VISCERAL AND CUTANEOUS PAIN: NEURAL CORRELATES AND PHARMACOLOGICAL INTERVENTION

by

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ABSTRACT (345 words)

VISCERAL AND CUTANEOUS PAIN: NEURAL CORRELATES AND PHARMACOLOGICAL INTERVENTION

Our brain is involved in processing pain, whether it is superficial cutaneous pain, caused by a scratch or a burn, or deep internal pain, caused by heartburn or gas in the intestines. Moreover, activation of a common cortical network is suggested during different types of pain in humans, implying that as long as the stimulus is painful it will be processed similarly in the cerebral cortex. However, no one has yet made direct comparison between superficial and deep pain of similar intensity and location; direct comparison is necessary in order to see how superficial pain relates to a more clinically relevant deep pain and to further our understanding of the latter.

In three separate studies, the perception of visceral and cutaneous pain in humans was examined using psychophysical, brain imaging and pharmacological approaches, respectively. The first study revealed that for a similar given intensity, duration and location, visceral pain is more unpleasant, more varied qualitatively, more diffuse and more persistent after stimulation has ended, suggesting that there are some significant distinctions in the neural processes of external and internal pain in humans. The second study examined such processes with functional magnetic resonance imaging (fMRI), disclosing substantial differences in cortical processing of sensory information from skin and viscera, including limbic areas associated with the emotional component of pain (anterior cingulate and insular cortices), and sensory areas (primary somatosensory cortex). In addition, several similar cortical areas were activated by both superficial and deep pain, consistent with the existence of a common pain network independent of the nature of pain. The final study examined a possible divergence in pharmacological processes underlying deep and superficial pain, which could arise from differences in neuronal processing. The findings revealed that NMDA-receptors mediate both visceral and cutaneous pain in humans, yet the affect of visceral pain might be more susceptible

to their blockers, which may be a potential explanation for different treatments of visceral and cutaneous pains.

Together these studies provide direct evidence of the differences and similarities between visceral and cutaneous pain in humans within the perceptual, physiological and pharmacological domains.

RESUMÉ

DOULEURS VISCÉRALES ET CUTANÉES : CORRÉLATS NEURAUX ET INTERVENTION PHARMACOLOGIQUE

Notre cerveau est impliqué dans le traitement de la douleur, qu'il s'agisse de douleur superficielle cutanée causée par une éraflure ou une brûlure, ou de douleur profonde, causée par des brûlement d'estomac ou des gaz intestinaux. De plus, l'activation d'un réseau cortical commun est suggérée durant l'expérience de différents types de douleurs chez l'humain, ce qui implique que tant que le stimulus est douloureux, il sera traiter de façon similaire par le cortex cérébral. Cependant, personne auparavant n'a comparé directement les douleurs superficielle et profonde d'intensité et de location similaires. Une comparaison directe est nécessaire afin de déterminer le rapport entre la douleur superficielle et la douleur profonde cliniquement plus significative et, afin d'augmenter nos connaissances de cette dernière.

La perception des douleurs viscérale et cutanée chez l'humain a été examinée au moyen de trois études distinctes utilisant respectivement une approche psychophysique, l'imagerie cérébrale et une approche pharmacologique. La première étude a révélé que, pour une intensité, une durée et une location similaire, la douleur viscérale est plus déplaisante, plus variée qualitativement, plus diffuse et plus persistante suite à la terminaison de la stimulation, ce qui suggère qu'il existe certaines distinctions dans les processus neuraux des douleurs externes et internes chez l'humain. La seconde étude a examiné ces processus neuraux au moyen de l'imagerie par résonance magnétique fonctionnelle, révélant des différences substantielles du traitement cortical de l'information sensorielle provenant de la peau et des viscères, incluant des différences au niveau des aires limbiques, associées au traitement de la composante émotionnelle de la douleur, et des aires sensorielles. De plus, plusieurs aires corticales étaient activées de façon similaire par les douleurs superficielle et profonde, ce qui est compatible avec l'existence d'un réseau commun du traitement de la douleur, indépendant de la nature de la douleur. La dernière étude a examiné la divergence possible dans les processus

pharmacologiques qui sous-tendent les douleurs profonde et superficielle, qui pourrait survenir à cause des différences au niveau du traitement neuronal. Les résultats démontrent que chez l'humain, les récepteurs NMDA sont impliqués dans les douleurs viscérale et cutanée bien que la dimension affective de la douleur viscérale semble être plus susceptible à leur blocage, ce qui expliquerait possiblement les différents traitements des douleurs viscérale et cutanée.

Dans l'ensemble, ces études apportent une évidence directe des différences et similarités entre les douleurs viscérales et cutanée chez l'humain tant dans le domaine perceptuel, que physiologique et pharmacologique.

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Chapter 2 contains a manuscript in press in Pain entitled "Psychophysical analysis of visceral and cutaneous pain in humans". I conducted the study, analyzed the data and prepared the manuscript. Drs. Bushnell and Duncan provided theoretical collaboration and guidance throughout the study, including setup, data analyses, and the manuscript. Dr. Boivin provided time, staff and equipment for the gastroentorology part of the study, such as measurement of esophageal sphincter location, catheter placement etc.

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Chapter 1

INTRODUCTION TO VISCERAL AND CUTANEOUS PAIN

The idea of pain as a sensory experience has prospered since the recorded history. It continues to thrive today, somewhat successfully fighting its way through the barricades of XXI century analgesics. Some theories attribute this persistent nature of the pain experience to its complexity, taking into consideration the sensory-discriminative, affective-motivational and cognitive aspects; others ascribe pain's survival to its primitivism, which leads to unpredictable plasticity in the system, and therefore its inherent difficulty to be understood.

The question that comes to the mind of almost every individual interested in pain research is – What is pain? The International Association for Study of Pain (IASP) tried to answer this question by giving the following definition: **"An unpleasant sensory and emotional experience which we primarily associate with tissue damage or describe in terms of tissue damage, or both"**. According to this definition, pain is an entity, and irrespective of from where it originates – from inside, such as a stomachache, or from the outside, such as a scratch or a burn – or how long it lasts – one minute or one year – it will still be called pain. It is not surprising, therefore, that due to easier access, many researchers have focused primarily on developing numerous and elaborate models to study superficial pain, hoping that mechanisms underlying pain arising from skin would be applicable to pain arising from other structures, for instance, the viscera. It is called pain, after all. However, our personal experience indisputably indicates that sensibilities and responses associated with pain in visceral tissues, such as stomachache, heartburn or gas, are different from those associated with skin damage. Therefore, it is not illogical to conclude that, despite being called "pain", mechanisms underlying these two types of noxious sensations might very well differ, thus suggesting that not everything we know about the cutaneous modality applies to the visceral one.

Despite the lack of comprehensive investigative reports, the distinctiveness of visceral pain has long attracted the attention of numerous researchers and clinicians. Books describing bizarre qualities in the visceral domain date to the beginning of last century (Mackenzie, 1909) and perhaps even earlier. However, it is only recently that researchers started to seriously consider visceral pain as a subject of interest, and much more is now known about the anatomy and physiology of visceral tissues. Having this information at hand, researchers have attempted to contrast these new findings on visceral pain with the existing knowledge on cutaneous pain, anxiously trying to answer the questions of where and how they differ. Comparisons were made across studies that quite often employed different stimulation paradigms and parameters, thus considerably undermining the meaning of their conclusions. Therefore, in order to answer the question of how visceral pain is different from cutaneous pain, one absolutely needs to directly compare the two modalities in the same experimental sample, preferably equating the intensity, duration and location of painful sensation. Indeed, one needs to experience both, cross the Atlantic Ocean in the Titanic and fly across in a supersonic jet, in order to really know how and why they are different. So far, this kind of direct comparison between visceral and cutaneous pains has been lacking in the field of pain research.

Even though animal models provide a better system for studying the neurophysiolgical mechanisms of pain transmission using various intricate approaches, such as cellular unit recordings, immunocytochemistry and/or gene knockouts, human studies offer a superior method for studying behaviour and cognition. In addition, complications associated with species differences may be avoided when studying human subjects; this, in turn, has significant clinical importance. Indeed, "any evidence won from man is indubitably applicable to the human problem" (Lewis, 1942). One of the first steps to perform in humans when contrasting two modalities, visceral and cutaneous pain in this context, is a thorough psychophysical analysis, first described in 1957 by Stanley Smith Stevens (Stevens, 1957). This technique allows for accurate characterization of sensory and/or pain thresholds, the quality, onset, duration and progress of pain in both modalities. These could be contrasted directly thereafter, thus providing direct evidence for the differences and similarities between the perceptual qualities of visceral and cutaneous pains. Having determined the perceptual differences and/or similarities, the next logical step is to contrast the neuronal activity

following visceral and cutaneous pain, since perception is directly linked to the brain. In humans, this has become possible with the development of new brain imaging techniques, such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), event-related electrical potentials (ERPs), magnetoencephalography (MEG), and event-related optical imaging (EROS). These techniques measure changes in either blood flow or the electrical or magnetic signals originating from the brain following a particular arousing external stimulus. Correlation of the resultant brain activity, in our case following painful stimulation, with the previously evaluated perception, allows the determination of differences and similarities in brain areas involved in processing of visceral and cutaneous pains. In addition, this will provide the possibility to reflect on the role of each brain structure in sensory, motor, affective, cognitive and other aspects of visceral and cutaneous pain experiences. Several different approaches could be taken thereafter in the process of contrasting visceral and cutaneous pains, such as a more thorough assessment of neuronal processes with EEG or ERP, examination of pathophysiology and more. One approach that could be important clinically, however, is the analysis of pharmacological modulation of visceral versus cutaneous pain, especially by agents with potential analgesic properties. This could further reflect on the possible physiological mechanisms underlying visceral and cutaneous pain modalities and could bring research one step closer to identifying specific analgesic agents.

The work described in the present thesis concentrates on the comparison of normal visceral and cutaneous pain, which is felt by all (some rare exceptions apply) individuals and is evoked by activation of normal, intact anatomical sensory pathways. Specifically, my studies will contrast visceral and cutaneous pain in normal human subjects using the psychophysical, brain imaging and pharmacological approaches referred to above. First, a brief overview of the existing, mostly animal literature about visceral and cutaneous properties relevant to the perception of visceral and cutaneous pain will be given. This will be followed by three separate reports examining visceral and cutaneous pain in humans using psychophysical, brain imaging and pharmacological techniques, respectively. Then I will present final conclusions about visceral and cutaneous pain drawn from the performed studies and consolidated in order to answer the posed question of how pain from the viscera differs from pain from the skin.

1.1 PERCEPTUAL ATTRIBUTES OF VISCERAL AND CUTANEOUS PAIN

Early clinical and experimental observations indicate that superficial cutaneous and deep visceral pains have different sensibilities. These observations date to the early XXth century, when Livingston (1935) and then Lewis (1942) described pain from the skin to be precise and localized to the site of stimulation, while pain from visceral structures to be diffuse and not always localized. The observation that visceral pain may be referred to another area of the body, such as skin, muscle or both (e.g. tenderness in the left arm of a person having myocardial infarction), was described even earlier by several neurosurgeons, who already then attempted to explain the underlying mechanisms (Mackenzie, 1909; and see below). The phenomenon of "referred" pain is considered a bizarre, and somewhat unique, characteristic of visceral pain.

Earlier clinical observations also indicate that unlike cutaneous pain, visceral pain cannot always be evoked by conventional stimuli, but requires more elaborate and specific approaches. For example, direct trauma readily and effectively produces superficial pain but does not result in visceral pain; cutting, crushing and/or burning the healthy intestines does not evoke painful responses, as long as the traction of the mesenteries and stimulation of the body wall is avoided. This phenomenon of "visceral insensitivity" has been observed by numerous surgeons, who frequently tested it in their patients (Mackenzie, 1909; Livingston, 1935; Lewis, 1942; Livingston, 1998). It took several years to realize that "adequate stimuli for visceral afferents appear to be those arising from their own environment and especially their own activities" (Ruch, 1946). With this view in mind, dilatation or distention, spasms or strong contractions, especially when associated with ischaemia and chemical irritants, were found to be effective in producing pain in the visceral domain, thus providing definite evidence that "true" visceral pain exists.

Further observations associated visceral and cutaneous pains with highly divergent autonomic and behavioural responses. As described by Lewis (1942), cutaneous pain evokes quick protective reflexes, tachycardia, hypertension and increased alertness, while visceral pain produces quiescence, bradycardia, hypotension and loss of interest in the environment (Lewis, 1942). It also results in sweating, pilo-erection, and/or vasomotor changes (Ruch, 1946).

1.2 PHYSIOLOGICAL PROPERTIES

Perceptual differences alluded to in the previous section could be attributed to and explained by the divergence in the anatomical, physiological and perhaps neural mechanisms underlying visceral and cutaneous sensations. The following section will summarize findings from animal and some human studies examining such differences in the periphery.

1.2.1 Innervation

Five decades ago, Theodore Cedric Ruch wrote that "sensory innervation of the viscera is a somewhat larger question than visceral sensation" (Ruch, 1946), thereby implying the complexity of visceral innervation. Indeed, contrary to somatic structures which are supplied exclusively by the spinal nerves, visceral structures receive dual innervation from vagal and spinal primary afferent fibers (Kiernan, 1998; Iversen et al., 2000). It is believed that primary sensory neurons whose cell bodies lie in the dorsal root ganglia of the thoracic and upper lumbar nerves are involved in the transmission of visceral pain, whereas parasympathetic nerves are solely responsible for non-sensory or homeostatic visceral events. Yet the role of vagal visceral afferents in the control of nociception has long been a topic of debate. In rats, cervical vagal afferents have been shown to transmit noxious cardiac input following intrapericardial administration of algogenic chemicals (Qin et al., 2001). In addition, high threshold mechanosensitive gastric vagal afferents have been characterized, and c-fos expression in the nucleus of solitary tract (NTS), largely due to activation of vagal afferents, was shown to increase following noxious gastric distention (Traub et al., 1996), thus suggesting a nociceptive role. Specifically, due to the projection of NTS neurons via the parabrachial nucleus to limbic areas, visceral vagal afferents may influence emotional reactions to noxious visceral stimuli. Therefore, these are thought to play a more important role in the affective-motivational rather than sensory-discriminative aspect of pain (Traub et al., 1996). In addition, a modulatory, pain-reducing role for vagal afferents during mechanical hyperalgesia and allodynia in visceral and somatic tissues has been suggested (Janig et al., 2000), based on findings in rats that vagotomy selectively amplified the decrease in both rectal distention thresholds following intraperitoneal lipopolysaccharide application (Coelho et al., 2000), and the paw-withdrawal latencies to mechanical stimulation before and after intradermal injection

of bradykinin (Janig et al., 2000). On the other hand, low intensity vagal nerve stimulation in humans resulted in a significant decrease in the thermal pain thresholds, suggesting a pro-nociceptive role (Ness et al., 2000).

Skin is a highly innervated organ supplied by peripheral axons of up to 1-1.5 million spinal afferent neurons (Holmes & Davenport, 1940). According to the quantitative anterograde and retrograde tracing studies performed in the last decade with the horseradish peroxidase technique, the number of afferents that innervate visceral organs is considerably less. In a cat, the total number of primary afferent neurons signaling afferent information from abdominal and pelvic viscera, heart, thoracic large vessels and lungs is less than 30,000 (Oldfield & McLachlan, 1978; Morgan et al., 1981; Kuo et al., 1982; Kuo et al., 1983; Kuo & de Groat, 1985; Ruhle et al., 1985; Baron et al., 1985a; Baron et al., 1985b; Baron et al., 1985c). This number is even smaller in a rat, amounting to only about 20-40% of those in a cat in the pelvic, lumbar splanchnic and renal nerves (Hulsebosch & Coggeshall, 1982; Neuhuber, 1982; Ciriello & Calaresu, 1983). Therefore, a simple arithmetic computation suggests that, on average, visceral afferent innervation is relatively scarce, amounting to less than 5% of that found in the skin. Furthermore, unlike cutaneous nerves, this small number of visceral afferents enters the spinal cord through more than ten different segments (Cervero & Tattersall, 1986), thereby dispersing the originally weak message even further.

In addition to the poor innervation and high diffusion in the spinal cord, the relative distribution of fibers in visceral and cutaneous nerves is dissimilar. If a typical cutaneous nerve contains about 20-25% of large myelinated A-β fibers, 10-15% of small myelinated A-δ fibers, and 60-70% of small unmyelinated C-fibers (McMahon, 1997), the conduction velocities of most abdominal visceral afferent fibers lie in the A-δ and C-fiber range, with approximately a 10:1 ratio between unmyelinated and myelinated fibers, respectively (Cervero & Tattersall, 1986; Janig & Morrison, 1986). Less than 5% of A-β fibers have been identified in the greater splanchnic nerve of a cat (Kuo et al., 1982), and none in the pelvic nerve of a rat (Sengupta & Gebhart, 1994a; Sengupta & Gebhart, 1994b). Therefore, the majority of the visceral message is carried to the spinal cord via a small number of predominantly unmyelinated C-fibers, which

many researchers believe is one of the reasons for the dull, poorly localized and diffuse nature of visceral pain.

1.2.2 Sensory Receptors

Skin has quite an elaborate system of sensory receptors in terms of both number and function. Each receptor type is morphologically distinct and responds to a specific sensory modality (e.g. temperature, pressure) at a specific intensity. Consequently, cutaneous pain is a result of activity in specialized sensory receptors in the skin, called nociceptors, which are further subdivided into mechanical, thermal-mechanical or polymodal types (Gardner et al., 2000), thus increasing their specificity even further.

Contrary to cutaneous sensory receptors, the precise functional role of visceral receptors is still unknown and might differ depending on the organ of interest. The receptors of primary visceral afferent neurons are located in mucosa, muscle and serosa (mesentery) of hollow organs (e.g. gut, bladder). Two classes of visceral receptors capable of transmitting sensory information, including pain from the viscera, have been described. The first class, termed specific visceral nociceptors, is similar to specific nociceptors found in the skin. It has a high threshold for activation and responds to stimuli of noxious intensities; thus, it is specific to pain. To date, this type of receptor has been identified in the heart, veins, lung and airways, testes, esophagus, small intestine, colon, ureter, urinary bladder, uterus, and biliary system (Gebhart & Sengupta, 1994; Cervero, 1996). The second class of receptors, termed non-specific or intensity-encoding visceral receptors, has a low threshold to natural stimuli and responds to a variety of stimulus intensities ranging from innocuous to noxious. This type of visceral receptor is non-specific to pain and is unique to visceral structures. It has been identified in the gastrointestinal tract, specifically in the esophagus and colon, urinary bladder, heart and testes (Gebhart & Sengupta, 1994; Cervero, 1996).

Similar to cutaneous nociceptors that respond to mechanical, thermal and chemical stimulation in both humans (Torebjork, 1974; Hallin et al., 1982) and animals (Beitel & Dubner, 1976; Croze et al., 1976), a large proportion of visceral receptors is also polymodal in nature. Mechanosensitive visceral afferent fibers that also respond to thermal and chemical stimuli

have been identified in pelvic (Su & Gebhart, 1998), superior spermatic (Kumazawa & Mizumura, 1980a; Kumazawa & Mizumura, 1980b; Kumazawa et al., 1987), and splanchnic nerves (Adelson et al., 1997) in animals. In humans, only limited data are available on other than visceral mechanoreceptors. However, data from studies on acid sensitivity in the esophagus (Fass et al., 1998), and thermal sensitivity in the stomach and small intestine (Villanova et al., 1997) suggest that polymodal visceral receptors are present in the human gut.

Another interesting observation about visceral and cutaneous sensory receptors is that both skin and viscera contain "silent nociceptors" (Lynn, 1991; Cervero & Janig, 1992a). These receptors have no spontaneous discharge activity at rest, are unresponsive to mechanical stimuli, are sensitive to chemical stimuli, and are capable of developing spontaneous activity, as well as mechano- and thermal sensitivity following tissue injury and/or inflammation. Thus, based on their properties, "silent nociceptors" would be recruited in pathological conditions, such as irritable bowel syndrome, and it is believed that a significantly greater number of these receptors are found in viscera. "Silent nociceptors", first described in the knee joint of a cat, are also present in the skin (Schmidt et al., 1995), as well as in the urinary bladder (Habler et al., 1990; Habler et al., 1993), colon (Gebhart, 2000) and the heart (Pan & Chen 2002).

1.2.3 Viscero-Somatic Convergence

One of the physiological issues that frequently surfaces in the debate over visceral and cutaneous pain is the abundance of viscero-somatic convergent neurons in the spinal cord, as well as subcortical and even cortical areas (for review see Cervero & Tattersall, 1986; Foreman, 2000). In monkeys, viscero-somatic convergent neurons have been demonstrated at the cervical, thoracic, lumbar and sacral levels of the spinal cord (Milne et al., 1981; Foreman et al., 1981; Ammons et al., 1984a; Ammons et al., 1985; Bolser et al., 1991; Chandler et al., 1996). Specifically, the majority of cells excited by visceral stimuli, such as gallbladder distention (Ammons & Foreman, 1984b), electrical stimulation of the cardiopulmonary sympathetic (Bolser et al., 1991) or greater splachnic nerve afferents (Foreman et al., 1981) possessed cutaneous receptive fields. In addition, 84% of cells in the thoracolumbar and sacral spinal cord responded to both noxious cutaneous stimulation and urinary bladder distention (Milne et al., 1981). A similar proportion of viscero-somatic convergence has been shown in cats; 84%

of T2-T4 and 67% of T8-T12 spinal neurons responded to somatic inputs and electrical stimulation of the splanchnic nerve, and about 10% of L4-L6 spinal neurons had receptive fields in the skin and deep tissues (Ammons et al., 1984b; Cervero & Tattersall, 1987; Schaible et al., 1987). In addition, 86% of the upper thoracic neurons driven by electrical stimulation of the cardiopulmonary sympathetic afferents were classified as high threshold based on their responses to stimulation of their cutaneous receptive fields (Foreman et al., 1984). Furthermore, up to 40% of T2-T7 spinal neurons with convergent input from the distal esophagus, heart and somatic fields have been described in cats (Garrison et al., 1992), suggesting viscero-visceral convergence as well. Again, the majority (74%) of neurons excited by distention of distal esophagus and somatic stimulation were classified as high threshold cells (Garrison et al., 1992), suggesting that a high proportion of viscero-somatic convergent neurons are specific for pain processing.

Viscero-somatic convergence is not exclusive to the spinal cord. In squirrel monkeys up to 70% of neurons in the ventroposterolateral (VPL) nucleus of the thalamus and 34% of neurons in the primary somatosensory cortex (SI), one of the VPL projection sites (Gingold et al., 1991; Shi & Apkarian, 1995), receive viscero-somatic convergent input (Bruggemann et al., 1994; Bruggemann et al., 1997; Bruggemann et al., 1998). In addition, more than 50% of the right ventrobasal thalamic neurons in rat responded to baroreceptive and mechanical nociceptive stimulation; 78%, 7%, and 15% of these cells belonged to ventroposterolateral (VPL), ventroposterolateral parvicellular (VPLpc), and ventroposteromedial (VPM) nuclei, respectively (Zhang & Oppenheimer, 2000), while a higher proportion (74%) of rat ventrobasal thalamic neurons responded to both mechanical stimulation of the skin (brush, pressure, pinch) and to at least one visceral stimulation (distention of uterus, colon, mechanical vaginal probing, pressure again the cervix) (Berkley et al., 1993a). More than 20% of viscero-somatic neurons responded to noxious pinch, while 61% of all neurons were activated by stimulation of more than one viscus (Berkley et al., 1993a). Furthermore, neurons in the lateral thalamus of cat also exhibited convergence, being driven by stimulation of the urinary bladder, colon and/or esophagus and skin (Horn et al., 1999). Like the thoracic neurons described above (Garrison et al., 1992), up to 31% of these cells responded to distention of more than one viscus, demonstrating viscero-visceral convergence (Horn et al., 1999). In addition, neurons in

the rat thalamic nucleus submedius, thought to play an important role in nociception (Craig, Jr. & Burton, 1981; Dostrovsky & Guilbaud, 1988), were activated by noxious mechanical and/or thermal cutaneous stimulation, as well as chemical stimulation of muscle and/or visceral organs (Kawakita et al., 1993). Similar to the VPL-SI example, neurons in the ventrolateral orbital cortex of cat, which is a primary projection site of nucleus submedius (Craig, Jr. et al., 1982; Price & Slotnick, 1983; Yoshida et al., 1992; Coffield et al., 1992), also demonstrated viscero-somatic convergence, being driven by several noxious modalities, including skin heating and gall bladder distention (Snow et al., 1992). In addition, 66% of anterior hypothalamic neurons in rats were driven by electrical stimulation of the splachnic nerve and the skin (Snowball et al., 2000). Furthermore, the interconnectivity of the anterior hypothalamus with ventrolateral columns of the brainstem periaqueductal grey matter (PAG) (Snowball et al., 2000), which, in turn integrates visceral and deep somatic information (Keay et al., 1994; Clement et al., 1996), suggests that viscero-somatic convergence is common at all levels of the ascending pathways thought to be important in pain (Willis & Westlund, 1997; and see later). Finally, 80% of primate anterior insular cortex neurons were activated by both cutaneous pinch and baroreceptor challenge (Zhang et al., 1999), whereas right posterior insula in rat received direct input from thalamic viscero-somatic convergent neurons (Zhang et al., 2000), suggesting convergence in the insular cortex as well.

If the potential significance of viscero-visceral convergence is to integrate, coordinate and/or control functions of the various internal organs, for instance regularize uterine and colonic inputs in the thoraco-lumbar and lumbar-sacral spinal cord (Berkley et al., 1993b), that of viscero-somatic convergence is not completely understood. Furthermore, the vast amount of convergence between visceral and cutaneous information at all the levels of the spinal cord and the brain suggests that specific visceral messages are significantly blunted, if not almost completely lost, when they reach the spinal cord. This observation is even further substantiated by the magnitude of viscero-somatic convergence higher up in the periphery-brain axis. Indeed, only 6% of neurons in the lateral thalamus in squirrel monkey and 3% in cat do not possess a cutaneous receptive field, or are visceral-specific (Bruggemann et al., 1994; Horn et al., 1999). Therefore, together these data imply that Mackenzies's idea of the absence of "true visceral pain" (Mackenzie, 1909) has more basis than had previously been considered.

1.2.4 "Referred" Pain

The low density of innervation and receptor specificity, as well as the abundant convergence of sensory information in the spinal cord, might explain the diffuse and poorly localized nature of visceral pain. However, what are the mechanisms for "referred" pain, mentioned above, as the unique quality of visceral pain? It is important to note that according to several opinions, "referred pain" is a quality pertinent to the deep rather than exclusively visceral structures (Ruch, 1946), since pain from muscles and joints can also be referred (Fukui et al., 1996; Arendt-Nielsen & Svensson, 2001). However, the possible mechanisms underlying this phenomenon have similar causal pathways and will therefore be described in concert.

There are several popular theories explaining the nature of visceral pain referral to other tissues. None of the theories are mutually exclusive and none fully elucidate all the physiological and morphological aspects associated with this phenomenon. One of the most popular theories is the "convergence-projection" theory originally formulated by Theodore Cedric Ruch (1946). According to this theory, visceral and somatic primary sensory neurons converge onto common spinal neurons, thus, the signal is misconstrued as originating from other structures, such as the skin (Ruch, 1946). This model has received the most experimental support by far, since the number of viscero-somatic convergent neurons identified along the entire spinal cord is colossal (see above). However, there are several factors associated with the phenomenon of "referred pain" that cannot be explained solely on the basis of this theory. First of all, Ruch's theory does not address the delay in the development of "referred pain" observed frequently in patients with renal colic or anginal attack (Lewis, 1942). Likewise, several researchers have addressed the issue of the absence of bi-directionality in "referred pain", or as Thomas Lewis (1942) pointed out: "why pain arising from the somatic structures is not referred to the region of the viscus"; this has not been normally demonstrated, "yet there would be no apparent reason why the latter form of reference should not occur if reference were merely dependent upon extension of a commotion from one area of grey matter to the next" (Lewis, 1942). Interestingly, two anecdotal reports of such referral have been published several years ago. One case report described a patient with severe abdominal pain following latissimus dorsi muscle strain (Sandford & Barry, 1988), while another described a woman with lancinating pain in one dermatome consistently triggered by light stimulation of another

dermatome (Lee et al., 1991). Although somato-visceral and/or somato-somatic "referred pain" could create a great deal of excitement among researchers, the cases cited above are very unlikely examples; the lack of projection of the described structures to the same spinal segment (Garrison, 1989) makes them anatomically inconsistent with the convergence-projection theory. A more recent study demonstrated the referral of intradermal capsaicin injection onto the remote skin area; yet again it only occurred in one out of twelve subjects, who also developed pain referral following control substance injection (Witting et al., 2000).

