following intramuscular injection of hypertonic saline by subsequent injection of local anaesthetic into the primary site of noxious stimulation (Rubin, Gandevia et al. 2009) (See Chapter 3). However, we did note that referred pain continued in two subjects despite the complete abolition of local pain, suggesting the persistence of central plastic changes – albeit in the short term. Yet because referred pain can become chronic, and that it is believed that chronic pain can be established by plastic changes within the central nervous system following initial nociceptor stimulation, it is important to understand the means by which referred pain is brought about, including the time course over which referred pain develops.

In a recent investigation we observed a progressive change in the spatial expression of hypertonic-saline induced muscle pain over two consecutive experiments separated by several weeks. In two subjects the local pain from the injection into tibialis anterior disappeared completely, revealing only referred pain in the dorsum of the foot. Our initial observations suggest that the pattern of perceived pain can alter over time and may migrate away from the local area of pain during repeated nociceptor activation. In the present study we tested the hypothesis that identical injections of hypertonic saline into tibialis anterior (TA), once per week over four weeks, result in the attenuation of local pain and an increase in the expression of referred pain.

4.2 METHODS

4.2.1 Experimental Protocol

Thirty-five subjects (18 males, 17 females) aged 18 – 31 attended 5 sessions each separated by 7 days. Each session involved a single 1 ml bolus (over 3 s) injection of 5% hypertonic saline through an indwelling 23 G cannula. The first of each 40 min session involved an injection into the muscle belly of the flexor carpi radialis (FCR) of the arm contralateral to the dominant writing hand. This session was used to familiarise subjects to the quality and time-course of the pain, and to familiarise them with the method of reporting the intensity and area(s) of pain every 30s as described in 2.2. The subsequent four experimental sessions involved an identical injection into the belly of the tibialis anterior (TA) muscle. Leg injections were contralateral to the initial arm injection and within 10 mm of the initial injection. The TA of all subjects was palpated to
estimate the depth of muscle, the degree of sub-dermal adiposity and check for gross abnormalities or unknown conditions such as latent trigger points. The injection site was marked on the skin with a permanent marker and indicated on a transparent template of the lower leg, on which anatomical landmarks were also marked so that the injection site could be identified in subsequent experiments.

4.2.2 Analysis

Only subjects that completed the series of 5 experiments were included in the analysis. Intensities and areas from the scanned drawings were partitioned as local or referred pain on the basis of two definitions, as illustrated in Fig 2.1.

Area and pain intensity data from the 4 TA injections were entered into separate 3-way repeated measures ANOVAs using general linear modelling with SPSS for Windows (version 14.0 SPSS Inc. Chicago, IL). The within-subjects factors were Region (local & referred), Week (1 through 4) and Time. Using GraphPad Prism for Windows (Version 5.00 GraphPad Software, San Diego CA, USA), separate 2-way ANOVAs with Bonferroni corrections for multiple comparisons, to compare replicate means by row, were performed for each measure (area, intensity) at each region, for each definition, and for total area and maximal intensity.

4.3 RESULTS

Twenty one healthy subjects (9 males, 12 females), aged 18–28 years, completed the study. Two subjects were excluded because they experienced a maximal VAS rating <3 in their first TA injection. The remaining subjects were either unable to attend at 7 day intervals or had illness or injury during the course of the study.

Local pain induced by the intramuscular injection of hypertonic saline into TA generally radiated distally from the injection site and referred pain was frequently reported on the dorsum of the foot. Referred pain was less commonly reported in the toes, projecting to the knee or in the anteromedial aspect of the lower leg. Subjects who perceived pain in these regions consistently did so in subsequent experiments. The sensory descriptors most frequently chosen to describe the quality of pain were “dull”, “throbbing” and “aching”. On three occasions in two subjects reported a change in the quality of pain or the frequency of the throbbing that occurred simultaneously in local and referred regions.
While five subjects did develop minor superficial bruising by the third and fourth TA injection, there was no relation to the distribution, quality or magnitude of pain. Bruising, evident by the third week as slight discoloration within a 30 mm radius of the injection site was not painful to palpate. The vast majority of subjects (80%), however, reported deep tenderness in the area of the injections lasting 2-4 days after the initial injections. This tenderness had resolved by the time they returned for the subsequent injections at one week intervals. No superficial numbness to stroking or gently palpation around the injection site was reported by subjects.

![Figure 4.1](image-url)  

**Figure 4.1:** Mean total pain area and maximal intensity (±SE) irrespective of regional classification (n=21) in 4 consecutive experiments each separated by 1 week. Filled points indicate data significantly greater than 0, hollow points represent data not significantly greater than 0. Significance levels are indicated between week 1 and the week of the corresponding colour and obtained from 2-way ANOVAs with Bonferroni corrections for multiple comparisons. Trend lines are created with 6th and 7th order polynomials. These data demonstrate decreases in the overall percept of pain over consecutive weeks.

### 4.3.1 Changes in the spatial expression of pain over time

In all subjects the total areas of pain varied significantly over the course of the four TA injections (3-way ANOVA; F[1,21] = 9.43, p = 0.006, power = 0.832). A progressive decrease in total area (integration of time vs. mean area curve) of 47% over 4 weeks was observed, together with a progressive decrease in peak area of 32%. Likewise, maximal intensity from any location varied significantly (F[1,21] = 18.26, p < 0.0005, power = 0.982) over the duration of the experiment. A progressive decrease in total intensity (integration of time vs. mean intensity curve) of 43% was accompanied by a 21% progressive decrease in mean peak intensity over the 4 experiments, as shown in Fig. 4.1. After four weeks the peak total area and maximal pain intensity were significantly weaker than in week 1 (2-way ANOVA, p<0.001).
With the exception of one subject, each region of pain was separated by more than 10 cm. The remaining subject reported identical pain ratings at each of two to four closely separated regions in three of the four weekly TA injections. According to the standard definition (D1; see Methods) these regions were classified as areas of local pain. However, according to our spatially restrictive classification (D2), these regions were defined as being areas of referred pain.

Although the regional distribution of pain varied significantly over the course of the experiment, as determined by 3 way ANOVA (D2, Area ($F_{1,21} = 8.14$, $p = 0.010$, power = 0.775), intensity ($F_{1,21} = 26.23$, $p < 0.0005$, power = 0.998), D1, Area ($F_{1,21} = 8.85$, $p = 0.007$, power = 0.808), intensity ($F_{1,21} = 14.87$, $p = 0.001$, power = 0.956) there were still a number of distinct and significant trends. In the first two weeks, all subjects perceived local pain irrespective of classification method (D1 or D2). In the third and fourth weeks, however, some subjects experienced only referred pain (Table 4.1), with a net increase in the frequency of referred pain being apparent.

![Graphical data from one of these subjects are shown in Fig 4.2.](image-url)

**Table 4.1:** Frequency of local and referred pain after HS injections in 21 subjects over 4 weeks as depicted by two definitions of referral. A minor yet clear trend of decreased referral and decreased local pain is evident over the subsequent injections.

All but one individual who had initially experienced referral went on to display referred pain in the final experiment (week 4). The increased numbers of referral captured in D2 relative to D1 indicate a greater abundance of distinct secondary regions of pain, or a movement of pain away from the stimulus site over time. Two subjects displayed a progressive movement of pain away from the injection site over the four weeks. Graphical data from one of these subjects are shown in Fig 4.2.
**Figure 4.2:** 3D projection of raw data from four experiments on the same subject, each separated by one week. Individual local and referred areas, drawn every 30 s, are displayed beside the schematic of a leg. Vertical lines indicate the distance from the stimulus site and pain intensity is represented by colour. This reveals the increase in referral that was, in two subjects, progressive over the course of the four trials.

With the standard (D1) classification of pain area, the overall area and intensity of local pain showed a progressive 50% reduction over the course of the experiment, together with a 20% reduction in referred overall area. In contrast, the restricted definition of pain area (D2) revealed decreases in total area (61%) and intensity (55%) of local pain yet a 42% increase in total referred area, coupled with a minor (10%) increase in intensity of referred pain over the four weeks. Statistical analyses (separate 2-way ANOVAs with Bonferroni corrections for multiple comparisons) are illustrated in Fig. 4.3.
Figure 4.3; Mean pain area and intensity (± SE) in local regions and referred regions (n=21) as defined by definition 1 and definition 2 in 4 consecutive experiments, each separated by 1 week. Filled points indicate data significantly greater than 0, hollow points represent data not significantly greater than 0. Significance levels are indicated between week 1 and the week of the corresponding colour and obtained from 2-way ANOVAs with Bonferroni corrections for multiple comparisons. Trend lines are created with 6th and 7th order polynomials. While the local pain percept is attenuated
irrespective of regional classification, definition 2 depicts a distinct increase in referred area and reduced but still significant increase in referred intensity.

Figure 4.4 shows the relationship of mean normalised area and intensity between local and referred regions over the four TA injections; data are normalised to the maximal value of the first occurrence of pain within each region for each subject.

4.3.2 Changes in the duration of local and referred pain over time
The mean duration of pain induced by each injection progressively fell (by 25%) from 8 minutes in week 1 to 6 minutes in week 4. However, analysing the temporal changes in the expression of pain emphasised the differences between the two definitions of local and referred pain. While D1 showed a progressive 32% decrease in the mean duration of local pain and a 5% increase in duration of referred pain, D2 revealed a progressive 39% decrease in the duration of local pain yet a progressive 27% increase in the mean duration of referred pain.

4.4 DISCUSSION
This is the first study to have shown consistent decreases in the perception of local pain and increases in the perception of referred pain following repeated intramuscular injections of hypertonic saline, delivered over four weeks. Irrespective of how the regions of local and referred pain were defined, significant progressive reductions were observed in maximal intensity and total area of perceived local pain; there were also reductions in the duration of pain over time. This would suggest that the efficacy of the
stimulus was decreasing over time (or the subjects were habituating to the repeated stimuli), but we do not believe this reflects the total pain experience. Rather, we see a change in the spatiotemporal expression of local and referred pain over time: coinciding with this progressive decrease in local pain was an increase in the incidence, area and intensity of referred pain, when measured according to our spatially restrictive definition of referred pain (D2). Our results support the notion that the location of perceived pain may not coincide with the region of nociceptor activation. Indeed, our results suggest that the more frequent the nociceptor activation the greater the chance that a mismatch between the location of perceived pain and nociceptor activation will occur.

It is now commonly accepted that referred pain results from central, rather than peripheral mechanisms. Indeed, the changes in perceived pain reported here mirror the changes in receptive field properties described in previous animal investigations. Hoheisel and Mense demonstrated that following injections of the pro-inflammatory algesic peptide bradykinin into the gastrocnemius-soleus and semitendinosus muscles of cats, dorsal horn neurons developed new receptive fields in response to subsequent electrical stimulation and repeated injection of bradykinin (Hoheisel and Mense 1989). Further, they have reported that intramuscular injections of bradykinin evoke distal expansion and the development of new receptive fields in dorsal horn neurons that receive group III and IV afferents (Hoheisel, Mense et al. 1993). In rodents, Cook and colleagues showed that brief inputs from peripheral unmyelinated afferent fibres can result in substantial cutaneous receptive field changes that can last more than an hour (Cook, Woolf et al. 1987). Although these animal studies did not explore receptive field changes during repeated transient acute pain stimuli separated by days, they clearly demonstrate that the receptive fields of superficial dorsal horn neurons display a level of plasticity and provide a potential basis for the changes in pain perception described in this investigation.