Another popular theory is the axon reflex theory proposed by Sinclair and colleagues (Sinclair et al., 1948). According to the authors, "this theory depends essentially upon the existence of axon branching among the sensory pathways conveying the sensation of pain" (Sinclair et al., 1948). In other words, there exist some primary sensory neurons with branching, dichotomizing, or bifurcating axons that innervate both somatic and visceral targets, leading to the ambiguous afferent message source. Support for this mechanism came from several studies using electrophysiological (Bahr et al., 1981; Pierau et al., 1982; Devor et al., 1984; Habler et al., 1988), double-labeling (Taylor & Pierau, 1982; Schmid et al., 1983; Borges & Moskowitz, 1983; Pierau et al., 1984; Alles & Dom, 1985; Dawson et al., 1992), and electron micrography techniques (Langford & Coggeshall, 1981). Dichotomization between the intercostal and splanchnic nerves (Dawson et al., 1992), as well as bifurcating axons in the lumbar spinal nerve innervating both intervertebral discs and the groin skin (Takahashi et al., 1993), were demonstrated in the rat using fluorecent dyes to trace the intraneural connections. In addition, more than 70% of lumbosacral dorsal horn neurons in cat could be driven by receptors in skeletal muscle and other tissues, such as tendon, joint and/or bone (Hoheisel & Mense, 1990). On the other hand, the occurrence of dichotomizing unmyelinated afferents supplying pelvic viscera and perineum is rare in the sacral segments of the cat, amounting to less than 0.5% of the afferent neurons (Habler et al., 1988). Likewise, only about 3% of dorsal root ganglion neurons in the lumbar spine of rats had dichotomizing axons projecting to both the lumbar facet joint and the sciatic nerve (Sameda et al., 2001). Taking into account the low proportion of sensory neurons with bifurcating axons compared to the frequency of the "referred pain" occurrence in clinical practice, axon-reflex theory might only be a contributing

factor to the phenomenon of pain referral. In addition, this theory does not support the time delay in the evolution of "referred pain".

Yet another theory, that of convergence-facilitation, was developed by James Mackenzie (1909), who supported the idea of visceral insensitivity, or the absence of "true" visceral pain. He thus proposed that impulses from viscera, when reaching the spinal cord can create an "irritable focus" capable of sensitizing sensory neurons for the afferent somatic inputs (Mackenzie, 1909). Although this theory was not generally accepted, due to the simple fact that "true" visceral pain does exist, Mackenzie's proposition became the underlying explanation of central sensitization and/or wind-up, clinically manifest as hyperalgesia and allodynia in both visceral and somatic structures (Mayer & Gebhart, 1994).

Other theories, such as interaction at the supra-spinal level (Theobald, 1941; Theobald, 1949), or the expansion of receptive fields (Hoheisel et al., 1997), have been proposed as the underlying causes of pain referral. Although none of the theories described to date fully explain the "referred pain" phenomenon, it is clear that both central and peripheral mechanisms are involved. The contribution of peripheral sources is evident from local anaesthesia studies, where local application of anaesthetic cream results in a significant reduction in "referred pain" intensity (Laursen et al., 1997). Moreover, a recent study by Laursen et al. (1999), who performed either partial or complete nerve blocks with tourniquet or regional intravenous lidocaine, respectively, found similar effects on referred pain, thus proposing that the peripheral component of referred pain is associated with intact myelinated fibers (Laursen et al., 1999). However, peripheral components cannot be solely responsible for the referral, since distal occurrence, hyperalgesia, as well as the reduction instead of eradication of referred sensation with local anaesthetics, all point towards central involvement.

1.2.5 Ascending Pathways

The idea of a direct pathway carrying sensory information about pain from the periphery to the brain dates back to René Descartes (1664). However, it took years of challenging and intensive anatomical and neurophysiological testing before the majority of ascending sensory pathways was identified. New findings are still emerging.

The complexity of sensory pathways is striking, especially with the new discoveries of collateral connectivity between some of them (Djouhri et al., 1997). However, it is generally accepted that there are two main systems that relay sensory information from the periphery to the higher brain structures. Depending on their anatomical position in the human spinal cord, these pathways are considered to be a part of either the anterolateral system, that contains spinothalamic, spinomesencephalic, spinoreticular and spinolimbic tracts, or the dorsal column-medial lemniscus system, that contains spinocervicothalamic and post-synaptic dorsal column pathways.

The spinothalamic tract (STT) is by far the major pathway carrying nociceptive information from the skin. Its numerous anatomical connection sites, as well as its relay and termination nuclei, have been identified and described in detail elsewhere (for review see Willis, Jr., 1986; Willis & Westlund, 1997). The largest input to STT neurons comes from cutaneous nociceptors (Kenshalo, Jr. et al., 1979), yet animal studies in monkey, cat, and rat identified STT neurons responsive to visceral nociceptive inputs as well (Foreman et al., 1981; Rucker & Holloway, 1982; Rucker et al., 1984; Ammons, 1989; Al-Chaer et al., 1999). Although the majority of visceral responsive cells possess somatic receptive fields, STT has long been considered the predominant pathway relaying nociceptive information from viscera.

The spinomesencephalic tract is another sensory pathway that plays a role in nociceptive processes. Several classes of neurons have been identified, yet high threshold and wide-dynamic range neurons constitute the majority (Yezierski & Schwartz, 1986; Yezierski, 1988; Yezierski & Broton, 1991). The function of the spinomesencephalic neurons in visceral nociception is unclear, yet its projections to the periaqueductal grey, parabrachial nuclei and amygdala (Willis & Westlund, 1997) may lead to new discoveries, especially in its possible contribution to the affective dimension of pain (Yezierski et al., 1991; Basbaum & Jessell, 2000),.

The majority of spinoreticular tract neurons have also been characterized as nociceptive specific, suggesting their involvement in pain processes (Ammons, 1987; Fields et al., 1975; Fields et al., 1977). A similar laminar distribution to that of the spinothalamic tract neurons, as well as thalamic and limbic projections further support its nociceptive role (Kevetter et al.,

1982). Although studies of spinoreticular neurons predominantly employed stimulation of the somatic structures, some data show that these neurons can be excited by inputs from both visceral and somatic afferent fibers (Foreman et al., 1984; Ammons, 1987), suggesting a possible role in the processing of pain from both modalities.

Pathways with projections to limbic structures include spino-parabrachio-amygdalar, spino-ponto-amygdalar, spino-parabrachio-hypothalamic, and spinohypothalamic tracts. Spinohypothalamic neurons in the sacral spinal cord, as well as spino-ponto-amygdalar neurons in the parabrachial area, respond to convergent noxious visceral and somatic inputs (Bernard et al., 1994; Katter et al., 1996), while spino-parabrachio-amygdalar neurons receive nociceptive projections from deep and superficial cutaneous fields (Neugebauer & Li, 2002). , Their role in the affective-motivational aspect of pain has been proposed based on their projections to areas of the limbic system (Bernard et al., 1994; Katter et al., 1996; Neugebauer et al., 2002). In addition, the new discovery of a spino-parabrachio-hypothalamic nociceptive pathway, probably involved in motivational responses to noxious cutaneous and/or visceral events (Bernard et al., 1994), further supports the complexity and poor specificity of the sensory ascending system.

The dorsal column-medial lemniscus system is thought to play a major role in conveying information about tactile sensation and limb proprioception (Gardner et al., 2000), although some argue for its function as a somato-visceral system as well (Willis, Jr.,1986). One of the pathways ascending in the dorsal columns - not very prominent in man - is the spinocervicothalamic tract (Willis, Jr., 1986; Willis & Westlund, 1997). Despite numerous electrophysiological studies that have been performed on spinocervical tract cells in animals (Brown et al., 1975a; Brown et al., 1975b; Brown et al., 1976; Brown et al., 1980; Brown, 1981; Brown et al., 1986; Brown et al., 1987), their nociceptive role is still under question. Nevertheless, some strongly argue for the existence of considerable A-δ inputs, and believe the role of this pathway in nociception is underestimated (Kajander & Giesler, Jr., 1987a; Kajander & Giesler, Jr., 1987b). The role that the spinocervicothalamic neurons play in visceral sensation is even less clear, yet some studies have demonstrated that a few neurons in the

thoracic spinal cord and lateral cervical nucleus respond to both somatic and visceral inputs (Cervero, 1983; Meng & Lu, 2000).

For a long time, no compelling evidence existed for a role of the postsynaptic dorsal column pathway (PSDC) in visceral sensation (Willis, Jr., 1986). However, recent and quite intriguing observations from human and animal studies have shown otherwise, suggesting this polysynaptic tract plays a very important and almost specific function in visceral pain.

The idea of the involvement of the PSDC in visceral pain originated from anecdotal surgical findings. Several surgical procedures have been used to alleviate intractable pain of visceral origin. Bilateral anterolateral cordotomies, in which ascending tracts in the anterolateral quadrant of the human spinal cord are interrupted, have proved to be successful in relieving diffuse pelvic visceral cancer pain (White & Sweet, 1969). These, however, resulted in highly debilitating side effects, including extremity paresis, respiratory complications, bladder, bowel and sexual abnormalities, hypotension, and high mortality rates. Another procedure, the commissural myelotomy, in which a longitudinal incision extending over several spinal segments is used to interrupt decussating axons of the spinothalamic tract, resulted in less severe, but still incapacitating side effects. As early as 1970, a procedure called limited midline myelotomy was introduced (Hitchcock, 1970). In this procedure, a small midline stereotactic lesion is made at the C1 or T10 level without interrupting the axons of the spinothalamic tract. Surprisingly, this lesion resulted in significant relief of pelvic cancer pain in humans (Gildenberg & Hirshberg, 1984), suggesting a role for dorsal quadrant pathways in visceral pain.

The PSDC pathway describes a large population of neurons postsynaptic to primary afferent fibers, the axons of which ascend in the dorsal columns. The PSDC pathway arises from cells distributed medially to laterally in lamina III in the dorsal horn, as well as from a few cells just lateral to lamina X (Willis & Westlund, 1997). The PSDC pathway projects to the dorsal column nuclei, nucleus gracilis (NG) and nucleus cuneatus (NC), which in turn project to ventral posterior lateral (VPL) nucleus and the medial part of the posterior complex (PO_m) in the thalamus. The trajectories of the PSDC fibers are somatotopically organized in the dorsal columns, such that NG receives projections from lumbar and sacral segments of the spinal

cord, while information from thoracic and cervical segments of the spinal cord terminates in NC.

Several animal studies have examined the role of the dorsal column pathway in visceral pain. Extracellular recordings from neurons in the VPL nucleus of the thalamus, nucleus gracilis in the medulla, and the PSDC neurons in the lumbo-sacral spinal cord in rats and primates have shown that these cells similarly respond to graded cutaneous and graded visceral mechanical, as well as chemical visceral stimuli (Hirshberg et al., 1996; Al Chaer et al., 1996a; Al Chaer et al., 1996b; Al Chaer et al., 1997; Al Chaer et al., 1998; Al-Chaer et al., 1999), supporting the idea that they are components of the same nociceptive pathway. Interestingly, lesions of the dorsal column at the T10 level abolished the responses of VPL neurons to colorectal distention (CRD) and to innocuous mechanical stimuli applied to the skin, without changing the response of these cells to noxious cutaneous stimulation (Al Chaer et al., 1996b). Conversely, lesions to the ventrolateral column at the same level, which presumably interrupt the spinothalamic tract, markedly reduced responses to noxious cutaneous stimuli compared to innocuous cutaneous stimuli and CRD (Al Chaer et al., 1996b), thus suggesting that the dorsal columns contain a pathway that is important in transmitting nociceptive information from viscera but not skin. Furthermore, from the minor effects of morphine and the non-NMDA receptor antagonist CNQX, both of which limit synaptic transmission, on the activity of NG and PSDC neurons following cutaneous stimulation but not CRD, Al-Chaer et al. (1996) concluded that visceral input to NG is mediated by PSDC neurons, while cutaneous input is mediated, at least in part, by unmyelinated primary afferent fibers (Al Chaer et al., 1996a). This finding further suggests that, with respect to nociceptors, PSDC is visceral specific. Further support comes from a recent study that examined the effects of dorsal and ventral lateral column lesions on behaviour following either intradermal capsaicin injection or colonic inflammation in rats (Palecek et al., 2002). Palecek and colleagues found that ventral lateral, but not dorsal column, lesions at C1 eliminated reduction in exploratory activity induced by capsaicin, while the same dorsal column lesion abolished behavioural changes induced by colonic inflammation and distention without affecting those of capsaicin injection (Palecek et al., 2002).

Several human studies demonstrated the analgesic role of surgical interruption of the post-synaptic dorsal column pathway in patients with intractable pain. Punctuate midline

myelotomies performed at T3-T10 levels, that specifically disrupted the PSDC fibers, have been shown to greatly alleviate pain due to cervical, stomach, colon, rectal, lung, and/or ovarian cancers (Hirshberg et al., 1996; Nauta et al., 1997; Becker et al., 1999; Nauta et al., 2000; Kim & Kwon, 2000). This surgical procedure dramatically decreased the intensity of pain and the use of pain medication, led to a significant improvement in daily activities, and did not cause extra neurological deficits (Hirshberg et al., 1996; Becker et al., 1999; Kim et al., 2000). The findings provide further evidence for the existence of a visceral pain pathway that lies in the midline of the posterior column and is separate from other conventional pain pathways, such as the spinothalamic tract.

The animal and human experiments summarized above provide compelling evidence for the importance of the post-synaptic dorsal column pathway in visceral pain, especially that of pelvic origin. Although some data in animals indicate that dorsal column lesions at the cervical level suppress epigastric nociception induced by duodenal distention (Feng et al., 1998), as well as reverse pancreatitis-induced decrease in rearing behaviour (Houghton et al., 1997), further research is needed to elucidate the role of this pathway in signaling visceral pain of non-pelvic origin.

1.2.6 Neurochemistry

1.2.6.1 NMDA-Receptors

Excitatory amino acids, such as glutamate and aspartate, seem to be the principal compounds mediating fast neurotransmission in both visceral and cutaneous sensory neurons (Kandel & Siegelbaum, 2000). Therefore, it has been generally accepted that visceral and cutaneous afferents do not fundamentally differ in the chemistry of fast neurotransmission (McMahon, 1997). However, recent findings on the involvement of NMDA receptors (NMDA-Rs) in the development of wind-up and/or central sensitization, which are thought to be the underlying mechanisms of allodynia and hyperalgesia during persistent pain states, have led to a more thorough analysis and have shed some light on the potential differential role of NMDA receptors in visceral and cutaneous pains. According to several reports, this differential role is particularly pertinent to acute, or short-lasting pains, given that NMDA-Rs seem to be similarly involved in mediating inflammatory pain states arising from visceral and somatic structures.

Several animal reports have suggested that acute visceral pain is mediated by NMDA-Rs. Intravenous ketamine, a non-competitive NMDA-R inhibitor, dose-dependently inhibited noxious mechanical stimulation of the rat ureter (Olivar & Laird, 1999); likewise intravenous memantine, a less potent NMDA-R antagonist, inhibited responses associated with noxious colorectal distention (CRD) (McRoberts et al., 2001). In addition, both intravenous and intrathecal application of a more potent non-competitive NMDA-R channel blocker dizocilpine maleate (MK-801) attenuated responses of CRD-sensitive neurons (Ji & Traub, 2001). The same study demonstrated that a spinally, but not systemically, administered competitive NMDA-R antagonist APV also has an effect in attenuating CRD-sensitive neuronal responses to noxious and innocuous stimulation (Ji et al., 2001), suggesting a spinal site of action of NMDA-Rs in mediating sensory transmission from visceral tissues. Nevertheless, one study that recorded from the CRD-responsive neurons in lumbosacral spinal cord failed to show the analgesic effects of MK-801 during noxious stimulation (Kozlowski et al., 2000). However, the drug concentrations used in this study were much lower than those shown to produce analgesia to acute visceral stimulation with the same NMDA-R antagonist (Ji et al., 2001), which could explain the lack of effect.

Contrary to the majority of observations from visceral organs, some studies failed to find a role for NMDA in acute somatic pain. In one study, ketamine applied intrathecally had no effect on tail flick latency (Lutfy et al., 1997), while in another, intrathecal AP-5 administration failed to produce analgesia in a hot-plate test in rats (Nishiyama et al., 1998). Furthermore, other data indicate that the lack of analgesia in these studies was not related to the route of administration. Olivar and Laird (1999) found that intravenous ketamine injection did not affect pressor responses evoked by noxious pinch (Olivar et al., 1999).

Overall, the majority of findings from animal studies support a differential involvement of NMDA-Rs in acute visceral and cutaneous pains, yet the role of NMDA-Rs in acute human pain still remains controversial, and will be directly addressed and discussed in more detail in Chapter 4.

1.2.6.2 Neuropeptides

Visceral afferents have a larger proportion of peptide-containing cell bodies, marked by neuropeptides, such as substance P (SP) and calcitonin-gene-related-peptide (CGRP), compared to those of cutaneous afferents (McMahon, 1997; Cervero & Laird, 1999). Data from the ureteric primary afferent fibers in guinea pigs and the splanchnic nerve in rats show that up to 80% of visceral primary afferent neurons are marked by SP, compared to only 20-25% of the somatic primary afferents (Semenenko & Cervero, 1992; Perry & Lawson, 1998). This is despite a wider distribution of the somatic afferents in the spinal cord – the peptide-containing somatic afferents terminate in lamina I, lamina II and lamina V, while those from viscera terminate in lamina I and lamina V of the dorsal horn spinal cord (Snider & McMahon, 1998).

The possibility that neuropeptides, in particular SP, might be more important in the transmission of information from the viscera has been examined in transgenic mice lacking the substance P receptor (De Felipe et al., 1998; Laird et al., 2000; Laird et al., 2001). This group and others have previously shown that substance P does not play a role in the acute somatic pain sensation or the development of somatic hyperalgesia after induction of hind paw inflammation with complete Freund's adjuvant, but is involved in the hyperalgesic processes following intraplantar capsaicin injection (De Felipe et al., 1998; Cao et al., 1998; Mansikka et al., 1999). Similarly, substance P receptor knock-out mice showed comparable behaviour to wild-type animals following acute visceral nociception induced by mechanical stimulation, yet they demonstrated reduced sensitivity to the same mechanical stimuli following intracolonic instillation of chemical irritants; primary and referred hyperalgesia to mechanical stimuli were absent as well (Laird et al., 2000). However, from the knock-out studies it is still not evident whether there are substantial differences in the relative contribution of substance P to visceral and somatic pains; substance P may only become important whenever neurogenic inflammation is present irrespective of the stimulus origin. This potential lack of specificity might explain the involvement of substance P in both visceral and somatic (capsaicin only) hyperalgesia. Nevertheless, one potential role substance P may play specifically in visceral pain is suggested by studies showing the involvement of NK-1 receptors in processes mediating stress in animals and anxiety in humans (Hill, 2000). Since visceral pain is more susceptible to

stress and is associated with significantly higher anxiety than pain of somatic origin (Mayer, 2000; Strigo et al., 2002), it is very likely that substance P receptor antagonists might play a significant role in clinical visceral pain, despite the lack of consistent effects in somatic pain states (Dionne, 1999).

1.3 BRAIN IMAGING OF PAIN

As was mentioned in the very beginning, studying neuronal processes underlying human pain perception became significantly easier with the development of new brain imaging techniques. In particular, the discovery of functional magnetic resonance imaging (fMRI) has allowed a thorough examination of areas involved in sensory, motor, cognitive and other brain functions. All functional neuroimaging is based on the assumption that increases in neuronal activity are coupled to the increases in blood flow. The main principles underlying fMRI methods will be briefly described.

1.3.1 Functional Magnetic Resonance Imaging (fMRI)

Several different methods are used to obtain signal intensity changes associated with physiological brain function in MRI; all these are termed "functional MRI", or methods that attempt to localize brain function during brain stimulation. Some of these methods use exogenous intravascular contrast agents with high magnetic moments (e.g. supermagnetic iron oxide) or exogenous freely diffusible traces (e.g. trifluoromethane). However, by far the most widely used fMRI technique is blood-oxygenation-level-dependent (BOLD) MRI. The BOLD contrast effects are based on the same contrast mechanisms, yet instead of exogenous paramagnetic agents, BOLD uses an endogenous one. Specifically, the BOLD principle is based on the fact that deoxygenated hemoglobin (HbR) is paramagnetic, while oxygenated hemoglobin (HbO_2) is diamagnetic; thus HbR can act in a fashion analogous to that of the exogenous contrast agents (Ogawa et al., 1990; Ogawa et al., 1993).

Changes in the concentration of the contrast agents per voxel, or the 3D picture element, determine the stimulation-induced signal intensity changes. Exogenous contrast agents generally induce signal loss in the fMRI images, thus increases in their concentration in local

plasma volume during functional activation are accompanied by decreases in signal intensity. HbR concentration, however, drops following functional brain activation (Villringer & Dirnagl, 1995), and is thus accompanied by an increase in fMRI signal intensity.

The relationship between the BOLD signal and the physiological event underlying this signal, or the relationship between neuronal activation and the vascular response (neuronal-vascular coupling), has been the subject of long debate. In fact, neuronal-vascular coupling, or changes in cerebral blood flow with local activity was suggested more than a century ago by Roy and Sherington (1890). By definition, BOLD reflects hemodynamic changes and can be explained on the basis of changes in blood cell flux and velocity (Villringer & Dirnagl, 1995). Specifically, one of the consequences of neuronal-vascular coupling would be to increase oxygen supply to the activated brain areas, which, in turn, would lead to an increase in deoxygenated hemoglobin, yet according to the BOLD principle HbR concentration decreases. This has been explained on the basis of no or little actual increase in oxygen metabolism during activation compared to the increases in local blood flow, which is, in turn, due to arteriolar dilatation following activation. Significantly higher increases in local blood flow than in local oxygen consumption would lead to a large delivery of HbO_2 ("arteriolarization" of blood) to the capillaries and veins surrounding activated areas, resulting in the dilution of the HbR, and decreasing its local concentration. Several possible factors have been proposed to mediate arteriolar dilation: decreases in glucose concentration (Duckrow et al., 1985), increases in adenosine (Rubio et al., 1975), nitric oxide (Goadsby et al., 1992) and/or lactate concentration and subsequent fall in pH (Niwa et al., 1993), potassium-induced vasorelaxation (Kuschinsky et al., 1972), or even neurogenic mechanisms (Lou et al., 1987) have all been implicated.

In actuality, hemodynamic changes reflected in the BOLD fMRI signal are not linear. Three phases in the hemodynamic response to transient increases in neuronal activity have been described. First, a small decrease in image intensity, or "dip" in oxygenation, within the first seconds of activation occur, followed by a large increase above baseline due to the oversupply of oxygenated blood, and then a final decrease below baseline due to the diminished oxygenated blood supply (Fox et al., 1988; Ernst & Hennig, 1994; Buxton et al., 1998; Vanzetta & Grinvald, 1999; Friston et al., 2000). A simplistic overview of the hemodynamic response is demonstrated in Figure 1-1.

A more controversial issue in the BOLD fMRI, as well as other neuroimaging methods, has been the relationship between hemodynamic changes and neuronal activity. Several recent studies have provided compelling evidence that blood flow changes are in fact well correlated with neuronal activity. Specifically, when BOLD fMRI timecourse following visual stimulation was correlated with single- and multi-unit activity, as well as local field potential (LFP) recordings from the primate visual cortex, the relationship was "roughly" linear (Logothetis et al., 2001). Provided that microelectrode recordings measure the interaction of various synaptic and cellular mechanisms, this suggests that neuronal activity is indeed reflected in the hemodynamic changes. Therefore, the authors concluded that increases in BOLD contrast directly and "unequivocally" reflect an increase in neuronal activity (Logothetis et al., 2001). Moreover, the stronger correlation with LFP suggested that BOLD specifically represents synaptic activity in a population of cells, rather than individual neuronal firing rates (action potentials) (Logothetis et al., 2001). Further evidence for hemodynamic and neuronal correlation has come from evoked potential studies in humans (Arthurs et al., 2000) and in animals (Ogawa et al., 2000; Tsubokawa et al., 1980; Mathiesen et al., 1998; Ngai et al., 1999; Brinker et al., 1999).

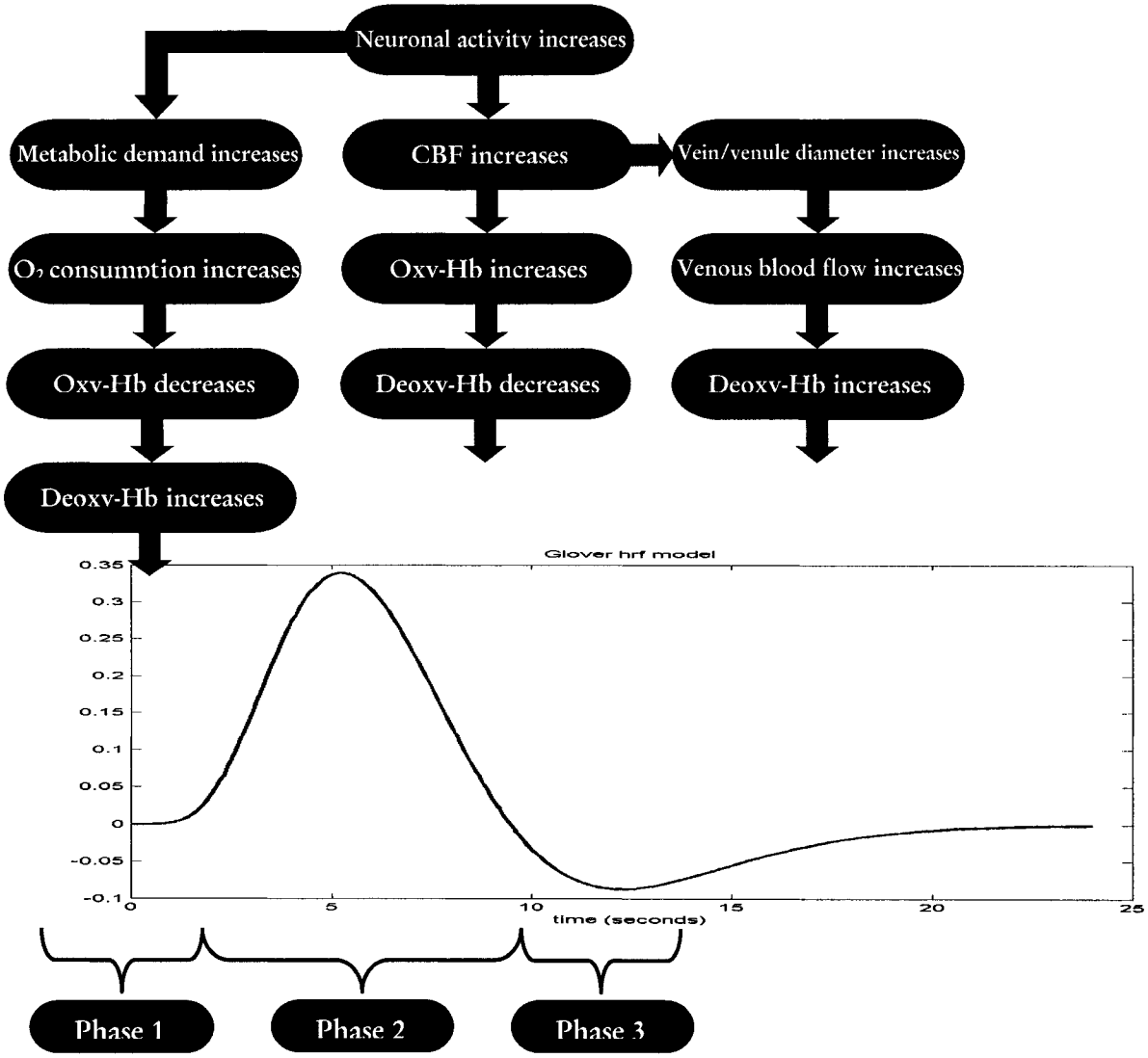
1.3.2 Advantages and Limitations of fMRI

One of the remarkable advantages of BOLD-fMRI is its non-invasiveness due to the absence of contrast agent administration. Since the temporal resolution of this method is not dependent on the half-lives of the injected tracers, but rather on the time course of HbR concentration changes (approximately 2 sec), the number of measurements is not limited by the maximally tolerated dose of the contrast agents. Thus BOLD fMRI allows the study of individual subjects/patients, thereby providing information on across-subject variability. In addition, high spatial resolution (down to 1mm) is achievable with new powerful magnets.

Even though BOLD fMRI has revolutionized the field of sensory and cognitive neuroscience, it has its limitations. It is susceptible to various thermal and physiological artifacts, such as noise due to thermodynamic processes, field inhomogeneities, pulsatile blood flow, respiration, head motion, as well as the type of scanner or imaging sequence used, and the magnetic field strength. Consequently, there are still several questions that remain unanswered about BOLD

fMRI. One of them is the sensitivity of this method to changes in small capillaries versus large venous vessels. The latter has the potential to create a false-positive activation, especially with low intensity magnets, which can be distant from the actual neuronal activity site. Another disadvantage of the method, raised by several recent editorials (Arthurs & Boniface, 2002; Heeger & Ress, 2002), is based on the sensitivity of BOLD to synaptic activity in a population of cells, rather than individual spiking mentioned above. This raises the issue of whether small changes in a large population of cells can be distinguished from large changes in a small population of cells, or whether actual activity can be isolated from the background activity and/or modulatory inputs from other sources. Finally, it is still debatable whether BOLD is sensitive specifically to excitatory synaptic activity, or can detect inhibitory potentials as well (Arthurs & Boniface, 2002).

Figure 1-1: Simplistic overview of hemodynamic response function



Three phases of a hypothetical hemodynamic response to brief neuronal stimulation. Phase 1 – "dip" – the increase in neuronal activity results in an initial increase in oxygen consumption, leading to changes in the concentration of oxy- and deoxy-hemoglobin in the nearby vasculature. Phase 2 – "increase" – the increase in neuronal activity then triggers a large increase in local blood flow leading to the local oversupply of the oxygenated blood. Phase 3 – "undershoot" – an increase in blood flow causes vasodilation of venules and veins, leading to an increase in venous blood volume and deoxygenated blood. HRF timecourse adapted from Glover et al., 1999 (Glover, 1999).

Chapter 2

PSYCHOPHYSICAL ANALYSIS OF VISCERAL AND CUTANEOUS PAIN IN HUMAN SUBJECTS

As was mentioned in the previous chapter, clinical observations indicate that sensibilities evoked by visceral and cutaneous pain diverge to a great extent in several domains, including qualitative, spatial, and autonomic. However, studies directly comparing visceral and cutaneous pain are lacking, and are necessary for identifying specific differences. This chapter describes the first experiment, performed with sensitive psychophysical methods in healthy human subjects, in a series of studies on the direct comparison of visceral and cutaneous pain.

2.1 ABSTRACT

Clinical evidence suggests that cutaneous and visceral pain differ in sensory, affective and motivational realms, yet there has been little comparative characterization of these types of pain. This study uses psychophysical measures to compare directly visceral and cutaneous pain and sensitivity.

Healthy subjects (11 M, 7F, age 19-29) evaluated perceptions evoked by balloon distention of the distal esophagus and contact heat on the upper chest. Subjects gave continuous ratings of pain intensity using an on-line visual analog scale (VAS), reported maximum pain intensity and unpleasantness on printed VASs, chose phrases from the McGill Pain Questionnaire and Spielberger State-Trait Anxiety Inventory, and drew the area of perceived sensation.

For esophageal distention, the threshold for pain intensity was higher than that observed for unpleasantness, whereas for contact heat, pain and unpleasantness thresholds did not differ for either phasic (10 sec) or tonic (36 sec) stimulus application. The relative unpleasantness, calculated as the difference between the unpleasantness and the intensity ratings, was higher during esophageal distention than during either phasic or tonic cutaneous heat; this difference in relative unpleasantness was seen at all intensities of esophageal stimulation. Subjects chose significantly more affective words and reported more anxiety during visceral pain than during phasic cutaneous heat pain. A similar tendency was observed when visceral pain was compared to tonic cutaneous heat pain. Subjects also chose a wider range of words to describe visceral than cutaneous pain. On-line VAS ratings revealed greater pain sensation after stimulus termination during visceral than during phasic cutaneous pain; likewise, a similar tendency was observed between visceral and tonic cutaneous pain. Finally, visceral pain led to a more spatially diffuse sensation and was referred to the entire chest and sometimes to the back.

Our results show that visceral pain is more unpleasant, diffuse and variable than cutaneous pain of similar intensity, independent of the duration of the presented stimuli. The data suggest the likelihood of both similarities and differences in the neural substrates underlying visceral and cutaneous pain.

2.2 INTRODUCTION

Common experience and clinical observations suggest fundamental differences in the sensibilities of cutaneous and visceral tissues. Sensations arising from somatic structures are normally local and precise, and a diversity of intense stimuli readily produces pain if applied to normal skin (Lewis, 1942). In contrast, sensations arising from visceral stimulation are generally more difficult to localize, and fewer types of experimental stimuli can effectively produce visceral pain (Livingston, 1935; Lewis, 1942; Livingston, 1998). These disparities in sensory experience could arise from differences in the peripheral innervation of visceral and cutaneous tissues or in the central processing of this sensory input.

Although cutaneous pain has been well characterized in controlled experimental conditions, our understanding of visceral pain is mainly based on clinical observations. A few studies of visceral pain have examined the effects of experimental stimuli, such as electric shock (Frobert et al., 1995), chemical exposure (Fass et al., 1998), and mechanical distension (Barish et al., 1986; Ness et al., 1990; Ness et al., 1998); however, no studies have directly compared the human experience of pain evoked from visceral and cutaneous structures. Likewise a number of studies have examined possible cortical mechanisms underlying visceral pain, but none of these has directly compared the effects of visceral and cutaneous simulation, and none has thoroughly examined possible cerebral correlates of sensory and affective dimensions of visceral pain.

Considering the lack of comparison studies of visceral and cutaneous sensations, we performed a psychophysical experiment to evaluate the perceived intensity and unpleasantness, as well as other qualitative attributes of visceral and cutaneous perception, in a group of normal subjects.

2.3 METHODS

Two psychophysical studies were performed in separate subject samples. The first study compared esophageal distention to phasic cutaneous heat pain, whereas the second study compared esophageal distention to tonic cutaneous heat pain.

2.3.1 Study 1

2.3.1.1 Subjects

With the approval from the McGill Institutional Review Board and the Ethical Review Board of St. Luc Hospital, we studied 15 healthy volunteers (9 males and 6 females), ranging in age from 19 to 29 years (mean age 23.7 yr). During the preliminary session all volunteers received a modified version of the gastrointestinal reflux disease questionnaire (Locke et al., 1994) to rule out any gastrointestinal and esophageal symptoms, or episodes of chest pains. None of the subjects was obese (mean BMI 22.7) or taking any medication. Subjects were excluded from the study if they were under 18 or over 35 years of age, pregnant or breast-feeding, had cardiovascular, neurological disease, or any chronic pain condition. After preliminary testing, the presence of strong gag reflex and inability to use rating scales were added to the exclusion criteria in this study. Five volunteers did not complete all aspects of the experiment and were completely (3 males, 1 female) or partially (1 female) excluded from the analysis.

2.3.1.2 Intraesophageal Balloon-Catheter:

A custom-designed polyethylene balloon (square type) 8 cm in length, 6 cm in diameter, with a maximum volume of 70-80 ml was attached to a multilumen polyvinyl esophageal catheter 10 cm above the tip (Mui Scientific, Mississauga, ON, Canada). The catheter was attached to a pump via an 800-cm long tube (the long tube allowed the placement of the pump in an adjacent room for use in the MRI environment). One of the three lumens in the catheter was connected to a pressure transducer; the second lumen was attached to the piston on a pump (G&J Electronics, Toronto, ON, Canada) which was used to inflate the balloon with air at a rate of ~ 50 ml/sec; and the third lumen served as the motility port to measure the position of the lower esophageal sphincter.

2.3.1.3 Study Protocol:

2.3.1.3.1 Preliminary determination of thermal stimulus intensities

The general study protocol is summarized in Table 2-1. The experiments were conducted in early morning after an overnight fast. Subjects were dressed in a hospital gown and lay comfortably on a hospital bed. After the experimental procedures were explained and informed consent obtained, thermal sensitivity and pain thresholds were determined on the chest using a 9-cm2 Peltier-type contact thermode (Medoc, Ramat Yishai, Israel). Ten-sec heat stimuli were presented using an ascending series of predetermined constant stimuli (range from 40 to 50 °C). Heat pulses were separated by at least 60 sec during which time the baseline was 30 °C. The rate of temperature increase was always 5°C/sec. After each stimulus, subjects were asked to rate on separate visual analog scales (VASs) the maximum sensation of warmth (if the stimulus produced no pain), the maximum pain sensation intensity (if the stimulus was painful), and the maximum unpleasantness evoked by the stimulus. All scales ranged from 0 ('no sensation', 'no pain sensation' or 'not at all unpleasant'), to 100 ('extremely warm sensation' - described as pain threshold, 'extremely intense pain sensation', or 'extremely unpleasant').

Table 2-1: Experimental Design

CHRONOLOGICAL ORDER	EXPERIMENTAL STEPS
1.	Explanation of experimental procedures and informed consent
2.	Determination of cutaneous stimulus intensities (Post-stimulus Visual Analog Scales)
3.	McGill Pain Questionnaire, Spielberger State-Trait Anxiety Inventory, pain referral drawing
4.	Determination of esophageal stimulus intensities (Post-stimulus Visual Analog Scales)
5.	McGill Pain Questionnaire, Spielberger State-Trait Anxiety Inventory, pain referral drawing
6.	Psychophysical comparison of esophageal and cutaneous sensations (On-line and post-stimulus Visual Analog Scales)

2.3.1.3.2 Preliminary determination of esophageal distention intensities

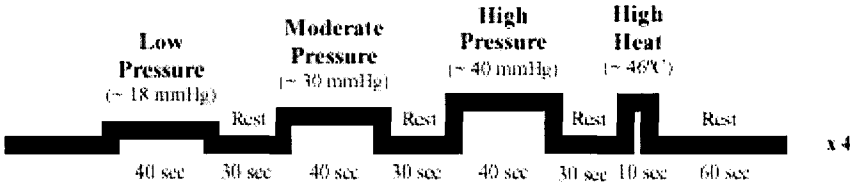
Next, a short-acting local anesthetic spray ('Xylocaine') was applied to the throat in the area of the epiglottis, and the esophageal balloon catheter was passed orally into the esophagus to a position 5 cm above the lower esophageal sphincter. The position of the balloon was verified using a four-channel motility measurement system (MMS-100, Narco-Bio Systems, Austin, TX, USA). Testing started after the effects of the anesthetic had worn off. However, even during the anesthetic period the esophageal sensations were not affected. Before determining esophageal sensitivity and pain threshold, we adjusted the baseline pressure for each subject so that the balloon was inflated but not perceived. Then a series of 40-sec predetermined stimuli were administered to assess the sensory detection and pain thresholds for each subject. At the end of each distention, subjects were prompted to rate the maximum sensation attained during the stimulation period. Some subjects rated the stimulus using a 5-button response pad to avoid throat irritation, whereas others rated the stimuli using the VAS scales described above (where 'pressure' was substituted for 'warmth').

2.3.1.3.3 Comparison of esophageal and cutaneous sensations

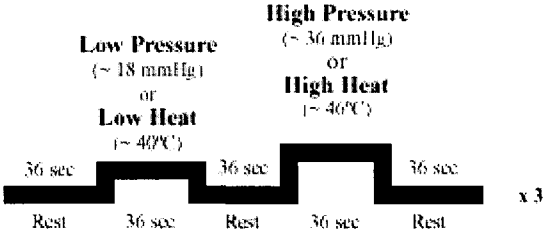
The final sequence, shown in Figure 2-1a, was created individually for each subject and consisted of three distension pressures followed by one heat stimulus applied to the subject's chest. Three distension pressures were chosen based on the preliminary evaluation and corresponded to three pressures that produced 'first sensation', 'mild pain sensation' and 'moderate pain sensation'. The thermal stimulus intensity was chosen based on the cutaneous sensitivity evaluation and corresponded to the temperature that produced 'moderate pain sensation'. Each distension was 40 sec long followed by a 30-sec rest period, whereas each cutaneous stimulus was 10 sec long followed by a 60-sec rest period. These stimulus durations were chosen to allow direct comparison with previous psychophysical studies.

Figure 2-1: Experimental paradigm

A.



B.



Stimuli were presented in quasi-random and counterbalanced order during both Study 1 (a) and Study 2 (b)

2.3.1.4 Response Measures:

2.3.1.4.1 Qualitative Evaluation

Following the preliminary determination of thermal and esophageal stimulus intensities (refer to Table 2-1), subjects were asked to complete 1) the short form of McGill Pain Questionnaire (MPQ) to describe qualitatively the painful sensations produced by esophageal distension and thermal stimulation of the skin, and 2) the 'State' part of Spielberger's State-Trait Anxiety Inventory (STAI) to record changes in anxiety that might be evoked by noxious stimuli. They were also asked to indicate on a drawing of the human body where they felt pain during cutaneous and visceral stimulation.

The short form of MPQ consists of 20 groups of subjective descriptors that are divided into four categories – sensory (groups 1-10), affective (groups 11-15), evaluative (group 16) and miscellaneous (groups 17-20) (Melzack, 1987). The state portion of STAI consists of 20 items describing the present state of the subject where each item is given a weighted score of '1' to '4' with '4' corresponding to high level of anxiety. Anxiety is measured by adding the weighted scores for each subject to obtain the total anxiety score, which can vary from a minimum of 20 to a maximum of 80 (Spielberger, 1983).

2.3.1.4.2 On-line and Post-Stimulus VAS

After each stimulus in the comparison sequence (refer to Table 2-1), subjects were asked to rate the sensations they experienced on the 'warmth/pressure', 'pain intensity' and 'unpleasantness' VASs described above. In addition, during the final comparison sequence (refer to Table 2-1), subjects made continuous on-line VAS ratings to report pain sensation experienced throughout the experimental trials. The subject moved a sliding bar along a pain scale (from 'no pain sensation' to 'extremely intense pain sensation ') according to the sensation produced by the stimuli. The output was sampled at approximately 10 Hz and recorded by a computer.

2.3.1.5 Statistical Analysis

A repeated measures analysis of variance (ANOVA) was used to assess the significant effects due to the intensity level or rating of the stimulus. Post-hoc contrast analyses and Student's t-

tests were used to examine specific differences. Non-parametric statistical analysis with Wilcoxon signed-rank test was applied to McGill Pain and Spielberger's anxiety questionnaires. Visceral and cutaneous pain intensity and unpleasantness thresholds were determined using graphic interpolation. Results are expressed as mean $\pm$ SEM. Results were considered to be significant if the probability scores (p) were less than 0.05.

2.3.2 Study 2

In Study 1, the duration of the esophageal and cutaneous stimuli differed, and the order of stimulus presentation was fixed. In order to determine if these variables could account for the observed perceptual differences, we conducted a second experiment in seven more normal subjects (5 males, 2 females, age 19-28) using stimuli of equal duration, presented at different times. Otherwise, the experimental protocol was similar to that of Study 1. Thresholds for pressure, warmth, pain intensity and unpleasantness were determined separately for cutaneous and visceral stimuli, each followed by McGill Pain, Spielberger State-Anxiety and Area of Pain Sensation Questionnaires (Table 2-1). Two sequences of cutaneous thermal stimulation and two sequences of visceral distensions were then applied in quasi-random order. Each sequence, shown in Figure 2-1b, contained 3 low- and 3 high-intensity stimuli (corresponding to 'first sensation' and 'moderate pain sensation', respectively); all stimuli were of equal duration (36 sec). Response measures employed in this experiment were identical to those described in Study 1. Two subjects did not complete all aspects of the experiment and were completely (1 male) or partially (1 male) excluded from the analysis.

2.4 RESULTS

2.4.1 Pain Intensity and Unpleasantness Thresholds

Pain intensity and unpleasantness thresholds from Study 1 and Study 2, determined by graphic interpolation for each subject, are shown in Table 2-2. In both experiments, the mean visceral unpleasantness thresholds were significantly lower than the mean visceral pain intensity thresholds ($p < 0.05$, paired student t-test), indicating that distension pressures rated as non-painful on the pain intensity scale were considered unpleasant. Thresholds for heat pain intensity and unpleasantness did not differ for either phasic (Study 1) or tonic (Study 2) cutaneous thermal stimuli (p's = 0.2 and 0.3, paired Student t-test), indicating that temperatures became painful and unpleasant at similar intensities. We also observed no difference in either pain intensity or unpleasantness thresholds between the two studies (p's = 0.9 and 0.8 – visceral, and 0.5 and 0.4 – cutaneous, two-sample Student t-test). In addition, gender had no significant effect on the pain and unpleasantness thresholds during visceral (p's = 0.2) or cutaneous testing (p's = 0.3, two-sample Student t-test) when the corresponding data from Study 1 and Study 2 were pooled to increase the statistical power.

2.4.2 Post-Stimulus VAS Ratings

The mean post-stimulus intensity and unpleasantness ratings for visceral and cutaneous pain from Study 1 are shown in Figure 2-2a. Both intensity and unpleasantness ratings increased with more intense visceral stimuli. Statistical analysis showed a highly significant effect of stimulus intensity on subjects' ratings of the intensity and unpleasantness evoked by visceral stimuli ($p < 0.0001$, $n = 10$, repeated measures ANOVA). At all three intensities of pressure distension, the unpleasantness ratings were higher than the corresponding intensity ratings ($p < 0.05$, $n = 10$, repeated measures ANOVA). In contrast, unpleasantness ratings for the cutaneous heat stimuli were somewhat lower than the corresponding intensity ratings, but this difference did not reach statistical significance ($p = 0.2$, $n = 10$, paired t-test). Figure 2-2c shows mean pain intensity and unpleasantness ratings for visceral and cutaneous stimuli from Study 2. As in Study 1, stimulus intensity had a significant effect on subjects' intensity and unpleasantness ratings for both visceral ($p < 0.05$, $n = 5$, repeated measures ANOVA) and

cutaneous stimuli ($p < 0.05$, $n = 5$, repeated measures ANOVA). There was a strong tendency for the unpleasantness ratings to be higher than the pain intensity ratings for visceral stimuli ($p = 0.06$, $n = 5$, repeated measures ANOVA). Evaluating each level separately, there was a significant effect during low-pressure stimulation ($p < 0.05$, $n = 5$, paired student t-test), but not during high-pressure stimulation ($p = 0.1$, $n = 5$, paired student t-test). In contrast, neither level of cutaneous thermal stimulation was rated as more unpleasant than painful. During low cutaneous heat stimulation the intensity and unpleasantness ratings did not differ, and during high cutaneous heat stimulation the mean unpleasantness rating was lower than the intensity rating, but this difference did not reach statistical significance ($p = 0.1$, $n = 5$, repeated analysis of variance ANOVA).

2.4.3 Relative Unpleasantness

In order to compare two unrelated stimulus modalities (temperature and pressure), the subjects' ratings of unpleasantness were normalized relative to the perceived intensity of stimulation. Thus, relative unpleasantness was calculated by subtracting subjects' estimates of pain intensity from the corresponding ratings of unpleasantness for each visceral and cutaneous stimulus. The relative unpleasantness for all given stimuli, represented as the 'difference', is shown in Figure 2-2b for Study 1 and Figure 2-2d for Study 2. As can be seen in Figure 2-2b the relative unpleasantness for all three levels of visceral stimuli was positive, indicating that during esophageal balloon distension the subjects' estimates of unpleasantness were higher than their corresponding intensity ratings. There was no significant effect of stimulus intensity on the relative unpleasantness during visceral stimulation ($p = 0.6$, $n = 10$, repeated measures ANOVA), suggesting a proportional increase in both the intensity and the unpleasantness ratings with stimulus intensity. Conversely, during cutaneous heat the relative unpleasantness was negative, indicating that the subjects' estimates of unpleasantness were lower than the corresponding intensity ratings. Repeated analysis of variance (ANOVA), followed by post-hoc contrasts analyses, showed a significant difference between the relative unpleasantness associated with the visceral stimuli compared to that observed for the cutaneous stimulation (p's < 0.01, $n = 10$) (Figure 2-2b).

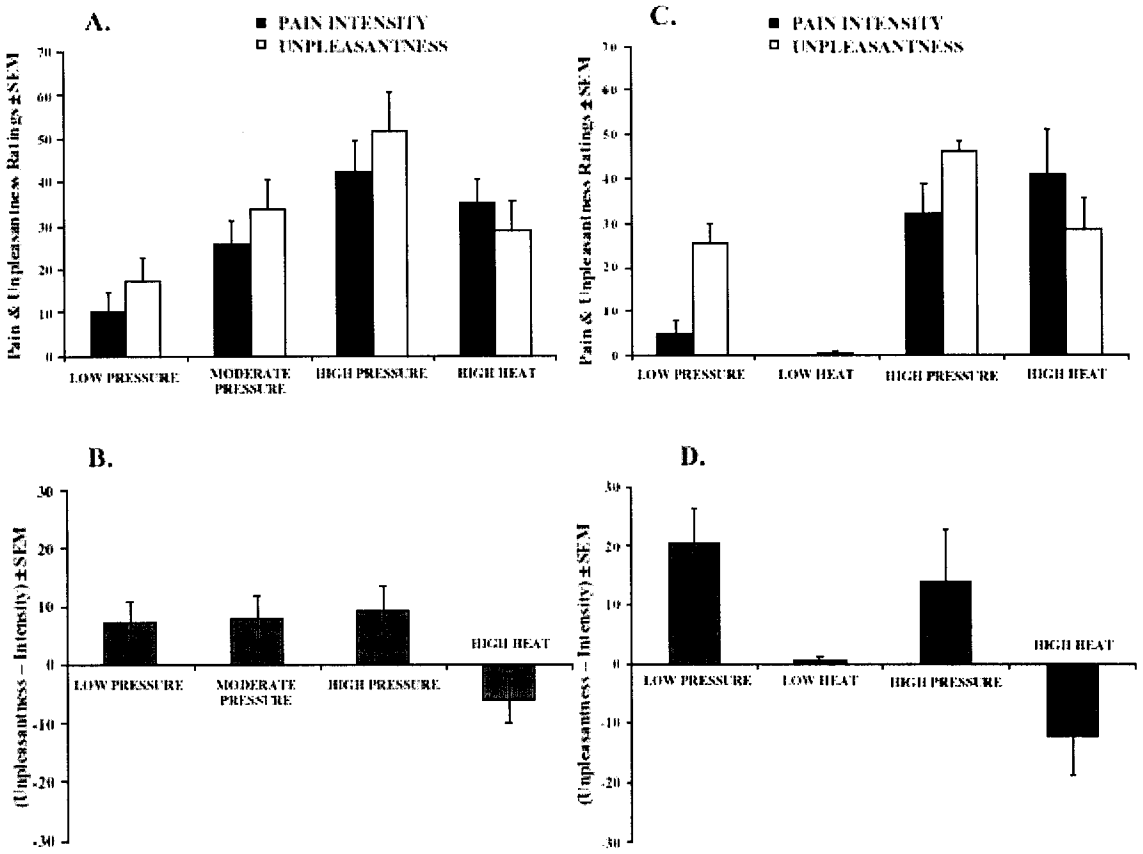
Similar findings were observed in Study 2 (Figure 2-2d). Relative unpleasantness was positive for both intensities of visceral stimuli and negative for the high cutaneous heat stimulus. Similar to previous findings, there was a significant effect of stimulus modality on relative unpleasantness for all levels of stimulation, with the relative unpleasantness for visceral stimuli being significantly higher than for the cutaneous stimuli ($p < 0.05$, $n = 5$, repeated measures ANOVA followed by contrast analyses). This once again suggests higher relative unpleasantness of visceral compared to cutaneous sensitivity, independent of stimulus duration. Similar to Study 1, we observed no effect of stimulus intensity on relative unpleasantness ($p = 0.3$, $n = 5$, repeated measures ANOVA). Low cutaneous stimuli were generally neither painfully intense nor unpleasant, and although it seemed that the low level of cutaneous heat evoked less relative unpleasantness, compared to that calculated for the high level of cutaneous heat stimulation, this difference did not reach statistical significance ($p = 0.1$, $n = 5$, repeated measures ANOVA). In order to examine gender effects in the relative unpleasantness, the corresponding data from Study 1 and Study 2 were again grouped to increase the sample size. Careful examination showed no difference in the relative unpleasantness during high cutaneous ($p = 0.6$) or low visceral ($p = 0.5$) stimulation. Whereas relative unpleasantness associated with high visceral pressure seemed higher in female subjects, it did not reach statistical significance ($p = 0.1$; two-sample Student t-test).

Table 2-2: Pain intensity and unpleasantness thresholds to visceral mechanical and cutaneous thermal stimuli

	VISCERAL		CUTANEOUS	
STUDY-SUBJECT	INTENSITY (mmHg)	UNPLEASANTNESS (mmHg)	INTENSITY (°C)	UNPLEASANTNESS (°C)
1-1	7	13	41	47
1-2	35	24	-	-
1-3	37	30	-	-
1-4	26	7.5	45.1	45.5
1-5	31.5	32	-	-
1-6	40	5	-	-
1-7	24.5	12	45.1	45.1
1-8	34.5	28.5	47.2	47.1
1-9	7	2	45.1	47.1
1-10	12	8.5	43.5	43.5
1-11	26.5	24.5	40.5	40.5
1-12	-	-	45.2	45.1
STUDY1: MEAN ± SEM	25.54 ± 3.6 *	17.0 ± 3.3	44.1 ± 0.8	45.1 ± 0.8
2-1	23	16	44.75	45.1
2-2	37.5	30	46.75	46.2
2-3	22	12	45.9	45.2
2-4	28	20.25	44.7	43.2
2-5	19.6	12.25	44.2	45.1
2-6	22.75	6.15	42.8	40.3
STUDY 2: MEAN ± SEM	25.47 ± 2.7 *	16.2 ± 3.4	44.85 ± 0.6	44.18 ± 0.9

* $p < 0.05$ (paired sample t-test)

Figure 2-2: Post-stimulus pain intensity and unpleasantness ratings

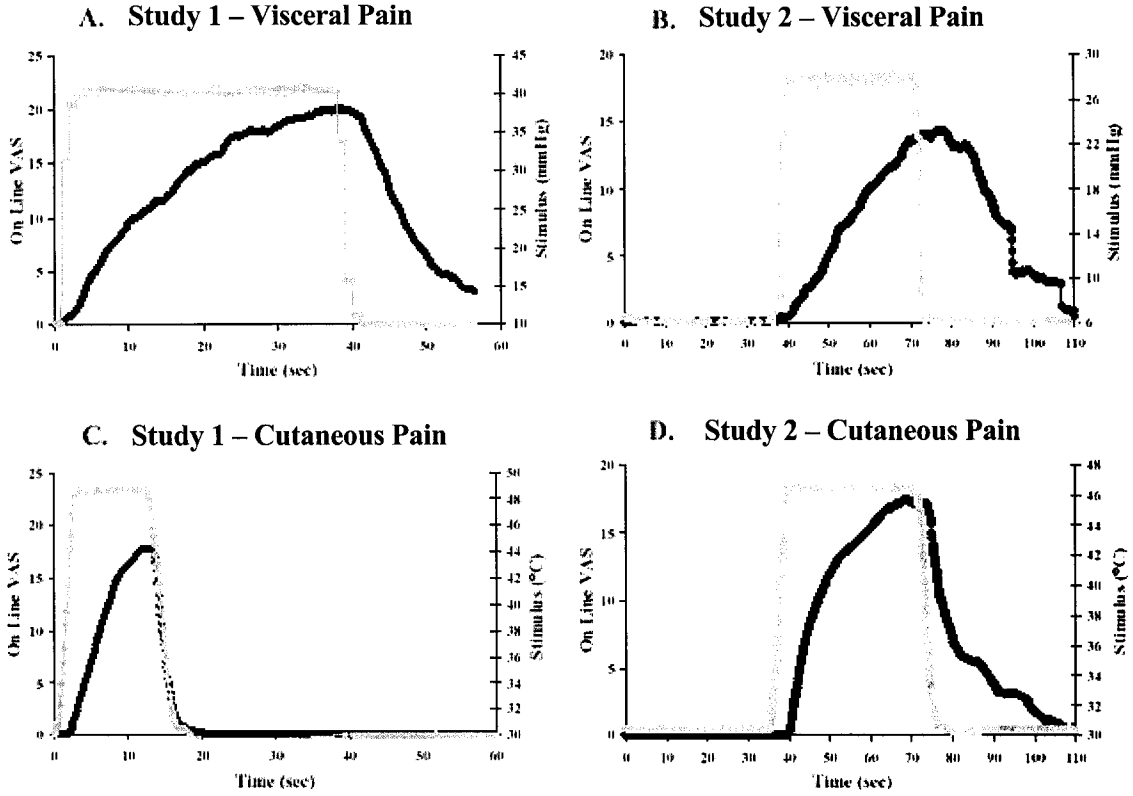


Median post-stimulus VAS ratings from Study 1 **(a, b)** and Study 2 **(c, d)**. **a)** Median post-stimulus unpleasantness ratings were higher than the corresponding pain intensity ratings for all levels of visceral stimulation ($p < 0.05$, repeated measures ANOVA, $n = 10$) but not for heat pain stimulation ($p = 0.2$, paired student t-test, $n = 10$); **b)** Relative unpleasantness, calculated as the difference between unpleasantness and corresponding pain intensity ratings, was positive for all levels of visceral stimuli and was significantly higher than the relative unpleasantness of the cutaneous stimulus ($p < 0.05$, repeated measures ANOVA, followed by post-hoc contrast analysis), which was negative. **c)** Median post-stimulus unpleasantness ratings were higher than the corresponding pain intensity ratings for the low level visceral stimulus ($p < 0.05$, $n = 5$, paired Student t-test) but not for the high level visceral stimulus ($p = 0.1$) nor for the thermal stimulation ($p = 0.2$, repeated measures ANOVA, $n = 5$); **d)** Relative unpleasantness was positive for both levels of visceral stimulation and was significantly higher than the relative unpleasantness of cutaneous stimuli ($p < 0.05$, repeated measures ANOVA, followed by post-hoc contrast analysis, $n = 5$). Error bars represent SEM.