In humans, it has been shown that experimental muscle pain can be affected by temporal summation. Graven-Nielsen and colleagues shown that an individual had a greater tendency to perceive referred pain during sequential hypertonic saline injections given at 90 second compared to 360 second intervals (Graven-Nielsen, Arendt-Nielsen et al. 1997). Furthermore, the authors reported that when the hypertonic saline injection into muscles of the anterior leg compartment were repeated 1 and 4 hours after the initial injection, subjects - on average - reported an increase in referred pain intensity of 84% and referred pain area of 165%. However, the authors also noted that these values decreased to initial injection levels following another
hypertonic saline injection 24 hours later. Contrasting our findings, two studies were unable to find significant differences between two subsequent trials of hypertonic saline separated by multiple days (Wolff BB 1965; Graven-Nielsen 1997). An insignificant (7.5%) reduction in overall maximal intensity has been reported after two 0.5 ml injections of hypertonic saline (5%) separated by one week (Graven-Nielsen 1997). With subjects completing one drawing of the pain distribution per experiment, this study reported increases in local and referred pain area of 16% and 12%, respectively. The difference in findings may be reconciled by the limited number of subjects, limited trials, and the involvement of 80-100% of the subjects in three other experiments in the same publication. Indeed, the present study specifically used subjects who had not previously participated in any other pain studies. Although there appears to be some variation in the timing and degree of the changes in pain perception with multiple intramuscular hypertonic saline injections, the results highlight the plastic nature of pain processing.

Habituation is by no means a novel process; indeed it underpinned the studies that drove our modern understanding of neuroplastic processes (Pinsker, Kupfermann et al. 1970). In this respect it is surprising that there is a paucity of information regarding the central mechanisms of habituation to pain. A recent functional study examined the effects of repeated noxious thermal stimuli once a day for 8 days. Functional MRI revealed consistent decreases throughout the pain neuromatrix, particularly in the thalamus, anterior insula and SII, correlating with a progressive decrease in VAS score form the noxious stimuli (Bingel, Schoell et al. 2007). Interestingly the only area that demonstrated an increase in BOLD activity at the end of the 8 day paradigm was the rostral sAAC. As noted above, this area is known to play an essential role in endogenous pain control systems (Petrovic, Petersson et al. 2000; Bantick, Wise et al. 2002; Legrain, Guérit et al. 2002; Ohara, Crone et al. 2004; Ohara, Crone et al. 2004; Valet, Sprenger et al. 2004). This site has also been correlated with placebo induced analgesia (Wager, Rilling et al. 2004; Zubieta and Stohler 2009) and distraction induced analgesia(Kulkarni, Bentley et al. 2005;Brooks, 2002 #582). In a recent extension by the authors of the aforementioned functional study, the somatotopic and pharmacological specificity of inhibition(Rennefeld, Wiech et al. 2010). A similar stimulus was found to result in an opioid independent significant habituation at the site of stimulus and to a lesser extend the other limbs.

It is now well documented that persistent pain is also associated with brain plasticity. For example, we and others have shown that persistent neuropathic pain is associated with reorganization of the primary somatosensory cortex, the magnitude of which is correlated to the intensity of on-going pain (Flor, Elbert et al. 1995; Wrigley, Press et al.
Since pain relieving strategies can reverse at least part of this somatosensory reorganization in minutes, it has been proposed that cortical plasticity associated with persistent pain results at least partly from the unmasking of latent connections (Birbaumer, Lutzenberger et al. 1997). Similarly, it has been proposed by Hoheisel and Mense, that pain referral during acute muscle nociceptor activation may also be due to the recruitment of latent connections (Hoheisel, Mense et al. 1993). It may be the case that acute activation of the same group of nociceptors over a period of weeks results in the strengthening of these latent connections and a resultant increase in the likelihood that referred pain will be expressed. Decreases in local muscle pain may result from similar endogenous pain control mechanisms to that described above from repeated heat pain stimuli.

4.4.1 Methodological considerations

While local pain is frequently said to incorporate the site of stimulus, definitions for its boundaries are somewhat arbitrary. Furthermore, there is little consensus over the definition of referred pain, except that it must not include the site of stimulus. In the present study, we employed two definitions of local and referred pain so as to facilitate comparison with other studies and enable a more accurate description of the dynamic spatiotemporal expression of local and referred pain. The first was a definition (D1) of Schulte and colleagues (Schulte, Graven-Nielsen et al. 2003), where pain in the ankle and foot was classified as referred pain and any pain proximal to this was defined as local. For D2 we classified local and referred pain in terms of separation from the injection site and the existence of distinct islands of perceived pain. The estimates of referral are in fact conservative since pain that extended beyond the area of nociceptor activation were entirely classified as local rather than partially referred.

The different classifications did however depict dramatic differences in local and referred pain. Both definitions depicted trends of decreased area, intensity and duration in the local region. However, D2 depicts a significant increase in referred area, intensity and duration while D1 did not. We suggest that future studies interested in defining changes in referred muscle pain employ the second definition, as it appears more sensitive to changes in the intensity and area of referred pain perception. The hypertonic-saline model of muscle pain is frequently used to assess somatosensory changes in pain processing over repeated injections (Arendt-Nielsen, Graven-Nielsen et al. 1997; Graven-Nielsen, Arendt-Nielsen et al. 1997; Graven-Nielsen 1997; Laursen, Graven-Nielsen et al. 1999; Stohler and Kowalski 1999; Schulte, Graven-Nielsen et al. 2003; Ge, Madeleine et al. 2004; Schulte, Segerdahl et al. 2006), and it is common practice to reuse the same groups of subjects for subsequent experimental
studies. Our data suggests that previous local noxious stimuli must be taken into account when studying the long-term effects of acute nociceptor activation.

4.4.2 Conclusions
The characterisation and validation of musculoskeletal pain models is fundamentally important to derive meaningful correlates to the perception of pain and the underlying neural mechanisms. Since some chronic pain conditions are defined by the presence of referred pain, it is imperative that we understand the mechanisms involved in this dynamic process in order to treat the underlying cause. These studies pave the way for the investigation of the underlying mechanisms responsible for referred muscle pain. This study conclusively demonstrates plasticity in the expression of hypertonic-saline induced muscle pain. Although this plasticity imparts limitations to our experimental systems, it is likely to underlie clinical conditions and thus must be studied in its own right.
CHAPTER 5

A COMPARISON OF THE SPATIOTEMPORAL
EXPRESSION OF LOCAL AND REFERRED ACUTE
MUSCLE PAIN IN THE ARM AND LEG
5.1 INTRODUCTION

Estimates for the prevalence of chronic muscle pain in the population range from 4.2% (Lindell, Bergman et al. 2000) to 11.4% (Bergman, Herrstrom et al. 2001). The majority of human muscle pain models frequently result in pain some distance from the noxious stimuli known as referred pain. This is likely to underlie a large proportion of clinical complaints, the majority of which are idiopathic. While initially described as somatic pain associated with noxious stimulation of the viscera (Head 1893; Mackenzie 1893), referred pain has also been reported to occur during noxious stimulation of ligaments, tendons and joints (Steinbrocker, Isenberg et al. 1953). It has been reported during noxious stimulation of numerous skeletal muscles, including tibialis anterior, flexor carpi radialis, brachioradialis, third dorsal interosseous, infraspinatus, sartorius, adductor longus, gastrocnemius, tensor fasciae latae, peroneus longus, multifidus, temporalis and trapezius (Lewis 1939; Feinstein, Langton et al. 1954; Wolff BB 1965; Jensen and Norup 1992; Graven-Nielsen 1997; Ge, Madeleine et al. 2003).

Data from numerous human studies (Doran and Ratcliffe 1954; Whitty and Willison 1958; Graven-Nielsen, Arendt-Nielsen et al. 1997) and animal models (Cook, Woolf et al. 1987; Hoheisel and Mense 1989; Hoheisel, Mense et al. 1993) suggest that referred pain is, at least in part (Hockaday and Whitty 1967; Laursen, Graven-Nielsen et al. 1999), centrally mediated. Indeed, current theories suggest that referred pain is a form of central sensitisation, resulting from the unmasking and sensitization of inactive synaptic connections (Mense 1994). If central sensitization does indeed underlie the phenomenon of referred pain, we would expect its characteristics to be similar during identical noxious stimuli delivered to skeletal muscles in different body segments. To test this we employed the model of hypertonic-saline induced muscle pain to produce identical noxious stimuli in the tibialis anterior (leg) and flexor carpi radialis (forearm) muscles in the same subjects (Kellgren 1938; Kellgren 1938). We hypothesized that the relationships between local and referred pain percepts (incidence, duration, area and intensity) would be identical in the upper and lower limbs. Furthermore, we tested the hypothesis that the relationships between local and referred muscle pain in a given subject are independent of the stimulus intensity.

While local pain is frequently said to incorporate the site of stimulus, definitions for its boundaries are somewhat arbitrary. Furthermore, there is little consensus over the definition of referred pain, except for the notion that it must not include the site of stimulus. In the present study we used two definitions of local and referred pain to quantify the spatiotemporal profile of pain. The first was a definition based on Schulte
and colleagues (Schulte, Graven-Nielsen et al. 2003), where pain in the ankle and foot were classified as referred leg pain and pain in the hand and fingers were classified as referred arm pain. The second, is a more stringent (Rubin, Gandevia et al. 2009) adaptation of the classical definition that classified local and referred pain on the basis of separation from the stimulus site. This allowed quantification of discrete “islands” of pain often experienced within regions classically defined as local pain.

5.2 METHODS

5.2.1 Experimental Protocol
Thirty-six subjects (21 male, 15 female), aged 18 – 31 years attended two sessions, each separated by 7-10 days. Each session involved a single 1 ml bolus injection (over 3 s) of either 5% (n=21) or 10% (n=15) hypertonic saline through an indwelling 23 G butterfly cannula. The first session involved an injection into the muscle belly of the left flexor carpi radialis (FCR) while the second involved an identical injection into the rostral belly of the right tibialis anterior (TA), superficial to the aponeurosis. In order to avoid history-related effects of injecting the same muscle in the same subject (see Discussion), different sets of subjects were used for the 5% and 10% injections. Subjects reported the quality and distribution of their sensations every 30 seconds until the sensation of pain had ceased.

5.2.2 Analysis
Only subjects that completed both TA and FCR experiments were included in the analysis. Intensities and areas from the scanned drawings were partitioned as local or referred pain on the basis of two definitions, as illustrated in Fig 2.1. The first is a definition (D1) based on Schulte and colleagues (Schulte, Graven-Nielsen et al. 2003). In order to deal with the incidence of referral separately to, and independently of, the magnitude of referred pain, we grouped referrals and non-referrals separately. In this manner only zero values from subjects who experienced referred pain in at least one experiment or local pain in at least one experiment were included in means and subsequent graphs. Only pain sensations experienced for a total of 60 seconds or more (i.e. two data points) were included in the analysis. This reflected the actual pain experience of those subjects who did experience referral. In each subject pain intensity was normalized to the maximum intensity of pain experienced by that subject over all experiments. Templates of the respective limbs covered most of an A4 page. As this resulted in the arm having a greater ratio of represented area to actual area than the
leg, area data from the TA were normalised to the FCR with an arm : leg ratio of 23:39. These data were expressed in arbitrary units. Statistical analysis was performed using Prism 5 (GraphPad Software Inc). Both FCR – TA comparison and 5% - 10% comparisons were analysed with unpaired separate 2-way ANOVAs, to compare replicate means by row, for each measure (area, intensity) at each region (and for each definition), and maximal intensity. Local and referred intensity comparisons were displayed using linear regressions and analysed with a 1-way ANOVA and Newman-Keuls post-tests to compare intensity.

5.3 RESULTS

Of the 36 students recruited into the study, 29 (21 male, 8 female) completed the study. Five subjects withdrew after the first session and two subjects were excluded because they experienced a maximum pain rating below 3/10 on the visual analogue scale (VAS). A total of three subjects in 4 experiments experienced FCR pain in the local (n=2) and referred (n=2) regions for a total of less than 60 seconds. These four anomalous data points of low intensity (VAS<2.5) were excluded from analysis. Each of these subjects experienced significant pain in an alternate region and thus were not excluded in their entirety.