2.4.4 On-line VAS Ratings

Average continuous ratings of perceived visceral and cutaneous pain are shown in Figures 2-3a and 2-3b from Study 1 and Figures 2-3c and 2-3d from Study 2. The perceived intensity of both visceral and cutaneous pain continued to gradually increase well after the stimuli had reached their maximum levels; peak intensity ratings were generally coincident with the stimulus offset. In Study 1, when the short cutaneous stimulus was used, subjects' intensity ratings of the cutaneous pain temporally paralleled the stimulus temperature changes, while visceral pain outlasted the stimulus in eight out of nine subjects ($p < 0.05$, $n = 9$, paired student t-test). A similar temporal pattern was observed in Study 2, although a slight pain sensation persisted some time after the stimulus offset when longer cutaneous stimuli were used. These temporal differences between visceral and cutaneous pain perception were confirmed by analysis of the areas under the VAS curves. As indicated in Table 2-3, for Study 1 the area under the on-line VAS ratings curve following stimulus offset was significantly larger for visceral than for cutaneous pain ($p < 0.05$, $n = 9$, paired Student t-test). A similar pattern was observed for visceral and cutaneous pain in Study 2, as indicated by the trend for the area under the VAS curve to be greater during visceral than during cutaneous pain (Table 2-3, $p = 0.09$, $n = 5$, paired Student t-test). In fact, all but one subject showed more pain sensation during visceral pain following stimulus offset. We observed no differences in peak intensity ratings between visceral and cutaneous pain within either Study 1 (21.9 vs. 18.6, $n = 9$, $p = 0.5$, paired student t-test) or Study 2 (16.1 vs. 17.7, $n = 5$, $p = 0.8$, paired student t-test); this uniformity of response allowed for direct comparison of the two modalities. Likewise, no differences were observed between the two studies in ratings of visceral ($p = 0.5$) or cutaneous ($p = 0.9$) pain, suggesting that the observed differences between visceral and cutaneous pain do not appear to be substantially influenced by stimulus duration.

Figure 2-3: On-line VAS pain ratings



On-line VAS ratings from Study 1 (**a, b**) and Study 2 (**c, d**). Gray and black lines represent stimulus intensity and ratings of pain intensity, respectively. In Study 1, continuous VAS ratings indicated greater total pain sensation following offset of the moderate visceral stimulus (**a**), compared with that seen following offset of the moderate cutaneous stimulus (**b**) ($p < 0.05$, paired student t-test, $n = 9$). Similarly, in Study 2, pain sensation following stimulus offset was greater for moderate visceral stimulation (**c**) than for moderate cutaneous stimulation (**d**) in four out of five subjects ($p = 0.09$, paired student t-test, $n = 5$).

Table 2-3: Area under the on-line VAS curve after stimulus termination

STUDY – SUBJECT	VISCERAL PAIN	CUTANEOUS PAIN
1-1	N/A	N/A
1-2	37.8	0
1-3	2.9	0
1-4	153.1	17.9
1-5	0	0
1-6	166.7	0
1-7	228.5	0.6
1-8	475.5	6.1
1-9	417.7	0
1-10	23.0	0
1-11	N/A	N/A
STUDY 1: MEAN ± SEM	167.3 ± 59.4	2.7 ± 2.0 *
2-1	66.0	61.0
2-2	N/A	N/A
2-3	339.9	342.9
2-4	295.1	53.0
2-5	638.3	236.2
2-6	172.8	1.9
STUDY 2: MEAN ± SEM	302. 4 ± 96.7 $^{p=0.09}$	139.0 ± 64.5

* $p < 0.05$ (paired sample t-test)

2.4.5 Qualitative Pain Evaluation

2.4.5.1 McGill Pain Questionnaire

Nine subjects in Study 1 and all subjects in Study 2 filled out the McGill Pain Questionnaire (MPQ) following cutaneous heat stimulation of the chest and esophageal balloon distention. The MPQ was analyzed by assessing the number of words chosen in each of the four categories (sensory, affective, evaluative and miscellaneous) and the total number of words chosen in all categories.

Figures 2-4a and 2-4b summarize the number of words chosen to describe visceral and cutaneous pain overall and in each of the four categories described by the MPQ in Study 1 and Study 2, respectively. In each of the studies, subjects chose a similar distribution of words. The total number of words chosen in all four categories was higher for visceral than for cutaneous pain in Study 2 ($p < 0.05$, $n = 6$, Wilcoxon Signed Rank Test), with a similar tendency in Study 1 ($p = 0.07$, $n = 9$, Wilcoxon Signed Rank Test). Likewise, the number of affective words chosen to describe visceral pain was significantly greater than that ascribed to cutaneous pain of similar intensity in Study 1 ($p < 0.05$, $n = 9$, Wilcoxon Signed Rank Test), with a similar tendency in Study 2 ($p = 0.1$, $n = 6$, Wilcoxon Signed Rank Test). Furthermore, more miscellaneous words were chosen during visceral than during cutaneous pain in both studies (p's < 0.05; Wilcoxon Signed Rank Test), suggesting that visceral pain evokes more varied sensations. Finally, there was a tendency for the number of sensory words chosen to be higher during cutaneous than during visceral pain in Study 1 ($p = 0.07$), but this tendency was not observed in Study 2 ($p = 1.0$; Wilcoxon Signed Rank Test).

The subjects' choice of sensory and affective words to describe the two modalities of stimulation was consistent with higher visceral affect. More than half of the subjects chose affective words to describe visceral pain (56% - Study 1, 50% - Study 2), while only one subject in each study chose affective words to describe cutaneous pain. The most frequently chosen affective word to describe visceral pain in both studies was 'suffocating' (45% - Study 1, 33% – Study 2). Conversely, all subjects in both studies chose sensory words to describe both visceral and cutaneous pain. Not surprisingly, the most frequently chosen sensory words were

'pressing' (56% - Study 1, 67% - Study 2) to describe visceral distention pain and 'hot' (45% - Study 1, 100% - Study 2) to describe cutaneous heat pain.

All subjects chose miscellaneous words to describe visceral pain and about 80% of subjects to describe cutaneous pain. 'Spreading' and 'nagging' were the most frequently chosen words for visceral pain in Study 1 and Study 2, respectively. It is of interest to note that 30% of subjects in Study 1 described their esophageal pain as 'burning' or 'hot'.

Once again, data from Study 1 and Study 2 were grouped to examine the possible influence of gender. The total number of words chosen during visceral pain was higher in female compared to male subjects ($p = 0.05$). However, no significant differences were observed in the number of words chosen in the sensory ($p = 1.0$), evaluative ($p = 0.9$), or miscellaneous ($p = 0.5$) categories, and only a trend toward significance was noted in the affective category ($p = 0.1$; two-sample Kolmogorov-Smirnov test). We also did not observe any effects of gender on the total ($p = 0.4$), sensory ($p = 0.4$), affective ($p = 1.0$), evaluative ($p = 0.5$) or miscellaneous ($p = 0.4$) number of words chosen during cutaneous pain.

2.4.5.2 Spielberger's State-Anxiety Questionnaire

Nine subjects in Study 1 and all subjects in Study 2 completed the 'state' portion of Spielberger's State-Trait Anxiety Inventory (STAI) following heat stimulation of the chest and distension of the esophageal balloon. Results in the two studies were similar ($p = 0.9$, Kolomogorov-Smirnov two sample test), and these are shown in Table 2-4. The median anxiety score attained by the subjects was significantly higher for visceral than for cutaneous pain during Study 1 (50 vs. 39; $p < 0.05$, $n = 9$, Wilcoxon Signed Rank Test); a similar tendency was observed during Study 2 (45.5 vs. 41; $p = 0.07$, $n = 6$, Wilcoxon Signed Rank Test). These results indicate that for a similar intensity of visceral and cutaneous stimuli, the anxiety associated with visceral pain is higher. Examination of gender effects showed no difference in the anxiety scores during visceral ($p = 0.4$) or cutaneous pain ($p = 0.2$, two-sample Kolmogorov-Smirnov test).

2.4.5.3 Area of Pain Sensation

Nine subjects in Study 1 and all subjects in Study 2 indicated the area of pain sensation on a drawing of the human body following cutaneous heat and visceral distension stimuli. To compare the areas of pain sensation each drawing was scanned and subsequently analyzed for the number of pixels in each shaded area. Cutaneous and visceral pain sensation areas are shown in Figure 2-5a and 2-5b, respectively. Because the areas were not normally distributed, non-parametric tests were used to compare the two groups. A typical subject's drawings are shown in Figure 2-5c and 2-5d for visceral and cutaneous pain, respectively. As indicated by the drawings, all nine subjects in Study 1 localized the sensation evoked by phasic cutaneous heat stimulation precisely to the area where the heat was applied. Tonic heat used in Study 2 also evoked precise sensations although the areas of sensation varied slightly among subjects. Nevertheless, no significant differences were observed between the areas of perceived pain during phasic and tonic heat (0.8x103 vs. 2.0x103 pixels, $p = 0.3$, two-sample Kolmogorov-Smirnov test). Conversely, subjects' drawings following esophageal balloon distension showed high variability in the size and the location of the areas of sensation in both studies (no significant difference between the two studies: 3.2x103 vs. 3.8x103; $p = 0.2$, two-sample Kolmogorov-Smirnov test). Generally balloon distension produced pain in the entire chest spreading along the midline in some subjects and laterally across the chest in others. Two subjects in Study 1 and two subjects in Study 2 reported visceral pain referred to the back. Statistical analyses showed that the visceral pain was perceived over a significantly larger area than cutaneous pain evoked by either phasic (3.2x103 vs. 0.8x103 pixels, $n = 9$, $p < 0.01$, Wilcoxon Signed Rank Test) or tonic cutaneous stimulation (3.8x103 vs. 2.0x103 pixels, $n = 6$, $p < 0.05$, Wilcoxon Signed Rank Test). Finally, we did not observe gender effects in the area of sensation following visceral ($p = 0.8$) or cutaneous pain ($p = 0.9$; two-sample Kolmogorov-Smirnov test).

Figure 2-4: Words chosen from MPQ

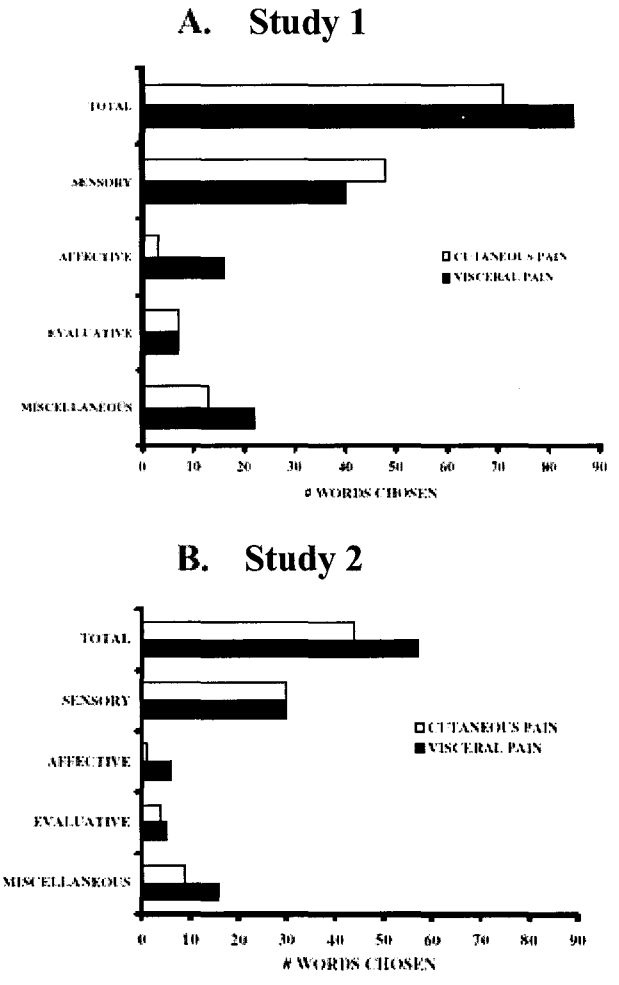
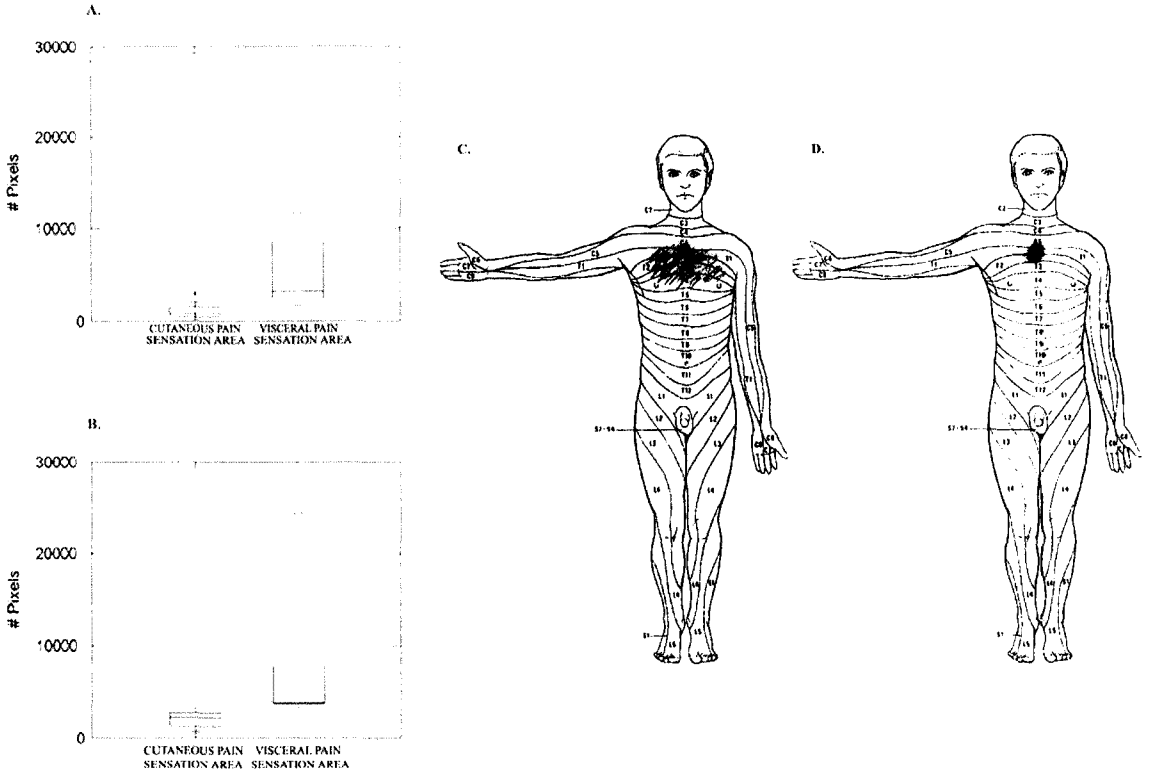


Table 2-4: Spielberger's State Anxiety Inventory following visceral and cutaneous pain

STUDY – SUBJECT	VISCERAL PAIN	CUTANEOUS PAIN
1-1	38	25
1-2	N/A	N/A
1-3	N/A	N/A
1-4	38	32
1-5	45	42
1-6	59	39
1-7	62	35
1-8	59	58
1-9	50	44
1-10	35	39
1-11	76	72
STUDY 1: MEDIAN	50 *	39
2-1	41	34
2-2	39	32
2-3	50	41
2-4	37	41
2-5	55	52
2-6	60	54
STUDY 2: MEDIAN	45.5 $^{p=0.07}$	42

* $p < 0.05$ (Wilcoxon Signed Rank Test)

Figure 2-5: Area of pain sensation



(a, b) Box plots illustrating total area of pain sensation. Horizontal lines mark the median surface area of pain sensation recorded by the subjects; boxes represent values ranging from 25th to 75th percentile. The median area of pain sensation during visceral stimulation was larger than that of cutaneous stimulation in both Study 1 **(a)** ($p < 0.05$, Wilcoxon signed rank test, $n = 9$) and Study 2 **(b)** ($p < 0.05$, Wilcoxon signed rank test, $n = 6$); **(c, d)** Drawings produced by a single subject indicating the site of sensation evoked by esophageal balloon distention **(c)** and cutaneous heat application **(d)**.

2.5 DISCUSSION

Results of the present study indicate that visceral pain is associated with higher unpleasantness than is cutaneous heat pain of equal perceived intensity, independent of the stimulus duration or the order of stimulus presentation. This greater degree of relative unpleasantness associated with visceral pain is especially evident at low stimulus intensities. As a consequence, visceral, but not cutaneous, stimulation is associated with unpleasantness thresholds that are lower than those for pain.

2.5.1 Perceived unpleasantness and stimulus duration

One potential explanation for possible differences in the magnitude of unpleasantness evoked by visceral and cutaneous stimuli hinges on the proposal that longer periods of stimulation (typical of most experimental visceral stimuli) may be associated with an increase in perceived affect or unpleasantness, compared with that observed with short, phasic stimulation paradigms. Our results demonstrate, however, that visceral extension is perceived as more unpleasant than cutaneous heat, even when the duration of stimulation is equivalent. Moreover, comparison of the subjects' ratings for contact heat stimuli shows no difference in the relative unpleasantness evoked by the phasic heat stimulation paradigm of Study 1 (10-sec stimulus duration) and that evoked by the tonic heat paradigm of Study 2 (36-sec stimulus duration). Rainville et al. (1992) had used short-duration painful heat (5 sec) and electric shock as phasic stimuli, whereas cold-pressor (applied for 5 minutes) and ischaemia (applied for 15 minutes) were used as tonic stimuli (Rainville et al., 1992). These modalities of tonic stimulation are more likely to produce a deep painful sensation and thus resemble visceral stimulation more than the superficial pain evoked by electric shock or cutaneous heat, regardless of their duration. Therefore, it is plausible that differences in relative unpleasantness observed by Rainville et al. (1992) may have been a consequence of deep versus superficial sensations rather than the duration of the painful stimulus. This interpretation is consistent with the higher relative unpleasantness associated with visceral pain observed in both present studies, as well as the similarity in relative unpleasantness evoked by phasic and tonic heat stimuli presented in Studies 1 and 2, respectively.

2.5.2 Descriptive measures of pain unpleasantness

Our subjects' direct estimates (VAS) of higher unpleasantness associated with visceral pain are consistent with results obtained from the McGill Pain Questionnaire's descriptor checklist. Subjects chose more affective words to describe visceral pain compared to phasic cutaneous pain of similar intensity and tended to do so when compared to tonic cutaneous pain as well. We also found that visceral pain was described with a wider range of words from the miscellaneous component of the MPQ, when compared to those chosen for cutaneous pain of similar intensity. Even from the miscellaneous category, subjects tended to associate cutaneous heat stimuli with descriptors related more to the sensory dimension of pain, such as 'radiating', 'piercing' and 'penetrating'. During visceral pain, however, the subjects' most frequently chosen words from the miscellaneous category reflected unpleasant sensations, such as 'nauseating' and 'nagging'; this latter descriptor was drawn from a group of 'affective tension' words in the original MPQ by Melzack and Torgerson (Melzack & Torgerson, 1971). This again suggests a higher affect for visceral pain, which does not seem to be dependent on the duration of the cutaneous stimulus.

2.5.3 Relationship between pain and anxiety

In the present study, we found a higher level of anxiety associated with visceral pain, compared to that attributed to cutaneous pain of similar intensity. Furthermore, anxiety scores recorded during visceral stimulation correlated highly with ratings of pain unpleasantness (Pearson's $r = 0.6$, $p < 0.05$), but not with pain intensity (Pearson's $r = 0.3$, $p = 0.2$); no such correlation was observed during cutaneous stimulation (Pearson's $r = 0.4$, $p = 0.2$). Taken together, these results suggest that the higher levels of anxiety associated with stimulation of the viscera are directly linked to the increased perception of unpleasantness or negative affect evoked by this mode of stimulation.

The association between anxiety and pain has been addressed in several investigations, but the exact relationship between the two measures is still unclear. Some studies of postoperative or chronic pain have found that state-anxiety was a predictor of pain affect (Scott et al., 1983), in agreement with results from the present study using experimental stimuli; others, however,

have suggested that state-anxiety influences both affective as well as sensory dimensions of pain (Gaskin et al., 1992). These latter results could imply that fear of surgery or emotional distress associated with chronic disease can heighten the sensory dimension of pain; however, clinical findings from Scott et al (1983) and Wade et al. (1990) argue against this interpretation (Scott et al., 1983; Wade et al., 1990). Alternatively, the conflicting results may be explained by the different measures used to evaluate sensory and affective pain perception; our study, as well as that of Scott et al. (1983) and Wade et al. (1990), used direct parametric measures of both intensity and unpleasantness, whereas those of Gaskin et al. (1992) employed only the McGill Pain questionnaire which requires a less sensitive, nonparametric, analysis of verbal descriptors.

Results of the present study may shed additional light on the specific relevance of anxiety and its modulation of pain perception. Weisenberg et al. (1984) proposed that anxiety related to the pain-inducing stimulus would intensify the painful experience, while anxiety irrelevant to the noxious stimulus would diminish pain (Weisenberg et al., 1984). Likewise, al Absi and Rokke (1991) demonstrated in normal volunteers that high levels of relevant anxiety (regarding cold pressor stimulation) increase pain, while high levels of irrelevant anxiety (regarding the possibility of an electrical shock when undergoing cold pressor stimulation) decrease the pain experienced (al Absi & Rokke, 1991). The present study extends these findings by demonstrating a more direct relationship between anxiety and one component of the pain experience—e.g. pain affect. The most parsimonious explanation for the source of anxiety in the present study points toward insertion of the esophageal tube. Within the context of esophageal pain, this relevant anxiety was associated with higher levels of perceived unpleasantness; likewise, during cutaneous pain, this anxiety, now considered as irrelevant to the painful stimulus, was associated with decreased levels of unpleasantness evoked by the noxious cutaneous stimulation. Therefore, in the present study, increased anxiety scores during esophageal distention argue both for the relevance of this procedure as an antecedent to the anxiety itself, and for the direct relationship between the relevance of anxiety and heightened emotional responses (unpleasantness and negative affect) evoked by the visceral stimulation.

2.5.4 Discrimination of visceral and cutaneous stimulation

In our study, the majority of subjects had difficulty discriminating between the intensities of visceral but not somatic stimuli, and visceral pain produced less defined sensations than cutaneous pain of similar intensity. We also found that visceral pain was perceived as more diffuse than cutaneous pain of similar intensity since visceral sensations were referred to a significantly larger area.

These results are in agreement with the literature; visceral pain, unlike pain from the skin, is generally considered to be difficult to localize and not well correlated with the intensity of stimulation—qualities consistent with information encoded by unmyelinated afferent fibers (Ranson, 1915; Cervero, 1985). Intensity-coding of noxious stimuli applied to the esophagus is thought to be carried by the splanchnic nerve which contains 90% unmyelinated C fibers (Kuo et al., 1982; Sengupta et al., 1990; Sengupta & Gebhart, 1994). In addition, innervation of the viscera (Kuo et al., 1982; Cervero & Connell, 1984a) and the distal esophagus is very sparse (Christensen & De Carle, 1974; Christensen, 1984; Neuhuber, 1987). Therefore, the density of visceral projections into the spinal cord is much lower than that of their somatic counterparts (Cervero et al., 1984a; Cervero & Connell, 1984b). Furthermore, in contrast to the periphery, a large number of spinal cord dorsal horn neurons receive input from both skin and viscera (Cervero et al., 1986; Garrison et al., 1992; Laird et al., 1996). Somatovisceral convergent neurons have been found in the ascending pathways of the spinal cord that are known to transmit sensory information and pain, in particular to the higher brain structures (Foreman & Weber, 1980; Foreman et al., 1984; Al-Chaer et al., 1999). Only a small number of visceral-specific cells in the spinal cord and thalamus of animals has been identified (Rucker et al., 1984; Berkley et al., 1993a; Bruggemann et al., 1994; Al-Chaer et al., 1999). As a result, poor visceral innervation, the loss of specificity in the ascending pathways, and the predominance of unmyelinated afferents in the visceral nerves are all contributing factors that may explain the more diffuse visceral sensations compared to sensations arising from skin.

Esophageal pain is often confused with cardiac pain (Roberts et al., 1975; Henderson et al., 1978). Similar sensations and referral to the same somatic areas have been described during cardiac and esophageal chest pains (Morrison & Swalm, 1940; Kramer & Hollander, 1955; Lee

et al., 1985). Clinical esophageal pain, which varies from a 'tight squeezing' sensation to a 'stabbing' or 'tearing pain', predominantly distributes to the epigastric and retrosternal regions. More severe pain radiates to the back in the majority of patients, and less frequently to the left arm, neck or jaw (Henderson & Marryatt, 1981; Lee et al., 1985). In our study, experimentally induced esophageal pain showed a similar distribution to that found in patients, i.e. to the entire chest in all subjects and to the back in four subjects. The sensations produced were also similar, generally described as 'pressing', 'spreading', 'nagging' and 'suffocating', suggesting that esophageal balloon distention closely mimics clinical esophageal pain. Furthermore, following esophageal balloon distention, about 30% of subjects in Study 1 described their pain as 'burning' or 'hot'. 'Burning' is one of the characteristic qualities of pain in patients with gastroesophageal reflux disease and in normal subjects following balloon distention or electrical stimulation of distal esophagus (Mehta et al.,1995; Fass et al., 1998). This quality seems to be unique to the esophagus since other organs of the alimentary tract do not evoke this type of sensation (Ness et al., 1990). Another visceral organ that seems to produce burning pain sensation is urinary bladder (Ness et al., 1998), suggesting that esophagus and urinary bladder share similar family and/or proportion of sensory receptors. Two different classes of receptors, high-threshold and intensity-encoding, seem to exist in both urinary bladder and esophagus (Sengupta et al., 1989; Habler et al., 1990; Sengupta et al., 1990; Cervero et al., 1999).

2.6 CONCLUSION

In the present study, we have shown that esophageal distention is a reliable and reproducible model of visceral pain in normal subjects. Furthermore, in this direct comparison of visceral and cutaneous painful sensation, we have shown that for stimuli of similar intensity, duration and site of delivery, visceral and cutaneous pains are characterized by different relative unpleasantness, perception and distribution.

2.7 ACKNOWLEDGEMENTS

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Chapter 3

CEREBRAL ACTIVATION DURING PAINFUL STIMULATION OF SKIN AND VISCERA

The findings from the previous chapter indicate that the perception of visceral pain significantly differs from cutaneous pain in human subjects. Specifically, for a similar given intensity and duration, visceral pain is more unpleasant, more qualitatively variable, more persistent and more diffuse. As was mentioned in the beginning, the diffuse and poorly localized qualities of visceral pain can be attributed to differences in the properties of visceral and cutaneous afferents described in detail in Chapter 1. However, differences in perception cannot be solely explained by the differences in spinal cord activity, suggesting the involvement of the cerebral cortex. Since none of the studies thoroughly examined the neural correlates of visceral pain in relation to cutaneous pain, the following chapter describes the attempt to do so with the use of functional magnetic resonance imaging (fMRI) technique.

3.1 ABSTRACT

Physiological evidence indicates that visceral and cutaneous nociception are integrated in the CNS, yet their divergent behavioural components (freezing vs flight) suggest substantial differences in neural processing. Our study examines such differences by directly comparing human cortical processing of intensity-equated visceral and cutaneous stimulation.

Seven subjects (4M, 3F, age 19-34) underwent fMRI-scanning (1.5 Tesla, standard head coil) during visceral and cutaneous pain induced by balloon-distention of the distal esophagus and contact heat on the midline chest. Two stimulus intensities (producing non-painful and painful sensations), interleaved with rest periods, were presented in each functional run in a counterbalanced order using a block design paradigm. Analyses compare high to low intensity stimulation conditions.

A similar cortical network (including secondary somatosensory and parietal cortices, thalamus, basal ganglia, and cerebellum) was activated by visceral and cutaneous painful stimuli. Cutaneous pain, when compared to visceral pain, evoked significantly higher activation in the anterior insula in both hemispheres. Cutaneous heat, but not esophageal distention, produced frontal activation, despite higher affective scores associated with visceral pain. Visceral, but not cutaneous, pain activated bilateral inferior primary somatosensory cortex, bilateral primary motor cortex and a more anterior locus within the anterior cingulate cortex.

Our results demonstrate a common cortical network subserving cutaneous and visceral pain in humans; however, the data also suggest significant distinctions in the processing of these stimuli, including a differential involvement within insular, primary somatosensory, motor and frontal cortices.

3.2 INTRODUCTION

Analyses of the cerebral network underlying human pain processing have concentrated largely on cutaneous or superficial pain. Numerous studies have examined the mechanisms associated with pain from the skin using neurophysiological and brain imaging approaches. Several brain structures have been shown to constitute the network subserving cutaneous pain including primary and secondary somatosensory cortices (SI and SII), anterior cingulate cortex (ACC), and insular cortex (IC) (Jones et al., 1991; Talbot et al., 1991; Coghill et al., 1994; Hsieh et al., 1995; Casey et al., 1996). Fewer studies have examined the neural pathways associated with visceral pain in normal and pathological conditions. A wide pattern of visceral-related cortical activity consistent with the cutaneous pain network has been observed in some studies (Aziz et al., 1997; Binkofski et al., 1998; Baciu et al., 1999; Mertz et al., 2000). Despite the growing literature of basic reports and clinical research, evidence for the differential involvement of these or other cerebral structures in the processing of visceral pain is still lacking, yet divergent behavioural components, such as 'freezing' or 'flight' associated with visceral and cutaneous pain, respectively, suggests differences in the neural processing of these two systems.

Three studies have directly compared the neural processes underlying visceral and somatic sensations in humans. One study looked at esophageal sensation and two studies examined ano-rectal sensations, since these gastrointestinal organs contain both visceral and somatic tissues (Aziz et al., 2000; Hobday et al., 2000; Lotze et al., 2001). Several brain areas have been differentially activated by innocuous visceral and somatic stimulation including primary somatosensory (SI) and anterior cingulate (ACC) cortices in all three studies, and secondary somatosensory cortex (SII) in one study (Aziz et al., 2000; Hobday et al., 2000; Lotze et al., 2001). Since painful stimuli were not employed in these studies, it is difficult to speculate whether visceral and somatic pain would show similar differences in the cortical activation pattern. Indubitably, innocuous and noxious stimulation of the skin produces sensations that are processed differently by the cerebral cortex (Coghill et al., 1994; Casey et al., 1996; Davis et al., 1998).

Using psychophysical experimentation, we have recently shown that visceral and cutaneous pains of similar intensity and duration are characterized by different relative unpleasantness,

perception and distribution (Strigo et al., 2002). Previous imaging data and our psychophysical findings suggest the likelihood of both differences and similarities in neural processing underlying visceral and cutaneous pain. We therefore compared the cortical networks subserving visceral and cutaneous pains resulting from balloon distention of the distal esophagus and thermal heat application on the midline chest, respectively, employing functional magnetic resonance imaging (fMRI) technique.

3.3 METHODS

3.3.1 Subjects

With the approval of McGill Institutional Review Board and the Ethics and Research Committee of Montreal Neurological Institute, we studied seven healthy volunteers (4 males, 3 females), ranging in age from 19 to 34 years (mean age 25.8). None of the subjects was obese (mean BMI 23.6) or taking any medication, and all were free of esophageal symptoms. Each subject underwent esophageal manomentry (MMS-100, Narco-Bio Systems, Austin, TX, USA) to identify the distance of the lower esophageal sphincter to accurately position the probe.

3.3.2 Stimulation

Esophageal stimulation was performed by distending a custom-designed polyethylene balloon (square type) 8 cm in length, 6 cm in diameter, with a maximum volume of 70-80 ml, which was attached to a multilumen polyvinyl esophageal catheter 10 cm above the tip (Mui Scientific, Mississauga, ON, Canada). The catheter was attached to a pump via an 800-cm long tube (the long tube allowed the placement of the pump in an adjacent room for use in the MRI environment). One of the three lumens in the catheter was connected to a pressure transducer; the second was attached to the piston on a pump (G&J Electronics, Toronto, ON, Canada) which was used to inflate the balloon with air at a rate of ~ 50 ml/sec; and the third served as the motility port to measure the position of the lower esophageal sphincter. Thermal stimulation of the upper chest was performed with a 9-cm^2 Peltier-type contact thermode (Medoc, Ramat Yishai, Israel). The rate of temperature increase was 5°C/sec.

Esophageal balloon distention and thermal heat stimulation of the upper chest were presented in separate functional runs and in two different sessions in five subjects, and in one session in two subjects. At the start of the visceral experiment, the balloon catheter was passed perorally following the application of local anesthetic ('Xylocaine") and positioned in the distal esophagus 5 cm above the lower esophageal sphincter; at the start of the cutaneous experiment, the thermode was securely taped onto the upper midline chest. The stimulation sequences for visceral and cutaneous stimulation were identical and consisted of stimuli of two

different intensities – 'high', which produced moderate pain sensation in all subjects and 'low', which was perceived by all subjects but was not painful. The stimuli were given in quasi-random and counterbalanced order; each stimulus was presented three times and lasted approximately 36 sec (9 whole brain acquisitions). Each stimulus was interleaved with the baseline period of equal duration where no stimulation was applied (Figure 3-1). The stimulation parameters were individually determined in order to equate the perceived intensities of visceral and cutaneous stimuli.

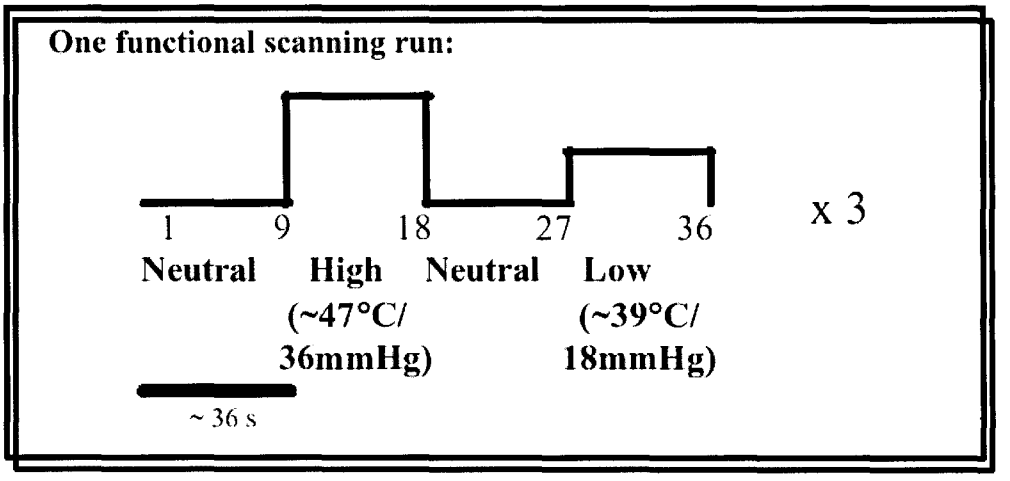
3.3.3 Imaging Procedure

MRI was performed using a 1.5 T Siemens Vision scanner (Siemens AG, Eriangen, Germany) with a standard head-coil. Each session consisted of one anatomical scan and 4-8 functional scanning runs. The anatomical scans were recorded using a high-resolution T1-weighted anatomical protocol (TR 22 ms, TE 20 ms, flip angle 30, FOV 256 mm). The functional scans were collected using a BOLD (blood oxygen level dependent) protocol with a T2*-weighted gradient echo-planar imaging (EPI) sequence (TR 4.0 s, TE 51ms, flip angle 90) yielding a 5x5 mm in-plane resolution.

The scanning planes were oriented parallel to the anterior commissure-posterior commissure line and covered the whole brain from the top of the cortex down to the base of the cerebellum (27 slices, 5 mm thickness, TR 4.0 s). The individual scans consisted of 126 whole brain volume acquisitions, divided in 12 cycles. Each cycle consisted of nine successive volume acquisitions or 36 sec without stimulation followed by 36 sec with either visceral or cutaneous stimulation, and ended with 36 sec of no stimulation again (Figure 3-1). Before being positioned in the scanner, all subjects were instructed to attend to the stimuli and refrain from movement as much as possible. To further prevent movement artifacts, the head was immobilized with padded earmuffs, a foam headrest, and a plastic bar across the bridge of the nose. Each subject was provided with earplugs to decrease the noise generated by the MRI machine. After each functional scanning run, the subjects rated the intensity and unpleasantness of experimental stimuli, as well as any pain or discomfort associated with nonspecific sources other than the stimulus using the fingers of one hand to avoid head movements. All scales ranged from 0 ('no sensation', 'no pain sensation' or 'not at all

unpleasant'), to 10 ('extremely warm/pressure sensation' – described as pain threshold, 'extremely intense pain sensation', or 'extremely unpleasant'). No affective ratings were requested for non-painful stimuli.

Figure 3-1: Experimental paradigm



Experimental paradigm during single functional scanning run for both visceral, and cutaneous stimulation. Stimuli were presented in quasi-random and counterbalanced order.

3.3.4 Image Processing and Analyses

Functional data were motion corrected and low-pass filtered with a 6-mm FWHM Gaussian kernel in order to increase the signal-to-noise-ratio. All images were resampled into stereotaxic space (Collins et al., 1994). Activation maps, comparing painful heat to non-painful conditions, were generated using fMRISTAT-MULTISTAT software developed at the Montreal Neurological Institute, Montreal, Canada. This analysis yields t-statistics based on a linear model using random field theory, correlated errors, and Bonferroni correction; data are also corrected for temporal correlation, artifactual drift and random effects. The procedures have been recently described in detail (Worsley et al., 2002; technical support available at http://www.math.mcgill.ca/keith/fmristat).

In brief, the design matrix for the linear model is based on a regressor defined by the external stimulus events convolved with a pre-specified hemodynamic response function. The analysis fits the linear model to a single run of fMRI data allowing for spatially varying autocorrelated errors. Statistical outputs from different runs during a session are then combined using a type of random effects analysis. Thresholds for peak and cluster size detection are set using random field theory (Worsley et al., 1996; Cao, 1999).

Only positive stimulus correlations were used in the analyses. For both visceral and cutaneous stimulation the resulting t-statistic image reflected the difference in activation between the painful and non-painful conditions. The volume of the whole brain was estimated to be 1200 cm^3 (150 000 voxels) yielding a threshold value of 4.5 for the global search. Directed searches were performed in SI, SII, IC, ACC, and MPFC. The following search volumes were used: SI – 12.1 cm^3 (1512 voxels), SII – 10.1 cm^3 (1260 voxels), IC – 6.2 cm^3 (780 voxels), ACC – 9.6 cm^3 (1200 voxels), and MPFC – 13.2 cm^3 (1650 voxels), as described previously (Olausson et al., 2001). For a directed search within these volumes the t-values for significant activation were calculated to be 3.3 for SI, SII, ACC, and MPFC and 3.1 for IC.

To directly compare brain activations produced by visceral and cutaneous stimulation, regions of interest (ROIs) were drawn in each area of the brain that showed differential activity in the t-statistics maps, namely anterior insula, frontal lobe, inferior SI, and MI. The original motion-

corrected raw data from functional runs of like modality and sequence were then averaged for each individual subject. Subsequently, the signal in the above areas was extracted for each individual subject's averaged raw data during high and low intensity stimulations, subtracted and averaged across subjects to give a number that corresponded to the activity in the area of interest. Basic statistical analyses were further used to identify significant differences in the activated signal.

3.4 RESULTS

3.4.1 Psychophysical Ratings

Figure 3-2 shows the average post-scan psychophysical ratings for painful (Figure 3-2a) and non-painful (Figure 3-2b) visceral and cutaneous stimuli. As we have shown previously (Strigo et al., 2002), the ratings of unpleasantness associated with visceral pain were higher than the corresponding ratings of intensity ($p < 0.05$, Wilcoxon Signed-Rank Test), while there was no difference between the intensity and unpleasantness ratings during cutaneous pain ($p = 0.8$) (Figure 3-2a). Moreover, the intensity ratings of high (painful - Figure 3-2a) and low (non-painful - Figure 3-2b) visceral stimuli were not different from the corresponding intensity ratings of cutaneous stimuli ($p = 0.8$ - high; $p = 1.0$ - low, Wilcoxon Signed-Rank Test), which allowed us to directly compare visceral and cutaneous stimulation.

3.4.2 Cerebral activity associated with visceral pain

Table 3-1 summarizes the regions of the increased BOLD responses, which reflects cerebral activity (Logothetis et al., 2001) during painful esophageal distention. Directed searches revealed significant activation in bilateral secondary somatosensory cortex (SII), which was also significant in a global search, left anterior cingulate cortex, right anterior insula, bilateral mid-insula, left posterior insula, and bilateral prefrontal cortex (Brodman Area (BA) 9/46). Clusters of global significance were seen bilaterally in cerebellum, inferior intra-abdominal region of the primary somatosensory cortex (SI), the face area of the primary motor cortex (BA 4), as well as in the supplementary motor area (BA 6/32), left parietal association cortex (BA 40), left putamen and the left thalamus (most probably ventrolateral nucleus).

3.4.3 Cerebral activity associated with cutaneous pain

Table 3-2 summarizes the regions of the increased cerebral activity during painful thermal heat stimulation of the chest. Directed searches revealed significant activity in bilateral anterior insula and left posterior insula, which were also significant in a global search, left anterior cingulate cortex, bilateral mid-insula and right posterior insula, bilateral secondary somatosensory cortex and right prefrontal cortex (BA 9/46). The areas of global significant

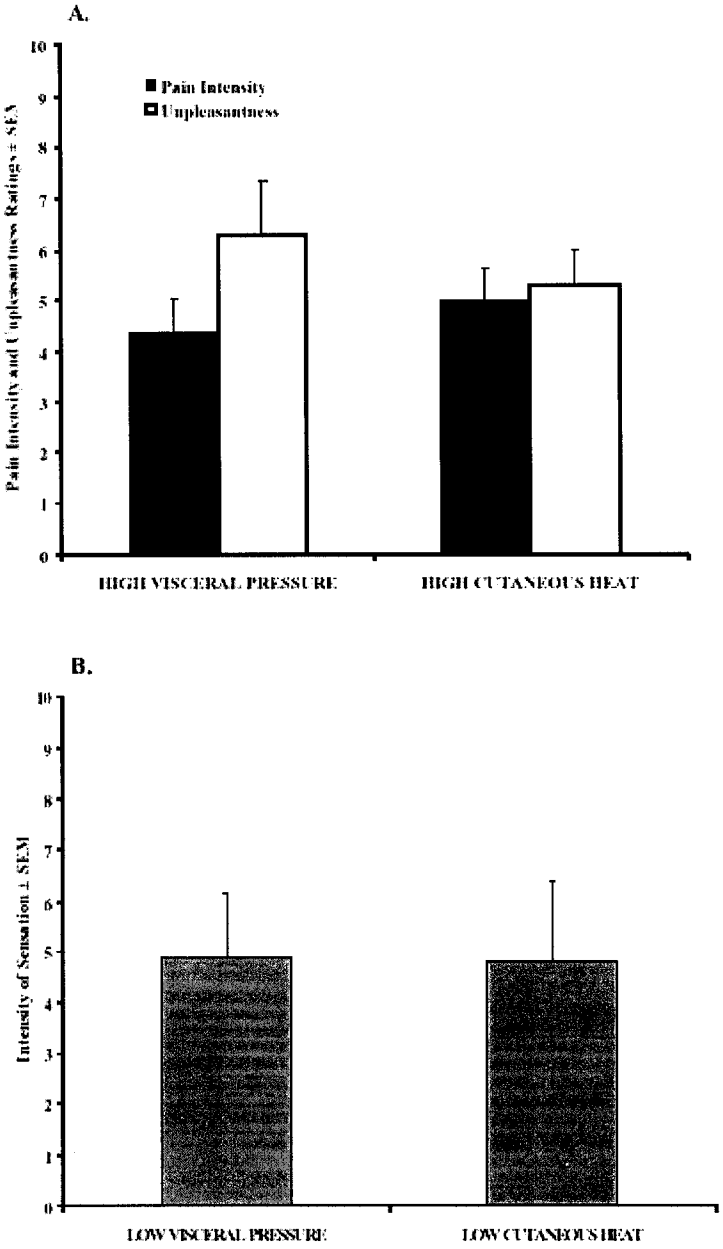
activation were observed in cerebellum and parietal association cortex (BA 40, 7) bilaterally, frontal cortex (BA 10/46) and right putamen, and thalamus (most probably ventrolateral nucleus) and left posterior cingulate (BA 23).

3.4.4 Comparison of visceral and cutaneous pain

Figure 3-3a and 3-3b illustrate brain regions that showed similar levels of significant activity during visceral and cutaneous pain, respectively. To identify brain regions that showed differential activity during visceral and cutaneous pain, region-of-interest (ROI) analyses were performed in areas that were activated by one condition only, or had a large difference (> 2) in their respective t-scores of activation, and are shown in Table 3-3. As can be seen in Figure 3-4a and 3-4b, cutaneous pain resulted in a significantly higher activity in the right ($p < 0.01$) and left ($p < 0.05$; paired Student t-test) anterior insula when compared to visceral pain. On the other hand, visceral but not cutaneous pain significantly activated the face area of the primary motor cortex on the right ($p < 0.01$) and showed a similar tendency on the left ($p = 0.1$, paired Student t-test) (Figure 3-4a and 3-4b). In addition, anterior cingulate cortex was differentially activated by visceral and cutaneous pain, and this is demonstrated in Figure 3-4a and 3-4b. Two different peaks of activation (at least 6xFWHM apart) were observed within the ACC, with visceral pain represented more rostrally.

Surprisingly, ROI analyses in the inferior intra-abdominal SI did not result in significant difference between visceral and cutaneous pain ($p = 0.2$, paired Student t-test), even though this region was not activated during the cutaneous stimulus (Figure 3-5a). Similarly, despite highly significant activation of the right frontal lobe during thermal heat stimulation and the absence thereof during esophageal distention (Figure 3-5b), ROI analysis did not result in significant differences between the two ($p = 0.2$, paired Student t-test).

Figure 3-2: Psychophysical ratings



Average post-run pain intensity and unpleasantness ratings following high **(a)** and low **(b)** intensity stimulation. **a)** Average post-stimulus intensity ratings following high visceral stimulation were not different from those following high cutaneous stimulation ($p = 0.8$), allowing for direct comparison; average ratings of unpleasantness following high visceral stimulation were higher than the corresponding pain intensity ratings ($p < 0.05$), while they were not different following high cutaneous stimulation ($p = 0.8$). **b)** Average post-stimulus intensity ratings following low non-painful visceral stimulation were not different from those following low non-painful cutaneous stimulation ($p = 1.0$, Wilcoxon signed rank test). Error bars represent SEM.

Table 3-1: Regions of increased neuronal activity during painful esophageal distention

Region – Brodman Area (BA)	Stereotaxic Coordinates			t-score
	M-L	A-P	S-I	
R. Secondary Somatosensory Cortex (SII)	52	-27	29	**5.4**
L. Secondary Somatosensory Cortex (II)	-52	-33	30	3.7[a]
R. Primary Somatosensory Cortex (SI) – intra-abdominal region	52	-18	25	**5.7**
R. Primary Somatosensory Cortex (SI) – trunk region	24	-32	64	2.8
L. Primary Somatosensory Cortex (SI) – intra-abdominal region	-60	-16	25	**5.4**
R. Anterior Insula	30	20	6	4.2[a]
R. Mid Insula	34	0	2	4.1[a]
L. Mid Insula	-36	2	6	4.4[a]
L. Posterior Insula	-26	-16	4	4.4[a]
R. Primary Motor Cortex (MI) – BA 4	42	-10	36	**5.0**
L. Primary Motor Cortex (MI) – BA 4	-50	-10	34	**4.9**
SMA (cluster) – BA32/6	2	10	48	**5.3**
R. Cerebellum (cluster)	-24	-60	-22	**6.5**
	-32	-56	-32	**6.4**
L. Cerebellum (cluster)	30	-56	-30	**5.2**
Cerebellar Vermis	0	-50	-22	**4.7**
L. Parietal Association Cortex – BA 40	-38	-58	52	**5.7**
– BA 7 (precuneus)	-14	-68	38	**4.6**
R. Parietal Association Cortex – BA 7 (precuneus)	4	-52	54	3.9
L. Basal Ganglia (Putamen)	-22	-2	4	**5.1**
R. Basal Ganglia (Putamen)	26	-12	4	**4.6**
L. Thalamus	-14	-14	6	**4.7**
R. Thalamus	10	-12	2	3.9
R. Prefrontal Cortex (BA 9/46)	38	42	30	3.7[a]
L. Prefrontal Cortex (BA 9/46)	-36	36	36	3.8[a]
Anterior Cingulate Cortex (BA 32)	-2	26	36	3.6[a]

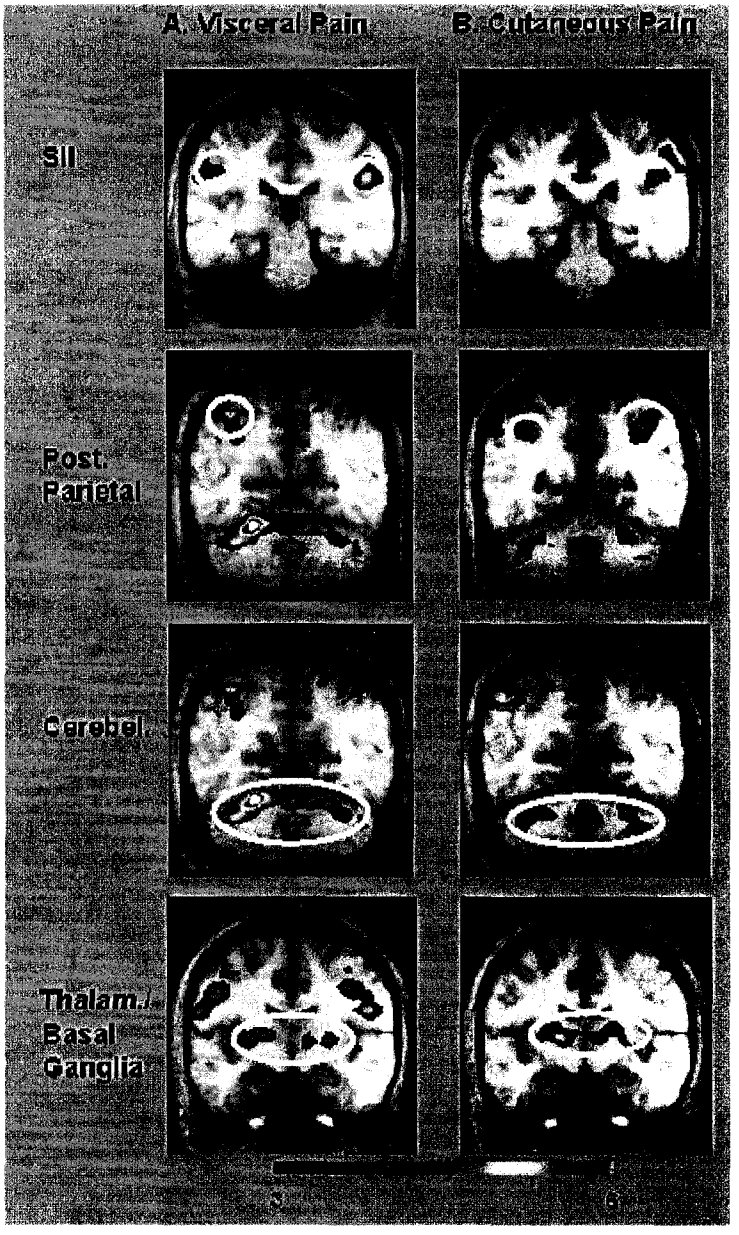
t-scores of global significance are indicated in bold; [a] indicates significance from the directed search; values that did not reach significance are placed for comparison

Table 3-2: Regions of increased neuronal activity during painful thermal heat stimulation

Region – Brodman Area (BA)	Stereotaxic Coordinates			t-score
	M-L	A-P	S-I	
R. Anterior Insula (cluster)	32	12	8	**6.3**
	32	24	6	**6.2**
L. Anterior Insula (cluster)	-36	2	16	**5.7**
	-36	12	10	**5.1**
R. Mid Insula	36	-4	-6	4.4[a]
L. Mid Insula	-42	0	2	4.1[a]
R. Posterior Insula	36	-20	14	3.6[a]
L. Posterior Insula	-28	-22	6	**4.9**
R. Secondary Somatosensory Cortex (SII)	54	-38	32	**4.6**
L. Secondary Somatosensory Cortex (SII)	-56	-24	16	3.7[a]
R. Primary Somatosensory Cortex (SI) – trunk region	18	-37	64	2.0
L. Primary Somatosensory Cortex (SI) – trunk region	-22	-40	64	2.0
R. Thalamus	12	-12	8	4.3
L. Thalamus	-16	-14	6	**5.4**
R. Frontal (BA 10/46)	42	50	-2	**5.3**
R. Cerebellum	36	-56	-44	**5.3**
	28	-56	-30	**5.0**
L. Cerebellum	-40	-52	-44	**4.9**
	-26	-66	-32	**4.5**
Cerebellar Vermis	4	-58	-30	**4.5**
R. Parietal Association Cortex – BA 40	54	-36	44	**5.0**
– BA 7 (precuneus)	38	-50	42	**4.9**
L. Parietal Association Cortex – BA 40	-60	-38	38	**4.8**
– BA 7 (precuneus)	-34	-48	38	**4.5**
Posterior Cingulate Cortex (BA 23)	-2	-26	28	**4.9**
R. Basal Ganglia (Putamen)	26	6	4	**4.9**
L. Basal Ganglia (Putamen)	-30	-6	6	**4.6**
R. Prefrontal Cortex (BA 9/46)	42	40	20	3.9[a]
Anterior Cingulate Cortex (BA 24)	-10	0	42	3.5[a]

t-scores of global significance are indicated in bold; [a] indicates significance from the directed searches; values that did not reach significance are placed for comparison

Figure 3-3: Pain-evoked cortical activity – Similarities



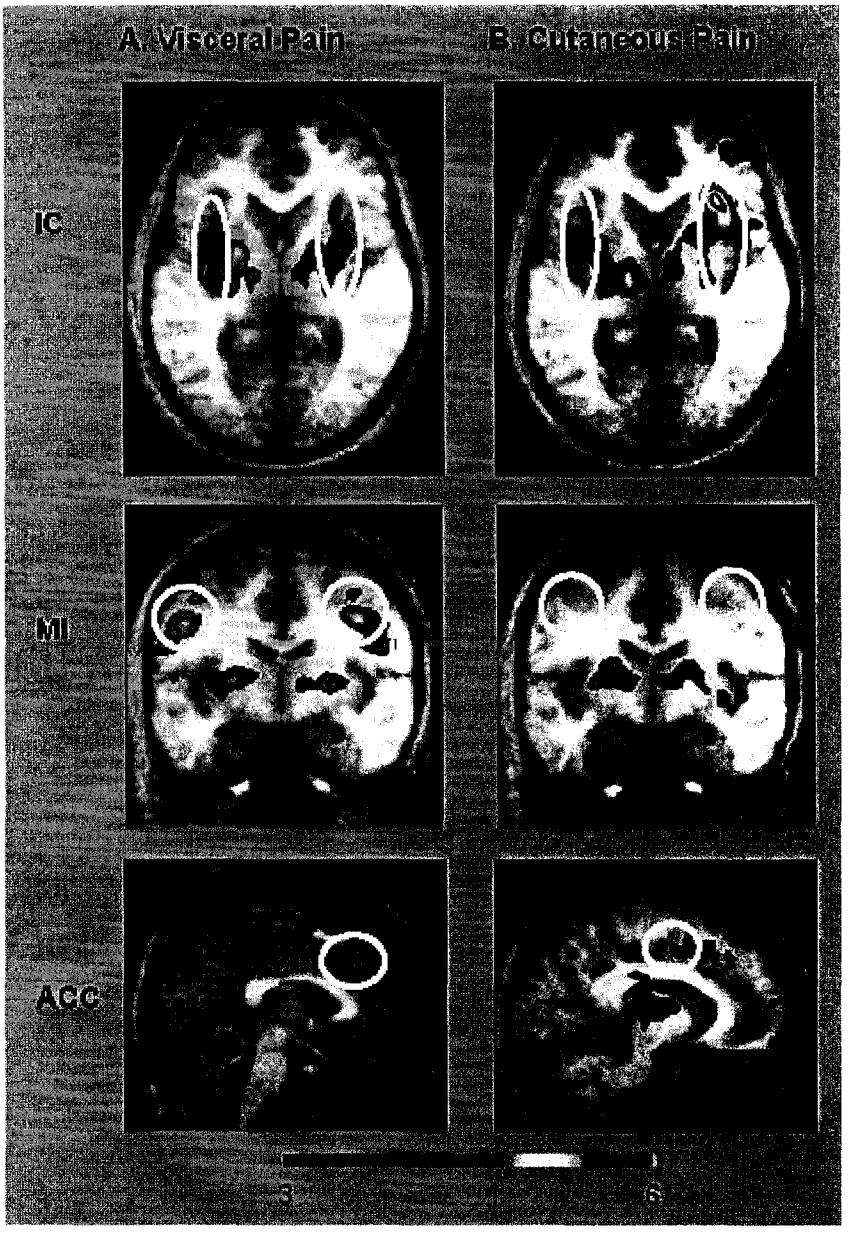
Cortical activation evoked by esophageal distention (**a**) and thermal heat (**b**). Both stimulations resulted in similar activity in the second somatosensory cortex (SII), posterior parietal cortex, cerebellum, thalamus and basal ganglia The right side of the images corresponds to the right side of the brain and white circles indicate regions of significant activation (c.f. Tables 1 and 2 for stereotaxic coordinates and t-values. Colour bars show t-values.