5.3.1 Incidence and distribution of pain

In all subjects, intramuscular injection of hypertonic saline (1 ml, 5%) into tibialis anterior (TA) induced local pain in the leg, frequently radiating towards the ankle. Referred pain was often perceived in the ankle and less frequently in the toes. Likewise, the local pain induced by intramuscular injection into flexor carpi radialis (FCR) was most commonly perceived to radiate distally through the forearm. The presence or absence of referred pain in an individual after injection into FCR was, to an extent, a predictor of the percept induced by the TA injection. With D1, 67% and 73% of subjects had equal occurrences or non-occurrences of referral after 5% and 10% injections, respectively. With D2, 86% and 67% of subjects had equal occurrences or non-occurrences of referral after 5% and 10% injections. Referred pain from FCR typically manifested in the wrist and medial digits, while it was less frequently perceived in the thumb or proximally within the upper arm. Pain was only perceived in the injected limb and the area of local pain was almost always greater than that of the referred pain (Table 5.1).
Table 5.1; Incidence of pain in local and referred regions after injection in the tibialis anterior (TA) and flexor carpi radialis (FCR) muscles with 5% and 10% hypertonic saline, for both definition 1 (D1) and 2 (D2). Numbers refer to number of subjects in absolute and percentage terms.

Apart from 2 TA injections and 3 FCR injections (classified by D2), all other experiments resulted in the perception of local pain irrespective of regional classification. As shown in Table 2, the mean duration of pain was similar in the forearm and leg, irrespective of region (local vs referred) or classification (D1 vs D2).

Table 5.2; Mean duration of pain in local and referred regions after injection in the tibialis anterior (TA) and flexor carpi radialis (FCR) muscles with 5% and 10% hypertonic saline, for both definition 1 (D1) and 2 (D2). The data do not include “0” values, where pain was not perceived in that region.

5.3.2 TA–FCR comparison

Figure 5.2 shows the mean pain profiles for all subjects displaying similar time-courses were observed for injections into both the TA and FCR. These similarities were particularly striking in the local regions that displayed no significant differences (p<0.5) in pain intensity, irrespective of regional classification. Mean pain intensity was highly correlated between the two injection sites despite originating from stimuli delivered on separate occasions. Nevertheless, there were some differences, particularly in relation to the area of perceived pain. Higher correlations were observed between local areas of pain in the two muscles ($R^2 > 0.99$) than referred regions ($0.72 \leq R^2 \leq 0.96$), irrespective of how local and referred pain were defined. Indeed, the only significant difference when comparing mean intensity trends with 2-way repeated measures ANOVAs was observed between referred regions of the TA and FCR after 10% injections. Though higher correlations were observed between areas of pain at local regions ($0.96>R^2>0.84$) than referred regions ($0.91>R^2>0.41$) including both definitions,
these data were far more varied over the course of the experiments than respective intensity scores.

**Figure 5.2:** Comparison of mean pain area and intensity (± SE) in local regions and referred regions after injection in the tibialis anterior (TA) and flexor carpi radialis (FCR) muscles as defined by definition 1 and definition 2 between 5% and 10% HS concentrations. Trend lines are created with 6th and 7th order polynomials.
5.3.3 Stimuli comparison

The effects of stimulus intensity were examined in two groups of subjects – one receiving intramuscular injections of 5% hypertonic saline, the other 10%. This avoided history-dependent changes. As expected, peak pain intensities were higher following injection of 10% hypertonic saline into either TA or FCR. Local pain from injections of the 10% solution into either muscle was significantly higher than that produced by injection of the 5% solution (P<0.001), regardless of how local pain was defined (D1 or D2). The mean intensity of referred pain was consistently greater from 10% injections (p<0.005) in both the TA and the FCR. Intensity in the referred (D1) region of the FCR did not differ (p=0.85) between the two stimuli, indeed it was highly correlated between the two injections $R^2 = 0.89$ The mean changes in intensity of referred pain for TA and FCR are shown for the two stimulus intensities and two definitions in Fig. 3. While the duration of local pain induced from the stronger stimulus tended to be longer, the difference was not significant (p=0.055; Table 1).

![Referred Intensity](image)

**Figure 5.3;** Comparison of mean pain intensity (± SE) in referred regions as defined by definition 1 and definition 2 between injections in the tibialis anterior (TA) and flexor carpi radialis (FCR) muscles. Trend lines are created with 6th and 7th order polynomials.

5.4 DISCUSSION

The primary purpose of this study was to characterise the differences in referral between two muscles, one in the arm (flexor carpi radialis; FCR) and one in the leg (tibialis anterior; TA), using an identical bolus injection (1 ml) of hypertonic saline into the same subjects. Additionally, we used two groups of subjects to examine the effect of stimulus intensity (5% vs 10% hypertonic saline) on the expression of local and
referred pain. With this approach we have shown, for the first time, a differential effect of identical noxious stimuli on the expression of referred muscle pain in the leg and the arm. While many other studies have shown that the concentration of hypertonic saline affects the global magnitude of pain (Jarvik 1962; Jensen and Norup 1992; Graven-Nielsen, McArdle et al. 1997; Graven-Nielsen 1997), we show concentration-dependent, differential effects on the expression of referred pain from intramuscular injections of hypertonic saline into TA and FCR.

Higher concentrations (10%) of hypertonic saline consistently induced a greater intensity of local pain in both TA and FCR. For a given stimulus intensity (5% vs 10%), local pain ratings were nearly identical for both muscles. However the expression of referred pain did differ between the two muscles: a stronger stimulus induced more intense referred pain in the TA than the FCR, though this difference was only apparent when referred pain was classified according to definition 2. Given the absence of these effects in definition 1, specifically the presence of pain that is local in D2 but referred in D1, it can be deduced that these effects represent an increase in contiguous regions of pain that extend into the ankle, as shown in Fig 1 panel D. Indeed, 6 subjects showed this pattern in TA compared with three from 10% injections into the FCR. These findings may represent a decreased sensitivity of FCR-induced referred pain to high-intensity painful stimuli, or an increased sensitivity of the TA to high-intensity stimuli. Conversely, these results could derive from a “ceiling effect” within the FCR, such that the incidence of referred pain reaches its maximum at lower stimulus intensities.

While not significant, Graven-Nielsen et al. (Graven-Nielsen 1997) did observe increases in pain duration, area under the intensity vs time curve and local pain area when comparing the effects of 5.0% and 11.5% hypertonic stimuli. Here, we demonstrate a significant increase in local pain intensity from the higher-intensity stimulus that is consistent in two different muscles. If stimuli in the two muscles are the same, and we have no reason to believe they are not, noxious stimulation of TA is somehow able to induce contiguous regions of pain that extend into the distal limb more consistently than similar injections into FCR.

Our observation that increased concentrations can have dramatically different effects on referred pain while maintaining a consistent perception of local pain may suggest that the maximum intensity of local pain is associated with “saturation” in perception rather than a saturation of the afferent barrage. Furthermore, these data suggests that the relationship between perceptual saturation of local pain and the expression of referred pain may well vary across muscle groups. Our finding of nearly identical
measures of local pain intensity between TA and FCR may, at each concentration of hypertonic saline, suggest common ceiling effects in the perception of local pain. In contrast, the same stimuli (10%) induced higher referred intensities in TA relative to FCR.

5.4.1 Methodological considerations
This study utilised two explicit definitions to distinguish pain as either local or referred: the first D1 is more conservative, whereas the second D2 considers any island of pain that is sufficiently removed from the site of the injection as being referred pain, even if it lay within the area classically defined as “local”. Given that a 1 ml bolus injection of hypertonic saline into the rostral forearm or leg could not enter the anatomical compartments of either the hand or foot, it is reasonable to conclude that the expression of referred pain remote to the site of injection represents central (spinal and/or supraspinal) neural processes.

The complement of sensory receptors in each muscle and their density are assumed to be similar. It is, of course, possible that the two muscles studied have different densities of muscle nociceptors, though to date there have been no physiological studies demonstrating this. Furthermore, the nearly identical local intensity plots with correspondingly high correlations demonstrate a consistent pain percept between the two muscles regardless of the individual afferent drive. Though the increase in pain induced by injections of the stronger hypertonic saline concentration may be expected, this effect could be a result of the different subject populations. Unfortunately, it is difficult to control for this given our previous demonstration of history-dependent changes in the spatiotemporal expression of muscle pain when the same subjects are studied in a longitudinal design (CH 4, Journal of Pain, in press 2010).

5.4.2 Conclusions
Measured in the same subjects, this study has shown a consistent similarity in the local pain response to intramuscular injection of hypertonic saline between FCR and TA. It has been assumed that the magnitude of referred pain is primarily driven by the intensity of the stimulus in a manner dependent on the afferent barrage. Our observation that increasing concentrations can have dramatically different effects on referred pain suggest that local pain may be related to a saturation in the perception of local pain rather than a saturation of afferent barrage. Furthermore, the relationship between the perceptual saturation of local pain and the expression of referred pain may well vary from muscle group to muscle group.
CHAPTER 6

WITHIN-LIMB SOMATOTOPIC REPRESENTATION OF ACUTE MUSCLE PAIN IN THE HUMAN DORSAL POSTERIOR INSULAR CORTEX
6.1 INTRODUCTION

It is well established that the insular cortex is involved in the processing of noxious stimuli. It has been proposed that the dorsal posterior part of the insular cortex receives noxious information via direct, somatotopically organized projections from a pain-specific region of the thalamus; the posterior portion of the ventromedial thalamic nucleus (VMpo) (Craig, Bushnell et al. 1994; Blomqvist, Zhang et al. 2000; Craig 2003; Craig and Zhang 2006). Indeed, our laboratory has recently shown that the insula is, at least crudely somatotopically organized for noxious inputs, with noxious stimulation of the forearm evoking activation that was located immediately anterior and lateral to the region activated by noxious stimuli applied to the leg, although an area of overlap was also evident (Brooks, Zambreanu et al. 2005; Hua, Strigo et al. 2005; Henderson, Gandevia et al. 2007).

Interestingly, it has recently been revealed that the dorsal posterior insula also displays a somatotopic organization for the processing of gentle touch, mediated by C tactile afferents (Björnsdotter, Löken et al. 2009). Indeed, the organization of functional activation evoked by gentle touch applied to the forearm and thigh was almost identical to that reported in an earlier investigation from our laboratory (Henderson, Gandevia et al. 2007). In addition, the authors report that in a subject lacking large-diameter afferents, insula somatotopy is preserved, suggesting that insula organization may underlie the patient’s ability to correctly localize gentle touch; a previous study from this group had shown that C tactile afferents do not activate the primary somatosensory cortex (Olausson, Cole et al. 2008). Although it was suggested that the dorsal posterior insula may contain only a crude somatotopic map, it is possible that there is a preservation of afferent fiber organization from the body, through the VMpo to the dorsal posterior insula.

Knowing the degree of spatial detail within the insula may have significant implications for the potential role of the dorsal posterior insula in the processing of noxious stimuli. For example, it is well-established that the primary somatosensory cortex (SI) has a fine somatotopic organization and it is thought that this detail allows for the precise localization of somatosensory stimuli (Penfield and Boldrey 1937; Penfield and Rasmussen 1950). Indeed, we have recently shown that within SI, the degree of perceived referred pain following an intramuscular injection of hypertonic saline is represented by the extent of spread of SI activation (Macefield, Gandevia et al. 2007). In contrast to SI, it has been shown in rodents that in phylogenetically older brain regions, such as the midbrain periaqueductal gray matter, noxious afferent inputs are...
also somatotopically organized, although this organization appears to be rather crude compared to SI (Keay and Bandler 2001). Indeed, this relatively crude body representation of sensory input is presumably all that is required for the body to organize and direct the relatively gross behavioural responses to pain such as fight and flight. If a similar crude map exists within the dorsal posterior insula then it may be the case that this region is not involved in processing aspects of the pain experience that require precise stimulus localization, but instead is involved in processing more global aspects of the pain experience, such as the direction of behavioural responses.