Table 3-3: Regions of interest analyses (ROI) in areas of significant activation

BRAIN REGION	VISCERAL PAIN	CUTANEOUS PAIN
R. Anterior Insula	0.410±0.44	1.869±0.30 **
L. Anterior Insula	0.379±0.59	1.921±0.35 *
R. MI	1.067±0.51 **	-1.129±0.62
L. MI	0.686±0.62	-0.998±0.62
R. SI (intra-abdominal)	1.755±0.77	0.544±1.35
L. SI (intra-abdominal)	1.326±0.82	-1.286±1.83
R. Frontal (BA 10/46)	1.046±1.38	2.032±0.94

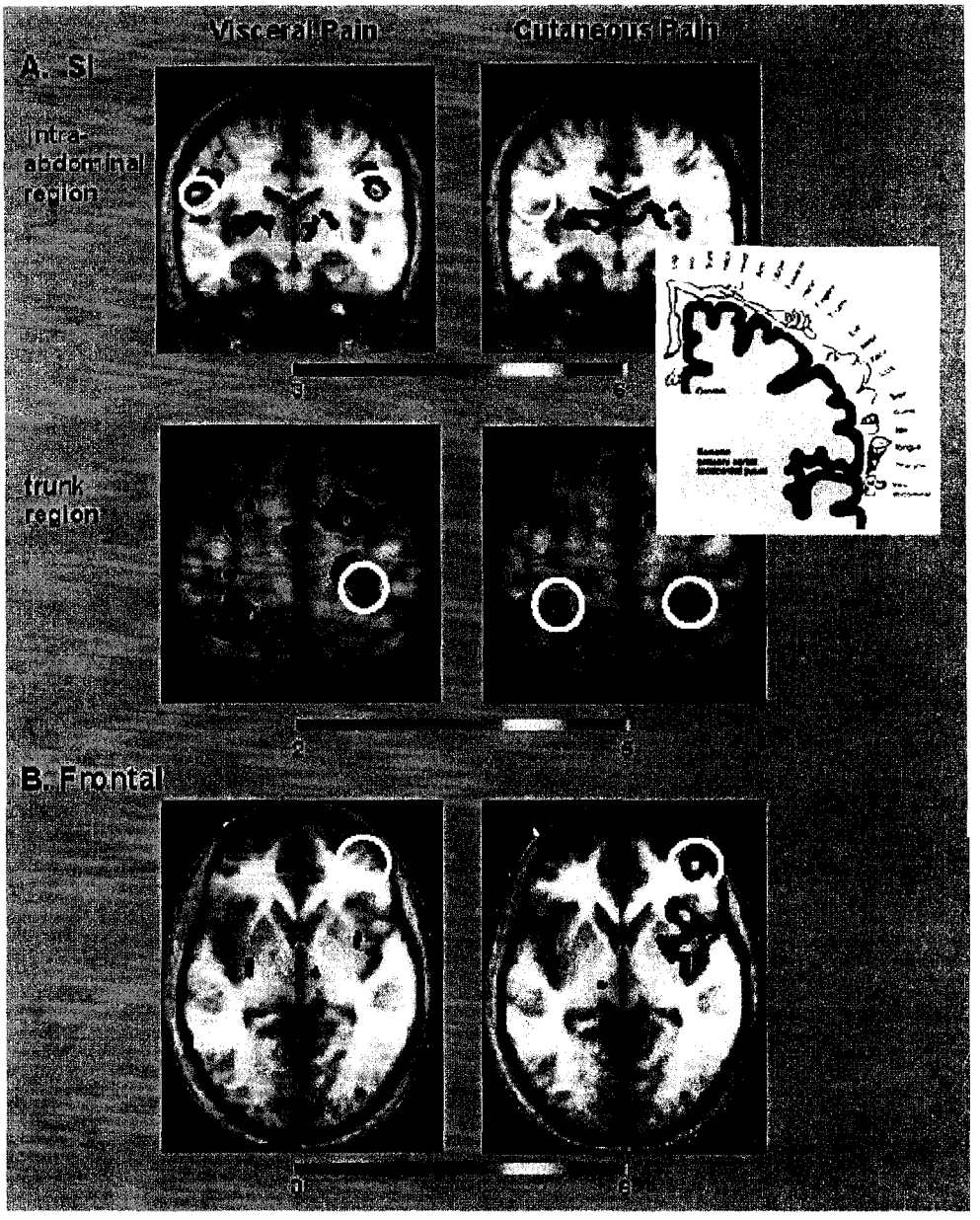
** $p < 0.01$ (paired Student t-test)
* $p < 0.5$ (paired Student t-test)

Figure 3-4: Pain-evoked cerebral activity - Differences



Cortical activation evoked by esophageal distention (**a**) and thermal heat (**b**). **a**) Painful visceral but not cutaneous stimulation significantly activated primary motor cortex (MI, face area) on the right ($p < 0.05$) with a similar tendency on the left ($p = 0.1$, paired t-test), and more rostral part of the anterior cingulate cortex (ACC); **b**) Painful cutaneous but not visceral stimulation resulted in significantly higher activity in bilateral anterior insula ($p < 0.05$, paired t-test); more posterior part of the ACC was activated by cutaneous heat. (c.f. Tables 1 and 2 for stereotaxic coordinates and t-values). Colour bars show t-values.

Figure 3-5: Pain-evoked cortical activity – Differences (cont'd)



Cortical activation evoked by esophageal distention and thermal heat in primary somatosensory (SI) (**a**) and frontal (**b**) cortices. **a**) Visceral but not cutaneous stimulation significantly activated intra-abdominal region of SI, while both types of stimulation showed small activation in the trunk region of SI (heat – bilateral, balloon – right); **b**) Cutaneous but not visceral stimulation activated right frontal cortex (BA 10/46). (c.f. Tables 1 and 2 for stereotaxic coordinates and t-values. Colour bars show t-values.

3.5 DISCUSSION

Results of the present study indicate that in humans, visceral and cutaneous pain of similar intensity activate a wide cortical and subcortical network, including secondary somatosensory, parietal association and medial prefrontal cortices, as well as basal ganglia, thalamus and cerebellum; this is consistent with the complex nature of the pain experience and the pattern of activation found in previous pain studies. However, in addition to these similarities, a number of limbic areas, including insular and anterior cingulate cortices, primary somatosensory, motor and frontal cortices, show differential involvement during painful stimulation of skin and viscera; this is consistent with divergent qualitative sensations, emotional experiences and behavioural reactions associated with visceral and cutaneous pain.

3.5.1 Limbic System

3.5.1.1 Insular Cortex:

Our results demonstrate a differential involvement of the anterior insula in the processing of visceral and cutaneous pain. Painful visceral stimulation was associated with activation of the right anterior insula, whereas painful cutaneous heat resulted in significantly higher and bilateral activation of anterior insula. The majority of PET and fMRI studies in humans show activity in anterior insula following painful tonic and phasic heat stimulation (Coghill et al., 1994; Svensson et al., 1997; Derbyshire & Jones, 1998; Becerra et al., 1999). Furthermore, anterior insula was the only brain region significantly activated in a study by Coghill et al. (1994) when painful and vibrotactile stimulations were directly compared, suggesting direct involvement of this brain structure in nociceptive processes. This is also consistent with the existence of nociceptive neurons in this region seen with the neurophysiological recordings in primates (Craig & Dostrovsky, 1999; Craig, 2000). Furthermore, its anatomical connections with primary and secondary somatosensory cortices and anterior cingulate cortex (Mufson & Mesulam, 1982), as well as several thalamic nuclei known to contain a high number of nociceptive neurons (Craig et al., 1994), additionally support the role of anterior insula in nociceptive processing.

Activity in anterior insula is also observed during innocuous warm and cool temperatures (Craig et al., 1996; Davis et al., 1998; Craig et al., 2000; Fulbright et al., 2001). Craig et al. (2000), when examining cerebral activity during innocuous cooling, proposed that the activation of anterior insula is a result of 'subjective evaluation of thermal stimuli', and they also found a significant correlation between right anterior insula activity and stimulus temperature, suggesting that this region might play a role in thermal evaluation as well. Furthermore, the anterior insula has strong connections with the posterior part of the ventromedial thalamic nucleus (VMpo), which in turn contains thermoreceptive neurons and evokes thermal sensations during electrical stimulation in humans (Lenz et al., 1993; Craig et al., 1994). Finally, VMpo-anterior insula projections are thought to constitute an important temperature pathway involved in temperature inhibition of pain (Craig et al., 1996), which was corroborated by findings from the study on thalamic microstimulation in patients with chronic pain (Duncan et al., 1998).

Our results support the nociceptive role of the anterior insula, since both visceral and cutaneous pain resulted in its activation. However, while pain intensities were identical during visceral and cutaneous stimulation, the activity following thermal pain in anterior insula was significantly higher than that following esophageal distention; this makes the preferential role of the anterior insula in temperature sensation a likely possibility. Furthermore, the activation of the right anterior insula during visceral pain could also result from the heart-burn-like sensation produced by the esophageal balloon that we have previously observed (Strigo et al., 2002).

We also found that visceral pain activated left, and cutaneous pain bilateral posterior insula; and both conditions resulted in activity in the mid-insula. Generally, insular cortex is associated with autonomic responses to various stimuli and is thus considered to play a major role in visceral sensations (Augustine, 1996). Furthermore, anatomical studies on anterograde and retrograde labeling in rats show that special visceral sensations (i.e. gustatory) are represented in the anterior insula, while general visceral sensations (i.e. cardiovascular, respiratory and gastric) are located more posteriorly (Cechetto & Saper, 1987). In addition, extracellular unit recordings from rat insula confirm this viscerotopy, yet also demonstrate that many neurons receive

convergent inputs from baroreceptor, chemoreceptor, gustatory, and nociceptive organs in the region between the taste and visceral areas (Hanamori et al., 1998). Human data using direct intraoperative stimulation of the insular region mapped a wide pattern of activation throughout the entire insular region, including visceral and somatic sensory as well as motor responses (Penfield & Faulk, 1955). Finally, a study analyzing field potentials in rats showed the possible representation of somatic pain within the visceral or posterior insula (Ito, 1998). These data suggest that visceral and somatic representation is quite complex within the insular cortex, which would explain the wide activation pattern within the area during both visceral and cutaneous pain found in our study.

3.5.1.2 Anterior Cingulate Cortex

Cutaneous pain activated the more posterior, or midcingulate, part of the ACC compared to visceral pain, which in turn, resulted in more rostral ACC activity. Similar topography has been observed by Lotze et al. (2001) during visceral and somatic stimulation, where visceral stimulation induced by rectal balloon-distention activated a more anterior part of the ACC when compared to the somatic stimulation induced by the distention of the anal canal (Lotze et al., 2001).

Experimental evidence suggests that the midcingulate region is involved in response selection, cognition and pain (Devinsky et al., 1995; Vogt et al., 1996). Activation within this region is consistently seen in human imaging studies with PET and fMRI following painful stimulation (Talbot et al., 1991; Coghill et al., 1994; Casey et al., 1996; Svensson et al., 1997; Svensson et al., 1998). Furthermore, in animals, this region is known to contain nociceptive neurons and receive significant inputs from the thalamic nuclei containing nociceptive neurons (Craig, Jr. et al., 1981; Craig, Jr. et al., 1982; Sikes & Vogt, 1992; Vogt et al., 1993). In addition, surgical lesions within this region relieve chronic pain in humans and result in decreased pain sensitivity in animals (Hurt & Ballantine, Jr., 1974;Vaccarino & Melzack, 1989). However, there is a strong possibility that the role the midcingulate plays in nociception is very closely related to the affective-motivational component of pain (Vogt et al., 1996). Several reports have confirmed activity in this region during tasks with strong emotional value, such as visual recognition of emotional faces or Stroop interference task using sad words (George et al., 1993;

George et al., 1994). Similarly, activity in this region strongly correlated with unpleasantness to noxious heat stimulus (Tolle et al., 1999).

On the other hand, the part of the ACC activated by visceral pain in our study is located more rostrally in the cingulate gyrus, closer to the perigenual region, which is thought to be responsible for visceromotor control, vocalization and affect (Devinsky et al., 1995; Vogt et al., 1996). Electrical stimulation of the ventral part of the perigenual region (BA 25) (just anterior to the activity seen in this report) produces various visceral responses including nausea, vomiting, salivation and others (Pool & Ransohoff, 1949; Lewin & Whitty, 1960), which would be consistent with the responses associated with esophageal stimulation in our study. The important role that the perigenual cortex plays in affect is shown in studies of electrical stimulation of the human cingulate, which evokes various emotional responses including fear, pleasure, and agitation (Meyer et al., 1973; McGraw et al., 1976). Furthermore, human brain imaging studies implicate the rostral ACC in the emotional experience associated with guilt, anger, and recollection of traumatic events in trauma-exposed individuals (Dougherty et al., 1999; Shin et al., 1999; Shin et al., 2000).

Indeed, activation of the 'affective' division of the ACC by visceral pain is consistent with the higher unpleasantness ratings observed in our study. Moreover, activation of the same ACC region has been reported by Rainville et al. (1997) in which hypnotic suggestions were given to increase the unpleasantness of painful stimuli in healthy human subjects. This region was also activated by tonic, but not phasic, painful heat, when capsaicin was applied to subject's foreheads, and nitroglycerin-induced cluster headache (Hsieh et al., 1996; Svensson et al., 1998; May et al., 1998). All these stimuli would be considered more unpleasant than any short-lasting, noxious stimuli. In addition, recent animal data show that destroying neurons in the rostral ACC only diminishes affect associated with pain but not acute pain behaviours (Johansen et al., 2001).

3.5.2 Somatosensory System

Both visceral and cutaneous pain resulted in similar activity in the secondary somatosensory cortex (SII); this result was anticipated due to the involvement of parietal opercula in pain processing. SII activation is consistently seen in human brain imaging studies following both visceral and somatic painful stimulation (Talbot et al., 1991; Coghill et al., 1994; Aziz et al., 1997; Binkofski et al., 1998). Although animal data suggest that less than 3% of SII neurons respond to noxious stimulation (Robinson & Burton, 1980), a recent human study that recorded evoked potentials in the area following cutaneous laser stimulus argues for the existence of direct nociceptive input into SII (Lenz et al., 1998). Furthermore, data from fMRI and MEG studies suggest that SII is the primary cortical target for esophageal afferent fibers (Binkofski et al., 1998; Schnitzler et al., 1999); non-painful esophageal stimulation produces activity within SII cortex as well (Binkofski et al., 1998; Kern et al., 1998). In addition, non-painful rectal distention similarly activates the SII area (Aziz et al., 2000; Hobday et al., 2001). Therefore, if the SII cortex does indeed play a more important role in visceral sensation, it could perhaps explain the slightly higher t-values of SII activation associated with visceral stimulation in our study.

Distal esophageal distention activated the inferior part of the primary somatosensory cortex (SI), which represents the intra-abdominal region in the sensory homunculus (Penfield & Rasmussen, 1955). A recent histological study showed that this area receives extensive vagal projections and likely represents a visceral part of SI (Ito, 2001). Similar activation has been observed following non-painful distention of the distal esophagus and the rectum (Aziz et al., 2000; Hobday et al., 2001), suggesting that it is indeed a site of visceral representation. However, ROI analyses of the visceral SI in our study did not demonstrate a significant difference between visceral and cutaneous pain, despite the absence of significant activity in this region during the latter condition. This result is most likely attributable to variable activation within this region and its proximity to the extensive activation of the secondary somatosensory cortex by cutaneous stimulation. Interestingly, esophageal distention resulted in a sub-threshold activity in the trunk area of SI ($t = 2.8$), suggesting the possibility of the referral of the esophageal pain to the chest, a phenomenon which we have previously observed psychophysically (Strigo et al., 2002).

3.5.3 Motor System

3.5.3.1 Primary Motor Cortex and Supplementary Motor Area

Painful visceral stimulation resulted in bilateral activity in the primary motor cortex (MI), which was higher than that observed during painful cutaneous stimulation on the right and left sides. The area of the MI activated by esophageal distention most likely corresponds to the area responsible for vocalization and salivation (Penfield & Rasmussen, 1955), and has previously been activated by distal esophageal distention (Aziz et al., 2000). The higher MI activity found in our study following visceral pain is not surprising since esophageal balloon distention induces salivation and peristaltic contractions (Yamamoto et al., 1998), and is more likely to produce a desire to vocalize than thermal heat. Moreover, a similar area of MI has been activated by swallowing (Hamdy et al., 1999), another consequence of balloon-distention.

Visceral pain also resulted in higher activation of a supplementary motor cortex (SMA) as compared to cutaneous pain, the activation of which was just below significance ($t = 4.2$). This area is thought to be responsible for motor control, motor planning and execution (see Picard & Strick, 1996 for review). Moreover, its activation has previously been observed in other pain studies and is probably the result of a desire to avoid noxious stimuli (Coghill et al., 1994; Iadarola et al., 1998; Becerra et al., 1999; Kwan et al., 2000), which would explain the higher activity following visceral stimulation.

3.5.3.2 Cerebellum, Thalamus and Basal Ganglia

Both types of stimulation resulted in similar bilateral cerebellar activity. Several loci of activation were noted during visceral and cutaneous pain, which is consistent with the absence of topography in this brain structure (Shambes et al., 1978; Bower & Kassel, 1990). The cerebellum has been implicated in the control of various functions, including motor, sensory, cognitive, and, according to the recent evidence, nociceptive (Ekerot et al., 1991; Gao et al., 1996; Bower, 1997; Saab et al., 2000). Several human and animal imaging studies reported activity in the cerebellum following painful somatic and visceral stimulation (Casey et al., 1994; Svensson et al., 1997; Derbyshire et al., 1998; Iadarola et al., 1998; Becerra et al., 1999; Saab et al., 2000; Mertz et al., 2000). Furthermore, in a recent study, electrical and chemical stimulation

of cerebellar cortex suggested that the cerebellum plays a role in the modulation of visceral and somatic nociceptive responses (Saab et al., 2001), and it is very likely that this modulatory role is similar during pain arising from both skin and viscera (Saab & Willis, 2001; Saab et al., 2001). Our results did not show differential activation in the cerebellum following visceral and cutaneous pain, suggesting that cerebellar activation most probably does not depend on the type and origin of stimulated fibers, but rather that it plays a modulatory role in nociception as well as cognition and associated motor responses.

In the present study, both visceral and cutaneous pain resulted in similar thalamic activity, which was more pronounced on the left side. According to the stereotaxic coordinates (Talairach & Tournoux, 1988), this activation most probably corresponds to the ventral lateral nuclei. These nuclei are part of the ventrolateral group of thalamic nuclei thought to be involved in motor control and information relay among the basal ganglia, cerebellum and motor cortex (Amaral, 2000). Therefore, the cerebellar activation noted above, as well as the activity observed in the putamen during visceral and cutaneous pain, is probably a part of the same circuit and is most likely due to the suppression of motor events in the fMRI scanner.

3.5.4 Frontal Cortex

Cutaneous, but not visceral pain highly activated the right frontal lobe, consistent with Brodman Area 10/46. Three out of seven subjects showed activity in this region following cutaneous heat stimulation, suggesting the involvement of this area in cognitive, rather than nociceptive, processes. Similar frontal activity has been previously observed in human pain studies. Coghill et al. (1999) found a negative correlation between frontal activation and the perception of pain intensity evoked by noxious heat stimuli, attributing it to episodic memory associated with rating the stimulus (Coghill et al., 1999). Furthermore, Hsieh et al. (1999) found decreased activity in this region when they compared an habitual-pain state to a pain-alleviated state in trigeminal neuropathy patients (Hsieh et al., 1999); this is consistent with an involvement of the medial frontal lobe in stimulus evaluation, as proposed by Coghill et al. (1999). Our data suggest a more precise role for this region in stimulus evaluation related to a spatial component. Both the cutaneous stimulation in this study and the stimuli used by Coghill et al. (1999) were well localized, and both result in activity in the right BA 10/46. On the other

hand, both the painful conditions studied by Hsieh et al. (1999) and the visceral stimulation in our study did not have a precise spatial localization, and therefore, either resulted in a decrease or no change in signal in this area.

In addition to the right frontal cortex, medial prefrontal cortex (MPFC) was activated by visceral pain bilaterally and by cutaneous pain on the right. Activity in the MPFC is generally associated with emotions and has been shown in human imaging studies when pleasant, unpleasant, happy, sad and disgusting visual stimuli were compared to neutral (Lane et al., 1997a; Lane et al., 1997b; Teasdale et al., 1999). Based on these studies, activity in this area has been allocated to a general hedonic value irrespective of the valence associated with it. Several pain studies also observed MPFC activity following phasic and tonic heat stimulation in normal subjects, and in patients with atypical facial pain (Derbyshire et al., 1994; Xu et al., 1997; Derbyshire et al., 1998;Paulson et al., 1998), which could also be a result of the hedonic value of painful experience. Therefore, it is plausible that the bilateral activation of the MPFC during visceral pain stimulation results from higher unpleasantness associated with it in our study as compared to the cutaneous stimulation, which was less bothersome and thus only activated medial prefrontal cortex on the right.

3.5.5 Hemispheric Lateralization

Both visceral and cutaneous pain resulted in higher t-scores for activation in the right compared to the left hemisphere in SII and the anterior insula, as well as frontal cortex during the cutaneous pain condition. Since we stimulated the midline during both conditions, we did not expect a significant hemispheric lateralization of the activation. Furthermore, a recent study comparing heat pain-related activation of the two hemispheres found no hemispheric preference in the processing of painful heat in the somatosensory and insular cortices (Coghill et al., 2001).

Hemispheric lateralization following visceral stimulation is not clear since the majority of organs studied are midline organs, which would assume bilateral brain activity. As a result, previous MEG studies have shown that the distal esophagus is represented bilaterally in insular cortex and SII (Furlong et al., 1998; Schnitzler et al., 1999). On the other hand, preferential

activation of right anterior insula following painful distal esophageal distention has been observed with PET and fMRI techniques (Aziz et al., 1997; Binkofski et al., 1998), while painful rectal balloon distention also resulted in the predominant activity in the right insula (Baciu et al., 1999). In addition, more neurons with convergent visceral and somatic inputs have been identified in the right anterior insula of primates (Zhang et al., 1999), which could perhaps explain the lack of activity in the left anterior insula during esophageal-balloon distention in our study.

3.6 CONCLUSION

The results of the present study demonstrate that visceral and cutaneous pain of similar intensity are represented differently in the somatosensory, motor and limbic areas of the brain, which could explain the diverse reactions associated with stimulation within skin and viscera. However, the overlap in activation sites observed in the present study supports the existence of a common cortical network independent of the nature of the painful stimulus.

In addition, we have shown that esophageal balloon distention and thermal stimulation of the midline chest are comparable visceral and cutaneous pain models, respectively, that can be reliably used for the further comparison studies.

3.7 ACKNOWLEDGEMENTS

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Chapter 4

THE EFFECTS OF RACEMIC KETAMINE ON PAINFUL STIMULATION OF SKIN AND VISCERA IN HUMAN SUBJECTS

One of the major clinical challenges presented by visceral pain is the difficulty in finding adequate treatments. This is why it is important to look for new, more effective and specific analgesic drugs. NMDA-receptor antagonists provide such an opportunity, since recent evidence suggests their involvement in the processes mediating hyperalgesia and allodynia. From the discussion in previous chapters, we have seen that visceral and cutaneous pain elicit different perceptual qualities that can be explained by differences in cerebral processes seen in Chapter 3. Differences in cerebral processing of visceral and cutaneous pain may, in turn, suggest differential pharmacological mechanisms underlying the two. NMDA-receptors offer a suitable model for studying NMDA-related analgesic responses, since they have a wide distribution in the periphery, spinal cord and the brain, and it is still not evident whether they play a similar or differential role in the processes underlying visceral and cutaneous pain in humans. The following chapter describes a comparison between visceral and cutaneous pain dependence on NMDA-receptor-mediated processes with the use of the non-competitive NMDA-receptor antagonist, ketamine.

4.1 ABSTRACT

Evidence suggests that NMDA-receptors may play a differential role in visceral and somatic pain. Specifically, animal data indicate an analgesic role for NMDA-R antagonists in acute visceral, but not acute somatic pain. In humans, analgesic effects are documented in acute somatic pain, but only anecdotal reports exist for visceral pain. We therefore conducted a study comparing the role of ketamine in acute experimental visceral and cutaneous pain in humans.

In a double-blind, randomized, cross-over study, 12 healthy volunteers (3M, 9F) underwent i.v. ketamine or saline infusion in two sessions. Subjects evaluated perceptions induced by balloon distention of the distal esophagus and contact heat on the upper chest during continuous computer-controlled infusion of either ketamine (60 and 120 ng/ml) or saline. Two stimulus intensities producing non-painful and painful sensation were used. Subjects reported maximum pain intensity and unpleasantness on visual analog scales (VAS) and chose phrases from the McGill Pain Questionnaire (MPQ).