The aim of this investigation was to use high-resolution functional magnetic resonance imaging to determine if a fine somatotopic organization of noxious stimuli applied to muscles within a single limb (the arm) exists within the dorsal posterior insular cortex. Furthermore, we reanalyzed earlier data to create a whole-body map of noxious inputs arising from skeletal muscles within the dorsal posterior insula.

6.2 METHODS

6.2.1 Subjects
Thirteen healthy subjects (9 males) aged 19-48 years participated in this study. All procedures were carried out with the understanding and written informed consent of each subject. All procedures were approved by local institutional Human Research Ethics Committees (University of Western Sydney, and the University of Sydney) and were conducted in accordance with the conditions established by the Declaration of Helsinki.

6.2.2 Stimulus and MR imaging
With subjects in a supine position, a fine plastic cannula (23 gauge), attached to a 3ml syringe containing sterile hypertonic (5%) saline was inserted deep into the central belly of the left deltoid muscle, then into the belly of the left flexor carpi radialis muscle (FCR), and a thirdly into the belly of the left first dorsal interosseus muscle (FDI). Scanning was not initiated for five minutes after completion of the previous scan, once any minor pain from insertion of the needles had undeniably subsided. Continuous series of 288 gradient echo image sets using Blood Oxygen Level Dependent (BOLD) contrast were then collected using a 3 Tesla, Siemens Magnetom Trio scanner (32 axial slices, TR=4s, TE=40ms, flip angle=90 degrees, FOV=220x220, raw voxel size=1.96x1.96x4mm thick, 0.4mm interslice gap). Following a 70 volume baseline, subjects received an intramuscular injection of hypertonic saline (1.0 ml) into one of the
three muscles. Subjects were not made aware of when the injection was to be administered or in which order it was to be presented. Each subject received injections into each of the three muscles during subsequent fMRI scans. During each scan, subjects were instructed to press a button with their right thumb to indicate when they (i) felt the onset of pain, (ii) the pain began to subside from its peak, and (iii) the pain had ceased. Immediately following each fMRI scan and while still inside the scanner, subjects were read a linear 10 point pain intensity scale (0=no pain, 10=maximum imaginable pain) and to draw the area of perceived pain onto standard picture of the arm. A 3-D T1 weighted anatomical scan (voxel size=0.8x0.8x0.8mm) was also collected. During a separate scanning session, in two subjects, the same procedures were repeated but the hypertonic saline injections were made into the right arm.

6.2.3 MRI analysis
Using SPM5 (Friston, Holmes et al. 1995), all functional image sets were motion corrected and only subjects with movement parameters less than 1mm in the X, Y and Z planes were used for analysis. Following realignment, individual's functional images were spatially normalized to the Montreal Neurological Institute template (MNI), global signals removed using the technique described by Macey et al. (Macey, Macey et al. 2004) whereby a mask was placed around the insula such that the dorsal posterior insula was defined in each subject according to standard anatomical landmarks. Images were then spatially smoothed with a 6mm full-width-at-half-maximum Gaussian filter.

Significant changes in signal intensity were determined on a voxel-by-voxel basis using a box-car model (convolved with a haemodynamic delay) which approximated to the period of perceived pain (70 volume baseline, 30 volume on). One-sample t-tests were performed to determine significant signal intensity increases and decreases during each of the three stimulation paradigms (p<0.05 random effects, corrected for multiple comparisons, minimum cluster size 10 voxels). The resulting statistical maps were then overlaid onto a T1-anatomical image set.

In addition, individual subject analyses were performed that were restricted to the contralateral posterior insula. The most significantly activated voxel within the contralateral posterior insula was determined in each subject for each of the three muscle pain stimuli. The co-ordinates of these most significantly activated voxels, in MNI space, were determined and the mean (±SEM) values plotted onto an individual subject's T1-weighted anatomical image. Significant differences in these MNIs during each of the three paradigms were determined (p<0.05, 2-tailed t-test). The X and Y co-
ordinates in each subject, for each of the three stimulation paradigms, were plotted and the 3-dimensional distances between each of the maximally activated voxels during each stimulation paradigm determined using Euclidean geometry. In the two subjects in which hypertonic saline injections were made into both the left and right upper limbs, the activation maps within the dorsal posterior insular during all 6 noxious stimulation paradigms were overlaid onto their T1-weighted anatomical images and the changes in signal intensities plotted over time.

Finally, in previous investigations we used fMRI to determine the patterns of brain activity evoked by hypertonic saline injections into the leg (tibialis anterior muscle, n=19) and face (maseter muscle, n=17) (Henderson, Gandevia et al. 2007; Nash, Macefield et al. 2009). We performed an identical processing and statistical analysis procedure on these previously obtained data and determined the maximally activated voxels within the contralateral dorsal posterior insula in each subject.

6.3 RESULTS

6.3.1 Pain perception and spread

In all subjects, injection of hypertonic saline evoked pain which began within 5-10 seconds of the injection began, reached a peak within approximately 30 seconds, and remained above baseline for at least 4 minutes. The mean (±SE) maximum intensities of pain, rated on a 0-10 point VAS scale, were: deltoid: 5.0±0.5, FCR: 5.9±0.5 and FDI: 5.9±0.6. There was no significant difference between the maximum pain intensities during each of the three paradigms (t-test; p>0.05). It can be seen in Figure 6.1 that, in the vast majority of subjects, injections of hypertonic saline into the deltoid and FDI muscles resulted in a relatively restricted pattern of perceived pain spread, i.e., immediately surrounding the injection site. In contrast, in a number of subjects, hypertonic saline injection into FCR muscle resulted in pain that was perceived as radiating distally, sometimes extending into the wrist and hand. All subjects reported that the pain from each injection was deep not superficial.
Figure 6.1: Individual pain referral patterns overlaid onto a standard picture of the arm following intramuscular hypertonic saline (5%) injection into the deltoid, flexor carpi radialis (FCR) and first dorsal interosseus (FDI) muscles.

6.3.2 Signal intensity changes

Group analysis:

Group analysis revealed that muscle pain was associated with significant increases in signal intensity in a number of brain regions. Following hypertonic saline injection into the deltoid, FCR and FDI muscles, significant increases in signal intensity occurred in the anterior cingulate, anterior insular, contralateral dorsal posterior insular, cerebellar and secondary somatosensory cortices (Figure 2). Overall, the pattern of brain activation was similar during all three sources of muscle pain.
Figure 6.2; Cortical and sub-cortical regions which show increases (hot colour scale) in signal intensity during pain in the deltoid (top row), flexor carpi radialis (FCR; middle row) and first dorsal interosseus (FDI; lower row) muscles. The slice locations in Montreal Neurological Institute (MNI) space are indicated in the bottom left of each image in the lower row. ACC: anterior cingulate cortex; SII: secondary somatosensory cortex; dp Insula: dorsal posterior insula.

6.3.3 Individual subject analysis

Within the contralateral posterior insula, an analysis of individual subject data revealed a clear somatotopy. Hypertonic saline injection in the deltoid muscle evoked signal intensity increases that were located medial to those evoked by FDI injections, and anterior to those evoked by FCR injections (Table 1; Figure 6.3). This somatotopic organization was confirmed in the two individual subjects in which hypertonic saline was administered to both the left and right arms (on different days). That is, deltoid injections evoked increases in signal intensity within the contralateral dorsal posterior insula which were located medial and anterior to the signal increases evoked by FDI and FCR injections, respectively (Figure 6.4).
Table 6.1; Mean (±SEM) X, Y and Z co-ordinates in Montreal Neurological Institute space within the contralateral dorsal posterior insular cortex following injection of hypertonic saline (5%) into the maseter, deltoid, flexor carpi radialis, first dorsal interossous and tibialis anterior muscles. Images used for the analysis of maseter and tibialis anterior muscle pain were collected in previous studies.

Figure 6.3; Plot of the mean (±SEM) locations of the most significantly activated voxels within the contralateral dorsal posterior insula, following injection of hypertonic saline.
into the deltoid, flexor carpi radialis (FCR) and first dorsal interosseous (FDI) muscles. The slice location in MNI space are indicated in the bottom left of the image. Below is a plot of the mean (±SEM) X and Y co-ordinates in MNI space of these activated voxels. Considering only the X and Y planes, the mean location during deltoid pain is located anterior to the mean location during FCR pain (p<0.05).

Figure 6.4; Locations of significant signal intensity increases in the dorsal posterior insula cortex in two subjects in which hypertonic saline injections were made into the left deltoid, flexor carpi radialis (FCR) and first dorsal interosseus muscles (FDI) during one session and then the right deltoid, FCR and FDI in a second session. Activations on the left side occurred during injections made into the right side of the body and vice versa. The slice locations in MNI space are indicated in the bottom right of each image. To the left and right are plots of the percentage signal intensity changes over time, relative to the baseline period, for each significantly activated region. The vertical dashed lines indicate the scan at which each hypertonic saline injection was delivered. Note that within the contralateral dorsal posterior insula, deltoid pain activated a region medial to that activated by FDI pain and anterior and medial to that activated by FDI pain.
Scatter plots of the differences in X and Y co-ordinates of maximally activated voxels revealed that, in the vast majority of subjects, hypertonic saline injection into the FCR resulted in a mean maximal increase in signal intensity that had either the same X and Y co-ordinates (n=5), or was located posterior to the location of signal intensity increases evoked by injection into the deltoid (n=7), (Figure 6.5). Furthermore, in only 3 subjects did deltoid and FCR injections evoke maximal signal increases in the same location, with a mean distance between the two loci of 4.4±0.9mm. Similarly, in the majority of subjects, hypertonic saline injection into FCR evoked maximal increases in signal intensity that were located posterior and medial to the location of signal intensity increases evoked by injection into FDI (n=7). In only 1 subject did FDI and FCR injections evoke maximal signal intensity increases in the same location, with a mean distance between the two signal increases of 5.1±0.9mm. Finally, in the majority of subjects, hypertonic saline injection into the deltoid resulted in a maximal signal intensities that were located lateral to the location of signal intensity increases evoked by injection into FDI (n=9). Furthermore, in only 2 subjects, FDI and deltoid injections evoked maximal signal intensity increases in the same location, with a mean distance between the two signal increases of 5.9±1.1mm.
Figure 6.5: Two-dimensional scatter plots of differences in the X and Y co-ordinates (left column) and 3 dimensional distances (±SEM; right column) of the most significantly activated voxel during flexor carpi radialis (FCR) relative to deltoid pain (upper panel), FCR relative to first dorsal interosseous (FDI) pain (middle panel), and deltoid relative to FDI pain (lower panel). Note that in most subjects FCR pain activated a region posterior to that activated by deltoid pain and posterior and medial to that
activated by FDI pain. Further, in the vast majority of subjects, deltoid pain activated a region medial to that activated during FDI pain.

In addition to determining the locations of maximally activated voxels within the contralateral dorsal posterior insula during muscle pain applied to the upper limb, we have previously mapped activity within the dorsal insula during muscle pain applied to the face (masseter) and leg (tibialis anterior). Using these images we found that the mean (±SEM) X, Y and Z co-ordinates evoked by leg pain (tibialis anterior muscle) were 36.1±0.6, -18.5±1.0, 14.7±0.8 and face pain (masseter muscle) were 37.3±0.8, -15.4±0.8, 12.2±0.8. Furthermore, on average, masseter pain evoked signal intensity increases within the dorsal posterior insula that were 3.1mm, 4.7mm, 3.2mm and 4.2mm away from deltoid, FCR, FDI and tibialis anterior pain, respectively. Similarly, tibialis anterior pain evoked signal intensity increases that were 3.2mm, 1.9mm and 2.9mm away from deltoid, FCR and FDI pain, respectively.

6.4 DISCUSSION:

Using high-resolution fMRI we have shown, for the first time, that within the dorsal posterior insular cortex, muscle pain applied to different muscles of a single limb evokes somatotopically organized increases in signal intensity. Signal intensity increases evoked by deltoid pain were located medial and anterior to those evoked by muscle pain in the forearm and hand, respectively. Combined with our previous data, it appears that, in humans, the dorsal posterior insular cortex contains a whole-body somatotopic map of noxious inputs arising from skeletal muscles. Furthermore, this somatotopic representation cannot be considered coarse.

We have previously used fMRI to determine the patterns of brain activity evoked by hypertonic saline injections into the leg and forearm (Henderson, Gandevia et al. 2007). Using high resolution fMRI this previous study involving 23 subjects demonstrated that pain originating in muscle or skin of the leg evoked increases in signal intensity in the contralateral dorsal posterior insula that were located medial and posterior to those evoked by forearm pain. Though this study utilised a different scanner running a TR of 3s and a TE of 50ms the data collection and subsequent analysis was practically identical. Using the images collected in this and another study (Henderson, Gandevia et al. 2007; Nash, Macefield et al. 2009), we performed an identical processing and statistical analysis procedure to that of employed in the current investigation and determined the maximally activated voxels within the
contralateral dorsal posterior insula in each subject. Following spatial normalization we found excellent co-registration between functional images collected on different scanners in the same individual (n=3). Further, since we did not compare the magnitudes of signal intensity changes but instead restricted our analysis to the maximally activated voxels, we are confident that the whole body map shown in Figure 6 is accurate. Indeed, this combined analysis reveals that the body schema within the contralateral dorsal posterior insula is not as crude as has been previously suggested, but instead contains separate representations for muscles within a single limb.

Figure 6.6; Plots of the mean (±SEM) X, Y and Z co-ordinates of the most significantly activated voxels within the contralateral dorsal posterior insula, during shoulder (deltoid), forearm (flexor carpi radialis; FCR) and hand (first dorsal interosseous; FDI) pain assessed in this investigation. Also included are the X, Y and Z co-ordinates of the most significantly activated voxels during leg (tibialis anterior; TA) and face (masetter) muscle pain determined using data from two previous investigations (Henderson, Gandevia et al. 2007; Nash, Macefield et al. 2009).
It has been previously shown by us and others that the dorsal posterior region of the insular cortex is activated by noxious cutaneous and noxious muscle stimuli (Vogel, Port et al. 2003; Brooks, Zambreanu et al. 2005; Henderson, Gandevia et al. 2007). Although not universally accepted, it has been proposed that the dorsal posterior insula receives noxious information via direct, somatotopically organized inputs from the ventromedial thalamic nucleus (VMpo) (Craig and Blomqvist 2002; Craig 2003). In addition to noxious stimuli, a recent human brain imaging investigation by Björnsdotter and colleagues (Björnsdotter, Löken et al. 2009) revealed that crude light touch, mediated by low-threshold C fibres (C tactile afferents), also activates the dorsal posterior insula in a somatotopic fashion. Indeed, the authors report a seemingly identical organization of arm and leg activation patterns to that which we previously reported during noxious forearm and leg cutaneous and muscle pain, i.e. leg medial and posterior to arm (Henderson, Gandevia et al. 2007). Further, this somatotopically organized activation also occurred in an individual lacking large-diameter myelinated afferents, suggesting that, in healthy individuals, dorsal posterior insula activation during crude light touch results from activation of C tactile afferents (Björnsdotter, Löken et al. 2009).

It has been reported that in two individuals that lack A-beta fibres, light crude touch can be localized (Olausson, Cole et al. 2008). Since light crude touch in A-beta deficient subjects does not evoke signal intensity increases within the primary or secondary somatosensory cortices, rather causing decreases (Olausson, Cole et al. 2008), it was proposed that the dorsal posterior insula provides the neural substrate via which light crude touch is localized. Although it is possible that the dorsal posterior insula may contain only a very crude body map for light touch, our data reveals that it contains a rather fine within-limb representation for skeletal muscle pain. How fine this is would need to be assessed by examining noxious inputs from more closely placed muscles, but there is no doubt that there is spatial differentiation between noxious inputs from muscles located in each of the three segments of the upper limb. This extends our previous work and suggests that the dorsal posterior insula may be involved in the accurate localization of muscle pain and possibly other somatosensory stimuli. This idea is supported by the results of an investigation by Mazzola and colleagues (Mazzola, Isnard et al. 2009) in which they report that stimulation of a single posterior insula site can evoke sensory changes in a very discrete region of the body. Magneto- and electro-encephalographic studies report that both the SI and operculo-insula regions are the earliest activated structures following noxious stimulation (Ploner, Schmitz et al. 1999; Frot and Mauguiere 2003); it is possible that the dorsal posterior insula acts in concert with the primary and secondary somatosensory cortices to
provide an individual with an ability to accurately localize noxious stimuli that arise from deep body structures such as muscle and perhaps viscera. Indeed we are currently performing a detailed examination of within-limb somatotopy for the primary and secondary somatosensory cortices and for the cerebellar cortex.

Alternatively, the somatotopic organization within the dorsal posterior insula may provide a means by which an individual directs appropriate behaviours to noxious and non-noxious (pleasant touch) stimuli. For example, different motor and autonomic patterns are evoked by noxious stimuli originating in different body parts and different tissues in the same body region (Lewis 1939; Bandler, Price et al. 2000). Cutaneous pain evokes active coping responses such as flight and fight, whereas muscle and visceral pain evokes a passive coping response characterized by quiescence and hyporeactivity (Bandler, Price et al. 2000). In primates, the dorsal insula sends somatotopically organized inputs to regions of the striatum which contain nociceptive responsive neurons, receive primary sensory and motor inputs and are involved in stimulus-response associations and novelty (Chudler and Dong 1995; Chikama, McFarland et al. 1997). Furthermore, it has been reported that the putamen is activated in a somatotopic fashion during noxious cutaneous stimuli; it was suggested that this organization reflects pain-related motor responses (Bingel, Lorenz et al. 2004).

Although it is possible that the dorsal posterior insula is involved in mediating motor responses, the results of investigations into the insular representation of light touch, suggest that, in addition to stimulus localization, the dorsal insula is involved in processing the emotional component of somatosensory stimuli. The activation of CT afferents by light touch evokes a pleasant emotional response and activates the dorsal posterior insula (Björnsdotter, Löken et al. 2009). Similarly, it is thought that the emotional component of noxious stimuli is processed within the insular cortex since lesions encompassing the insula can result in pain asymbolia, a condition in which the intensity and quality of noxious stimuli are preserved, but patients appear “unable to recognize the disagreeable nature of painful or threatening stimuli” (Berthier, Starkstein et al. 1988). Indeed, it has been hypothesized that the dorsal posterior insula is an “interoceptive” cortex; that is, a region that monitors the internal state of the body (Craig and Blomqvist 2002). These previous reports, in combination with the data presented here, suggest that the dorsal posterior insular cortex may process the emotional component of both non-noxious and noxious stimuli.

6.5 CONCLUSIONS
We have shown for the first time that the contralateral dorsal insula receives noxious inputs from muscles in the upper limb in a relatively fine somatotopic fashion, arguing for a role of the dorsal posterior insula in stimulus localization.
CHAPTER 7

GENERAL DISCUSSION
7.1 GENERAL DISCUSSION

As outlined in the introduction to this thesis, muscle pain is of significant clinical interest, given the prevalence of clinical conditions such as fibromyalgia and myofascial pain syndromes, and the fact that such deep pain often leads to chronic pain. Moreover, these deep sensations are often accompanied by referred pain, which must be distinguished from any local focus of noxious stimuli. The nature of this referral is poorly understood, and I believe that the work presented in my thesis has contributed to the body of knowledge on the aetiology of referred muscle pain.

In this thesis I have explored the spatiotemporal expression of acute muscle pain, both with respect to its measurement and to its representation within the brain. I have further refined the well-documented model of muscle pain induced by intramuscular injection of hypertonic saline: I have increased the spatial and temporal resolution of the pain profile and analysed the data with two different definitions of local and referred pain. These studies involve bolus injections of 5 and/or 10% hypertonic saline, inducing a deep, dull pain that lasts for some 10 minutes. By recording the pain distribution and intensity every 30 seconds, rather than the standard approach of recording only once in an experiment, I have been able to document the dependence of referred pain on local pain (Chapter 3), demonstrate consistent long term plastic changes in a human muscle pain model for the first time (Chapter 4) and differentiate the perception of referral from muscle pain within the arm and leg (Chapter 5). Furthermore, using fMRI I show distinct somatotopy of muscle pain from three different foci within the upper limb.

7.2 THE DEPENDENCE OF REFERRED PAIN ON LOCAL PAIN

In Chapter 3, I presented a study that examined the relationship between local and referred muscle pain. By injecting local anaesthetic into the same intramuscular site in which preceding injections of hypertonic saline had induced local and referred pain, I showed a parallel decline in local and referred pain. Previous studies have demonstrated that referred pain requires an ongoing stimulus from the local initial noxious stimulus (Whitty and Willison 1958; Hockaday and Whitty 1967). Indeed, Hockaday and colleagues (Hockaday and Whitty 1967) showed that intramuscular injection of 2% lignocaine was able to consistently abolish both local and referred pain within a “few seconds,” though it was noted that severe referred pain returned within minutes. Unfortunately, neither the injectate volume nor the dynamics of these interactions were documented.
Saline-induced referred pain is typically observed to follow local pain by about 20 seconds (Hockaday and Whitty 1967; Graven-Nielsen, Arendt-Nielsen et al. 1997), suggesting the involvement of central processes such as temporal summation (Laursen, Graven-Nielsen et al. 1997). Furthermore, the decline in referred pain is seen to precede that of local pain (Graven-Nielsen, Arendt-Nielsen et al. 1997). Though it has been suggested that this delay may result from inhibitory processes (Graven-Nielsen 2006), these observations could just as easily be explained by a form of activity-dependent normalisation of wind-up process such as LTP. Given that lignocaine can abolish C and Aδ mediated activity (Tanelian and Maclver 1991), and that referred pain is at least somewhat dependent on the magnitude of local stimuli (Laursen, Graven-Nielsen et al. 1997), it is perhaps not surprising that local and referred pain decline in parallel in the presence of local anaesthetic. One simple interpretation of these observations may involve a normalisation of wind-up process at a set of central synapses that convey divergent information to higher brain centres. This supports the idea that referred pain depends on an ongoing noxious stimulus, removal of which alleviates both the local pain and the referred pain.

While there were no significant differences over the initial stimulus period between stimuli + anaesthetic and stimuli + sham experiments there were consistent differences in the first 90 seconds of the experiments. Specifically, referred pain area and intensity in this initial timeframe was greater in the second injections and local pain was less. Furthermore, in two repeat experiments referred pain persisted following the abolition of local pain and developed without any local pain at all in two other subsequent experiments on the same subject. This suggested the possibility of long-lasting, presumably central, processes that can support the perception of pain in the absence of an ongoing noxious input.