Ketamine decreased, in a dose-dependent fashion, the VAS ratings of pain intensity and unpleasantness evoked by both visceral and cutaneous pain. Ketamine also reduced the number of sensory and affective words chosen for visceral pain, and the number of sensory words for cutaneous pain. Ketamine had no effect on either innocuous esophageal or cutaneous stimulation. Finally, most subjects reported minimal side effects, which included insobriety, light-headedness and indifference.

Our results confirm the analgesic effects of low-dose ketamine, with minimal side effects, on cutaneous heat pain and indicate a similar effect on acute visceral pain. The reduced verbal scores for affect in visceral, but not cutaneous, pain may reflect a differential effect of NMDA-R antagonists for the two pain states observed in animal models.

4.2 INTRODUCTION

Recent evidence indicates that NMDA receptors (NMDA-Rs) are involved in pain processing. Specifically, it has been shown that NMDA-R transmission is one of the major mechanisms in the development of central sensitization and wind-up, resulting in allodynia and hyperalgesia associated with chronic pain (Woolf & Thompson, 1991; Fisher et al., 2000). In animals, both systemic and spinal administration of NMDA-R antagonists inhibit wind-up in normal, monoarthritic and neuropathic rats (Qian et al., 1996; Laurido et al., 2001; Suzuki et al., 2001), while subcutaneous injection reduces inflammation associated with arthritic dorsal flexion pain (Wang et al., 2000). Furthermore, pretreatment with an NMDA-R antagonist reduces c-*fos* expression in the lumbar spinal cord induced by lower urinary tract irritation with acetic acid (Birder & de Groat, 1992), as well as repetitive noxious and innocuous colorectal distention (Zhai & Traub, 1999). In humans, NMDA-R antagonists reduce wind-up associated with repetitive electrical nerve stimulation, as well as the intensity and spread of pain (Guirimand et al., 2000), while intravenous and epidural injections reduce hyperalgesia and allodynia associated with neuropathic pain following nerve and/or burn-injuries, as well as topical capsaicin application (Andersen et al., 1996; Ilkjaer et al., 1996; Warncke et al., 1997; Takahashi et al., 1998; Leung et al., 2001). Subcutaneous, oral, and intramuscular applications, however, produce variable effects in patients with chronic orofacial pain and following capsaicin injection (Mathisen et al., 1995; Rabben et al., 1999; Gottrup et al., 2000), suggesting that the administration route plays a role.

The involvement of NMDA-Rs in acute visceral and somatic nociceptive processes is more obscure. In animals, the majority of recent reports show that intravenous and/or intrathecal application of various NMDA-R antagonists decreases acute visceral nociceptive responses following colorectal and/or ureter distention (Iwasaki et al., 1991; Olivar et al., 1999; McRoberts et al., 2001; Ji et al., 2001; Kozlowski et al., 2000), but does not affect acute somatic responses induced by graded pinch, hot plate or formalin injection (Nishiyama et al., 1998; Olivar et al., 1999; McRoberts et al., 2001). A similar lack of effect by an NMDA-R channel blockers has been observed during the tail flick test following intrathecal application in mice

(Lutfy et al., 1997), but not following intraperitoneal injection in rats (Iwasaki et al., 1991), thus suggesting the importance of route of administration during acute pain states as well.

In humans, the role of NMDA-R related transmission in acute, short-lasting pain has not been clearly identified. Only two reports have examined acute somatic pain in humans. Contrary to the majority of animal findings, they both found analgesic effects of intravenous application of NMDA-R blockers on electrical, thermal and pressure pain (Arendt-Nielsen et al., 1996; Smith et al., 2001). None of the studies published to date examined the role of NNDA-Rs in acute visceral pain transmission.

Ketamine is one of the most potent clinically available NMDA-R antagonists for use in humans. Its analgesic role is most probably due to the partial blocking of NMDA-Rs (Hustveit et al., 1995; Fisher et al., 2000). Since intravenous application seems to produce the most consistent results in both animals and humans, we used systemic ketamine in this study to directly compare the role of NMDA-Rs in acute cutaneous and acute visceral pain in human volunteers.

4.3 METHODS

4.3.1 Subjects

With approval from the McGill University Institutional Review Board, we studied 13 healthy volunteers (3 males, 10 females) ranging in age from 18 to 38 years (mean age 28.2). None of the subjects was obese (mean BMI 23.6). Subjects were excluded from the study if they were under 18 or over 40 years of age, pregnant or breast-feeding, had a history of cardiovascular, gastrointestinal, neurological disease or any chronic pain condition, were taking any medication or had a history of substance abuse. Additional exclusion criteria were the presence of a strong gag reflex and frequent nausea. Each subject underwent esophageal manomentry (MMS-100, Narco-Bio Systems, Austin, TX, USA) to identify the position of the lower esophageal sphincter in order to accurately position the probe. One female subject did not complete the experiment due to discomfort caused by the balloon and was not included in the analysis.

4.3.2 Intraesophageal Balloon-Catheter

A custom-designed polyethylene balloon (square type) 8 cm in length, 6 cm in diameter, with a maximum volume of 70-80 ml was attached to a multilumen polyvinyl esophageal catheter 10 cm above the tip (Mui Scientific, Mississauga, ON, Canada). The catheter was attached to a pump. One of the three lumens in the catheter was connected to a pressure transducer, the second lumen was attached to the piston on a pump (G&J Electronics, Toronto, ON, Canada) which was used to inflate the balloon with air at a rate of ~ 50 ml/sec, and the third lumen served as the motility port to measure the position of the lower esophageal sphincter.

4.3.3 Drug Infusion and Assays

Infusion of racemic ketamine (Ketalar®, Park-Davis) or saline was performed with a computer-controlled pump (Stanpump, Harvard 22 Basic Syringe Pump®, Harvard Apparatus, South Natick, MA, USA) according to Domino's weight-adjusted parameters (Domino et al., 1984). The infusions were randomized by the hospital pharmacy. The target plasma concentrations set on the pump for the ketamine/placebo infusions were 60 $ngmL^{-1}$ and 120 $ngmL^{-1}$, during periods II and III, respectively (see Figure 4-1). Plasma samples were analyzed with an enantio-

selective high performance liquid chromatography method for S-ketamine, R-ketamine, nor-S-ketamine and nor-R-ketamine.

4.3.4 Study Protocol

4.3.4.1 Baseline Determination of Thermal Stimulus Intensities

Each subject participated in two separate experimental sessions (drug vs. placebo) conducted on two different days in the early morning after an overnight fast in a double-blind, randomized, cross-over approach. The experimental sessions were identical except for two factors: the drug/placebo infusion, and the baseline determination of thermal and esophageal stimulus intensities (see below), which were performed only on the first day. During each of the two study days subjects were dressed in a hospital gown and lay comfortably in a reclining chair (Care-cliner, Winco, Ocala, FL). On the first study day subjects received a thorough explanation of the experimental procedures and signed the informed consent. Then the thermal sensitivity and pain thresholds were determined on the chest using a 9-cm^2 Peltier-type contact thermode (Medoc, Ramat Yishai, Israel). Ten-sec heat stimuli were presented using an ascending series of predetermined constant stimuli (range from 40 to 50 °C). Heat pulses were separated by at least 60 sec, during which time the baseline was 30 °C. The rate of temperature increase was always 5°C/sec. After each stimulus, subjects were asked to rate the maximum sensation of warmth (if the stimulus produced no pain), the maximum pain sensation intensity (if the stimulus was painful), and the maximum unpleasantness evoked by the stimulus on separate visual analog scales (VASs). All scales ranged from 0 ('no sensation', 'no pain sensation' or 'not at all unpleasant'), to 100 ('extremely warm sensation' – described as pain threshold, 'extremely intense pain sensation', or 'extremely unpleasant').

4.3.4.2 Baseline Determination of Esophageal Distention Intensities

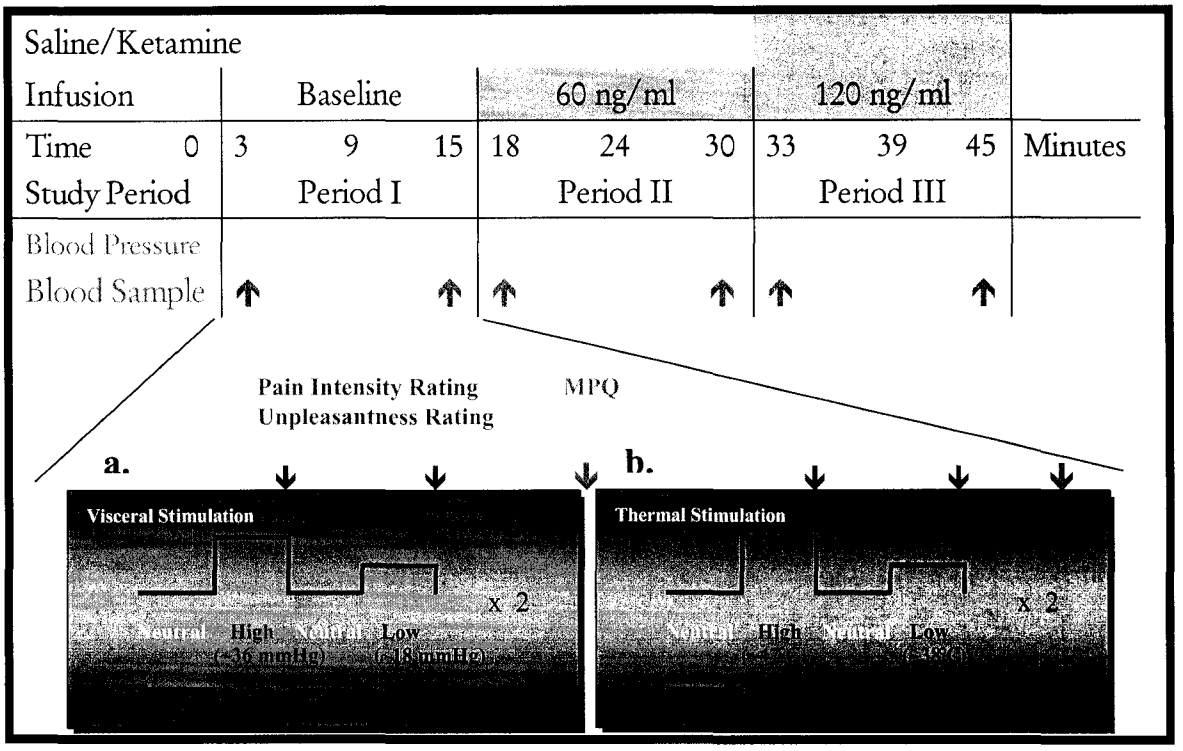
A short-acting local anesthetic ('Xylocaine') was sprayed on the pharyngeal mucosa. The esophageal balloon catheter was then passed orally into the esophagus to a position five cm above the lower esophageal sphincter. Testing started after the effects of the anesthetic had worn off. Before determining esophageal sensitivity and pain threshold, we adjusted the baseline pressure for each subject so that the balloon was inflated but not perceived. Then a

series of 30-sec predetermined constant stimuli (range from 12 to 44 mmHg) were administered to assess the sensory detection and pain thresholds for each subject. Stimuli were separated by at least 60 sec during which time the baseline was 6 mmHg. At the end of each distention subjects were prompted to rate the maximum sensation attained during the stimulation period using the VAS scales described above (where 'pressure' was substituted for 'warmth').

4.3.4.3 Comparison of Esophageal and Cutaneous Sensations and Drug Administration

After the thermal and esophageal sensitivities had been determined, two intravenous cannulae were inserted, one in each arm, for ketamine/saline infusions and for drawing blood samples, respectively. The experiment that followed consisted of three periods (Figure 4-1), during which no ketamine/placebo, (60 $ngmL^{-1}$ target level concentration) ketamine/placebo or (120 $ngmL^{-1}$ target level concentration) ketamine/placebo were administered to each subject intravenously. Except for the drug concentration, the periods were identical: each lasted approximately 15 min and consisted of an esophageal distention sequence followed by a thermal stimulation sequence. The sequences, individually created for each subject, were identical for esophageal and cutaneous stimulation and are shown in Figure 4-1a and 4-1b, respectively. Each sequence consisted of stimuli of two different intensities (high – always painful and low – perceived but not painful) presented in quasi-random and counterbalanced order and repeated two times. Each stimulus was equal in duration and was presented for 30 sec. A non-invasive sphygonamoneter device was placed on the arm used for blood-sampling and a pulse oximetry probe was positioned on a finger of the same arm. Blood samples were drawn and systolic/diastolic blood pressure was measured at the beginning and the end of each period (Figure 4-1), while heart rate and oxygen saturation were monitored continuously throughout the experiment.

Figure 4-1: Experimental design



Stimulation sequences for esophageal-balloon distention (**a**) and thermal heat (**b**). Stimuli were presented in quasi-random and counterbalanced order.

4.3.4.4 Response Measures

After each stimulus in the comparison experiment, subjects were asked to rate their sensation of pain or warmth/pressure and unpleasantness using visual analog scales (VASs) explained above (Figure 4-1). At the end of each stimulation sequence (i.e. esophageal and thermal) in each period, subjects were aslo asked to fill out a short form of the McGill Pain Questionnaire (MPQ) to evaluate the drug effects on the qualitative pain description.

4.3.5 Statistical Analysis

A two-way repeated analysis of variance (ANOVA) was used to determine the effects of drug concentration on pain or pressure/warmth and unpleasantness ratings following esophageal and cutaneous stimulation. The post-hoc Tukey test was used to determine the specific effects. Non-parametric analysis using Wilcoxon Signed Rank test was used to analyze the results from McGill Pain Questionnaire. Results are demonstrated as the mean $\pm$ SEM.

4.4 RESULTS

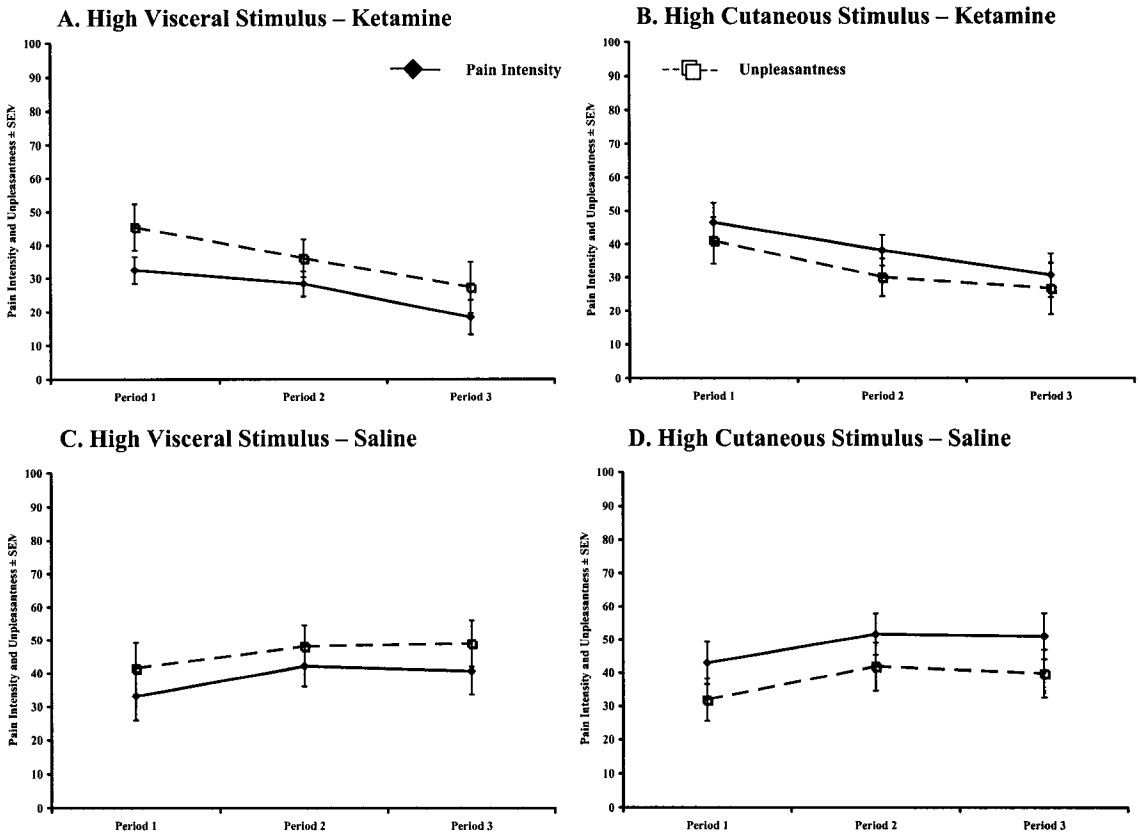
4.4.1 Post Stimulus VAS Ratings - High Intensity Stimulation

The effects of ketamine administration on pain intensity and unpleasantness ratings following high visceral and high cutaneous stimulation are shown in Figures 4-2a and 4-2b, respectively. A repeated measure ANOVA indicated a significant effect of ketamine dose on both pain intensity and unpleasantness ratings following high visceral ($p < 0.005$) and high cutaneous ($p < 0.005$, $n = 12$, repeated two-way ANOVA) stimulation. Post-hoc Tukey tests showed a significant decrease in pain intensity and unpleasantness perception during *Period 3* (120 $ngmL^{-1}$ ketamine) compared to the baseline *Period 1* (0 $ngmL^{-1}$ ketamine) during both visceral and cutaneous stimulation. Although the mean ratings were lower in *Period 2* (60 $ngmL^{-1}$ ketamine) compared to baseline, there was no significant decrease in the perception of pain intensity or unpleasantness during either visceral ($p = 0.5$) or cutaneous stimulation ($p = 0.2$, $n = 12$, Tukey HSD test).

The effects of saline administration on pain intensity and unpleasantness ratings following high visceral and high cutaneous stimulation are shown in Figures 4-2c and 4-2d, respectively. A repeated measure ANOVA revealed no significant effects of *Period* on the perception of pain intensity and unpleasantness during either high visceral ($p = 0.2$) or high cutaneous ($p = 0.1$, $n = 12$, repeated two-way ANOVA) stimulation, suggesting that the effects of ketamine described above were not due to a placebo effect.

In agreement with our previous findings (Strigo et al., 2002), we found a significant difference in the relationship between pain intensity and unpleasantness during visceral and cutaneous stimulation ($p < 0.05$, $n = 12$, repeated two-way ANOVA) during both ketamine and placebo sessions. In both sessions, pain intensity ratings of high visceral stimulation were lower than the corresponding unpleasantness ratings ($p < 0.05$), while those of high cutaneous stimulation were higher than the corresponding unpleasantness ratings (p's < 0.05 - high and low). The data confirm our previous findings of higher unpleasantness associated with visceral pain.

Figure 4-2: Average post-stimulus VAS ratings following high intensity stimulation



a) Average post-stimulus pain intensity and unpleasantness ratings following high visceral stimulation decreased in a dose dependent manner during ketamine administration ($p < 0.005$, repeated two-way ANOVA, $n = 12$); **b)** Similarly, average post-stimulus pain intensity and unpleasantness ratings following high cutaneous stimulation decreased in a dose dependent manner during ketamine administration ($p < 0.005$, repeated two-way ANOVA, $n = 12$); **c)** Saline administration had no effect on subjects' ratings of average pain intensity and unpleasantness following high visceral pressure ($p = 0.2$, repeated two-way ANOVA, $n = 12$); **d)** Likewise, saline administration had no effect on subjects' ratings of average pain intensity and unpleasantness following high cutaneous heat ($p = 0.1$, repeated two-way ANOVA); Mean post-stimulus unpleasantness ratings following high visceral stimulation were higher than the corresponding pain intensity ratings during both ketamine **(a)** and placebo **(b)** sessions ($p < 0.05$, repeated analysis of variance ANOVA, $n = 10$), while mean post-stimulus unpleasantness ratings following high cutaneous stimulation were lower than the corresponding pain intensity ratings during both ketamine **(c)** and placebo **(d)** sessions ($p < 0.05$, repeated analysis of variance ANOVA). Error bars represent SEM.

4.4.2 Post-Stimulus VAS Ratings – Low Intensity Stimulation

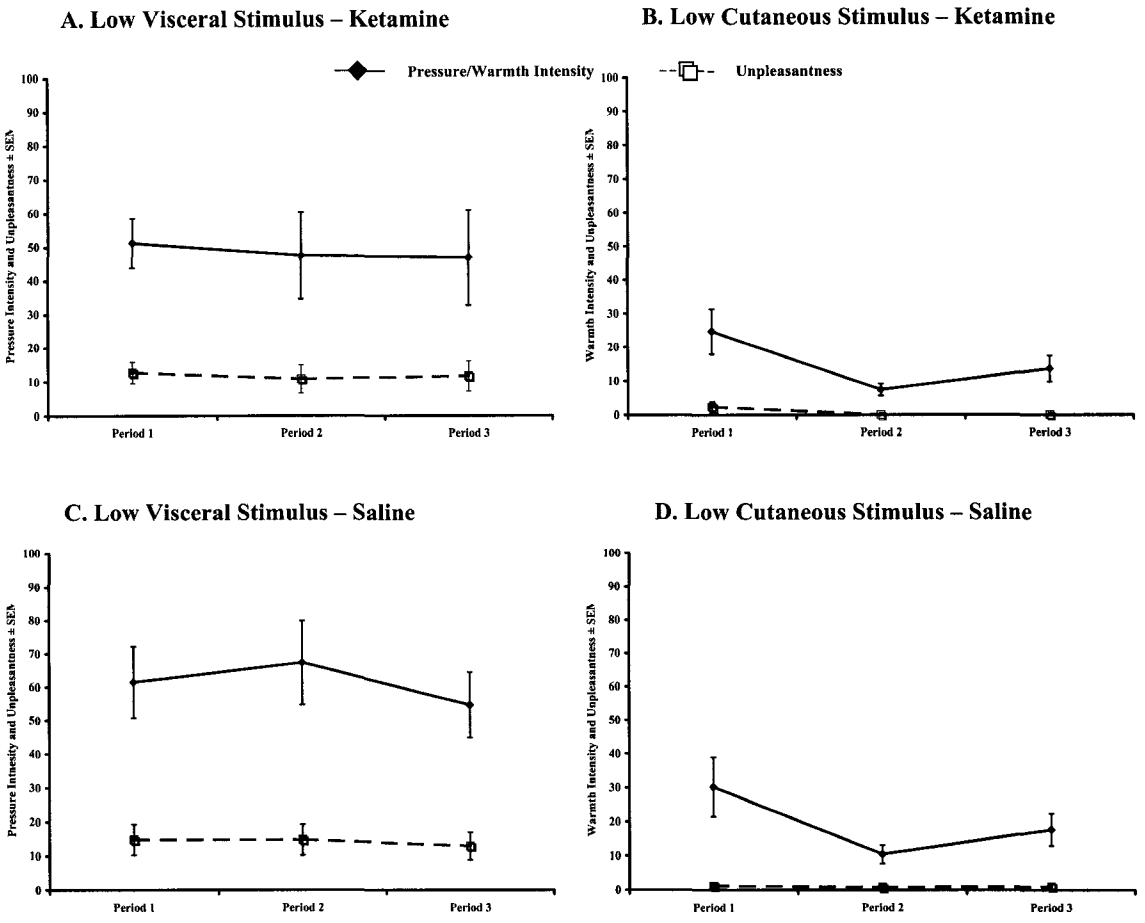
Perceived intensity ratings following low visceral and low cutaneous stimuli are shown in Figures 4-3a and 4-3b, respectively, for the ketamine session, and in Figures 4-3c and 4-3d, respectively, for the placebo session. On average, low intensity stimuli were not painful to the subjects, thus they used 'pressure' and 'warmth' scales (see METHODS) to rate the intensity of the stimulation, and these are illustrated in the figures.

As can be seen in Figures 4-3a and 4-3c, neither ketamine dose nor repeated saline administration affected the subjects' perception of pressure ($p = 0.9$ – ketamine; $p = 0.5$ – placebo) or unpleasantness ($p = 0.9$ – ketamine; $p = 0.7$ – placebo, repeated ANOVA) following low visceral stimulation.

Interestingly, when the effects of ketamine dose on warmth ratings (Figure 4-3b) following low non-painful cutaneous stimulation were examined, a significant decrease in the subjects' perceptions was observed ($p < 0.05$, repeated ANOVA). Post-hoc Tukey tests indicated a significant decrease in warmth ratings in *Period 2* compared to *Period 1* ($p < 0.05$), but not in *Period 3* compared to *Period 1* ($p = 0.1$), or *Period 3* compared to *Period 2* ($p = 0.5$). However, the same effects were seen during saline administration as well (Figure 4-3d). In other words, saline administration significantly lowered ratings of warmth in *Period 2* compared to *Period 1* ($p < 0.05$) but not in *Period 3* compared to *Period 1* ($p = 0.2$), or *Period 3* compared to *Period 2* ($p = 0.5$; repeated ANOVA followed by post-hoc Tukey). This observation suggests that the decrease in warmth perception was not specific to the drug administered, and thus most likely was not due to ketamine-produced analgesia. Similar to the findings from low visceral stimulation, neither ketamine nor saline affected the unpleasantness ratings to low cutaneous stimulus ($p = 0.2$ – ketamine, $p = 0.8$ – placebo, repeated ANOVA).

It is interesting to note that in accordance with our previous observation (Strigo et al., 2002), subjects rated low, generally non-painful visceral pressure stimuli as unpleasant ($p < 0.01$, repeated ANOVA).

Figure 4-3: Average post-stimulus VAS ratings following low intensity stimulation



a) Ketamine administration did not affect post-stimulus pressure and unpleasantness ratings following low visceral stimulation ($p = 0.9$, repeated two-way ANOVA, $n = 12$); **b)** Average post-stimulus warmth and unpleasantness ratings following low cutaneous stimulation decreased during low ketamine dose (60 $ngmL^{-1}$) in *Period 2* ($p < 0.05$, $n = 12$, repeated ANOVA followed by post-hoc Tukey test) but were not different during high ketamine dose (120 $ngmL^{-1}$) in *Period 3* ($p = 0.1$, $n = 12$, repeated ANOVA followed by post-hoc Tukey test); **c)** Saline administration had no effect on subjects' perception of low visceral pressure ($p = 0.7$, repeated two-way ANOVA, $n = 12$); **d)** Similar to the ketamine session, saline administration decreased subjects' perception of warmth during low ketamine dose (60 $ngmL^{-1}$) in *Period 2* ($p < 0.05$, $n = 12$, repeated ANOVA followed by post-hoc Tukey test) but not during high ketamine dose (120 $ngmL^{-1}$) in *Period 3* ($p = 0.2$, $n = 12$, repeated ANOVA followed by post-hoc Tukey test); Low cutaneous stimulus was generally not unpleasant, while low, non-painful visceral pressure resulted in moderate unpleasantness in all subjects during both ketamine (a) and placebo (c) sessions (p's < 0.05, repeated ANOVA, $n = 12$). Error bars represent SEM.