7.3 LONGITUDINAL CHANGES IN THE PERCEPT OF LOCAL AND REFERRED PAIN

This was explored in Chapter 4, where a longitudinal design involving weekly injections of hypertonic saline resulted in an overall decline in the area and intensity of local pain yet an overall increase in the area and intensity of referred pain. These results were somewhat unexpected. If, as suggested by the previous study, referred pain is dependent on ongoing noxious input from the local site, this percept would parallel the percept of local pain. In addition, many studies suggested that this model was
repeatable on the basis of no significant differences between two subsequent trials of hypertonic saline separated by multiple days (Wolff BB 1965; Graven-Nielsen, Arendt-Nielsen et al. 1997). Furthermore, some subjects experienced local signs of bruising indicative of an inflammatory response that would be expected to sensitize, rather than desensitize; i.e. pain would be expected to increase over time. In this respect it is likely that our stimulus was not exclusively characterised by the C and Aδ barrage from the hypertonic saline (Laursen, Graven-Nielsen et al. 1999) but additionally the inflammation and resultant bruising caused by the insertion of the needle and the act of injecting fluid into the muscle. Histological analysis of rat skeletal muscle has confirmed the presence of several inflammatory markers after just one isotonic injection (McMahon, Wells et al. 1998). Indeed, bleeding was seen in the majority of subjects following withdrawal of the needle post-injection but not prior to delivery. While superficial bruising was evident in 5 subjects, tenderness was reported by the vast majority of subjects in the days following injection. Importantly, however, this tenderness had resolved prior to the subsequent injection seven days later. Nonetheless, this is the first study using any model of muscle pain to show consistent decreases in mean local pain percepts and increases in referred pain percepts as a result of identical repeated trials in humans. Irrespective of regional classification the area and intensity of pain decreased significantly over the course of the four identical weekly injections.

This study utilises two explicit definitions to distinguish pain as either local or referred, nonetheless this is a simplistic representation of phenomena that cannot be defined so simply as contiguous areas of local and referred pain are frequently observed. While these findings are likely to add to a mounting body of evidence suggesting that referral is central in nature and dynamic in expression, they are novel in their depiction of a decreasing local percept of local pain. While the founding studies (Kellgren 1938) suggested that the spread of algogenic fluids in muscle were minimal and constrained, more recent analysis (Graven-Nielsen, McArdle et al. 1997) suggests a wider distribution, though this is likely to vary extensively between subjects and individual trials. With this in mind, the distinction between local and referred pain in homographic tissues is likely to resemble a complex interplay between the diffusion of stimulus, central sensitisation and descending inhibition. In contrast, local pain is either present at the site of stimulus or not. My second definition exploits this more binary concept to define local pain with the arbitrary constraints that the sensation is not felt within 2 cm of the stimulus. While it could be argued that the two-point discrimination of muscle is larger, this is an entirely different cognitive appraisal.
7.4 COMPARISON OF PAIN PERCEPTS IN DIFFERENT LIMBS WITH DIFFERENT STIMULI

In chapter 5 I conducted a repeated-measures study in order to validate subsequent trials of the hypertonic saline-induced pain model within the same region, a practice often utilised as experimental controls. Another commonly used control involves injection into alternate muscles. In chapter 5 I compared identical low and high concentration hypertonic-saline injections into an upper limb muscle (FCR) and a lower limb muscle (TA) in the same subjects. This study demonstrated a near identical magnitude of local pain for a given stimulus (irrespective of the definition of local pain) in either limb. However, significant differences were observed in the manifestation of referred pain. While 5% hypertonic saline induced a comparable level of pain in the two limbs, the 10% solution resulted in a markedly increased response in TA. Application of two definitions of local and referred pain facilitated a distinction in the spatial distribution of pain within the different limbs. Specifically, 10% injections into the TA saw greater numbers experiencing single contiguous bands of pain that extended from the injection site down to the ankle. This may represent a decreased local inhibition in the TA and an increased sensitivity. This study was conducted with knowledge of the results of Chapter 4, such that different subjects were specifically used for comparisons between the 5 and 10% injections within the same limb, so as to avoid longitudinal changes. However, it is possible that the first injection (FCR) influenced the perception of pain from TA, studied a week later.

7.5 SOMATOTOPY OF MUSCLE PAIN

Using functional magnetic resonance imaging (fMRI) I showed in Chapters 6 and 7 that the pain induced by injections of hypertonic saline into three muscles of the upper limb – the deltoid (shoulder), flexor carpi radialis (forearm) and first dorsal interosseous (hand) - is reflected in activation of the known sites within the brain that comprise the pain neuromatrix. While differences in the location of the primary somatosensory cortex across individuals prevented our seeing group differences in activation from the three muscles, we could define - for the first time - intra-limb somatotopy of muscle pain within the insula. This fits with the earlier demonstration of somatotopy of muscle (and cutaneous) pain within the insula following injections into the leg and arm (Henderson, Gandevia et al. 2007).
7.6 A MECHANISM OF REFERRED PAIN

Several mechanisms have been suggested to explain the appearance of referred pain. Both the convergence projection theory (Ruch 1947), and the bifurcation (Sinclair 1948) of axons from both local and referred regions, fail to explain the temporal (Hockaday and Whitty 1967; Graven-Nielsen, Arendt-Nielsen et al. 1997) and stimulus-dependent dynamics (Laursen, Graven-Nielsen et al. 1997) or the observation of unidirectionality. Based on a number of animal experiments, in which noxious stimuli resulted in expansion and development of new receptive fields within the dorsal horn (Cook, Woolf et al. 1987; Hoheisel and Mense 1989; Hylden, Nahin et al. 1989; Laird 1989; Hoheisel, Mense et al. 1993; Ren, Williams et al. 1994), it has been proposed that referred pain is the result of heterosynaptic activity-dependent plasticity or more simplistically a form of central sensitisation. While neither term is precisely explicit, nor provides a detailed model that explains all associated phenomenon in all cases, caution should be exercised when using the term “central sensitization” as there is a great deal of confusion in the literature. The IASP defines central sensitisation as “an enhanced responsiveness of nociceptive neurons in the CNS to their normal afferent input”. The perception of pain results from interplay between nociception and antinociception, as such, nociceptive neurons may impose an inhibitory effect. Indeed, in this respect our observation of decreased sensation at the local site may also be referred to as being driven by central sensitisation.

While numerous studies probe increased sensitisation to pain, a common reaction in both humans and animals involves a decreased response to subsequent noxious stimuli in a process known as habituation (LeBlanc and Potvin 1966; Becerra, Breiter et al. 1999; Bingel, Schoell et al. 2007; Rhudy, Bartley et al. 2009). These influences are believed to result from a complex interplay between ascending stimulatory and descending inhibitory systems (Bingel, Schoell et al. 2007; Rhudy, Bartley et al. 2009) and may well underlie the decrease in local pain observed in Chapter 4. While adaptation in pain is poorly researched, E. R. Kandel’s study of the withdrawal reflex adaptation in aplysia has become the foundation of our understanding of cellular and molecular plasticity throughout the nervous system. Long-term potentiation (LTP) most commonly refers to an NMDA-dependent increase in excitatory postsynaptic potentials after a synchronous or intense parasynaptic activation. Indeed, these processes are likely to underlie the phenomenon observed in Chapter 4, though they are however outside the scope of this thesis. Nevertheless, if referred muscle pain is a result of some form of NMDA-dependent central sensitisation, such an increase should be at least somewhat attenuated in the presence of an agonist such as ketamine. Support for this comes from the observation that the expanded referred pain areas in fibromyalgia
patients were partly inhibited by ketamine (Graven-Nielsen, Aspegren Kendall et al. 2000).

While single neurone recordings from anaesthetized cats have demonstrated desensitisation after repeated stimulation (Paintal 1960), this phenomenon, together with the observation of decreased pain intensity following two subsequent injections of hypertonic saline, has been observed to occur over the course of hours rather than weeks. Inflammation is known to stimulate an early increase in descending facilitation from the RVM (Urban and Gebhart 1999) that may decrease over time as descending inhibition increases, leading to a net enhancement of antinociception, in an NMDA-dependent manner (Terayama, Guan et al. 2000).

7.7 FUTURE DIRECTIONS

Anaesthesia of the referred pain area has been observed to have varying effects on the percept of referred pain, from a significant reduction or complete block (Whitty and Willison 1958; Hockaday and Whitty 1967; Laursen, Graven-Nielsen et al. 1997) to having no effect on the intensity of referred pain (Kellgren 1938; Feinstein, Langton et al. 1954; Whitty and Willison 1958). Amidst these vastly differing effects of regional anaesthesia some studies have sought to elucidate the local and referred somatosensory changes from experimentally-induced muscle pain (Kosek and Hansson 2002) others. Is non-noxious peripheral input magnified or sensitised by convergent pathways involved in the processing of pain, or are these more subtle sensations occluded by such intense painful stimuli? During noxious intramuscular electrical stimulation, one particular study concluded that bilateral innocuous cold, warm and pressure pain thresholds were elevated, suggesting the effect of endogenous pain inhibitory systems (Kosek and Hansson 2002). This is not, however, a simple story; if we are to view the latter as an indicator or hypoalgesia, there are many other papers that demonstrate hyperalgesia or allodynia. As summarized in table 3 of Graven-Nielsen et. al (Graven-Nielsen 2006), there is a great deal of conjecture regarding the precise interaction between painful and non-painful sensations. In order to dissect the peripheral interaction between painful and tactile sensations and the fibre groups involved we have developed a simple model whereby tactile stimuli (low amplitude 200 Hz vibration) is applied in the presence of constant TA pain, induced by infusion of hypertonic saline. This innocuous vibration is detected by different mechanoreceptors in hairy skin (HFA) and deep tissue (FAII), but can be independently and progressively blocked by cutaneous anaesthetic or compression block of the nerve supplying the deep tissue. This work is presented in the Appendix. We know that
compression block acts on large-diameter fibres before small-diameter fibres, while local anaesthetic acts first on the smaller C fibres and then progressively on the larger group III, II and I myelinated fibres. With these techniques we have demonstrated that superficial (cutaneous) inputs mediate the vibration-induced allodynia and that deep inputs mediate the vibration-induced hypoalgesia. Thus, the relative expression of tactile-modulated perception of muscle pain is dependent upon the central integration of superficial (excitatory) and deep (inhibitory) inputs, and this differs across individuals (Stawowy, Rossel et al.). We are currently extending this work to glabrous skin in the upper limb, in addition to demonstrating a clinical relevance by applying similar methodology to a model of DOMS.

7.8 CONCLUDING REMARKS

While there have been many studies to have used the intramuscular hypertonic-saline model of muscle pain, none have measured pain with the high temporal resolution I developed in this thesis. By measuring the area and intensity of pain every 30 s I have been able to show the time-course over which muscle pain develops and abates with greater temporal resolution. Moreover, by developing a new method of quantifying the area of local and referred pain, I have been able to improve our understanding of how referred pain develops. Indeed, in Chapter 4 I presented evidence to show that, when an identical bolus injection of hypertonic saline is delivered to the same site of the tibialis anterior muscle over four weeks, the site of local pain can migrate distally. This would never have been identified had we simply relied on the classic definition of local and referred pain, in which the boundaries are defined somewhat arbitrarily. In addition, I have compared the spatiotemporal expression of local and referred pain in two muscles (FCR and TA) in the same subjects, and demonstrated that a subject who experiences referred pain following intramuscular injection of hypertonic saline into FCR is also likely to experience referred pain following injection in TA. Finally, using fMRI, I have extended our understanding of the central processed involved in the perception of muscle pain. Importantly, I have shown for the first time that there is a within-limb somatotopy of muscle pain in the insular cortex; it remains to be seen whether it is possible to differentiate sites of local and referred pain within this structure.
CHAPTER 8

APPENDIX
VIBRATION-EVOKED ALLODYNA AND HYPOALGESIA DURING MUSCLE PAIN

Saad Nagi, Troy Rubin, Vaughan G. Macefield and David A. Mahns

8.1 INTRODUCTION

It is widely appreciated that tactile and pain sensations rely on limited classes of mechanoreceptors and sensory nerve fibres. However, it is unclear whether these distinct sensations result from the activation of a single class of afferent fibre or the convergence of inputs from multiple classes. In pathological conditions such as allodynia, tactile stimuli frequently manifest as painful sensations. In many pain states, it is not possible to resolve whether the observed allodynia, or hypoalgesia for that matter, reflects an alteration in the peripheral responsiveness (sensitisation) of nociceptive afferents or a perturbed central integration of pain and tactile modalities. Therefore, an increased understanding of the interplay between nociceptive and non-nociceptive inputs is fundamentally important if we are to improve the treatment of numerous musculoskeletal and pain pathologies.

Transcutaneous Electrical Nerve Stimulation (TENS) and other pain-relieving techniques have been widely used to treat chronic pain conditions (Hamalainen, Subiesta et al. 2009; Wu, Erickson et al. 2009; Kang, Wu et al. 2010). Pain relief is typically ascribed to the activation of large diameter tactile afferents leading to spinal (central) inhibition of nociceptive processing (Ochoa and Torebjork 1989; Marchettini, Simone et al. 1996; Melzack 1999). Although pain relief is frequently reported, i.e. hypoalgesia, there have been reports of exacerbated pain conditions, or allodynia, during afferent stimulation (Wu, Gavva et al. 2008; Kang, Wu et al. 2010). Consistent with this view, several studies in patients with chronic pain conditions have suggested that allodynia is mediated by peripheral inputs from large diameter (Group II) tactile afferent fibres (Jin, Keller et al. 2007; Swiderski, Gong et al. 2007; Banik and Brennan 2008; Cameron, Brennan et al. 2008; Kang and Brennan 2009; Spofford, Ashmawi et al. 2009). In contrast, several psychophysical and microneurographic studies involving patients with chronic pain conditions have argued in support of non-Group II afferents in mediating allodynia (Spofford, Ashmawi et al. 2009) (Cline, Ochoa et al. 1989) (Wu, Gavva et al. 2008). Thus, the exact role of peripheral afferent fibres in mediating allodynia remains unclear.
In many pain studies, both noxious and innocuous stimuli are applied to the same or adjacent regions of skin (Cline, Ochoa et al. 1989; Arnér, Lindblom et al. 1990; Gracely, Lynch et al. 1992; Koltzenburg, Torebjork et al. 1994). This approach can lead to ambiguity as it remain unclear whether any change in pain perception reflects a sensitised state of nociceptive afferents or a perturbed central integration of pain and tactile modalities. To resolve this ambiguity, we simultaneously infused a noxious hypertonic saline stimuli to a deep muscle of the leg (Tibialis Anterior) and applied innocuous vibration to the hairy skin overlying the tibia. Within regions covered by hairy skin, dynamically sensitive (Group II) tactile afferents are associated with Hair Follicles (HFA) and Pacinian Corpuscles (PC). HFAs are located in the superficial layers of hairy skin and respond to focal vibration. In contrast, PC are located beneath (deep) the hairy skin and are excited by mechanical waves travelling through the skin and underlying tissues (Xu, Richebe et al. 2009). This anatomical separation of tactile afferents within regions covered by hairy skin allows a blockade of skin (superficial) inputs, with local anaesthetic, while preserving inputs from beneath the skin (PC) in order to investigate the differential role of superficial and deep afferent fibres to vibration-evoked responses during muscle pain.

8.2 METHODS

A total of 19 healthy human subjects aged 20-45 years, with no reported musculoskeletal disorders, took part in this study. Informed consent was obtained from each subject. All experiments were approved by UWS Human Ethics Committee (HREC 06/179). Each experiment examined the impact of cutaneous vibration (200 Hz, 200 µm, 30 s) on muscle pain induced by infusion of hypertonic saline (HS) into Tibialis Anterior (TA). In 14 subjects, intradermal anaesthesia (2.0 % Xylocaine) was used to test the contribution of afferent fibre classes located in superficial and deep tissues by comparing the effects of cutaneous vibration, on HS-induced muscle pain, before and after intradermal anaesthesia. In all experiments, subjects sat comfortably on a chair with both legs horizontally stretched out and supported on both sides. The anatomical boundaries of TA were readily identified by palpation during inversion of the foot and dorsiflexion of the ankle joint.

8.2.1 Hypertonic saline-induced muscle pain

Infusion of hypertonic saline (HS: 5 % sodium chloride) into the TA was used to induce muscle pain in conscious human subjects. A needle was inserted, through the skin, into the TA (=60 mm distal to the tibial tuberosity and =30 mm lateral to the anterior border of tibia) and connected to an infusion pump (Harvard Apparatus, model 55-2226, USA)
containing HS. The initial infusion rate was set at 200 µl/min and, once the subject identified the onset of the pain, the infusion rate was adjusted (where needed) in order to maintain a constant pain rating of 4-6 on a Visual Analog Scale (VAS). The VAS was divided into ten equal segments within a range of 0 (no pain) to 10 (worst pain). Beyond these initial adjustments, a stable baseline muscle pain was maintained throughout the duration of HS-infusion, i.e. ≈10-20 min, without further adjustments to the infusion rate.

8.2.2 Cutaneous vibration

A circular perspex probe with a rounded 4 mm diameter tip was placed in contact with the skin overlying TA without compressing the underlying structures. The probe was positioned perpendicular to the skin surface ≈150 mm distal to the tibial tuberosity and ≈15 mm lateral to the anterior border of tibia. The probe was attached to a feedback controlled sinusoidal stimulator as in (Wu, Boustany et al. 2007). Vibration was applied prior to, during and following HS-induced muscle pain. Vibration lasted 30 s and was repeated at 45 s intervals in order to provide sufficient time between trials to prevent desensitisation of the peripheral afferent fibres. The frequency (200 Hz) and amplitude (200 µm) parameters were chosen, as the resulting stimulus is unequivocally innocuous and is capable of activating primary afferent fibres located in and beneath the skin (Baddeley 2000; Wu, Boustany et al. 2007). Subjects were instructed to use the VAS to report any change (increase, decrease or no change) in the intensity of HS-induced muscle pain during vibration. At least three consistent and consecutive responses to vibration were required before progressing with the experiment. White noise was delivered through headphones to ensure that auditory cues associated with mechanical stimulator were not detected by the subjects (Wu, Boustany et al. 2007; Xu, Richebe et al. 2009).

8.2.3 Intradermal anaesthesia

After recording three consistent vibration-evoked responses during HS-induced muscle pain, 0.2-0.4 ml of local anaesthetic (Xylocaine 2 %) was injected intradermally using a sterile needle and syringe. This effectively blocked inputs from superficial afferent fibres within an area of 2-3 cm surrounding the vibration probe without affecting inputs arising from deep afferent fibres (Arnér, Lindblom et al. 1990; Wu, Boustany et al. 2007). The blockade of superficial afferent fibres was verified by the insensitivity of subjects to light mechanical stimulation of the hairs (or skin) and pinprick stimuli in the anaesthetised region (Wu, Boustany et al. 2007). Following intradermal anaesthesia, vibration was applied a minimum of three times to the anaesthetised skin region.
8.2.4 Statistical analysis

Data are presented as mean ± standard error of the mean (±SEM). In each individual, the VAS recorded during vibration (Vibration) was compared to HS-induced muscle pain (Base) measured immediately preceding the vibration. In each subject, triplicate Base and Vibration values were analysed as independent, sequential events. The responses to vibration were also expressed as percentage of the HS-induced muscle pain (Base) observed immediately preceding vibration. Significant changes were detected using a repeated-measures, one-way analysis of variance (ANOVA); (Rubin, Henderson et al. 2010). Where significant differences were indicated (P < 0.05), individual groups were compared using a Newman-Keuls multiple comparison test. All statistical comparisons were made using Graph Pad Prism.

8.3 RESULTS

19 experiments were performed with the aim of identifying the contribution of afferent fibres located in (superficial) and beneath (deep) the skin to the modulation of underlying muscle pain during innocuous cutaneous vibration. Prior to the induction of muscle pain, all subjects reported that the vibration evoked sensations of localised pressure, at the vibration site, and a diffuse sense of vibration within the leg, both of which were non-painful, i.e. VAS = 0. Infusion of HS into the TA evoked sensations of dull aching muscle pain that radiated from the injection site down the leg and often extending beyond the ankle. Once established, the intensity of the muscle pain (triplicate Base values) remained steady over the course of the ≈10-20 min HS-infusion and did not significantly differ between the alldynia and hypoalgesia groupings (ANOVA: F = 0.051, 6, P = 0.998; n = 17).

During the HS-induced muscle pain, subjects could be divided into three distinct groups based on their response to innocuous cutaneous vibration: i. those who consistently reported an increase in pain (alldynia) during vibration (n = 8); ii. those who consistently reported a decrease in pain (hypoalgesia) during vibration (n = 9); and iii. those in which the response varied from trial-to-trial (n = 2, data not shown). Following the cessation of the HS-infusion, the muscle pain abated (VAS = 0) at which point all 19 subjects reported that vibration was non-painful.

8.3.1 Vibration-evoked alldynia

Following the onset of muscle pain, vibration-evoked alldynia was observed in 8 of 19 subjects. The mean data (±SEM, n = 8) presented in figure 1 demonstrates that HS-induced muscle pain (Base: 4.5 ± 0.2, 4.4 ± 0.2, 4.4 ± 0.2) increased significantly
during vibration (Vibration: 5.2 ± 0.2, 5.1 ± 0.2, 5.0 ± 0.2; P < 0.001; ANOVA: F = 10.2, 6, P < 0.001; n = 8). Consistent with the stability of HS-induced muscle pain, and the reproducibility of vibration-evoked allodynia, no changes in the underlying muscle pain (Base) or vibration-evoked allodynia were observed (P > 0.05). Moreover, allodynia initiated during one vibration abated before the onset of subsequent vibration trains.

**Figure 8.1:** Vibration-evoked (a) allodynia and (b) hypoalgesia during hypertonic saline-induced muscle pain. Each panel includes a raw trace and mean data (±SEM). Vibration-evoked responses (Vibration: hatched bars) were compared to the baseline HS-induced muscle pain (Base: open bars). A; In eight subjects cutaneous vibration evoked a significant and reproducible increase in HS-induced muscle pain. B; In nine subjects cutaneous vibration evoked a significant and reproducible decrease in HS-induced muscle pain. Baseline HS-induced muscle pain remained constant throughout the experiment and did not significantly differ when the data of allodynia and hypoalgesia groups were pooled. **P<0.01; ***P<0.001

Following intradermal anaesthesia, the subjects were insensitive to light touch, pinprick stimuli and reported a qualitative change in the sense of vibration, such that the sense of local pressure, at the vibration site, was abolished while the diffuse sense of vibration persisted (figure 2). Prior to intradermal anaesthesia, subjects reported that vibration significantly increased the HS-induced muscle pain by ≈1 VAS unit or 15% (Base: 100%; Vibration: 115.5 ± 2.8%, 115.6 ± 3.1%, 114.4 ± 3.5%; P < 0.05). Following intradermal anaesthesia, the vibration-evoked allodynia was abolished (Base: 100%; Vibration: 101.6 ± 3.9%, 101.4 ± 4.0%, 103.3 ± 4.1%; P > 0.05; ANOVA: F = 5.96, 9, P = 0.001; n = 8). Furthermore, in three subjects, intradermal anaesthesia abolished the observed allodynia revealing an underlying hypoalgesia. These results
are consistent with our hypothesis that the activation of afferent fibres in the skin alone is sufficient to mediate allostynia. Furthermore, the emergence of an underlying hypoalgesia, following intradermal anaesthesia, suggests that the activation of underlying (or deep) afferent fibres may preferentially mediate hypoalgesia.