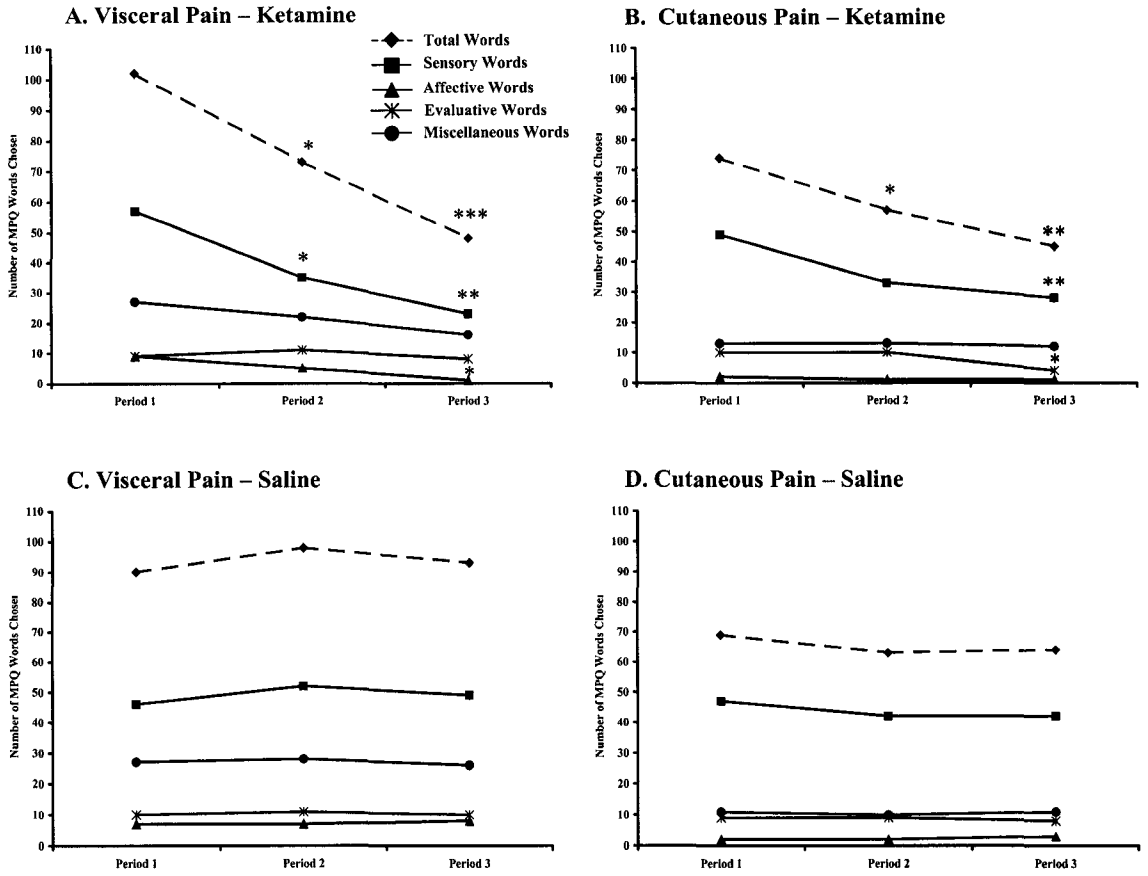
4.4.3 McGill Pain Questionnaire

At the end of the visceral and cutaneous stimulation sequences in each period and in both sessions subjects completed the McGill Pain Questionnaire (MPQ) during which they chose the words that most closely described the sensation of pain produced by either stimulation. The MPQ was analysed by assessing the number of words chosen in each of the four categories (sensory, affective, evaluative, and miscellaneous) and the total number of words chosen in all categories.

Figures 4-4a and 4-4b summarize the number of words chosen to describe visceral and cutaneous pain overall and in each of the four categories after each of the three examination periods during ketamine injection. Ketamine administration resulted in a dose-dependent decrease in the total and sensory number of words chosen during visceral pain (p's < 0.05; Wilcoxon Signed Rank with correction for multiple comparison). During cutaneous pain, ketamine also dose-dependently decreased the total number of words (p's < 0.05) and the number of sensory words at the highest concentration ($p < 0.01$; Wilcoxon Signed Rank with correction for multiple comparisons). In addition, the highest dose of ketamine decreased the number of affective words chosen during visceral ($p < 0.05$) but not cutaneous pain ($p = 0.4$), and the number of evaluative words during cutaneous ($p < 0.05$) but not visceral pain ($p = 0.4$; Wilcoxon Signed Rank with correction for multiple comparison). There was also a tendency for the number of miscellaneous words chosen to decrease during visceral ($p = 0.06$) but not during cutaneous pain ($p = 0.9$), suggesting that ketamine affected most of the qualitative components of visceral stimulation.

Figures 4-4c and 4-4d summarize the number of words chosen to describe visceral and cutaneous pain during the placebo session. Saline had no effect on the total number or the number of words chosen in any of the MPQ categories during either visceral or cutaneous pain (p's > 0.5; Wilcoxon Signed Rank Test), suggesting that the effects seen with the MPQ are not due to the placebo effect.

Figure 4-4: Average number of words chosen from McGill Pain Questionnaire



a) Ketamine dose-dependently decreased the number of total and sensory words chosen following high visceral stimulation, as well as the number of affective words chosen at the highest dose administered (120 $ngmL^{-1}$) (p's < 0.05, $n = 12$, Wilcoxon signed rank test with correction for multiple comparisons); The number of miscellaneous words chosen at the higher dose tended to decrease as well ($p = 0.06$, $n = 12$, Wilcoxon signed rank test with correction for multiple comparisons); **b)** Ketamine dose-dependently decreased the number of total words chosen following high cutaneous stimulation, as well as the number of sensory and evaluative words chosen at the highest dose administered (120 $ngmL^{-1}$) (p's < 0.05, $n = 12$, Wilcoxon signed rank test with correction for multiple comparison); c, d) Saline administration did not affect the number of total, sensory, affective, evaluative, or miscellaneous words chosen during either high visceral (**c**) or high cutaneous (**d**) stimulation (p's > 0.5, $n = 12$, Wilcoxon signed rank test).

4.4.4 Sensitization Effects

As indicated in the introduction, an important role of NMDA-Rs in the development of allodynia and hyperalgesia has been proposed (Woolf et al., 1991). In view of the fact that some subjects experienced sensitization effects, i.e. a significant increase in ratings to the same stimulus with time, to painful visceral and cutaneous stimulation during the placebo session, the effects of hyperalgesia were factored into the analyses to examine whether ketamine effects were more pronounced in the sensitized subjects.

Six subjects (3 females) developed hyperalgesia to high visceral and five subjects (all females) to high cutaneous stimulation. None showed signs of allodynia to either low visceral or low cutaneous stimulation. On the contrary, significant habituation to low non-painful cutaneous stimuli was seen in *Period 2* of the placebo session in ten subjects, which is indicated by the decrease in the warmth rating mentioned above. A repeated measure ANOVA showed no significant effects of either cutaneous ($p = 0.7$) or visceral ($p = 0.4$) sensitisation on the analgesic effects of ketamine at either concentration, suggesting a similar response to the drug in both sensitised and non-sensitised subjects.

4.4.5 Gender Effects

Gender effects were examined in this study. However, because of the small number of male subjects (3) compared to female subjects (9), the results of this analysis should be interpreted with caution. We observed no interaction between gender and the effect of ketamine on the perception of pain intensity and unpleasantness following either visceral or cutaneous stimuli ($p = 0.5$, repeated ANOVA), suggesting that the drug affected both sexes similarly. Nevertheless, small gender effects in pain perception were noted. In particular, higher unpleasantness ratings were seen in female subjects following high cutaneous stimulation across all three periods in both sessions (p's < 0.05, Student t-test: grouping variable gender), while the corresponding intensity ratings were higher in females only during the second period of the ketamine session ($p < 0.05$) and were not different otherwise (p's > 0.2), thus suggesting that cutaneous pain is more unpleasant for female subjects. Moreover, these differences were not due to differences in stimulus intensities, since the intensities of both high and low visceral

and cutaneous stimuli did not differ between genders ($p = 0.5$ – low visceral; $p = 0.9$ – high visceral; $p = 0.5$ – low thermal; $p = 0.2$ – high thermal; Student t-test, grouping variable 'gender'). A different pattern, however, emerged during high visceral stimulation, where no gender differences were observed in either the intensity or unpleasantness ratings across both sessions (p's > 0.5, repeated ANOVA), suggesting that both genders are equally affected by visceral pain. In addition, there were no gender differences in the ratings of the low visceral and cutaneous stimuli in all periods of both ketamine and placebo session (p's > 0.3, repeated ANOVA, followed by Student t-test with gender as a grouping variable).

4.4.6 Subjective Side Effects

Since NMDA-R antagonists and ketamine, in particular, are associated with severe psychotomimetic side effects limiting their clinical use (Ghoneim et al., 1985), the subjects' mental state was monitored throughout the experiment. Ketamine doses used in the study were lower than those previously used in normal volunteers (Persson et al., 1999), thus producing minimal sedation in all subjects. Specifically, ketamine at doses employed in this study did not interfere with the ability of subjects to give prompt verbal responses to all presented stimuli, since the time allocated for the responses was constant throughout the periods and sessions. Moreover, all subjects were able to fill out the MPQ after each study period in both sessions. Since the MPQ requires a high level of alertness, these findings further suggest that sedation was minimal at the doses used.

The most common subjective side effects during ketamine and saline administration are summarized in Table 4-1. No side effects were reported during *Period 1* in either session. During ketamine administration 42% of subjects in *Period 2* (ketamine 60 $ngmL^{-1}$) and 67% in *Period 3* (ketamine 120 $ngmL^{-1}$) reported a feeling of insobriety. 33% and 42% of subjects reported light-headedness during *Period 2* and *Period 3*, respectively. Twenty-five percent of subjects during *Period 2* and 58% during *Period 3* experienced dizziness, and 33% of subjects reported indifference at both ketamine concentrations. Four subjects during *Period 2* and three during *Period 3* reported that 'things look strange' following ketamine administration. Only one male subject became moderately nauseated at the very end of ketamine administration in *Period 3*, which resulted in balloon evacuation. He was not disturbed by the episode, and described his

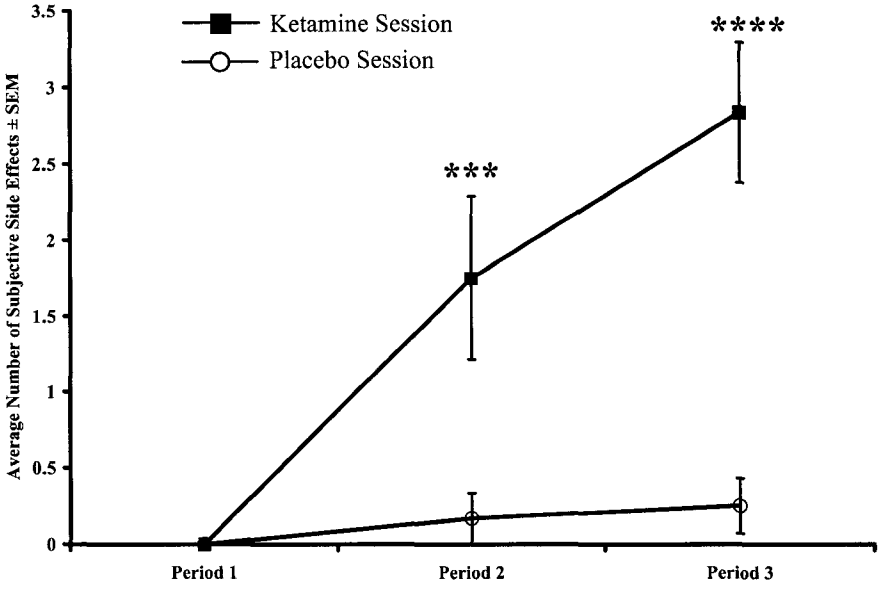
experience as having "no control over the balloon coming out". Generally, all of the subjects reported enjoying the ketamine experience.

To quantify the relationship between subjective side effects, drug/saline injection period and the experimental session, the number of subjective side effects was calculated for each subject within each ketamine/placebo period and is summarized in Figure 4-5. On average, subjects experienced significantly more subjective side effects during ketamine compared to the placebo session ($p < 0.0005$; repeated measures ANOVA). A repeated measure ANOVA also showed a dose-dependent increase in the number of subjective side effects during ketamine ($p < 0.00001$) but not during the placebo ($p = 0.4$) experimental session. Post-hoc Tukey tests showed significant increases in ketamine-related side effects in Period 2 ($p < 0.005$) and Period 3 ($p < 0.0005$) compared to the baseline Period 1, and there was a tendency for an increase in side effects in Period 3 compared to Period 2 ($p = 0.06$). Furthermore, there were no gender differences in side effects observed across periods ($p = 0.5$) or experimental sessions ($p = 0.9$; repeated measures ANOVA).

Table 4-1: Subjective Side Effects During Ketamine and Placebo Runs

	KETAMINE SESSION		PLACEBO SESSION	
	Side Effect (#subjects /total)		Side Effect (#subjects /total)	
PERIOD 1 (0 $ngmL^{-1}$)	-		-	
PERIOD 2 (60 $ngmL^{-1}$)	**Insobriety**	**(5/12)**	Giddy	(1/12)
	Light-Headed	**(4/12)**		
	Indifferent	**(4/12)**		
	Dizzy	**(3/12)**		
	Drowsy	(2/12)		
	Floating	(2/12)		
	Giddy	(1/12)		
PERIOD 3 (120 $ngmL^{-1}$)	**Insobriety**	**(8/12)**	Light-Headed	(1/12)
	Dizzy	**(7/12)**	Dizzy	(1/12)
	Light-Headed	**(5/12)**	Drowsy	(1/12)
	Indifferent	**(4/12)**		
	Floating	(3/12)		
	Drowsy	(1/12)		
	Giddy	(1/12)		

Figure 4-5: Average Number of Subjective Side Effects



The average number of subjective side effects during ketamine sessions (filled squares) increased in a dose-dependent manner with the ketamine administration ($p < 0.0001$, repeated measures ANOVA, $n = 12$), and was significantly higher than the average number of side effects during placebo sessions (open circles) ($p < 0.0005$, repeated ANOVA, $n = 12$), which was constant across session time ($p = 0.4$, repeated measures ANOVA, $n = 12$). Error bars represent SEM.

4.5 DISCUSSION

In this report, we demonstrated that low dose ketamine decreases perceived pain intensity and unpleasantness induced by both noxious visceral and cutaneous stimulation in a dose-dependent manner, but has no effect on the perception of innocuous visceral and cutaneous stimuli. However, ketamine altered the number of affective words chosen for visceral but not for cutaneous pain, suggesting the possibility of a greater role for NMDA receptors in affective visceral than cutaneous pain pathways.

4.5.1 Acute Visceral vs. Acute Somatic Effects

Several animal studies investigating the analgesic role of NMDA-receptors have proposed that NMDA-R-related transmission is more important in acute nociceptive responses involving visceral but not somatic tissues, which in turn, require inflammation and hyperalgesia (Lutfy et al., 1997; Nishiyama et al., 1998; Olivar et al., 1999; McRoberts et al., 2001; Ji et al., 2001).

Our findings on the analgesic effects of ketamine are in agreement with animal data during acute visceral but not acute cutaneous pain, since parallel effects were observed in this study. One of the possible explanations could be the duration of the somatic stimulation used. One could argue that the tail-flick, graded pinch or hot-plate tests used in animal studies (Lutfy et al., 1997; Nishiyama et al., 1998; Olivar et al., 1999) produce very transient pain, which would be much shorter compared to the sensation produced by 30 sec long noxious stimuli used in our study. This stimulus duration could, in turn, potentiate sensitisation and/or "wind-up", which are known to be susceptible to NMDA-R blockers. Indeed, five subjects in our study showed signs of hyperalgesia in response to noxious cutaneous stimulation. Nevertheless, we did not see differential involvement of NMDA-Rs mediating cutaneous pain in sensitised versus non-sensitised subjects in our experiment, since both populations were equally affected by ketamine. This suggests that the findings seen here and in animal studies are probably due to a differential role of NMDA-Rs in the transmission of short thermal pain between animals and humans.

This idea is additionally supported by the significant analgesic effects of low-dose ketamine on acute somatic pain observed in two human studies (Arendt-Nielsen et al., 1996; Smith et al., 2001), and a high tendency noted in another human study (Ilkjaer et al., 1996). Therefore, based on these observations, it is plausible to assume that in humans, unlike animals, both acute visceral and acute cutaneous nociceptive transmission involves NMDA-receptors-mediated processes.

Nevertheless, species differences in transmission of acute somatic pain might not be so straightforward, since even in animals intraperitoneal ketamine seems to attenuate acute somatic nociceptive responses following tail-flick, radiant heat, hot plate and tail immersion tests (Iwasaki et al., 1991; Kawamata et al., 2000; Sarton et al., 2001). Yet intraperitoneal injection of drugs would affect both visceral and somatic afferents innervating the peritoneum, making it difficult to isolate the site of action.

4.5.2 Subjective Pain Responses

Despite the lack of differential effects of ketamine on visceral and cutaneous pain and unpleasantness perception in the VAS ratings, we observed some differences during the qualitative testing with the MPQ, which is a more subtle measure of pain quality. The main difference was seen in the affective and miscellaneous components of pain perception, which were diminished by ketamine during visceral but not during cutaneous pain.

Furthermore, our data suggest greater dissociation between sensory and hedonic value during visceral than cutaneous pain. This is evident from two findings: 1) unlike warmth, non-painful visceral pressure is highly unpleasant; 2) there is a higher correlation between intensity and unpleasantness ratings during cutaneous ($r = 0.82$) than during visceral ($r = 0.6$) stimulation. Therefore, despite a similar analgesic effect on unpleasantness ratings during visceral and cutaneous pain, which are expected to parallel the intensity ratings (Wade et al., 1996), in our study ketamine specifically targeted and attenuated the emotional valence of visceral pain, as measured by the MPQ. A high emotional component (e.g. anxiety, unpleasantness) associated with visceral pain (Strigo et al., 2002) is one of the main factors in its debilitating nature

observed clinically. Therefore, the fact that ketamine even at low-doses can attenuate the affect of visceral pain can potentially have significant clinical implications.

4.5.3 Subjective Side Effects

The major limiting factor in using ketamine for pain relief are its central nervous system side effects, which even under subanaesthetic dosages may include cognitive dysfunction, dizziness, blurred vision, hallucinations, paranoia, and/or balance disturbances (Ghoneim et al., 1985; Martin & Eisenach, 2001; Persson et al., 2002).

We did not observe serious side effects in our subjects at doses that produced significant acute pain reduction. Furthermore, recent studies by Schmid et al. (1999) and De Kock et al. (2000) concluded that ketamine doses resulting in post-operative analgesia do not cause more severe side effects than other commonly used analgesic drugs, such as opioids (Schmid et al., 1999; De Kock et al., 2001). We agree with the conclusions from these studies and even further support the use of ketamine as an analgesic drug, since at even lower doses than those used by Schmid et al. (1999), and De Kock et al. (2000), ketamine diminishes both acute visceral and acute cutaneous pain in humans.

4.5.4 Possible Mechanisms of Action

The exact mechanism of ketamine-produced analgesia is not presently known. Besides NMDA-R, ketamine is known to interact with several other receptor systems, including opioidergic (μ, δ, κ) cholinergic (muscarinic and nicotinic), and monoaminergic (Smith et al., 1980; Finck & Ngai, 1982; Pekoe & Smith, 1982; Hustveit et al., 1995), all of which could play a role in analgesic properties.

Ketamine interaction with NMDA-receptors is mediated via its binding to the phencyclidine binding site with a much higher affinity compared to other receptors or voltage-gated channels (Hustveit et al., 1995; Fisher et al., 2000), suggesting that at subanaesthetic doses, it is the primary binding site for ketamine and the one responsible for the resulting analgesia.

Some literature, however, argues that the analgesic effects of ketamine are unrelated to its binding to the NMDA-R, but rather are due to the activation of monoaminergic descending inhibitory pathways (Pekoe et al., 1982; De Kock et al., 2001). A recent study by Kawamata et al. (2000) clarified this issue to some extent by demonstrating that beneficial ketamine effects on inflammatory pain are NMDA-Rs mediated, while effects on acute, non-inflammatory pain involve the periaqueductal grey matter (PAG) descending inhibitory system (Kawamata et al., 2000). Since the stimuli used in our study most likely were not long enough to produce inflammation, the pain reduction seen here could be the result of activating the monoaminergic system. Administration of noradrenergic antagonists would be necessary to verify this conclusion.

Another mechanism possible leading to ketamine analgesia is an interaction with μ-opioid receptors. Several studies have examined this possibility using μ-opioid receptor antagonists, and obtained variable results. Some reports showed that ketamine analgesia is blocked by naloxone (Ryder et al., 1978; Pekoe et al., 1982; Crisp et al., 1991), while others did not demonstrate such inhibition (Fratta et al., 1980; Takahashi et al., 1987; Maurset et al., 1989; Mikkelsen et al., 1999), suggesting that ketamine analgesia is not a μ-opioid receptor-mediated process. Yet Hustveit et al. (1995), when examining an electrically induced contraction of guinea pig ileum preparation, showed partial naloxone antagonism, suggesting that μ-opioid receptors may be involved but are not the only source of ketamine-induced antinociception (Hustveit et al., 1995). This theory is somewhat strengthened by a recent study that examine the effects of S(+)ketamine in μ-opioid receptor knock-out mice (Sarton et al., 2001). Sarton et al. (2001) showed that spinal nociceptive mechanisms mediating tail-immersion tests cannot be blocked by the sole action of ketamine on NMDA-Rs but require an intact μ-opioid system, whereas supra-spinal nociceptive mechanisms mediating the hot-plate test can be blocked by ketamine action on NMDA-Rs alone (Sarton et al., 2001). We did not administer naloxone in our study, which would be necessary to nerify the importance of μ-opioid system in the analgesic effects observed in our experiment. These, in turn, may play a differential role in visceral versus cutaneous analgesia, if indeed the spinal component of NMDA-R induced analgesia were stronger in acute visceral compared to acute cutaneous pain as suggested previously (McRoberts et al., 2001; Ji et al., 2001).

4.6 CONCLUSION

The results of the present study demonstrate parallel analgesic effects of intravenous low-dose ketamine on visceral and cutaneous pain in healthy human subjects, and differential effects on affect associated with visceral and cutaneous pain. While the mechanism leading to attenuation of painful responses observed here cannot be precisely determined, central and peripheral action is a likely possibility. Finally, significant analgesia and minimal side effects observed at the administered doses support the use of this NMDA-R antagonist in a clinical setting for pain control.

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Chapter 5

FINAL CONCLUSIONS AND SUMMARY

Presented here is the majority of work I have performed in the comparison of two types of pain in humans; cutaneous pain – something that we all have experienced and know about, and visceral pain – something that is still an enigma of the physiological and psychological sciences. In fact, in 1935 William Kenneth Livingston wrote: "there is no subject in the field of medicine that offers the student more stirring problems than does that of visceral pain" (Livingston, 1935). Even though substantial progress has been accomplished in the field, visceral pain remains a "problem" three generations later. The focus of the above discussion, however, was to analyze thoroughly and systematically pain arising from viscera in relation to pain from the skin, directly compare the two at several different levels, and draw conclusions on *if*, *how* and *why* visceral pain is different from cutaneous pain in humans.

In the beginning I addressed the accuracy of the IASP definition of pain, suggesting that a stomachache should not be equated to a skin scratch. This suggestion was based on several types of evidence (e.g. differences in innervation, sensory receptors etc.), yet the strongest argument by far originated from personal experiences of others and myself. We know that we have stomachache: we feel it. Thus, despite the colossal amount of viscero-somatic convergence in the spinal cord and the brain, which undermines the specificity of visceral messages, visceral pain is different and distinguishable from pain arising from the skin. Moreover, I can now corroborate my argument with original findings described in the previous chapters, where we have seen that human visceral and cutaneous pain that feel equally intense, greatly diverge in several aspects, including perception, neural processing and pharmacology.

Specifically, Chapter 2 detailed psychophysical analyses showed that visceral pain induced by esophageal distention, when compared to cutaneous pain of similar intensity induced by

thermal heat stimulation, is associated with higher relative unpleasantness, anxiety and affect, as well as a more spatially diffuse sensation that persists after stimulus termination. In Chapter 3, the examination of cerebral activity with fMRI showed that visceral but not cutaneous pain activated bilateral inferior primary somatosensory cortex, bilateral primary motor cortex and a more anterior locus within the anterior cingulate cortex, while cutaneous but not visceral pain evoked higher activation in the bilateral anterior insula and, despite lower affective scores, resulted in activity in the right frontal lobe. Yet activation of a similar cortical network (including secondary somatosensory and parietal cortices, thalamus, basal ganglia, and cerebellum) was also observed following comparable visceral and cutaneous painful stimuli. Finally, in Chapter 4, intravenous ketamine administration demonstrated that low doses of a non-competitive NMDA-R antagonist decreased perception of noxious visceral and cutaneous stimulation in a similar and dose-dependent manner, while showing no effect on the perception of innocuous visceral and cutaneous stimuli. In addition, despite similar effects on the quantitative dimension, NMDA-receptors play a differential role in the qualitative aspect of visceral and cutaneous pain in humans.

5.1 Different Sensory Experience

Using psychophysical measures, we have established that visceral pain results in a different sensory experience than does cutaneous pain. Specifically, at a similar intensity visceral pain is more diffuse, persistent and poorly localized. Differences in the activation pattern in the primary somatosensory cortex, seen with fMRI, are probably responsible for the different localization of visceral pain. In other words, visceral activation of intra-abdominal SI, which is specific for the visceral stimulation, explains why we feel it in the first place, yet activation of trunk SI region, which is non-specific, explains the diffuse and poorly localized quality and radiation.

Likewise, slowly adapting responses to balloon distention of distention-sensitive afferent fibers (intensity-encoding) in the viscera (Cervero & Janig, 1992b; Sengupta, 2000) can explain the slower onset of visceral pain observed with on-line VAS. On the other hand, the activation of neurons showing sustained afterdischarges following termination of the distending stimulus can explain the slower offset of visceral compared to cutaneous pain. This type of cell has

been identified in the colon and the urinary bladder, yet is believed to exist in other visceral tissues (Ness & Castroman, 2001).

5.2 Different Emotional Experience

Using psychophysical measures, we also established that visceral pain is associated with different emotional experiences than is cutaneous pain. Specifically, for a similar intensity, visceral pain results in higher unpleasantness, anxiety and affect. Differences that we observed with fMRI in the activation pattern of the anterior cingulate and prefrontal cortices, the areas closely related to affect (Rainville et al., 1997; Teasdale et al., 1999), may be the underlying substrates of higher visceral unpleasantness.

The differences in emotional experience between visceral and cutaneous pain might also be related to a differential involvement of NMDA-receptors, especially at the central level. NMDA-receptors have a wide distribution in the central nervous system and the periphery (Hashimoto & Oka, 1997; Kinkelin et al., 2000; McRoberts et al., 2001). Specifically, they have been found in somatosensory, motor, cingulate, insular and prefrontal cortices (Wedzony & Czyrak, 1996; Shima & Tanji, 1998; Escobar et al., 1998; Kharazia & Weinberg, 1999). Almost all of these areas demonstrated activity during both visceral and cutaneous pain in our fMRI experiment. Therefore, similar effects of ketamine on both may be expected, if indeed ketamine exerts its analgesic action via the NMDA-receptor. Interestingly, similar effects of morphine and the μ-opioid receptor system on both intensity and unpleasantness to noxious, but not innocuous stimuli have been observed previously (Morin et al., 1999), consistent with parallel effects that we observed, suggesting similar mechanisms at the perceptual level. However, at the cortical level, dissociation between affective and sensory dimension in the μ-opioid receptor system has been shown with a ligand binding experiment, where sensory pain scores correlated with activity in thalamus, nucleus accumbens and amygdala, while affective pain scores correlated with activity in the anterior cingulate, thalamus, and nucleus accumbens (Zubieta et al., 2001). Therefore, it is plausible that the differential effect of ketamine on affective scores associated with visceral and cutaneous pain is the result of a differential activation within anterior cingulate and/or prefrontal cortices. Interestingly, a radiolabelled ligand study showed that NMDA-receptor antagonist increases the number of serotonergic

receptors in both frontal and cingulate cortices (Wedzony et al., 1997). Since serotonin plays a major role in visceral pathophysiology, and serotonin-related transmission is one of the main targets for functional visceral disorders associated with high discomfort (Gershon, 2000), it is possible that the decreased affect related to visceral pain following ketamine administration is due to its differential action on serotonergic transmission in cingulate and/or frontal cortices.

5.3 Final Conclusions

The work presented above indicates that visceral pain differs from cutaneous pain in several domains, and that the affective domain is likely the most significant. In addition, there is no single visceral pain center, as there is no cutaneous pain center, in contradiction to the specificity theory of pain proposed many years ago. On the contrary, a wide pattern of cortical areas is involved in the processing of visceral and cutaneous pain, consistent with the complexity of the pain experience. Differential activation within these and other areas clearly explains divergent reactions, and possibly diverse effective treatment, of visceral and cutaneous pain in humans.

Although, the existence of a single pain center would make the lives of individuals studying or experiencing pain much simpler – indeed, the target would be localized and easier to reach – there is still a vast number of opportunities to study the physiology and pharmacology of pain. In particular, drugs or alternative treatments that specifically target the emotional component of visceral pain might one day bring a relief to many people suffering from debilitating visceral pain syndromes.

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