**Figure 8.2; Effects of cutaneous anaesthesia on vibration-evoked (a) allodynia and (b) hypoalgesia.** Each panel includes a raw trace and the mean vibration-evoked response (±SEM; hatched bars) expressed as a percentage of the baseline muscle pain (open bars). A; Cutaneous anaesthesia significantly attenuated the vibration-evoked allodynia (*P<0.05; n=8). B; Cutaneous anaesthesia had no significant effect on the vibration-evoked hypoalgesia (P>0.05; n=6)

### 8.3.2 Vibration-evoked hypoalgesia

Following the onset of muscle pain, 9 of 19 subjects reported vibration-evoked hypoalgesia. In this group mean data (±SEM, n = 9) revealed that the background muscle pain (Base: 5.6 ± 0.2, 5.6 ± 0.2, 5.6 ± 0.3) was significantly reduced during vibration (Vibration: 4.8 ± 0.2, 4.9 ± 0.2, 4.8 ± 0.3; P < 0.001; ANOVA: F = 8.13, 6, P = 0.0001; n = 9). The intensity of the background muscle pain (Base) and the magnitude of the vibration-evoked hypoalgesia observed in consecutive vibration trains (n = 3) did not significantly differ over the ≈10-20 min HS-infusion (P > 0.05). Moreover, hypoalgesia initiated during one vibration abated before the onset of subsequent vibration trains (figure1).

In 6 of the 9 subjects who reported vibration-evoked hypoalgesia, the effect of intradermal anaesthesia on vibration-evoked hypoalgesia was tested. Prior to intradermal anaesthesia, cutaneous vibration reduced the intensity of the HS-induced
muscle pain by ≈1 VAS unit or ≈15% (Base: 100%; Vibration: 85.9 ± 2.8, 85.7 ± 2.0, 85.9 ± 4.5; P < 0.01; ANOVA: F = 11.01, 6, P = 0.001; n = 6). Following intradermal anaesthesia, subjects were insensitive to light touch, pinprick stimuli and reported a qualitative change in the sense of vibration such that the sense of local pressure, at the vibration site, was abolished, while the diffuse sense of vibration persisted. Moreover, following intradermal anaesthesia, the vibration-evoked hypoalgesia did not significantly differ from the pre-anaesthetic responses (Vibration: 85.7 ± 10.2%, 88.7 ± 10.4%, 98.1 ± 9.0%; P > 0.05; ANOVA: F = 5.45, 9, P = 0.003; n = 6). In contrast to the allodynia group, responses to vibration following intradermal anaesthesia were more variable; in three subject the vibration-evoked hypoalgesia was either preserved (n = 1) or intensified (n = 2, as shown in the traces of figure 2) suggesting that preferential activation of afferent fibres beneath the skin alone is sufficient to generate vibration-evoked hypoalgesia. In the remaining three subjects, intradermal anaesthesia either abolished the hypoalgesia (n = 1) or revealed an underlying allodynia (n = 2). Overall, in the majority of cases (6 of 9) where hypoalgesia was observed, intradermal anaesthesia demonstrated that hypoalgesia is evoked by the activation of deep afferents alone (3 subjects initially presenting with allodynia plus 3 of 6 presenting with hypoalgesia).

8.4 DISCUSSION

The aim of this study was to investigate the contribution of afferent fibres located in (superficial) and beneath (deep) the skin to vibration-evoked responses during underlying muscle pain. Based on subject responses to innocuous cutaneous vibration, two apposing mechanisms, i.e. allodynia (n = 8) and hypoalgesia (n = 9) were identified. In a further two subjects, the response varied from trial-to-trial with vibration increasing pain on some occasions, reducing pain or having no discernible effect.

In a review of the literature, it was unclear whether allodynia, or hypoalgesia, results from the activation of single class of afferent fibre or whether convergence of inputs from multiple classes is required. Furthermore, there is ambiguity in the literature as to whether such responses are consequence of a central change in the integration of sensory inputs or a peripheral change in primary afferent fibre responsiveness. This ambiguity is largely due to the confinement of noxious and innocuous stimuli to a single compartment (Cline, Ochoa et al. 1989; Arnér, Lindblom et al. 1990; Gracely, Lynch et al. 1992; Koltzenburg, Torebjork et al. 1994). We have attempted to resolve this lack of clarity by applying noxious stimuli HS to the muscle compartment and innocuous stimuli (vibration) to the skin compartment. Although the region of skin stimulation
overlies the muscle TA, a sheet-like fascia anatomically separates the TA from the skin. Furthermore, each compartment has separate vascular and nerve supplies. Thus, HS-induced peripheral sensitisation of afferent fibres is highly unlikely in this two-compartment model.

In subjects that showed allodynia, the vibration-evoked increase in muscle pain was abolished by intradermal injection of local anaesthetic. Furthermore, an underlying suppression of muscle pain was observed in 3 of 8 subjects. When administered intradermally, the local anaesthetic blocked both small (Group III and IV) and large (HFA) diameter afferent fibres in the superficial layers of skin without affecting the input from deep afferent fibres (PC). Prior to intradermal anaesthesia, all subjects described the vibration as a sensation of localised pressure at the point of contact and a diffuse sense of vibration. Following intradermal anaesthesia, the local pressure at the point of stimulation was abolished while the diffuse sense of vibration persisted. These qualitative reports support the differential action of local anaesthetic block. In two of the subjects that showed alldynia, vibration was also applied in the adjacent, non-anaesthetised region in order to demonstrate that the effect of intradermal anaesthesia was indeed due to the blockade of superficial afferent fibres and not a generalised decline in the capacity of the central nervous system (CNS) to support alldynia. Thus, the activation of afferent fibres in the skin alone is sufficient to mediate alldynia. Moreover, the underlying suppression of muscle pain suggests that even in subjects that demonstrated a reproducible increase in pain, the measured pain ratings were the result of an integration of inputs mediating alldynia and hypoalgesia.

Several studies involving models of experimental pain or patients with chronic pain conditions have suggested Group II afferent-mediated alldynia (Gracely, Lynch et al. 1992; Koltzenburg, Torebjork et al. 1994; Cervero and Laird 1996; Jin, Keller et al. 2007; Swiderski, Gong et al. 2007; Banik and Brennan 2008; Cameron, Brennan et al. 2008; Kang and Brennan 2009; Spofford, Ashmawi et al. 2009). In contrast, microneurographic studies have unequivocally demonstrated that stimulation of individual, Group II afferent fibres is always non-painful (Saïd, Hmani-Aifa et al.; Badre and D’Esposito 2009; Sasaki, Nanez et al. 2010). Other studies have suggested a role of non-Group II fibres in mediating alldynia (Cline, Ochoa et al. 1989; Wu, Gavva et al. 2008; Spofford, Ashmawi et al. 2009). It seems, however, possible that different psychophysically distinct types of alldynia can be evoked (Koltzenburg, Torebjork et al. 1994) depending on the nature of stimulus applied. A central integrating mechanism is supported by the observation that upon application of innocuous cutaneous vibration within the innervation territory of the sciatic nerve (TA is innervated by sciatic
afferents), subjects reported an increase in HS-induced muscle pain. Moreover, the capacity of intradermal anaesthesia to abolish vibration-evoked allodynia without abolishing the underlying muscle pain further supports a central mechanism.

Vibration-evoked allodynia may represent a change in the response properties of central neurons, which allows for an integration of pain and tactile modalities (Woolf 1983). This is in agreement with animal studies, which have demonstrated that the excitatory responses of central neurons following peripheral nociceptive input (Woolf 1983; Wall and Woolf 1984; Simone, Sorkin et al. 1991; Dubner and Ruda 1992; Mustapha, Fang et al. 2009) were amplified by concurrent non-nociceptive inputs (Mustapha, Beyer et al. 2007). Animal- and human-based studies have attributed allodynia to long-term changes in the central mechanisms (Dubner and Ruda 1992; Banik and Brennan 2008; Mustapha, Fang et al. 2009; van Strien, Cappaert et al. 2009). However, in the present experiments, vibration-evoked allodynia was short-lived and relied on peripheral nociceptive input for its initiation and sustainability. Thus, long-lasting plasticity changes cannot account for the rapid adaptability of vibration-evoked allodynia.

Intradermal anaesthesia had relatively variable effects in those subjects that displayed a reproducible fall in pain intensity during vibration. In the majority of cases where hypoalgesia was observed (6 of 9), intradermal anaesthesia demonstrated that hypoalgesia is evoked by the activation of deep afferent fibres alone. This is consistent with the Gate Control theory, which postulates suppression of the spinal (central) relay of nociceptive input, driven by the activation of large diameter tactile afferents, resulting in hypoalgesia (Ochoa and Torebjork 1989; Melzack and Wall 1995; Marchettini, Simone et al. 1996). On other occasions, the variability in the post-anaesthetic results may be attributable to variability in the local anaesthetic effectiveness. However, this seems unlikely due to the high concentration of Xylocaine (2 %) used and that the effectiveness of the intradermal anaesthesia was confirmed, by the loss of tactile and pinprick sensibility, before vibration was reapplied. The variability in outcomes following intradermal anaesthesia may be consistent with the overall pain rating being a reflection of the balancing, within the CNS, of afferent inputs arising from the skin that promote excitation (allodynia) and deep inputs that promote inhibition (hypoalgesia) of central neurons. This may explain the amplification of hypoalgesia (n = 2) following intradermal anaesthesia as the consequence of the abolition of underlying allodynia, or excitatory inputs, from the skin. However, these interactions may be subject to complex (and varied) temporal and spatial patterns of peripheral input (Banik and Brennan
Alternatively low pain ratings are inherently more subjective and thus more variable.

When vibration was reapplied upon cessation of HS-induced muscle pain, all subjects reported vibration as non-painful with the quality of sensation comparable with that prior to induction of muscle pain. Thus, afferent fibres mediating vibration-evoked responses cannot be regarded as nociceptive. Furthermore, these observations suggest that, initiation and maintenance of vibration-evoked responses always requires peripheral nociceptive input. When the peripheral nociceptive input is removed, central changes rapidly revert to normal. Comparable findings were reported when the nociceptive input was blocked in patients with chronic pain states and in models of experimental pain (Arnér, Lindblom et al. 1990; Gracely, Lynch et al. 1992; Koltzenburg, Torebjork et al. 1994). However, other studies have reported that central changes were triggered, but not maintained, by the nociceptive input (Wall and Woolf 1984; Moscovitch, Nadel et al. 2006). It is still unclear whether peripheral nociceptive input is capable of amplifying the central nociceptive relay independent of long-term changes that sustain central sensitisation.

8.5 CONCLUSION

The majority of studies have not been able to definitively assign an excitatory or inhibitory role of different afferent fibre classes. However, this study shows that both allodynia and hypoalgesia can be generated under similar experimental conditions involving activation of superficial (excitatory) and deep (inhibitory) inputs, respectively. Since noxious and innocuous stimuli were applied to different compartments, HS-induced sensitisation of superficial afferent fibres seems quite unlikely. In some cases, the blockade of allodynia, or excitatory inputs, upon intradermal anaesthesia revealed an underlying hypoalgesia. In other cases, the abolition of allodynia resulted in amplification of hypoalgesia, or inhibitory inputs. These observations imply that perceptual changes are a consequence of the integration, or balancing, within the CNS, of excitatory and inhibitory inputs. In the present experiments, these changes were short-lived, which dissipated when peripheral nociceptive input was removed.
CHAPTER 9

BIBLIOGRAPHY


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